Imperial College London

PhD Thesis - 2015-2019

Fertility Restoration

Benjamin P Jones MBChB BSc (Hons) MRCOG

Department of Surgery & Cancer

GMC number: 7039569 RCOG
College Number: 191294

PhD Student

Mr Benjamin P Jones MBChB BSc (Hons) MRCOG

Clinical Research Fellow

Division of Surgery and Cancer

Imperial College London, Hammersmith Hospital Campus

Du Cane Road, London, W12 0NN

Email: bpj15@ic.ac.uk; Tel: +44 (0) 7740358900

Supervisors

Mr J. Richard Smith MD FRCOG

Consultant Gynaecological Surgeon

West London Gynaecological Cancer Centre

Hammersmith Hospital

Du Cane Road, London, W12 ONN

Email: james.smith25@nhs.net; Tel: +44 (0)2077300431

Professor Sadaf Ghaem-Maghami PhD MRCOG

Clinical Senior Lecturer

Division of Surgery and Cancer

Institute of Reproductive & Developmental Biology

Imperial College London, Hammersmith Hospital Campus

Du Cane Road, London, W12 0NN

Email: s.ghaem-maghami@imperial.ac.uk; Tel: +44 (0)2083833267

Acknowledgements

There are many people who have contributed so much to my research in so many different ways. To Mr Smith, my primary supervisor, who has taught me so much, not just about research, or uterine transplantation or fertility preserving surgery, but so much more. When I temporarily left speciality training to undertake a PhD, in the context of me recently passed my membership exam I knew a lot of facts, and could regurgitate countless guidelines, but in the great scheme of things knew very little! Over the last 5 years he has nurtured me and helped me grow, by helping my brain evolve by giving me the insight to think 'outside the pelvis' and cross fertilise between disciplines and specialities. Most importantly he has taught me so much about love, respect, trust and honour, and for all of the above I will be eternally grateful. I know there will become a time when he will insist I call him Richard, but the respect and admiration I have for him means he will always be Mr Smith, or perhaps and deservedly Professor Smith!

'Dearest Dena', to which I introduced so many emails, and appropriately so! She is so dear to me, both personally and professionally. In the context of how busy she is, her unbelievable response rate and ability to get things done so quickly always astounds me. But after all the ups and downs, having her to reflect with, always helped me maintain the vision I had, to reach the goals I so desperately wanted to achieve. She is probably the one who has given so much, but received comparatively little in return, and I hope I manage to rectify the unbalance in the years to come!

To Joseph Yazbek, whose unwavering help, support and forward-thinking ideas gave me direction, and his wonderful work ethic and outlook on life gave me something to aspire to. He also introduced me to his wife, Jara Ben Nagi, a collaboration which has brightened my horizons and greatly potentiated my ambitions. Ms Ben Nagi is responsible for the elective oocyte cryopreservation aspect of this thesis, which is something I am now so passionate about. This was been made possible by her brilliant vision, excellent ideas and forward-thinking concepts, which I hope I can take forward, together with her, in the future.

To the team at the Polytechnic Institute in Valencia, Spain, who made the dream of endometrial transplantation turn to reality, I am hugely appreciative. Professor 'Paco' Jimenez especially, who was always so open minded and enthusiastic about our collaborations, and his hard work and support has been so important to me over this period of research. I am extremely grateful also to Ximo Garcia-Dominguez, Luís García-Valero, Professor Jose Vicente, and all the rest of the team, for their expertise, advice and amazing support with the animal studies. Further thanks also go to Dr Baljeet Kaur for her goodwill and help with histopathology.

To my friend Srdjan Saso, whose work I continued by taking uterine transplantation from research concept in an animal model, to clinical application in humans. To follow from someone who achieved so much, and published so prolifically, certainly inspired me to try to live up to his exceptional standards. He helped me learn the basics, and very quickly the more complex aspects of academia. More importantly, he contextualised research and made it exciting and worthwhile. He helped me see my research not as work that I used my brain for, but as a way of life that I felt in my heart. His motivation enabled me to truly feel the elation of manuscript acceptances, and despair at the rejections, which I inherently took so personally. Srdjan kept my feet on the ground, and helped me get back on the horse (laptop) when I needed him most. I am forever grateful for his help and support but most importantly his friendship, which I will forever cherish.

Finally, to those who I owe the most, my family; my wife Claire, and children Scarlett and Lucius. I am thankful for them putting up with the early morning phone calls, late-night responding to emails and the sheer time they lost me to my research. When I started my PhD, my primary motivation was to perform complex, novel and ground-breaking operations. If I am honest, the thought of uterine transplantation seemed quite absurd in the context of the associated risk, and the perceived availability of other options such as surrogacy. However, in the time I took undertaking this research, Claire conceived twice and gave birth to our two children. Together, we saw her abdomen grow, felt them move and kick, and held them both at birth, knowing our lives were changed inconceivably forever, with feelings of love and pride that I didn't know were possible prior to adulthood. The experiences are

etched into my heart where they will remain eternally, and I now truly understand the value of gestation; and I was only a bystander! The immeasurable emotion and pleasure they have all given me is indescribable and make the complexities and risks of uterine transplantation appear insignificant by comparison. I know truly empathise and understand the impact of infertility and have no regrets over committing my career to the cause of helping such women achieve their parenthood aspirations. Whilst my period of research has unquestionably been the best part of my career, I cannot pretend there weren't heart-breaking moments of disappointment, exhaustion or sheer frustration. In those times, it was my family who really kept me going, not just through their love and support but the realisation that whatever else happened didn't really matter, as long as I had them to come home to. This is not intended to belittle my research, but contrarily truly epitomises the significance of what I hope my research achieves.

Declaration of Originality

This thesis is submitted in fulfilment of the requirements for the degree of Doctor of Philosophy (PhD) at Imperial College London. The work presented herein is my own work except for where indicated.

Mr Benjamin P Jones MBChB BSc (Hons) MRCOG

March 2020

Copyright Declaration

The copyright of this thesis rests with the author. Unless otherwise indicated, its contents are licensed under a Creative Commons Attribution-Non Commercial 4.0 International Licence (CC BY-NC).

Under this licence, you may copy and redistribute the material in any medium or format. You may also create and distribute modified versions of the work. This is on the condition that: you credit the author and do not use it, or any derivative works, for a commercial purpose.

When reusing or sharing this work, ensure you make the licence terms clear to others by naming the licence and linking to the licence text. Where a work has been adapted, you should indicate that the work has been changed and describe those changes.

Abstract

Infertility not only results in the loss of reproductive function but has numerous psychological sequalae and is associated with significant long-term emotional burden. There is one group of women whose infertility was traditionally believed to be unconditional and absolute: those with absolute uterine factor infertility (AUFI). Whereas not absolute in nature, a major cause of subfertility, is the consequence to the physiological deterioration of ovarian reserve and oocyte quality that is associated with advancing age. This thesis summarises a series of interrelated studies which contribute significantly to the eventual aim of achieving fertility restoration in these groups.

Study 1 details extensive analysis of >200 women with AUFI who are interested in potentially undergoing uterine transplantation (UTx), which represents the largest study of its kind globally to date. It highlights important insights into the screening process of women with AUFI who desire to undergo the UTx, including their perceptions toward UTx and alternative options to acquire motherhood. Moreover, comprehensive pre-operative clinical, biochemical, radiological and psychological evaluation of the women selected to proceed with UTx is detailed, to allow comprehensive insight into this cohort.

Study 2 demonstrates novel insight into the motivation of women who wish to altruistically donate their uterus and highlights high levels of acceptability after becoming aware of the risks and expected recovery. Despite the significant associated risks and lengthy recovery process, women who donate their uterus expect to gain psychological and emotional benefit by enabling another woman to bear a child themselves. However, despite desire and motivation to donate, the selection criteria currently implemented universally reduce the number of potential donors significantly.

Study 3 presents novel data from 186 male to female (M2F) transgender women that clearly highlights a desire to experience physiological experiences that are unique to women, such as menstruation and gestation, as well as potentially having a physiologically functioning transplanted

vagina. The findings suggest transgender women believe the potential benefits of UTx outweigh the significant risks with which it is associated and may improve quality of life, happiness and dysphoric symptoms while enhancing feelings of femininity. This chapter also describes a series of feasibility studies including the demonstration of a technique to undertake transgender UTx in the rabbit model.

The final study on fertility restoration in AUFI introduces the concept of endometrial transplantation (ETx), including initial pilot studies that helped develop the initial surgical technique, as well as a short-term viability study, in endometrial autotransplantation. This study represents the first ETx trial undertaken globally, and demonstrated gross and microscopic evidence of viable endometrium following endometrial autotransplantation, along with the achievement of clinical pregnancies.

Finally, this thesis presents a detailed account of the prospect of elective oocyte cryopreservation, which currently represents an option to restore reproductive potential in women with age related reproductive decline. Novel insight is demonstrated into the perceptions of such women, having undergone the process, including quantitative analysis of how it impacts quality of life. Detailed outcomes of stimulation cycles are also presented, including new perspectives on markers of oocyte yield in such populations and reproductive outcomes including the attainment of livebirths.

Table of Contents

Publications and Presentations related and unrelated to Thesis **p.11**

Figure and Tables **p.21**

Abbreviations **p.27**

Introduction **p.30**

Adoption *p.34*
Surrogacy *p.36*
Uterine transplantation *p.39*

Critical review of human cases of uterine transplantation **p.43**

Case 1: Saudi Arabia *p.43*
Case 2: Turkey *p.47*
Cases 3-12: Sweden *p.51*
Case 13: China *p.55*
Case 14: Cleveland, USA *p.57*
Cases 15-23: Czech Republic *p.59*
Cases 24-29: Dallas, USA *p.63*
Cases 30-31: Germany *p.69*
Cases 32-35: India *p.72*

Overview of key aspects of uterine transplantation **p.78**

Recipients *p.78*
Donors *p.82*
Surgical considerations *p.87*
Immunological considerations *p.94*
Reproductive considerations *p.96*

Uterine Transplantation in male to female transgender women **p.111**

Psychosocial considerations *p.112*
Legal considerations *p.113*
Ethical considerations *p.115*
Anatomical considerations *p.117*
Hormonal considerations *p.122*
Reproductive considerations *p.123*
Obstetric considerations *p.125*
The future *p.126*

Elective oocyte cryopreservation **p.128**

Obstetric considerations *p.129*
Social factors *p.130*
Legal and Economic implications *p.132*

The Future *p.133*

Objectives of thesis **p.134**

Hypotheses **p.135**

Study 1a: Screening of uterine transplantation recipients **p.136**

Study 1b: Pre-operative investigation of potential uterine transplant recipients **p.160**

Study 2: Perceptions, acceptability and suitability of non-directed living uterine transplantation donors; a cross sectional study **p.181**

Study 3a: Uterine transplantation in transgender women; A cross-sectional survey assessing perceptions, acceptability and demand **p.200**

Study 3b: Uterine transplantation in transgender women; feasibility in animal models **p.215**

Study 4a: Preliminary pilot studies in endometrial transplantation **p.231**

Study 4b: Endometrial auto-transplantation in a rabbit model **p.245**

Study 5a: Elective oocyte cryopreservation; perceptions and regret following social egg freezing **p.264**

Study 5b: Elective oocyte cryopreservation; impact of social egg freezing upon quality of life **p.280**

Study 5c: Elective oocyte cryopreservation; laboratory, clinical and reproductive outcomes in social egg freezing **p.294**

The Future: The Future of Ovarian and Uterine Fertility Restoration **p.311**

Uterine Transplantation *p.312*
Tissue bioengineering *p.320*
Ovarian cortex cryopreservation *p.325*

Conclusion **p.329**

Appendices **p.334**

Peer Reviewed Publications related to Thesis

1. **Jones, BP**, Saso, S, Yazbek, J, Thum, M-Y, Quiroga, I, Ghaem-Maghami, S, Smith, JR, on behalf of the Royal College of Obstetricians and Gynaecologists. Uterine Transplantation. Scientific Impact Paper No. 65. *BJOG*. 2021; https://doi.org/10.1111/1471-0528.16697.
2. Kasaven LS, **Jones BP**, Keays R and Saso S. Anaesthetic considerations for fertility-sparing surgery and uterine transplantation. *Anaesthesia*. 2021 76: 46-55. DOI: 10.1111/anae.15389
3. **Jones BP,** Rajamanoharan A, Williams N, Vali S, Saso S, Mantrali I, Jalmbrant M, Thum MY, Diaz-Garcia C, Ghaem-Maghami S, Wilkinson S, Quiroga I, Friend P, Yazbek J and Smith JR. Uterine Transplantation Using Living Donation: A Cross-sectional Study Assessing Perceptions, Acceptability, and Suitability. *Transplantation Direct*. 2021: 7; 3 - p e673 doi: 10.1097/TXD.0000000000001124
4. **Jones BP**, Ranaei-Zamani N, Vali S, Williams N., Saso S, Thum MY, Al-Memar M, Dixon N, Rose G, Testa G, Johannesson L, Yazbek J, Wilkinson S and Smith JR (2021), Options for acquiring motherhood in absolute uterine factor infertility; adoption, surrogacy and uterine transplantation. *The Obstetrician & Gynaecologist.* 2021.
5. **Jones BP,** Rajamanoharan A, Vali S, Williams NJ, Saso S, Thum MY, Ghaem-Maghami S, Quiroga I, Diaz-Garcia C, Thomas P, Wilkinson S, Yazbek J, Smith JR. Perceptions and Motivations for Uterus Transplant in Transgender Women. *JAMA Netw Open*. 2021 Jan 4;4(1):e2034561. doi: 10.1001/jamanetworkopen.2020.34561.
6. **Jones BP**, Kasaven L, Vali S, Saso S, Jalmbrant M, Bracewell-Milnes T, Thum MY, Quiroga I, Friend P, Diaz-Garcia C, Ghaem-Maghami S, Yazbek J, Lees C, Testa G, Johannesson L, Jones B, Smith JR. Uterine Transplantation; Review of Livebirths and Reproductive Implications. *Transplantation*. 2020 Dec 10. doi: 10.1097/TP.0000000000003578.
7. **Jones BP**, Rajamanoharan A, Kasaven L, Jalmbrant M, Green J, Mahmoud M, Odia R, Saso S, Serhal P & Ben Nagi J. The novel use of fertility quality of life (FertiQoL) treatment subscale to

assess treatment acceptability in social egg freezing. *Hum Fertil* (Camb). 2020 Sep 3:1-9. DOI: 10.1080/14647273.2020.1815242

8. **Jones B.P**. Saso, S.; Bracewell-Milnes, T.; Thum, M.Y.; Nicopoullos, J.; Diaz-Garcia, C.; Friend, P.; Ghaem-Maghami, S.; Testa, G.; Johannesson, L.; Quiroga, I.; Yazbek, J.; Smith, J.R. Human Uterine Transplantation: A Review of Outcomes From the First 45 Cases, *Obstetric Anesthesia Digest*: 2020: 40: 3;121-122 doi: 10.1097/01.aoa.0000693664.92902.20
9. **Jones BP**, Saso S, Quiroga I, Yazbek J, Smith JR. Limited Availability of Deceased Uterus Donors: A UK Perspective. *Transplantation*: 2020; 104: 8; 250-251 doi: 10.1097/TP.0000000000003282
10. **Jones, B. P.,** Vali, S., Saso, S., Garcia-Dominguez, X., Chan, M., Thum, M. Y., Ghaem-Maghami, S., Kaur, B., García-Valero, L., Petrucci, L., Yazbek, J., Vicente, J. S., Quiroga, I., Marco-Jiménez, F., & Smith, J. R. (2020). Endometrial autotransplantation in rabbits: Potential for fertility restoration in severe Asherman's syndrome. *Eur J Obstet Gyn R B*, *248*, 14–23. https://doi.org/10.1016/j.ejogrb.2020.03.011
11. **Jones, B. P.,** Kasaven, L. , L'Heveder, A. , Jalmbrant, M. , Green, J. , Makki, M. , Odia, R. , Norris, G. , Bracewell Milnes, T. , Saso, S. , Serhal, P. and Ben Nagi, J. (2019), Perceptions, outcomes and regret following social egg freezing in the UK; a cross-sectional survey. Acta Obstet Gynecol Scand. Accepted Author Manuscript. doi:10.1111/aogs.13763
12. **Jones BP**, Saso S, Bracewell-Milnes T, Thum MY, Nicopoullos J, Diaz-Garcia C, Friend P, Ghaem-Maghami S, Testa G, Johannesson L, Quiroga I, Yazbek J, Smith JR. Human uterine transplantation: a review of outcomes from the first 45 cases. BJOG. 2019 Oct;126(11):1310-1319. doi: 10.1111/1471-0528.15863.
13. **Jones BP,** Saso S, Yazbek J, Smith JR. Re: UK Criteria for Uterus Transplantation: A review. 2019 BJOG: Int J Obstet Gy, 126: 1507-1508. doi:10.1111/1471-0528.15912
14. **Jones BP**, Saso S, L'Heveder A, Bracewell-Milnes T, Thum MY, Diaz-Garcia C, Quiroga I, Ghaem-Maghami S, Testa G, Johannesson L, MacIntyre D, Bennett P, Yazbek J, Smith JR. The vaginal microbiome in uterine transplantation. BJOG. 2019 2019 Aug 8. doi: 10.1111/1471-0528.15881.

15. **Jones BP**, Alghrani A, Smith JR. Re: Uterine Transplantation in Transgender Women: Medical, Legal and Ethical Considerations. BJOG. 2018 Nov 21. doi: 10.1111/1471-0528.15558.
16. **Jones BP,** Serhal P, Ben Nagi J. Social egg freezing: Early is not always best. Acta Obstet Gynecol Scand. 2018 Dec;97(12):1531. doi: 10.1111/aogs.13467.
17. **Jones BP**, Williams NJ, Saso S, Thum MY, Quiroga I, Yazbek J, Wilkinson S, Ghaem-Maghami S, Thomas P, Smith JR. Uterine Transplantation in Transgender Women. BJOG. 2018 Aug 20. doi: 10.1111/1471-0528.15438.
18. **Jones BP**, Serhal P, Ben-Nagi J. Social egg freezing should be offered to single women approaching their late thirties: FOR: Women should not suffer involuntary childlessness because they have not yet found a partner. BJOG. 2018 Aug 2. doi: 10.1111/1471-0528.15291.
19. **Jones BP,** Saso S, Mania A, Smith JR, Serhal P, Ben Nagi J. The dawn of a new Ice Age: social egg freezing. Acta Obstet Gynecol Scand. 2018 Feb 26. doi: 10.1111/aogs.13335.
20. Saso S, Clancy, N, **Jones BP**, Bracewell-Milnes T, Al-Memar M, Cannon EM, Ahluwalia S, Yazbek J, Thum MY, Bourne T, Elson DS, Smith JR & Ghaem-Maghami S. Use of biomedical photonics in gynecological surgery: a uterine transplantation model. Future Sci 2018 Feb. 10.4155/fsoa-2017-0129
21. **Jones BP**, Saso S, Yazbek J, Smith JR. Uterine transplantation: past, present and future. *BJOG: An International Journal of Obstetrics & Gynaecology* 2016. DOI: 10.1111/1471-0528.13963

Peer Reviewed Abstracts related to Thesis

1. Tiemann TT, **Jones BP,** Saso S, Yazbek J, Rom J, Smith JR, Schott S, 2019, Perceptions on uterine transplantation among obstetricians and gynaecologists across Europe. BJOG, Pages: 125-125, ISSN: 1470-0328
2. **Jones BP**, Odia R, Green J, Petrie A, Norris G, Saso S, Serhal P, Ben-Nagi J, 2019, Predictors of oocyte yield in women embarking on social egg freezing. BJOG: 205-205, ISSN: 1470-0328
3. **Jones BP,** Saso S, Chan M, Yazbek J, Bracewell-Milnes T, Thum M-Y, Ghaem-Maghami S, Quiroga I, Friend P, Smith JR, 2019, Implementing uterine transplantation into multi-organ retrieval setting: specialist nurse in organ donation and intensive care nurse perspectives, BJOG: 195-195, ISSN: 1470-0328
4. **Jones BP**, Saso S, Chan M, Bracewell-Milnes T, Thum M-Y, Yazbek J, Ghaem-Maghami S, Quiroga I, Friend P, Smith JR, 2019, Uterine transplantation in the context of a multi-organ retrieval in the UK. BJOG, 192-192, ISSN: 1470-0328
5. Saso S, Al-Memar M, **Jones BP**, Chan M, Bracewell-Milnes T, Patterson E, Thum M-Y, Yazbek J, Smith RJ, Bourne T, 2019, Performing ultrasonography in patients awaiting uterine transplantation: the UK cohort. BJOG: 127-127, ISSN: 1470-0328
6. Chan M, Keane T, **Jones BP,** Smith JR, Saso S, Stevens M, 2019, Endometrial extracellular matrix as a biomaterial for endometrial regeneration. BJOG: 204-204, ISSN: 1470-0328
7. Chan M, Keane T, **Jones BP,** Smith JR, Stevens M, Saso S. 2019, Bioengineering approaches for female fertility preservation and infertility treatment. BJOG: 196-196, ISSN: 1470-0328
8. Jalmbrant M, **Jones BP**, Saso S, Bacarese-Hamilton T, van den Akker O, Smith R. A survey of the psychological attributes of women waiting for uterus transplants: General and fertility-specific quality of life and psychological distress,: Wiley: 2017; 10-11, ISSN: 1470-0328
9. Jalmbrant M, **Jones BP**, Saso S, van den Akker O, Smith R 2017, Developing a psychological screening protocol for the UK uterine transplant trial. Wiley. 2017 10-10, ISSN: 1470-0328
10. Saso S, **Jones BP**, Clancy N, Tziraki M, Al-Memar M, Bracewell-Milnes T, Thum M-Y, Elson D, Smith JR. Imaging of organ viability during deep pelvic surgery: The use of biomedical

photonics to assess uterine perfusion and vessel integrity in uterine transplantation models. Stream 4 - Ultrasound and Imaging (FC4). BJOG: Int J Obstet Gy. 2016 123: 19–23. doi:10.1111/1471-0528.14083

11. Saso, S., Clancy, N.T., **Jones, BP.,** Al-Memar, M., Bracewell-Milnes, T., Thum, M., Bourne, T., Elson, D. and Smith, J. OC13.05: Assessing uterine graft viability using multispectral imaging during uterine transplantation. Ultrasound Obstet Gynecol 2015; 46: 29. doi:10.1002/uog.15036
12. Saso, S., Clancy, N.T., Tziraki, M., **Jones, B.,** Al-Memar, M., Bracewell-Milnes, T., Thum, M., Bourne, T., Elson, D. and Smith, J. OC24.07: Use of endoscopic laser speckle contrast analysis to assess uterine perfusion and blood flow in a uterine transplantation model. Ultrasound Obstet Gynecol 2015; 46: 53. doi:10.1002/uog.15106

Presentations related to Thesis

Oral Presentations

1. **Jones BP.** Uterine Transplantation. British Fertility Society (BFS) Virtual Study week. 15th June 2020.
2. **Jones BP**. & Smith JR. Uterine Transplantation; the past, present and future. The ethics and regulation of reproductive donation, Lancaster. 12th September 2019
3. **Jones BP.** Uterine transplantation in the context of a multi-organ retrieval. RCOG World Congress. London, UK. (**Prize - Best oral presentation)**
4. **Jones BP.** Uterine Transplantation. British Fertility Society (BFS) Study week. 6th June 2019
5. **Jones BP.** Uterine Transplantation. London School of Obstetrics & Gynaecology (LSSOG) annual conference 8th March 2019
6. **Jones, BP** & Smith JR. Uterine Transplantation. NHSBT / BTS Multi-organ retrieval masterclass. 8th January 2019
7. **Jones BP.** Uterine Transplantation. British Fertility Society (BFS) Study week. 5th June 2018.
8. **Jones, BP** & Smith JR. Uterine Transplantation. NHSBT / BTS Multi-organ retrieval masterclass. 19th December 2017
9. **Jones, BP** Uterine Transplantation. British Society of Histocompatibility and Immunogenetics (BSHI) Annual Conference 2017. 3rd October 2017
10. **Jones, BP.** Uterine Transplantation. Medical Student lecture for MSc in Reproductive Health University College London. 25th April 2017
11. Jalmbrant, M., **Jones, B.,** Saso, S., van den Akker, O., Smith, R. (2017) Developing a psychological screening protocol for the UK uterine transplant trial. RCOG 2017 Congress. Cape Town. 20-22 March.
12. Jalmbrant, M., Bacarese-Hamilton, T., **Jones, B.,** Saso, S., van den Akker, O., Smith, R. (2017) A survey of the psychological attributes of women waiting for Uterus Transplants: general and

fertility specific quality of life and psychological distress. RCOG 2017 Congress. Cape Town. 20-22 March.

13. **Jones, BP**. & Smith JR. 'Uterine Transplantation'. BTS RINTAG Stakeholder Day. London. 19th January 2017
14. **Jones, BP**, Saso S, Smith, JR. 'Psychological issues associated with absolute uterine factor infertility and attitude of patients towards uterine transplantation'. British Transplant Society Annual Congress, Harrogate. 2nd March 2017
15. Smith JR & **Jones BP**, 'The Ethics of Uterine Transplantation.' British Transplant Society Annual Congress, Harrogate 2nd March 2017
16. Smith JR & **Jones BP**, 'Uterine Transplantation'. Clinical Trials Forum. British Transplant Society Annual Congress, Harrogate. 3rd March 2017.
17. **Jones, BP**. & Smith JR Uterine Transplantation; In the context of Multi-organ Retrieval. South East Collaborative Meeting. 6th December 2016
18. **Jones, BP**. Uterine Transplantation; In the context of Multi-organ Retrieval. South Central Collaborative Meeting, Oxford. 14th November 2016
19. **Jones BP**. & Smith JR. UK Uterine Transplantation Programme, NHSBT RINTAG Meeting, London. 8th November 2016
20. **Jones BP**. & Smith JR. Uterine Transplantation. Uterine Transplantation Ethics Symposium, Lancaster. 16th September 2016
21. Saso S, **Jones BP**, Clancy N, Tziraki M, Al-Memar M, Bracewell-Milnes T, Thum M-Y, Elson D, Smith JR. Imaging of organ viability during deep pelvic surgery: The use of biomedical photonics to assess uterine perfusion and vessel integrity in uterine transplantation models. *RCOG World Congress*. Birmingham, UK.
22. Saso S, **Jones BP**, Bracewell-Milnes T, Al-Memar M, Thum MY, Yazbek J, Clarke A, Smith JR. Attitudes of Patients towards Uterine Factor Infertility and UTx: A Face-to-Face Consultation. *RCOG World Congress*. Birmingham, UK.

23. Saso S, **Jones BP,** Yazbek J, Thum MY, Smith JR. Uterine Transplantation in the UK: The move from the Animal Model into the Human Setting – Surgical, Reproductive & Clinical Aspects. *RCOG World Congress*. Birmingham, UK.
24. **Jones BP**. & Smith JR. UK Uterine Transplantation Programme, NHSBT NODC Meeting, London. 14th June 2016
25. **Jones BP**. & Smith JR. UK Uterine Transplantation Programme, NHSBT RINTAG Meeting, London. 10th May 2016
26. **Jones, BP.** Uterine Transplantation. Medical Student lecture for MSc in Reproductive Health University College London. 26th April 2016
27. **Jones, BP**. & Smith JR. Uterine Transplantation; Context of Multi-organ Retrieval. National Retrieval Group Meeting, London. 9th March 2016
28. **Jones, BP**. Uterine Transplantation in the UK. Inaugural International Society of Uterine Transplantation Conference. Gothenburg, January 2016.
29. **Jones, BP.** Womb for rent. Innermost Learning Symposium. Cardiff, Wales. 24th November 2015 (**Prize - Most highly rated lecture**)
30. **Jones, BP.** Fertility Preservation and Restoration. Medical Student lecture for BSc Students Surgery and Anaesthesia Imperial College London. 6th November 2015.
31. Saso S, Clancy N, **Jones B,** Al-Memar M, Bracewell-Milnes T, Thum M, Bourne T, Elson DS, Smith J. Assessing uterine graft viability using multispectral imaging during uterine transplantation, 25th World Congress on Ultrasound in Obstetrics and Gynecology (ISUOG), Montreal, Canada.
32. Saso S, Clancy N, Tziraki M, **Jones B**, Al-Memar M, Bracewell-Milnes T, Thum M, Bourne T, Elson DS, Smith J Use of endoscopic laser speckle contrast analysis to assess uterine perfusion and blood flow in a uterine transplantation model, 25th World Congress on Ultrasound in Obstetrics and Gynecology (ISUOG), Montreal, Canada.

Poster Presentations

1. **Jones BP,** Jalmbrant M, Green J, Norris G, Odia R, Saso S, Serhal P, Ben Nagi J. Perceptions, outcomes and regret following social egg freezing in the UK. ESHRE Annual Meeting 2019. Vienna, Austria.
2. Green J, **Jones BP,** Jalmbrant M, Norris G, Odia R, Saso S, Serhal P, Ben Nagi J. Predictors of oocyte yield in social egg freezing. ESHRE Annual Meeting 2019. Vienna, Austria.
3. **Jones BP,** Tiemann T, Saso S, Yazbek J, Rom J, Smith JR, Schott S. Perceptions on Uterine Transplantation amongst Obstetricians and Gynaecologists across Europe. RCOG World Congress 2019. London, UK.
4. **Jones BP,** Saso S, Bracewell-Milnes T, Nicopoullos J, Thum MY, Diaz-Garcia C, Friend P, Ghaem-Maghami S, Quiroga I, Yazbek J, Smith JR. Human Uterine Transplantation. RCOG World Congress 2019. London, UK.
5. **Jones BP,** Odia R, Green J, Petrie A, Norris G, Saso S, Serhal P, Ben Nagi J. Predictors of oocyte yield in women embarking on social egg freezing. RCOG World Congress 2019. London, UK.
6. **Jones BP,** Jalmbrant M, Green J, Norris G, Odia R, Saso S, Serhal P, Ben Nagi J. The novel use of the Fertility Quality of Life (FertiQoL) treatment subscale in social egg freezers. RCOG World Congress 2019. London, UK.
7. Saso S, Al-Memar M, **Jones B,** Chan M, Bracewell-Milnes T, Patterson E, Thum M-Y, Yazbek J, Smith RJ, Bourne T. Performing ultrasonography in patients awaiting uterine transplantation: the UK cohort. RCOG World Congress 2019. London, UK.
8. Chan M, Keane T, **Jones B,** Smith JR, Stevens M, Saso S. Bioengineering approaches for female fertility preservation and infertility treatment. RCOG World Congress 2019. London, UK.
9. Tiemann TT, **Jones BP**, Saso S, Yazbek J, Rom J, Smith JR, Schott S, 2019, Perceptions on uterine transplantation among obstetricians and gynaecologists across Europe. RCOG World Congress 2019. London, UK.
10. Chan M, Keane T, **Jones B,** Smith JR, Saso S, Stevens M. Endometrial extracellular matrix as a biomaterial for endometrial regeneration. RCOG World Congress 2019. London, UK.

11. Saso, S., Al-Memar, M., **Jones, B.,** Bracewell-Milnes, T., Thum, M., Yazbek, J., Smith, J. and Bourne, T. (2017), OC04.05: Pelvic ultrasonography of patients awaiting uterine transplantation: the UK cohort. Ultrasound Obstet Gynecol, 50: 8. doi:10.1002/uog.17589
12. Saso S, **Jones BP**, Bracewell-Milnes T, Al-Memar M, Thum MY, Yazbek J, Smith JR. A UK survey assessing the perceptions of healthcare professionals towards uterine transplantation: Level of support amongst colleagues. British Transplant Society Annual Congress, Harrogate. 1st- 3rd March 2017.
13. **Jones BP**, Saso S, Jalmbrant M, Yazbek J, Smith JR 'The value of gestation; Patient perspectives of uterine transplantation. British Transplant Society Annual Congress, Harrogate. 1st- 3rd March 2017.
14. **Jones BP**, Saso S, Yazbek J, Giele H, Quiroga I, Friend P, Smith JR Uterine transplantation in the UK; SNOD and ITU nurse perspectives. British Transplant Society Annual Congress, Harrogate. 1st- 3rd March 2017.
15. Saso S, **Jones BP,** Thum MY, Yazbek J, Ghaem-Maghami S, Smith JR. The immunology of uterine transplantation: Applying the animal model to the human setting. British Transplant Society Annual Congress, Harrogate. 3rd March 2017.
16. Saso S, **Jones BP,** Thum MY, Yazbek J, Smith JR. Uterine transplantation in the United Kingdom: The move from the animal model into the human setting. British Transplant Society Annual Congress, Harrogate. 1st- 3rd March 2017.
17. **Jones BP**, Saso S, Jalmbrant M, Yazbek J, Smith J R. Fertility options in Absolute Uterine Factor Infertility; Patients' perspective. RCOG World Congress. Birmingham, UK.
18. Saso S, **Jones BP,** Thum MY, Del Priore G, Yazbek J, Smith JR. Endoscopic Laser Speckle Contrast Analysis: A Novel Method to Assess Blood Flow in Gynecologic Surgery. San Diego, USA.

Figures and Tables

Figures

Figure 1: *Classification of ovulation disorders, as defined by the World Health Organisation……p.30*

Figure 2: *Causes of absolute uterine factor infertility……p.33*

Figure 3: *Geographical representation of international regulation of surrogacy……p.38*

Figure 4: *Chronological representation of uterine transplantation cases performed……p.41*

Figure 5: *Uterine transplant retrieval technique demonstrating bilateral long internal iliac arteriovenous pedicles……p.89*

Figure 6: *Uterine transplant graft following retrieval (not including optional ovarian veins)……p.89*

Figure 7: *Recipient anatomy following implantation demonstrating bilateral internal iliac to external iliac arterial and venous anastomoses……p.90*

Figure 8: *Diagrammatic representation of uterine graft utilising the modified surgical technique with internal iliac arterial input and utero-ovarian venous drainage……p.91*

Figure 9: *Recipient anatomy following implantation of uterine graft demonstrating internal iliac to external iliac arterial anastomoses, and utero-ovarian to external iliac venous anastomoses……p.92*

Figure 10*: Proposed utero-vaginal retrieval operation with main dissection markings including long vascular pedicles to origin of the anterior division of the internal iliac vessels, and a long vaginal cuff……p119*

Figure 11: *Proposed utero-vaginal transplant graft following retrieval……p.120*

Figure 12: *Male to female transgender anatomy following utero-vaginal transplantation demonstrating vascular and vaginal anastomoses……p.120*

Figure 13: *Proposed ligamentous support following implantation……p.121*

Figure 1a.1: *Inclusion and exclusion criteria for potential recipients in INSITU study……p.139*

Figure 1a.2: *Considerations, attempts and success at other options to acquire motherhood……p.147*

Figure 1a.3: *Reasons attributed to preference of uterine transplantation over adoption……p.148*

Figure 1a.4: *Reasons attributed to preference of uterine transplantation over surrogacy…… p.148*

Figure 1a.5: *Flowchart summarising process of systematic exclusion after implementing INSITU selection criteria ……..p.149*

Figure 1b.1: *Questionnaires utilised to help assess psychological wellbeing in selection process ……..p.165*

Figure 2.1: *Motivating factors for consideration for uterine donation……..p.191*

Figure 2.2*: Perceived importance of each factor influencing motivation to donate……..p.193*

Figure 2.3: *Systematic process of exclusion utilising the selection criteria for the UK living donor uterine transplantation programme……..p.195*

Figure 3a.1: *Contributing factors that influence motivation and desire to undergo uterine transplantation in male to female transgender women……p.209*

Figure 3a.2: *Perceived importance of each factor influencing motivation quantified to undergo uterine transplantation in male to female transgender women……..p.210*

Figure 3a.3: *Reasons attributed to influence decision to not cryopreserve sperm……p.212*

Figure 3b.1: *Uterine graft including two uterine horns, the main body of the uterus, a long vaginal cuff, the parametrium and surrounding vasculature to the level of the aorta and IVC……..p.223*

Figure 3b.2: *Vertical incision on scrotum, exposing the testis……..p.224*

Figure 3b.3: *Horizontal incision on testicular sac (top), with subsequently opening of proposed neovagina that leads through inguinal canal into the abdominopelvic cavity……p.225*

Figure 3b.4: *Creation of neovulva and external aspect of neovagina……..p.226*

Figure 3b.5 – *Exposing the aorta and inferior vena cava……..p.227*

Figure 3b.6 – *Neovaginal anastomosis.……..p.228*

Figure 3b.7: *Demonstration of cannulation placement to confirm patency of neovagina following neovaginal anastomosis.……p.229*

Figure 4a.1: *Midline incision of unilateral uterus (left) and attempted excision of endometrium as a tubular structure (right).……p.237*

Figure 4a.2: *Identification of endometrium (left) with subsequent resection of the endometrium from the underlying myometrium (right) ……p.238*

Figure 4a.3: Excision of the endometrium (left) and the *resected specimen post retrieval (right).p.238*

Figure 4a.4: *Midline incision (left), identification of endometrium (centre) and excised endometrium (right) ……p.239*

Figure 4a.5: *Midline incision (left), identification of uterus (centre) and use of atraumatic grasper to reveal section of uterus (right).……p.240*

Figure 4a.6: *Midline incision on uterine horn (left), identification and resection of endometrium (centre) and excised endometrium (right)……p241*

Figure 4a.7: *Horn of uterus closed, and haemostasis achieved……p.242*

Figure 4a.8: *Histological analysis of endometrial samples excised using surgical technique (LE; Luminar epithelium, SF; Stratum functionalis, SB; Stratum basalis, SM; Submucosa)……p.242*

Figure 4a.9: *Macroscopic appearance of endometrium removed from myometrium (top) and laid out ex-vivo (bottom) ……p.243*

Figure 4a.10: *Demonstration of increased uterine size 5 days post ovulation, compared to no preceding ovulation……p.245*

Figure 4a.11 – *Demonstration of increased uterine size and vascularity, in addition to endometrial thickness associated with post-ovulatory appearances……p.246*

Figure 4b.1: *Study design……p.251*

Figure 4b.2: *Endometrial transplantation surgical technique……p.253*

Figure 4b.3: *A) Demonstration of post-operative ultrasound technique and B) Measurement of maximal mid-sagittal uterine depth……p.254*

Figure 4b.4: *Comparison of haematological parameters and C-reactive protein (CRP) trends during first 96 hours between endometrial transplantation and endometrial resection groups…… p.258*

Figure 4b.5: *Comparison of maximum uterine depth between un-operated control uteri, endometrial transplantation and endometrial resection groups throughout study……p.259*

Figure 4b.6: *Laparoscopic identification of implantation during first IVF cycle, with evidence of pregnancies in both ETx uteri (A&B), no evidence of pregnancy in the ER uteri (C), and confirmation of pregnancies in the control uteri (D-F) ……p.260*

Figure 4b.7: *Laparoscopic identification of implantation during second IVF cycle, with evidence of pregnancies in one ETx uterus (A), no evidence of pregnancy in the other ETx or the ER uteri (B-C), and confirmation of pregnancies in the control uteri (D-F)……..p.261*

Figure 4b.8: *Comparison of the macroscopic appearance of the uterine cavity between A) 48 hours post endometrial transplantation and B) 96 hours post endometrial transplantation……p.262*

Figure 4b.9: *Comparison of the macroscopic appearance of the uterine cavity between A) an un-operated control uterus, B) 6 months post-operatively after IVF, C) 6 months post-operatively after IVF with presence of fetal tissue and D) 6 months after endometrial resection……..p.263*

Figure 4b.10: *Comparison of the macroscopic appearance of the uterine cavity between A) an un-operated control uterus, B) 6 months post-operatively after IVF, C) 6 months post-operatively after IVF with presence of fetal tissue and D) 6 months after endometrial resection……..p.264*

Figure 5a.1: *Contributing factors that influenced decision to undergo social egg freezing……p.272*

Figure 5a.2: *Perceived importance of each factor influencing decision across the participants…p.273*

Figure 5a.3: *Participants' perception of their chances of achieving a livebirth……p.274*

Figure 5b.1. *FertiQoL treatment module in context of social egg freezing……p.285*

Figure 5b.2. *Demographics of women undergoing social egg freezing……..p.288*

Figure 5c.1: *Estimated chance of livebirth utilising counselling tool by Goldman et al.2017, which quantities probability of livebirth based upon age and number of oocytes cryopreserved……p.304*

Figure 5c.2: *Number of patients per age group at first thaw cycle (successful livebirths vs unsuccessful cycles)……p.305*

Tables

Table 1: *Summary of recipient demographics and surgical details (Amended from Jones et al. 2019)……p.80*

Table 2: *Summary of donor demographics, surgical details and donor outcomes in cases to date (Amended from Jones et al 2019)……p.86*

Table 3: *Estimations of numbers of women of reproductive age undergoing solid organ transplantation, based on NHSBT annual figures……p.97*

Table 4: *Livebirths following UTx summary including neonatal outcome……p.99*

Table 1a.1: *Scoring system used to select recipients to proceed……p.140*

Table 1a.2: *Basic Demographic information of potential uterine transplant recipients……p.142*

Table 1a.3: *Indication for prior hysterectomy……p.143*

Table 1a.4: *Medical history of women seeking uterine transplantation……p.145*

Table 1a.5: *Psychological history of women seeking uterine transplantation……p.146*

Table 1a.6: *Previously reported pregnancies in women with non-physiological vaginas (Re-used with permission from Jones et al. 2019)……p.155*

Table 1b.1: *Serum biochemistry results for participants……p.168*

Table 1b.2: *Serum virology results for participants……p.169*

Table 1b.3: *WHOQOL-BREF, SF36 and HADS results for participants……p.171*

Table 1b.4: *FertiQoL Core, BMQ general, DAS, marital taxon and mMOS-SS scores for participants ……p.172*

Table 2.1: *Basic demographic information of uterine donor cohort……p.187*

Table 2.2: *Obstetric history of study cohort……p.188*

Table 2.3: *Medical history of study cohort……p.189*

Table 2.4: *Donor screening criteria for UK living donor uterine transplantation programme……p.196*

Table 3a.1: *Basic demographic information of uterine donor cohort……..p.207*

Table 4b.1: *Surgical data and post-operative data including reproductive outcomes……..p.257*

Table 5a.1: *Demographic data of women undergoing social egg freezing……..p.271*

Table 5a.2: *Participants perceptions to the future following social egg freezing……..p.276*

Table 5a.3: *Summary of studies that assess perceptions towards SEF across varying populations ……..p.278*

Table 5b.1: *Participants responses to FertiQol treatment module environment subscale ……..p.289*

Table 5b.2: *Participants responses to FertiQol treatment module tolerability subscale……..p.290*

Table 5b.3. *Subgroup analysis of FertiQol Treatment scores. ……..p.292*

Table 5b.4*: Summary of studies using FertiQoL treatment module in infertile populations……p.293*

Table 5c.1: *Demographics, baseline clinical data and stimulation outcomes……p.301*

Table 5c.2: *The incidence rate ratio (IRR), confidence interval and p-values to assess the effect of each variable on oocyte yield……..p.302*

Table 5c.3: *Mann Whitney U and confidence interval and p-values to assess the effect of each variable on oocyte yield on OHSS and Non-OHSS groups……..p.303*

Table 5c.4: *Predicted average chance of livebirth per age group……p.304*

Abbreviations

AFC	*Antral follicle count*
AMA	*Advanced maternal age*
AMH	*Anti-Mullerian hormone*
ART	*Assisted reproductive technologies*
AS	*Asherman's syndrome*
ATG	*Anti-thymocyte globulin*
AUFI	*Absolute uterine factor infertility*
BMI	*Body mass index*
BD	*Twice daily*
BP	*Blood pressure*
CAIS	*Complete androgen insensitivity syndrome*
CIN	*Cervical intraepithelial neoplasia*
CIT	*Cold ischaemic time*
CMV	*Cytomegalovirus*
CRP	*C reactive protein*
CTA	*Computed tomography angiography*
DAS	*Dyadic adjustment scale*
DBD	*Donation after brainstem death*
DUETs	*Dallas UtErine Transplant Study*
E2	*Estradiol*
EBL	*Estimated blood loss*
EBV	*Epstein Barr Virus*
ECM	*Extracellular matrix*
EOC	*Elective oocyte cryopreservation*
ER	*Endometrial resection*
ETx	*Endometrial transplantation*
F2M	*Female to male*
FBC	*Full Blood Count*
FSH	*Follicle stimulating hormone*
G&S	*Group & Save*
GDM	*Gestational diabetes*
GnRH	*Gonadotrophin releasing hormone*
GRS	*Gender reassignment surgery*
GUM	*Genitourinary medicine*
HADS	*Hospital anxiety and depression scale*
HCG	*Human chorionic gonadotrophin*
HFEA	*Human Fertilisation and Embryology Authority*
HIV	*Human immunodeficiency virus*
HLA	*Human leukocyte antigen*
HPV	*Human papilloma virus*
HR	*Heart rate*
HRA	*Human Research Authority*
HRT	*Hormone replacement therapy*

HVS	*High vaginal swab*
ICG	*Indocyanine green*
ICSI	*Intra-cytoplasmic sperm injection*
INSITU	*INvestigational Study Into Transplantation of the Uterus*
ISUTx	*International Society of Uterine Transplantation*
IUA	*Intra-uterine adhesions*
IV	*Intravenous*
IVF	*In-vitro fertilisation*
LD	*Living donation*
LFTs	*Liver Function Tests*
LH	*Luteinising Hormone*
LSCS	*Lower section Caesarean section*
M2F	*Male to female*
MDT	*Multi-disciplinary team*
MMF	*Mycophenolate mofetil*
mMOS-SS	*Eight-item modified Medical Outcomes Study Social Support Survey*
MRI	*Magnetic resonance imaging*
MRKH	*Mayer-Rokitansky-Küster-Hauser syndrome*
MRA	*Magnetic resonance angiography*
MSI	*Multi-spectral imaging*
NHSBT	*National Health Service Blood & Transplant*
NODC	*National Organ Donation committee*
NRG	*National Retrieval Group*
OC	*Obstetric cholestasis*
OD	*Once daily*
OFI	*Ovarian factor infertility*
OHSS	*Ovarian hyper stimulation syndrome*
OTC	*Ovarian tissue cryopreservation*
PCR	*Polymerase chain reaction*
PGT-A	*Pre-implantation genetic testing for aneuploidies*
POI	*Premature ovarian insufficiency*
PPROM	*Premature prelabour rupture of membranes*
QoL	*Quality of life*
RAT	*Radical abdominal trachelectomy*
RCOG	*Royal College of Obstetricians and Gynaecologists*
REC	*Research Ethics Committee*
RINTAG	*Research and INnovation Technology Advisory Group*
RR	*Respiratory rate*
SaBTO	*Safety of Blood, Tissues and Organs*
SC	*Subcutaneous*
SD	*Standard deviation*
SEF	*Social egg freezing*
SGA	*Small for gestational age*
SIS	*Small intestine submucosa*
SNOD	*Specialist nurse in organ donation*

SOT	*Solid organ transplantation*
TORCH	*Toxoplasmosis, rubella, cytomegalovirus, herpes simplex & HIV*
TB	*Tuberculosis*
U&Es	*Urea & Electrolytes*
UC-MSCs	*Umbilical cord-derived mesenchymal stem cells*
UK	*United Kingdom*
USS	*Ultrasound Scan*
USA	*United States of America*
UTI	*Urinary tract infection*
UTx	*Uterine Transplantation*
UW	*University of Wisconsin*
VTE	*Venous thromboembolism*
WBC	*White blood cells*
WIT	*Warm ischaemic time*

Introduction

Infertility has been estimated to affect one in seven couples in the United Kingdom (UK).[1] The term *infertility* in this context is potentially inaccurate, underpinned by the widely used clinical definition of infertility as "a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse".[2] The majority of women who are thereby clinically diagnosed as *infertile* may well have *disease* of the reproductive system that is impacting their ability to conceive, but the majority are not absolutely infertile. This inaccurate nomenclature is exemplified by the admission that approximately half of those individuals who do not conceive within the first 12 months, will do so in the following 12 months.[1] Pathology affecting the ovaries, Fallopian tubes, uterus, cervix and vagina may impact the ability to conceive. However, many of those who are infertile, have *disease* which is treatable, either surgically or by utilising artificial reproductive technologies, to restore fertility.

Group I:	*Hypothalamic pituitary failure*
Group II	*Hypothalamic-pituitary-ovarian dysfunction*
Group III:	*Ovarian failure*

Figure 1: *Classification of ovulation disorders, as defined by the World Health Organisation*[1]

As defined by the World Health Organisation, there are three main categories of ovulation disorder which are summarised in **Figure 1.**[1] Women with hypothalamic-pituitary failure may restore their fertility by moderating their energy balance if they are in a negative chronic energy balance or increasing their body weight if their body mass index (BMI) is <19kg/m^2. Otherwise ovulation induction can be undertaken with either gonadotrophin releasing hormone (GnRH) or gonadotrophins.[1] Those women with hypothalamic-pituitary-ovarian dysfunction, a group which

predominantly consists of women with polycystic ovarian syndrome, may restore ovulation with conservative measures such as weight loss if the BMI is >30kg/m^2.[3] Alternatively, ovulation can be induced using clomiphene citrate,[4] letrozole,[5] or metformin.[6] In those where these are ineffective, laparoscopic ovarian drilling may successfully restore ovulation,[7] or a combination of clomiphene citrate and metformin,[6] or gonadotrophins can be used for ovulation induction,[8] Conversely, in women with ovarian failure, there are no current ways to reliably restore fertility; introducing a group of women with ovarian factor infertility (OFI). Such women may have been rendered infertile by premature ovarian insufficiency (POI), which may be secondary to immunological, surgical or oncological aetiologies, or as a consequence of the physiological decline in oocyte quality and quantity. In the UK, there are now more women over the age of 45 years using donor oocytes than their own,[9] and given the ongoing global trend of deferring motherhood, it is likely OFI rates will increase in the future. Elective oocyte cryopreservation, or ovarian cortex cryopreservation, offers the capability to restore fertility, whilst enabling biological relation, but requires the foresight to plan and undertake the treatment, at a time when the ovary is still fully functioning. While considered experimental, novel techniques such as the use of tissue engineered scaffolds to create artificial ovaries,[10-12] or the use of stem cell therapy to restore ovarian function,[13] offers a potential possibility for fertility restoration in OFI in the future.

When considering the Fallopian tubes, or tubal factor infertility, proximal and distal tube occlusions can be treated with microsurgery to improve fertility rates,[14,15] whereas those that are untreatable, or in those following bilateral salpingectomy, the issue can be overcome by the use of in-vitro fertilisation (IVF).

Regarding the uterus, reversible causes of uterine factor infertility include endometrial polyps,[16] submucosal fibroids,[17] and intrauterine adhesions.[18] However, absolute uterine factor infertility (AUFI) is a term used to define women who cannot conceive, or carry pregnancy, because of the absence of a uterus or the presence of one that is anatomically or physiologically non-functional. It has been estimated that AUFI affects one in 500 women of childbearing age.[19] Causes of AUFI, as

summarised in **Figure 2**, can be categorised into congenital, such as Mayer-Rokitansky-Küster-Hauser Syndrome (MRKH) and acquired, after the development of Asherman's Syndrome or following hysterectomy to treat massive obstetric haemorrhage, benign gynaecological disease or gynaecological cancer. Whilst representing the aetiology of infertility in a minority of cases, cervical and vaginal factor infertility can generally be overcome by intra-uterine insemination or the use of IVF.

As such, there is a group of women whose infertility was traditionally believed to be unconditional and absolute: those with absolute uterine factor infertility (AUFI). Whereas not absolute in nature, a major cause of infertility is the consequence to the physiological deterioration of ovarian reserve and oocyte quality that is associated with advancing age. These two groups of women remain the main focus of this thesis. Following diagnosis of infertility women suffer not only the loss of bodily function, but the realisation of permanent and irreversible infertility, which is associated with significant long-term emotional burden.[20,21] As such management requires an integrated, multi-disciplinary approach, involving gynaecologists, psychologists and clinical nurse specialists.[22] Additionally, particularly in conditions such as MRKH, when the diagnosis is during a teenage girl's life when they are vulnerable and at significant risk of feeling isolated amongst their peers, counselling and patient support groups can be of particular benefit.[23]

Reproductive planning is a dynamic process, centralised around parenthood aspirations, but in the context of each individuals' available resources. Most women, in line with cultural norms and potentiated by social expectations, expect to acquire motherhood by conceiving naturally, carrying pregnancy themselves and giving birth to their own children. Women with absolute infertility typically have no option but to change their reproductive plans, and either experience involuntary childlessness or acquire parenthood through adoption or surrogacy. Women with OFI in addition can utilise IVF with donor oocytes, which enables them to experience gestation, but does not allow biological relation to subsequent offspring.

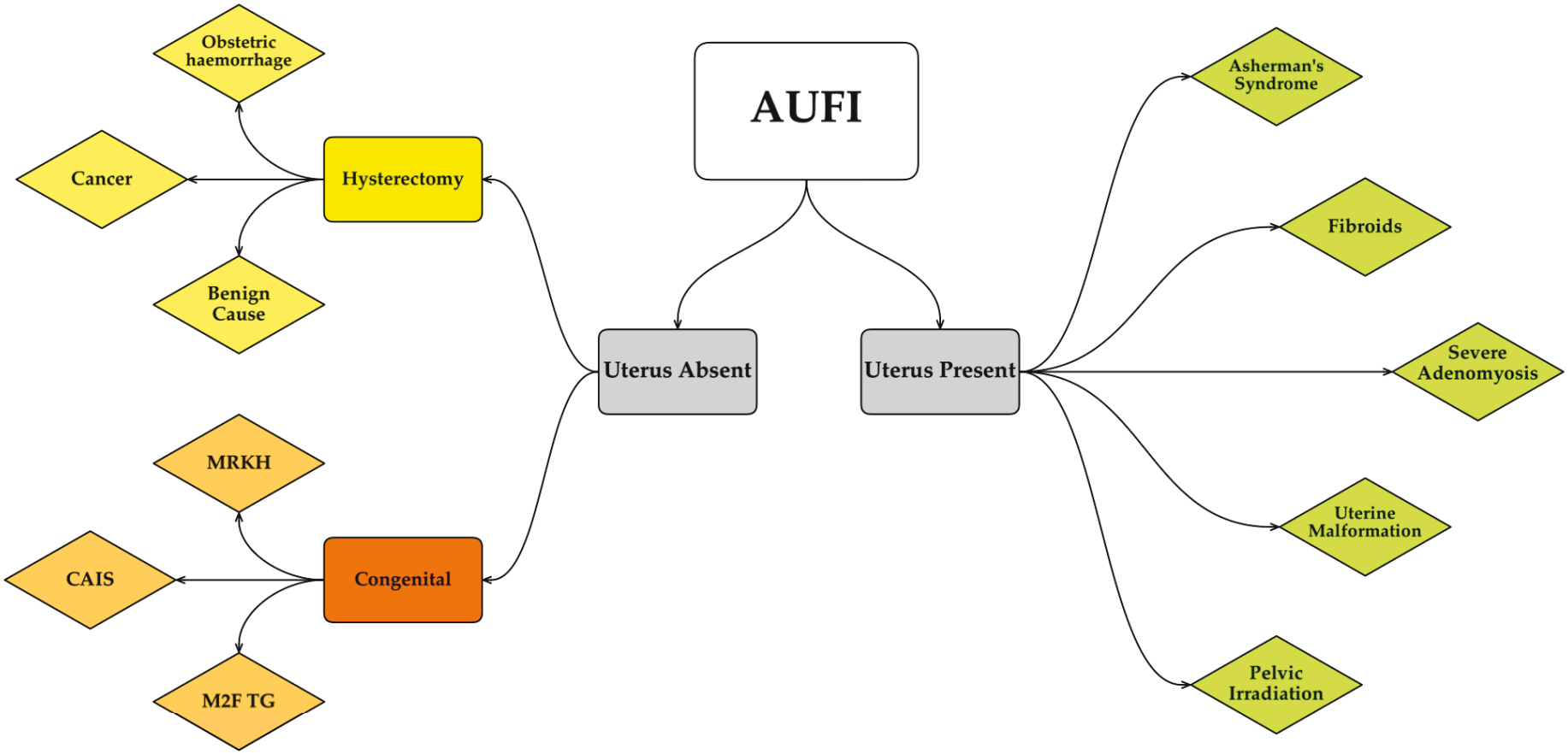


Figure 2: *Causes of absolute uterine factor infertility*

While some women may voluntarily remain childless,[24] many infertile women suffer significant psychological sequalae, such as anxiety, depression, low self-esteem, loss of gender identity, a decrease in their quality of life and an enduring sense of incompleteness and grief as a consequence.[25-28] Moreover, it has been demonstrated that infertile women who fail to conceive following assisted reproductive technologies (ART) suffer significantly worse from a psychological perspective than those who are successful.[29] In resource poor areas, there may also be associated socio-economic implications including a negative impact upon social status and worsening marital discourse.[30]

Adoption

Adoption is the irreversible process of transferring parental rights and responsibility from a child's birth parents to adoptive parents, creating a new family unit that will raise the child. For women with AUFI who desire parenthood, adoption may offer a sense of fulfilment and self-worth by allowing them to enhance the lives of children who have had a less fortunate start in life.[31] Moreover, it provides a safe home, with enhanced emotional, economic and social stability which creates a nurturing environment for children who cannot be cared for by their own biological parents.

In the UK, adoption came into legislation in 1926 with the inception of the *Adoption of Children Act 1926*. With this initial legal framework, the main purpose was to provide access to parenthood for infertile couples and to provide options for unmarried mothers. Although this encouraged the formalisation of adoption agreements, there was still concern over the lack of safeguarding legislation. These concerns prompted the development of *The Adoption of Children Act 1949,* with notable inclusions such as the supervision of adoption orders by local authorities, equality of inheritance rights between both adopted and birth children and the recommendation of the use of social workers to safeguard children's rights. After the number of adoptions in the UK hit a peak of 24,800 in 1968, the introduction of widely available contraception and access to safe termination of pregnancies saw a decline in the adoption rate. In the UK, the number of children defined under the care of local authorities has increased every year since 2013, primarily due to an increased number of care orders,

resulting in 72,670 children in the system in 2017.[32] In contrast to the increasing demand, the number of children who are adopted continues to decrease, with just 4,350 (6%) children being adopted in 2017.[32]

While adoption offers a mutually beneficial arrangement for both parents and their adopted children, it is often associated with a number of challenges or attachment-related difficulties which require consideration for prospective parents. Given that 62% of children who become looked after by the local authorities do so after suffering abuse or neglect,[32] it is unsurprising there is an increased risk of emotional, behavioural and relational difficulties in children who are adopted,[33,34] and they are more likely to access mental health services in the future.[31] Importantly, evidence suggests the negative sequalae are not a consequence to the process of being adopted, but secondary to traumatic early life experiences such as nutritional deficits, exposure to drugs and alcohol, inadequate shelter and neglect.[35] However, successful placements with adoptive families have also been found to ameliorate some behavioural issues.[35] Age at adoption, and therefore time spent in suboptimal surroundings, has also been identified to impact subsequent psychological development.[36] In a large Swedish cohort study, adoption at younger age (<2 years) was found to a protective factor against the development of subsequent depression when compared to children who spent longer in foster care.[37]

From an educational perspective, children who are adopted have been identified to not reach the same level of academic performance, when compared to their peers.[38] However, adoption has been shown to improve cognitive development and school performance in adoptees, when compared to siblings and peers who remained with their birth parents or in institutionalised care.[38] By being immersed in potentially more emotionally stable and cognitively stimulating environments, it is undeniable that adopted children are given an opportunity to flourish.

Surrogacy

Surrogacy refers to the process whereby a woman conceives, gestates and gives birth to a child with a pre-arranged plan of giving the child to another person or couple; the *intended* parents. Surrogacy is usually performed using gametes from the intended parents, which enables biological parenthood. Whilst AUFI is widely considered as the most acceptable indication for utilising surrogacy,[39] it is still associated with a multitude of religious, legal, economic and psychosocial factors that require consideration.

The regulation of surrogacy varies internationally and between states within the United States as represented in **Figure 3**. Whilst associated with moral and ethical issues, commercial surrogacy is permitted and legally enforceable in certain countries including Russia, Ukraine and Georgia. Commercial surrogacy on the other hand is forbidden, while altruistic surrogacy is acceptable in other countries such as the United Kingdom, Australia, Canada, Brazil, India and South Africa. Contrarily, any form of surrogacy is prohibited in many areas of the world including most of Western Europe, China, Japan, Pakistan, Turkey, Saudi Arabia and some areas of North America, excluding it as a possibility in more than a third of the world's population. A recent survey orchestrated by, the International Federation of Fertility Societies, including respondents from 65 countries, reported that surrogacy was permitted by statute or guideline in just 38% of the countries represented, and prohibited in 56%, with participants from 6% not answering.[40]

Although the UK was one of the first nations to introduce a regulatory framework for ART, subsequent legislative reforms have received criticism.[41] The Surrogacy Arrangements Act 1985 was heavily influenced by recommendations from the Committee of Inquiry into Human Fertilisation and Embryology 1984, referred to as the *Warnock Report*. The *Warnock Report* highlighted concerns about the potential for the use of financial incentives in commercialisation of surrogacy to exploit vulnerable women. The core principle of the Surrogacy Arrangements Act 1985 was to criminalise commercial surrogacy, however no safeguards were put in place for the rights of intended parents or

surrogates, nor was the welfare of subsequent children addressed. It wasn't until five years later, when a legal framework for transfer of parental rights from surrogates to the intended parents was established, following the creation of the Human Fertilisation and Embryology Act 1990. This Act stipulated that at least one of the intended parents must have a genetic link to the child, and that only *reasonable* expenses could be paid to the surrogate.

Currently the legal framework for international surrogacy in the UK is underpinned by Section 27 of the Human Fertilisation and Embryology Act of 1990 whereby the surrogate, regardless of the origin of the germ cells that created the embryo, is the legal mother of the child. Section 28 of the act details the legislation surrounding the legal father of the child; if the surrogate is married, then her husband, who has no genetic or physical relationship to the child, would be the legal father. Following the birth of the child, the intended parents are required to apply to the family court for a parental order. However, surrogacy arrangements are not legally enforceable in the UK, putting couples utilising surrogacy in a legally precarious position. Despite the potential legal ramifications, a recent systematic review concluded that most surrogacy arrangements are successfully implemented.[42]

While the rates of domestic surrogacy in the UK have remained relatively stable in recent years, there is a growing minority of prospective parents using international surrogates.[41,42] The increasing use of international surrogates has been attributed to less restrictive regulations, more favourable economic implications, better standard of care and secondary to domestic shortages of donors.[43,44] However, utilising international surrogates does not bypass the UK surrogacy legislation, and intended parents are still required to apply for a parenthood order upon their return to the UK.

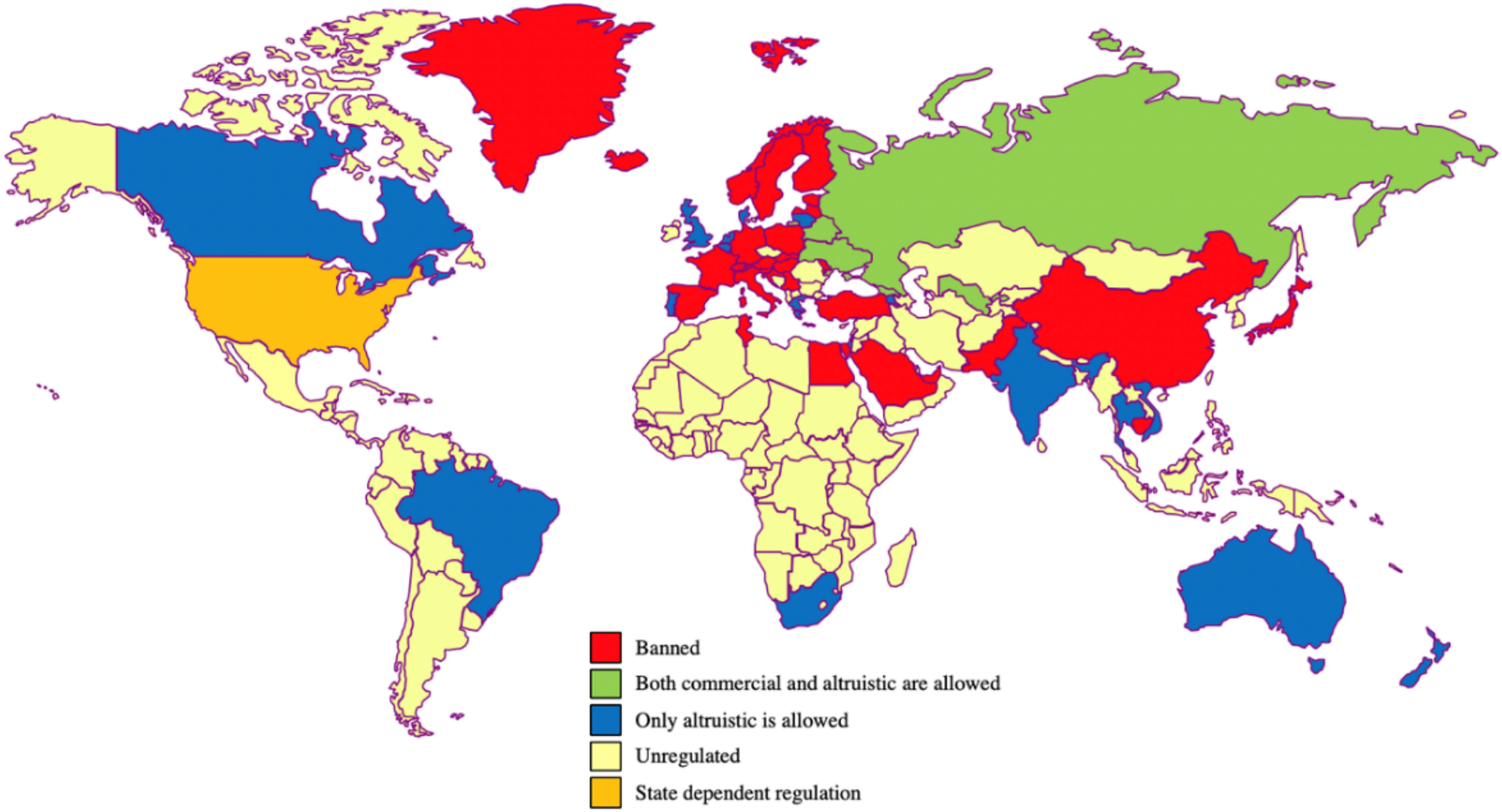


Figure 3: *Geographical representation of international regulation of surrogacy*

When considering the long term outcomes amongst children born to surrogates, a recent systematic review revealed similar perinatal outcomes to IVF with oocyte donation.[42] Moreover, there are no major differences in psychological development compared with children born to non-surrogates.[42] A 10 year prospective study in the UK showed that families generally maintain good relationships with surrogate families, and the majority of children were made aware of their mode of conception and did not suffer negatively as a consequence.[45]

The outcomes in surrogate mothers are also largely encouraging, with the majority finding the process to be a positive experience. Analysis of 16 studies that assessed long term psychological outcomes found no long-lasting serious psychopathology.[42] However, some surrogates found relinquishing care of their newborn child to the intended parents difficult. One study in particular demonstrated that more than a third (35%) of surrogate mothers had such difficulties,[46] although this reduced to 6% after 12 months.[46] Similarly, when considering long term psychological outcomes of intended mothers and their relationships with their children, no major differences have been shown when compared to mothers who conceive naturally.[42]

Uterine transplantation

UTx entails the transplantation of the uterus, including the cervix, as well as the surrounding ligamentous tissues and supplying and draining blood vessels. UTx is the only therapeutic intervention that restores reproductive anatomy and functionality in women with AUFI. This not only enables the experience of gestation, but allows biological, social and legal parenthood, thereby bypassing many of the aforementioned religious and legal implications in surrogacy.

UTx was initially considered in the 1960s and 1970s as a treatment for utero-tubal factor infertility.[47] However the subsequent success and inherent academic and clinical prioritisation of IVF, temporarily delayed advancement. It wasn't until the 1990s when research began to intensify, following the

development of the radical abdominal trachelectomy (RAT),[48] which provided a greater understanding of the vasculature of the uterus, which could be directly extrapolated into the field of UTx. Given the myriad of physiological adaptations that arise in a pregnant uterus, including an increase in uterine blood flow of 350%, it was initially uncertain whether or not the partially devascularised uterus following abdominal radical trachelectomy would be able to maintain adequate uterine perfusion to maintain a pregnancy until term. However, despite an association with preterm labour, likely owing to the anatomical absence of cervix, the majority of women who attempt conception post-RAT are successful.[49,50] When extrapolated into the prospect of UTx, it provided the expectation that UTx would be possible if supplied by two vessels, albeit through the uterine vessels, via the anterior division of the internal iliac, as opposed to the ovarian vessels which are used the RAT. Subsequent research was undertaken in small and large animal models to supplement knowledge of the essential anatomical, immunological, physiological and obstetric considerations necessitated by the complex multi-faceted nature of UTx, as summarised previously.[51]

It wasn't until 2000 when the first UTx was undertaken in Saudi Arabia using a living donor.[52] The recipient had undergone emergency post-partum hysterectomy six months previously. Unfortunately, inadequate pelvic support of the graft led to acute vascular thrombosis, and a hysterectomy was performed 99 days post-implantation.[52] More than a decade later the second UTx attempt was made, this time using a deceased donor and a recipient with MRKH.[53] Unfortunately in this case, while clinical pregnancies have been reported, a livebirth has not yet been confirmed, despite multiple embryo transfers and miscarriages.[54] In 2014, the first livebirth following UTx was achieved in Sweden,[55] in a series of nine UTx procedures, which demonstrated the feasibility of the procedure using living donors.[56] Eight livebirths have since been reported from this pivotal study,[57] the success of which has paved the way for UTx procedures to be undertaken globally. The first livebirth following UTx utilising a deceased donor was subsequently achieved in Brazil in 2017.[58] More than 70 UTx procedures have now been performed globally and the number of cases being performed is increasing exponentially (**Figure 4**).

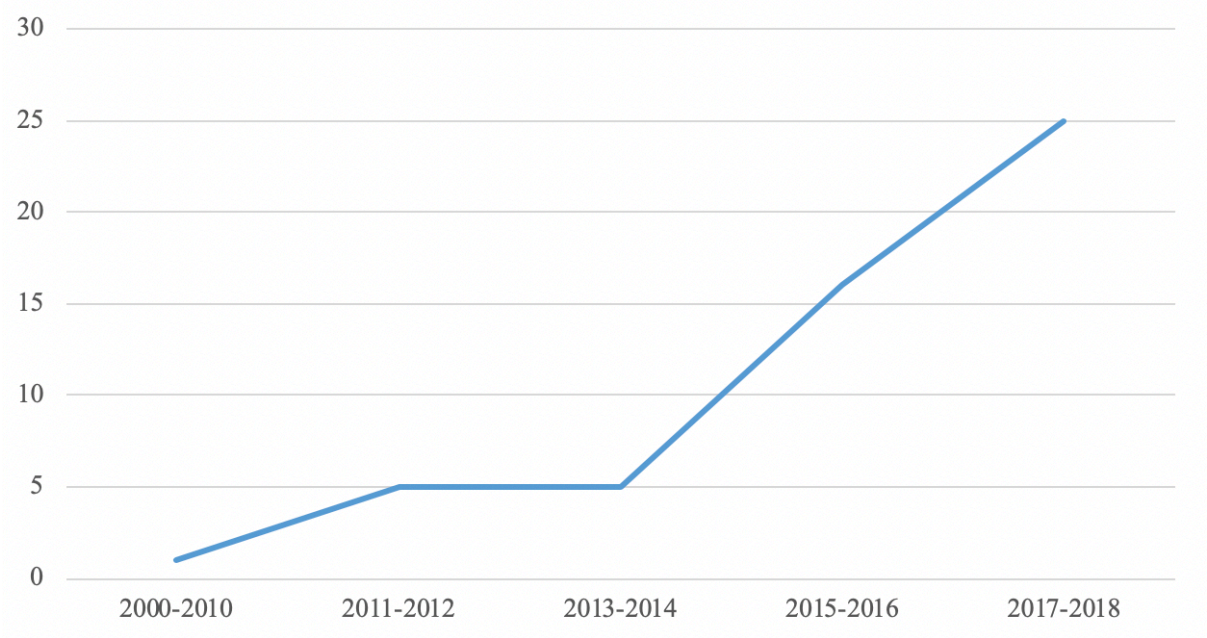


Figure 4: *Chronological representation of uterine transplantation cases performed*

Whilst the details from a number of cases remain unpublished, a recent review of 45 UTx cases revealed that nine livebirths have been reported.[59] However more than a quarter of cases required emergency hysterectomy, and an additional 10% suffered complications necessitating further surgical intervention, highlighting the associated surgical risk involved.[59]

Following implantation of the graft, immunosuppression is commenced and intensive follow up is essential to monitor recovery. After 6-12 months, following stabilisation on a non-teratogenic immunosuppression regimen, embryo transfers can be commenced.[60] Following conception, high risk pregnancy care ensues, utilising expert feto-maternal medicine input. Owing to concerns regarding structural support of the uterus, delivery is by Caesarean section as close to 37 weeks as possible, unless clinically indicated sooner. Post-delivery, depending on reproductive plans and clinical condition, repeat embryo transfer can be undertaken, or completion hysterectomy is carried out, which would allow transplant related medications and immunosuppression to be stopped, thereby reducing long-term immunosuppression related morbidity such as infection and neoplasia.[61,62]

UTx brings together complex bioethical considerations from both transplantation and ART fields.[63,64] Areas attracting the most attention have included the welfare of children born following UTx,[65,66] the values of reproductive autonomy and gestational parenthood,[67,68] and considerations of the alternative options to acquire motherhood such as surrogacy.[69-71] The first ethical principle '*primum non nocere'*, or *'first, do no harm'*, is seldom relevant in modern day clinical practice, as harm may arise from almost any clinical activity. As such, a more realistic reflection is the determination of whether or not there is overall net benefit whilst minimising risk of harm. The potential benefit from UTx will vary depending on each individual, governed by personal reproductive aspirations and available resources, in the context of their religious or cultural background. Only fully informed women can determine whether or not the potential benefit associated with UTx will outweigh the significant associated risk. It has been argued that if adoption and surrogacy were presented and perceived more favourably, fewer women may seek UTx. Moreover it is claimed that by providing UTx we thereby reinforce attitudes towards parenthood that may perpetuate discriminatory social biases, including pronatalism, gestationalism and geneticism.[72] These views have been utilised to counterargue consideration of publicly funding of UTx in countries with socialised medical care,[73,74] as well as in insurance-based or mixed systems.[75]

Critical review of human cases of uterine transplantation

Case 1: Saudi Arabia

In 2000, the first human UTx attempt was made in Saudi Arabia, using a living donor, from a team with no previously published research into the concept.[52] The recipient was a 26-year-old woman who underwent hysterectomy at the age of 20 following massive post-partum haemorrhage after Caesarean section. After 'thorough', albeit not described, evaluation she was found to be eligible.

The donor was a 46-year-old woman who underwent hysterectomy and bilateral salpingo-oophorectomy because of bilateral multiloculated ovarian cysts. The indication for the hysterectomy is dubious, as ironically this management should only be undertaken in cysts with a risk of malignancy index over 200. Clearly, if there was a suspicion of malignancy then the graft would be entirely unsuitable for UTx. The operation proceeded through a midline incision, and bilateral oophorectomy was performed with subsequent frozen section *confirming* their benign nature. Given that the specificity of assessing benign lesions through frozen section has been shown to be 99.2-100% and the sensitivity is 96.5-97%,[76,77] the accuracy associated with the use of frozen section is acceptable in this context. However, given that the implantation would have already happened prior to the full histological analysis being confirmed, the remote risk of implanting an organ from a woman with an active neoplasia, particularly in the context of immunosuppression, requires consideration. The full histological findings were not reported. The infundibulopelvic ligaments were clamped, divided, and sutured. No efforts were made to preserve the ovarian vasculature, but the Fallopian tubes were included as part of the graft. The uterosacral ligaments were then serially divided and sutured before the uterovesical peritoneum was incised and the bladder separated from both the cervix and vagina. She subsequently underwent modified hysterectomy, performed in such a way to preserve tissue and vascular integrity. This was facilitated by transecting the round ligaments as far laterally as possible, although the uterine vessels were clamped just an inch from the uterine body. The vagina was subsequently entered, utilising a circumferential incision. The graft was then removed, immersed

in cold saline and subsequently flushed with cold modified Euro Collins solution. In an attempt to extend the vascular pedicle, back table dissection and vascular reconstruction was undertaken, as the veins were extended using 6cm long segments of saphenous vein, while the arteries were extended using 8cm reverse segments. Whilst the report states that 'the ureters were identified and protected' throughout the procedure, there is no description of what measures were put in place to protect them. Of significance, a small laceration was subsequently identified on the anterior wall of the left ureter. It was splinted with a double J tube and sutured. The operation duration was not recorded.

The recipient operation began when 'uterine extirpation was imminent'. Whilst it is important to minimise ischaemic times where possible, the decision to begin the recipient operation before the back-table dissection, particularly when such extensive vascular reconstruction was planned, was perhaps premature. This is exemplified by a future case that was undertaken in Germany, where following retrieval, the implantation operation was cancelled amid concerns of significant perceived risk of graft thrombosis, as it was not possible to perfuse the graft during the back-table dissection.[78] The implantation continued, with mobilisation of the bladder and rectum, and subsequent excision of the cervical stump. The graft was introduced, and the *cervix* was anastomosed to the recipient's *vagina* using simple interrupted sutures, perhaps suggestive of a very small cuff of vagina from the donor. A subsequent case in Sweden was complicated by ascending infection, which was attributed to a short donor vagina cuff. However, the Swedish case was in a woman with MRKH, who may not have had a full-length vagina. Contrastingly, the recipient in this case underwent previous hysterectomy which should not have impacted her vaginal length. The decision to anastomose the vagina before the vessels would increase potentially damaging warm ischaemia time, but the additional structural support may facilitate the subsequent vascular anastomosis and ensure appropriate and realistic tension is placed on the vascular anastomoses, which makes it an acceptable strategy. The extended vascular pedicles of the donor graft were subsequently anastomosed end-to-side to the external iliac vessels, which is appropriate. There is no description of what structural support was utilised, although 'uterosacral shortening' was undertaken. Standard triple immunosuppression with cyclosporine, azathioprine, and corticosteroids was introduced. The

operation duration was not described, nor was the cold and warm ischaemia times or estimated blood loss (EBL). The lack of reporting of these valuable data highlight weaknesses in this case.

Post-operatively allograft rejection was monitored utilising ultrasound doppler, magnetic resonance imaging (MRI) and measurement of the CD4/CD8 ratio in peripheral blood samples. As there was no proven way to detect rejection at this time following UTx, these measures seem reasonable, although it is likely that if there was evidence of rejection on MRI or ultrasound (USS), it would be fairly advanced by that stage, and may not be possible to reverse. An episode of acute rejection was diagnosed on the ninth post-operative day, with a CD4/CD8 ratio of 3.4 and ultrasonographic evidence of myometrial oedema. Despite escalation of maintenance immunosuppression and an intravenous dose of methylprednisolone, the rejection did not reverse and a subsequent dose of anti-thymocyte globulin (ATG) was given, which subsequently worked, with a macroscopically normal cervix and improvement in the CD4/CD8 ratio to 1.3. Despite having normal ovaries, supplemental combined estrogen and progesterone hormone therapy was given, which the transplanted uterus responded to with two withdrawal bleeds, demonstrating uterine functionality. Unfortunately, the graft failed after 99 days secondary to acute vascular thrombosis which the authors attributed to 'inadequate uterine structure support, which led to probable tension, torsion, or kinking of connected vascular grafts'. There are multiple reasons which may have attributed to this outcome. Firstly, despite 'uterosacral shortening' there was no other description of techniques used to provide support to the uterus. Subsequent cases have provided lateral support with suturing of the broad and round ligaments, in addition to anterior support from the bladder peritoneum, which it appears was not undertaken in this case. In the discussion, it was suggested suspension of the uterus from the anterior abdominal wall could reduce the risk of subsequent torsion of the graft. However, given multiple subsequent successes after restoring normal structural support to the uterus, this seems unnecessary, and suspension sutures could result in adverse symptoms as pregnancy progresses. Another issue in this case is their planned technique of arterial and venous reconstruction, to extend the vascular pedicle. Whilst the use of larger vessels is essential to reduce the risk of subsequent thrombosis, in other solid organ transplants (SOT), ex-vivo vessel repair is associated with a greater risk of arterial thrombosis.[79] Whilst unplanned and

prompted by adversity, in a future UTx case in Dallas, an ex-vivo arterial reconstruction was undertaken using another branch of the internal iliac artery, which also resulted in thrombosis.[80] Therefore it should be avoided where possible in UTx, instead maximising the length and calibre of the arteries by taking healthy vessels as laterally as possible to maximise vessel diameter.

This reinforced the need for further, interlinked research studies in animal models to allow better understanding of vital anatomical, immunological and fertility aspects of UTx, and highlighted the attempt in the human model was perhaps premature. Macroscopic and histological examination revealed acute thrombosis in the vessels of the uterine body, with resulting infarction. It is of interest, and gives insight to the perfusion of the uterus, that despite the uterus becoming necrotic, the Fallopian tubes remained viable. It should be noted that there was no description of whether or not she underwent pre-operative IVF to ensure appropriate embryos post-operatively. However, as the Fallopian tubes were preserved and transplanted, the expectation may have been that she would eventually spontaneously conceive. Given that this case was undertaken when slow freezing methods were still widely used for embryo cryopreservation, with associated inferior outcomes compared to the newer vitrification methods, the potential benefit of using IVF may have been perceived to be less important. However, given that subsequent animal studies highlighted high rates of tubal blockage seen in non-human primates following utero-tubal auto-transplantation,[81] the risk of ectopic pregnancy may have been significant had the UTx been successful. However, no comment was made regarding tubal blockage from the case, suggesting that the same problem may not have been evident herein.

Subsequent further animal research continued, spearheaded by the UK, United States of America (USA) and Swedish teams, in both small and large animal studies, which gave a greater understanding on UTx and insight into how such knowledge could be extrapolated to the human model. Perhaps the most important are the vascular considerations necessary to enable sufficient blood supply to retain uterine viability and functionality. A microvascular technique was initially employed using a uterine vessel anastomosis, but thrombotic complications necessitated the development of a macrovascular

method,[82] in the form of a large vessel patch. Other important considerations include the use of cervical biopsies to detect rejection,[81] and the application of multi-spectral imaging to detect blood flow within the uterus.[83] Following pregnancies after UTx, in rabbit,[84] mouse,[85] non-human primate,[86,] and sheep models,[87] the next logical step was for further attempts in humans.

Case 2: Turkey

The second human UTx was performed in 2011 in Turkey, utilising donation after brainstem death (DBD).[11] It should be considered that this was another case that was undertaken by a team without previous research experience in the field of UTx. The recipient was a 21-year-old woman with MRKH. Notably, she had previously undergone intestinal vaginoplasty for vaginal reconstruction. Pre-operatively, the patient underwent two cycles of IVF, with an overall yield of eight grade-1 embryos of unspecified development. The donor was a 22-year-old nulliparous woman who was pronounced brainstem dead after a road traffic accident. The uterine graft, including a vaginal cuff were retrieved with a long vascular pedicle including bilateral common iliac and ovarian and arteries and veins. This dissection included by a wide excision of the broad, round and uterosacral ligaments, along with uterovesical peritoneum, which was subsequently used to enhance uterine structural support post-implantation. The uterine retrieval was carried out before the kidneys, liver and heart were retrieved. Whilst it was noted that the donor was kept haemodynamically stable throughout, undertaking the UTx retrieval before lifesaving organs is contentious, as in the event of unplanned bleeding, resultant haemodynamic changes could impact the subsequent quality and viability of the undoubtedly more important organs. Whilst the long vascular pedicles reduce the risk of thrombosis, it is worth noting that in the context of a multi-organ retrieval in the UK, including the pelvic vasculature to the level of the common iliac, would not be feasible, owing to the need to preserve the iliac 'Y graft' for use to extend the vascular pedicle for other transplants such as the pancreas. The operation took two hours.

The graft was removed and perfused with cold University of Wisconsin (UW) solution to ensure removal of all blood cells and fibrin and minimise ischaemic damage. Implantation ensued, with orthotopic replacement and fixation of the retrieved uterus, and anastomosis between the common iliac vessels of the graft and the respective external iliac vessels of the recipient in an end-to-side fashion. The implantation took 5h 30m. Warm and cold ischaemic times were not included. Induction immunosuppression with ATG was given and maintenance therapy was commenced with triple therapy; tacrolimus, prednisolone, and mycophenolate mofetil (MMF).

The post-operative recovery was uncomplicated, although the inpatient stay was prolonged, as the recipient was discharged at the end of the first post-operative month. Her menstrual cycles resumed, without the use of supplemental hormones, with menstruation 20 days post-operatively. Her menstruation was irregular for the next two menstrual cycles after the first menstrual cycle, and her endometrial thickness reached 21mm. Vaginal biopsy samples were taken every two weeks for the first three months, whereas endometrial biopsies were obtained every three months. The use of vaginal biopsies has not been shown to correlate with rejection of the uterus, so the use of these is questionable. Moreover, endometrial biopsies risk the introduction of bacteria into the cavity, which in the context of an immunosuppressed state, would be better avoided. This case subsequently reported the first clinical pregnancy after UTx in humans, which unfortunately resulted in miscarriage at six weeks gestation, after undertaking embryo transfer 18 months post-UTx.[54] Moreover, despite multiple attempted embryo transfers, and recurrent miscarriages, no livebirths have yet been achieved. It has also not yet been reported whether or not the graft has been removed.

The decision to use a nulliparous donor brings the additional risk of potentially implanting a uterus that has undiagnosed uterine factor infertility, although this risk would be remote in the context of a macroscopically and ultrasonographically normal uterus from a woman without adverse reproductive history.

The more significant contributing factor to the inability to maintain pregnancy, is the potential impact of the intestinal neovagina, with its resultant interrelationship with the vaginal microbiome, upon reproductive outcomes. The vagina plays an essential immunomodulatory role within the reproductive system. It is composed of stratified squamous non-keratinised epithelium, the most superficial layer of which desquamates, mechanically eliminating adherent micro-organisms,[88] whilst the deeper layers provide an impermeable barrier to pathogenic organisms.[89] The vaginal epithelium in addition expresses receptors that sense invading microorganisms,[90] and subsequently produces antimicrobial peptides and cytokines. This not only limits microbial invasion and replication but also modulates inflammation.[91,92] The vagina also contains T cells, neutrophils, macrophages and natural killer cells which additionally contribute to the immune response.[93] Furthermore, secretions from the vagina, which are rich in mucin and antimicrobial peptides, provide a mechanical and biochemical barrier.[94,95] These immunomodulatory roles help to modulate and maintain a commensal vaginal microbiome, which prevents microorganisms from ascending into the uterus. The vaginal microbiome is composed of a diverse array of bacteria, which are most commonly dominated by species of lactobacilli.[96] Through their lactic acid producing capability, lactobacilli dominated microbiota are acidic (pH <4.5), which further inhibit the proliferation of other bacteria.[97] In vaginal dysbiosis, lactobacillus dominance is lost which is associated with an increasingly alkaline environment and greater microbial diversity, which facilitates the proliferation of pathogenic organisms. Whilst the vaginal microbiome in the varying types of neovagina in women with MRKH has not yet been established, an 'optimal' vaginal microbiome is typically dominated by one or two species of lactobacilli,[98] with a pH of <4.5. Changes in vaginal microbiome composition have long been associated with changes in vaginal pH.[99] For example, transgender women with skin lined neovagina have been demonstrated to have an elevated pH, with colonisation of bacteria traditionally identified in skin, intestine or bacterial vaginosis dominated microbiota instead.[100] Since lactobacilli were identified in <5%,[100] it was initially believed that skin lined neovagina would not support the growth of lactobacilli. However, subsequent studies using molecular detection techniques demonstrated that lactobacilli species were in three quarters of transgender women with skin lined neovagina.[101] Studies that have analysed the bacteria microbiota within intestinal neovagina identified alkaline

environments, with a mean pH of 8, and microbiota more consistent with that found in bowel. Whilst lactobacilli species were actually identified in two thirds of cases, the presence of lactobacilli does not necessarily indicate normal vaginal physiology. Moreover, *Escherichia coli* (n=14/15) and *Bacteroides* spp. (n=13/15) were isolated in almost all cases.[102] The structure of the vaginal microbiome has been identified to effect IVF outcomes,[103,104] including pregnancy,[105] implantation and livebirth rates.[106] Vaginal dysbiosis or lactobacillus depletion is also implicated in both early and late miscarriage,[107,108] and also preterm birth.[109,110] During pregnancy, a healthy vaginal microbiome becomes increasingly lactobacillus dominant, and less diverse, as a consequence to oestrogen dominance. During pregnancy, a lactobacillus deplete or dysbiotic vaginal microbiome is a risk factor for preterm labour, and especially for preterm prelabour rupture of membranes.[111,112] As such, given the role of UTx is to attain a livebirth, consideration of the vaginal microbiome is essential. Data can be extrapolated from women with cervico-vaginal atresia following vaginoplasty as they generally have a normally functioning uterine body. Outcomes in women with bowel neovagina appear very poor with just one livebirth being reported,[113] with two early trimester miscarriages and one ectopic pregnancy, seen in other cases.[113-115]

The vaginal microbiome has also been implicated in the development of carcinogenesis in various gynaecological cancers.[116] In particular there is an interrelationship between the vaginal microbiome and high-risk strains of human papilloma virus (HPV). HPV infection is associated with dysbiotic vaginal microbiomes with greater microbial diversity, and clearance of the virus is linked to vaginal microbiome composition.[117] It is therefore unsurprising, given the association between high risk strains of HPV and cervical intraepithelial neoplasia (CIN), that women with CIN have lactobacilli deplete and more diverse vaginal microbiome than their healthy counterparts.[118,119] Furthermore, a recent meta-analysis confirmed a causal link between vaginal dysbiosis and cervical cancer through associated oncogenic HPV acquisition and persistence,[120] although further prospective studies comparing the vaginal microbiome between women with CIN and women without CIN with persistent oncogenic HPV infection would provide clarification. In the context of UTx, cancer is 2-4 times more common after solid organ transplantation than in the general population,[121] including those

that are HPV related,[122] so this requires particular consideration. Given that follow up after UTx necessitates routine cervical biopsies to detect for rejection,[123] frequent visualisation and histology of the cervix would likely ensure an early diagnosis should it arise.

The ephemeral nature of UTx creates a unique advantage over other organ transplants, because following completion of the recipient's family, the graft can be removed, and the previously essential immunosuppressive medications can be stopped. However, the poor reproductive outcomes demonstrated in this case raises the question of how long a uterus should remain in place following UTx, in the event successful livebirth is not achieved. Given that immunosuppression related complications are dose and duration dependant,[62] the cumulative risk increases as time progresses with the graft in situ, and it is essential that an end point is agreed, between the UTx multi-disciplinary team (MDT) and the recipient. Not only does physical risk increase, but the adverse psychological impact of multiple miscarriages and failed embryo transfers, including post-traumatic stress disorder, anxiety and depression, will undoubtedly be significant.[124]

Cases 3-12: Sweden

The following nine cases were undertaken by the Swedish team, led by Professor Brännström, all using live donors.[56] After extensive counselling and investigation, with consideration of the alternatives including adoption and surrogacy, eight women with MRKH were selected, in addition to another who previously underwent a radical hysterectomy for cervical cancer. All the donors were directed, the majority of whom were the recipients' mothers (n=5), where others consisted of an aunt (n=1), a friend (n=1), a sister (n=1) and a mother in law (n=1). The use of directed donors may have immunological benefit owing to familial relation, although has the additional considerations of coercion. Four of the donors were post-menopausal.

The retrievals were all undertaken via infraumbilical midline laparotomy. The graft was similar to that retrieved in the Turkish case, but the vascular pedicle was not as extensive, including the uterine

vasculature to the level of the anterior division of the internal iliac. The utero-ovarian veins were also preserved and included in the graft. It also included substantial retrieval of the round and uterosacral ligaments, as well as an extensive area of bladder peritoneum. This was undertaken to enhance the structural support post implantation, to prevent the adverse outcome seen in the first case. Bilateral salpingectomy was also performed, to reduce the aforementioned risk of ectopic pregnancy. A vaginal cuff of 10–15 mm was also included. Following removal, back table dissection was undertaken, and some cases had their utero-ovarian veins anastomosed to the uterine vein to promote venous drainage post implantation. The graft was flushed with HTK solution and kept on ice until implantation.

To minimise cold ischaemic time, a second team of surgeons opened and prepared the recipient for implantation, through a midline infraumbilical incision. Following dissection of the vaginal vault away from the bladder anteriorly and the rectum posteriorly, organ fixation sutures were placed in the round and uterosacral ligaments, and paravaginal areas. Those with uterine remnants underwent excision of the tissue in order to access the vagina. Preparatory vascular dissection was then undertaken to prepare the external iliac vessels for subsequent anastomosis. The uterine graft was subsequently brought into the pelvis in the normal anatomical position, and bilateral end to side anastomoses were undertaken between the anterior division of the internal iliac vessels in the donor and the external iliac vessels in the recipient. Following unclamping and confirmation of uterine perfusion with doppler probes, the vaginal anastomosis was undertaken. This technique reduces cold ischaemic time, compared to undertaking some structural support prior to revascularisation, as undertaken previously. The ligamentous support sutures were then tied, as well as suturing the extensive section of bladder peritoneum over the fundus of the bladder. Induction therapy was with ATG and maintenance therapy included tacrolimus, azathioprine and MMF, in combination with a tapered course of prednisolone. The intention was to cease MMF after 6-9 months, before embryo transfers were commenced 12 months post-operatively. The mean operation time in the donor was >12 hours. Whilst the donor time is directly related to the surgical complexity, particularly related to the intricate dissection of the unpredictable uterine venous plexus, it is clear this very long surgery is associated with major venous thromboembolic risk. Moreover, this complex dissection is associated

with significant surgical risk, as experienced in this series were one donor developed a ureterovaginal fistula. This was attributed to a difficult operation, which necessitated the use of bipolar diathermy, with subsequent thermal injury which was therefore not noted intraoperatively. Treatment necessitated an initial pyelostomy with definitive reimplantation of the ureter five months later. In addition, the mean EBL in the donor cases was >900mls, with two cases bleeding in excess of 2000mls. This degree of haemorrhage for a quality of life improving procedure is difficult to justify, and additional measures are required, such as the use of cell saver, to minimise the potential implications of anaemia in these women.

Two recipients underwent hysterectomy post-operatively due to complications. The first, in a recipient with heterozygous Protein C deficiency, developed acute uterine artery thrombosis on the third post-operative day. The second presented 33 days post-operatively with pelvic infection and despite treatment with antibiotics, developed a drainage refractory pelvic abscess necessitating hysterectomy 105 days post-operatively. All the implantation operations were between 4-6 hours in duration. The mean EBL was 672mls. The remaining seven transplanted uteri remained viable post-operatively, and all experienced menstruation. Following stabilisation on a non-teratogenic immunosuppression regimen, embryo transfers were commenced from 12 months post-operatively, in line with standard SOT guidance.[125] In September 2014 the first live birth after UTx was reported, signalling to the world that UTx is a potential treatment for AUFI.[55] The team have since reported 8 livebirths in total from their cohort of seven successful procedures,[126] highlighting an enormously successful study. From the seven successful procedures, six women gave birth, with two women having two children each. The only woman to not give birth, but who suffered five miscarriages, had a skin neovagina.[127] While it is likely a non-physiological skin neovagina may be associated with a dysbiotic environment, it should be noted that two women with skin neovaginas had successful livebirths, highlighting that it is possible. In those with skin neovagina, two (50%) achieved a single livebirth, one (25%) experienced multiple miscarriages, and the other (25%) underwent emergency hysterectomy three days post-operatively for graft thrombosis.[56,128] In the five recipients with physiological functioning vagina, whilst one (20%) underwent hysterectomy for persistent intra-uterine / pelvic infection, all the others

(80%) successfully achieved livebirths, with six livebirths in total. Whilst further data is needed before firm conclusions can be drawn, cases of UTx with normal mucosal vagina appear to have superior success rates (80% vs 50% livebirth rate: 1.2 vs 0.5 livebirth per UTx ratio). Another consideration is that whilst they achieved excellent pregnancy rates, the Swedish team used cleavage stage embryos. Cleavage stage embryos have been consistently shown to have significantly inferior pregnancy rates compared to blastocysts, with a recent study highlighting a 55% pregnancy rate with cleavage stage, and up to 71% pregnancy rates with blastocysts. Moreover, subsequent embryo transfers were not undertaken during stimulated cycles, and natural cycle IVF was undertaken. Given that natural cycle IVF is associated with a 30% longer time to achieve pregnancy,[129] owing to the need for 2-3 times more cycles than conventional IVF,[130] the decision to use such a protocol is open to debate. Reducing the time taken to achieve pregnancy would likely reduce the time the graft would remain in situ, and therefore cumulative immunosuppression exposure, and subsequent immunosuppressive related complication risk.

The Swedish team previously undertook research in a non-human primate model to develop a preliminary grading system for uterine allograft rejection, where they identified that cervical biopsies were a consistent and achievable method of detecting rejection in the graft.[131] This was subsequently extrapolated successfully in this study,[56] with subsequent revision, to include a borderline category to avoid excessive rejection diagnoses and overtreatment.[123] The use of cervical biopsies negates the need to take endometrial biopsies, which have been shown in the baboon model to be less consistent,[131] and are less likely to introduce bacteria into the cavity.

From a psychological perspective, in donors, there was no significant change in quality of life between baseline and one-year post-procedure, and no reported regret, even in those whose recipient subsequently underwent emergency hysterectomy, or suffered complications themselves.[132] moreover, following UTx donation, all women eventually returned to their pre-operative social and physical status.[132] With regards to recipients, the psychological well-being was monitored regularly throughout the trial using a research protocol which was adapted from a facial transplant study.[133] The

participants, as well as their partners, did not report any psychological issues at baseline, nor following the procedure, despite adverse events such as episodes of rejection. This was confirmed quantitively using the aforementioned questionnaires, where despite a significant transient deterioration in SF-36 for two parameters (bodily pain and physical function) at 3 months post-operatively, they had returned to baseline 3 months later. Many recipients were relieved, and expressed joy, by the onset of menstruation following UTx, as it not only demonstrated graft function but made them feel like every other woman. There was no significant impact following UTx upon sexual desire or satisfaction.

In summary, this series was very well thought through, was based on extensive personal animal research and experience, and delivered exemplary results. Many aspects of this study have been utilised to shape the protocols of other teams around the world.

Case 13 - China

The next case was undertaken in China.[134] The recipient was a 21-year-old woman with MRKH. In preparation for the UTx, she underwent preparatory vaginoplasty using a biological membrane. She was extensively investigated and counselled and found to be a suitable recipient. Preparatory IVF was undertaken pre-operatively with 22 oocytes being retrieved, which resulted in 14 embryos which were cryopreserved for use post-UTx. The donor was her mother, a 42-year-old woman who had 2 previous vaginal deliveries who was investigated using pelvic ultrasound, which was normal, and cervical cytology / HPV screening.

Following open Hasson port entry 4-5cm above the umbilicus, four further ports were inserted, and an intrabdominal pressure of 12mmHG was maintained throughout the operation. The operation proceeded in a similar fashion to that previously, with retrieval of the uterus, including the cervix and top part of the vagina, in addition to extensive lateral excision of the broad, uterosacral and round

ligaments, as well and the bladder peritoneum. The cardinal ligaments, in addition to a more lateral paravaginal excision were also taken in addition. A 1-1.5cm cuff of vagina was included in the graft. The main difference in surgical technique was the decision to use the lateral uterine arteries, 2cm distal to the anterior branch of the internal iliac, which made the arterial part of the graft relatively smaller in calibre to cases undertaken previously. This case also did not include retrieval of the uterine veins and instead, the sole venous supply was through the utero-ovarian veins. By utilising the ovarian/utero-ovarian veins, the surgical dissection negates the need of the aforementioned complex dissection around the unpredictable uterine venous plexus and ureter. This greatly reduces surgical risk, particularly in light of the two ureteric injuries seen in the first four cases performed globally, but also operative time, this case in particularly being undertaken in six hours, more than half of that of the nine cases undertaken in the Swedish series. Moreover, the EBL was just 100mls, which is almost a tenth of that seen in the Swedish cohort, exemplifying further the potential benefits of a minimally invasive approach.

Subsequent graft preparation included cannulation and perfusion of the graft with cold HTK solution, with storage on ice until the recipient was ready for implantation. The implantation operation was undertaken through an infraumbilical midline incision. The implantation technique was largely similar to those undertaken previously, with lateral uterine artery to external iliac artery anastomoses, but this time utero-ovarian to external iliac venous anastomoses. The EBL was <500mls and the operation duration was 8h 50m.

The post-operative course was largely uneventful. The donor was discharged after a five-day admission, with an indwelling catheter, which was successfully removed 2 weeks post-operatively, in line with the team's standard management of women following radical hysterectomy. Regarding the recipient, in the third post-operative week she presented with vague symptoms suggestive of rejection, with an elevated CD4:CD8 ratio of 2.2. Ultrasound findings were unremarkable. Following a three-day course of intravenous methylprednisolone, her symptoms resolved. No surgical complications were experienced. She subsequently experienced regular menstruation, and radiological evidence of

endometrial proliferation. Whilst not scientifically reported, there are media reports that a successful livebirth has since been achieved.

The main novel aspect of this case was the utilisation of minimal access retrieval techniques, in addition to refinement of the surgical technique to facilitate the use of robotic surgery. Following the Swedish cohort which demonstrated the concept, this early attempt to reduce donor risk is commendable at this early stage, and necessary to ensure the sustainability of using living donors. However, it is worth noting the donor in this case was premenopausal (42 years old) and the dissection utilised the full length of ovarian veins and necessitated bilateral salpingo-oophorectomy. The inevitable iatrogenic POI caused as a consequence to this technique is difficult to justify for a quality of life improving procedure in young women.

Case 14: Cleveland, USA

The next case was undertaken in Cleveland, USA, and used a 34-year-old multiparous DBD donor. Whilst the case has not been fully reported, a brief report,[135] and video describing the surgical technique,[136] has provided insight into the case. The recipient was a 26-year-old woman with MRKH. A similar surgical technique to that described in the initial DBD case and Swedish cases, with a uterine graft being removed including long vascular pedicles to the level of the internal iliac. Following mobilisation of the vagina away from the bladder and rectum, and exposure of the external iliac vessels bilaterally, the graft was implanted using an internal iliac to external iliac anastomosis. Following unclamping of the vessels the graft re-perfused. Extensive ligamentous support was implemented including round ligaments, uterosacral ligaments and the uterine remnants. The operation took approximately 7 hours.

Following an initially stable recovery, with uterine viability confirmed on cervical biopsy and using angiography, 12 days post-operatively the donor underwent emergency hysterectomy following

intrabdominal haemorrhage. Diffuse candida infection of the graft, including infiltration and interruption of one of the two arterial anastomoses, caused intra-abdominal haemorrhage which subsequently necessitated hysterectomy.[136]

Whilst candida species often reside as a harmless vaginal commensal organism, which is facilitated by their polymorphic potential enable them to tolerate a wide pH range, they have the potential to cause life threatening infections. Under normal conditions, the vaginal mucosal barrier is intact, and the aforementioned immunomodulatory role of the vagina is functioning. However, in the context of UTx, following transection of the vagina, the mechanical barrier helping to maintain homeostasis between host and candida species is disrupted, which has been shown to be a risk factor for systemic fungal infection.[137] Moreover, following the induction of an immunosuppressed state, and following the administration of steroids, which both increase the risk of invasive fungal disease,[137] it is unsurprising that invasive candidiasis is an established complication following SOT.[138] Risk increases further in the transplantation of organs where the presence of candida is common, such as the small bowel,[139,140] where a risk of 11.6% has previously been reported. The use of deceased donors in UTx may present additional risk. More than half of women on the intensive care unit have active infections, and almost three quarters receive antibiotics,[141] which creates significant potential for vaginal dysbiosis and subsequent Candida infection.[142] Moreover, given that one in five of such infections are fungal in nature,[141] particular consideration is required, in line with guidance from the Advisory Committee on the Safety of Blood, Tissues and Organs (SaBTO). Whilst SaBTO conclude that ‘organs and tissues from donors with superficial fungal infection of the skin or mucosa due to candida species are acceptable for donation’, appropriate investigation, including microbial evaluation of the preservation solution the graft is stored in, and the judicious use of antifungal prophylaxis is essential in UTx.[143] Closure of the vaginal cuff in transit can also be considered.

Cases 15-23: Czech Republic

The following nine cases were undertaken in the Czech Republic between April 2016 and November 2017 including five LD cases and 4 DBD cases.

All living donors were thoroughly investigated and assessed and were only deemed suitable if they were healthy. Both living and deceased donors were only selected if they had a normal uterus with no preceding history of cancer, diabetes, hypertension or infectious disease. These selection criteria are appropriate, with the aim of minimising risk for the recipient and optimising the rate of success. It is of interest that, in contrast to previous series', nulliparity was not an exclusion criterion. The donors were all aged between 18-60, even in the deceased donor group. Whilst the lower age bracket is suitable, the use of older donors brings additional complexity. In post-menopausal living donors, supplementation with pre-operative hormone replacement therapy (HRT) can be undertaken, but this is not possible in DBD. The use of HRT only allows the assessment of endometrial functionality, following the observation of withdrawal bleeding, and it also optimises tissue quality by acting on potential atrophic changes in the proposed graft. However, in living donors counselling is required, owing to the risk of venous thromboembolism (VTE) associated with oral HRT,[144] particularly in new users,[145] and in the context of major surgery which has long been considered a major risk factor for VTE.[146] Gynaecological surgery in particular is associated with a more than 20 fold increased risk in the first 6 weeks, compared to not having surgery.[147] Transdermal HRT should therefore be preferred, where the risk of VTE does not appear to be increased,[144] and consideration should be given to stopping the treatment 2-4 weeks prior to surgery, although each case should be individualised. However, the inability to demonstrate functional endometrium, and optimise tissue quality in deceased donors, raises questions over the use of menopausal deceased donors. Other selection criteria implemented in this study included a maximum of four deliveries, including one caesarean section. The stipulation of only including one previous Caesarean is understandable to reduce the additional risk of pregnancy associated complications following multiple previous Caesarean sections such as placenta praevia/accrete or scar pregnancy. However, it may limit further, an already selection

criteria induced depleted donor pool. Moreover, if a healthy donor who met all the other criteria presented, after having five normal vaginal deliveries without complication, it may be inappropriate to exclude such a donor. Pre-operative investigations included transvaginal ultrasound examination, hysteroscopy and cervical smear were carried out in both living and deceased donors. The decision to hysteroscope donors, in particular deceased donors, warrants further consideration. Whereas it allows direct visualisation of the cavity, the potential to introduce microorganisms such as bacteria or candida is significant, which as exemplified by the Cleveland case, can be a very serious issue. Moreover, there is an argument that the use of expert ultrasonography negates the need for direct visualisation of the cavity. A previous study investigated the efficacy of transvaginal ultrasound vs hysteroscopy in the diagnosis of submucosal fibroids, and found ultrasound to be 100% sensitive and 94% specific, compared to that of hysteroscopy which was 100% and 96% respectively.[148] Moreover, with other pathology that may impact suitability to donate, such as the presence of a structural abnormality, for example a bicornuate or subseptate uterus, transvaginal 3D ultrasound remains an accurate diagnostic modality, with a recent study showing a sensitivity of 96.7% and a specificity of 100%.[149] As such, the use of hysteroscopy in this context is debatable, and requires further consideration in future cases. Living donors in this study also underwent CT angiography to assess the uterine vasculature, which is certainly appropriate, to enable appropriate exclusion in the event of stenotic or atherosclerotic vessels, and to help plan the surgery.

The recipients were extensively counselled, including consideration of their pre-operative sex life. All recipients had MRKH. A minimum of 10 day 5 euploid blastocysts were frozen pre-operatively.

The surgical technique employed in the LD cases was similar to that described previously, with midline laparotomy, and dissection of the uterus, with bilateral uterine arteries and veins including patches of internal iliac. Ovarian veins were included in the graft in those who were peri or post-menopausal, thereby negating the potential issues associated with POI seen in the Chinese case discussed previously. The DBD technique was similar to described previously with extensive ligamentous dissection and long vascular ovarian and internal iliac artery and venous pedicles.[150] The

back table dissection was then undertaken, transecting all vessels not included in the graft, and leaving the ovarian veins long, along with the uterine arterial and venous vasculature including a cuff of internal iliac. Following perfusion of the graft, hysteroscopy is performed, as discussed previously. Whilst undertaking it ex-vivo negates issues relating to potential impact on dignity and psychological sequalae in the donor family if undertaken pre-operatively, the potential benefit is still difficult to justify compared to the potential risk of ascending infection. The operation times for the LD cases were between 5-7 hours and the EBL was 420mls.

The recipient surgery commenced once the graft was perfused on the back table and judged satisfactory to be implanted. A midline infraumbilical incision was undertaken, and in a similar fashion to that described previously, the external iliac vessels were dissected and mobilised away from each other and the vaginal vault was dissected away from the bladder. The uterus was then positioned orthotopically in the pelvis, bilateral end to side internal iliac to external iliac arterial anastomoses were undertaken. One case included an internal iliac to internal iliac anastomosis, although it is not described why this was the case. Regarding venous anastomoses, either internal iliac and/or ovarian veins were used, anastomosed to the external iliac, depending on quality. After perfusion is confirmed, the ligamentous connections were made, and the vagina anastomosed, using interrupted sutures. The implantation operations took 3-5 hours and the mean EBL per case was 700mls.

ATG induction immunosuppression was given, and maintenance therapy was given including tacrolimus and MMF for the first three months. Oral prednisolone was given for four weeks. 5 days of antifungal prophylaxis was given with fluconazole 400mg IV, and 24 hours of prophylactic antimicrobials were given. Three months of antiviral cover was given with 900mg valganciclovir daily. Aspirin was commenced.

Post-operatively, six recipients had functional uteri, and menstruation started 5-14 weeks post UTx. Mild rejection episodes were seen in three recipients, while a single moderate and severe episode

were also seen. Mild and moderate rejection was treated with three days of intravenous (IV) methylprednisolone. The severe case was treated with ATG for five days, with escalation of maintenance immunosuppression including azathioprine and prednisolone. In total, four of the women had azathioprine added, due to repeated rejection episodes. Two cases required emergency hysterectomy in the first two weeks due to graft thrombosis. Both presented with congested appearances to their cervices. During one case, from a DBD donor, the uterus appeared necrotic so was removed after 7 days. The other case, from a LD, had a congested but seemingly viable uterus at laparotomy, so thrombectomy and re-anastomosis was undertaken. Despite successful reperfusion, the graft re-thrombosed ten days later and was removed. The decision to attempt thrombectomy, in the context of the macroscopic findings appears reasonable, although the team acknowledge the high risk of subsequent graft failure in such cases. Moreover, their decision to immediately re-operate after visualising the congested cervix, without imaging should be commended. Potential delaying re-operating in such circumstances may result in an inability to salvage the graft, or potentially deterioration of the patient. Another graft was removed seven months post UTx due to HSV infection which obliterated the endometrial cavity rendering it dysfunctional. Whilst HSV serostatus was not reported in the donor and recipient, it may have been reactivation of the disease or primary infection from the allograft. Histological assessment of the graft post hysterectomy revealed fibrotic obliteration of the cervical and uterine cavities.

Regarding other complications, vaginal stenosis was seen in three recipients. Whilst this could be an immunological phenomenon between donor and host tissues, as it is not a complication commonly reported previously, it may be due to suturing technique used at the vaginal anastomosis. The use of interrupted sutures was implemented in this series. In abdominal radical trachelectomy cases, which includes a similar anastomosis, the parachute suture technique is commonly used, and vaginal stenosis is much less commonly reported, in approximately 10% of women.[151] Moreover, spatulating the vagina prior to anastomosis may also help reduce the risk of stenosis. Following treatment for vaginal stenosis, one woman suffered a bladder injury with subsequent formation of vesico-vaginal fistula. Following re-laparotomy and repair of the defect, the stenosed vaginal tissue was removed, and the

vagina re-approximated using a different suturing technique, albeit still using interrupted sutures. It is of interest that at the time of reporting, there had been no re-stenosis.

Two living donors suffered complications. One experienced an intraoperative ureteric injury, which was identified, sutured and stented, with an uneventful recovery. The second suffered bladder hypotonia, which resolved after treatment with a suprapubic catheter and bladder retraining. Complications from such invasive surgery are possible, which is essential to be included in the counselling of future living donors. The management of both complications herein was appropriate, and no long-term impact was caused, but efforts on prophylaxis to prevent future cases suffering similar complications must be implemented. The use of ureteric slings or stents can highlight the ureteric course intraoperatively. Moreover, every effort to preserve the major nerve plexuses must be made, to reduce the risk of post-operative bladder or bowel issues.

Regarding reproductive outcomes, four recipients subsequently underwent 12 embryo transfers in total, with no pregnancies reported at the time of publication. Given that the embryos were reportedly day 5 euploid blastocysts, the pregnancy rates are suboptimal, and require reflection once full data has been reported. In summary, this is an excellent study that added significantly to data in UTx worldwide and provided a number of learning points as discussed herein.

Cases 24-29: Dallas, USA

The next series of five cases were undertaken in Dallas, using multiparous living donors.[152] Inclusion criteria for donation included being between the ages of 40-65, a negative HPV test and genitourinary medicine (GUM) screen, a normal pelvic ultrasound and sufficiently meeting the psychological criteria. Women who were under the age of 40 were considered if they were absolutely certain they did not want further pregnancies. Exclusion criteria included being obese (BMI>30kg/m^2), having an active infection or having a significant medical comorbidity which may pose additional risk. These

selection criteria are sensible and logical, without overly excluding women, thereby optimising potential donor availability without compromising organ quality.

All the recipients were women with MRKH. Similar selection criteria to previous cases were implemented, but with an age range of being between 20-35. The decision to exclude women between 35-38 is understandable given the associated reproductive decline associated with women as they approach their late thirties.[153] However, given that women were funding their own IVF treatment in this series, that decision is perhaps difficult to ethically justify, as pregnancy outcomes would be largely similar following successful conception. All women underwent IVF pre-operatively.

The donor surgery was carried out through an infraumbilical midline incision. In a similar fashion to previous cases, the arterial pedicle included the uterine artery with either a patch or segment of internal iliac artery. The venous outflow included the uterine vein and/or utero-ovarian veins. Following transection of the vagina beneath the cervix and removal of the graft, the uterus was flushed on the back table with perfusion solution, using an angiocath placed in the arterial inflow. The mean operative time was 330 minutes and the mean EBL 900mls. The mean hospital stay was 6 days. One donor suffered a vaginal cuff dehiscence which required surgical repair. Other adverse events seen in donors included an episode of mild depression, temporary gluteal cramping which resolved three weeks post-operatively, a UTI and faecal impaction.

The recipient operation was similarly performed through a midline infraumbilical incision, in line with previous cases. The external iliac artery and vein were dissected along their respective course to prepare for anastomosis with the vasculature of the graft. The arterial anastomosis was then performed using the internal iliac artery patch of the donor to the external iliac artery of the recipient. The first two cases used the uterine artery with a smaller patch of internal iliac, whereas the latter three cases included a longer section of internal iliac. In the second case, who was the oldest donor (55 years old) there was a severely diseased uterine artery orifice which was not possible to cannulate and flush.[80] As such, an arterial reconstruction between the internal pudendal or obturator artery and the uterine

artery was undertaken. The venous anastomosis was undertaken using what was perceived to present the best venous outflow of the graft, using either the uterine or utero-ovarian vein medial to the ovary, end to side with the external iliac vein. The first three cases had both uterine and utero-ovarian venous outflows, whereas, the final two cases, in women aged 34 and 36 years old, used the utero-ovarian veins. By only utilising the vein medial to the ovary allowed ovarian preservation, in contrast to the aforementioned case in China, which utilised the full length in a pre-menopausal woman, thereby causing iatrogenic surgical POI. Whilst there may be concerns regarding potential positional and growth implications of the graft during pregnancy when utilising just the utero-ovarian veins, following two successful livebirths and the absence of significant complication in these cases,[154] this modification does not appear to impact the efficacy of the procedure. The concept of draining the uterus through the ovarian vessels is not new. Following the development of the abdominal radical trachelectomy more than two decades previously,[48] more than 400 infants have since been born in women who have undergone the procedure,[155] proving that uteri remain viable when drained by the ovarian vessels alone.[156] This has been since demonstrated in primate models using real-time indocyanine green (ICG) fluorescence to assess uterine haemodynamics, where blood flow from uterine arteries perfused predominantly to ovarian veins, rather than uterine veins. After the vascular anastomosis was completed, and perfusion assessed, the cervico-vaginal anastomosis was then carried out.

Regarding recipient outcomes, the first three cases failed, with hysterectomy being undertaken on days 6,12 and 14. Despite ultrasound doppler demonstrating blood flow, the appearance of the cervix in all three cases prompted further intervention. The first and third cases both had congested appearing cervices suggestive of an outflow problem, which was reaffirmed from subsequent histological assessment which showed necrotic haemorrhagic damage. During the first two cases, there was kinking noted on the uterine vein after pulling the graft caudally to perform the cervico-vaginal anastomosis. This prompted a change in position of the venous anastomosis to more medial, as opposed to superior wall of the external iliac vein, as is usually undertaken during kidney transplantation. An alternative may be to undertake the vaginal anastomosis, with some anchoring

sutures, for rough approximation, prior to undertaking the vascular anastomosis. Whilst this may add a few minutes onto the warm ischaemic time, it would undoubtedly facilitate more accurate and realistic tension to be placed upon the vascular anastomoses as they are being performed. This may help prevent subsequent 'kinking' or too much tension being placed on the vessels if the vaginal anastomosis is undertaken after the vessels are tied. The second case, who had the arterial reconstruction undertaken, had an ischaemic appearing cervix, which again was reinforced histologically where ischaemic necrosis was seen in the graft. This reinforces the aforementioned point from the Turkish case, where vascular reconstruction should be avoided if possible, owing to the increased risk of thrombosis and graft failure. Whilst this case was prompted by adversity, given the 'almost complete' occlusion of the uterine artery, it raises the question about whether or not the procedure should proceed with vascular reconstruction, or be abandoned. In this example, with the benefit of hindsight, in the context of the subsequent graft failure, the latter option now appears preferable. However, in future cases individualisation of each case is essential, as the risk-benefit ratio of relatively minor vascular reconstruction may be more advantageous than abandoning the procedure. In all three cases, protocol driven biopsies of the cervix identified necrotic areas. These findings prompted the undertaking of MRI scan, which further identified necrosis within the graft. As such, the use of MRIs post-operatively to assess uterine viability was implemented into their protocol. Moreover, whilst the presence of uterine artery doppler was falsely reassuring in these cases, there was lack of blood flow within the uterine parenchyma in those grafts which failed. As such, in future cases, if there is a lack of blood flow within the uterine parenchyma, urgent MRI or surgical exploration is warranted.

Case 29 – Brazil

This case was the 6th DBD UTx case reported to date.[58] The donor was a 45 year old multiparous woman who had three previous normal vaginal deliveries. The recipient was a 32-year-old woman with MRKH.

Recipient selection criteria for this study were similar to those utilised previously including being a woman with AUFI, between the ages of 21-38 years with a BMI <30kg/m^2. They were also required to have absence of significant medical disease that would preclude pregnancy and be human immunodeficiency virus (HIV) and Hepatitis B and C negative. Their decision to insist upon adequate ovarian reserve is seemingly appropriate in this research context, and in line with most other teams undertaking UTx globally. The alternative of using oocyte donation independently significantly increases risk of pregnancy complications including preeclampsia, preterm delivery and small for gestational age.[157] When the criteria for this study would have been established, the only published livebirth following UTx was born at 31^{+5}/40 after developing severe pre-eclampsia.[55] It was uncertain if this was due to precipitating factors associated with UTx, or because the recipient had unilateral renal agenesis. As such, the decision to exclude women requiring oocyte donation, to mitigate potential additional obstetric risk is justified, although it is likely this may be alleviated in future clinical programmes once detailed obstetric outcomes are known. Another inclusion criterion which requires consideration is the insistence upon being in a relationship for at least two years, thereby excluding single women. Moreover, women were also excluded if their partner did not have visible spermatozoa, suggesting women who were in same sex relationships, who may wish to use a sperm donor would not be considered. This decision to exclude such women the opportunity to conceive and insist them be in a relationship with a male, has ethical issues. There is no doubt social support positively influences transplant success and medication adherence, but published outcomes are conflicting and inconsistent. However, following stem cell transplantation, recipients with partners have been shown to successfully buffer the impact of stressful life events on psychological distress, whereas unpartnered recipients did not, exemplifying the benefit of being in a relationship.[158] Despite this, it is inappropriate and unjust to exclude single women, and potential recipients with appropriate social support should be eligible for UTx.

The donor criteria described were fairly minimalist, including just multiparous women who were at least 45 years old. The use of multiparous women is in line with previous studies to ensure the uterus is of proven fertility. However, the exclusion of women between the ages of 18-44 seems absurd,

particularly in light of the implications of donor age upon outcomes in solid organ transplantation and reproductive medicine. In other SOTs, increased donor age has been shown to be associated with worse outcomes.[159-161] Age has been shown to be an independent risk factor for calcification of both internal and external iliac arteries,[162] and arterial stiffness has been observed to increase as age advances.[163] While the use of marginal organs is acceptable in life saving SOTs, owing to the significant risk of death whilst on a waiting list for an organ,[164] it is difficult to justify the use of suboptimal grafts in quality of life improving transplants such as UTx, so to only use grafts from women who are perimenopausal, or post-menopausal, seems inappropriate. Moreover, from a reproductive medicine perspective, pregnancy outcomes have also been shown to be inferior in women > 45 years, compared to those <45 years, with significantly reduced implantation and pregnancy rates, along with higher miscarriage rates, despite similar age donor oocytes,[165] suggesting the use of older donors may additionally impact subsequent pregnancy outcomes following UTx.

The retrieval was carried out in a similar fashion to previous cases, utilising the macrovascular internal iliac patch bilaterally in addition to bilateral ovarian veins, and extensive ligamentous dissection and a 2cm cuff of donor vagina. The ovaries and Fallopian tubes were also removed with the graft, and subsequently removed at the back-table dissection, which lasted 90 minutes. The decision to remove the ovaries and discard them, seems unnecessary, and potentially confusing for donor family. Not only is additional counselling and consent required to remove extra organs, these organs contain germ cells that contribute the genetic material to future offspring, which may make donor family question the genetic makeup of the child subsequently born following UTx.

The implantation was carried out in a similar fashion to those previously, through an infraumbilical midline laparotomy, with the uterus being implanted in an orthotopic position with bilateral internal iliac to external iliac end to side anastomoses, with additional ovarian venous outflows into the external iliac veins. It should be noted that following revascularization, it took more than 60 minutes to achieve haemostasis of several small vessels. This is commonly performed during the back-table dissection, and potentially subjected the recipient to additional and unnecessary blood loss, which

ended up at 1200mls. The immunosuppression regime included ATG induction, with tacrolimus and initially MMF maintenance therapy, which was changed after five months to azathioprine and prednisolone for embryo transfers and throughout pregnancy.

The decision to undergo IVF pre-UTx is in line with other studies, and in this case 16 oocytes were aspirated, which were used to create eight good quality blastocysts which were subsequently vitrified. Following UTx, natural cycle IVF was used, with supplemental vaginal progesterone supplementation, and successful pregnancy was achieved. The pregnancy course was largely uncomplicated, apart from an episode of pyelonephritis, which required a two-day admission and ten-day course of ceftriaxone. A baby girl was born by Caesarean section at 35^{+3} weeks gestation in good condition weighing 2550g. Peripartum hysterectomy was undertaken at the time of Caesarean section, but the reason for this was not described. The uterine graft appeared normal histologically, although there was some intimal fibrous hyperplasia in the uterine arteries. The placenta was normal.

This case was a well thought through experiment, based on previous study protocols, and carried out with excellent outcomes. Ultimately, it proved the feasibility of undertaking UTx using deceased donors and provided an initial logistical workflow to undertake UTx as part of a multi-organ retrieval. In a similar fashion to the Swedish cohort, the use of natural cycle IVF may prolong the time taken to conceive, and number of cycles required.

Cases 30-31: Germany

The following two cases were performed in Tubingen, Germany. Both cases included directed donations, from Mother to daughter. Both donors were 46 years old multiparous women, with 2-4 previous normal deliveries, with no significant previous medical, surgical or obstetric issues. In addition to the routine pre-operative workup for organ transplantation, the donors underwent similar investigations to those undertaken previously, including ultrasound, MRA and CT scans of the abdomen, cervical smear and HPV tests. They also underwent psychological assessments, and those

under severe psychological strain were excluded. This robust investigation of the donors, in the context of their characteristics, make them ideal UTx donors.

The recipients were both 23 years old, with MRKH. One attained adequate vaginal length of approximately 9cm through dilator therapy whilst the other was following laparoscopic Vecchietti. One of the recipients had a history of relapsing remitting multiple sclerosis. Both recipients underwent pre-operative IVF in line with previous cases. In these cases, cleavage stage embryos were cryopreserved using slow freezing techniques. This is in contrast to the use of blastocysts, which were preferred in case series' performed in Czech Republic, Dallas and Brazil.[58,166,167] Blastocysts have various benefits over cleavage stage embryos including superior pregnancy and livebirth rates and are being increasingly utilised clinically,[168-170] although debate over their usage continues.[171] Moreover, this stage embryo facilitates the capability of performing pre-implantation genetic testing for aneuploidies (PGT-A), where embryo biopsy from the trophectoderm during the blastocyst stage is favourable.[172] PGT-A allows the detection of chromosomal abnormalities such as aneuploidy, which are accountable for more than half of miscarriages.[173] PGT-A has been shown to increase implantation and pregnancy rates whilst reducing the risk of miscarriage following single embryo transfer,[174,175] and has been shown to result in a higher livebirth rate per embryo transfer.[176] The majority of the data demonstrating the clinical efficacy of PGT-A is undertaken in young women who have good prognosis, which is directly extrapolatable to the UTx recipient population. Whilst double embryo transfer can produce equivalent implantation rates to single embryo transfer with PGT-A,[177] the transfer of a single euploid embryo virtually negates the risk of multiple gestation, which is higher than 50% following double embryo transfer. In UTx, multiple gestation would potentiate antenatal risk significantly with associated increased likelihood of preterm labour, miscarriage, pre-eclampsia and fetal growth restriction,[178,179] in addition to uncertainties surrounding structural support and uterine perfusion.

Whilst potentially promising, the use of PGT-A in UTx will continue to provoke debate, and further consideration should be given to which subgroups of women it would be most useful, in the context of

cost-effectiveness and potential false positives secondary to mosaicism. Optimising pregnancy rates may not only have psychological and economic benefits, but it could also reduce graft longevity and therefore cumulative immunosuppression exposure. Moreover, managing miscarriage following UTx brings additional complexities including a theoretical increased infection risk, so measures to reduce risk of miscarriage should be prioritised. However, it should be noted that zygotic splitting is possible, and has been shown to increase significantly after embryo biopsy, from 1.5% in non-PGT-A cycles to 2.4% in IVF cycles after PGT-A.[180] As such, it is only a matter of time before multiple gestation pregnancy is experienced in the context of UTx.

With regard to the method of cryopreservation, there is widespread evidence that vitrification is superior to slow freezing in terms of cryosurvival rates and reproductive outcomes.[181] Despite seemingly adequate numbers of embryos being stored from these cases (9-10), embryo shortages were encountered after just 1-3 cycles, owing to poor embryo survival on thawing. This necessitated fresh egg collections following UTx, which may be complicated by distorted anatomy and be associated with increased risk of infection owing to the immunosuppressed state. Despite this, in the two cases performed using fresh cycles, 14-16 oocytes were retrieved using long cycles, with subsequent pregnancies being achieved, in the absence of reported complication.[182]

The retrieval surgery entailed removal of the uterine graft, with long arterial pedicles to the level of the anterior internal iliac artery, the major uterine veins, the utero-ovarian veins and a 2cm cuff of vagina. In one donor, given there was an inadequate uterine vein on the right, the ovarian vein was used instead, necessitating unilateral oophorectomy. The removal of one ovary has been shown to bring forward the age of menopause by approximately 1 year, from an average age of 50.7 to 49.6 years, compared to women who have not had an ovary removed.[183] As such, removing one ovary is likely to be associated with less comorbidity than if both ovaries are removed. No surgical complications were experienced in the donors. The retrieval operative times were between 9-12 hours. Blood loss was not recorded.

The graft was implanted in an orthotopic position, in the same manner as described previously, with vascular anastomoses to the external iliac vessels, a cervico-vaginal anastomosis inferiorly, and surrounding ligamentous anastomoses. The implantations took between 4.5-6 hours. Blood loss was not published. ATG induction immunosuppression was given with maintenance tacrolimus, MMF and prednisolone. Both recipients resumed menstruation after 6 weeks and did not experience complication at the time of the report (3-6 months post op).

This series also included a donor retrieval which did not proceed to implantation. The intended donor was 61 years old with a previous medical history of metabolic syndrome including obesity, hypertension and type II diabetes. Despite acceptable pre-operative MRA and CT imaging of the uterine vasculature, along with the absence of palpable atherosclerosis intra-operatively, extreme resistance was encountered on multiple attempts to flush the uterus through the left uterine artery. As it was not possible to perfuse the graft, the implantation was aborted due to the perceived high risk of graft thrombosis.[78] Subsequent exploration of the graft revealed multiple strictures of the deep arteries, highlighting the caution needed to be taken when using older donors, particularly those with additional risk factors for atherosclerosis. This exemplifies the potential vascular implications of using older donors, particularly with risk factors such as hypertension, obesity and type II diabetes, and justifies almost all other UTx programmes that have excluded such women.

Cases 32-35: India

The final four cases published at the time of writing were undertaken in Pune, India.[184,185] Whilst there was no research experience from this team previously, they describe undertaking cadaveric dissections and 30 similar dissections in living cases whilst undertaking radical hysterectomy for cervical cancer. Specifically, they describe the use of injecting methylene blue in the back table through the uterine arteries, whilst clamping the uterine veins, and observing the flow out of the uterus through the utero-ovarian veins.

Three recipients had MRKH whilst one had severe Asherman's Syndrome and were all between the ages of 21 and 30. All donors were Mothers to the recipients, who were all parous (3-4 previous deliveries) between the ages of 42-48. All recipients and donors were extensively investigated in a similar fashion to those described previously. Recipients underwent pre-operative IVF, with day 3 cleavage stage embryos stored for future use post-UTx. As described previously, this stage embryo is associated with suboptimal success compared to blastocyst stage embryo's and does not allow the optimal use of PGT-A, although this was not used in these cases. The first two cases stored eight and four embryos respectively. The number cryopreserved in the third and fourth cases is unknown. IVF was undertaken four weeks pre-operatively. This seems appropriate, as the hypervascularity and potential hypercoagulable state associated with ovarian stimulation should have resolved by then. The use of ultrasound two weeks after egg collection confirmed normal ultrasonographic appearance of the ovaries.

All of the dissection for the retrievals were undertaken laparoscopically, with the graft subsequently being delivered through a mini-laparotomy incision. Following creation of pneumoperitoneum through Veress needle entry via Palmer's point, the infundibulopelvic ligaments were skeletonised, and the ovarian vessels were dissected to their entry into the infundibulopelvic ligament. The pararectal and paravesical spaces were then opened, and the pouch of Douglas was dissected. Careful ureteric dissection was undertaken until they were lateralised and completely denuded. The anterior branch of the internal iliac artery along with the uterine artery were skeletonised. The internal iliac vein was dissected from the origin to the insertion of the uterine veins. Vessels not included in the graft were ligated. The uterosacral ligaments were cut approximately 2 to 2.5 cm away from the uterus and supporting ligaments were separated prior to colpotomy with 1.5cm of donor vagina. The cardinal ligaments were not included, because of the vessel that run through them. Following mini-laparotomy, with a 5-7cm transverse abdominal incision the vessels were then clamped at the internal iliac artery, the uterine vein and the ovarian artery and veins. Bilateral oophorectomy was performed for both donors. For the latter two cases, the ovarian tissue was subsequently placed in the retroperitoneum. Whilst usually undertaken for oncology patients attempting to preserve fertility, the

use of ovarian tissue autotransplantation, as demonstrated herein, could be used to prevent POI, where it is felt necessary to remove the ovaries to maximise the length of ovarian veins retrieved with the graft. In data to date, following the first livebirth 15 years ago,[186] more than 130 livebirths have now been achieved following ovarian cortex reimplantation.[187] Ovarian function is restored in 95% of cases, and whilst the mean duration of ovarian function is approximately 5 years, they can function for up to 7 years,[188] or as long as 11 years if the procedure is repeated.[189] Whilst all the ovarian tissue was re-implanted, depending on the donor age, and typical number of premenopausal years remaining, a proportion of the ovarian cortex could be cryopreserved for future use, in case POI arose following the initial re-implantation.

Whilst the graft could have been removed through the vagina, the authors attribute the use of a mini-laparotomy to reduce the potential for microorganism contamination of the graft. This is reasonable, although the use of a specimen bag, closed, prior to removal through the vagina may suffice for that role. Despite this, the removal of the graft vaginally may increase risk of trauma to the delicate vessels during removal, and whilst the mini-laparotomy may increase recovery in the donor, it's use is probably justified. The estimated blood loss in all four cases was 100mls. The mean operative time was 3 hours 30 minutes. There were no donor complications. The significant reduction in reported blood loss, operative time, and lack of complications perhaps highlight the advantages to the use of minimal access retrieval techniques, although little can be drawn from such a small number of cases.

Following removal of the graft, it was flushed with 1-2l cold preservation solution through all four arterial inflows and stored on ice. Bilaterally, the internal iliac and ovarian vessels were identified, dissected, skeletonised, and the openings were prepared for anastomosis. In the first two cases, the uterine (including internal iliac), arterial lengths were 8.5-9cm, whereas the corresponding veins were 9-10cm in length. The ovarian arteries were 4-11cm, whilst the veins were 11cm. The latter two cases measurements were not recorded. The use of Ligaclips (Ethicon, Cincinnati, OH, USA) were used in the latter two cases to facilitate the ligation of smaller vessels not included in the graft, to reduce

subsequent blood loss in the recipient following revascularisation. For the latter two cases, the ovarian arteries and uterine veins were ligated on the back table.

The recipient procedures were undertaken simultaneously with the donor operations. Following laparoscopic entry, the peritoneum was incised between round ligaments, and the bladder and rectum were dissected away. If the uterine buds were located centrally, they were excised to access the vagina. One case, with Asherman's syndrome, required concomitant hysterectomy. This was completed laparoscopically, in a standard fashion, but in addition the round ligaments were kept long, and the uterosacral ligaments were cut close to the uterus, both to optimise subsequent structural support following implantation. The peritoneum overlying the sacral promontory was then entered and extended unilaterally across to the common iliac vessels. Following the opening of the pararectal space, the external iliac and internal iliac vessels were identified, and the ureter was dissected away. This dissection was repeated on the contralateral side. A hollow plastic tube was inserted into the vagina to elevate the overlying pelvic peritoneum and following a transverse incision on the vagina, the rectum and bladder were dissected away. Support sutures were inserted through the round ligaments bilaterally and a suture was inserted in the anterior longitudinal ligament over the sacral promontory for further support. Following this pre-operative laparoscopic dissection, the abdomen was opened using a vertical midline incision and the graft was brought into the pelvis. The vascular anastomoses were then carried out. The first two cases used the same anastomoses as described previously, connecting the internal iliac vessels of the graft to the external iliac vessels of the recipient in an end to side manner. In addition, the ovarian veins were anastomosed to the external iliac vessels, similarly to cases undertaken previously. The latter two cases instead anastomosed the arterial vessels of the graft to the internal iliac arteries of the recipient using an end to end technique, whilst the venous anastomoses were only performed using the ovarian veins, to the external iliac veins (end to side). This ensured exclusive arterial supply to the uterus and continued the evolution of the surgical technique with the isolated venous drainage through the ovarian veins. This use of these veins alone, decrease surgical risk significantly, and reduces operative time, with a mean of 3 hours for the retrieval of these two latter cases being reported. Following approximation and suturing of the

supporting ligaments, routine closure was undertaken. All four implantation operations were between 4-4.5 hours. The EBL was between 300mls-1000mls with a mean of 625mls.

All recipients were given induction immunosuppression, but there were differences between the first and second two cases. The first two cases received the standard immunosuppression regime previously described, with ATG and methylprednisolone induction, and tacrolimus, MMF and prednisolone maintenance. Two episodes of rejection were noted histologically, with unspecified severity, which was successfully treated with escalation of the maintenance immunosuppression, and a course of steroids respectively. The first was diagnosed using a cervical biopsy on post-operative day 7, whereas the latter episode, was diagnosed using a hysteroscopic guided endometrial biopsy. Whereas the use of hysteroscopic guided biopsies allows visualisation of the cavity, it clearly increases the theoretical risk of ascending infection, and is of debatable necessity in light of the already established use of cervical biopsies to detect rejection described previously.[123] However, judgement should be reserved until detailed immunological outcomes and correlation between endometrial and cervical biopsies are published. The immunosuppression regime was changed for the second two cases, with basiliximab and methylprednisolone induction, and the addition of everolimus in the maintenance. Whilst there was no reason cited for changing the immunosuppression regimen, it may be a reaction to the rejection episodes experienced in the first two cases. It is of interest that no rejection episodes were described in the latter two cases, although the period of follow up is not described. Post-operatively all four recipients menstruated, without hormonal stimulation in the second post-operative month. No surgical complications were experienced. While no pregnancies have been published yet, the media have reported a livebirth has subsequently been achieved following a case performed in India.

These cases highlight important evolutionary progress with regards to key surgical aspects of UTx. It provides further evidence that the uterus remains viable when drained solely by the ovarian vessels, but also demonstrates the potential utilisation of minimal access surgical techniques, during both the retrieval and implantation. Whereas the benefits are currently negated by the necessity to implant the

organ through an open incision, further evolution may enable the procedure to be entirely undertaken laparoscopically. Whilst the authors describe the post-operative courses of both donors and recipients in the latter two cases ‘flawless’, further data and future outcomes are essential to verify such positive outcomes.

Overview of key aspects of uterine transplantation

At the time of writing, 35 cases have been published in peer reviewed journals. The outcomes of an additional ten unpublished cases which have been undertaken in Dallas, have been communicated personally, the results of which have been published in the British Journal of Obstetrics and Gynaecology.[59] The following summary, reviews the outcomes from these 45 cases, to establish trends and best practice, as the transition between research concept and established clinical procedure approaches. According to media reports, other unpublished cases have been performed in Serbia, Lebanon, Belgium, France, Spain,

Recipients

UTx is a potential treatment for women with AUFI, who are of reproductive age and have not yet completed their family. Aetiologies of AUFI where the uterus is absent, can be categorised into congenital, such as MRKH or complete androgen insensitivity syndrome (CAIS) and acquired, following hysterectomy to treat post-partum haemorrhage, benign gynaecological disease or gynaecological cancer. Causes of AUFI characterised by a non-functioning uterus include Asherman's Syndrome (AS), severe inoperable fibroids, adenomyosis or radiation damage and congenital structural abnormalities. The mean age of the recipients in the UTx cases performed to date is 27.8 years (n=45; range 20-38; SD 4.5). **Table 1** summarises the key surgical information from the cases performed to date.

Team	Year	Author	Cause of AUFI	Age (y)	Surgical route	WIC	CIC	Duration	Arterial anastomosis	Venous anastomosis	Blood loss (mls)
Saudi Arabia	2000	*Fageeh et al 2002.*[52]	PPH - Hyst	26	Open	N/R	N/R	N/R	UA extended with 8cm reversed SV lengths to EI	UV extended with 6cm SV lengths to EI	N/R
Turkey	2011	*Ozkan et al 2013.*[54]	MRKH	21	Open	N/R	N/R	5h 30m	CI – EI	CI – EI	N/R
Sweden	2012 - 2013	*Brannstrom et al 2014.*[56]	Cervical Ca – Hyst	33	Open	1h 18m	1h 30m	4h 10m	II to EI	II to EI +/- OV to UV/EI	400
Sweden	2012 - 2013	*Brannstrom et al 2014.*[56]	MRKH	38	Open	1h 38m	1h 47m	4h 17m	II to EI	II to EI +/- OV to UV/EI	1600
Sweden	2012 - 2013	*Brannstrom et al 2014.*[56]	MRKH	28	Open	1h 34 m	1h 4m	4h 50m	II to EI	II to EI +/- OV to UV/EI	800
Sweden	2012 - 2013	*Brannstrom et al 2014.*[56]	MRKH	27	Open	1h 17m	57m	5h 4m	II to EI	II to EI +/- OV to UV/EI	250
Sweden	2012 - 2013	*Brannstrom et al 2014.*[56]	MRKH	35	Open	1h 13m	1h 6m	4h 55m	II to EI	II to EI +/- OV to UV/EI	750
Sweden	2012 - 2013	*Brannstrom et al 2014.*[56]	MRKH	27	Open	1h 24m	2h 0m	4h 30m	II to EI	II to EI +/- OV to UV/EI	700
Sweden	2012 - 2013	*Brannstrom et al 2014.*[56]	MRKH	28	Open	1h 15m	54m	4h 44m	II to EI	II to EI +/- OV to UV/EI	300
Sweden	2012 - 2013	*Brannstrom et al 2014.*[56]	MRKH	33	Open	1h 14m	56m	5h 56m	II to EI	II to EI +/- OV to UV/EI	750
Sweden	2012 - 2013	*Brannstrom et al 2014.*[56]	MRKH	35	Open	1h 32m	1h 28m	4h 31m	II to EI	II to EI +/- OV to UV/EI	500
Xian, China	2015	*Wei et al 2017.*[134]	MRKH	22	Open	1h 32m	3h 33m	8h 50m	UA to EI	OV – EI	490
Clevelan d, USA	2016	*Flyckt et al 2017.*[136]	MRKH	26	Open	N/R	7h 14m	N/R	UA to EI	DUV to EI	N/R
Czech Republic	2016 - 2018	*Chmel et al 2018.*[166]	MRKH	30	Open	N/R	5h 20m	3h 30m	II to II	OV to II	1200
Czech Republic	2016 - 2018	*Chmel et al 2018.*[166]	MRKH	26	Open	N/R	6h 30m	3h 50m	II to EI	OV to EI	600
Czech Republic	2016 - 2018	*Chmel et al 2018.*[166]	MRKH	23	Open	N/R	5h	5h	II to EI	OV to EI	700
Czech Republic	2016 - 2018	*Chmel et al 2018.*[166]	MRKH	26	Open	N/R	4h	3h 40m	II to EI	DUV to EI	200
Czech Republic	2016 - 2018	*Chmel et al 2018.*[166]	MRKH	26	Open	N/R	4h 50m	3h 40m	II to EI	DUV to EI + OV to EI	600
Czech Republic	2016 - 2018	*Chmel et al 2018.*[166]	MRKH	30	Open	N/R	4h 40m	4h	II to EI	DUV to EI + OV to EI	200
Czech Republic	2016 - 2018	*Chmel et al 2018.*[166]	MRKH	26	Open	N/R	2h 30m	4h 10m	II to EI	UV to EI	1000
Czech Republic	2016 - 2018	*Chmel et al 2018.*[166]	MRKH	25	Open	N/R	9h	5h	II to EI	DUV to EI + OV to EI	500
Czech Republic	2016 - 2018	*Chmel et al 2018.*[166]	MRKH	33	Open	N/R	4h	4h	II to EI	II to EI + OV to EI	300
Dallas, USA	2016	*Testa et al 2017.*[167]	MRKH	32	Open	1h 14m	3h	6 h	Patch of II to EI	DUV to EI + UOV to EI	2000

Dallas, USA	2016	*Testa et al 2017.*[167]	MRKH	33	Open	1h 8m	4h 30m	6 h	Patch of II to EI / UA reconstruction to EI	DUV to EI + UOV to EI	500
Dallas, USA	2016	*Testa et al 2017.*[167]	MRKH	34	Open	1h	3h	5 h 30m	Whole segment of II to EI	DUV to EI + UOV to EI	500
Dallas, USA	2016	*Testa et al 2017.*[167]	MRKH	28	Open	1h 7 m	3h	4 h 30m	Whole segment of II to EI	UOV to EI	500
Dallas, USA	2016	*Testa et al 2017.*[167]	MRKH	28	Open	40m	4h	4 h 30m	Whole segment of II to EI	UOV to EI	300
Dallas, USA	2017	*Jones et al. 2019.*[59]	MRKH	34	Open	21m	4h 29m	4h 30m	Whole segment of II to EI	DUV to EI + UOV to EI	500
Dallas, USA	2017	*Jones et al. 2019.*[59]	MRKH	24	Open	1h 2m	4h 13m	5h 11m	Whole segment of II to EI	DUV to EI	700
Dallas, USA	2017	*Jones et al. 2019.*[59]	MRKH	28	Open	1h 16m	7h 14m	7h 3m	Whole segment of II to EI	DUV to EI + DUV to EI + UOV to EI	400
Dallas, USA	2018	*Jones et al. 2019.*[59]	MRKH	22	Open	16m	3h 9m	5h 23m	Whole segment of II to EI	DUV to EI + UOV to EI	250
Dallas, USA	2018	*Jones et al. 2019.*[59]	MM - Hyst	29	Open	1h 5m	1h 17 m	5h 9m	Whole segment of II to EI	UOV to EI	500
Dallas, USA	2018	*Jones et al. 2019.*[59]	MRKH	20	Open	1h 31m	3h 30m	5h 11m	Whole segment of II to EI	DUV to EI + UOV to EI	1400
Dallas, USA	2018	*Jones et al. 2019.*[59]	MRKH	23	Open	1h 17m	6h 56m	7h 43m	Whole segment of II to EI	DUV to EI + DUV to EI	500
Dallas, USA	2018	*Jones et al. 2019.*[59]	MRKH	30	Open	60m	4h 40m	4h 48m	Whole segment of II to EI	UOV to EI	300
Dallas, USA	2018	*Jones et al. 2019.*[59]	MRKH	21	Open	1h 7m	3h 27m	5h 50m	Whole segment of II to EI	UOV to EI + UOV to EI + DUV to EI	200
Dallas, USA	2019	*Jones et al. 2019.*[59]	MM - Hyst	31	Open	40m	3h 17m	4 h 1m	Whole segment of II to EI	DUV to EI + UOV to EI	600
Dallas, USA	2019	*Jones et al. 2019.*[59]	MRKH	30	Open	1h	2h 31m	4h 30m	Whole segment of II to EI	OV to EI + UOV to EI + DUV to EI	600
Brazil	2016	*Ejzenberg et al 2018.*[58]	MRKH	32	Open	1h 25m	6h 25m	11h	N/R	II to EI + OV to EI	1200
Germany	2017	*Brucker et al 2017.*[78]	MRKH	23	Open	47m	N/R	5h 57m	II to EI	II to EI + UOV to II of graft	N/R
Germany	2017	*Brucker et al 2017.*[78]	MRKH	23	Open	1h 7m	N/R	4h 31m	II to EI	II to EI + UOV to II of graft	N/R
India	2017	*Puntambekar et al 2018.*[184]	MRKH	21	Open	35m	45m	4h	II to EI	II to EI + OV to EI	1000
India	2017	*Puntambekar et al 2018.*[184]	AS	26	Open	35m	34m	4h	II to EI	II to EI + OV to EI	300
India	2018	*Puntambekar et al 2018.*[185]	MRKH	30	Open (prepared lap)	52m	34m	4h	II to II	OV to EI	400
India	2018	*Puntambekar et al 2018.*[185]	MRKH	24	Open (prepared lap)	46m	33m	4h 30	II to II	OV to EI	800

Table 1: *Summary of recipient demographics and surgical details (Amended from Jones et al. 2019)*[59]

Mayer-Rokitansky-Küster-Hauser Syndrome

The overwhelming majority (n=40; 88.9%) of UTX cases performed to date have been undertaken in women with MRKH. MRKH affects 1 in 5000 newborn females,[190] and is the second most common cause of primary amenorrhea.[191] As a consequence to the failure of the Müllerian ducts to develop, the uterus, fallopian tubes and upper vagina are absent. Women with MRKH have a normal female karyotype (46XX) and have normal secondary sexual characteristics. MRKH is categorised into two subtypes; Type 1 and Type 2. Type 1 incorporates isolated uterovaginal aplasia or hypoplasia, whereby type 2 incorporates the addition of renal, ovarian, hearing or skeletal abnormalities. MRKH has traditionally been considered a sporadic condition, owing to previously reported discordance between identical twins, and no confirmed cases of females with MRKH being born from surrogate pregnancies using oocytes from women with MRKH.[192,193] In such cases, the recurrence risk of between 1-5% is usually quoted to affected women.[194] However, familial cases have also been reported affecting both males and females.[195,196] Recent technological advancement in sequence technologies have enabled the demonstrated the partially genetic makeup to MRKH.[197-199] In familial cases, the syndrome appears to be inherited in an autosomal dominant fashion, with incomplete penetrance and variable expression, resulting in a theoretical inheritance rate of up to 50%. As such genetic counselling is essential and exome sequencing should be considered in familial cases.

Hysterectomy

Four (8.9%) cases have been undertaken so far in women following previous hysterectomy because of cervical cancer (n=1),[56] post-partum haemorrhage (n=1),[52] and following unsuccessful myomectomy (n=2), although other indications include benign gynaecological disease such as adenomyosis or dysfunctional

Asherman's Syndrome

One UTx case (2.2%) has been performed for severe AS.[184] Asherman's Syndrome affects up to 1.5% of women of reproductive age.[200] The mainstay of treatment includes the restoration of uterine

function using operative hysteroscopy and adhesiolysis. However, high rates of infertility, miscarriage, poor implantation and abnormal placentation can persist.[201] Novel therapeutic modalities, such as the use of stem cells appear promising, with studies describing resumption of menstruation,[202] successful conception with IVF,[203] and successful spontaneous pregnancies,[204] in previously treatment refractory cases following treatment with autologous stem cells. UTx, with concomitant hysterectomy immediately prior, should be reserved for treatment refractory Asherman's, after the exhaustion of all other treatment options.

Donors

UTx is a non-life saving, quality of life improving SOT. Unlike quality of life improving vascular composite allograft transplants, such as face, upper limb, abdominal wall and penis, the non-essential nature of a uterus after the completion of one's family, allows the potential use of both living and deceased donors. Each donor type presents ethical, logistical, physiological, immunological and anatomical advantages and disadvantages that continue to stimulate debate.[205-208] The mean age at donation is 44 years (n=45, range 20-62; SD 10.2). Where the parity of the donor is reported the majority have been multiparous (n=40; 93.2%) with three (6.8%) being nulliparous. The mean donor parity is 2.5 (range 0-7; SD 1.3). A summary of the donor demographics and surgery details and outcomes is displayed in **Table 2**.

Living Donation

80% (n=36) of UTx cases reported so far have been performed using living donors in Saudi Arabia,[52] Sweden,[56] China,[134] Czech Republic,[166] the United States,[80] Germany,[78] and India.[184,185]

The use of living donors offers organisational advantages, allowing the elective planning of a complex operation which demands multi-disciplinary expertise. This allows abundant time for pre-operative counselling and investigation of the donor. Additional investigations such as magnetic resonance angiography (MRA) and computed tomography angiography (CTA), can also be undertaken, which

provide data on morphology, calibre and patency of the vessels that will subsequently be transplanted as part of the graft. CTA has widely been used pre-operatively in the assessment of living donors, to visualise the arterial vasculature to the 1-2mm terminal uterine artery branches.[209] However, following the first 5 cases in the Dallas series, where CTA did not accurately assess the venous outflow, the use of MRA with delayed venous phase was recommended in addition.[210]

The close proximity and timing of retrieval and implantation operations in living donation results in a reduction in cold ischaemic time (CIT), which reduces risk of potential ischemia-reperfusion injury, which has been shown to increase risk of acute and chronic rejection as well as chronic graft dysfunction.[211,212] A reduction in CIT has been demonstrated in UTx cases performed so far, where the mean CIT in deceased donor cases is double that of living donors (5h 42m+/- 2h 7m; n=8 vs 2h 50m +/- 1h 47m; n=33). The CIT experienced in the only successful DBD case that has resulted in a livebirth so far was 6 h 30, demonstrating in a human model that this CIT acceptable.[58] However, uterine grafts have been demonstrated to tolerate CITs of up to 24 hours in both animal and human models,[213-215] so the extended CIT experienced using deceased donors is of uncertain clinical significance at present.

Just over half of living donors used so far have been directed donations from biological relatives (n=19; 52.8%), with either first degree (Mother; n =16, Sister n = 1), or second degree (Aunt; n = 2) relation. 17 (29.4%) cases used donors that were unrelated, with directed donation from close friends (n=2) or extended family (n=1) or directed altruistic donation from women with no pre-existing genetic or emotional relationship to the recipient (n=14). The majority (n=13) of those non-directed cases were from cases performed in Dallas, utilising predominantly healthcare workers from the hospital where the surgeries were performed.[80,216]

The use of first-degree relatives as donor may have a beneficial immunological match. This has been demonstrated in a study comparing outcomes between related and unrelated kidney donors over almost 40 years, where significantly more episodes of rejection were seen in the unrelated group

(46.3% vs 40.7% p=0.03). However, a significant difference in graft survival was not observes, raising uncertainty regarding overall benefit.[217] Owing to the need to have completed one's family prior to donation, the most likely related donor, as exemplified in the cases performed so far, is maternal relation. The average age of living donor is 45.4 +/- 8.6 (range 30-62), in contrast to deceased donors which is 38.3 +/- 14.3 (range 20-57).

The major disadvantage of using living donors is the associated risk involved. Regarding donor surgical morbidity in the cases performed so far, as per the Clavien-Dindo classification of surgical complications, 11.1% (n-4) donors suffered 4 grade IIIb complications. Two donors suffered ureteric injuries which were repaired intra-operatively during the retrieval,[52,166] whilst one donor re-presented post-operatively with a ureterovaginal fistula.[56] The other Grade IIIb complication was vaginal cuff dehiscence which subsequently required surgical repair.[80] 27.8% (n=10) donors suffered lesser significant Grade I-II complications including wound infection,[56] bladder hypotonia,[166] urinary tract infections (UTI; n=3), constipation, leg/buttock pain, depression,[80] intubation related respiratory failure and anaemia.[59] The mean donor blood loss in reported cases so far has been 600mls +/- 581mls (n=29; range 100-2400mls).

Team	Year	Author	Donor Type	Age (y)	Donor parity	Route	Duration	Blood loss (mls)	Donor complication
Saudi Arabia	2000	*Fageeh et al 2002.*[52]	LD - unrelated	46	N/R	Open	N/R	N/R	Intraoperative ureteric laceration - repaired
Turkey	2011	*Ozkan et al 2013.*[54]	DBD	22	0	Open	2h	N/A	N/A
Sweden	2012 - 2013	*Brannstrom et al 2014.*[56]	LD – Mother	52		Open	10h 24m	300	Nil
Sweden	2012 - 2013	*Brannstrom et al 2014.*[56]	LD - Mother	58		Open	12h 37m	2400	Wound infection, Ureterovaginal fistula D16
Sweden	2012 - 2013	*Brannstrom et al 2014.*[56]	LD –Aunt	54		Open	12h 53m	800	Nil
Sweden	2012 - 2013	*Brannstrom et al 2014.*[56]	LD - Mother	50		Open	10h 34m	600	Nil
Sweden	2012 - 2013	*Brannstrom et al 2014.*[56]	LD - Friend	61	3 (mean)	Open	10h 17m	600	Nil
Sweden	2012 - 2013	*Brannstrom et al 2014.*[56]	LD - Mother	53		Open	10h 52m	700	Nil
Sweden	2012 - 2013	*Brannstrom et al 2014.*[56]	LD - Mother	50		Open	10h 17m	400	Nil
Sweden	2012 - 2013	*Brannstrom et al 2014.*[56]	LD - Sister	37		Open	11h 23m	400	Nil
Sweden	2012 - 2013	*Brannstrom et al 2014.*[56]	LD – Mother-in-law	62		Open	13h 8m	2100	Nil
Xian, China	2015	*Wei et al 2017.*[134]	LD - Mother	42	2	Robot assisted	6 h	100	Nil
Cleveland, USA	2016	*Flyckt et al 2017.*[136]	DBD	34	3	Open	N/R	N/A	N/A
Czech Republic	2016 - 2018	*Chmel et al 2018.*[166]	LD - Mother	53	2	Open	5h 20m	100	Nil
Czech Republic	2016 - 2018	*Chmel et al 2018.*[166]	LD - Mother	58	2	Open	6h 10m	800	Nil
Czech Republic	2016 - 2018	*Chmel et al 2018.*[166]	LD - Aunt	47	1	Open	7h 10m	100	Nil
Czech Republic	2016 - 2018	*Chmel et al 2018.*[166]	LD - Mother	49	3	Open	5h 30m	100	Bladder hypotonia (resolved 3 months post-op)
Czech Republic	2016 - 2018	*Chmel et al 2018.*[166]	LD - Mother	49	2	Open	5h 30m	1000	Intraoperative ureteric laceration - repaired
Czech Republic	2016 - 2018	*Chmel et al 2018.*[166]	DBD	57	1	Open	N/R	N/A	N/A
Czech Republic	2016 - 2018	*Chmel et al 2018.*[166]	DBD	24	0	Open	N/R	N/A	N/A
Czech Republic	2016 - 2018	*Chmel et al 2018.*[166]	DBD	20	0	Open	N/R	N/A	N/A
Czech Republic	2016 - 2018	*Chmel et al 2018.*[166]	DBD	56	1	Open	N/R	N/A	N/A
Dallas, USA	2016	*Testa et al 2017.*[167]	LD – Non-directed	42	2	Open	8h	400	Leg/buttock pain

Dallas, USA	2016	*Testa et al 2017.*[167]	LD – Non-directed	55	3	Open	9h	1000	UTI
Dallas, USA	2016	*Testa et al 2017.*[167]	LD – Non-directed	45	3	Open	8h	1300	Depression, vaginal cuff dehiscence
Dallas, USA	2016	*Testa et al 2017.*[167]	LD – Non-directed	34	2	Open	8h	1750	UTI
Dallas, USA	2016	*Testa et al 2017.*[167]	LD – Non-directed	36	4	Open	8h	250	Faecal impaction
Dallas, USA	2017	*Jones et al. 2019.*[59]	DBD	39	2	Open	N/R	N/A	N/A
Dallas, USA	2017	*Jones et al. 2019.*[59]	LD – Non-directed	38	3	Open	7h 7m	1100	Nil
Dallas, USA	2017	*Jones et al. 2019.*[59]	DBD	48	1	Open	N/R	N/A	N/A
Dallas, USA	2018	*Jones et al. 2019.*[59]	LD – Non-directed	34	7	Open	6h 38m	600	Nil
Dallas, USA	2018	*Jones et al. 2019.*[59]	LD – Non-directed	48	2	Open	6h 12m	400	Intubation related respiratory failure
Dallas, USA	2018	*Jones et al. 2019.*[59]	LD – Non-directed	31	4	Open	7h 34m	750	Nil
Dallas, USA	2018	*Jones et al. 2019.*[59]	LD- Friend	33	2	Open	6h 27m	1500	Anaemia
Dallas, USA	2018	*Jones et al. 2019.*[59]	LD – Non-directed	39	4	Open	5h 33m	600	UTI
Dallas, USA	2018	*Jones et al. 2019.*[59]	LD – Non-directed	31	1	Open	5h 13m	450	Nil
Dallas, USA	2019	*Jones et al. 2019.*[59]	LD – Non-directed	42	2	Open	6h 10m	800	Nil
Dallas, USA	2019	*Jones et al. 2019.*[59]	LD – Non-directed	30	1	Robotic	9h 25m	100	Nil
Brazil	2016	*Ejzenberg et al 2018.*[58]	DBD	45	N/R	Open	N/R	N/A	N/A
Germany	2017	*Brucker et al 2017.*[78]	LD - Mother	46	4	Open	12h 7m	N/R	Nil
Germany	2017	*Brucker et al 2017.*[78]	LD - Mother	46	2	Open	9h 3m	N/R	Nil
India	2017	*Puntambekar et al 2018.*[184]	LD - Mother	45	4	Lap-assisted	4h	100	Nil
India	2017	*Puntambekar et al 2018.*[184]	LD - Mother	42	3	Lap-assisted	4h	100	Nil
India	2018	*Puntambekar et al 2018.*[185]	LD - Mother	48	3	Lap-assisted	2h 40	100	Nil
India	2018	*Puntambekar et al 2018.*[185]	LD - Mother	47	4	Lap-assisted	3h 20	100	Nil

Table 2: *Summary of donor demographics, surgical details and donor outcomes in cases to date (Amended from Jones et al 2019)*[59]

Donation after Brainstem Death

Donation after brainstem death (DBD) refers to the retrieval of the uterus during a multi-organ retrieval. 9 (20%) UTx procedures have been undertaken using deceased donors in Turkey,[53] the United States,[136,210] Brazil,[58] and the Czech Republic.[166] The first livebirth following DBD UTx was achieved in Brazil in 2017,[58] which demonstrated UTx is feasible in this model. The main advantage of using DBD is the complete elimination of physical and psychological risk in the donor. However, organisationally this presents logistical difficulties. An on-call rota, made up of individual teams with the necessary expertise, is necessary to provide cross-cover to prevent interference with everyday schedules. For a novel procedure, this is a significant investment in resources, which may not be possible in a number of countries, or healthcare systems. The UTx team in Limoges, France has had to suspend their UTx activities until they resolve the logistical difficulties associated with using the DBD model.[208]

It is well known that transplant outcomes in other SOTs are superior in living donors, when compared to deceased donors,[218] even when adjusted for differences in ischaemia times.[219] This has been attributed to physiological instability following brainstem death, and organ deterioration caused as a consequence to brain-death associated systemic inflammation.[220] Physiological optimisation therefore should be undertaken, as demonstrated by the Brazilian DBD UTx case where norepinephrine and vasopressin were used for four hours prior to retrieval.[58]

Surgical considerations

The majority of donor surgeries performed have so far been undertaken utilising a midline laparotomy approach (n=36). Minimally invasive surgical techniques have been used more recently in living donors (robot-assisted =2, laparoscopy =4),[184,185,221,222] highlighting that the transition into adopting minimally invasive surgical techniques is currently in progress.

From a vascular perspective, the arterial input of all cases performed so far has been through the uterine arteries, with varying degrees of laterality and often including varying lengths of the anterior division of the internal iliac arteries (n=45; 100%). The graft arterial supply is mostly anastomosed end-to-side with the recipient's external iliac vessels (n=42; 93.3%). However, three cases (6.6%) anastomosed the graft arteries to the internal iliac arteries,[166,185] two of which used an end-to-end technique, allowing exclusive arterial supply to the uterus.[185]

The venous drainage has predominantly utilised the uterine veins, to the level of the anterior division of the internal iliac vein, with or without the addition of the ovarian veins. This early surgical technique, demonstrated in **Figures 5-7**, used the largest vessels possible, which minimises the risk of graft thrombosis post-operatively. However, the complex ureteric dissection around the tortuous and unpredictable uterine venous complex poses significant surgical risk and a time-consuming retrieval. This was exemplified by two ureteric injuries being sustained in the first four cases. Moreover, with a mean operative time of >12 hours utilising this technique in the Swedish cohort,[56] given that the risk of VTE increases with surgical duration,[223] the risk of potentially life-threatening VTE is significant, and difficult to justify in a quality of life improving organ transplant.

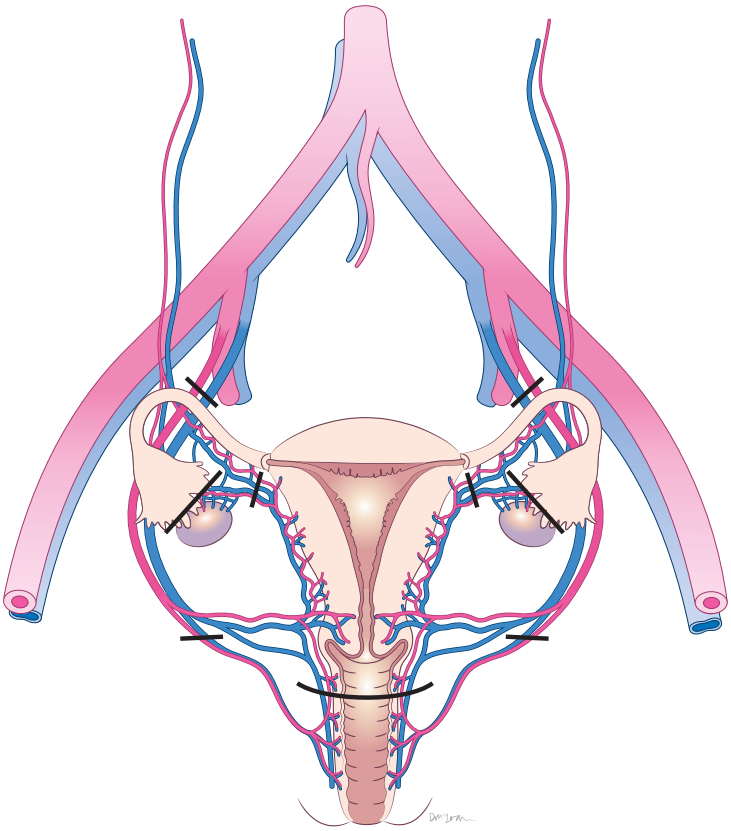


Figure 5: *Uterine transplant retrieval technique demonstrating bilateral long internal iliac arteriovenous pedicles (Reproduced with permission from Jones et al. 2019)*[59]

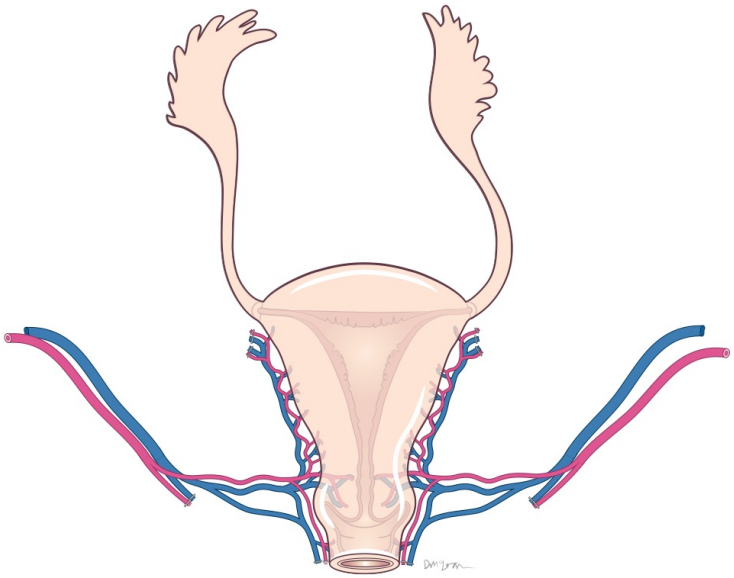


Figure 6: *Uterine transplant graft following retrieval (not including optional ovarian veins) (Reproduced with permission from Jones et al. 2019)*[59]

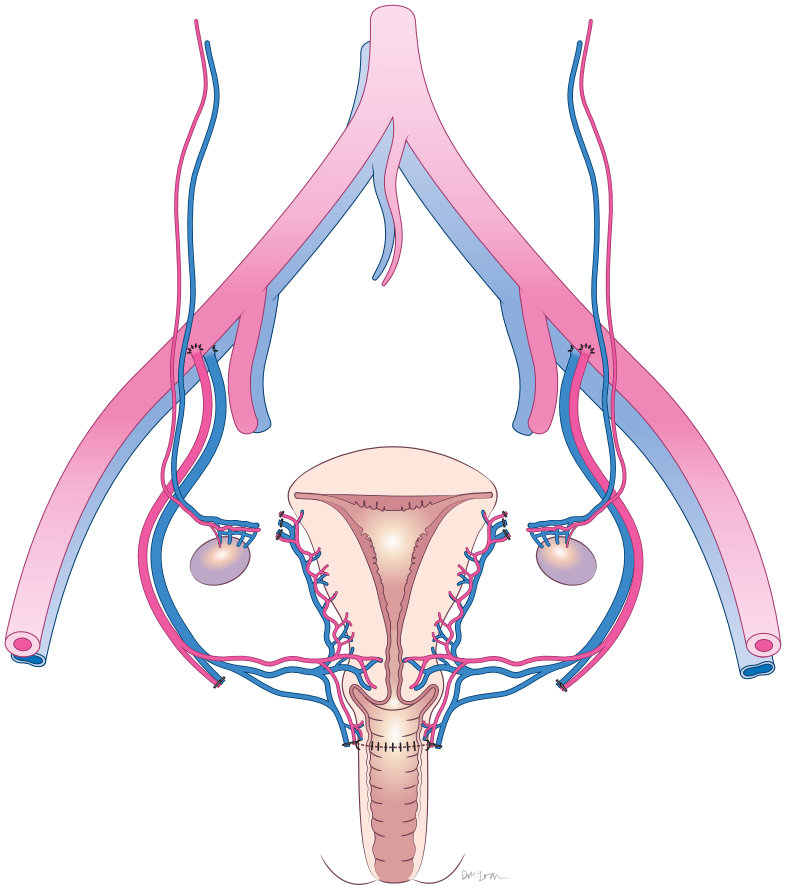


Figure 7: *Recipient anatomy following implantation demonstrating bilateral internal iliac to external iliac arterial and venous anastomoses (Reproduced with permission from Jones et al. 2019)*[59]

Recent evolution in surgical technique, with modification to the venous drainage in cases performed in China,[134] Dallas,[80] the Czech Republic,[166], and India.[185] By utilising the ovarian/utero-ovarian veins instead of the uterine veins as demonstrated in **Figures 8** and **9**, the surgical dissection negates the need of the aforementioned complex dissection around the complex and unpredictable uterine venous plexus and ureter. The mean operative time for the cases using this technique is 5h 51m +/- 1h 46m (n=10; 2h40m-8h) which is almost 3 hours faster than those performed in living donors using the initial surgical technique (n=25, mean; 8h 39m +/- 2h 46m, range; 4h-13h 8m). However, in most of these cases the uterine veins were retrieved in addition to ensure the best quality veins could be selected,[166,167] so the evolved surgical technique procedure time is expected to decrease in the future if only the utero-ovarian/ovarian veins are retrieved. This reduces surgical risk as well as operative time,

which subsequently theoretically reduces VTE risk, increasing the sustainability of using living donors in UTx. This was exemplified by the two most recent cases undertaken in India, where a mean retrieval time of 3 hours was reported.[185] Reducing the number of venous anastomoses from four to two will not only reduce implantation time, but also the potentially harmful warm ischaemic time (WIT). Hypothermia reduces metabolic turnover and thereby reduces oxygen requirements, which subsequently limits tissue ischaemia. However, as the organ warms, metabolic demands increase, and in the context of an organ which is still oxygen and nutrient deplete, there is accelerated functional and structural cell deterioration. As such, the effects of warm ischaemia have been demonstrated to be much more damaging than CIT in other SOTs.[224,225] The mean WIT in UTx cases so far is 1h 6m +/- 21m. However, it is worth considering that a study in primates identified that WITs of up to 4 hours may be acceptable in UTx.[226]

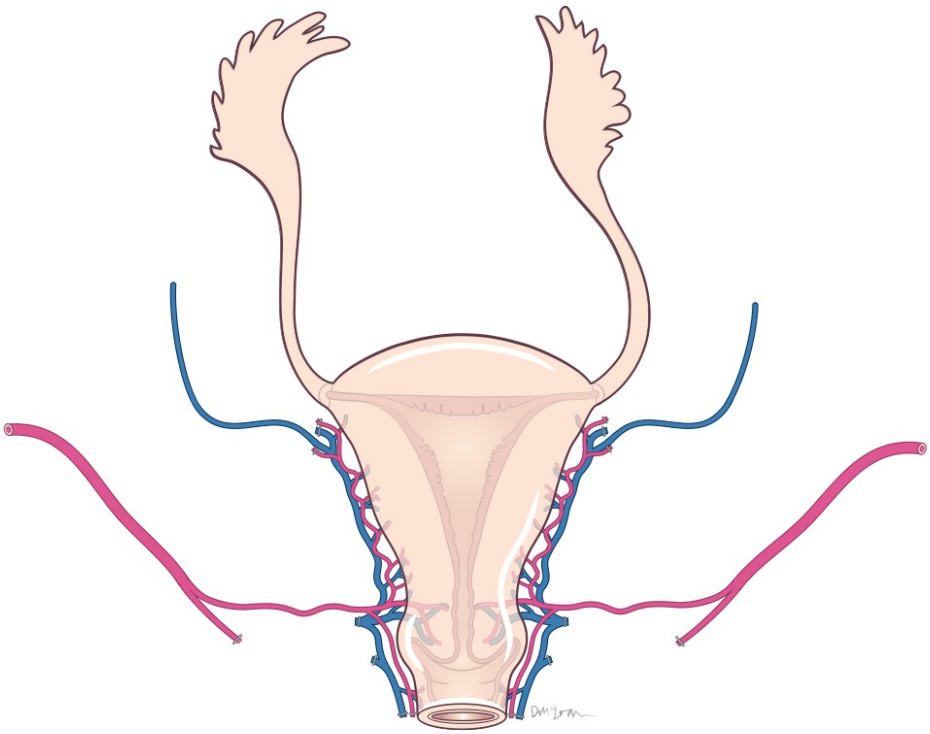


Figure 8: *Diagrammatic representation of uterine graft utilising the modified surgical technique with internal iliac arterial input and utero-ovarian venous drainage (Reproduced with permission from Jones et al. 2019)*[59]

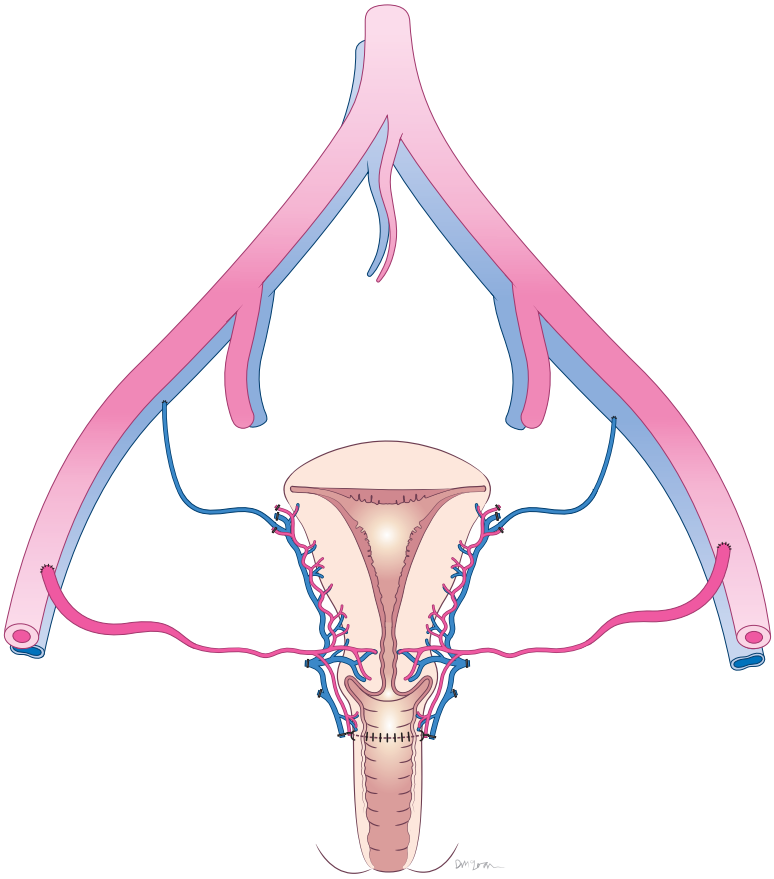


Figure 9: *Recipient anatomy following implantation of uterine graft demonstrating internal iliac to external iliac arterial anastomoses, and utero-ovarian to external iliac venous anastomoses (Reproduced with permission from Jones et al. 2019)*[59]

In the deceased donor model, where donor risk is not a concern, there is no reason why the initial surgical technique using the uterine +/- ovarian veins should not continue to be adopted. This technique can also be undertaken harmoniously in the context of a multi-organ retrieval where the proximal iliac vessels can still be used for vascular reconstruction in other organ transplants. In pre-menopausal living donors, ovarian preservation should be standard practice, utilising the utero-ovarian veins medial to the ovary if ovarian venous drainage is employed. In post-menopausal living donors, consideration should be taken to retrieve maximal lengths of ovarian veins, facilitated by concomitant bilateral salpingo-oophorectomy, in appropriately counselled and consented women. The

use of MRA with delayed venous phase can help provide information to plan the venous dissection in the pre-operative workup of the living donor.[167]

Following the retrieval, preparatory back table dissection is required to optimise the graft for implantation. The graft is cannulated and flushed with perfusion solution. Following ligation of all leaking vessels and visualisation of perfusion solution flowing through the graft to the outflowing veins, the uterus is then placed orthotopically in the pelvis of the recipient. Vascular anastomoses can then be performed, and structural support obtained using the uterosacral, round and broad ligaments laterally, the bladder peritoneum anteriorly and the vagina and paravaginal tissues inferiorly. The mean operative time for the recorded implantations performed to date is 5h 5m (n=43; range - 3h 30m -11h; SD 1h 25m). The mean blood loss at implantation is 632mls +/- 397mls (n=40; 200-2000mls).

With regard to graft survival, of the 45 cases reported so far, 13 (28.6%) required emergency hysterectomy, mostly secondary to graft thrombosis (n=7; 53.8%), with three (23.1%) due to infection (pelvic bacterial infection/abscess; n=1, candidal vasculitis of the arterial anastomosis; n=1, herpes simplex infection of the graft; n=1) and two (15.4%) secondary to unspecified graft ischaemia.[59] One hysterectomy (7.7%) was undertaken following post-operative haemorrhage from a branch of the internal iliac, which likely led to uterine hypoperfusion and resultant graft failure. The majority of emergency hysterectomies were undertaken in the first 15 days post-operatively (n=10; 76.9%), whereas two were undertaken during months 3-4 (15.4%) and one during month 7 (7.7%).[59]

All successful UTx procedures with sufficient follow up resulted in menstruation without the need for supplementary hormone therapy (n=30), predominantly during the second post-operative month (n=24; 80%), whilst three (10%) bled in the first month and three menstruated between months 3 and 4 (10%).

Seven (15.6%) women have had planned completion hysterectomies, six of whom (85.7%) achieved successful livebirths. More than half (n=25; 55.4%) continue to have functioning grafts. The majority

of recipients have not suffered any reported significant medical or surgical complication post-operatively (n=25; 55.6%). Other complications, in addition to the emergency hysterectomies, include six (13.3%) grade IIIb complications as per the Clavien-Dindo classification.[227] These include a vesicovaginal fistula (n = 1), vaginal cuff dehiscence (n = 1) and vaginal stenosis which required stenting (n = 4).[166] Five (11.1%) recipients suffered grade I-II complications, including UTIs (n = 3),[166,228] and post-operative pleural effusions (n = 2).[56]

Immunological considerations

In the cases performed so far, the polyclonal antibody ATG has been most commonly used for induction immunosuppression (n=42; 93.3%),), with or without the addition of methylprednisolone. One (2.2%) case used cyclosporin with methylprednisolone. One (2.2%) case used cyclosporin and two cases (4.4%) received the monoclonal antibody basiliximab (n=2; 4.4%), all in combination with methylprednisolone. Alemtuzumab is also commonly used in kidney transplantation, with less biopsy proven rejection when compared to basiliximab,[229] and ATG,[230] particularly in recipients at low risk of rejection,[230] as would be the case in UTx. It has also been demonstrated to reduce maintenance immunosuppression and steroids exposure post-transplantation.[229] As such, whilst it is not licensed to be used in SOT, it may also be a suitable induction agent.

Following the transplantation of most solid organs, a combination of a calcineurin inhibitor such as tacrolimus or cyclosporin and an antimetabolite like mycophenolate mofetil or azathioprine are usually used, with or without the addition of corticosteroids. In UTx, the effective use of calcineurin inhibitor monotherapy following UTx has been demonstrated in both small animals,[231] and large animals.[87] Whilst the first human case used cyclosporin,[52] the majority since have used tacrolimus in combination with MMF (n=33; 97.1%). Four cases have also utilised everolimus, in addition to tacrolimus, with the aim to reduce the dose of tacrolimus being administered.[185] Steroids are usually initiated and subsequently weaned as clinically appropriate, and reintroduced in cases where rejection

is experienced. MMF is stopped prior to embryo transfer owing to its teratogenic nature,[232] where it is usually replaced with azathioprine (n=7; 31.8%), or an attempt to use tacrolimus monotherapy is made (n=15; 68.2%). The last ten cases (22.2%) performed in Dallas did not receive MMF, and instead received tacrolimus and azathioprine as initial maintenance therapy. This has enabled earlier embryo transfers to be undertaken, which could reduce graft longevity, cumulative immunosuppression exposure and therefore immunosuppressive related burden.

In the available data from human cases to date, 17 women (45.9%) experienced 26 episodes of rejection in total, while 20 women (54.1%) have not yet had any. The majority were histologically proven (24 episodes in 15 women), whilst two were diagnosed based on clinical findings and raised serum lymphocyte subpopulations. In those diagnosed histologically, 15 (62.5%) were categorized as grade 1 (Mild), six (25%) were grade 2 (Moderate) and three (13.5%) were grade 3 (Severe).

Grade 1-2 episodes of rejection were treated with three-day courses of intravenous methylprednisolone and temporary or permanent escalation of maintenance immunosuppression, usually with the addition of azathioprine and prednisolone. All three episodes of grade 3 rejection were treated with ATG, although one required plasmapheresis and intravenous immunoglobulin, in a recipient who had developed donor specific antibodies.

Rejection data is known in 9 pregnancies that have resulted in livebirth.[58,123,154] In those cases, just one (12.5%) experienced an episode of rejection antenatally, which was asymptomatic, with no impact on fetal wellbeing. Histological resolution was achieved with IV methylprednisolone, and the addition of azathioprine and prednisolone into the maintenance immunosuppression.[123]

The most common life threatening complication after organ transplantation is infection.[233] In the context of UTx, 9 recipients (22%); have suffered infection related complications, the aetiology of which may have been contributed to by the immunosuppression. Three women (7%) suffered severe infections of the graft which resulted in hysterectomy including bacterial infection / pelvic abscess

(n=1),[56] chronic herpes simplex virus infection of the graft (n=1),[166] and candidal vasculitis of the arterial anastomosis (n=1).[136] Six women (15%) suffered urinary tract infections (UTIs) which mostly responded to oral antibiotics, although one required 10 days of intravenous antibiotics for pyelonephritis during pregnancy. Two of these women also developed lower genital tract infections, including a symptomatic enterococcus vaginal infection which responded to oral antibiotics, and another developed necrotising fungal cervicitis which was successfully treated with miconazole and fluconazole.[234]

The ephemeral nature of UTx creates a unique advantage over other organ transplants, because following completion of the recipient's family, the graft can be removed, and the previously essential immunosuppressive medications can be stopped. Immunosuppression related complications are dose and duration dependant,[62] so UTx recipients are theoretically at less risk of the complications seen with lifelong immunosuppression. Reducing the dose of immunosuppression has been shown to reduce malignancy related complications but is expectedly associated with an increase in number of rejection episodes.[235] The overall aim of immunosuppression in UTx is therefore to prevent rejection episodes, using the lowest dose of the least number of agents possible, whilst minimising the longevity of the graft in the context of the recipient's reproductive plans. As such data is needed on tacrolimus levels at times of rejection and complication, in relation to the longevity of the graft survival, to optimise future reference ranges and suggested timeframes specific to UTx.

Reproductive considerations

Given the primary goal of UTx is the delivery of a healthy baby, consideration of potential obstetric issues, and the inclusion of feto-maternal medicine expertise in the process of UTx is essential. At a time when there is no question of the feasibility of the procedure, and as the number of livebirths begins to increase in line with the number of cases being undertaken, detailed assessment of such outcomes is vital to reflect upon to facilitate future management of such cases.

	Number of transplants (n)	Proportion female (%)	Proportion aged 18-49 (%)	Proportion both female and aged 18-49 (%)	Approx. number of women
Kidney (DBD)	*2577*	*37*	*37*	*14*	*357*
Kidney (LD)	*1017*	*36*	*50*	*18*	*183*
Liver	*981*	*37*	*28*	*10*	*98*
Pancreas	*204*	*42*	*69*	*29*	*59*
Cardiothoracic	*348*	*41*	*50*	*21*	*73*
Total	*5127*				*770 (15%)*

Table 3: *Estimations of numbers of women of reproductive age undergoing solid organ transplantation, based on NHSBT annual figures*.[236]

More than half a decade has passed since successful pregnancies have been achieved following SOT,[237] and more than 14,000 women have since given birth worldwide following SOT.[238] The number of transplants being undertaken in females is increasing, and in the UK 36-42% of SOTs are undertaken in women, with between 28-69% being of reproductive age.[236] Based on NHSBT figures, as summarised in **Table 3,** approximately 15% of all SOTs are undertaken in women of reproductive age,[236] of which a minority will subsequently have babies.

Following SOT, and subject to the intended improvement in end stage organ disease for which the transplant was undertaken, most SOT recipients subsequently resume normal ovulatory cycles within 6-9 months,[239] thereby restoring reproductive potential. The majority of fetal and maternal outcomes

in pregnancies following SOT are ascertained from data from multiple national transplantation pregnancy registries, which are comprised of predominantly liver and renal recipients, owing to their relatively greater preponderance to being performed. When compared to the general population, most studies have identified an increased risk of pre-term delivery, fetal growth restriction, low birth weight and hypertensive disorders of pregnancy, but in the absence of an increased risk of congenital malformation.[240] In addition to the increased feto-maternal risks, graft function often deteriorates during pregnancy, with evidence suggesting one third of women experience a deterioration in graft function following renal transplant, two thirds of whom fail to recover.[241] This highlights that pregnancy following SOT represents a cruel gamble between acquiring motherhood, and preserving life sustaining organ function.

UTx differs entirely from other SOTs because it is undertaken directly for restoration of fertility, in women, who are fit and healthy with normal ovulatory cycles, without significant medical comorbidity. As such, extrapolating from women with end stage organ disease, which is often associated with multiple medical comorbidities, who have undergone lifesaving SOT, is inappropriate. UTx has different fetal and maternal implications during pregnancies that warrant specific consideration and reflection.

Team	Author	Donor age (y)	Donor parity	Donor type	Recipient age (y)	Time interval (months)	Antenatal complications	Rejection in pregnancy	Gestation	Sex	Apgar Scores	Weight (g)	Centile	Neonatal complications	Congenital malformation	Completion hysterectomy?
Sweden	*Brannstrom et al 2014.*[55]	61	2	LD	35	12	PET (SK), Anaemia	18/40– Resolved with steroids	31^{+6}/40	M	9-10-10	1775	63rd	RDS + Jaundice	Nil	3 months PN
	Brannstrom et al 2016.[242]	50	3	LD	28	12	OC, Anaemia	No	34^{+4}/40	M	9-10-10	2335	57th	Mild RDS	Nil	3 months PN
	Castellón et al 2017.[243] *Molne et al 2017.*[123]	53	2	LD	32	N/R	Nil	No	35+1/40	M	8-8-8	2700	76th	N/R	Nil	N/R
		N/R	N/R	LD	N/R	N/R	PET (SK), OC, PPROM	No	34^{+5}/40	M	3-7-10	3074	96th	N/R	Nil	3 months PN
		N/R	N/R	LD	N/R	N/R	PET (SK)	No	35^{+3}/40	F	9-10-10	2552	58th	N/R	Nil	3 months PN
	Akouri, 2017.[244]	53	2	LD	32	N/R	Nil	N/R	37^{+1}/40	F	9-10-10	2600	27th	N/R	Nil	N/R
	Brannstrom et al. 2020.[245]	62	3	LD	33	10	Minor APH	No	36+1/40	M	9-10-10	2894	62nd	Mild RDS	Nil	N/R
Dallas, USA	*Testa et al 2018.*[154]	34	2	LD	30	8.5	SCH 13/40 – 22/40	No	33^{+1}/40	M	8-9	1995	57th	Mild RDS	Nil	Peri-partum
	Pers. Comm. (Johannesson 2020))	36	4	LD	28	6.5	Nil	No	36^{+6}/40	F	9-9	2920	60th	Nil	Nil	At second delivery
	Pers. Comm. (Johannesson 2020)	36	4	LD	28	30	Nil	No	38+0	F	9-9	3370	74th	Nil	Nil	Peri-partum
	Pers. Comm. (Johannesson 2020))	39	3	LD	25	17	GDM	No	35+6	F	8-8	2860	74th	Mild RDS	Nil	3 months post-delivery
	Pers. Comm. (Johannesson 2020)	35	7	LD	24	6.8	USS indicated cervical cerclage 23/40, PTL	No	30+6	F	7-8	1770	83rd	Mild RDS	Nil	7 months PD
	Pers. Comm. (Johannesson 2020))	32	5	LD	20	4	Nil	No	37+2	M	8-8	3140	62nd	Mild hypoglycemia	Nil	N/A
	Pers. Comm. (Johannesson 2020)	39	4	LD	31	3	Nil	No	37+0	M	8-9	2960	51th	Nil	Nil	2 months PD
Brazil	*Ejzenberg et al 2018.*[58]	45	3	DBD	32	7	Pyelonephritis 32/40	N/R	35^{+3}/40	F	9-10-10	2550	58th	Nil	Nil	Peri-partum
Czech Republic	*Kristek et al 2019.*[246] *Chmel et al 2018.*[166]	N/R	24	LD	26	17	GDM	Nil	35/40	M	N/R	N/R	N/R	N/R	Nil	N/R
Cleveland	*Flyckt et al 2020.*[247]	24	1	DBD	33	11	Placenta accreta / membranacea	12/40– resolved with steroids	34^{+2}/40	F	8-9	1930	26th	Nil	Nil	Peri-partum

Table 4: *Livebirths following UTx summary including neonatal outcomes*

APH; Antepartum haemorrhage, **DBD;** Donation after brainstem death, **F**; Female, **GDM;** Gestational diabetes, **LD**; Live donation **M;** Male**, N/R;** not recorded, **OC;** Obstetric Cholestasis**, Pers. Comm.;** Personal communication, **PET;** Pre-eclampsia**, PN;** Post-natal, **PPROM;** Premature Pre-labour rupture of membranes, **PTL;** Preterm labour, **RDS**; Respiratory distress syndrome, **USS;** Ultrasound, **SCH;** Subchorionic haematoma, **SK;** Single Kidney

15 livebirths have now been reported in peer reviewed publications following UTx. A further six have been personally communicated from the medical director of uterine transplantation at Baylor University Medical Centre, Dallas from the Dallas UtErine Transplant Study (DUETS) (Johannesson, 2020). The key details from cases which have led to the successful livebirths are summarised in **Table 4**. 19 women have given birth to 21 infants following UTx. Of the 21 livebirths, 19 were achieved following UTx using LD undertaken in Sweden, Dallas, Serbia, the Czech Republic, and Germany.[55,154,242-244,246,248] Two livebirths have been confirmed following DBD in Brazil and Cleveland.[58,247] Further livebirths have been reported in the media from cases undertaken in both China and India, but detailed, published outcomes are still awaited. Of those cases where outcomes are known in detail (n=16), insight can be drawn from the pregnancy outcomes so far.

Fertility considerations prior to uterine transplantation

The creation and cryopreservation of embryos is currently a requisite prior to UTx due to multiple reasons. This is primarily necessitated by the inclusion of bilateral salpingectomy as part of the current surgical technique in UTx which is undertaken to reduce the future risk of ectopic pregnancy. Utero-tubal transplantation has previously been undertaken, with successful pregnancies reported in animal models.[47,249] In humans, a single case included retrieval and implantation of the fallopian tubes as part of the graft,[52] which was unsuccessful after being complicated by thrombosis due to poor structural support. Of potential importance, the fallopian tubes appeared to remain viable. Recent studies have demonstrated that the Fallopian tubes are supplied by the utero-ovarian vasculature alone,[250] which reinforces the well-known fact that Fallopian tubes remain viable following uterine vessel ligation as part of radical trachelectomy. As such, if bilateral utero-ovarian arterial and vein anastomoses are subsequently used in UTx, then the Fallopian tubes could potentially be preserved. Such a technique has been employed in primates, with successful spontaneous conception, and livebirths reported.[251] However, evidence of uteroplacental insufficiency, characterised by placental fibrosis, impaired fetal growth and still births, raises questions over whether this anastomosis provides sufficient uterine perfusion in this model.

Data on reproductive outcomes following UTx is limited, although the fertility rate in the Swedish data from seven successful UTx procedures is 100%.[252] However, in contrast the data presented in the Czech series, despite 12 frozen embryo transfers, across 4 different UTx recipients, no pregnancies have yet been achieved.[166]

As discussed previously during the critical review of human cases performed so far, other relevant fertility related issues relate to the stage of embryo (cleavage stage vs blastocyst), the use of PGT-A, and the use of stimulation during embryo transfers (natural cycle vs conventional IVF).

Pre-conception

Conception following UTx, much like other SOTs, should be carefully planned once graft function is stable, once doses of immunosuppression have been minimised without evidence of rejection, following appropriate anti-viral prophylaxis, and after potentially teratogenic medications have been stopped.[60] In cases so far, the immunosuppressive agent MMF has been commonly used for the first 3-6 months. Owing to its teratogenic potential, with increased risk of miscarriage and congenital abnormality,[253,254] MMF should be stopped three months prior to conception. Moreover, following an appropriate course of anti-viral cytomegalovirus (CMV) prophylaxis, which should be individualised depending on risk, medications such as ganciclovir, valganciclovir, cidofovir, and foscarnet should be stopped owing to their teratogenic and toxic effects.[255] Low dose aspirin is routinely commenced following UTx, owing to its positive impact upon prolonging allograft survival and improving graft function in other SOTs.[256] Aspirin is safe to continue during pregnancy, where it has the additional benefits where it may reduce the risk of pre-eclampsia (PET),[257] and improve neonatal outcome in cases at risk of preterm delivery.[258]

General guidance in renal transplantation was originally to wait at least two years post-transplantation before attempting to conceive.[259] However, more recent consensus reduced this timeframe to 12 months,[260] as evidence suggests this to be associated with less episodes of rejection, along with superior maternal and neonatal outcomes, compared to conception within the first post-transplant

year.[261] In reality the number of years between transplant and pregnancy varies widely. A systematic review of >4000 kidney transplants demonstrated a mean transplant-pregnancy interval of 3.2 years,[262] but studies in other SOTs have identified mean intervals of up to 10 years following cardiac,[263] and 12 years after liver transplantation.[264] The prolonged timeframes most likely reflect the time needed for graft function to stabilise, but also to await the resumption of fertility following clinical improvement. Moreover, it may also signify the potential negative impact a life-threatening illness has on a women's reproductive aspirations, many of whom would have been so unwell that having children may have been a distant and unrealistic dream.

In contrast, UTx is undertaken in fit and healthy women with immediate plans to achieve pregnancy. The time interval between pregnancy and conception was originally 12 months following UTx.[265] However, following rationalisation of immunosuppression regimens, including the replacement of MMF with azathioprine, the time between UTx and embryo transfer has reduced as low as three months in recent cases.[60] Whereas a longer period gives an extended timeframe to observe graft function, and ensure immunosuppression stability, it prolongs the time needed to complete the recipient's reproductive aspirations, thereby increasing cumulative exposure to immunosuppression, along with the inherent associated risks this brings. As such, UTx should not automatically follow the guidance in the context of other SOTs, but instead each case should be individualised. Following physical recovery from the surgery, removal of teratogenic medications and after radiological and clinical demonstration of graft function, it is reasonable to commence embryo transfers.[60]

Antenatal complications

When considering outcomes in other SOTs, meta-analyses including 4706 pregnancies after renal transplantation and 450 pregnancies after liver transplant have identified that 27% of renal transplant and 22% of liver transplant recipients develop pre-eclampsia.[262,266] However, as a large proportion of renal transplant recipients have associated comorbidity such as pre-existing hypertension, they are inherently predisposed to antenatal complications such as PET, preterm delivery, and small for gestational age (SGA).[267] Given that the incidence of pre-existing hypertension has been demonstrated

in 54.2% following renal transplant and 27.2% of liver transplant recipients,[262,266] it is unsurprising PET is so prevalent in such cases following pregnancy. The implications of the impact of pre-existing comorbidity on pregnancy outcomes can further be demonstrated following analysis of the Swedish Health register which assessed outcomes from 980 infants born before SOT and 152 following SOT, and demonstrated the increased risk of PET, preterm birth and SGA was observed both before and after SOT.[268] As pre-existing hypertension would represent a contraindication to undergoing UTx, the significant prevalence of PET seen in other SOTs is unlikely to be replicated in UTx cases with normal renal anatomy.

In UTx cases undertaken so far, three women (18.8%) developed PET necessitating pre-term delivery between 31^{+6} and 35^{+3} weeks' gestation.[269] While it could be argued that this may be a consequence to other factors associated with UTx, such as IVF,[270] embryo cryopreservation,[271] the usage of immunosuppression,[272] or the transplant of post-menopausal, less elastic uteri,[273] it is important to note that all three women had single kidneys. Whilst one woman with a single kidney did not develop PET, the 75% risk of PET in women with single kidneys following UTx is higher than the previously reported 35% risk.[274,275] The fact PET has not been reported in pregnancies following UTx in recipients with normal urological anatomy is reassuring, and intimates it may be more likely as a consequence to the underlying congenital renal anomaly, than specific to the process of UTx. As around a quarter of women with MRKH have unilateral renal agenesis,[190,194] excluding such women may exclude a large number of potential recipients who are otherwise suitable. As such, following extensive counselling of the additional risks, and after detailed assessment of renal function, it is reasonable to consider undertaking UTx in women with single kidneys who are accepting of the increase antenatal risks, if their renal function is normal. Those with pre-existing renal impairment are not suitable to proceed and should consider other options to acquire motherhood, not only due to the increased risk of adverse pregnancy outcomes,[276] but also prevent further deterioration in renal function as a consequence to potential immunosuppression related nephrotoxicity,[277] and pregnancy.[278,279] Given the potential risk of hypertensive disorders developing, measurement of blood

pressure, and urinalysis +/- protein-creatinine ratio if indicated, should be undertaken at each antenatal visit, and in the eventuality of any symptoms suggestive.

Gestational diabetes (GDM) has also been seen in two UTx cases so far (12.5%). When compared to the baseline risk of approximately 5% of the general population,[280] the risk of GDM following UTx therefore appears raised. This is similar to other abdominal SOTs, where up to 13% of kidney and liver transplant cases are complicated by GDM,[262,266] with even higher rates observed in heart (15%) and lung transplants (33%). Given the selective inclusion criteria predominantly utilised in UTx programmes, recipients will not have many of the risk factors for GDM associated with other solid organ recipients. However, the increased risk associated with the use of steroids and tacrolimus,[281] which in addition to the impaired glucose tolerance associated with pregnancy, necessitates a pro-active strategy of surveillance following UTx, including routine oral glucose tolerance tests at 16 and 28 weeks' gestation.

Obstetric Cholestasis (OC) has also been diagnosed in in two cases (12.5%) following UTx so far, which is around ten times the risk compared to the background incidence of 1-1.5%.[282] OC has been shown to impact up to 22% of pregnancies following kidney transplant,[283] and between 7.7% - 27% of liver transplant cases.[264,284] However data from liver transplantation should be interpreted with caution owing to diagnostic difficulties due to the overlap with the biochemical deterioration associated with graft dysfunction. Moreover, many liver transplant recipients take ursodeoxycholic acid, which may in addition mask the diagnosis. The use of cyclosporin has been implicated as a cause of OC and was used in the above studies. However, there is no convincing evidence suggesting a similar association with tacrolimus, and animal model research has identified that tacrolimus does not have the same negative effect on canalicular function as cyclosporin.[285] Whilst the aetiology of OC in these cases is unclear, routine testing of bile acids and liver function is advisable in future cases.

Two pregnancies were complicated by antenatal anaemia, the first at 8.4g/dl at 8 weeks' gestation, and the second at 7.9g/dl at 18 weeks' gestation, both of which improved were treated with

intravenous iron replacement.[55,242] Anaemia following SOT is common, affecting up to 48% of renal transplant recipients.[286] This is due to multifactorial reasons, some of which, such as issues related to the production of erythropoietin are kidney specific and therefore not relevant in UTx.[287] However, the use of anti-metabolite immunosuppressants, such as azathioprine and MMF can cause anaemia as a consequence to their myelosuppressive effect.[286] Although tacrolimus does not cause bone marrow suppression, it's usage has also been demonstrated to be associated with anaemia.[288] Both azathioprine and tacrolimus were being taken in the cases where anaemia was observed, which in combination with the haemodilutional adaptation to pregnancy, may have been attributable. As such, proactive surveillance and replacement is necessary, both pre-conception and antenatally, to prevent the potential pregnancy associated complications of anaemia such as small for gestational age.[289]

Other complications in cases so far including premature prelabour rupture of membranes (PPROM) in one case,[269] pyelonephritis at 32 weeks' gestation requiring intravenous antibiotics,[58] and a subchorionic haematoma between 13-22 weeks' gestation, which resolved spontaneously.[154]

Immunosuppression

During pregnancy following UTx, tacrolimus has so far been the immunosuppressant of choice, either as monotherapy or in combination with azathioprine and/or prednisolone. Data in women taking tacrolimus and azathioprine during pregnancy have consistently shown that they are safe to take in pregnancy with no increased risk of congenital abnormality.[290,291] Whereas some studies have previously identified transplant recipients taking azathioprine and tacrolimus to be associated with preterm delivery and low birth weight,[292] these risks are similar across all transplant patients, irrespective of which specific immunosuppressant is being taken. As such, it is more likely to be related to underlying maternal condition, as opposed to a direct consequence of the immunosuppression.[279,293,294] During pregnancy, doses of tacrolimus often need to be increased, and decreased in the post-partum period in order to achieve the desired levels.[295] Target levels following UTx have been 10–15 ng/mL during the 1st four weeks post-operatively and 5–10 ng/mL thereafter.[56]

As such, regular surveillance of tacrolimus levels is essential, to ensure appropriate levels are being maintained.

Rejection

Whereas pregnancy is typically a heightened state of physiological immunological tolerance, there remains a possibility that the antigenic stimulus provided by the fetus may trigger graft rejection following SOT. Rejection rates during pregnancy do not seem to increase compared to comparable non-pregnant recipients.[296,297] Rejection following SOT often results in deterioration in graft function. In other SOTs this refers to the worsening function of vital organs, but there is no direct impact upon pregnancy itself. In UTx, graft dysfunction directly impacts the intrauterine environment, and may therefore cause specific pregnancy complications, thereby highlighting the need to promptly diagnose and manage rejection during pregnancy following UTx. In UTx cases so far, two cases of rejection during pregnancy have been reported (9.5%). The first was observed during the Swedish series of nine cases, where one recipient experienced asymptomatic, biopsy proven grade 2 rejection at 18 weeks' gestation. Following a three-day course of IV methylprednisolone and increased dose of maintenance prednisolone, the episode resolved without complication.[123] Following detailed assessment of rejection during this series, the frequency and severity of pre-conception episodes of rejection were not perceived to influence pregnancy outcomes, thereby raising uncertainty as to the necessity of the proposed histological grading system of diagnosis.

The second case of rejection experienced during pregnancy following UTx was seen in a case undertaken in Cleveland, USA, which subsequently resulted in the livebirth of the second infant following UTx using a deceased donor.[247] Six months prior to conception, grade 3 rejection was diagnosed histologically. The presence of a plasma cell rich inflammatory cell infiltrate, and the development of donor specific antibodies, raised suspicion of potential antibody mediated rejection. A subsequent endometrial biopsy identified diffusely necrotic endometrium. Aggressive therapy was implemented including ATG, methylprednisolone, increased maintenance tacrolimus, plasmapheresis and IV immunoglobulin. Histological resolution was confirmed three months later. Following a

suboptimal attempt at priming, prior to embryo transfer, a further attempt was made, which resulted in an 8mm endometrial thickness after 23 days of oestrogen priming. Following conception and confirmation of viability on ultrasound, a cervical biopsy at 12 weeks revealed grade 2 rejection, which was successfully treated with IV methylprednisolone and increased maintenance tacrolimus dosing. At 21 weeks, central placenta previa with accreta was diagnosed. A healthy neonate was subsequently delivered at 34 weeks' gestation, at the time of Caesarean hysterectomy, where a 2000mls blood loss was seen.

Placenta accreta entails morbid adherence of the placenta to the myometrium which arises due to an absence of decidua basalis and imperfect development of Nitabuch's layer. The defect within the endometrial–myometrial interface leads to abnormal decidualisation, which allows abnormally deep placental anchoring villi and trophoblast infiltration. Known risk factors include previous Caesarean section, advanced maternal age and previous curettage.[298] Whilst none of those factors were evident in this case, an endometrial biopsy was taken three months previously using an undescribed surgical technique. Whilst it is highly likely this was a hysteroscopy guided targeted biopsy, curettage may have been undertaken, thereby presenting a potential risk factor. However, the background risk of placenta accreta in the absence of a previous Caesarean section is 1 in 33,000.[299] However, the episode of rejection in the first trimester, at a time when the placenta is undergoing extensive vascular remodelling, given the eventual outcome, is likely to be highly significant in this context. Grade 2 uterine rejection entails a moderate inflammatory cell infiltrate, dominated by lymphocytes with some neutrophils, and the surface epithelium becomes thinner and oedematous.[123] Grade 3 rejection includes more diffuse inflammatory exudate, and the addition of surface erosions and ulceration. It is highly likely that if the surface epithelium becomes thinner and oedematous, or ulcerated, that abnormal trophoblast infiltration can occur. As such, this highlights the importance of optimising the immunosuppression in the first trimester at this critical stage. Whilst suboptimal immunosuppression may risk rejection, which is often asymptomatic and therefore difficult to promptly detect, increasing immunosuppression risks toxicity, and may facilitate CMV reactivation as higher blood

concentrations of immunosuppression are associated with higher CMV viral loads, both of which are best avoided in the first trimester during organogenesis.

Mode of delivery

SOT itself is not an indication for Caesarean section and vaginal delivery is generally encouraged. However, the Caesarean section rate is generally high amongst SOT recipients (range 23-100%), which is often attributed to associated maternal complications such as deterioration of renal function or the development of PET.[279] The mode of delivery in all livebirths following UTx to date is by Caesarean section. In contrast to other SOTs, this is undertaken due to uncertainty of how the structural support to the graft and vaginal anastomosis would respond to the mechanical stress of vaginal delivery, in addition to concerns regarding the ability of the vascular anastomosis to cope with the additional uterine blood supply response to uterine contractions

Normal uterine neurophysiology includes the perception of contractions via Frankenhauser's plexus, which lies just lateral to the uterosacral ligaments and medial to the uterine arteries, whereas pain sensation related to cervical dilatation is from T11 to T12. Complete neurectomy of all sensory pathways from the uterus is undertaken as part of the retrieval and no attempt is made to re-anastomose nerves during the implantation. As such, labour is painless, which may therefore be unnoticed by the recipient. Given the concerns regarding maintenance of structural integrity of the graft during labour, measures to avoid labour are appropriate, such as delivery at early term gestations in those cases which have an uncomplicated antenatal course. However, elective delivery by Caesarean section at 37 weeks is still associated with an increased risk of neonatal respiratory morbidity compared to vaginal delivery, with five times the risk of serious respiratory morbidity when delivered at 37 weeks, which reduces to a two-fold increase at 39 weeks.[300] Moreover, children born after 39 weeks have higher cognitive outcomes when compared to offspring delivered at 37-38 weeks.[301] As such, uncomplicated cases could be monitored closely, either as an inpatient with regular cardiotocography, or as an outpatient in well selected cases following education on how to identify signs of labour. This includes self-palpation and the need to attend if regular tightening's are

experienced, although this may be difficult to differentiate between physiological Braxton-Hicks contractions.

In the 16 reported livebirths, 75% of livebirths were born prematurely (<37/40), with a mean gestation at delivery was 35^{+1} weeks, highlighting that pre-term delivery may remain an issue following UTx. However, only one case presented with findings directly related to the pathogenesis of pre-term labour. A case, from then DUETs study, was noted to have a short cervix with a cervical length of 17mm at 23^{+4} weeks' gestation, and subsequently underwent an ultrasound indicated cervical cerclage. She subsequently went into pre-term labour at 30 weeks' gestation and was delivered by Caesarean section at 30^{+6}/40 following steroid maturation. Whereas the donor only previously had term deliveries, it should be noted that she had seven deliveries previously. Whilst data is conflicting, grand multiparity has previously been associated with increased risk of pre-term delivery,[302 303] which presents the only potential risk factor in the donor. In other cases, antenatal complications such as OC, PET and placenta accreta influenced management decision making to prompt premature delivery in some cases. This is exemplified by the fact that in cases where antenatal complications were experienced, the mean gestation at delivery was 34 weeks +/- 12 days (n=10). This compares to those cases where no antenatal issues were experienced, which were electively delivered once a clinician perceived *safe* gestation was reached, with a mean gestation of 37 weeks +/- 7 days (n=6).

Caesarean hysterectomy was undertaken in four cases (19%), two of which were undertaken secondary to complications such as placenta accreta (n=1),[247] and deterioration in renal function (n=1), whereas the other two were performed electively following the completion of the recipients reproductive aspirations (n=2).[58] In other cases, elective completion hysterectomy has been undertaken at least three months post-partum.

Whereas undertaking Caesarean hysterectomy reduces cumulative immunosuppression exposure and number of operations, there is undoubted additional surgical risk. However, quantification of such risk is difficult to determine, as extrapolation from emergent and unplanned cases undertaken as a

lifesaving treatment for massive obstetric haemorrhage would offer a different risk profile than planned completion hysterectomy following UTx with appropriate expertise present. As such, in the context of antenatal complication, the risk-benefit ratio favours undertaking completion hysterectomy peripartum. However, in straightforward cases where reproductive aspirations have been met, each case should be individualised, after detailed counselling and with consideration of the wishes of the recipient.

Neonatal outcomes

All infants born following UTx have been born in good condition with normal Apgar scores after 10 minutes (>7). There have been no reports of congenital abnormality. The mean birth weight of the babies reported so far is 2589g (SD 500g). The mean growth centile of each case, using standardised pre-term growth charts,[304] is on the 61st centile (SD 18). This suggests that unlike in reproductive outcomes in other SOTs, low birth weight does not appear to be an issue in UTx, reaffirming maternal condition to be more likely the responsible aetiology, as opposed to the use of immunosuppression. Regarding the potential impact of tacrolimus on the fetus, there is a risk of reversible neonatal hyperkalaemia and renal impairment, which requires consideration post-natally. Whilst tacrolimus is excreted in breast milk, infant ingestion is < 1% of the maternal dosage,[305] and breastfeeding has not been shown to contribute to infant tacrolimus concentrations after delivery.[306]

Uterine Transplantation in male to female transgender women

Gender dysphoria, defined as the persistent discomfort with one's gender identity or biological sex, affects between 0.5%-1.4% of adult males.[307] Treatment aims at congruence, to allow those who experience it to find comfort within their gendered self, which optimises psychological wellbeing and self-fulfilment.[308] Whilst many experiencing gender dysphoria require partial treatment or social transition, others only find comfort following surgical intervention to change their external genitalia and sexual characteristics. Traditionally, infertility has been an unfortunate consequence of the realignment of a transgender person's body with their gender identity.

Traditionally, iatrogenic infertility has been an unfortunate consequence to the realignment of a transgender person's body with their gender identity. Those desirous of parenthood therefore had no alternative but to consider adoption or surrogacy. Whilst anyone wishing to acquire motherhood through these options may be deterred, or excluded, by the associated financial, legal, ethical and religious factors, transgender women are additionally hindered by social and legal discrimination. In recent years, raising awareness, acknowledgement and acceptance of transgender individual's rights has led to recognition that they have interest in childbearing and require access to fertility services.[309] Simultaneously with this cultural change, the advancement and evolution in fertility preservation techniques have now made it a realistic possibility.

Following the rapid development of UTx and following the proven feasibility of the procedure in women assigned female at birth,[59] speculation has escalated regarding the possibility of UTx being performed in male to female (M2F) transgender women to enable them to gestate and give birth to their own children.[310-312] However, there are numerous psychosocial, ethical and legal considerations to establish the need and context for UTx in this model. Moreover, there are significant anatomical and physiological issues, in addition to fertility and obstetric considerations that require critical reflection to help determine whether performing UTx is feasible in M2F transgender women.

Psychosocial considerations

The ability to conceive and gestate is not universally essential for a good quality of life. Men, for example, scarcely consider the possibility of gestating their own offspring. Moreover, voluntary childlessness is sometimes chosen by both genders for multifactorial reasons including lack of interest in parenthood,[24] environmental and political concerns,[313] and independence and freedom from childcare responsibility.[314] However, many women experience significant psychological harm as a consequence to an inability to fulfil their own reproductive aspirations, exacerbated by social and cultural attitudes, norms and expectations concerning the value of reproduction and parenthood.[315]

Until recently women with AUFI have been restricted to adoption and surrogacy to acquire motherhood. However, whilst many find these satisfactory options, they are not universally acceptable, owing to a multitude of moral and ethical factors as well as complex legal, financial and religious issues. Even amongst those in a position to consider adoption or surrogacy, they may still find either option undesirable for the same personal and social reasons that underlie their fertile counterpart's decisions to reproduce instead of utilise adoption or surrogacy. Such factors include a desire to share a genetic relationship with their offspring, the potential to bond with their child in utero and the ability to experience gestation and childbirth. These factors may also be secondary to the ideology of meeting social expectations or conforming to gender norms, or because such women personally view pregnancy as an important component of womanhood or being a parent.

The development of UTx was primarily motivated by its potential to ameliorate the suffering caused by a discrepancy between procreative ability and reproductive aspirations, by providing women with AUFI the opportunity to conceive, gestate and give birth to genetically related offspring themselves. In a similar fashion, M2F transgender women may receive significant psychological and social benefits following UTx, particularly when parenting alone has been identified as a protective factor in suicide risk in transgender women.[73,316] Numerous studies indicate that approximately half of transgender women desire parenthood.[317,318] Whilst adoption or surrogacy may satisfy this desire in

some, other M2F transgender women may "regard pregnancy as the final step in realigning their life".[310] Gestation has been shown to often play a vital role in conveying and consolidating a female identity,[312] and is considered by many women to constitute a transformative experience.[319] The importance of gestation was highlighted in a survey of 121 M2F transgender women where many women were more interested in the prospect of UTx than sperm cryopreservation, holding pregnancy and delivery to be superior to biological relation.[317] As such, it could be argued that, in a similar fashion to the way desire to experience gestation and psychological sequelae spurred on UTx research in cisgender women with AUFI, UTx as part of gender reassignment surgery (GRS) in transgender women should be considered in the same light.

Legal considerations

Transgender women are considered like cisgender women in a full moral sense. As such there is little reason morally to treat them differently from cisgender women with AUFI with respect to their reproductive aspirations. Legally, under the Equality Act (2010) transgender individuals, regardless of whether they have been granted a gender recognition certificate, are afforded explicit protection from both direct and indirect forms of discrimination through the characterisation of 'gender reassignment' as a protected characteristic. As such individuals who are proposing to undergo, currently undergoing, or have undergone a process of gender reassignment cannot be subjected to discrimination from individuals, employers or services on the basis of this characteristic. Likewise, section 9 (1) of the gender recognition act 2004 stipulates that "where a full gender recognition certificate is issued to a person, the person's gender becomes for all purposes the acquired gender" and as such M2F transgender women have a legal right to be treated the same as their natal female counterparts, and thus be entitled to the same treatment, if it is indeed feasible. Subsequently, if UTx becomes an established treatment option for women with AUFI, based on EU and UK legislation it will, in the absence of the provision of compelling justification, be legally impermissible not to perform UTx in transgender women, solely because of their gender identity.

A potentially complicating factor however arises when the legal implications of subsequent fertility treatment are considered. It has been argued that M2F transgender women would be unable to undergo embryo transfer after UTx, as under current legislation it may be illegal.[320,321] The relevant section of the Human Fertilisation and Embryology Act 1990, Section 3(2) states 'no person shall place in a woman a live embryo other than a human embryo'. Of crucial importance, it is not specified in this section that the woman receiving the embryo needs to have been born female. As rapid progress in reproductive medicine propelled us further into uncharted territories, the aforementioned legislation soon became outdated and was subsequently amended, with the introduction of the Human Fertilisation and Embryology Act 2008. Instead of section 3 (2) referring to 'human' embryos, the updated legislation referred to 'permitted' embryos. To define 'permitted' a separate section (3ZA) was inserted which states that the germ cells must be obtained from ovaries of women, and testis of men respectively. It is in this section where it states that a 'woman and man include respectively a girl and boy (from birth)'. As 'woman' refers to a female adult, this addition appears to relate to the inclusion of young females who have not yet reached adulthood. Owing to the growing recognition and demand for fertility preservation in girls with childhood cancer,[322] this inclusion now rightly permits the future use of cryopreserved oocytes or ovarian tissue in *girls* rendered infertile by gonadotoxic therapy or surgery. Moreover, in M2F transgender women, in the context of the growing trend of transitioning at younger ages,[3] and the necessity to preserve germ cells prior to the onset of hormone therapy, this addition is actually essential to ensure that future embryos in this population are 'permitted'.

Even if the legislation did indeed make embryo transfers in M2F transgender women illegal, this would conflict with the Gender Recognition Act 2004, which states a person's gender becomes, for all purposes, the acquired gender, once a full gender recognition certificate has been issued. A transgender woman seeking UTx may also challenge the aforementioned provisions on the grounds it breaches her Article 8 and 14 rights under the European Convention of Human Rights, in the absence of any persuasive reason or legitimate aim which justifies the interference. As such, if UTx proves feasible in

M2F transgender women as proposed, it is essential that governing regulations evolve concomitantly to meet the demands and needs of modern-day families.

Ethical Considerations

The potential benefits M2F transgender women may gain from UTx are similar to those sought by women with AUFI who are assigned female at birth: overcoming involuntary childlessness, restoring the ability to conceive and gestate; and reducing dysphoria resulting from perceived misalignment between their bodies and their identities. Nevertheless, the risks UTx pose to transgender women currently remain unquantified, although may potentially be even greater than in women assigned female at birth. To ascertain an accurate reflection of the extent of such risks, it may only be realised after undertaking a series of cases. As such, it could be argued that provided there is sound scientific basis to hold that UTx in transgender populations is feasible, there seems little reason to refrain from undertaking research to determine the feasibility.

As with any new ART, physicians considering performing UTx in transgender women are both ethically and legally obliged to take account of the welfare, both physical and psychological, of the children who may be born as a result. Thus, if it can be shown that children born to transgender women through UTx are likely to suffer serious harm this would cast serious doubt on the appropriateness of the procedure.

In the context of undertaking UTx in women assigned female at birth, concerns have been raised regarding the physical risk exposed to the fetus as a result of gestation inside a donated uterus.[65] Feto-maternal risks may be further increased, or indeed decreased, by unknown factors following UTx in transgender women. However, it is likely the risks posed to the developing fetus are unlikely to be greater than those in women with chronic medical conditions such as renal disease, diabetes, autoimmune diseases and hypertensive disorders. Thus, provided that prior to conception and throughout pregnancy, appropriate steps are taken to optimise the gestational environment for the

developing fetus, UTx in M2F transgender women is unlikely to prove any less ethically acceptable than other high-risk pregnancies.

Concerns may, however, be raised regarding potential psychological harms on children born following UTx. In the context of UTx in women with AUFI, it has been noted that children born following UTx may, like the first IVF babies, experience unwanted media attention, confusion or abuse regarding their origin and perceived differences.[323] However, concerns may also arise regarding the possible harms that a parent's transgender identity may have on a child's psychology including self-perception, identity development, family relationships and social relationships. Yet, whilst such concerns warrant consideration there is a distinct absence of evidence to conclude that harm is caused to children born to transgender parents.[324] Available studies on outcomes for children of transgender persons suggest that transgender parents are no less likely to exhibit supportive, stable and loving parenting than cisgender parents.[325] Moreover, they are no more likely than other children to fail to meet developmental milestones,[326] or exhibit distress and confusion regarding their gender or sexual identities.[327] They are also unlikely to be harmed by knowledge of their parent's gender identity, provided it is disclosed early in childhood.[326] Instead it is more likely that psychological distress reported by such children may be secondary to harassment or discriminatory behaviour outside of the family unit. Such harms, as with racism and sexism, are best prevented through education of those who harass or discriminate, and not through preventing the births of those who are discriminated against or harassed. Moreover, given that children who are younger at the time of their parent's transitioning demonstrate better adaptation and maintain healthier relationships with their parents,[328] it is likely outcomes would improve further if they were born into their post-transition family unit. As such there is also no compelling reason to refrain from trialling UTx in transgender women on the basis of child welfare.

Anatomical considerations

In women with AUFI, as previously described, UTx entails the transplantation of the uterus, including the cervix and a cuff of vagina, along with the major blood vessels and surrounding ligamentous and connective tissues. The uterus is placed orthotopically in the pelvis of the recipient, where it is structurally supported using the uterosacral, round and broad ligaments laterally, the bladder peritoneum anteriorly and the vagina and paravaginal tissues inferiorly. Consideration of these anatomical components is essential when considering the feasibility of UTx in M2F transgender women.

Vascular anastomosis

The majority of UTx procedures performed so far have utilised internal iliac to external iliac end-to-side arterial anastomoses, although some have utilised the smaller lateral uterine arteries. The venous outflow predominantly utilised is the internal iliac vein to external iliac vein end to side anastomosis, with or without additional ovarian vein drainage to the external iliac vein.[59]

To determine the plausibility of retrieving a graft from a female woman, with subsequent implantation in a natal male pelvis, the intersex differences in relevant vascular anatomy require consideration. Fătu C, et al (2006) assessed the morphometry of 100 internal iliac arteries, 60 in males and 40 in females. The data presented revealed no difference in internal iliac artery length between sexes, with a mean length of 49mm.[329] However, the calibre of the vessels was 16mm wider in females compared to males (62mm vs 78mm). While a significant funnelling effect could predispose to vessel thrombosis, this difference could be negated by anastomosing further proximally or distally, where the vessels are similarly sized. With regards to the external iliac arteries, Timaran et al (2001) did not reveal a significant difference in external iliac artery calibre between sexes whilst evaluating lower limb angioplasties.[330] The lack of significant intersex differences in pelvic vasculature should not pose any issues utilising an internal iliac to external iliac anastomosis in M2F transgender UTx.

Vaginal anastomosis

In all the UTx cases performed so far, the recipient vagina has been anastomosed to a vaginal cuff from the uterine donor graft. Not only does this restore reproductive anatomy and allow physiological excretion of discharge and menstruation, but it allows direct visualization of the cervix and access to take biopsies, which is the only way to reliably detect rejection following UTx.[123] In the M2F transgender model, it would only be possible in those who have undergone GRS including vaginoplasty. Traditionally GRS consists of orchidectomy, penectomy, clitoroplasty and labiaplasty, with the subsequent creation of a neovagina utilising either penile and scrotal skin, skin from elsewhere or a segment of intestine. However, even in those who have undergone vaginoplasty, the absence of physiologically functioning vaginal mucosa could be problematic. The vagina is lined by multiple layers of stratified squamous epithelium, the top layer of which desquamates into the vaginal lumen approximately every 4 hours,[88] which removes micro-organisms that adhere to it. Vaginal epithelium also contributes to the innate immune response by facilitating the recognition of pathogens and stimulating the production of antimicrobial peptides and pro-inflammatory cytokines.[92] These protective mechanisms contribute to the creation of a commensal microflora, predominantly consisting of lactobacilli, which provide a physiologically stable environment to prevent infection and facilitate pregnancy. In M2F transgender women, the pH in penile skin-lined neovaginas has been shown to be persistently elevated when compared to natal women's vaginas, owing to the inability of the neovagina to support the growth of acidic lactobacilli.[100] Instead, colonisation with bacterial species naturally from skin or intestinal microfloras was observed. In M2F transgender women following UTx, the presence of a skin or intestinal neovagina, particularly in the context of immunosuppression, may therefore increase susceptibility to recurrent neovaginal infections post-operatively, and create a hostile environment that is incapable of sustaining pregnancy. This was exemplified in the second UTx case performed globally, where the recipient was a woman with MRKH syndrome who had an intestinal neovagina.[54] Despite multiple embryo transfers and at least five early pregnancy miscarriages, she has yet to achieve a live birth.

To overcome this anatomical hindrance in the M2F transgender model, a pioneering utero-vaginal transplant could be considered,[331] utilising as much donor vagina as possible, en-bloc with the uterus (**Figure 10**). The graft for implantation is displayed in **Figure 11**, which would subsequently be anastomosed to the recipients neovagina, which may require shortening to accommodate the transplanted vagina (**Figure 12**). Technically this would be achievable, using a similar technique to that employed at radical hysterectomy, with preservation of the vaginal branches of the uterine vessels. A longer vaginal cuff would therefore necessitate retrieval from deceased donors, excluding the use of living donors in this context. An alternative donor pool may be female to male (F2M) transgender men undergoing hysterectomy and vaginectomy as part of GRS. However, the increased radicality of the hysterectomy, including extensive peritoneal excision, may not be acceptable.

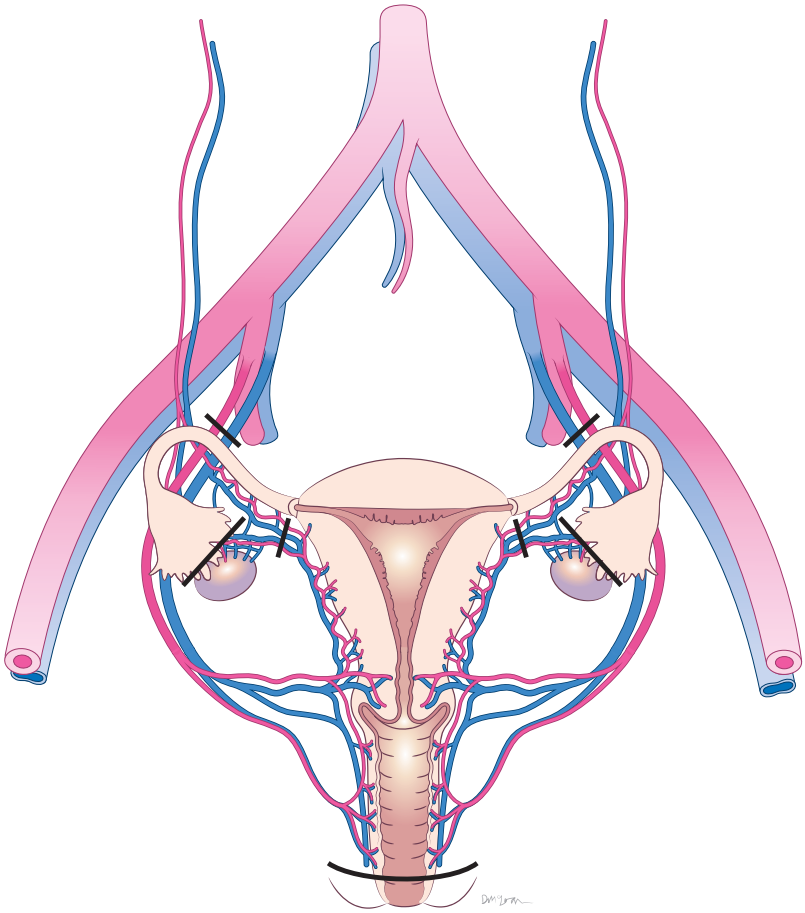


Figure 10: *Proposed utero-vaginal retrieval operation with main dissection markings including long vascular pedicles to origin of the anterior division of the internal iliac vessels, and a long vaginal cuff (Reproduced with permission from Jones et al. 2018)*[331]

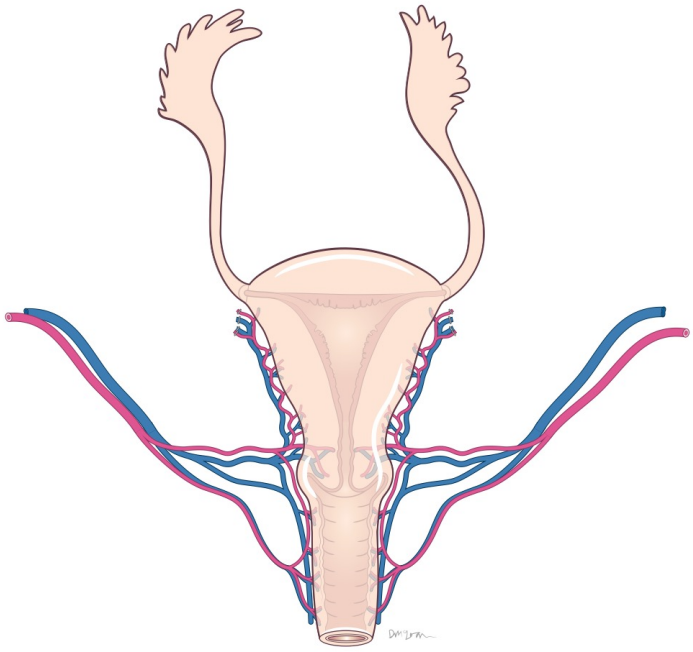


Figure 11: *Proposed utero-vaginal transplant graft following retrieval (Reproduced with permission from Jones et al. 2018)*[331]

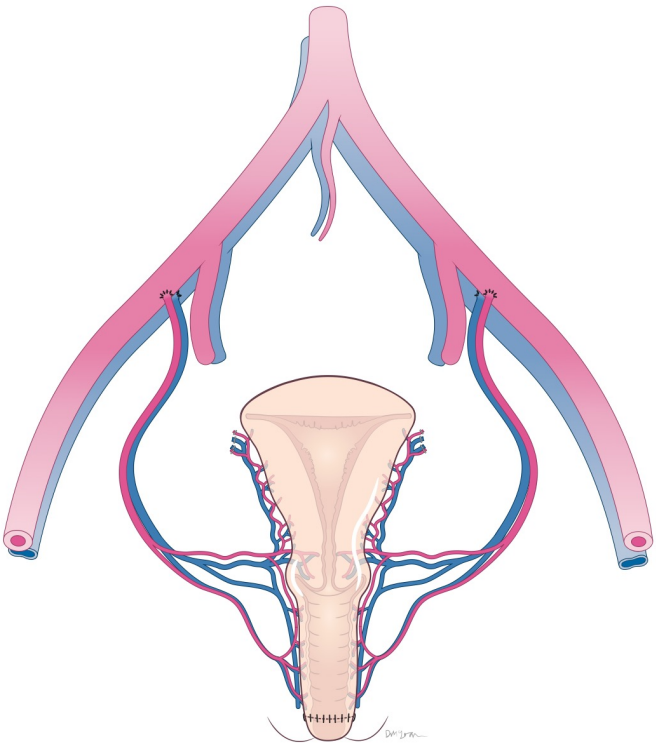


Figure 12: *Male to female transgender anatomy following utero-vaginal transplantation demonstrating vascular and vaginal anastomoses (Reproduced with permission from Jones et al. 2018)*[331]

Another consideration surrounds the potential implications of the prostate being left in place. Prostatectomy is not routinely undertaken in GRS, owing to the short and long-term potential risks. However, the oestrogenised environment in transgender women has been shown to cause prostatic atrophy,[332] and therefore should not create a structural hindrance in UTx, where it would lie anterior to the vagina. Alternatively, prostatectomy could be carried out if requested by the recipient, or if it caused a structural hindrance.

Ligamentous support

The ligamentous and soft tissue support is provided anteriorly by retrieving the bladder peritoneum as part of the graft and attaching it to the bladder peritoneum in the donor. This technique would be directly transferable to the M2F transgender model. Postero-laterally, in women with AUFI, the broad and uterosacral ligaments from the donor are connected to the pelvic side wall and uterosacral remnants respectively in the recipient. Whilst M2F transgender women would not have uterosacral remnants, a more radical ligamentous retrieval of the uterosacral ligaments would be sufficient with subsequent anastomosis to the recipient paraneovaginal region (**Figure 13**).

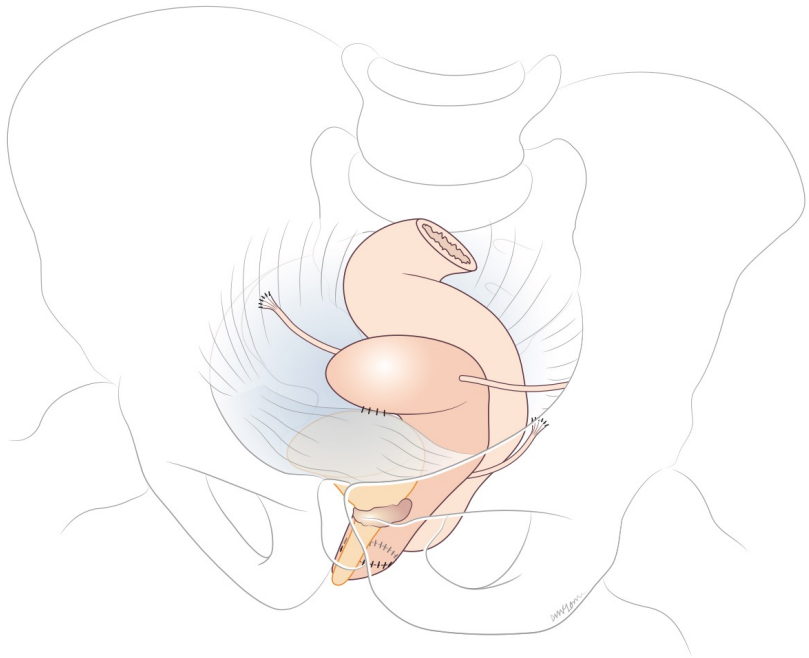


Figure 13: *Proposed ligamentous support following implantation (Reproduced with permission from Jones et al. 2018)*[331]

Hormonal considerations

Hormonal therapy is a mainstay of treatment for many transgender women. Exogenous oestrogen optimises the development of female secondary sexual characteristics whilst an anti-androgen, such as spironolactone or finasteride, minimises male characteristics. Progestogen is not routinely administered as its role as part of feminisation currently remains unclear.[333] However, when considering that unopposed oestrogen is a significant risk factor for endometrial hyperplasia and endometrial carcinoma in post-menopausal women,[334,335] additional progestogen supplementation would become essential following UTx. This is of particular relevance in the context of organ transplantation given the risk of cancer with immunosuppression is 100 times greater than the general population, at approximately 6%.[336] In addition, following solid organ transplantation women are also at risk of endometrial hyperplasia. An analysis of 45 women following renal transplantation, who presented with abnormal vaginal bleeding following renal transplant revealed a two-fold increased risk (69% vs 33%),[337] highlighting the need for appropriate surveillance following UTx.

Whilst continuous HRT is the usual regimen prescribed in M2F transgender women, following UTx a combined sequential HRT regimen would be more appropriate. Not only is menstruation, or withdrawal bleeding, an important clinical sign of graft function, but it is part of being female and contributes to gender identity, which may be beneficial psychologically.[338]

In the context of the requirement for exogenous hormone supplementation, the risk of VTE requires consideration. Oral oestrogen was previously implicated to cause an increased risk of VTE, affecting 6.4% of M2F women.[339] However, more recent data revealed the increased risk was associated with the use of ethinyl estradiol, a particularly thrombogenic oestrogen that is no longer routinely used.[340] This is reaffirmed by more recent data from 2236 M2F transgender individuals receiving hormone therapy, where there was no increased risk in VTE, apart from in those taking ethinyl estradiol, where an 6-8% incidence was apparent.[340] Similarly low VTE rates were seen in another retrospective study assessing 676 transgender women, a mean of 1.9 years after commencing feminising hormone

therapy, where just 0.15% of the population were affected.[341] Further studies have since suggested that VTE risk is lower in M2F transgender women taking transdermal oestrogen, as opposed to the oral route.[342] This coincides with findings from retrospective data and cohort studies in post-menopausal women, where although oral HRT is associated with an increased risk of VTE, the transdermal route is consistently not shown to be thrombogenic.[343,344] Given that graft thrombosis is one of the most common serious complications following organ transplantation, being responsible for up to 7% of kidney allograft failures, it requires particular attention in this patient group should they undergo UTx. A multi-faceted approach to reduce VTE risk would be necessary, starting pre-operatively, including the cessation of hormone therapy, the optimisation of lifestyle factors such as smoking cessation and screening for thrombophilias. Post-operatively subcutaneous low molecular weight heparin should be given for at least four weeks, until mobility is fully restored, and aspirin therapy should be continued for the longevity of the graft, as routinely undertaken following UTx.[56] Combined sequential hormone therapy should recommence post-operatively when normal mobility is restored and VTE risk has normalised. Oral ethinyl estradiol should be avoided, and the transdermal route should be used where possible. It should continue for at least six months post-operatively, prior to consideration for fertility treatment, once all potentially teratogenic medications have been stopped.

Reproductive considerations

Fertility preservation should be discussed in all M2F cases prior to the commencement of hormone therapy or contemplation of GRS.[308] Exogenous oestrogen can influence fertility by impairing spermatogenesis through morphological changes in the testicular tissue including an absence of typical Leydig cells.[345] M2F transgender women can preserve their fertility prior to transition utilising the established technique of sperm cryopreservation, with subsequent IVF or intra uterine insemination.

However, sperm cryopreservation is not possible in those who request feminising treatment prior to the onset of sexual maturity, owing to the absence of mature sperm. As such they have no option but delay transition, which may prolong suffering and risk potentiating psychological sequelae, or undergo the experimental technique of testicular tissue cryopreservation. This entails cryopreserving a biopsy of the immature testicular tissue, with subsequent thawing and in-vitro spermatogenesis in the future. Whilst promising, with success in various animal models,[346] this technique is still highly experimental with as yet undetermined success in humans.[347] Whilst these fertility preservation and restoration options exist for M2F transgender women, there is still suboptimal uptake prior to transition. Factors which may influence the decision in M2F transgender women, include cost, invasiveness of treatment, and desire to not delay transition.[348] In addition, some feel it may conflict with their core female identity knowing they are a biological *father*. Moreover, increasing evidence of a genetic component to gender dysphoria, may also impede uptake in sperm cryopreservation amid concerns future offspring may be similarly affected. Varying data from an 33% observation of concordance of transgender identity in monozygotic twins assigned male at birth, to more recent evidence identifying an oligogenic component involving several genes related to sex hormone–signalling, suggest genetics play a role.[349,350]

The current inclusion criteria for UTx in the UK stipulate that women under consideration for UTx need to have satisfactory ovarian reserve, with at least ten embryos cryopreserved as a pre-requisite.[351] Whilst this cannot be achieved in transgender women in the traditional way, biological relation is still possible if attempts at fertility preservation have been made. In M2F women who have sperm cryopreserved, those in a relationship with biological females could have entirely biological related offspring. However, in such scenarios the natal female partner, subject to the presence of a functional uterus, would clearly be the most appropriate partner to gestate. In transgender women who subsequently have male partners, the creation of embryos would necessitate the use of donor oocytes, in combination with cryopreserved sperm from themselves or fresh sperm from their partner. Given that some M2F transgender women feel that preserving sperm conflicts with their core female identity, some may even be content to forgo genetic relation altogether. To put these concepts into

perspective, a study of 232 M2F transgender women revealed that whilst 54% were attracted to women before GRS, this reduced to just 25% after surgery.[352] Conversely, 9% were attracted to men pre-operatively whilst this increased to 34% following transition, demonstrating the dynamic nature of sexual orientation following GRS.

Following UTx, similarly to women assigned female at birth, embryo transfer should not be attempted until at least six months post-operatively, once the graft function is stable, doses of immunosuppression have been minimised without evidence of rejection and after potentially teratogenic medications have been stopped. This is described in detail on pages 98-99. Specifically in M2F transgender women, if anti-androgens such as finasteride or spironolactone are being taken, these should also be stopped, owing to their potentially teratogenic effect.[353,354] Achieving pregnancy in M2F transgender women following UTx should be achievable utilising hormone regimens that have been used with success in women with POI.[355] All women should have undergone orchidectomy as part of the vaginoplasty, with resultant low testosterone levels. Following the withdrawal bleed on sequential combined HRT, oestrogen supplementation will need to be commenced to stimulate the endometrium. Once it is greater than 7mm in thickness, progesterone therapy should be supplemented to maintain the endometrial lining for implantation. Embryo transfer, using a single embryo, could then be attempted.

Obstetric considerations

Male pelvises differ from that of their female counterparts, to an extent that they can be used to determine gender at autopsy.[356] This dimorphism has arisen due to evolutionary adaptation to sex specific selection pressures. Natal males need a pelvis suitable for bipedal locomotion, whereas the female pelvis must also accommodate a foetus during pregnancy and be adequately capacious for childbirth. Whilst most male skeletal measurements are larger in males than females, the true pelvis of the female has evolved to become larger and broader. This dimorphism is most marked at the anteroposterior diameter of the pelvic inlet, the transverse diameter of the midplane between the

ischial spines, and the transverse diameter of the pelvic outlet.[357] Moreover, the pelvic inlet is oval shaped in females, whereas in males it is heart shaped. These differences in pelvic morphology between sexes would make M2F females increasingly susceptible to true cephalopelvic disproportion should labour be attempted. However, as the requisite mode of delivery in women following UTx is Caesarean section, owing to concerns regarding the ability of the graft to cope with the mechanical strain of labour, this should also be the case in M2F transgender women.

Sexual dimorphism arises predominantly due to the outcome of gender determined autosomal genes, which are regulated by sex-specific hormones and influenced by hormone receptor sensitivity.[358] This has been demonstrated in the pelvis, where despite similar growth patterns between sexes throughout childhood, it isn't until the onset of puberty when the growth trajectory increases in females, and it isn't until the late twenties when the pelvis attains the most favourable obstetric dimensions. As such if M2F transgender women undergo hormone therapy as part of the transition process at a younger age, they may develop similar pelvic morphology to natal females. Whilst in the absence of evidence, encouragement can be taken from biometric analysis of pelvic characteristics in F2M men whose pelvic measurements revealed 'masculinisation' following hormonal treatment.[359]

The Future

UTx appears to be a viable treatment option for women with AUFI. M2F transgender women should morally be considered women with AUFI, and their reproductive aspirations deserve equal consideration to women assigned female at birth. Moreover, once M2F transgender women have fully transitioned they are legally considered women in the UK, and as such are entitled to the same treatment as natal women, should it be feasible. There have so far not been any cases of M2F transgender UTx undertaken in any model. As hypothesised herein, despite a number of anatomical, hormonal, fertility and obstetric considerations that require consideration, there does not appear to be an overwhelming clinical argument against performing UTx in M2F transgender women. However, the increased radicality associated with the retrieval operation in this context, including a longer

vaginal cuff and more extensive ligamentous dissection, will almost certainly necessitate the use of deceased donors.

However, whilst this is so, such research must conform with existing ethics guidance such as Moore's 3 criteria, to minimise the risks of innovative surgical procedures.[360] Firstly, prior to any human research "extensive laboratory work on the technique, anatomy, physiology, biochemistry, and pathology" of the procedure must be undertaken in computer and animal models. While UTx has now been performed in more than 60 women assigned female at birth, because of the undetermined degree of uncertainty regarding the ability of genotypically male individuals to conceive and maintain pregnancy, performing research initially in animal models would be an appropriate next step. Moreover, prior to this thesis, there is no evidence on desire or motivations for M2F transgender women, which is essential to gauge to determine a need for investigating this concept further. The second point described by Moore (2000) stipulates that researchers must have access to input and collaboration from researchers and physicians from relevant disciplines. A multi-disciplinary approach in M2F transgender UTx is essential, with collaboration between gynaecologists, urologists, transplant surgeons, obstetricians, fertility specialists, psychologists, endocrinologists, plastic surgeons, dermatologists, speech and language therapists and psychotherapists. Lastly, the research must take place in a stable and supportive institutional setting.

Elective oocyte cryopreservation

Over the last half a century, societal perceptions and norms have evolved, resulting in the advancement of gender equality and progression of women's rights across the globe. Access to family planning, in combination with increased access to educational and professional opportunity has enhanced female economic independence and resulted in greater empowerment for women. Analogous with this advancement, the age of first-time motherhood is rising, and recent figures highlight that more women over the age of 40 are now giving birth than those under the age of 20.[361]

Ovarian reserve declines with age, from 6-7 million oogonia whilst in utero to less than 500,000 at the onset of puberty.[362] From the age of 37 years onwards the decline becomes exponential, owing to a rapid increase in follicle atresia, when just 25,000 oocytes remain.[3] In association with the decrease in quantity, oocyte quality additionally deteriorates as maternal age advances, which subsequently increases the risk of aneuploidy.[363] This clinically manifests as significantly reduced fecundity and in those who do become pregnant, there is a dramatically increased risk of miscarriage, from around 10% in a woman's thirties, to >90% once aged >45 years.[364] The risk of chromosomal disorders, including Down's Syndrome also increases as age advances, from 1 in 1500 at 20 years to 1 in 35 in women aged 45 years.[365] In light of this irreversible age-related deterioration in ovarian reserve, it is unsurprising the societal trend of delaying motherhood has been associated with an increased proportion of women who remain involuntarily childless.[366] This has resulted in a situation whereby more women over the age of 45 are using donor oocytes than their own.[9]

Since its introduction more than forty years ago, an estimated five million babies have been born following IVF globally, which now represents 2% of childbirths in the UK.[9] Whilst IVF overcomes infertility related to numerous causes, it cannot overcome the irreversible decline in oocyte quality and quantity associated with advancing age. Oocyte cryopreservation was first reported more than thirty years ago, after successful pregnancies were achieved following the novel application of a slow freeze and rapid thaw technique.[367] However, as a consequence of low pregnancy rates, a faster

cryopreservation technique, was developed; oocyte vitrification. Oocyte vitrification has greatly improved both oocyte survival and subsequent pregnancy rates.[368] Moreover, recent data has demonstrated that there is no difference in pregnancy, miscarriage or livebirth rates between the use of fresh and frozen transfers.[369] This has established oocyte cryopreservation as a realistic treatment option preservation option for women undergoing gonadotoxic treatment for cancer or those with a high risk of POI or complex medical problems. Moreover, it has provided an opportunity for women to electively freeze oocytes at a younger age for 'social' reasons, such as not being in a relationship; thereby introducing the concept of social egg freezing (SEF).

Elective oocyte cryopreservation (EOC) prior to the physiological decline in ovarian reserve nullifies the future age-related increased risk of miscarriage and congenital abnormality, maintaining the reproductive potential of the age when they were frozen, thereby optimising the chances of a future successful live birth. It has been identified that undergoing EOC prior to 36 years has the highest discriminative capacity to predict livebirth.[370] This is further exemplified by another study, where following vitrification, women aged 30-36 had a more than two-fold increase in implantation, pregnancy and livebirth rates than those aged 37-39.[371]

EOC empowers women with the opportunity to delay their childbearing years, whilst theoretically reducing, but not eliminating, the risk of permanent involuntary childlessness. However, utilising SEF as reproductive insurance is a potentially costly reproductive gamble associated with significant risk. There are multiple obstetric, psycho-social, economic, legal and ethical issues that require further discussion.[372]

Obstetric considerations

Whilst pregnancy rates are superior and early pregnancy complications reduced using oocytes cryopreserved at an earlier age, the increased maternal morbidity associated with advanced maternal age (AMA) remains. Mortality is increased by more than 300% in women over the age of 40, when

compared to women aged 20-24 years.[373] Moreover, in addition to a higher prevalence of chronic medical conditions in women of AMA,[374] there is also increased risk of maternal and obstetric complications. Women over the age of 40 years have an increased risk of developing hypertensive disorders of pregnancy, with almost double the risk of pre-eclampsia.[375] However, when considering the use of donor oocytes is an independent risk factor for pre-eclampsia itself,[376] SEF may be associated with less risk than using donor oocytes, which is the other option to use in such women to overcome OFI. AMA is also well established as a risk factor for fetal growth restriction,[377] and stillbirth,[378] in addition to gestational diabetes, pre-term birth and operative delivery.[374]

When considering neonatal outcomes, while higher morbidity has previously been implicated in older women, more recent evidence suggests that in the context of intensive antenatal care, similar neonatal outcomes to younger mothers can be achieved.[379] This has even been demonstrated in women at the extremes of reproductive age (>50 years), where appropriately screened women can still expect successful neonatal outcomes.[380] Although lack of long term outcomes means it is at present not possible to assess the lasting impact to children born following vitrification, it is reassuring that congenital abnormality rates are similar to those with natural conception,[381] and there has been no observed deleterious impact upon development for the first three years.[382]

Social Factors

SEF empowers women and allows greater reproductive autonomy. By allowing women to extend their reproductive potential into their forties, when men traditionally remain fertile, it helps facilitate gender reproductive equality. However, it has been argued that technological solutions to societal problems represses, as oppose to liberates, those women involved.[383] As such SEF may augment the societal issues for which it is being considered, with women instead relying upon it as ‘reproductive insurance’. This may also be reinforced by women feeling pressured to delay motherhood following pressure from their employers. This be intensified further by reports of companies such as Facebook and Apple offering to fund SEF as an employment benefit. In women who have the option to procreate earlier,

utilising SEF as 'reproductive insurance' is risky owing to the fact the number of oocytes in storage is finite.[384] Whilst this risk can be reduced by freezing more oocytes at younger ages,[385] this will have significant economic implications and success can still not be guaranteed.

The risk of perpetual involuntary childlessness is 6% when childbearing is deferred until the age of 30, which increases almost six-fold to 35% if postponed until aged 40.[386] The most common indication for SEF is not having a partner,[387] which has been shown to account for up to 88% of women undergoing EOC.[388] Moreover, in the aforementioned study by Cobo et al (2016), whilst 79% were single at the time of EOC, almost half (47%) had found a partner when they returned to thaw their oocytes, highlighting the potential societal impact of SEF in single women who are approaching the age associated with an exponential decline in their ovarian reserve.[385]

Other reasons to defer childbearing may be to seek high level education, to focus on their career, or ironically due to economic uncertainty. There are also potential advantages to postponing parenthood including higher household income, better quality of living, greater family stability and associated superior parenting practices.[389] It has also been demonstrated to result in superior outcomes in offspring, including better educational and psychosocial outcome,[390] and higher self-sufficiency in adulthood.[391]

Even in populations that have a good understanding of age related infertility, women continue to defer childbearing.[388] In a survey of more than 1000 women across the UK and Denmark, the vast majority (89%) considered reproductive planning an acceptable indication to undergo SEF.[392] Such positive attitudes towards the concept are reflected in practice by an almost three-fold increase in the number of oocytes frozen in the UK from 2,476 in 2008 to 7,047 in 2013.[9] Moreover, the number of women freezing oocytes for future personal use has risen 25-30% each year, and the number of clinics offering oocyte storage facilities is also increasing.[9] Whilst these figures include cases frozen for medical indications in addition to social reasons, the majority of cases over the age of 38 cited no partner as the reason for storage, alluding that SEF is the main contributor.

Reproductive planning is a dynamic concept, that evolves around each individual's values, which may be influenced by sociocultural expectations, in the context of the resources available. Single women, as they approach their late thirties, have two alternatives to EOC. Firstly, wait, as their ovarian reserve declines, until they find a partner they are content to have children with. However, this is a cruel reproductive gamble, with increasingly worse odds as time passes, along with potential heartbreak and financial expense associated with multiple failed cycles of IVF. The alternative option would be to consider the use of donor oocytes, thereby eliminating biological relation in subsequent offspring. This option similarly results in women becoming pregnant at AMA, but with the additional physical risk and financial expense associated with using donor oocytes. Alternatively, they could break social and cultural norms by becoming a single mother using donor sperm. Whilst this would result in pregnancy at a younger age, it is unlikely to be acceptable to many and may even adversely influence their ability to find a lifelong partner.

Legal and Economic Implications

In the UK, Human Fertilisation and Embryology Authority (HFEA) regulations allow the storage of gametes for 10 years.[393] While it may be extended if the individual has or is likely to become prematurely infertile, this would not be feasible in the context of physiological decline in ovarian function. As such, it requires consideration when women undergo EOC, particularly in women who voluntarily cryopreserve at an earlier age to optimise future success. In this scenario, it may be counterproductive, necessitating the need to prematurely use a sperm donor, or face the oocytes being discarded. The fact that these women could subsequently use donor oocytes, instead of their own, exemplifies the perverse nature of this regulation, which may need updating once the practical implications it has on SEF are exposed.

The 10 year storage limit has received criticism and at the time of writing this thesis, was being reconsidered.[393] If the primary role of the storage limit is to safeguard against women becoming pregnant outside of their natural reproductive years, it may be logical to instead implement legislation

limiting the age at which future utilisation of stored oocytes can be undertaken. This may prevent the significant adverse social implications, legal ramifications and financial repercussions associated with raising children at retirement age, particularly when the risk of personal ill-health approaches.

The Future

Spontaneous conception during a woman's most fertile reproductive years is unquestionably the safest and most efficacious way to acquire motherhood. However, in the context of a society where the age of motherhood and rates of involuntary childlessness continue to increase, SEF offers a prophylactic opportunity to negate age related OFI if necessary. However, the process is associated with risks such as drug reactions, surgical complications and ovarian hyperstimulation syndrome (OHSS) and is physically and psychologically demanding. At a time when UTx, along with the associated significant risk, is being undertaken to allow women with AUFI to become parents, the risk profile of EOC for women at risk of pathological or physiological OFI is much more favourable.

Women with absolute indications to freeze their oocytes, such as those approaching their late thirties who are not in a relationship and want future biologically related children, should consider EOC before their ovarian reserve deteriorates irreversibly to an extent where they have OFI. SEF extends the window of opportunity for single women to find a partner but retains the option to use a sperm donor in the future if they remain single. SEF in women with relative indications to delay childbearing, such as those who decide to focus on their career or prioritise their education, is without question a reproductive gamble. Extensive counselling is paramount to ensure the women are fully informed, including the possibility of unsuccessful treatment in the future with a finite number of stored oocytes, as well as the pregnancy related risks associated with advanced maternal age. Ultimately, each case should be individualised, with consideration of physiological, legal and economic aspects, to optimise timing of oocyte vitrification and determine how many oocytes to store.

Objectives of thesis

The overriding objective of this thesis is to study a series of interrelated concepts underlying fertility restoration including UTx, ETx and exploration of ovarian restoration through the use of elective oocyte cryopreservation.

Uterine Transplantation

1. To critically appraise the anatomical, surgical and physiological aspects necessary for a successful UTx in humans.
2. To screen and investigate potential recipients to undergo UTx, including clinical, biochemical and radiological evaluation, as well as a comprehensive quantitative psychological assessment.
3. To critically appraise the anatomical, surgical and physiological aspects necessary for a successful UTx in transgender women, to help determine feasibility in this model.
4. To assess the demand for UTx amongst M2F transgender women.
5. To understand the motivations, perceptions, acceptability and suitability amongst non-directed UTx donors.

Endometrial Transplantation

1. To assess the feasibility of ETx, by assessing clinical, radiological, biochemical, reproductive and histological outcomes of a short-term viability autotransplant study in the rabbit model

Elective oocyte cryopreservation

1. To assess perceptions, outcomes and regret following elective oocyte cryopreservation, including impact on quality of life
2. To evaluate laboratory, clinical and reproductive outcomes following elective oocyte cryopreservation, including the determination of factors that impact oocyte yield in elective oocyte cryopreservation cycles

Hypotheses

1. Uterine transplantation is a feasible fertility restoring procedure in women with absolute uterine factor infertility.
2. Male to female transgender women desire to undergo the process of uterine transplantation in order to acquire motherhood and relieve gender dysphoria.
3. Endometrial transplantation is a feasible procedure, which may have fertility restoring implications for women with Asherman's syndrome.
4. Elective oocyte cryopreservation is an acceptable treatment to restore fertility in women following the physiological decline in oocyte quality and quantity associated with advancing age.

Study 1a

Screening of uterine transplantation recipients

Introduction

Whereas it has been estimated that AUFI affects one in 500 women of childbearing age,[19] there is limited data on potential demand to undergo UTx in this population. A previous study in the UK demonstrated that 97.5% of women with AUFI would choose UTx, over surrogacy and adoption, after becoming aware of the additional risks posed by UTx.[22] Another study, specifically assessing perceptions in women with MRKH, showed almost two thirds of participants were motivated to undergo UTx, after becoming aware of the associated risks involved.[394] This is similar to the findings of a questionnaire in 60 women with AUFI in France, which demonstrated that 58.3% would partake in a clinical trial in UTx.[395] As such, there appears to be sufficient demand for UTx, and following more than 70 cases, and 23 livebirths having been reported, demand is likely going to increase further.

Despite a number of published studies summarising case series or UTx research programmes, there is little known about how to screen and prioritise women with AUFI. Two studies have reported details of their selection processes. The DUETS study reported on their full selection process, from pre-screening 179 potential recipients using a questionnaire, of which 18 women were eventually chosen by the principal investigator to proceed in an extensive medical and psychological objective evaluation process.[152] Decision to proceed was based upon meeting the selection criteria implemented for the trial, but there was no further elaboration on the method used to prioritise which participants that met the criteria proceeded. In a study based in Germany, 108 women were screened, 15 of whom proceeded for further evaluation. Apart from meeting the selection criteria, and having a potential living donor, no further explanation was given to elaborate upon how potential recipients were prioritised for further evaluation.

The INvestigational Study Into Transplantation of the Uterus (INSITU) is a clinical trial involving a cohort of ten women, who will undergo UTx using donors following brainstem death. The selection process entails multiple stages, including an initial pre-screen, which includes a detailed questionnaire which assesses the key background information required to determine suitability to proceed in light of

the selection criteria for the study. The aim of this study was to gauge interest amongst women with AUFI to undergo UTx, characterise their background demographics, determine their reasons for considering UTx over adoption and surrogacy, and ultimately assess their suitability to proceed.

Methodology

Recruitment commenced from a population of women with AUFI who contacted Womb Transplant UK (registered charity number 1138559) or were referred to the UK UTx research team for consideration for UTx over an eight-year period between 1st November 2011 and 1st November 2019. There was no public advertisement for the study. The first part of the selection process entailed a screening process. Potential participants were sent a consent form (**Appendix 1a.1**), a participant information leaflet (**Appendix 1a.2**) and a screening questionnaire (**Appendix 1a.3**) via email over a six-month period between 1st May 2019 and 1st November 2019. The questionnaire ascertained suitability to partake in the study by eliciting key demographic information before determining background medical, surgical, psychiatric, obstetric, gynaecological and social history. Further questions ascertained perceptions to adoption and surrogacy and reasons why UTx was preferable. Following receipt of the signed consent form and a completed questionnaire, each application was assessed, using a two-step process. The initial step utilised implementation of selection criteria, as are displayed in **Figure 1a.1** to determine absolute suitability to proceed in the process. The selection criteria were decided and agreed upon, following an international symposium on uterine transplantation hosted by our team, in London on 12th June 2015. It was attended by UTx specialists from a variety of disciplines, including gynaecological surgeons, transplant surgeons, psychologists, ethicists, reproductive medicine specialists, early pregnancy specialists and specialists in novel imaging modalities, from the UK, Sweden and the USA. It was organised to discuss, critique and verify the protocols planned for use in the INSITU study in light of the wealth of knowledge gleaned from animal studies and the human data ascertained from the cases undertaken by the Swedish team. Those participants who met the selection criteria, had their applications scored, using a scoring system

(**Table 1a.1**), which was agreed upon at the meeting, to facilitate prioritisation of those most suitable to undergo the process.

Inclusion	Exclusion
• Female • Age – 25-38 (or 40 if eggs frozen <38 years) • AUFI • Normal length vagina • BMI – <30 • American Society of Anaesthesiologists grade I • Meet psychological criteria • Meet HFEA criteria for IVF • Eligible for NHS treatment • Speak fluent English	• Unsuccessful oocyte retrieval • Previous multiple/significant surgeries • Skin graft or intestinal neovagina • Severe endometriosis • Cancer patients <5 years in remission • Previous living childbirth or adopted child or child born by surrogacy • HIV positive • Hepatitis B and/or Hepatitis C positive

Figure 1a.1: *Inclusion and exclusion criteria for potential recipients in INSITU study*

The remaining participants were then ranked, and the highest ten scoring respondents were invited to enrol in the main part of the INSITU study. They were then invited to consent to participate in the INSITU study, and for detailed counselling, assessment and subsequent investigation. This detailed assessment and evaluation of potential candidates is described fully in **Section 1b**.

Criteria		Score
Age	*36-40* *24-36*	0 1
BMI	*25-30* *<25*	0 1
Past medical history	*Mild chronic disease* *No medical problems*	0 1
Allergies	*Multiple drug allergies* *Single drug allergy or NKDA*	0 1
Previous surgery	*Previous Total abdominal hysterectomy* *Previous Subtotal Hysterectomy* *No previous major abdomino-pelvic surgery*	0 1 3
IVF score	*No frozen eggs* *Previous frozen eggs 36-38* *Previous frozen eggs <36 years*	0 1 3
Alcohol intake	*>14 units/week* *<14 units/week*	0 1
Smoking	*Ex-smoker + Smoker* *Non-smoker*	0 1
Relationship duration	*Single* *<1 year* *1-2 years* *>3 years*	0 1 2 3
Role in raising awareness	*No role* *Local / national fundraising events / social media involvement*	0 1
Adherence and commitment	*<1 year* *1-2 years* *3-4 years* *>5 years*	0 1 2 3

Table 1a.1: *Scoring system used to select recipients to proceed*

Statistics

SPSS version 24 software (SPSS, Chicago, Illinois, USA) was used for analysis. Descriptive statistical analysis was described as mean +/- SD or median +/- range.

Ethics approval

Human Research Authority (HRA) Research Ethics Committee (REC) approval was received (REC: 18/LO/0217) on 28th February 2018. Study start approval from received from the sponsor, Imperial College Healthcare NHS Trust, on 19th February 2019.

Results

962 women had previously expressed an interest in UTx or had been referred to the research team over the designated time period and were subsequently contacted to participate. 210 women responded, consented and returned the questionnaire, resulting in a response rate of 21.8%. The demographics of the cohort are summarised in **Table 1a.2**. 175 (83.3%) were UK residents, who lived a mean distance of 137 +/- 106 miles from the host hospital. The most prevalent age group was between 30-39 years (n=108, 51%). The mean BMI was 25.5 +/- 7.7 kg/m^2. The majority of participants were of white ethnicity (n=142, 68%), fluent in English (n=205, 98%), and either employed or self-employed (n=188, 90%). The majority of applicants were either married or in a relationship (n=195, 93%), the mean duration of which was 5.6 +/- 4.5 years.

Medical History

The aetiology of AUFI in more than two thirds of the cohort was MRKH (68%; n=143). 118 (83%) had isolated uterovaginal hypoplasia (type I) whereas 25 (17%) had additional associated urological, ovarian or skeletal conditions (type II). 29% (n=61) of women had previously undergone hysterectomy (**Table 1a.3**). Of the remaining five women, two (1%) were M2F transgender women, two had severe AS (1%) and one had CAIS (0.5%).

			Number (n)	%
Age (years)	20-29		65	31
	30-39		108	51
	40-49		34	16
	50-59		3	1
Body Mass Index Kg/m2	<18.4	Underweight	4	2
	18.5-24.4	Normal	104	50
	25-29.9	Overweight	59	28
	30-34.9	Obesity I	27	13
	35-39.9	Obesity II	3	2
	>40	Obesity III	10	5
Ethnicity	White		142	68
	Asian		23	11
	Black		8	4
	Mixed		1	0
	Other		9	4
	Would rather not say		27	13
Language	English		172	82
	Non-English but fluent in English		33	16
	Non-English but not fluent in English		5	2
Employment Status	Employed full/part time		186	89
	Self Employed		2	1
	Housewife		11	5
	Unemployed		6	3
	Student		5	2
Relationship Status	Single		12	6
	In a relationship		123	59
	Married		72	34
	Divorced		3	1
Smoking Status	Never smoked		152	72
	Ex-Smoker		29	14
	Current Smoker		29	14
Alcohol intake (units per week)	0		114	54
	≤14		95	45
	>14		1	1

Table 1a.2: *Basic Demographic information of potential uterine transplant recipients*

	Number (n)	%
Cervical cancer	22	35
Caesarean hysterectomy	12	20
Endometriosis	7	11
Dysfunctional Uterine Bleeding	5	8
Endometrial cancer	4	6
Ovarian cancer	2	3
Pelvic Inflammatory Disease	2	3
Placenta accreta	1	2
Adenomyosis	1	2
Fibroids	1	2
Bowel cancer	1	2
Placental site trophoblastic tumour	1	2
RTA (trauma)	1	2
Vaginal prolapse surgery complication	1	2
Benign ovarian teratoma	1	2
Total	**62**	**30**

Table 1a.3: *Indication for prior hysterectomy*

Of the women with MRKH (n=144), 49% (n=70) did not undergo previous treatment to optimise vaginal length. 58 women (40%) had previously used dilator therapy whereas 11% (n=16) underwent surgical intervention after unsuccessful dilator treatment. Six women underwent laparoscopic Vecchietti procedure, whereas five had a neovagina created using bowel (n=2), buccal mucosa (n=1), peritoneum (n=1), or perineal skin (n=1). Five women did not specify the surgical method used.

Of the 62 women who had undergone hysterectomy, 48% (n=30) were undertaken because of an oncological diagnosis, the most common of which was cervical cancer (n=22; 73%), with smaller numbers of women reporting previous ovarian cancer (n=2; 7%), endometrial cancer (n=4; 14%), bowel cancer (n=1; 3%) and placental site trophoblastic tumour (n=1; 3%). Two thirds (n=20; 66%) had been in remission for ≥5 years and the remaining 10 (33%) had been in remission <5 years. In those hysterectomies undertaken for benign disease (n=32), the most common indications were massive obstetric haemorrhage (n=13; 41%), and treatment for chronic pelvic pain (n=8; 25%)

Other than their cause of AUFI, 52 women (25%) had previous medical history, as summarised in **Table 1a.4**. The most frequently reported medical condition was asthma, which affected 6% (n=13) of the cohort. In addition, 8% of the cohort (n= 17) reported congenital kidney abnormalities, the most common of which was unilateral renal agenesis (n=14). One woman had a pelvic kidney, another had a duplex kidney and one had a horseshoe kidney. 15% of the cohort (n=32) reported previous mental health issues, the majority of whom had a diagnosis of depression (n=18, 9%). A summary of reported significant psychological history within the cohort is demonstrated in **Table 1a.5**.

14% (n=29) of participants were current smokers, and the same number were ex-smokers (n=29; 14%), with a mean cessation period of 4 years (±4.4 SD). The remainder had never smoked (n=152, 72%). 54% (n=114) did not consume any alcohol, whereas 45% (n=95) consumed <14 units per week and one woman (1%) consumed in excess of 14 units per week. One (1%) woman used recreational drugs.

	Number (n)	%
Asthma	13	6
Hypothyroidism	9	4
Premature ovarian insufficiency	7	3
Fibromyalgia	5	2
Epilepsy	2	1
Hypermobility	1	0.5
Glaucoma	1	0.5
Previous pancreatitis	1	0.5
Degenerative disc disease	1	0.5
Lymphoedema (legs)	1	0.5
Leukaemia (in remission)	1	0.5
Rheumatoid arthritis	1	0.5
Osteopenia	1	0.5
Ulcerative colitis	1	0.5
Anaemia	1	0.5
Hypertension	1	0.5
Type 2 Diabetes	1	0.5
Migraine	1	0.5
Irritable Bowel Syndrome	1	0.5
Eating disorder	1	0.5
Joint hypermobility	1	0.5
Total	52	25%

Table 1a.4: *Medical history of women seeking uterine transplantation*

	Number (n)	%
Depression	18	9
Anxiety	4	2
Post-Traumatic Stress Disorder	3	1
Gender Dysphoria	1	0.5
Cyclothymia	1	0.5
Body dysmorphic disorder	1	0.5
Borderline personality disorder	2	1
ADHD	1	0.5
Psychosis	1	0.5
Total	32	15

Table 1a.5: *Psychological history of women seeking uterine transplantation*

Reproductive history

10% (n=21) of the participants had previously been pregnant, which resulted in either miscarriage or termination of pregnancy. 16% (n=33) were parous. Four women (2%) previously had a stillbirth, and all four underwent Caesarean hysterectomy at the time due to massive obstetric haemorrhage. 28 (13%) of applicants had previously cryopreserved embryos at a mean age of 28 years. The mean number of embryos stored was 6.5 +/- 5.1. 182 (87%) women did not have embryos cryopreserved.

Motivations towards UTx

Figure 1a.2 summarises the previous considerations, attempts and successful livebirths amongst the cohort through the processes of adoption and surrogacy. With regard to adoption, 63% (n=132) of the cohort had previously considered it as an option to acquire motherhood, 5% (n=11) had formally attempted to adopt, and two (1%) had successfully adopted. 119 women supplied reasons for wishing

to undergo UTx rather than pursuing adoption. The most common reason cited was to experience gestation (n= 63; 53%), while 37% (n=44) wanted a biologically related child (**Figure 1a.3**).

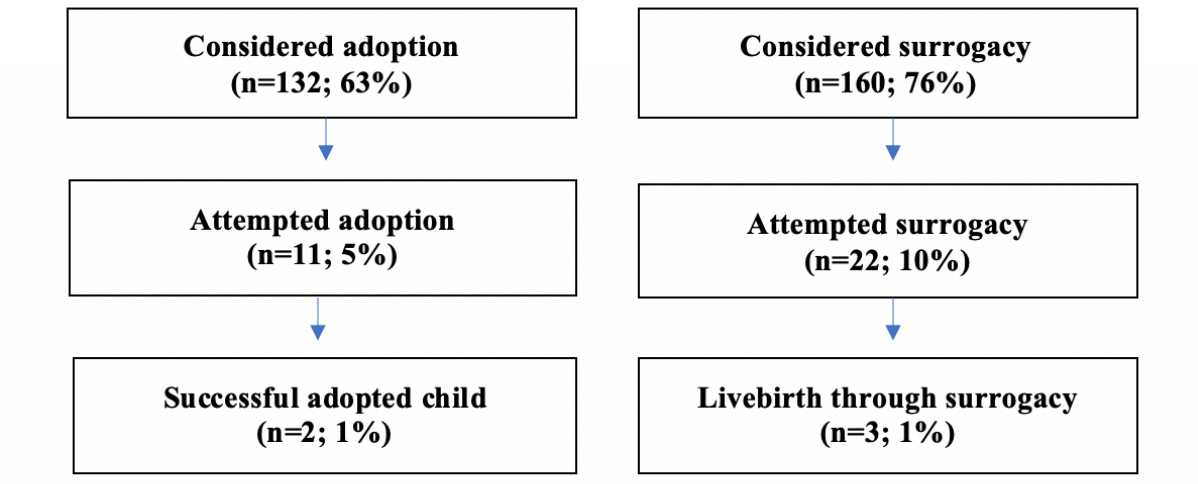


Figure 1a.2: *Considerations, attempts and success at other options to acquire motherhood*

76% (n=160) of participants had previously considered surrogacy, 22 (10%) had attempted it and two (1%) had successfully acquired motherhood using a surrogate. Of those who gave a reason for wishing to undergo UTx over surrogacy (n=143), as summarised in **Figure 1a.4**, the most common reason was to experience gestation (n=77; 54%). 15% of women (n=21) were concerned about the legal implications, 14% (n=20) identified the financial cost as a barrier and 8% (n=12) could not consider surrogacy due to religious reasons. 7% (n=10) reported a lack of trust over the surrogate. 8% (n=11) stated interest in surrogacy if unsuccessful in their pursuit of a UTx.

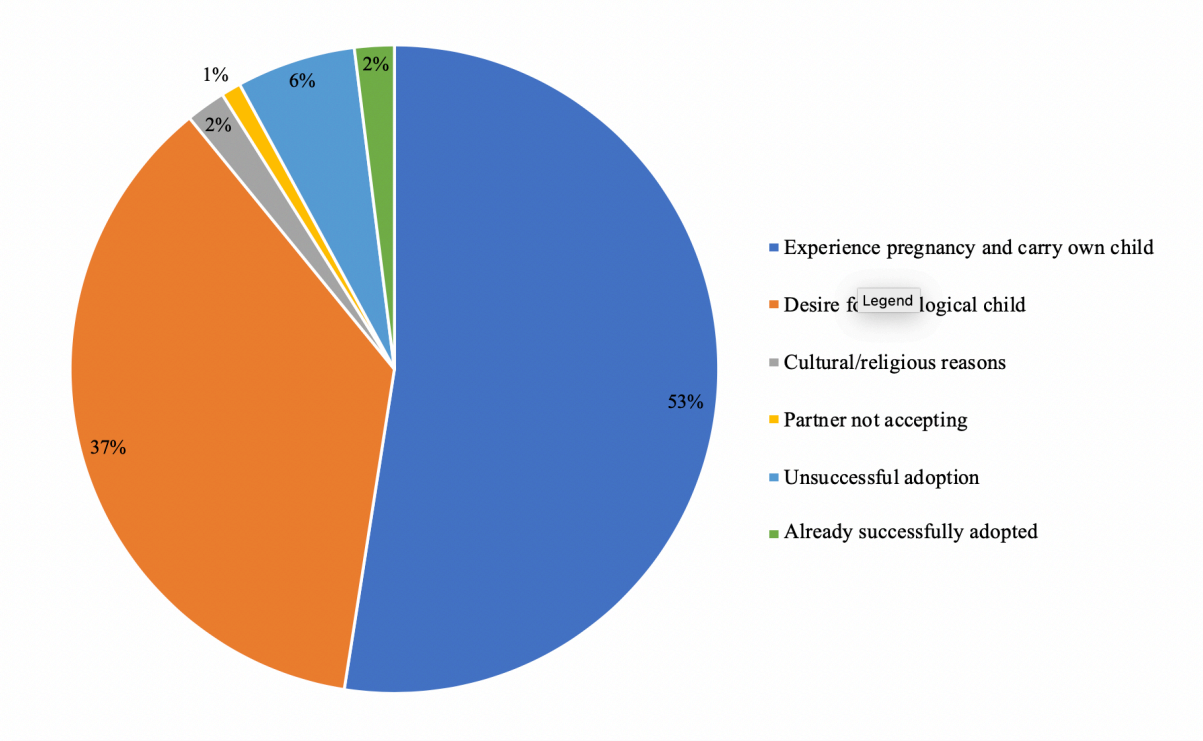


Figure 1a.3: *Reasons attributed to preference of uterine transplantation over adoption*

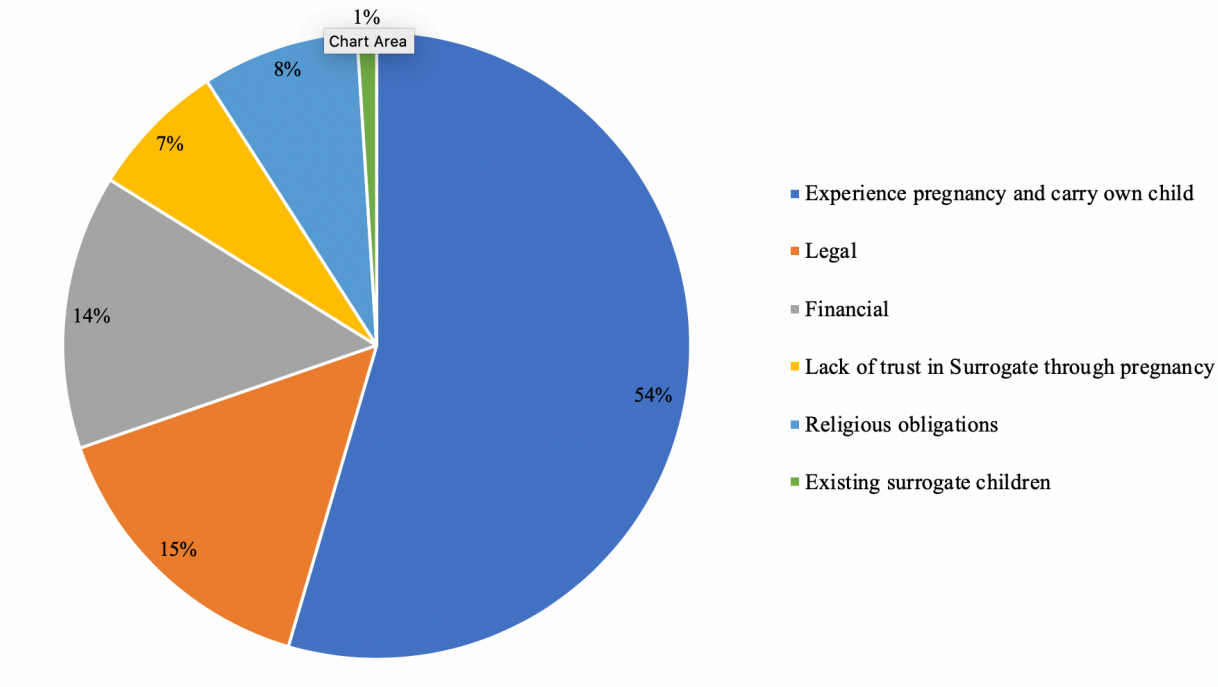


Figure 1a.4: *Reasons attributed to preference of uterine transplantation over surrogacy*

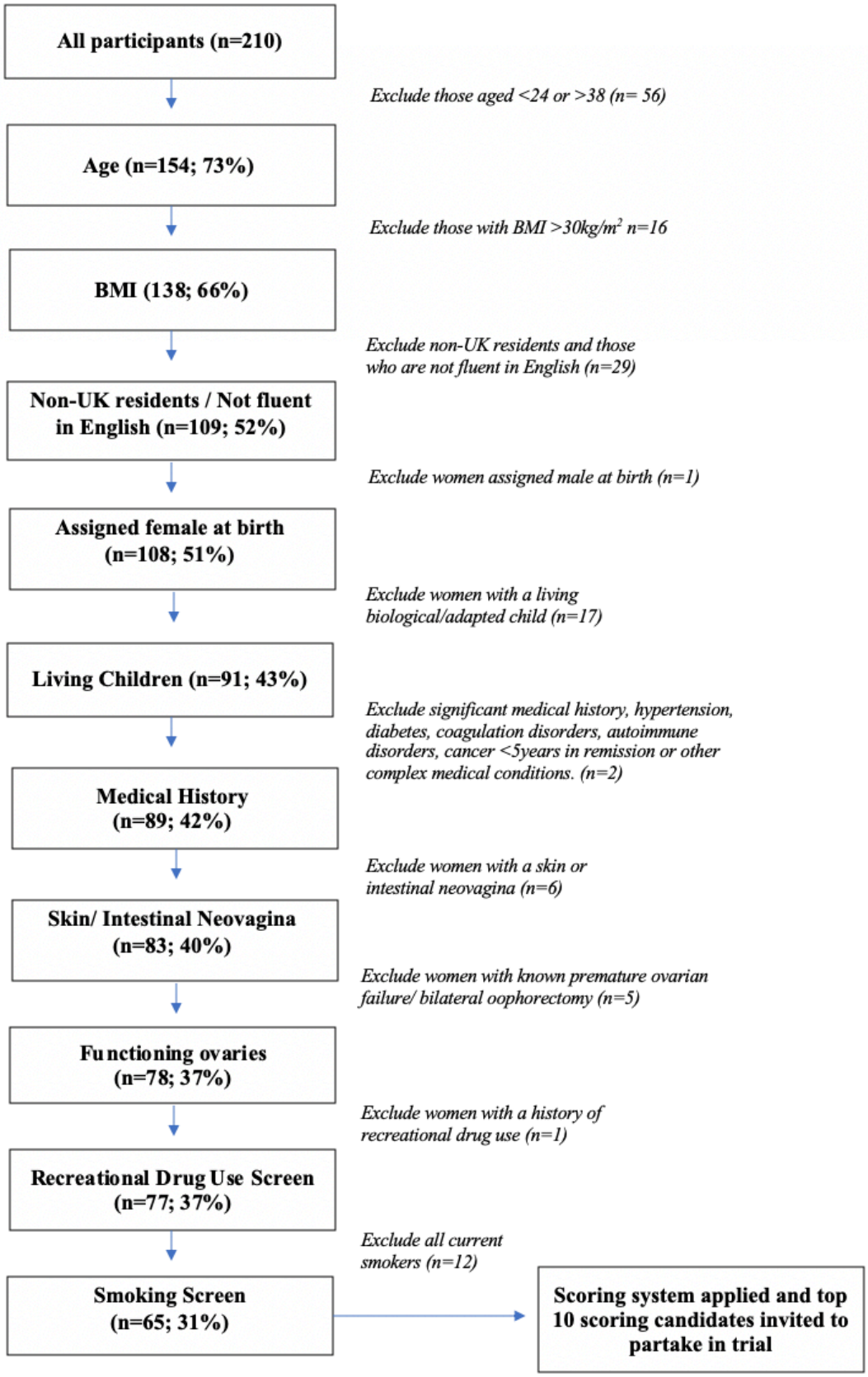


Figure 1a.5: *Flowchart summarising process of systematic exclusion after implementing INSITU selection criteria*

Participant selection

Figure 1a.5 outlines the process of systematic exclusion after implementing the selection criteria demonstrated in **Table 1a.1**. 56 (27%) women were excluded due to being <24 years or >38 years. 16 (7%) were subsequently excluded for having a BMI>30kg/m^2. A further 26 (12%) were not considered because they were not UK residents, whilst three were excluded for not being fluent in English. One woman was excluded because she was assigned male at birth. 17 (8%) women were not eligible due to already having living children. Two were excluded due to previous significant medical problems or due to being <5years in remission from cancer whereas six more (3%) had a skin intestinal or unspecified neovagina. An additional five women (2%) were deemed unsuitable due to a history of POI or no functioning ovaries. One woman had a history of recreational drug use and another 12 women were current smokers and as such, not considered further. Following application of the exclusion criteria, 65 (31%) applicants were deemed suitable to proceed with the trial.

Discussion

The data presented herein contains the largest and most detailed screening analysis of women with AUFI who have requested to be considered to undergo UTx. It demonstrates the characteristics of such women, highlights their suitability with regard to the selection criteria of the INSITU study, and provides novel insight into their perceptions of UTx, contextualised with respect to their other options to acquire motherhood.

The seemingly poor response rate may be attributable to multiple reasons. First and foremost, most women who did not meet the criteria were responded to at the time of their initial contact, primarily to enable them to readjust their reproductive aspirations and plans accordingly. Moreover, as women were being approached who contacted initially 8-9 years previously, it is likely that UTx may not now be a suitable option or they may have already achieved their family using alternative means. In addition, the contact details supplied at their initial contact may not have been active.

Whereas the majority of UTx cases have been undertaken in women with MRKH,[59] it is unknown if this is owing to proportional demand amongst women, their suitability or clinician preference. This data identifies that the most prevalent group of women with AUFI who desire UTx in the UK are women with MRKH. This is similar to a UTx screening process in 46 potential recipients in Germany, that identified women with congenital AUFI or MRKH made up 85% of their cohort.[396]

Women with MRKH are ideal candidates to undergo UTx. Owing to the 100% specificity and sensitivity of MRI,[397] and equivalent, although expert reliant combination of clinical examination and ultrasound,[398] surgery is rarely needed in the diagnosis of such women. As such there may be less pelvic adhesions and surgical complexity compared to other causes of AUFI such as after hysterectomy or following radiotherapy. Women with MRKH also tend to have normal ovarian function. They also appear to have reproductive benefits over other causes of AUFI, as data suggests significantly more oocytes are retrieved and fertilised with the subsequent creation of more suitable embryos to transfer in women with congenital absence of the uterus, compared to women who had previously undergone hysterectomy, despite being of similar age.[399]

The data also highlights that the majority of women who applied for UTx in this cohort had isolated uterovaginal hypoplasia (Type 1 MRKH). Whereas previous data has shown that women with type 1 constitute approximately 44% of all MRKH cases,[400] they made up 83% of the women who applied to be screened for UTx. Contrastingly, type 2 MRKH accounts for 56% of cases,[400] yet only 17% of the women with MRKH had other associated atypical features. This may demonstrate insight in these women, that women with type 1 are more suitable to undergo UTx than those with type 2, which has helped formulate their reproductive plans by taking this into account. Women with type 1 have been shown to have superior reproductive capacity over women with type 2, with significantly more follicles, oocytes, mature oocytes, fertilisations and cleavage stage embryos being demonstrated, utilising less gonadotrophins and stimulation days, although with similar pregnancy rates subsequently demonstrated in the surrogates.[401] Women with type 2 are also more likely to have concomitant medical issues that may preclude them from undergoing UTx, such as significant

renal/urological manifestations, which affect between 28-34% of women with MRKH.[190,402,403] Those who have a pelvic kidney, which affects approximately 17%,[190] are unsuitable if it acts as a structural hindrance during implantation. 23-28% of women with MRKH have unilateral renal agenesis,[190,194] which is associated with an approximate 35% risk of hypertensive disorders in pregnancy including pre-eclampsia.[274,275] Whilst the presence of unilateral kidney agenesis requires consideration, and warrants additional obstetric counselling, if the renal function is entirely normal, it is reasonable for such women to still be considered for UTx.

However, there is geographical variation internationally with regards to the most common aetiology for AUFI. In the United States for instance, programmes based in Dallas and Cleveland identified that the majority of potential recipients cause of AUFI was due to prior hysterectomy, which was seen in 63% and 64% of women respectively.[152] Given that in these US studies, 50-73% of hysterectomies were undertaken for benign indications may be reflective of very different clinical practices between Europe and the United States. In contrast 29% (n=62) of recipients in this cohort had undergone previous hysterectomy, with just 30% of those being undertaken for benign gynaecological reasons. 14% of the cohort presented herein (n=30) underwent hysterectomy due to a previous history of gynaecological cancer. In a similar fashion to all SOT recipients, UTx recipients will be susceptible to the well-established risk of cancer post-transplant,[235,404] owing to the immunosuppression induced physiological reduction in ability to destroy neoplastic cells. Data suggests immunosuppression may facilitate the growth of pre-existing dormant cancer cells. Recipients with a pre-transplant history of cancer have been demonstrated to be at a 30% increased rate of overall death.[405] This was reaffirmed by a recent meta-analysis which established that all-cause mortality, cancer-specific mortality and the risk of new primary malignancy was higher in recipients with a pre-transplant diagnosis of cancer, compared with those with history of malignancy.[406] Cervical cancer specifically, is 5 times more likely following kidney transplant, compared to the general population.[62] This is of particular relevance in this cohort, as the primary cancer in the vast majority of cases was the cervix (n=22; 73%). The greatest increased mortality has been shown to be in the first 5 years following cancer diagnosis.[405] As such, many UTx teams have stipulated selection criteria that preclude performing

UTx until at least 5 years following cancer diagnosis.[80,351] The recurrence risk also appears to be greater for more aggressive cancers, such as sarcoma.[336] As such, it may be prudent for women with previous hysterectomy for uterine sarcoma to be cancer free for longer periods before consideration for UTx, and surrogacy or adoption should be advised if acceptable to the woman. The only case performed with a previous history of cancer, was undertaken in Sweden, in a woman who had a previous radical hysterectomy as treatment for cervical cancer. She has since had two children, and has undergone completion hysterectomy, with no reported recurrence or new diagnosis of cancer.

Another consideration in women with MRKH is that they have varying degrees of vaginal shortening that often requires treatment to optimise diameter and depth, to enable adequate sexual function. Vaginal restoration techniques include conservative dilator therapy or surgical intervention using either the Vecchietti procedure, or the creation of a neovagina into the rectovesical space lined with skin, peritoneum or intestine.[407] Whilst the traditional aim of creating a functional vagina is to optimise sexual function, the development and progress in UTx now necessitates consideration for how the neovagina may impact future potential pregnancies. The only options that result in a physiologically functioning vagina, in the normal anatomical plane, are the use of dilator therapy and the Vecchietti procedure. Owing to the excellent outcomes, cost-effectiveness and low complication rates, dilator therapy is considered the first line treatment approach in such women.[408,409] The laparoscopic Vecchietti procedure should be considered where dilation is unsuccessful, with the creation of peritoneal or intestinal neovaginas reserved as a last resort.[409] This data confirms this is the clinical reality, as the majority of those who underwent vaginal restorative treatment used dilators, with a minority undergoing surgical intervention, after unsuccessful dilator therapy. However, three women had a bowel or skin neovagina, excluding them from proceeding in the process. In the context of UTx cases that have been published to date, the majority of recipients had a physiologically normal vagina (n=36, 80%).[56,58,166,167,184,410] Around a third (n=14; 31.1%) of women did not require any treatment as they had adequate vaginal dimensions from birth. A further third (n=14; 31.1%) used dilator therapy whilst 8 women (17.8%) underwent laparoscopic Vecchietti procedures. Four women (8.9%) had a skin neovagina,[56] one (2.2%) had a sigmoid neovagina,[54] and one (2.2%) had an

acellular porcine small intestine submucosa graft implanted pre-operatively.[134] One (2.2%) underwent unspecified vaginoplasty,[184] whilst details from two (4.5%) cases remain undisclosed.[52,136]

Given the previous elaborated potential implications of the vaginal microbiome upon UTx outcomes, as exemplified by the UTx case performed in Turkey,[54] it is vital that consideration of the technique utilised to acquire vaginal length in women with congenital forms of AUFI is given in the pre-operative assessment process, as it may directly impact outcomes. Reproductive outcomes following vaginoplasty are limited, mainly because women with MRKH have uterine hypoplasia and the aim of vaginoplasty is to facilitate sexual intercourse, not achieve pregnancy. However, women with cervico-vaginal atresia following vaginoplasty have a normally functioning uterine body, so data can be extrapolated from this population. However, such women have high rates of pathology associated with infertility such as endometriosis, which requires consideration. The small number of reported pregnancies and livebirths reported so far in such cases are displayed in **Table 1a.6**. Six successful livebirths have been reported in women with skin neovagina,[411-415] created using a modified McIndoe technique, reaffirming that livebirths are possible in women with skin neovagina. However, an early and mid-trimester miscarriage were also reported.[415,416] Three successful livebirths have also been achieved in women with neovaginas created using amniotic membrane.[417,418] With regard to bowel neovagina, reproductive outcomes appear less promising with just one livebirth being reported,[113] with two early trimester miscarriages and one ectopic pregnancy.[113-115]

Author	Case	Age (years)	Diagnosis	Type of neovagina	Further intervention	Pregnancies	Conception	Antenatal complications	Livebirth achieved?	Gestation (weeks)	Mode of delivery
Hampton et al., 1990.[411]	1	11	Vaginal agenesis (cervix markedly dilated and cystic)	Skin	No	1	Spontaneous	No	Yes	38	LSCS
Nargund & Parsons 2006.[412]	1	36	Cervico-vaginal agenesis with left ectopic ureter and vesical atresia	Skin	Rectovaginal fistula: revision surgery undertaken	1	IVF	No	Yes	32	LSCS
Acien et al 2008.[413]	1	14	Complete cervicovaginal agenesis	Skin	Two further uterine resections and re-implantation of uterine corpus secondary to stenosis and haematometra	1	Spontaneous	No	Yes	36	LSCS
Kriplani et al 2012.[416]	1	19	Cervical agenesis	Skin	n/r	1	Spontaneous	Mid-trimester miscarriage	No	21	n/a
Bastu et al 2012.[414]	1	n/r	Isolated vaginal agenesis	Skin	No	1	Spontaneous	n/r	Yes	36	LSCS
Rezaei et al 2015.[415]	1	12	Cervical agenesis (partial/complete vaginal aplasia)	Skin	n/r	3	Spontaneous Spontaneous Spontaneous	First-trimester miscarriage n/r n/r	No Yes Yes	12 35^{+6} 37	n/a LSCS LSCS
Chakravarty et al., 2000.[417]	1	12 -20*	Complete cervicovaginal atresia	Amniotic membrane	n/r	1	Spontaneous	n/r	Yes	n/r	n/r
	2	12 -20*	Partial cervicovaginal atresia	Amniotic membrane	n/r	1	Spontaneous	n/r	Yes	n/r	n/r
Zafar et al 2007.[418]	1	25	Vaginal atresia secondary to mismanaged spontaneous vaginal delivery	Amniotic membrane	No	1	Spontaneous	No	Yes	38	LSCS
Yang et al 2013.[113]	1	16	Vaginal agenesis with cervical remnant	Sigmoid colon	No	1	Spontaneous	PPROM	Yes	38	LSCS
	2	19	Vaginal agenesis with cervical remnant	Sigmoid colon	No	1	Spontaneous	First-trimester miscarriage	No	n/r	n/r
Kisku et al 2015.[114]	1	n/r	Vaginal agenesis	Sigmoid colon	n/r	1	n/r	First-trimester miscarriage	No	10	n/a
Sato et al 2000.[115]	1	24	Cloacal malformation	Sigmoid colon	No	1	Spontaneous	Ectopic pregnancy	No	6	n/a

Table 1a.6 - Previously reported pregnancies in women with non-physiological vaginas (Re-used with permission from Jones et al. 2019)[410]

Key: LSCS; Lower segment Caesarean section, **IVF**; In-vitro fertilisation, **n/a**; not applicable, **n/r**; not recorded, **PPROM**; premature rupture of membranes. ***** age unspecified

The selection criteria were carefully chosen for this study, initially based upon the Indianapolis Consensus,[419] but with additional insight from the preliminary UTx outcomes in Sweden, which were shared at a multi-disciplinary academic symposium in 2015.[351] The criteria were selected exclusively for this study, to minimise confounding variables and help accurately demonstrate outcomes following UTx. However, it is worth considering that some criteria have been considered from a legal perspective,[420] and found to not legally be justifiable to implement into future clinical programmes. Whilst '*the requirement for the recipient to produce her own ovum is not legally justified'*,[420] it most certainly is medically justified. Oocyte donation independently and significantly increases risk of pregnancy complications including preeclampsia, preterm delivery and small for gestational age.[157] When the criteria were established, the only published livebirth following UTx was born at 31^{+5}/40 after developing severe pre-eclampsia.[55] It was uncertain if this was due to precipitating factors associated with UTx, or because the recipient had unilateral renal agenesis. As such, the decision to exclude the use of oocyte donors to mitigate potential additional obstetric risk is justified, although this will may be alleviated in future clinical programmes once detailed obstetric outcomes are known.

Whilst selection criteria can be used to minimise risk and optimise outcomes, where selection criteria are based primarily on value judgements or are excessively more stringent than necessary, it has been argued that such decisions should be reconsidered in order to avoid unjust discrimination.[311,421,422] Organ allocation in UTx, unlike lifesaving SOTs, will not be governed by clinical urgency or survival benefit.[206] As such it is expected that age,[423] tissue matching and previous childbirth,[206,422] may impact prioritisation in the future, following the transition into clinical practice. Given that the selection criteria used for the INSITU study excluded 69% of women on their history alone, alleviation of the selection criteria should open up the possibility of UTx to many more women with AUFI, should it be demonstrated that safety and efficacy is not impacted.

As this cohort was self-selected, it is inappropriate to generalise perceptions of UTx, over adoption and surrogacy, from this cohort to the general population. However, the data presented herein provides novel insight into the reason's women with AUFI may choose to undergo UTx given the

availability of other alternatives to acquire motherhood. This data identifies that the predominant reason for women wishing to undergo UTx, over both adoption and surrogacy, is to experience gestation. With regards to adoption, the experience of gestation was more frequently reported than biological relationship to the child, suggesting this may be considered more valuable in such women. As UTx is the only therapeutic intervention that restores reproductive anatomy and functionality in women with AUFI, the perceived benefit of being able to gestate oneself, may be preferable for some women with AUFI, despite the additional associated risks involved. The associated legal issues associated with surrogacy were cited as the primary motivating factor to undergo UTx in 15% of this cohort. The vast majority of those (n=19; 90%), lived in the UK, thereby referring to the fact surrogacy arrangements are not legally enforceable in the UK, putting couples utilising surrogacy in a legally precarious position. The other two women lived in countries where surrogacy was illegal.

The economic implications of surrogacy were also cited by 14% of this cohort as reasons to undergo UTx. UK legislation states that only *reasonable* expenses can be paid towards the surrogate, and anything that exceeds this amount needs authorisation by the Court. The average cost of undergoing surrogacy in the UK was shown in a recent cross-sectional study to be approximately £25,000. However, the costs associated with surrogacy vary dramatically internationally from the UK being the cheapest, to the USA where the median associated cost was £120,000.[43] However, whilst the costs of this research study are charitably funded, UTx will represent a similarly, if not more costly alternative to surrogacy. Moreover, it is unlikely such as procedure will be funded by insurance companies, and it is currently unknown whether or not the National Health Service would fund such a programme through NHS England as a highly specialised service. As such, it is likely UTx will necessitate self-funding, which will be prohibitive for the majority of women.

Religious factors were responsible for 2% of cohorts' motivation to undergo UTx over adoption. Whilst adoption is readily accepted in Christianity and Hinduism, it is less readily accepted in other major religions. In Islam, whilst the act of fostering and looking after children in need is encouraged, the formal recognition of adoption as a legal entity is not recognised.[424] Similarly, despite a strong

ethical mandate towards the care of orphans, adoption is not formally recognised as a legal institution in Judaism.[425]

Religious factors were cited by 8% of the cohort as reasons to undergo UTx instead of utilising surrogacy. Surrogacy is associated with various religious complexities. In Islam, childbearing forms an important part of the Muslim identity and therefore the use of ART is generally accepted. The main stipulation for ART is that it takes place within a marriage and that the embryo is biologically related to both the husband and wife.[426] As such, traditional Islamic bioethics cannot accept surrogacy because it utilises male sperm in a woman to whom that male is not married, thereby mixing gene lineage. As such, surrogacy is not permitted in Islam. In Judaism, there are differing views surrounding ART across the major movements (Orthodox, Conservative and Reformed). IVF and egg freezing are generally considered as acceptable in order to fulfil a commandment in Genesis 9:1, which instructs Jews to "be fruitful and multiply". However, most Rabbis do not consider surrogacy as acceptable.[427] Of those Rabbis who do accept surrogacy, they stipulate that only full gestational surrogacy is permitted and preferably the surrogate would be a single Jewish woman, so as to not cause any marital discourse, and facilitate future Jewish identify in the offspring.[428] In Christianity, the Catholic church is generally opposed to all forms of ART including surrogacy. The main opposition to surrogacy orientates around the dissociation of husband and wife, by the intrusion of a person other than the couple, which is seen as immoral. This stance is not as uniform in the Protestant church, where many find IVF acceptable, as long as the couple are married.[427] Surrogacy, and most ART, is readily accepted in Hinduism.

From a religious perspective, UTx itself is generally accepted by most major religions. In Islam, the transplantation of organs that do not carry genetic traits such as the uterus is permissible. Moreover, the necessary use of IVF and embryo cryopreservation is acceptable,[426] but only if the gametes are from a lawfully married couple. In Judaism, the pursuit of a cure for infertility is seen as noble, given the centrality of children within the marital relationship. However, deceased organ donation is unacceptable in Jewish law, thereby UTx is restricted to the use of living donors. The

currently adopted surgical technique utilises prophylactic bilateral salpingectomy to reduce risk of ectopic pregnancy, necessitating the use of IVF. In Catholicism, whereas the transplantation of the uterus itself would be acceptable,[429] owing to the dissociation of husband and wife, the use of IVF is seen as gravely immoral, thereby precluding those of fixed Catholic faith. Whilst UTx may be acceptable if natural conception were possible following UTx, given the high rates of tubal blockage seen in non-human primates following utero-tubal auto-transplantation,[81] concerns would remain about future tubal patency and potential risk of ectopic pregnancy. There do not appear to be any issues of UTx in Hinduism.

Conclusion

This study summarises a detailed screening analysis of more than two hundred women with AUFI who applied to partake in the INSITU deceased donor UTx research study. It demonstrates the characteristics of such women, and highlights that the majority of women who wish to undergo UTx in the UK are women with Type I MRKH. This study also provides insight into the reasons why women would choose to undergo a procedure associated with significant physical risk, over the other options to acquire motherhood, and identifies the most common reason is to personally experience gestation. Ultimately this study highlights the attrition rate of women following screening, utilising commonly used selection criteria, and demonstrates that more than two thirds of women who requested to undergo the process were excluded. As such, as more studies present outcomes following UTx, critical assessment of the selection criteria utilised is warranted to ensure potential recipients are not being unnecessarily excluded, whilst maintaining those important to optimise success rates.

Study 1b

Pre-operative investigation of potential uterine transplant recipients

Introduction

Whereas UTx offers the opportunity to restore fertility in women with AUFI, it is associated with significant risk, as evaluated in detail from previous cases on pages 91-92,[59] including at least three major operations and the transient use of immunosuppression. As demonstrated in **Study 1a**, more than two thirds of women who applied for consideration of UTx as part of INSITU were excluded based on selection criteria alone. The implementation of selection criteria is not only to optimise outcomes, but to ensure that appropriately healthy women undergo the process, therefore minimising the potential adverse impact of associated risk. The recipients of lifesaving SOTs often have significant medical co-morbidities and therefore present much greater medical and surgical risk, but the potential lifesaving benefit often warrants the risk associated with the intervention. UTx differs by being a quality of life improving SOT, which remains associated with significant risk, but the potential benefit of the opportunity to gestate and give birth to biologically related offspring offers objectively less benefit than that which lifesaving SOTs offer. Whilst individual perceptions and circumstances vary, and access and acceptability to alternative options to acquire motherhood such as adoption and surrogacy differ, UTx may therefore represent an undesirable and risky option for many, but conversely may be the only acceptable or available option to acquire motherhood for others. Irrespective of desire, selection criteria have so far restricted UTx to healthy women with no significant medical comorbidities. Extensive screening and detailed evaluation processes have subsequently been implemented to ensure potential recipients are suitable, often incorporating a strategy from least to most invasive examinations.[152,396] A thorough assessment of the potential recipients performance status and comorbidities is essential to determine and mitigate intra-operative and post-operative risk where possible.

Extensive counselling is also an essential component in the pre-operative assessment of potential UTx recipients. Counselling is fundamental to ensure each woman is fully aware of the process, including the significant risks involved. The capacity to consent relies on the patients' cognitive state at the time and their ability to weigh up the benefits and disadvantages of the procedure. This is increasingly

difficult with novel procedures when the probability of success and risks are largely unknown and difficult to quantify. In such cases, individuals may be prone to decision-making biases, such as focusing on potential positive outcomes and minimising the perceptions of potential risk. On rare occasions, there may be elements of coercion impacting the ability to consent freely, or doubts about a person's mental state or cognitive capacity. Any suspicion of either would indicate a need for further assessment and may result in not proceeding if this eventuality arose. It is also important to explore how the couple plan to cope if the procedure is unsuccessful, if they have any alternative plans to achieve parenthood, and how they feel about childlessness. Moreover, consideration of the alternative options to acquire motherhood is essential, including both adoption and surrogacy.

Psycho-social assessment of UTx donors and recipients is a vital part of the overall suitability assessment. The initial psycho-social protocol for UTx was derived from face transplantation.[430,431] A key difference between UTx recipients and other VCA recipients is that the UTx group have lower levels of previous trauma and physical disability,[133,432] and most are generally assumed to be medically and psychologically well prior to the transplant. Nevertheless, psychological morbidity is common in normal populations with a weekly prevalence rate of 16.7%.[433] Ongoing or recent mental health problems may impact an individual's capacity to consent and cope with stressors throughout the transplant process. In the Swedish UTx series active psychiatric illness, lack of social support, unsolvable relationship difficulties, substance abuse, or unrealistic expectations were considered reasons for exclusion if it was not possible to ameliorate these issues through support.[432] However, in a study of 123 SOT recipients, there was no association between pre-transplant psychological morbidity and posttransplant distress,[434] suggesting individuals with mental health problems need not be excluded. However, comprehensive psychosocial assessment is essential to identify recipients of higher psychological need for the provision of additional support or treatment.

The aim of this study was to comprehensively evaluate those women with AUFI who were selected to proceed with the INSITU study from **Study 1a**, to determine suitability to undergo the procedure.

Methodology

Clinical protocol

Following the screening and pre-evaluation process described in **Section 1a**, the applicants were scored and ranked using the previously described scoring system summarised in **Table 1a.1.** The highest ten scoring respondents were invited to enrol in the main part of the INSITU study. A further ten were placed in a reserve group, who would be utilised if those in the top 10 were unsuitable on further investigation or decided not to proceed following counselling.

The participants were referred by their general practitioner, thereby ensuring eligibility to NHS care, and were reviewed in a gynaecology outpatient setting at Charing Cross Hospital in London. Those selected were evaluated extensively, including a detailed history and examination. The history focused on gynaecological, obstetric (if relevant), medical, surgical, and social history. Further details were elicited about previous fertility treatment, current medications being taken and any relevant allergies. The examination included an abdominal examination as well as vaginal and speculum examinations to ascertain vaginal dimensions.

Extensive counselling was undertaken, to ensure participants were fully informed and agreeable to proceed with the process. Recipients received detailed verbal and written information regarding the multifaceted process associated with UTx, including the necessary pre-operative assessment process and investigations. Detailed information was given surrounding the operation, potential complications, postoperative care, and necessary post-operative medications and immunosuppression (**Appendix 1b.1**). Additional information about fertility treatment (**Appendix 1b.2**), antenatal care (**Appendix 1b.3)**, Caesarean section (**Appendix 1b.4**). and hysterectomy (**Appendix 1b.5**). The recipient's partner was also counselled and received a specific written information leaflet (**Appendix 1b.6**) and subsequently signed a consent form (**Appendix 1b.7**). At the first appointment, each participant was discussed with the Chief Investigator and lead surgeon (Mr Richard Smith), to ensure that the selection criteria were met, and the participant was deemed suitable to proceed. If so, and

after all the written information was read and an opportunity was given to ask questions, each participant and their partner provided written consent to partake in the study.

Following consent, subsequent investigations were undertaken to confirm suitability to proceed. Blood tests were undertaken including a full blood count (FBC), urea and electrolytes (U&Es), liver function tests (LFTs), clotting, a group and save (G&S), tissue typing and a thrombophilia screen. An infection screen was also undertaken including HIV, Hepatitis B & C, EBV antibodies, Cytomegalovirus antibodies, herpes simplex antibodies, Syphilis and a tuberculosis (TB) Elispot (Oxford Immunotec, Abingdon, UK). A HPV test +/- cytology, and a GUM screen including chlamydia, gonorrhoea and trichomonas vaginalis (TV) were also undertaken, as well a high vaginal swab (HVS). Imaging of the pelvis was undertaken, including a transvaginal ultrasound, and an MRI.

In a concurrent fashion with the medical assessment, a detailed psychological evaluation was also undertaken. The participants completed a number of questionnaires to provide further insight into their baseline psychological condition, as summarised in **Figure 1b.1**.

Ethics approval

HRA (REC approval was received (REC: 18/LO/0217) on 28th February 2018. Study start approval from received from the sponsor, Imperial College Healthcare NHS Trust, on 19th February 2019.

Statistics

SPSS version 24 software (SPSS, Chicago, Illinois, USA) was used for analysis. Descriptive statistical analysis was described as mean +/- SD or median +/- range.

WHOQOL-BREF	*This questionnaire is a well-known measure of quality of life, which is particularly useful in assessing treatment efficacy.[435] It consists of 26 items measuring physical health, psychological health, social relationships, and the environment and has good psychometric properties, making it an ideal questionnaire to use in the process of UTx.*
FertiQoL	*FertiQoL is the first internationally validated psychometric tool that objectively measures the quality of life of individuals experiencing issues related to fertility [436]. The core sections consist of 24 items measuring emotional, mind-body, relational and social quality of life. The scale also includes 10 items measuring tolerability of infertility treatment and treatment environment, making it an ideal tool to monitor acceptability to treatment in UTx.*
Hospital Anxiety and Depression Scale (HADS)	*This is a very well validated scale consisting of 14 items that help screen for anxiety and depression.[437]*
Dyadic Adjustment Scale (DAS)	*This acts as a measure of relationship adjustment, and assesses relationship satisfaction and the perception of their relationship.[438] It consists of 32 items measuring dyadic consensus, dyadic satisfaction, affectional expression and dyadic cohesion.*
36-item Short Form Survey (SF-36)	*This health survey consist of 36 items which measure functional health and well-being.[439] The questions relate to eight scales including physical health, bodily pain, general health perceptions, vitality (energy/fatigue), social functioning, role limitations due to emotional health, and general mental health.*
Marital taxon measure	*This is a 10-item screening measure that has been shown to reliably assess marital discord. It can be administered as an interview measure or a self-reported measure.[440]*
Modified Medical Outcomes Study Social Support Survey (mMOS-SS)	*This is an 8-item abbreviated form of the MOS-SS, which is composed of two subscales that cover emotional and instrumental social support and have been shown to have excellent psychometric properties.[441]*
Beliefs about Medicines Questionnaire – General (BMQ-General)	*This questionnaire is an 8-item measure of general attitudes towards medications.[442] The scale has demonstrated stability of beliefs over time and is associated with medication adherence. Given the importance of taking medications following UTx, such as the essential use of immunosuppression, this is an ideal tool to use in the pre-operative selection process, to identify women at risk of non-adherence.*

Figure 1b.1: *Questionnaires utilised to help assess psychological wellbeing in selection process*

Results

Eleven women were selected and assessed in total for consideration for the INSITU trial. All 11 women (100%) were UK residents with a median age of 30 (24-39). The mean BMI was 24.2 +/- 2.9 kg/m^2. All participants were of white ethnicity and the majority were employed (n=10, 90.9%). All participants were married or in a relationship with a mean duration of which was 7.5 +/- 6.0 years. The most prevalent cause of AUFI in the cohort was MRKH (n=10, 90.9%) with one woman having AS following a previously unsuccessful myomectomy (9.1%). Apart from their AUFI, the vast majority of women did not have any significant previous medical history (10, 90.9%), although one reported suffering from previous recurrent UTIs and an overactive bladder (9.1%). One woman reported a prior history of depression, which was now inactive and not medicated (9.1%). Apart from the one woman (recipient 7) who took tolterodine for her overactive bladder (9.1%), none of the remainder of the cohort took any regular medications (90.9%). All but one of the cohort did not report any allergies (n=10, 90.9%), whereas one woman (9.1%) reported an adverse reaction to opiates. Five of the women had not previously undergone any surgery (45.5%). Three women (27.3%) had previous undergone laparoscopy, one of which was to undertake laparoscopic cystectomy for a dermoid cyst, whereas the other two were undertaken to investigate the cause of AUFI. One woman (9.1%) had previously undergone laparotomy, which was undertaken for open myomectomy, which resulted in AS. Two women (18.2%) had undergone previous breast augmentation surgery. Of those women with MRKH (n=10), seven (70%) previously underwent vaginal dilator therapy to obtain adequate vaginal length, whereas the remaining three (30%) did not require treatment.

None of the cohort had living children, either themselves or through adoption or surrogacy. Recipient 1, who had AS, had been pregnant previously but underwent termination of pregnancy. None of the cohort smoked, although four were ex-smokers, who had all stopped smoking >12 months previously, with a median pack year history of 1.5 (1-6). Three women (27.3%) did not consume any alcohol. The remainder all drank within the WHO recommended weekly intake of 14 units or less with a median of 3.5 units (1-14 units).

Clinical examination was unremarkable in all 11 women. Abdominal examination did not reveal any abnormalities, and vaginal and speculum examination identified a normal appearing cervix in the woman with AS and normal appearing vaginal apices in the women with MRKH. All women had acceptable vaginal dimensions, with length greater than 7cm in all cases.

Laboratory investigations

Routine baseline haematology, biochemistry and coagulation profiles did not reveal any significant findings as summarised in **Table 1b.1**. The most common blood groups were blood group A (n=5; 45.5%) and blood group O (n=4; 36.4%) with blood group B being present in two women (18.2%).

All participants were negative for syphilis, hepatitis B, hepatitis C and HIV. Virology results are demonstrated in **Table 1b.2**. In those where an unequivocal result was obtained, half (n=5;50%) of the cohort were seropositive for CMV, whilst the other half (n=5;50%) were seronegative. One woman had an equivocal result which required repeating. All of the women were seropositive for EBV (n=11; 100%) and the majority (n=8; 72.7%) were seropositive for HSV. Nine (81.8%) of the women had a non-reactive TB Elispot, whilst two (18.2%) had borderline reactive results.

At the time of writing, thrombophilia screening had been undertaken in six women, including lupus anticoagulant, protein S free antigen, protein C free level, Factor V Leiden status and antithrombin III activity, which did not reveal any evidence of thrombophilia. AMH levels were >10 in all but one woman (Recipient 7), who had a level of 2.8pmol/l, which was 4.2pmol/l on a second sample. The mean AMH was 20.5 +/- 9.6 pmol/l.

	Hb (g/l)	WCCs (x109/L)	Plts (x109/L)	Fib (s)	PT (s)	APTT (s)	Na^{2+} (mmol/l)	K^{+} (mmol/l)	Creat µmol/L	Urea (mmol/l)	eGFR (ml/ min/ 1.73m^2)	ALT (U/l)	ALP (U/L)	Alb (g/l)	Bili (µmol/L)
Range	*115-165*	*4-11*	*140-400*	*1.5-4.5*	*10-14*	*22-36*	*133-146*	*3.5-5.3*	*45-84*	*2.5-7.8*	*>90*	*<35*	*30-130*	*35-50*	*<21*
Recip. 1	151	9.7	265	3.52	12.8	33.6	140	4.2	63	4.3	>90	19	61	43	4
Recip. 2	146	13.7	312	3.88	14.5	29.4	140	4.3	69	5.3	>90	16	51	43	8
Recip. 3	137	6.6	227	2.65	13.2	32.7	138	4	63	3.5	>90	25	61	44	11
Recip. 4	145	9.6	252	4.42	13.6	27	141	4.1	76	6	>90	29	21	42	20
Recip. 5	138	11.6	230	3.24	12.8	30	141	4.1	68	3.7	>90	13	72	41	18
Recip. 6	150	8.7	235	2.2	11.6	26.6	140	4.5	72	5.2	>90	18	48	45	8
Recip. 7	128	5.5	160	3.34	13.3	27.6	136	4	54	2.9	>90	38	63	41	11
Recip. 8	121	6.8	220	2.6	13.7	30.8	138	4	63	5.7	>90	13	55	44	6
Recip. 9	132	8.3	260	3.53	13.2	29.1	139	4.2	69	4.5	>90	20	56	44	7
Recip. 10	139	5.9	240	2.17	14.3	32.5	140	4.2	69	4.4	>90	26	44	42	7
Recip. 11	120	7.8	267	3.77	15	33.5	136	3.7	57	4.1	>90	15	63	40	13

Table 1b.1: *Serum biochemistry results for participants*

Alb*; Albumen,* **ALP***; Alkaline phosphatase,* **ALT***; Alanine aminotransferase,* **APTT***; Activated partial thromboplastin time,* **Bili***; Bilirubin,* **Creat***; Creatinine,* **eGFR***; estimated glomerular filtration rate,* **Fib***; Fibrinogen,* **Hb***; Haemoglobin,* **Na^{2+}***; Sodium,* **Plts***; Platelets,* **PT***; Prothrombin time,* **Recip***.; Recipient*

	CMV		EBV		HSV	HIV	Hep B	Hep C
	IgG	*IgM*	*IgG*	*IgM*	*IgG*		*Anti-HBc*	*Ab*
Recip. 1	Not detected	Not detected	Detected	Not detected	Detected	Negative	Not detected	Not detected
Recip. 2	Not detected	Not detected	Detected	Not detected	Detected	Negative	Not detected	Not detected
Recip. 3	Not detected	Not detected	Detected	Not detected	Detected	Negative	Not detected	Not detected
Recip. 4	Detected	Not detected	Detected	Not detected	Not detected	Negative	Not detected	Not detected
Recip. 5	Equivocal	Not detected	Detected	Not detected	Not detected	Negative	Not detected	Not detected
Recip. 6	Detected	Not detected	Detected	Not detected	Detected	Negative	Not detected	Not detected
Recip. 7	Detected	Not detected	Detected	Not detected	Detected	Negative	Not detected	Not detected
Recip. 8	Detected	Not detected	Detected	Not detected	Detected	Negative	Not detected	Not detected
Recip. 9	Not detected	Not detected	Detected	Not detected	Detected	Negative	Not detected	Not detected
Recip. 10	Not detected	Not detected	Detected	Not detected	Detected	Negative	Not detected	Not detected
Recip. 11	Detected	Not detected	Detected	Not detected	Not detected	Negative	Not detected	Not detected

Table 1b.2: *Serum virology results for participants*

CMV; *Cytomegalovirus,* **EBV***; Epstein Barr virus,* **Hep***; Hepatitis,* **HIV***; Human immunodeficiency virus,* **HSV***, Herpes simplex virus,* **N/P***; Not performed,* **Recip***.; Recipient*

Of the ten women who underwent gonorrhoea and chlamydia testing, 100% (n=10) were negative. Trichomonas vaginalis species were not seen in any of women (n=11; 100%). The majority (n=9; 81.8%) were negative for high risk species of HPV, whereas two were positive (18.2%), one of which had borderline cytology (Recipient 6), and the other had low grade dyskaryosis, suggestive of vaginal intraepithelial neoplasia (VAIN) I (Recipient 7). Vaginoscopy was subsequently performed in both women, but no significant lesions were identified after application of acetic acid. Subsequent biopsies identified normal epithelium and did not reveal any evidence of intraepithelial neoplasia. Recipient 6, who had borderline cytology findings in the context of high-risk HPV infection subsequently underwent

topical treatment with topical imiquimod 5% twice weekly for eight weeks. Repeat HPV testing three months following treatment was negative.

Psychological evaluation

The quantitative data elicited from the questionnaires are summarised in **Table 1b.3** and **Table 1b.4**. The results indicated good quality of life, functional health and wellbeing amongst all the recipients, low levels of marital discourse, a good level of social support, healthy attitudes towards medications amongst all participants. On the hospital depression and anxiety scale (HADS) however, one recipient screened positive for anxiety, and another was borderline.

Participant selection stage 1

Following MDT discussion, including review of the clinical history and in light of the laboratory findings and psychological evaluation, it was agreed that all participants were suitable to proceed with radiological assessment apart from recipient 7. Given her history of overactive bladder and recurrent UTIs, which may be exacerbated following UTx, in addition to the VAIN I and low ovarian reserve, she was unsuitable to proceed. With regards to recipient 6, who had high risk HPV in the context of borderline cytology, it was agreed that as she was HPV negative after imiquimod treatment, it is reasonable to proceed with further evaluation. Following the exclusion of one participant from the study, a further participant was invited for assessment from the reserve list.

	WHOQoL-BREF				SF36									HADS	
	Domain 1 Physical	*Domain 2 Psychological*	*Domain 3 Social*	*Domain 4 Environ.*	*Physical Function*	*Role limited (physical health)*	*Role limited (emotional health)*	*Energy / Fatigue*	*Emotional wellbeing*	*Social functioning*	*Pain*	*General health*	*Health change*	*Depression score*	*Anxiety score*
Recip. 1	94.3	93.3	93.3	90	100	100	100	80	88	100	100	100	50	0	4
Recip. 2	97.1	93.3	86.7	95	100	100	100	85	96	100	100	90	50	0	5
Recip. 3	94.3	86.7	80	97.5	95	100	100	75	88	100	100	90	50	0	4
Recip. 4	88.6	76.7	86.7	87.5	100	100	100	65	72	100	100	80	50	3	12
Recip. 5	97.1	90	86.7	85	100	100	100	75	92	100	90	85	50	1	8
Recip. 6	91.4	76.7	93.3	82.5	100	100	100	65	84	100	100	90	100	0	0
Recip. 7	100	90	100	92.5	100	100	100	80	88	100	100	90	50	0	2
Recip. 8	94.3	96.7	93.3	90	100	100	100	50	92	100	100	80	100	0	0
Recip. 9	94.3	80	73.3	87.5	100	100	100	80	72	100	90	95	50	2	6
Recip. 10	97.1	66.7	73.3	85	100	100	66.7	60	76	87.5	100	90	75	0	2
Recip. 11	88.6	80	86.7	80	100	100	100	75	80	100	100	80	75	0	4
Mean +/- SD	94.3 +/- 3.6	84.5 +/- 9.2	86.7 +/- 8.4	88.4 +/- 5.3	99.5 +/- 1.5	100 +/- 0	97.0 +/- 10.0	71.8 +/- 10.6	84.4 +/- 8.3	98.9 +/- 3.8	98.2 +/- 4.0	88.2 +/- 6.4	63.6 +/- 20.5	0.5 +/- 1	4.3 +/- 3.5

Table 1b.3: *WHOQOL-BREF, SF36 and HADS results for participants*

Environ.; *Environmental***, Recip**.*; Recipient,*

	FertiQoL Core					BMQ general		DAS					Marital taxon	mMOS-SS
	Mind/body	*Emotional*	*Relational*	*Social*	*Core*	*General harm*	*General overuse*	*Consensus*	*Satisfaction*	*Cohesion*	*Affectional expression*	*Total*		
Recip. 1	70.8	54.2	79.2	83.3	71.9	10	9	63	44	16	11	134	0	80
Recip. 2	95.8	87.5	95.8	91.7	92.7	10	5	68	45	20	11	144	0	95
Recip. 3	62.5	62.5	87.5	79.2	72.9	5	5	59	47	16	8	130	2	100
Recip. 4	91.7	66.7	95.8	91.7	86.5	11	8	67	46	23	11	147	1	100
Recip. 5	79.2	62.5	100	87.5	82.3	12	7	66	40	21	11	138	0	100
Recip. 6	87.5	66.7	95.8	70.8	80.2	9	6	61	46	19	10	136	0	95
Recip. 7	100	75	91.7	83.3	87.5	11	8	60	48	21	10	139	0	87.5
Recip. 8	75	75	66.7	66.7	70.8	9	6	60	47	20	10	137	0	100
Recip. 9	87.5	70.8	79.2	54.2	72.9	12	6	62	42	18	11	133	0	97.5
Recip. 10	66.7	54.2	75	79.2	68.8	7	6	68	40	21	12	141	1	87.5
Recip. 11	95.8	95.8	70.8	83.3	86.5	11	7	75	33	20	12	140	0	100
Mean +/- SD	83.0 +/- 12.8	70.1 +/- 12.9	85.2 +/- 11.5	79.2 +/- 11.3	79.4 +/- 8.2	9.7 +/- 2.1	6.6 +/- 1.3	54.5 +/- 4.8	43.5 +/- 4.4	19.5 +/- 2.2	10.6 +/- 1.1	138.1 +/- 4.9	0.4 +/- 0.7	94.8 +/- 6.8

Table 1b.4: *FertiQoL Core, BMQ general, DAS, marital taxon and mMOS-SS scores for participants*

Recip.*; Recipient*

Radiological investigations

All recipients underwent abdomino-pelvic imaging including MRI and ultrasound assessments. Nine of the women (81.2%) had normal abdominal viscera, but two had previously unidentified urological system abnormalities; one had unilateral kidney agenesis with a solitary right sided kidney (recipient 9), whilst the other had a pelvic kidney (recipient 2). The woman with AS (candidate 1) had a multifibroid uterus measuring 9.8 X 9.0 X 7.9 with complete obliteration of the endometrial cavity. In the ten women with MRKH, of the nine who underwent MRI, five had the presence of uterine anlage. All five were bilateral in iliac fossae, caudal to the ovaries. Only one participant had evidence of endometrium within the anlage. All participants had radiologically normal appearing ovaries bilaterally, normal vertebrae and normal major vessel vasculature in the pelvis. All participants subsequently underwent pelvic ultrasound, which reaffirmed the MRI findings and did not reveal any other pathology.

Participant selection stage 2

Following further MDT discussion in light of the radiology findings and further investigation it was agreed that nine out of the remaining ten candidates were immediately suitable to proceed further in the trial. With regards to recipient 2, it was agreed that further evaluation with laparoscopy was needed to determine whether or not the pelvic kidney would be a structural hindrance to UTx, and that it would not be adversely affected during pregnancy. With regard to recipient 9, whilst she had unilateral renal agenesis, as her baseline renal function was normal, following MDT discussion it was agreed it was reasonable to proceed, following obstetric counselling regarding the increased risk of hypertensive disorders in pregnancy, in addition to the potential nephrotoxic impact of the required immunosuppression. Regarding recipient 1, it was agreed that should she proceed with UTx, she would undergo concomitant hysterectomy immediately prior to UTx, following confirmation that the graft being retrieved was suitable for implantation.

Following selection, each participant was planned to meet the IVF team, with a view to undergoing planned embryo preparation at The Lister Hospital, London. Finally, each participant would meet the

transplant team (Miss Isabel Quiroga) at the Churchill Hospital in Oxford, and the obstetric team (Miss Bryony Jones) at Queen Charlotte's and Chelsea Hospital for further counselling and to conclude the selection process. Following this selection process, each recipient would be 'on call', awaiting a suitable donor to be identified. This was in progress during the writing up of this thesis but is currently on hold as a consequence to the COVID-19 pandemic.

Discussion

This study demonstrates detailed physical and psychological evaluation of potential UTx recipients. Whereas UTx recipients do not have end stage organ disease associated with multiple comorbidities, given that the procedure and process entails significant surgical and medical risk, and in the context of it being a quality of life improving procedure, it is essential it is undertaken in women who are physically suitable, to minimise cumulative risk. The investigations were undertaken to not only ensure medically fit women undergo the process of UTx, but also reduce the chances of the complications seen in cases undertaken so far. Despite the extensive assessment process demonstrated herein, only one of eleven women (9.1%) selected to proceed following the screening process in Study 1a was found to be unsuitable.

The one woman who was unable to proceed in the study was multi-factorially unsuitable but did not meet the inclusion criteria for INSITU as she was high risk HPV positive with abnormal cytology. In addition to infection, cancer is another major immunosuppression associated morbidity,[61] which is 2-4 times more common after SOT than in the general population. The associated spectrum of cancer largely resembles those associated with viral aetiology in HIV syndrome,[121] including Hodgkin's Lymphoma, Non-Hodgkin's lymphoma, ano-genital cancers and Kaposi's sarcoma. However, despite the association with HPV, cervical and vaginal cancer rates have not been shown to be elevated post transplantation,[121] which is likely be secondary to successful HPV screening programmes. Whilst so far cancer has not been a complication experienced following UTx, one patient (2.2%) developed CIN II, in the presence of HPV subtype 31, which was treated by small loop excision 2 weeks later which

revealed normal epithelium.[265] Therefore in the context of UTx, it is essential that screening +/- monitoring for carcinogenic viruses, including HPV, EBV, as well as hepatitis B and C is undertaken. Whilst it could be argued that as the follow up after UTx entails frequent visualisation of the cervix routinely, this would facilitate frequent screening in high-risk cases and prompt recognition of pathology should it arise.

The decision to undertake thrombophilia screening of the more frequent but less thrombosis provoking Factor V Leiden, and the higher risk states such as protein S and protein C deficiency, was largely prompted following the Swedish case that resulted in thrombosis who was heterozygous for Protein C deficiency.[56] Given that thrombosis has been identified to be the most common reason for graft failure identified so far following UTx,[59] and that it is intended that all women subsequently become pregnant, which is in itself a prothrombotic state, it is a reasonable precaution to take in these early cases. However, it is likely that given the cost involved, as UTx transcends into clinical practice, a focused VTE related family history may instead be undertaken, with thrombophilia testing reserved for cases with risk factors identified.

This data highlights that 50% of the potential UTx recipients of this cohort are seronegative for CMV. This is more than in other SOTs where approximately 20-30% of SOT recipients are seronegative. CMV is transmitted through contact and is passed in secretions such as saliva, urine, or through sexual intercourse. Of significance in UTx, it can be acquired through the transplant of solid organs. Moreover, it can also be transmitted via the transplacental route, and is the most common global cause of congenital infection and leading infective cause of sensorineural hearing loss.[443] Following primary infection, it establishes lifelong latency, which can subsequently reactivate in later life. CMV infection is the most common viral infection experienced in SOT recipients,[444] where it can have severe consequences including CMV syndrome and invasive tissue disease,[445] but may also indirectly promote allograft rejection,[446] and the development of diabetes mellitus.[447] Infections can be following primary infection, or following reactivation, which is more likely in immunosuppressed patients. CMV seronegative patients in receipt of a SOT from a seropositive donor have the highest risk of

acquiring a primary CMV infection post-transplantation, which may be transmitted from the donor organ.[444] High risk cases such as this require prophylactic antiviral therapy for 100-200 days to cover the period of heightened immunosuppression burden, where it has been shown to lead to a reduction in CMV disease, mortality and graft rejection.[448] Where both the donor and recipient are seronegative, no prophylaxis is generally required. In cases of intermediate risk, where both donor and recipient are seropositive or the donor is negative and the recipient is positive, then prophylaxis is generally advisable if T cell depleting immunosuppression is used. It has been suggested that 70–90% of patients undergoing SOT will be infected with CMV without the use of appropriate antiviral prophylaxis.[449] In addition to the use of anti-viral therapy, the main therapeutic challenge is to strike a balance between maintaining immunosuppression to prevent allograft rejection but to still maintain innate and adaptive immunity.

CMV deserves special attention in UTx, as in contrast to other SOTs where a minority of recipients are females of reproductive age who subsequently achieve livebirths, it is intended that 100% of UTx recipients will subsequently become pregnant. As such, consideration of the potential fetal risks is essential. Offspring delivered following pregnancies affected by a primary CMV infection have a risk of congenital infection between 30–40%, of which approximately 13% will be symptomatic at birth.[450] Conversely, secondary CMV infection due to reactivation or reinfection during pregnancy carries a lower risk of transmission of 1-2%.[451] Infection in the first trimester is associated with a greater risk of more severe harm to the fetus.[452] In offspring who are symptomatic at delivery, up to 58% subsequently develop permanent sequalae, such as sensorineural hearing loss, visual impairment and cerebral palsy.[453] Given the potential maternal and fetal implications of CMV infection following UTx, and in light of the fact that recipients are more likely to be seronegative as demonstrated herein, the use of anti-viral prophylaxis is essential. Where possible, CMV negative recipients should receive grafts from seronegative donors. Following completion of prophylaxis where necessary, embryo transfers can be undertaken. During pregnancy, close monitoring of CMV polymerase chain reaction (PCR) is warranted, as is prompt intervention following detection of viraemia or the development of symptoms suggestive of CMV infection.

Two of the women in this cohort has borderline reactive Elispot tests. Undertaking a quality of life improving procedure in an individual who has previously been exposed to TB risks reactivation of latent TB, as a consequence to the increased risk associated with taking immunosuppressive medications.[454] The presence of a borderline result necessitates the evaluation of a repeat sample. In the context of both individuals not having any risk factors, or previous history of an active TB infection, it is likely the repeat test will be negative. This is demonstrated by a previous study which identified that after a borderline result, almost half (47%) have a negative result, whereby less than a quarter (23%) progress to a positive result.[455]

From a psychological perspective, the process of UTx involves a number of known and unknown stressors. There is evidence of an interaction between ongoing stress and immunosuppressant medication non-compliance.[456] A psychosocial assessment should include an assessment of how recipients have managed stressful events in the past, their coping styles, and their overall level of resilience to stressors. A multitude of psychometric assessments were undertaken to achieve this by establishing a quantitative assessment of QoL and functional health and wellbeing, perceptions toward their relationships and social support and attitudes to medications.

Regarding QoL, the two measures used to quantify QoL in this population concur that the potential recipients all have a good quality of life. With regards to WHOQoL-BREF, when compared to the population norms for each domain of 73.5 for physical, 70.6 for psychological, 71.5 for social and 75.1 for environmental subscales,[457] all recipients scored higher than these population norms in every domain with 94.3, 84.5, 86.7 and 88.4 respectively. With regards to the FertiQoL core, the results were all very favourable compared to previous samples of women with fertility issues across all core subscales, with an overall core score of 79.4, compared to 54.6 in infertile women.[436] Regarding functional health and wellbeing, the SF36 findings presented herein suggest the cohort present well compared to similar aged females amongst the general populations of both the UK and Europe,[458,459] with higher mean scores seen in all subscales. When considering the hospital anxiety and depression (HADS) scale, it has been identified to have good psychometric properties in the screening of both

anxiety and depression.[460] Whereas all the potential recipients in this study screened negative for depression, two tested positive for anxiety; one of which was classified as mild, the other moderate. Whilst, from a transplant perspective this is less ideal, it is largely unsurprising given the wealth of evidence that has identified large proportions of women with infertility have depression or anxiety.[461] A large study in 352 women in the USA identified that more than three quarters of infertile women suffer with symptoms of anxiety.[462] Whilst this needs further assessment and possibly intervention in this cohort prior to undergoing UTx, it is reassuring to observe that both recipients scored the highest points available (100) in the SF36 assessment for social functioning and role limitation secondary to emotional factors.

One of the main preventable reasons SOTs fail is deliberate or accidental medication non-adherence.[463,464] A history of medication non-adherence has been associated with a substantial increase in post-transplant immunosuppressant non-adherence.[465,466] Other factors of relevance are self-efficacy for taking medicines,[467] and the experience of side effects.[468] Medication adherence has been identified to be associated with individuals beliefs about medicines, which formulated the basis for the development of the beliefs about medicines questionnaire (BMQ). It is well recognised that individuals with stronger beliefs about the necessity of their prescribed medications, and less concerns, are more likely to adhere to the medications prescribed.[469] Whilst interventions to improve adherence may include medication self-administration training,[470] and planning coping strategies to improve adherence,[471] it has been shown that attitudes toward medicines often remain stable over time.[472] It is therefore of importance to establish the attitudes and beliefs of potential UTx recipients. Higher scores in both the general-harm and general overuse assessments indicate a negative attitude towards medications. This cohort have favourable beliefs about medicines, with a mean general overuse score of 6.6 and general harm score of 9.7, compared to 10.5 and 10.9 respectively in normal unmedicated populations,[473] suggesting adherence issues are less likely in this population.

Availability of social support has been linked to better medication adherence,[465,471,474] better mental health during the pregnancy and early parenting phase,[475,476] and positive psychological health

overall.[477,478] Ensuring that the recipient and donor have access to emotional and tangible support is vital. This cohort appear to have good social support, as exemplified by the mean eight-item modified Medical Outcomes Study Social Support Survey (mMOS-SS) score of 94.8, compared to other European data in women without children, where the mean score was 84.9.[479]

UTx is a fertility restoring treatment and clinicians must ensure the well-being of future offspring are considered. This includes an assessment of the commitment to raise the child, the suitability of the parents, and a consideration of genetic or environmental factors that may affect the child.[387] This assessment should include exploration of the recipient and their partners perceptions towards parenthood, motivations for seeking UTx and consideration of alternative means to achieve parenthood.[480] Whereas having a partner is not a requisite to undergo UTx, assessment of relationship stability is important, particularly when considering that embryos created prior to the UTx will render them unusable in the event of relationship breakdown if one of the parties withdraws their consent for them to be used. Relationship stability has also been associated with a better relationship with the medical transplant team,[481] and typically facilitates better access to social support.[466] Moreover, there are additional emotional benefits to being in a relationship. For example, following stem cell transplantation, recipients with partners have been shown to successfully buffer the impact of stressful life events on psychological distress, whereas unpartnered recipients did not.[158] In this cohort, all the potential recipients were in relationships or were married, with a mean relationship duration of 7.5 years. The marital taxon measure has been identified to be a good screening tool for marital distress, with a score of ≥4 suggesting further assessment and potentially intervention is warranted. As no potential recipients in this cohort scored a score over 2, with a mean of 0.4, it is suggestive of good relationship stability. Moreover, it is reaffirmed by the dyadic adjustment score (DAS) findings, where higher scores suggest better satisfaction and lower scores are indicative of conflict. The mean total score in this cohort was 138.1, which is much higher than previously identified cut offs between 92 and 107 which suggest marital distress.[482]

Conclusion

To summarise, following comprehensive evaluation of the recipients, the majority of participants were suitable to proceed with UTx following the pre-screening process described in study 1a. The extensive physical assessment including numerous haematological, biochemical, microbiological and radiological investigations identified one participant who was unsuitable to proceed. With the remaining women, it has provided valuable insight to help match donors appropriately once they become available. Whilst the extensive psychological assessment did not exclude any women, it has highlighted those who may need additional support throughout the process and provided a baseline to facilitate monitoring as the study proceeds, to ensure appropriate management and support is available should it be needed.

Study 2

Perceptions, acceptability and suitability of non-directed living uterine transplantation donors; a cross sectional study

Introduction

In 2014, almost 50 years after the initial concept was first considered, the first livebirth following UTx was reported.[55] More than 70 cases have now been performed worldwide, and outcomes have been recently reported from 45 cases, which have so far yielded nine livebirths.[59] This demonstrates that UTx is a feasible fertility restoring intervention for women with AUFI which allows them to conceive themselves and experience gestation. UTx is a non-life saving, quality of life improving solid organ transplant. Unlike other quality of life improving transplants, such as face, upper limb, abdominal wall and penis, the non-essential nature of a uterus after the completion of one's family allows the potential to use both living donors (LD) and DBD. Each donor type presents ethical, logistical, physiological, immunological and anatomical advantages and disadvantages that continue to stimulate debate.[205,208] LD can either be directed or non-directed in nature. Directed organ donations encompass the donation of an organ that is intended for a specific individual, most commonly a close family member or friend. Conversely, non-directed donations are when the recipient is unknown and not specified by the donor.

Compared to DBD, the use of LD has logistical advantages, including abundant time to investigate and prepare the recipient and donor preoperatively, and electively plan and coordinate operations in adjoining theatres. The close proximity this affords, along with the ability to optimally time retrieval and implantation operations, has been shown in cases so far to reduce cold ischaemic time when compared to DBD,[59] thereby reducing the potential for ischemia-reperfusion injury. Moreover, other solid organ transplant outcomes have been demonstrated to be superior in LD compared to DBD,[218] even when controlling for ischaemia times.[219] Contrarily, DBD has other advantages over LD. The mean age of deceased donors in cases performed to date is seven years younger than LD cases. Increased donor age is associated with major vessel calcification and greater arterial stiffness,[162-163] and has been demonstrated to be associated with worse outcomes in the transplantation of other solid organs.[159-161] Moreover, pregnancy outcomes have also been shown to be inferior in women >45 years, compared to those <45 years, when controlling for oocyte quality by using donor oocytes from

women of the same age, with significantly reduced implantation and pregnancy rates, along with higher miscarriage rates.[165] Given that the average age of donors in LD UTx cases so far has been 45 years, whilst those in DBD is 38 years,[59] the use of older donors may not only impact surgical success rates, but also subsequent pregnancy outcomes. The overwhelming advantage of using DBD however, is the complete removal of physical and psychological risk to the donor. In LD UTx cases so far, more than one in ten donors have suffered a complication necessitating further surgical intervention,[59] including ureteric injuries,[52,166,56] faecal impaction requiring digital evacuation under anaesthesia,[216] and vaginal cuff dehiscence.[80] Around a quarter of donors have suffered less serious complications including wound infection,[56] bladder hypotonia,[166] urinary tract infection,[59] leg/buttock pain, depression,[80] intubation related respiratory failure and anaemia.[59] However, recent evolution of the surgical technique has reduced potential donor risk. Modification of the vascular dissection, prioritising ovarian venous drainage where possible,[80,134,166,185] as opposed to the more complex and time consuming uterine venous complex,[56] has reduced surgical risk and decreased operative times significantly,[59] which should reduce potentially life or graft threatening venous thromboembolic risk. Moreover, the implementation of minimally invasive surgical techniques in recent cases, offers further risk reduction and an enhanced recovery process.[184,185,221]

Ethically, concern has been expressed regarding acceptable levels of donor risk,[483,484] ensuring valid consent, and issues surrounding the potential for donor regret.[63,485,486] The ethical arguments surrounding the use of LD hold substantial weight, and it has been proposed that DBD should be preferred provided there is sufficient availability of donor uteri, demonstrated similarity in success, and no negative impact during retrieval of lifesaving organs.[206,323,420] Where these conditions are not met, as is the case in other solid organ transplants such as kidney, LD can be justified provided that the donor provides informed and voluntary consent, the risks are proportionate to potential benefits which fall below an acceptable absolute threshold, and the utmost effort is made to minimise donor risk.[323] Given the improvement in donor risk profile, and anticipated shortage of suitable deceased donors,[487] it is anticipated a combination of LD and DBD will be needed to maintain future sustainability in UTx.[488] However, with regards to LD, reliance solely on directed donations may

exclude perfectly suitable women with AUFI, purely because they do not have someone willing to donate their uterus. As such, as has been the case in other solid organ transplants, the use of non-directed altruistic donations may facilitate future sustainability as UTx transcends from research concept to clinical procedure.

The aim of this study was to investigate the suitability of potential altruistic living donors and identify their motivations and perceptions towards undergoing uterine donation. Secondarily, determination of acceptability and willingness to proceed was evaluated, after potential donors were made aware of the risks, expected recovery and potential impact upon their lives.

Methodology

An electronic questionnaire was sent to all the women who contacted Womb Transplant UK (Registered Charity no.1138559) to offer their uterus for donation between July 2012 and 1st November 2019. An email containing the link to the questionnaire, as well as two information leaflets, was sent to potential participants. One information leaflet detailed the conduct of the study whilst the other explained the proposed process of LD UTx (**Appendix 2.1**). The questionnaire contained an initial 4-question consent process, which required agreement prior to proceeding to the 58-question questionnaire (**Appendix 2.2**). The online platform SurveyMonkey was used to distribute the questionnaire, over a six-month period between 1st June 2019 and 31st November 2019. Initial questions elicited demographic information before determining background medical, surgical, psychiatric, obstetric, gynaecological and social history. Further questions ascertained perceptions to adoption and surrogacy and knowledge and opinions of UTx. Subsequent questions specifically assessed acceptability of the necessary pre-operative investigations, the potential need for hormone supplementation in those who were post-menopausal, and after considering the risks and likely expected recovery, their keenness to still donate their womb. The final questions determined knowledge and perceptions of potential recipient risk, opinions regarding being informed of recipient progress after donation, and financial considerations. Most questions were closed, utilising tick boxes,

with the option to include further comment in cases where further description was warranted. Likert scales were used in the questions related to perceptions.

Statistics

SPSS version 24 software (SPSS, Chicago, Illinois, USA) was used for analysis. Descriptive statistical analysis was described as mean +/- SD or median +/- range. The Likert scale responses were quantified using a weighted ranking system to ascertain the most influential perceived factor (0 = not at all; 4 = definitely).

Ethics approval

Ethics approval was received from Imperial College REC on 30th May 2019 (ICREC reference: 19IC5202)

Results

385 women contacted the charity to request further information about altruistically donating their uterus. 152 women subsequently completed the questionnaire, resulting in a response rate of 39.5%. The cohort comprised of 151 women and one female to male transgender man. The demographics of the respondents are summarised in **Table 2.1**. The most prevalent age group was 30-39 years old (33%; n=50). The majority of respondents were Caucasian (94%; n=143), fluent in English (99%; n=151), married (50%; n=76) and in either full/part time employment or self-employed (68%; n=103). A minority (16%; n=24) were current smokers, 30% were ex-smokers (n=45) and the remainder had never smoked (55%; n=83).

			Number (n)	%
Age (years)	16-19		2	1
	20-29		36	24
	30-39		50	33
	40-49		43	28
	50-59		14	9
	60+		7	5
Body Mass Index Kg/m2	<18.4	Underweight	5	3
	18.5-24.4	Normal	54	36
	25-29.9	Overweight	45	30
	30-34.9	Obesity I	27	18
	35-39.9	Obesity II	11	7
	>40	Obesity III	10	7
Ethnicity	White		143	94
	Asian		4	3
	Black		2	1
	Mixed		2	1
	Other		1	1
Language	English		143	94
	Non-English but fluent in English		8	5
	Non-English but not fluent in English		1	1
Religion	Christianity		43	28
	Hinduism		2	1
	Islam		2	1
	Atheism		61	40
	Other		31	20
	Would rather not say		13	9
Employment Status	Employed Full Time		57	38
	Employed Part Time		26	17
	Self Employed		20	13
	Housewife		26	17
	Unemployed		12	8
	Student		5	3
	Would rather not say		6	4
Relationship Status	Single		43	28
	Living with partner		19	13
	Married		76	50
	Divorced		9	6
	Separated		2	1
	Would rather not say		3	2
Smoking Status	Never smoked		83	55
	Ex-Smoker		45	30
	Current Smoker		24	16
Alcohol intake (units per week)	0		69	45
	≤14		79	52
	>14		4	3

Table 2.1: *Basic demographic information of uterine donor cohort*

Medical history

Table 2.2 summarises the obstetric background of this cohort. Over a third were nulliparous (37% n=56). Of those who had children previously (n=96), around three quarters (73%; n=70) delivered their children vaginally, whilst the remainder had at least one child born by Caesarean section (27%; n=26). The majority of those who had previous Caesarean sections had one (13%; n=12); or two (11%; n=11); whereas three (3%) had ≥ 3. More than three quarters of those with previous pregnancies did not suffer significant antenatal or postnatal complication (n=74; 77%). The most frequently encountered obstetric complication was pre-term delivery, which impacted 11% (n=11).

		Number (n)	%
Number of Miscarriages (n=152)	0 1 2 3+	115 19 10 8	76 13 7 5
Number of Children (n=152)	0 1 2 3 4+	56 11 36 30 19	37 7 24 20 13
Number of Caesarean Section (n=96)	0 1-2 >2	70 23 3	73 24 3
Problems encountered in pregnancy (n=96)	No problems Pre-term delivery <37 weeks Pre-eclampsia Gestational diabetes Heavy bleeding after delivery Obstetric Cholestasis	74 11 3 4 2 2	77 11 3 4 2 2

Table 2.2: *Obstetric history of study cohort*

Gynaecologically, around a quarter of our cohort had previously experienced miscarriages (24%; n=37), eight of whom (5%) had ≥3. 17% (n=26) of individuals were post-menopausal, including one with POI. The majority of those still menstruating reported regular cycles (n=106; 84%) whereas 16% (n=20) had irregular cycles. Nearly a quarter of participants had previous abnormal cervical cytology (22% n=34), half of whom subsequently underwent surgical treatment (n=17; 50%), whilst half were monitored conservatively (n=17 = 50%). The vast majority had normal cytology since (97% n=33).

		Number (n)	%
Reported medical disorders	Yes	59	39
	No	93	61
Medical disorders (n=59)	Asthma	12	20
	Hypertension	4	7
	Diabetes	4	7
	Thyroid disorders	11	19
	Clotting disorder	1	2
	Neurological disorders	3	5
	Rheumatology disorders	10	17
	Inflammatory bowel disease	1	2
	Hepatobiliary disorders (minor)	2	3
	Other minor medical disorders	29	49

Table 2.3: *Medical history of study cohort*

Table 2.3 summarises the medical background of this cohort. The mean BMI of participants was 28.4kg/m^2 +/- 8.56. Nearly two thirds of participants did not report any significant medical history (61% n=93). Of those who reported medical comorbidities (39%; n=61), asthma was the most prevalent specified illness (20% n=12), followed by thyroid disorders (19% n=11) and rheumatological disorders (17% n=10). Four women reported a history of type II diabetes (7%), and the same proportion had hypertension (n=4; 7%). Three women reported neurological disorders including epilepsy, a surgically operated brain aneurysm and a functional neurological disorder. One individual reported having Factor V Leiden thrombophilia, whilst another had ulcerative colitis. Only one individual reported a previous history of malignancy, which affected her breast. None of the women in this cohort were known to be HIV positive, nor did any have syphilis or hepatitis. Around half reported other minor medical conditions, which would not impact suitability to donate their uterus (49% n=29). Over three quarters of participants did not have any previous psychiatric history (76% n=116). Of those 36 participants (24%) who did, the most prevalent were depression (n=26; 17%) and anxiety (n=18; 12%), with bipolar disorder (n=2; 1%), post-traumatic stress disorder (n=5; 3%), obsessive-compulsive disorder (n=1;1%), borderline personality disorder (n=5; 3%) and autism (n=2; 1%) being less prevalent.

The majority of the cohort had not previously undergone abdomino-pelvic surgery (59% n=90). 11 women (7%) previously had a single laparotomy, 10 (7%) of which were Caesarean sections, whilst

another had an explorative laparotomy. 11 women (7%) had previously undergone two open surgeries for Caesarean sections and three women (2%) had three previous laparotomies for due to previous Caesarean sections. A larger proportion of women had undergone a single previous laparoscopy (n=28; 18%), a quarter of which were for sterilisation (n=7). Two women had undergone two previous laparoscopies and four participants underwent a combination of laparotomy and laparoscopies.

Understanding and perceptions to uterine transplantation

Although 79% (n=120) of individuals 'strongly agree' or 'agree' that adoption and surrogacy are suitable methods to have children, all participants (n=152; 100%) felt that women should be freely able to voluntarily donate their uterus for UTx if fully informed of the process. The majority of respondents reported knowing 'a fair amount' or 'a lot' about UTx (58% n=88), around a third had 'heard it discussed only a few times' (n=52; 34%) and a minority admitted 'knowing nothing' (n=12; 8%). The majority of individuals 'strongly agreed' or 'agreed' that they understood the potential benefits (94% n=143) and risks (86% n=131) of donating their uterus.

Nearly all women in this cohort 'strongly agreed' or 'agreed' to consent to undergoing the necessary pre-operative investigations (98% n=150) and were similarly agreeable towards the expected recovery process including a four-day inpatient admission and an 8-week recovery period (97% n=148). All but one of the participants understood that future pregnancies in the recipient would not be genetically related to the donor (99% n=151). All participants reported completion of their family (n=152), with over a third of these being nulliparous (36% n=55). All those who were premenopausal understood that this operation would cease their ability to have children in the future (n=126). The majority of these understood that the intended operation involved ovarian conservation, which would result in normal hormone levels post-operatively (96% n=121). Moreover, 96% (n=122) 'strongly agreed' or 'agreed' that donation of their uterus would cease future menstruation.

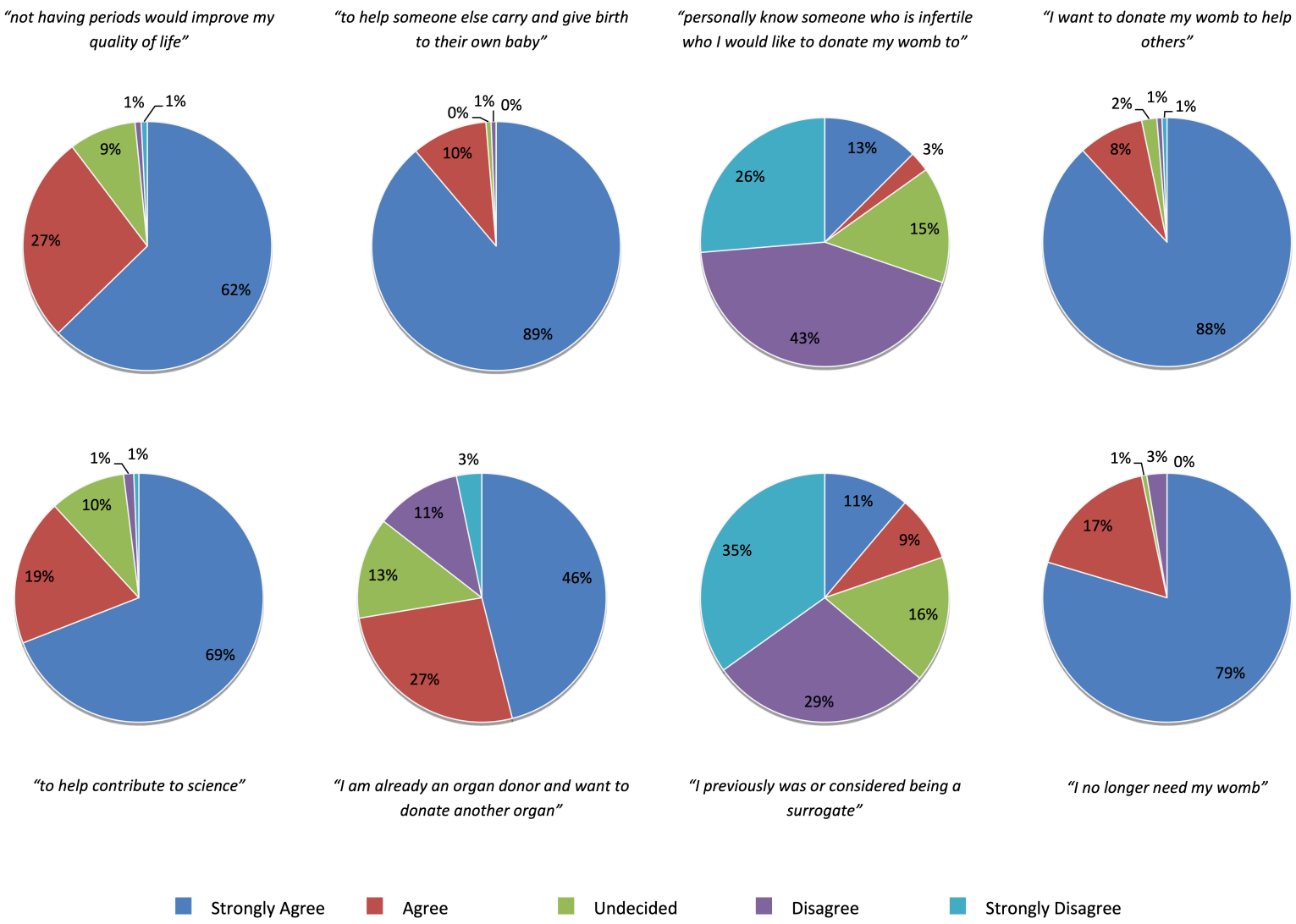


Figure 2.1: *Motivating factors for consideration of uterine donation*

The majority of these 'strongly agreed' or 'agreed' that cessation of menstruation would improve their quality of life (89% n=113). Of those participants who were postmenopausal (n=26), the majority 'strongly agreed' or 'agreed' to accept 3-6 months of hormone replacement therapy to demonstrate endometrial function by inducing withdrawal bleeds pre-operatively (92% n=24).

Motivations for uterine donation

Influencing factors for considering becoming a UTx donor are depicted in **Figure 2.1**. The overwhelming majority of respondents 'strongly agreed' or 'agreed' in wanting to donate to help someone else carry and give birth to their own baby (n=150; 99%), to help others (n=147 ;97%), and because they no longer needed their womb (n=147; 97%). Nearly three quarters of the cohort 'strongly agreed' or 'agreed' that they were already an organ donor and wanted to donate another organ (72% n=110). Contrastingly, only 20% (n=30) agreed to the same extent in wanting to donate due to previously considering or being a surrogate. Only 15% (n=23) of the cohort 'strongly agreed' or 'agreed' to personally knowing someone infertile who they wanted to donate their uterus to. Using a weighted scoring system, **Figure 2.2** represents the importance placed on each influencing factor. The two most influential factors were desire to help someone else carry and give birth to a child and wanting to help others. The least influential factors were personally knowing someone who was infertile that they wanted to donate to and wanting to donate due to considering or being a surrogate previously.

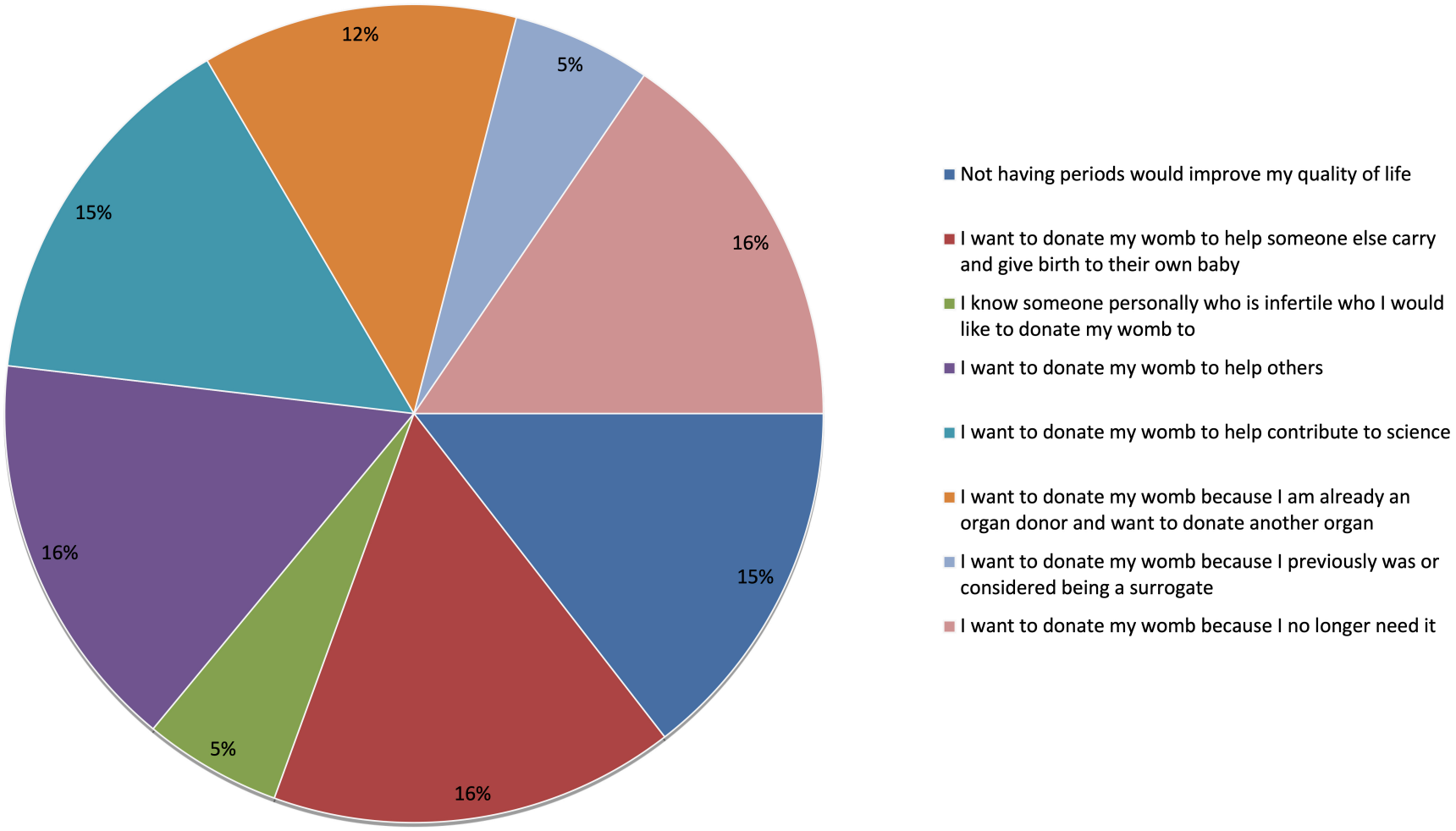


Figure 2.2: *Perceived importance of each factor influencing motivation to donate*

More than three quarters (n=117; 77%) of respondents were aware that the UTx recipient would be at greater risk of malignancy and infection due to the immunosuppressive medications required to be taken post-operatively. However, just 9% (n=14) agreed or strongly agreed that this knowledge would impact their decision to donate. More than two thirds of the cohort wanted to be kept informed of future progress of their uterine recipient (69% n=105). However, around a quarter (n=40; 27%) were undecided and 3% (n=5) would not want to know. Most respondents 'strongly agreed' or 'agreed' to understanding they would not receive any payment for donating their uterus (95% n=145), however four (2%) individuals 'strongly disagreed' or 'disagreed'. Given the likely media interest in the process due to its novelty, the majority (85%; n=129) 'strongly agreed' or 'agreed' in acknowledging the risk of unwanted media attention.

After reading the information and considering the risks, nearly all participants were still keen to donate their uterus (95% n=144). Eight (4%) remained undecided, with the majority further elaborating a desire to know further information, whilst one participant (1%) disagreed, citing the desire for further counselling to help not develop an attachment to the recipient's family.

Suitability

Figure 2.3 summarises a systematic process of exclusion, utilising the screening donor selection criteria we have implemented for the UK UTx LD programme (**Table 2.3**). 25 (17%) women were excluded due to age ≥60 or BMI>35kg/m^2. 17 (12%) more women would be excluded for being current smokers. A further 11 (8%) would be excluded due to significant pre-existing medical conditions and four (3%) more would be excluded due to multiple or significant previous surgical history. More than a quarter (n=38; 26%) would not be eligible due to nulliparity, or previous preeclampsia or preterm delivery. Three (2%) potential donors would not be suitable due to recurrent miscarriage, and four (3%) would not proceed due to previous cervical surgery for abnormal cells, or a recent abnormal cytology result. Following implementation of the selection criteria, 42 women (29%) would be suitable to proceed with further clinical evaluation, counselling and further investigation to determine suitability to donate.

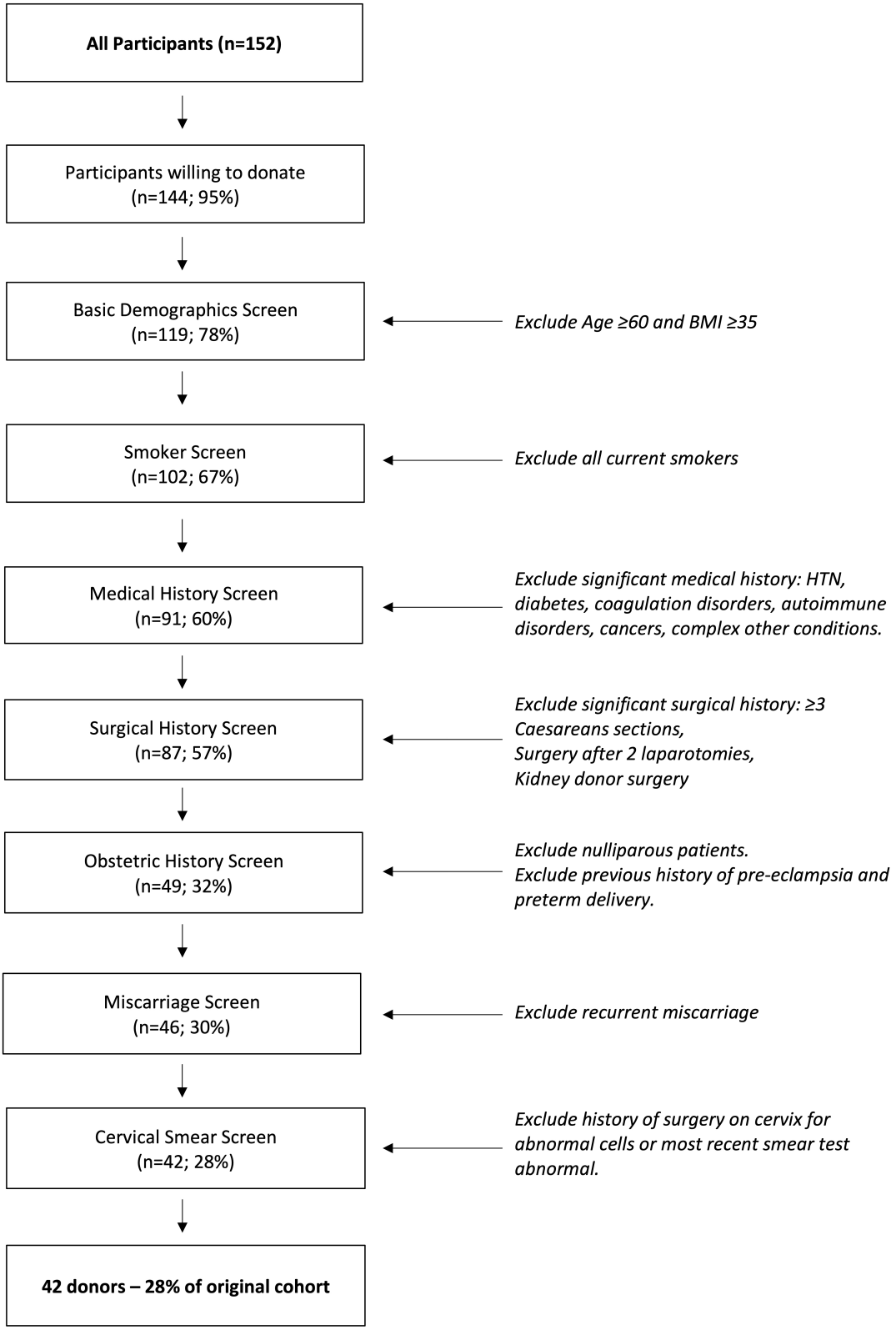


Figure 2.3: *Systematic process of exclusion utilising the selection criteria for the UK living donor uterine transplantation programme*

Inclusion	Aged 18-60 Multiparous BMI <35kg/m^2
Exclusion	History of cancer Previous multiple / significant uterine surgery 2 X Caesarean section acceptable No previous myomectomies Previous significant cervical surgery (Cone biopsy or Large loop excision of the transformation zone) HPV positive or abnormal cervical cytology Significant systemic disease (Diabetes, hypertension, autoimmune conditions etc.) Previous obstetric problems including pre-eclampsia and delivery <37/40 Previous recurrent miscarriages (3 or more) Current IV drug abuse Active bacteremia / fungaemia Active smoker

Table 2.4: *Donor screening criteria for UK living donor uterine transplantation programme*

Discussion

The data presented herein demonstrates novel insight into the perceptions of potential UTx living donors and portrays robust, multifactorial motivation and desire to proceed with donation following understanding of the risks involved and a realistic awareness of the expected process and recovery. This reinforces the generally supportive previously reported perceptions towards UTx amongst the public and in potential donors. When considering the public, a web-based questionnaire completed by 1247 US residents identified that more than three quarters (78%) supported UTx.[489] Similar levels of support was seen in a European study including 2000 Swedish women, where 80% were in favour of UTx and found it more acceptable than surrogacy.[490] However, a study in 3098 women of reproductive age in Japan demonstrated less favourable support, with just 44.2% being in favour of UTx. However, 47.5% had no opinion, which highlights a potential lack of awareness of UTx in those surveyed, particularly when the vast majority (93.3%) felt that UTx should be permitted either with, or without further discussion.[491] In the context of DD, more than a decade ago, before the first livebirth following UTx was reported,[55] just 6% of donor families agreed to uterine procurement as

part of deceased donation.[492] However, 10 years later, attitudes to uterine donation appear to have improved, with three quarters of women agreeing to donation of their uterus at the time of their death, which increased to 87% after becoming informed about the purpose of the transplant [493] In addition, a recent European study demonstrated that donation of the uterus was readily accepted, with no refusals observed in seven cases.[213]

In the context of LD, it is essential to assess motivations for donating, including ensuring that no financial incentives are involved. If of childbearing age, future reproductive plans need to be explored, particularly if their situation changed and they subsequently met a new partner who did not have children in the future. We demonstrate that the most common motivations for donation is to help someone else carry and give birth to a child and wanting to help others, which is consistent with the primary reasons to donate in the first six non-directed UTx donors in the DUETS study.[494] Interestingly, 10 of the first 13 living donor transplants from DUETS used donors who worked in the healthcare profession, the majority of whom were nurses. Altruistic non-directed organ donations from healthcare professionals have been reported previously. In 1996, a Professor in Germany donated a kidney to a patient unknown personally to him, while waiting for a cadaveric donor.[495] Three years later in the United States, a nurse similarly non-directly donated a kidney to a 13 year old child.[495]

In the context of UTx, psychological outcomes in donors have been reported following the Swedish series and DUETS.[132,216] In the Swedish study of nine transplants, two donors experienced a deviation from their baseline quality of life scores (SF-36), one of which coincided chronologically with both herself suffering complication with a ureterovaginal fistula in addition to the graft loss in the recipient, her daughter. However, there was no significant change in quality of life between baseline and one-year post-procedure, and no reported regret, even in those whose recipient subsequently underwent emergency hysterectomy, or suffered complications themselves.[132] Following UTx donation, all women eventually returned to their pre-operative social and physical status.[132] In DUETS, out of 13 cases, one donor experienced two episodes of depression in the first 12 months

post-operatively.[216] However, this donor had a pre-donation history of depression, and did not attribute the post-operative episodes to be related to the donation process, still referring to the donor experience as being 'amazing'.[216] These findings resonate with the positive psychosocial outcomes demonstrated following lifesaving solid organ donation.[496,497]

We also clearly demonstrate the anticipated decline in numbers of potential donors in the context of using proposed standard donor criteria. These figures can be used to estimate eventual donor availability and also manage expectations in potential UTx donors. In DUETS, 79 women initially expressed interest in donating their womb and 62 were subsequently screened by telephone.[152] Following pre-screen, 30 donors completed a detailed health history questionnaire, in a similar fashion to our study, where 12 women eventually proceeded to be clinically evaluated.[152] This presents an overall conversion from screening to being clinically evaluated of 19%, which compares to 28% in our cohort.

In DUETS, following clinical evaluation, 50% of potential donors eventually proceeded with donation (n=6), due to a combination of unsuitability or self-withdrawal. Thereby, within our cohort, utilising a 50% dropout rate, it would be anticipated that approximately 21 women may eventually be suitable to proceed with donation from the initial 152 women.

Whilst the use of marginal organs is acceptable in life saving organ transplants, owing to the significant risk of death whilst on a waiting list for an organ,[164] it is difficult to justify the use of suboptimal grafts in quality of life improving transplants such as UTx. However, defining which donor selection criteria result in a suboptimal graft is currently difficult to determine. Whilst various selection criteria have been utilised in trials to date, ongoing discussion and debate in the context of outcomes following UTx is required to determine which criteria optimise graft quality, and which criteria are of negligible benefit and therefore unnecessarily limit donor availability. Recent donor criteria have been suggested for implementation in the context of DD.[487] If extrapolated into LD, their suggested standard donor criteria, which excludes women with miscarriage or Caesarean section,

would reduce the number of suitable donors to just 25, before comprehensive clinical evaluation and investigation, representing just 16% of the initial cohort. Given that the majority of miscarriages are sporadic and due to problems completely unrelated to the uterus, such as embryonic aneuploidy,[498] and that 15-20% of women miscarry,[499] accepting donors with previous sporadic miscarriages may increase donor availability without detracting from the quality of the graft. Moreover, given that the mode of delivery in around a quarter of deliveries in the UK are by Caesarean section, alleviating this as an exclusion would improve donor availability as demonstrated herein.

This is the largest study of its kind to determine the background, perceptions and motivations of potential UTx living donors. The findings of this study demonstrate altruistic donation using non-directed donors could be a sustainable solution to the anticipated shortage of deceased donors, and lack of availability of directed donors for women with AUFI. A weakness of this study includes the fact participants were self-selected, all of whom directly contacted Womb Transplant UK to enquire about uterine donation. As such, the results presented herein are not directly extrapolatable to all women. Additionally, the exclusive use of self-reported data introduces the potential for bias.

Conclusion

Whilst the use of DD negates potential donor risk, the associated logistical difficulties are so complex they can compromise the viability of UTx programmes.[208] Moreover, even in healthcare systems with well-established DD programmes, concerns have been expressed regarding the potential availability of donors to meet future demand.[487,488] Whilst increasing donation after brain death conversion rates is essential, increasing potential DD supply may also be possible by considering uterine donation after cardiac death (DCD). However, this is a prospect that requires further research, to determine if the prolonged warm ischaemic time in such cases is detrimental to UTx outcomes. Given the willingness of women to altruistically donate their uteri demonstrated herein, the use of non-directed UTx donors is a readily available donor poor that should be explored further. It is important that potential donors' motivations for donating are established to ensure no financial incentives are involved. If of

childbearing age, the donor's future reproductive plans need to be explored, especially if their situation changed and they met a new partner without children in the future.

Study 3a

Uterine transplantation in transgender women; A cross-sectional survey assessing perceptions, acceptability and demand

Introduction

Gender dysphoria is defined as a persistent discomfort with one's gender identity or biological sex. Despite an absence of formal epidemiological evidence, it is estimated to affect up to 1.4% of adult males.[500] Management of gender dysphoria in M2F transgender women is complex and requires individualised multi-disciplinary care between various medical, psychological, and surgical specialists. Treatment typically includes one or a combination of psychological input, hormonal therapy or GRS.

Reproductive planning is a dynamic construct and individual reproductive aspirations are greatly influenced by the context in which they arise with anatomical, physiological, social, cultural, legal and economic factors all playing a central role in their development. For M2F transgender women, infertility is an unfortunate but unavoidable consequence of undergoing GRS. Supplemental estrogen has been shown to reduce sperm counts and motility,[501] highlighting the adverse reproductive impact associated with physical realignment with gender identity. Fertility preservation, by cryopreserving sperm, should be offered in those with reproductive aspirations of biologically related offspring after transition,[502] with subsequent use with donor oocytes in a surrogate or with a future female partner. However, while approximately half of transgender women desire biologically related children in the future,[317] fertility preservation rates remain low, likely owing to financial barriers to access and unwillingness to delay transition.[348,503] While the ability to gestate is not necessarily essential for a good quality of life, many women do experience significant psychological and social harms as a consequence of an inability to fulfil their own reproductive aspirations. These harms are exacerbated by social and cultural attitudes, norms and expectations which valorise reproduction, genetic relatedness, and parenthood.[315,319] Moreover, many women report distress, depression, loss of gender identity, and an enduring sense of incompleteness and grief as a consequence of being infertile.[25]

UTx has been demonstrated to be a viable fertility restoring treatment for women assigned female at birth with absolute uterine factor infertility (AUFI). Its development was primarily motivated by the potential to ameliorate suffering caused by a discrepancy between procreative ability and reproductive

aspirations, thereby providing women with AUFI the opportunity to conceive, gestate and give birth to genetically related offspring themselves. While more than 70 cases have now been undertaken worldwide, detailed outcomes have now been reported from 45 cases, including nine livebirths.[59] Rapid advancements in UTx research, as well as considerations of fairness and equality in reproductive care, have now led to discussions of the possibility of UTx in M2F transgender women. UTx in transgender women seeks to align reproductive capacity with aspiration, and alleviate the dysphoria that may arise as a result of being unable to attain parenthood.[310,312] Significant additional psychological and social benefits following UTx could also be received, particularly when considering that parenting has been identified as a protective factor in suicide risk for transgender women.[316]

Moreover, reproductive rights are recognised as human rights,[504] and legislation in the UK suggests it would be illegal to refuse to perform UTx in transgender women on the basis of their gender identity.[68,321,331] The UK's Gender Recognition Act (2004), for example, states that an individual's gender becomes, for *all* purposes, the acquired gender, once a gender recognition certificate has been authorised. Moreover, the Equality Act (2010) affords transgender people explicit protection from both direct and indirect discrimination related to their gender reassignment regardless of whether they have applied for/been granted a gender recognition certificate.[68,321] As such, unless there is compelling justification, M2F transgender women are entitled to the same treatment as women assigned female at birth.

UTx for M2F transgender women is undoubtedly more complex than for women assigned female at birth and is currently a prospect with unproven feasibility requiring significant further research. A number of anatomical, hormonal, fertility, and obstetric issues contribute to the associated increased complexity, but there is no overwhelming clinical argument contradicting the feasibility of UTx in this model.[331] However, despite the notion of performing UTx because of considerations of equality or justice, or to alleviate the suffering caused from the mismatch between reproductive aspirations and physiological capacity, there is limited data on perceptions of and potential demand for UTx in transgender women. The objective of this study is to investigate the reproductive aspirations of M2F

transgender women and identify their perceptions towards UTx. This is essential to establish desire and demand for UTx in this population, before the necessary animal and cadaveric studies are undertaken to establish the feasibility of the procedure in this model.

Methods

Study Design

The study was initially advertised throughout transgender support groups in the UK, including those on social media. The only inclusion criteria were being a M2F transgender woman and being over the age of 16 years. A consent form and an information leaflet, detailing the study and the proposed process of UTx in the context of transgender women (**Appendix 3a.1**), were sent to willing participants. Following receipt of consent, an electronic questionnaire was sent to all participants. It consisted of 27-questions (**Appendix 3a.2**) and was distributed via e-mail through SurveyMonkey over a six-month period between 1st May 2019 and 1st November 2019. The questions recorded demographic information, background to their dysphoric symptoms and gender reassignment, as well as any previous attempts at fertility preservation. Further questions ascertained reproductive aspirations, perceptions to adoption and surrogacy and opinions towards UTx. All the questions were closed, utilising tick boxes, with the option to include further comments where further description was warranted. Likert scales were used in questions related to perceptions.

Data analysis

SPSS version 24 software (SPSS, Chicago, Illinois, USA) was used for analysis. Descriptive statistical analysis was described as mean +/- SD or median +/- range. To quantify the Likert scale responses to ascertain the most influential perceived factor, a weighted ranking system was utilised, assigning a score of 0 to 4 for each answer to the various influencing factors (0 = not at all; 4 = definitely).

Results

186 M2F transgender women consented to partake in the study. 182 subsequently completed the questionnaire in its entirety, resulting in a response rate of 97.8%. The demographics of the cohort are summarised in **Table 3a.1**. The majority (n=109; 59.9%) were between the ages of 20-29 years, single (59% n=108) and defined themselves as atheist (56% n=102). More than half of respondents were attracted to both men and women (n=104; 57%), whereas lesser proportions were attracted exclusively to women (n=42; 23%), and men (n=26; 14%). Around a third (35% n=65) reported discomfort with their assigned gender at birth for <10 years, with similar proportions disclosing dysphoric symptoms for 11-20 years (35% n=63) and > 20 years (30% n=54). Regarding hormone therapy, 56 (31%) women had taken it for <1 year, and 79 (43%) participants had been using it for 1-5 years. 15 (8%) had taken it for 6-10 years and 5 (3%) for >10 years. 26 (14%) had not previously used hormone therapy. 11% (n=20) of participants had undergone GRS, with three quarters of whom (n=15; 75%) doing so in the last five years.

Reproduction aspirations

The vast majority of participants did not have children prior to transitioning (92% n=167) and only one woman reported having a child since. However, 95% (171) expressed a desire to have children in the future. Of the 15 (8%) participants who had children before transitioning, almost all (n=14; 93.3%) wanted further children in the future. Half of the cohort (50% n=90) 'strongly agreed' or 'agreed' that the current options of adoption or surrogacy are suitable methods for M2F transgender women to have children. Of the 55 (30%) individuals who 'disagreed' or 'strongly disagreed', almost half (n=24; 43.6%) specifically expressed that these options did not enable them to gestate. Around three quarters of the cohort felt that M2F transgender women were discriminated against compared to other women in the process of adoption (n=144; 79%) and surrogacy (n=128; 70%).

		Number (n)	%
Age (years)	16-19	24	13
	20-29	109	60
	30-39	41	23
	40-49	5	3
	50+	3	2
Religion	Christianity	20	11
	Hinduism	1	1
	Atheist	102	56
	Other	45	25
	Would rather not say	14	8
Sexual Orientation	Attracted to women	42	23
	Attracted to men	25	14
	Attracted to both women and men	105	58
	Would rather not say	10	5
Relationship Status	Single	108	59
	Living with partner	35	19
	Married	21	12
	Divorced	5	3
	Separated	1	1
	Widowed	1	1
	Would rather not say	11	6
Number of children	Before Transitioning		
	0	167	92
	1	9	4
	2	5	3
	3	0	0
	4+	1	1
	After transitioning		
	0	181	99
	1	1	1

Table 3a.1: *Basic demographic information of uterine donor cohort*

Perceptions of uterine transplantation

59% (n=105) of respondents felt they knew 'a lot' or 'a fair amount' about UTx, while 41% had 'heard it discussed' before. Most respondents 'strongly agreed' or 'agreed' that they understood the benefits (97%; n=177) and risks (89% n=160) respectively and the vast majority (n=173; 95%) believed the associated risks of UTx were outweighed by its potential benefits. The factors perceived to influence desire for UTx are demonstrated in **Figure 3a.1**.

The vast majority (n=171; 94%) 'agreed' or 'strongly agreed' that the ability to become pregnant and give birth to their own children would make them feel like 'more of a woman'. A similar proportion (n=161; 90%) expressed agreement/strong agreement regarding the potential impact of having the

ability to menstruate. Additionally, 88% (n=160) expected to feel greater satisfaction with their desired gender after UTx. Similarly high proportions of participants 'strongly agreed' or 'agreed' that having a transplanted, functioning vagina as part of UTx, would improve their sex life (90% n=163) and quality of life (90% n=163) while also enabling them to feel 'more of a woman' (93% n=168). Using the weighted scoring system, **Figure 3a.2** represents the relative proportion of each influencing factor, with all representing similar weightings of 14-15% of each.

Nearly all respondents (99% n=180) believed UTx would lead to greater happiness in M2F transgender women. The overwhelming majority (n=181; 99%) felt that if UTx was feasible for women assigned female at birth who do not have a uterus, then it should be offered to M2F transgender women. Of those who are sexually attracted to women, 79.5% (n=124) 'strongly agreed' or 'agreed' that despite having a partner with a functioning uterus, they would still want to undergo the process of UTx themselves. 27 (17.3%) women were undecided, while a small minority (n=5; 3%) disagreed or strongly disagreed. When considering the long-term implications of UTx, 61% (n=112) of participants 'strongly agreed' or 'agreed' that they would undergo hysterectomy after completing their family, to avoid the long-term

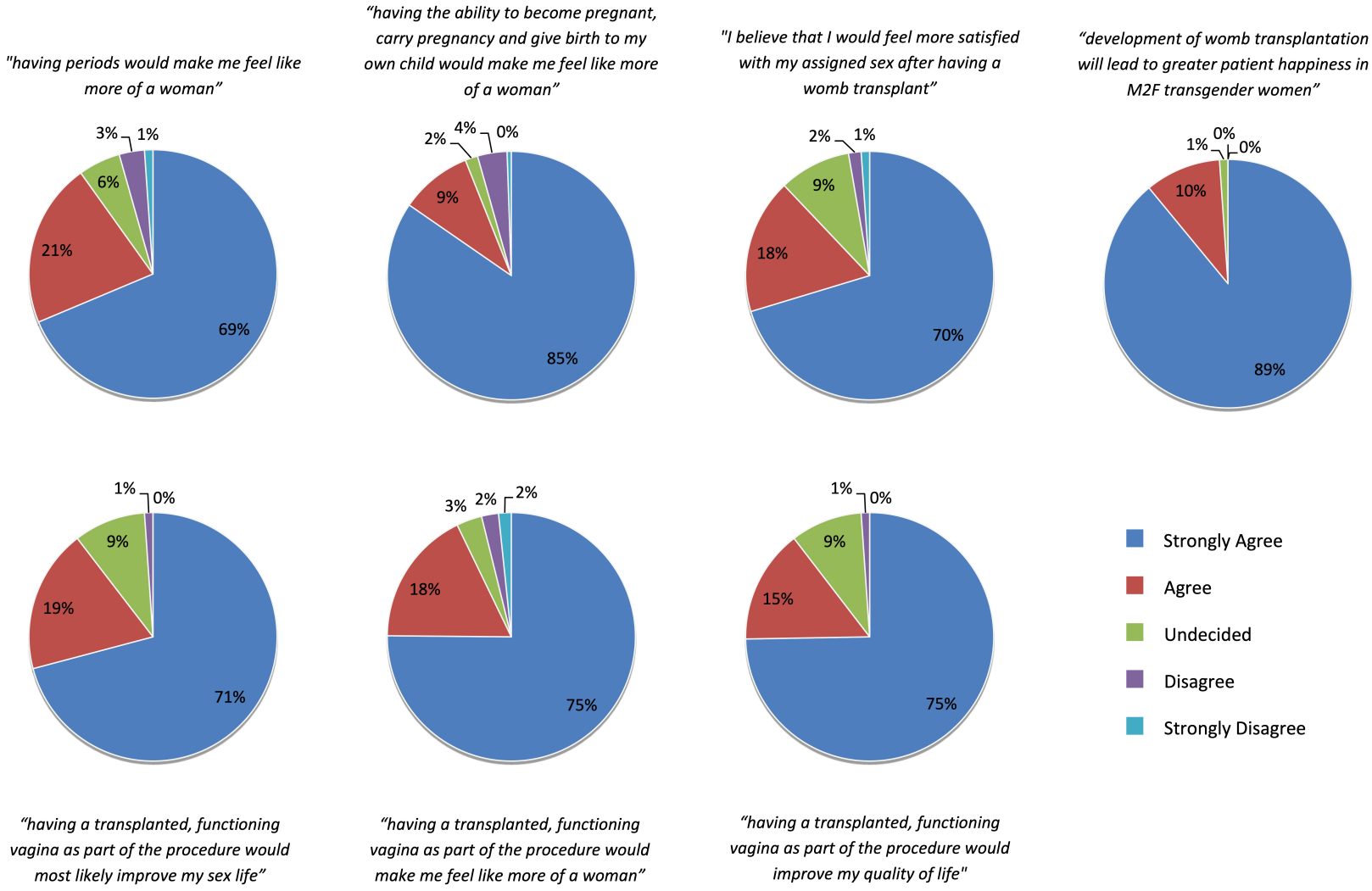


Figure 3a.1: *Motivating factors for undergoing uterine transplantation in male to female transgender women*

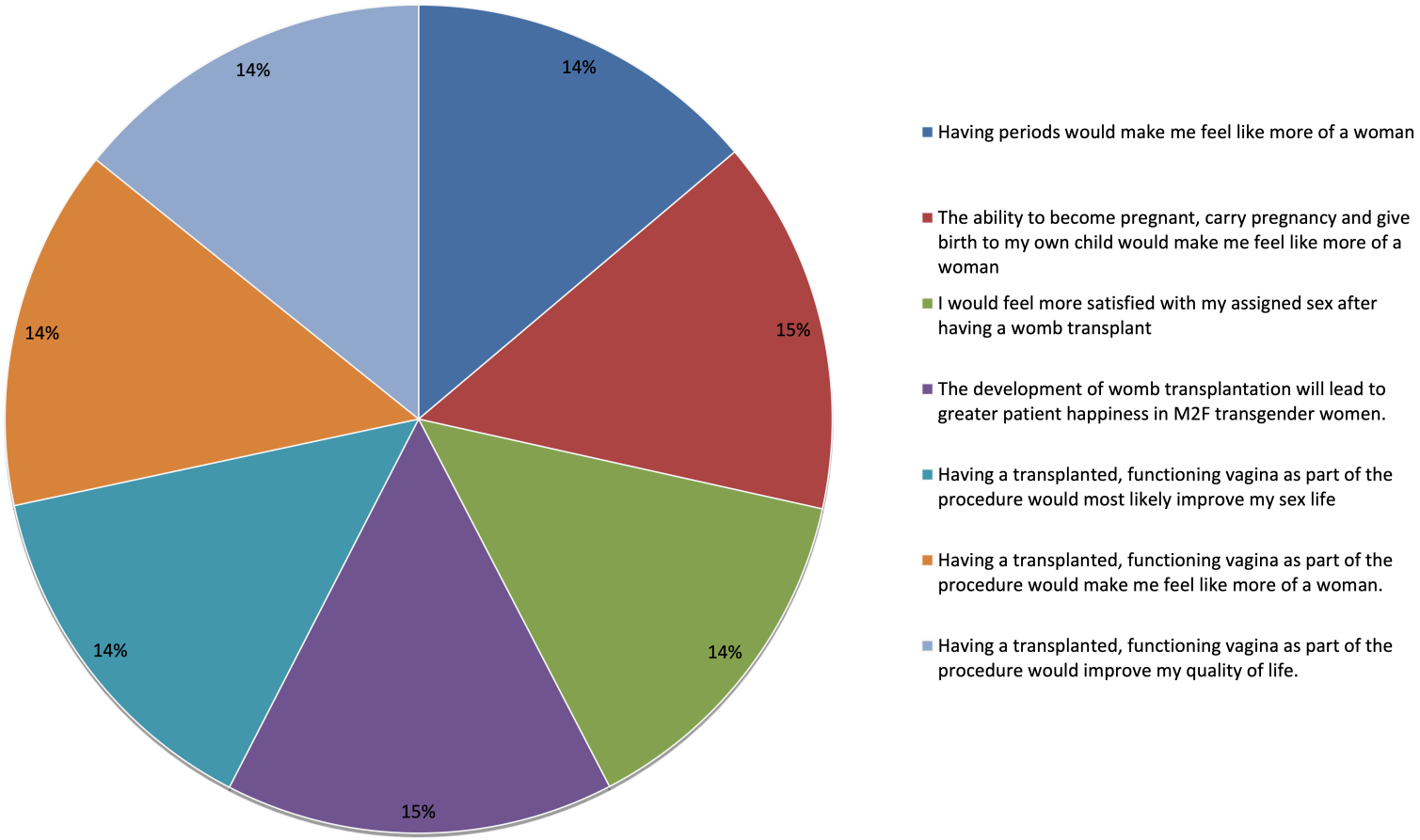


Figure 3a.2: *Perceived importance of each factor influencing motivation to undergo uterine transplantation in male to female transgender women*

risks of immunosuppressants. However, 30% (n=55) remained undecided and 8% (n=15) 'disagreed' or 'strongly disagreed'. All those who disagreed elaborated further that their unwillingness would be due to the perceived potential worsening of dysphoric symptoms following hysterectomy.

Fertility preservation

Of the 182 women in our cohort, just over half (56%; n=102) were offered sperm cryopreservation, whilst around a fifth (22%; n=40) had previously cryopreserved sperm for fertility preservation purposes. Of the 142 who had not cryopreserved sperm, the most commonly cited reasons for not doing so included financial implications (37% n=52), not wanting to delay hormonal/surgical treatment (18% n=25) and not wanting to genetically father a child (16% n=23). **Figure 3a.3** summarises the reasons this cohort provided for not cryopreserving their sperm. Since storage, one (0.5%) person returned to use their cryopreserved sperm in their partner which has resulted in the birth of two children. When asked if respondents would be more inclined to cryopreserve sperm if UTx became a realistic treatment option for M2F transgender women, more than three quarters (n=140; 77%) of the cohort 'strongly agreed' or 'agreed', 12% (n=21) were undecided and a further 12% (n=21) either 'strongly disagreed' or 'disagreed'.

Discussion

The data presented herein provides novel insight into the reproductive aspirations of M2F transgender women and demonstrates robust, multifactorial motivation and desire to undergo UTx, should it eventually be proven feasible. These findings compare similarly to perceptions of women assigned female at birth. For example, 95% of respondents to a UK study exploring the attitudes of women with AUFI towards UTx stated that despite the additional risks posed by UTx, they would choose UTx over surrogacy and adoption.[22] Another study, specifically assessing perceptions in women with MRKH, showed that almost two thirds of participants were motivated to undergo UTx, after becoming aware of the associated risks involved.[394] Similarly, the results of a questionnaire answered by 60 women with AUFI in France demonstrated that 58.3% would partake in a clinical trial in UTx.[395]

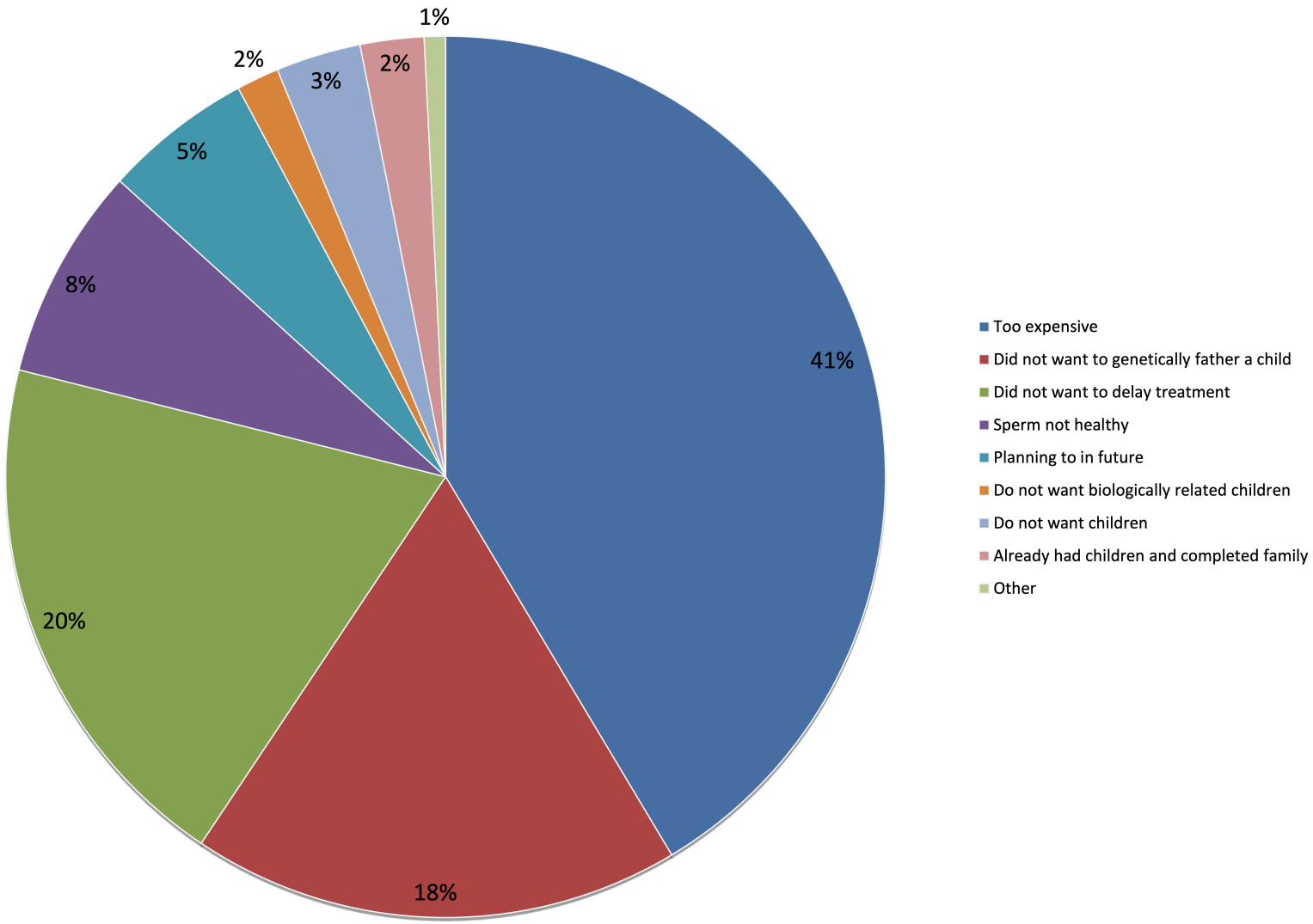


Figure 3a.3: *Reasons attributed to influence decision to not cryopreserve sperm*

In our cohort, <10% had children prior to transitioning. This is far lower than reported in previous studies undertaken between 2003-2008 where between 22-64% of transgender women had children prior to transition.[317,328,505-507] Conversely, 95% of our cohort reported a desire to have children in the future, which is higher than in a previous studies where approximately half of respondents reported a desire for parenthood.[317] These differences can be explained by the fact that respondents to this survey were much younger, where 73% were aged between 16-30 and 26% were aged 30-50, as opposed to the respondents in previous studies where 70% were aged between 30-50.[317] This epitomises the clinical implications associated with the trend of earlier presentation for gender dysphoria, which is substantiated by the ongoing exponential rise in the number of adolescent's assigned male at birth being referred to gender identity services.[508,509]

This shift of focus from childbearing prior to transition, to following transition highlights the importance of fertility preservation counselling and the offer of sperm cryopreservation before medical or surgical intervention, as recommended by The World Professional Association of Transgender Health, and the American Society for Reproductive Medicine.[308,309] However, fertility preservation remains low with around a fifth of our cohort utilising this opportunity. Uptake in fertility preservation among transgender youth has been demonstrated to be consistently low with rates varying between 1-38%.[348,503,510] Barriers have been identified including apprehension toward invasive procedures, financial implications, lack of awareness and desire to not delay transition.[348,511] In addition, almost a fifth of our cohort felt that preserving sperm would conflict with their core female identity, most attributing expected worsening of dysphoria knowing they were a biological father. More than three quarters of our cohort would, however, preserve sperm if UTx became an option. This suggests that respondents to the survey were not overly concerned about inheritance of gender dysphoria in their offspring, despite increasing evidence of a genetic component to gender dysphoria.[349,350]

Despite adoption and surrogacy being the most readily accessible routes to parenthood, our findings also identify that respondents to our survey feel discriminated against in both processes, which may help explain why <1% of our cohort had children since transitioning. While adoption or surrogacy may

allow a proportion of M2F transgender women to meet their reproductive aspirations, previous research suggests that gestation could play a vital role in conveying and consolidating a female identity,[312] and may therefore facilitate the alleviation of gender dysphoria in transgender women. This is substantiated by our findings, where the vast majority of respondents (94%) felt the ability to gestate would enhance their feelings of femininity. This is further supported by results showing that the majority of respondents attracted to females (79.5%) would still consider undergoing UTx if they were with a fertile female partner. These results correlate with those from an earlier survey of 121 M2F transgender women where many respondents were more interested in the prospect of UTx than sperm cryopreservation, holding pregnancy and delivery to be superior to biological relation.[317]

Our findings also show that transgender women expect the ability to menstruate to enhance satisfaction with their desired gender after UTx and anticipate improvements in perceptions of their femininity. The potential for having a functional vagina transplanted as part of the graft may also enhance sexual function, quality of life, and further optimise perceptions of femininity. This raises the prospect of transgender women wishing to undergo UTx primarily to relieve their dysphoric symptoms, not for the sole purpose of childbirth as is intended in women assigned female at birth. The intended ephemeral nature of UTx creates a unique advantage over other organ transplants, because following completion of the recipient's family, the graft can be removed, allowing the cessation of immunosuppressive medications. While essential to prevent rejection when the graft is in situ, the use of immunosuppression is associated with a number of risks, including infection and cancer.[61,512] Immunosuppression related complications are dose and duration dependant.[62] Therefore, minimising the time the transplanted uterus is in place is an important means of reducing the level of risk of UTx for recipients. If UTx was performed to allow women to experience menstruation, and enhance perceptions of femininity, the duration of the graft would likely increase, significantly worsening its risk-benefit profile. Our data identifies that while the majority of respondents (61%) would agree to hysterectomy following completion of their family, more than a third (39%) were undecided or disagreed. Given that all those who disagreed felt that it would exacerbate their dysphoria symptoms, it is clear that a proportion, after

extensive multi-disciplinary counselling, could opt to keep their uterus despite the ongoing cumulative risk related to immunosuppression exposure.

The possibility of permanent UTx in transgender women raises ethical concerns, as indeed it would in any woman. The production of a favourable risk-benefit ratio is a key component of any justified medical procedure. The additional risks caused by *permanent* UTx and life-long immunosuppression would therefore need to be considered and weighed against the likely benefits for the recipient. Thus, it may well be that despite the additional benefits that permanent UTx could provide to some transgender women, the risks of *permanent* UTx are too great. It is important, however, to conduct research into this possibility and to ensure that decisions about permanent UTx are not clouded by any individual and social biases.

This is the largest study of its kind to determine the reproductive aspirations of M2F transgender women, and the first to assess detailed perceptions and motivations toward UTx. The findings of this study affirm the need for further animal or cadaveric research to determine the feasibility of performing UTx in transgender women. A weakness of this study includes the fact participants were self-selected, were predominantly recruited via support groups and through social media, meaning that the results are not be directly extrapolatable to all M2F transgender women. Additionally, the exclusive use of self-reported data introduces the potential for bias.

Performing UTx in transgender women inherently increases the complexity of an already highly complex procedure. Concerns surrounding the neovaginal anastomosis, differing pelvic size and shape and hormonal variation question whether or not the procedure is feasible.[331] In particular, the lack of a physiological functioning vaginal mucosa may prove problematic utilising the traditional UTx surgical technique, highlighting the need for consideration and potential manipulation of the neovaginal microbiome in those which are found to be dysbiotic.[410] Otherwise, as hypothesised by our team previously, transplantation of a longer vaginal cuff may enable a physiologically functioning vagina,[331]

which may offer additional advantages such as improved sexual function and enhanced feelings of femininity.

Conclusion

Our findings suggest that UTx in M2F transgender women could facilitate the achievement of their reproductive aspirations while concomitantly alleviating dysphoric symptoms, enhancing feelings of femininity and potentially improving happiness and quality of life, despite the significant associated risks involved. The demonstration of desire and willingness to undergo UTx in this population validates the need for further animal and cadaveric model research to assess the feasibility of performing UTx in transgender women.

Study 3b

Uterine transplantation in transgender women; feasibility in animal models

Introduction

Contemplation of previous animal model research in UTx is necessary when attempting to consider the feasibility of M2F transgender UTx using animal models. After initial experiments using dogs,[513] the majority of initial studies utilised rodent models, before the knowledge gleaned from these experiments was extrapolated into large animal and primate settings.

In small animal models such as rodents, the uterine vessels are too small to dissect accurately and subsequently anastomose. A such, the surgical techniques employed generally used macrovascular patch which included preservation of the uterine vessels within the parametria which were included up to the level of to the internal iliac vessels. The vascular part of the graft that was subsequently used in such small animals was the aorta to aorta and inferior vena cava to inferior vena cava, or common iliac to common iliac arteriovenous anastomoses.[514] Multiple studies reported successful UTx utilising a macrovascular graft in mouse,[85] rat,[515] and rabbit models.[84] With regard to the vaginal anastomosis, in the mouse model, the transplanted cervix was initially left unattached in the abdomen,[516] but later techniques saw the creation of a cervico-cutaneous stoma.[85] The cervix was subsequently attached to the vagina in future studies in rats,[517] which allowed spontaneous conception and bears closer resemblance to the UTx procedure that is now being undertaken in humans.

Following the success in small animal models, the natural progression onto large animal research ensued, a model that greater resembles that of humans. Autotransplant studies were undertaken in pigs to assess the technical aspects of the surgery using the smaller uterine vessels, as opposed to the macrovascular anastomosis technique employed in the small animals. However, results were initially poor, owing to thrombosis, and ischaemia-reperfusion injury.[518,519] The use of smaller calibre vessel anastomosis predisposed to grafts to thrombosis due to the markedly reduced flow through the sites of anastomosis. This reaffirmed that the smaller vessel technique is inappropriate in UTx, even in the animal models where it is technically possible to do. A further allotransplant study using a macrovascular patch technique (internal to external iliac) showed more positive results, with 50% of

the animals still alive, after successful treatment of rejection episodes after 12 months.[520] In this series, the vagina was externalised as a cutaneous stoma in the right lower quadrant, which allowed endoscopy and biopsies to be taken. On reflection, whilst the porcine model bears greater resemblance to human anatomy and physiology than the smaller animal models, and availability is widespread with less ethical implications compared to using dogs or primates, it is technically difficult due to the large size of the uterus (approximately 1 metre long horns) in correlation to the small size of the vessels available for anastomosis.[521] Moreover, there appears to be a high susceptibility of the animal to ascending infection via the vagina or through the cutaneous stoma, owing to its general state of cleanliness.[520]

The use of sheep models has also been used in the preparatory research for UTx. This model is appropriate as both the body and pelvis size is closer to that of a human, with similarly sized corresponding vasculature. Uterine autotransplantations were initially undertaken, using an internal iliac to external iliac anastomosis, with successful surgical outcomes,[522] and subsequently pregnancy outcomes,[523] being reported. Subsequent allotransplant studies using a similar surgical technique revealed viable uteri and patent vessels in 60% of animals,[524] and another achieved successful pregnancies and livebirths.[87]

Following on from the successes seen in large animal models, the final experimental model utilised was that of non-human primates. A number of UTx auto-transplantation studies have been undertaken, in both baboons,[52,81,525] and macaques.[86] Prior to the first UTx attempt in Saudi Arabia, limited results are described by Fageeh et al from an autotransplant study in baboons.[52] The initial arterial anastomosis was between the uterine artery to uterine artery, using an end to end method. However, the majority of these were complicated by thrombosis. They subsequently revised the technique to include a uterine artery to internal iliac arterial anastomosis, with vascular patency being reported as 90% after 6-12 weeks.[52] A subsequent study in Sweden, again in baboons, included an auto-transplant of a graft including the uterus, Fallopian tubes and ovaries.[525] The vascular dissection included arterial pedicles incorporating the full length of the uterine arteries and a cuff of the anterior

division of the internal iliac artery. During the back-table dissection, the arteries and veins were anastomosed side to side to create a single artery and a single vein, which were both subsequently anastomosed unilaterally to their corresponding external iliac vessel. 90% of animals survived long term. The inclusion of the ovaries enabled monitoring of hormones post-operatively to help identify viability of the graft, with 50% demonstrating hormonal function. 20% of the animals subsequently menstruated.[525] Following on from these promising results, the experiment was repeated in a further six baboons.[81] Two thirds (n=4) survived the operations, all of which demonstrated cyclical hormone activity, but none of which subsequently menstruated. Histology revealed small fibrotic uteri, but normal ovaries. This highlighted clinically and histopathologically, that in the context of both the uterus and ovary being supplied by the same artery, either the ovary withstands ischaemia better, or its ability to induce neovascularisation was superior. Modification to the surgical technique included taking a longer cuff of internal iliac to the main branch, and further vascular reconstruction was undertaken to connect the larger arterial end of the graft to the contralateral posterior division of the internal iliac in an end to end manner, before anastomosing the extended vascular pedicle to end to end to the internal iliac artery. The results improved dramatically, with all animals surviving, 80% displaying cyclical ovarian function, and 60% demonstrating menstruation.[81] A final series of UTx autotransplant studies in Japan resulted in the first livebirth following UTx in a non-human primate model. An initial series of two cases were undertaken in cynomolgus macaques. Bilateral uterine artery to external iliac artery anastomoses were undertaken. Whilst one died intraoperatively, the other survived and resumed menstruation after 4-5 months. The same model and surgical technique was subsequently repeated on two further macaques, with spontaneous conception and successful livebirth reported.[86] A previous attempt to utilise unilateral artery and venous anastomoses was employed by the same team in four macaques. Unfortunately, 75% of uterine grafts failed, highlighting the need for bilateral arteriovenous anastomoses.

The utilisation of the outcomes and learning points from these animal model UTx studies is essential when planning further animal model research to prove the feasibility of UTx in M2F transgender women. It is clear, bilateral arterial and venous anastomoses, utilising the largest calibre vessels as

possible is essential. Moreover, the use of a vaginal-cutaneous stoma, or vaginal anastomosis in the orthotopic position is preferable to help assess outcomes, and subsequently facilitate natural mating, or IVF. The aim of these pilot studies is to assess the feasibility of performing UTx between female and male animals, to help plan future short-term feasibility studies to assess clinical and reproductive outcomes in this model.

Feasibility Study

A pilot study was undertaken in two parts to determine the feasibility of the proposed surgical technique necessary to undertake UTx in small and large animal models. These included a case in a large animal model using a goat, and a small animal model with a rabbit. The aim of both of these terminal experiments was to isolate a uterine graft with long, vascular pedicles including uterine arteries, leading to at least the anterior division of the internal iliac arteries and accompanying veins, as well as the cervix, vagina and both uterine horns (without the oviducts and ovaries), and implant it into a corresponding male. All procedures were performed in accordance with Directive 2010/63/EU EEC for animal experiments. Following discussion with the local ethics committee, it was agreed formal ethics approval was not required.

Feasibility study 1 – *Fresh cadaveric goat*

The decision to use a goat was made following the pilot study using a goat for endometrial transplantation, which is described further in chapter 4a. It was noted during the dissection that the pelvis was much shallower than that seen in during previous sheep studies on UTx,[526] and as such would facilitate the deep pelvic dissection required to retrieve and implant a uterus. Moreover, in a similar fashion to the sheep, the pelvic anatomy closely resembles that of a human, and the extensive previous research experience of the team in sheep would be directly transferable owing to the significant crossover in species.

A female adult donor weighing 40kg was euthanised. The anterior abdominal wall was cleaned and shaved. the abdomen was opened through a midline incision. The uterus was identified and mobilised. It was observed that the rumen reticulum was distended, which increased the difficulty of the dissection. The pelvic side wall was opened, and the major vasculature was identified. The pararectal and para vaginal spaces were opened, the bladder was dissected off anteriorly and the rectum was mobilised posteriorly as far as the pelvic floor. The common iliac vessels were identified, and a ligature was placed across the external iliac and lower internal iliac leading on to the uterine artery and large ovarian veins. Vaginal examination was undertaken concomitantly to facilitate the vaginal incision, which was made within 2cm of vaginal introitus. The specimen was removed, which included 5cm of vagina retrieved below the cervix.

An adult male goat, weighing 45kg was euthanased, and the anterior abdominal wall was cleaned and shaved. A midline incision just lateral to the penis. The majority of the penis was noted to be mostly under the skin and measured 11-12 inches long. The sigmoid flexure of the penis was identified, which is where abdominal entry was made. Following reflection of the bladder and attempted mobilisation of the rectum, it was noted there was extremely limited space in between, making the male pelvic completely infeasible with regards to implanting a utero-vaginal transplant. The procedure was therefore abandoned.

Feasibility study 2 – *Fresh cadaveric rabbit*

The decision to use a rabbit was made owing to previous surgical experience within the team with previous UTx research,[84,527] and the ETx studies detailed in chapter 4.

A 5.5kg female donor rabbit was euthanased. The anterior abdominal wall was cleaned and shaved before the abdomen was opened through a midline incision. The uterus was identified, and subsequent hysterectomy was undertaken, including a 8cm section of vagina and the most lateral aspects of the

uterine horns together with the aorta and vena cava, including the common iliac, internal iliac and uterine arterio-venous tree (**Figure 3b.1**). It was noted that the perforator vessels posterior to the IVC and the presacral artery and vein appeared engorged.



Figure 3b.1: *Uterine graft including two uterine horns, the main body of the uterus, a long vaginal cuff, the parametrium and surrounding vasculature to the level of the aorta and IVC*

Following the retrieval of the graft, a cadaveric dissection of the male was then undertaken. A midline laparotomy was performed. The bladder and ureters were easily identifiable, as was the prostate. The sigmoid colon and rectum were easily mobilised to open a plane behind the prostate in front of the rectum. However, it was observed that the subsequent space created was narrow, with limited options to creating an external stoma or neovagina without damaging the bowel or bladder. As such, the decision was made to make use of the anatomy and physiology of the male rabbit to facilitate the technique. It is well known that adult male rabbits, during acts of aggression, retract their testicles into their abdomen, suggesting sufficient calibre to facilitate the potential development of a neovagina, which leads directly to the abdomino-pelvic cavity. As such, a separate vertical incision on the

scrotum, with care taken not to incise the vaginal tunic, which is the sac enclosing the testicle, which leads to the inguinal canal into the abdomen (**Figure 3b.2**).

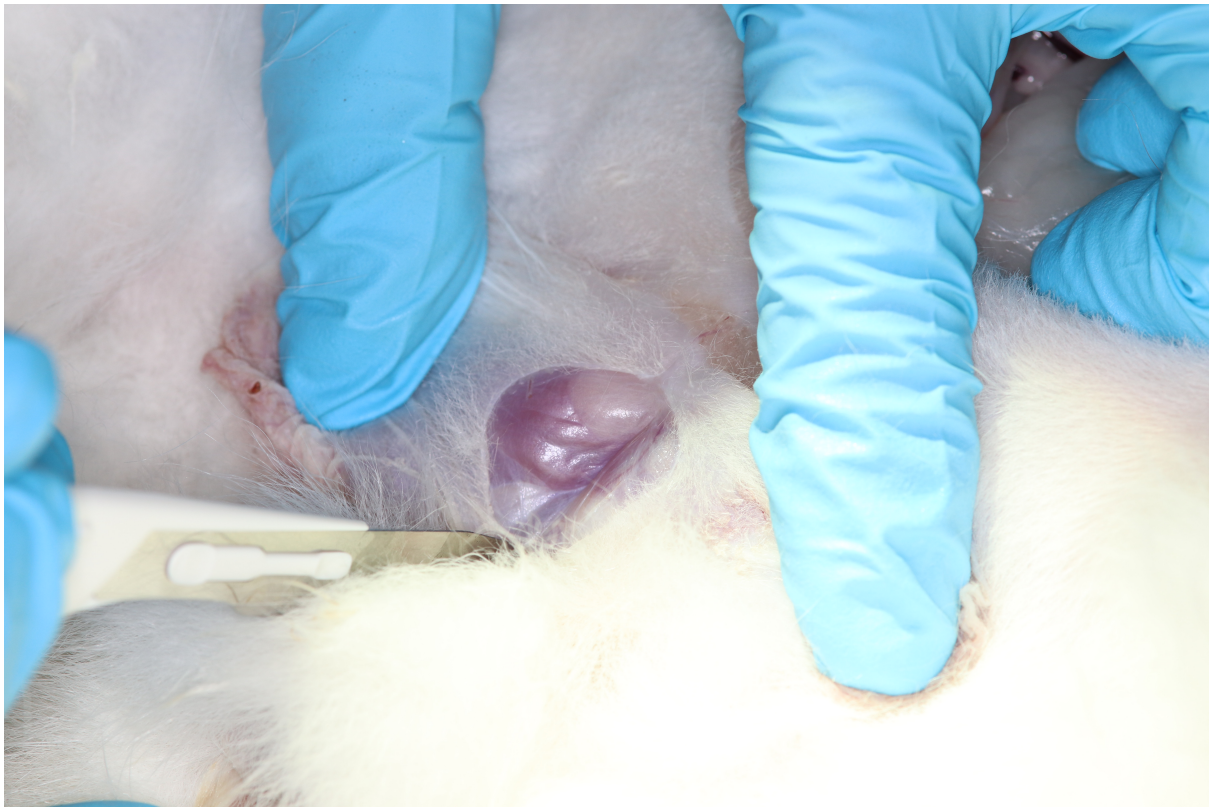


Figure 3b.2: *Vertical incision on scrotum, exposing the testis*

Following careful opening of the scrotum, the vaginal tunic was dissected away from the inferior margin, and subsequently mobilised superiorly toward the inguinal canal. The scrotal skin was then trimmed and inverted interiorly slightly, forming the proposed neovulva (**Figure 3b.3**). A single horizontal incision was then made on the vaginal tunic, and an opening formed, which would subsequently be anastomosed to the scrotal neovulva. This created a tunnel leading directly through the inguinal canal into the abdominopelvic cavity.

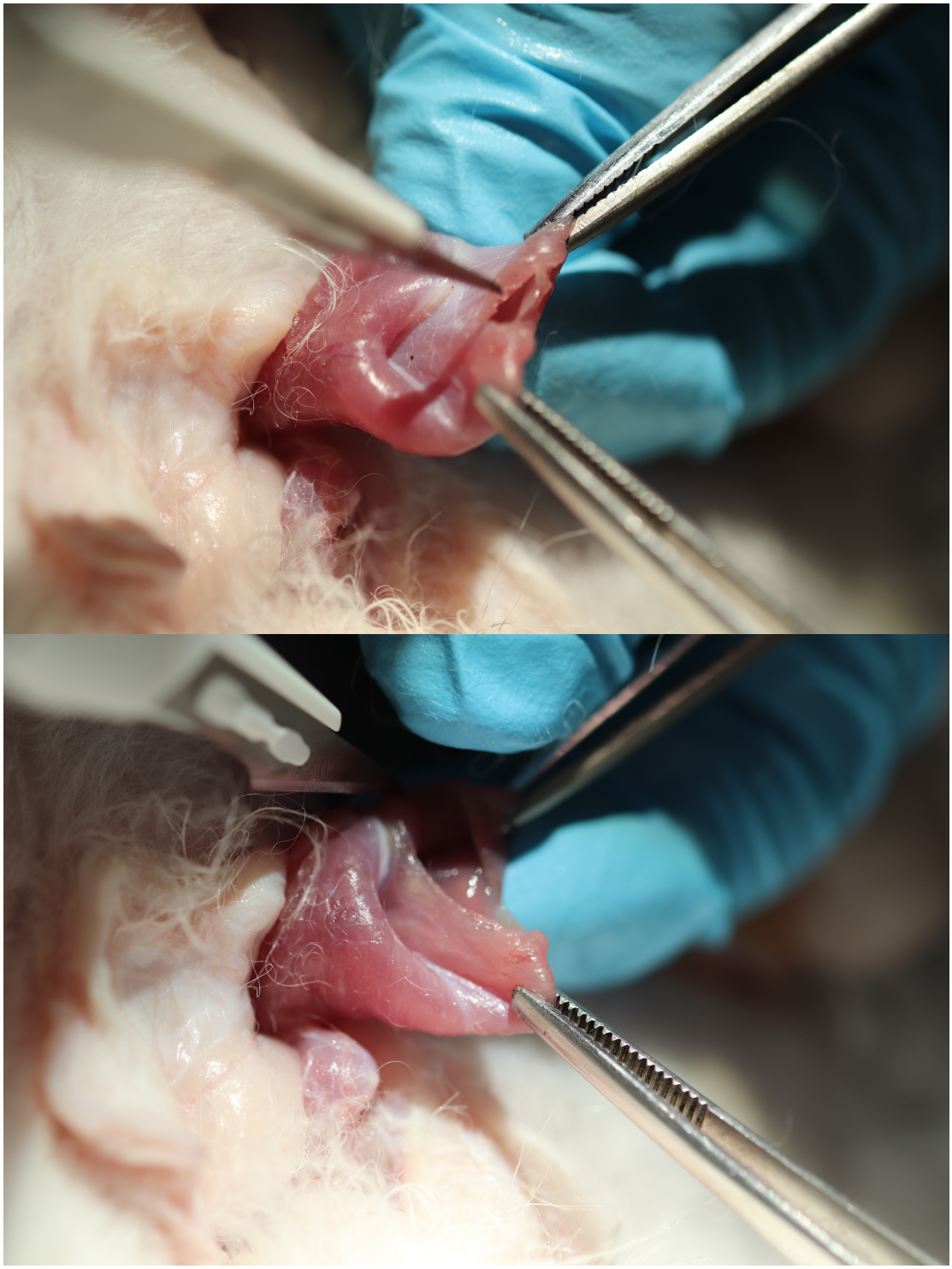


Figure 3b.3: *Horizontal incision on testicular sac (top), with subsequently opening of proposed neovagina that leads through inguinal canal into the abdominopelvic cavity*

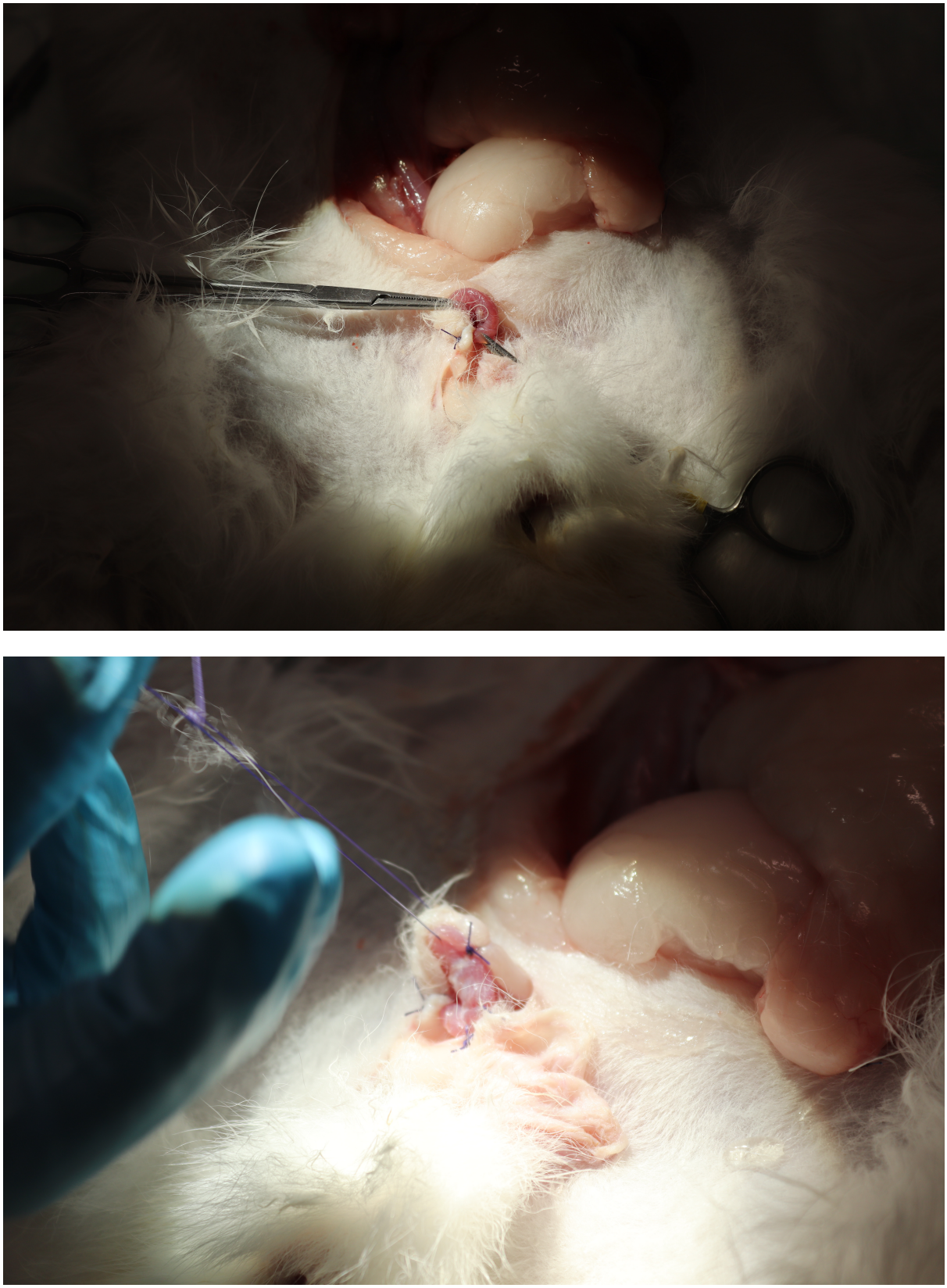


Figure 3b.4: *Creation of neovulva and external aspect of neovagina*

The neovaginal anastomosis was subsequently undertaken using circumferential interrupted sutures.

Following completion of the 'bottom end' procedure, further dissection was undertaken intra-abdominally, through the midline incision, to mobilise the pelvic anatomy and vasculature for implantation. The connective tissue overlying the infra-renal aorta and IVC were removed to expose approximately 4cm area of the aorta and IVC which would be underrun and clamped, ready for an end to side anastomosis (**Figure 3b.5**).

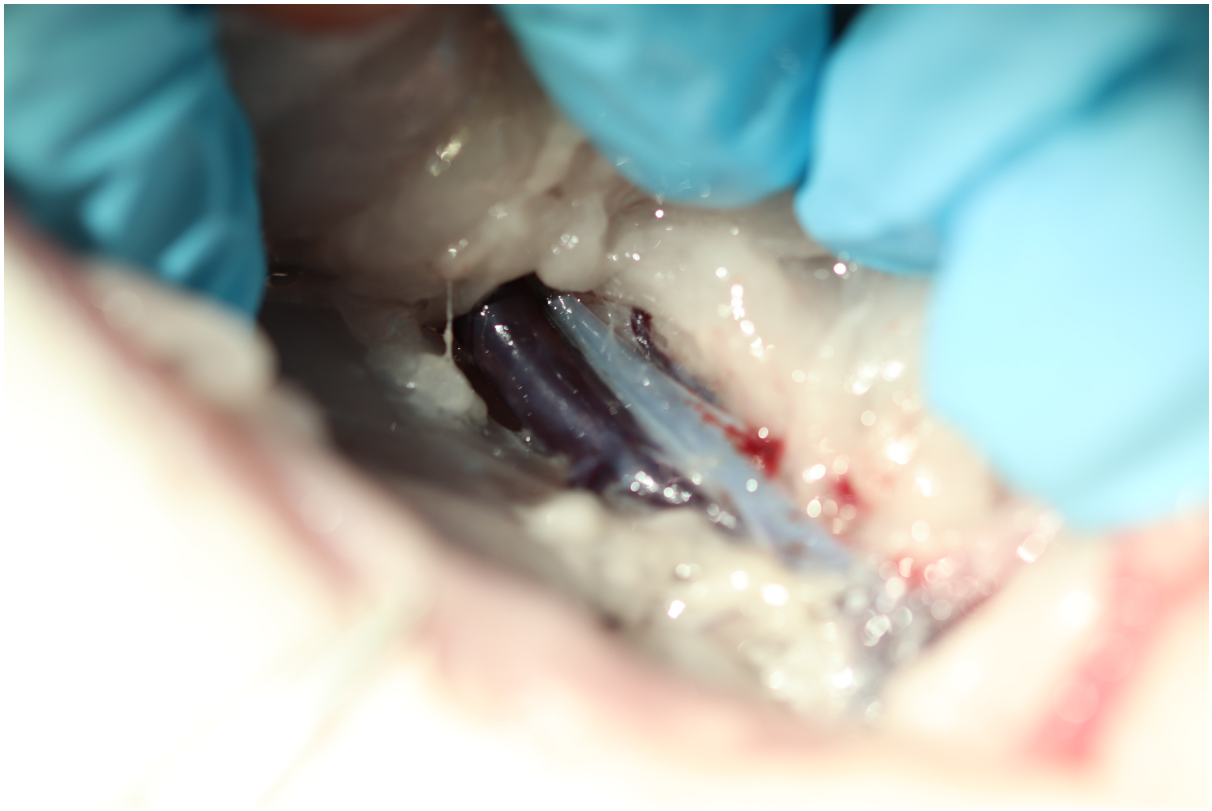


Figure 3b.5 – *Exposing the aorta and inferior vena cava*

The uterine graft was subsequently introduced into the abdomen. The inferior neovaginal anastomosis was undertaken (**Figure 3b.6**). As the experiment was terminal, and the vessels were not perfused, vascular anastomosis was not undertaken, but once the graft was in the proposed anatomical position, there was sufficient length and calibre of vessel to undertake the subsequent vascular anastomosis required, without excessive tension. The anastomosis would be undertaken with prolene 6-0 sutures, as per previous UTx studies in the rabbit model.[527] Whilst a more recent study used smaller (size 8-10),[528] the animals used in their study are smaller than that used in this study (3.8-4.7kg vs 5-6kg).

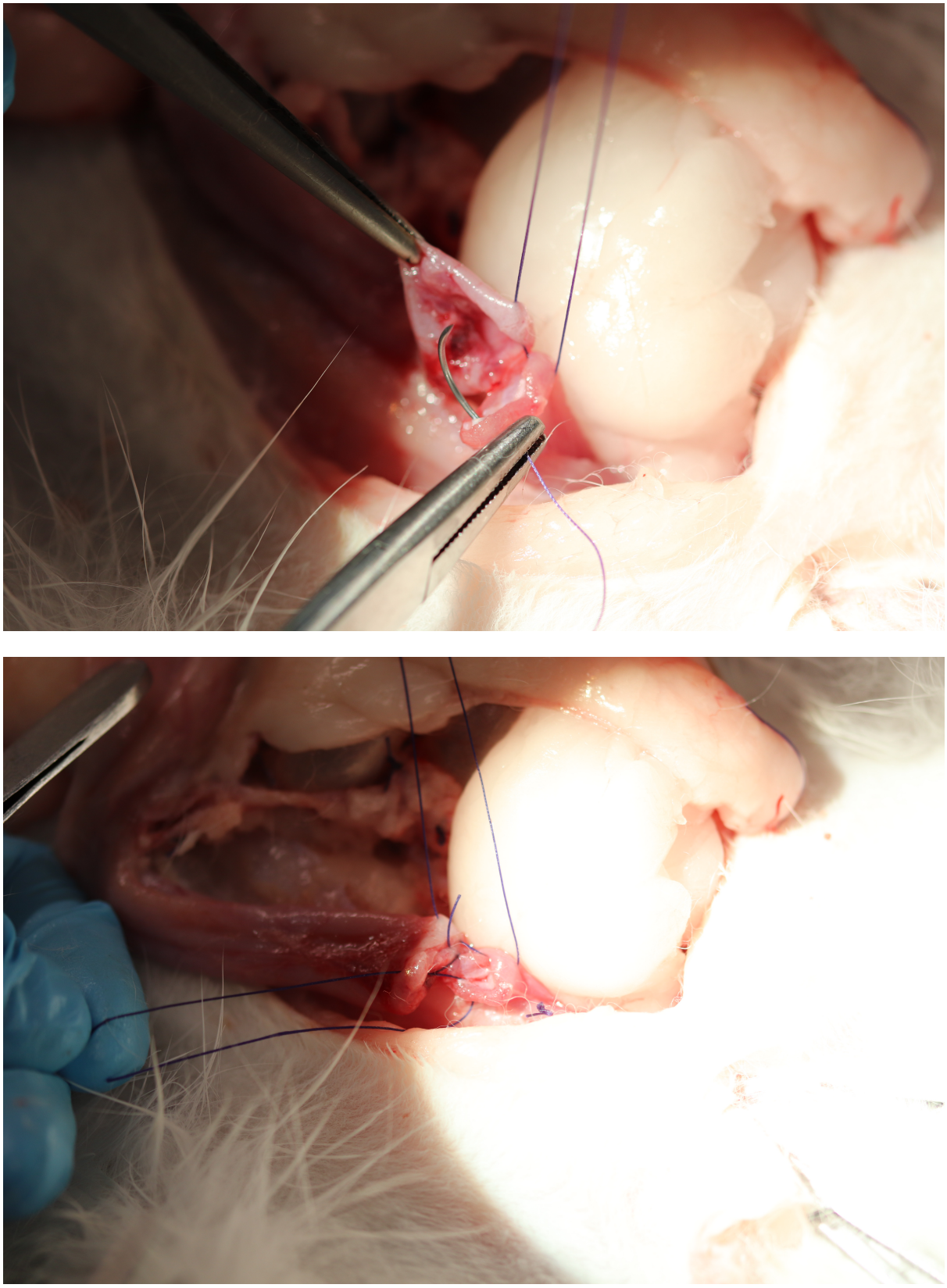


Figure 3b.6 – *Neovaginal anastomosis*

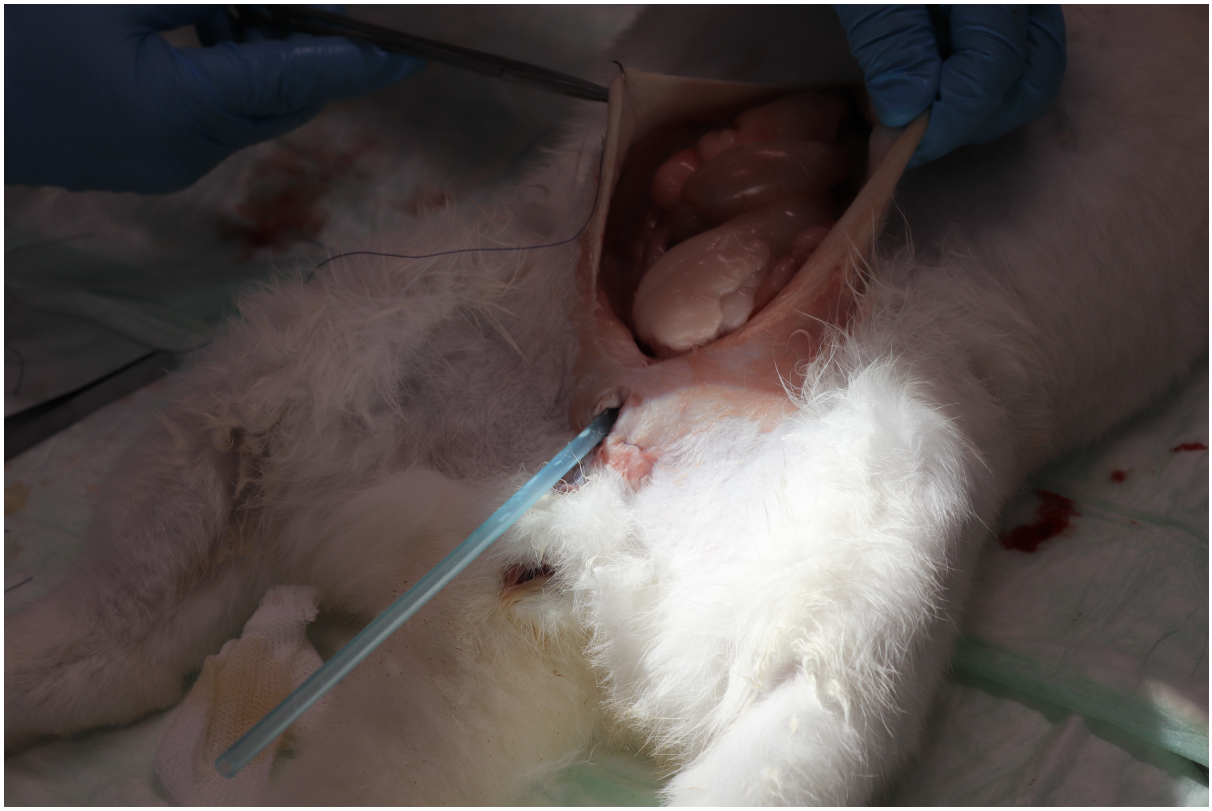


Figure 3b.7: *Demonstration of cannulation placement to confirm patency of neovagina following neovaginal anastomosis*

Prior to abdominal closure, a cannula was passed through the neovagina, and palpated within the intra-abdominal portion of the vagina, to ensure patency through the anastomosis (**Figure 3b.7**).

Discussion

This pilot study identified a potential small animal model which may be suitable to undertake a feasibility study to assess the clinical and reproductive outcomes following the implantation of a uterus into a male rabbit. Not only would this provide important scientific information regarding subsequently undertaking this in male to female transgender populations, but it may also give insight into undertaking UTx in women with complete androgen insensitivity syndrome, although there are fewer complicating factors in this model.

Rabbits make an ideal model for reproductive studies, as the gestation period lasts for approximately 30 days, which facilitates prompt outcomes following relatively short term follow up. This proposed model includes the creation of a neovagina, which could be used to access the uterus non-invasively. The rabbit model is favourable, as the comparatively long vagina facilitates the donor retrieval and gives options and flexibility with regard to the creation of a neovagina in this model. The method presented herein maintains sufficient neovagina length, which would reduce the risk of ascending infection and reduce the risk of inadvertent trauma. However, there are a number of associated limitations. Firstly, whilst bilateral orchidectomy was undertaken, the penis was not removed. While this is not essential to address the main objective, it would almost certainly be necessary in human models, most likely due to patient preference, but also due to it being an anatomical hindrance. Moreover, it should be considered that the neovagina creation method bears little resemblance to the penile inversion method commonly employed in male to female transgender women. In addition, an orthotopic transplant should be the primary choice as the lower pelvis allows for natural growth of the uterus during pregnancy without interference with other organs such as bowel.[520] As presented herein, the position is not orthotopic, and as it is slightly unilateral, structural support may eventually complicate short term viability studies. Moreover as the inguinal canal will not dilate to accommodate childbirth, delivery by Caesarean section is required, which would be required regardless following UTx in any model. In terms of pelvic dimensions, radiological data has shown that whilst female rabbits have a significantly greater interacetabular width, all other dimensions remain similar.[529] The decreased interacetabular width in male rabbits should not impact the procedure, nor the ability to gestate, and as delivery is by Caesarean section, the potential impact upon childbirth does not warrant consideration. Ultimately, while this method would provide valuable insight into research in this model, and despite the plausible surgical technique demonstrated herein, the decision to use the rabbit model may not be appropriate, owing to previous poor success rates in UTx in this model.[84,527,528]

Multiple feasibility studies have been undertaken in UTx in the rabbit model by our research team in the UK.[82,84,527,530] Following the identification that the use of a macrovascular patch technique in this model was acheievable,[82] an initial allotransplant study, using a macrovascular aorto-caval

anastomosis, was undertaken in a series of 6 rabbits to assess the technical feasibility. 100% of the animals survived the operation. After post-mortem, macroscopically and histologically there was no evidence of graft thromboses. However, post-operative complications including pulmonary emboli, intraperitoneal haemorrhage and limb paraplegia highlighted the additional vulnerability associated with using rabbits in this model. A follow up study was undertaken to assess the longer term outcomes following UTx, using a modified protocol similar to the previous surgical technique.[530] Greater effort was made to maintain the natural curvature of the spine intraoperatively, less pressure was used during perfusion of the graft at the back table, and increased doses of anticoagulation were utilised. Five uterine allotransplants were undertaken using the same macrovascular aorto-caval anastomosis. While all animals survived intraoperatively, 60% (n=3) died within the first four post-operative days. In the two remaining animals, spontaneous conception was not possible, which was attributed to lack of patency of the uterine cornua at the point of anastomosis.

A subsequent study was undertaken with the inclusion of IVF to negate the issues experienced previously.[527] In a series of nine cases, three recipients died intra-operatively. One died following blood aspiration following biting its tongue intra-operatively which resulted in cardiopulmonary arrest. Another demised as a consequence to intrabdominal haemorrhage, and the final one died with a ventricular haematoma being found on post-mortem. Five further animals died within the first 24 hours post-operatively, three of which were due to major vessel anastomosis breakdown, and the two others from medical comorbidities including pneumonia and pulmonary embolism.[84] Only one recipient survived long-term, which resulted in a survival rate of 11%. This clearly highlights the complexities to using rabbits as a UTx model. It would be expected that given the additional complexities associated with the proposed model, that the survival rate would be even worse. Moreover, given that the single surviving recipient eventually conceived, a livebirth was not obtained, and given the additional reproductive implications in the male model, the overall chance of successfully achieving a livebirth in a male model is remote.

A further, and more recent attempt at UTx in the rabbit model was made in China by Liu et al 2019.[528] Following the successful undertaking of many procedures, including livebirths in humans, it was surprising that a further study in a small animal model was undertaken. Six procedures were undertaken, utilising the same macrovascular patch graft. Despite 100% surviving intra-operatively, short term survival was poor, with only one surviving the first post-operative week, before demising on day 8 post-operatively. Whilst only one demised as a consequence to breakdown of the major vascular anastomosis, the others died following intestinal necrosis, bradycardia, respiratory failure, intestinal obstruction and pneumonia. No rabbits survived long term to assess reproductive outcomes. In summary, despite the achievable theoretically feasible UTx technique demonstrated herein to achieve UTx in a male rabbit, given the poor long-term survival demonstrated in female rabbits to date (0-40%), and extremely poor reproductive outcomes, the rabbit model is probably unsuitable to pursue this concept further. Given that only one pregnancy has been achieved from 20 cases, and associated failure to achieve a livebirth, given the additional complexities in the male model, successive outcomes would be highly unlikely.

Conclusion

Given the wealth of data in small and large animal models, and emerging data in humans, it is debatable about whether or not further animal research is needed, as the extrapolation between female human to male human may well be closer than animal male to human male. Further cadaveric work in humans would be essential, to demonstrate the technical feasibility of the procedure. If any further animal studies are undertaken, whilst the chance of success in the rabbit model remains low owing to the aforementioned complexities, the surgical technique demonstrated herein remains a feasible option. Whilst non-human primates have a closer resemblance to human anatomy and physiology, the additional ethical complexities associated with this model provides a significant barrier. Otherwise, undertaking the procedure in a woman with CAIS, which has less associated additional complexity and uncertainty, may provide the necessary steppingstone, should it be feasible. This is a prospect which requires significant further clinical and ethical debate.

Study 4a

Preliminary pilot studies in endometrial transplantation

Introduction

Uterine synechiae, as a consequence of trauma to the endometrium, were first described in 1894 following postpartum curettage.[531] It wasn't until more than 50 years later when the association of uterine synechiae, or intra-uterine adhesions (IUA), with preceding trauma was identified, and the term Asherman's Syndrome (AS) was coined.[532] IUA are characterised by fibrous bands of tissue on opposing endometrial walls leading to partial or complete obliteration of the cavity.[533] Whilst the pathophysiology of AS remains poorly understood, it is widely thought that it is provoked by trauma to the basal layer of the endometrium. This frequently arises following endometrial curettage during pregnancy, to manage retained products of conception postpartum or following miscarriage.[534] The risk appears to increase with recurrent procedures, with a previously reported prevalence of up to 40% in women who have undergone a repeat curettage for incomplete miscarriage or a secondary procedure for removal of placental remnants postpartum.[535] Other less common precipitating factors include endometritis, myomectomy or Caesarean section.[534,536,537]

AS has been demonstrated to affect 1.5% of women of reproductive age,[200] and is the responsible aetiology in up to 5% of infertile women.[538] A proportion of women with AS have some functional endometrium, which may cause cyclical pelvic pain, retrograde menstruation, irregular menstrual bleeding or subfertility. Others have a complete absence of functional endometrium and subsequently develop amenorrhea and are diagnosed with AUFI. It has previously been identified that infertility is present in 43% of women with AS.[534] Those who conceive may have suboptimal reproductive outcomes, as highlighted by a systematic review that identified that one in five women who miscarry have evidence of IUA.[539]

The mainstay of treatment includes the restoration of uterine function using hysteroscopic adhesiolysis.[540] Secondary prevention utilising a physical barrier such as a catheter balloon or intra-uterine device is also frequently undertaken.[540] Improvement in fertility and menstrual regularity have been demonstrated in several small uncontrolled retrospective series' following hysteroscopic

adhesiolysis.[541,542] However, high rates of infertility, miscarriage, poor implantation and abnormal placentation often continue,[201] with some studies identifying less than 25% of women achieve livebirth following hysteroscopic adhesiolysis.[541,543] The novel application of autologous stem cell treatment has also been utilised as an effective therapeutic modality, with reports of resumption of menstruation,[202,544] as well as successful pregnancies following spontaneous conception and IVF in treatment refractory cases.[203,204]

Recently, UTx following immediately preceding hysterectomy was undertaken for a woman with AUFI secondary to AS.[184] However, the presence of a structurally supported and vascularised uterine structure raises the possibility of ETx as a potentially less immunogenic option with less invasive surgery. Moreover, it has long been known that endometrium can be repaired by suturing it back onto the myometrial surface, as has been demonstrated after myomectomy or following the Modified Strassman Procedure,[545] when entering the cavity is unavoidable, with resultant good hysteroscopic appearance and clinical outcomes. As such, transplantation of the endometrium, could instead be an option for women with AS. Prior to this thesis, there have been no previous attempts of ETx reported in published literature.

The development of such a novel surgical technique needs to consider and implement the IDEAL guidelines, to ensure a methodical and scientific approach to surgical innovation.[546] The IDEAL guidelines are orientated around on five principles: Idea, Development, Exploration, Assessment, and Long-term study. The concept was developed by an MDT to optimise the production and dissemination of data, whilst evaluating quality research in surgery.

The objective of this study was to undertake a series of pilot studies to determine the feasibility of the proposed surgical technique necessary to undertake ETx, to determine if the ‘idea’ was suitable to proceed to the ‘development’ and ‘exploration’ phases, in the context of the IDEAL guidelines.

Feasibility Studies

A series of coordinated and interrelated pilot studies were undertaken in cadaveric goat, cadaveric rabbit and a live rabbit models, to help provide proof of concept and refine surgical technique. An additional experiment was undertaken to assess the potential hormonal impact upon the uterine dimensions, and impact upon endometrial retrieval. All procedures were performed in accordance with Directive 2010/63/EU EEC for animal experiment. Following discussion with the local ethics committee, it was agreed formal ethics approval was not required.

Feasibility study 1 – *Fresh cadaveric goat*

The reproductive anatomy of a female goat consists of a uterus which is made up of two horns, with a common uterine body. Oviducts extend from each uterine horn laterally, which facilitate transfer of oocytes from the ovaries to the uterine body. The uterine body connects to a cervix, which leads to the vagina.

A 40kg recently euthanased doe was opened through a midline laparotomy. The uterus was readily identified and mobilised. Sharp dissection was used to dissect away the bladder, identify the ureters and the supplying vasculature including the common, external and internal iliac vessels. The uterus was subsequently removed including a 1cm cuff of vagina. A midline incision was made on one of the uterine horns and the endometrium was attempted to be removed as a tubular structure, in a similar fashion to the technique implemented during ovarian cystectomy (**Figure 4a.1).** However, this proved technically difficult, particularly when attempting re-implantation

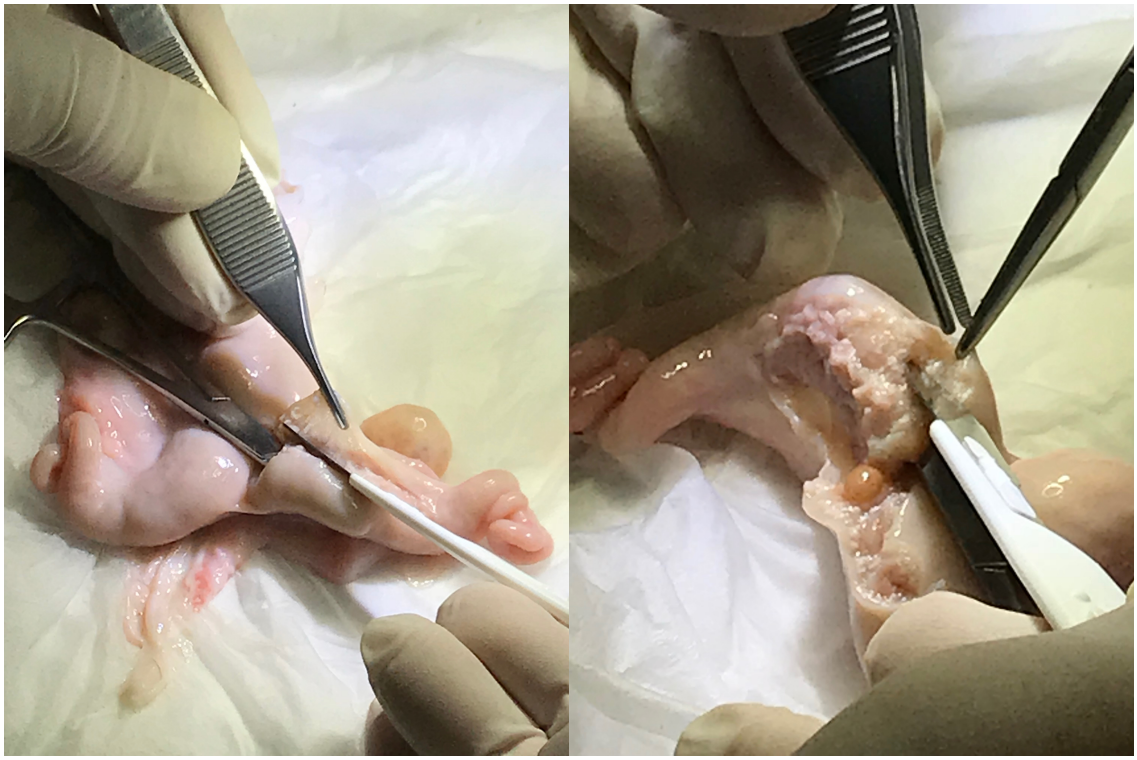


Figure 4a.1: *Midline incision of unilateral uterus (left) and attempted excision of endometrium as a tubular structure (right)*

A different approach was utilised for the contralateral horn. A midline uterine incision was performed, including excision through the endometrium. Forceps were used to facilitate the identification of the plane between the myometrium and the endometrium. Sharp dissection was subsequently used to resect the endometrium from the underlying myometrium (**Figure 4a.2**). The endometrium was subsequently removed, laid out and inspected (**Figure 4a.3**). It was noted that following resection it may be difficult to orientate which way to re-implant the endometrium, so a practical tip is to always lay it with the endometrium facing superiorly.

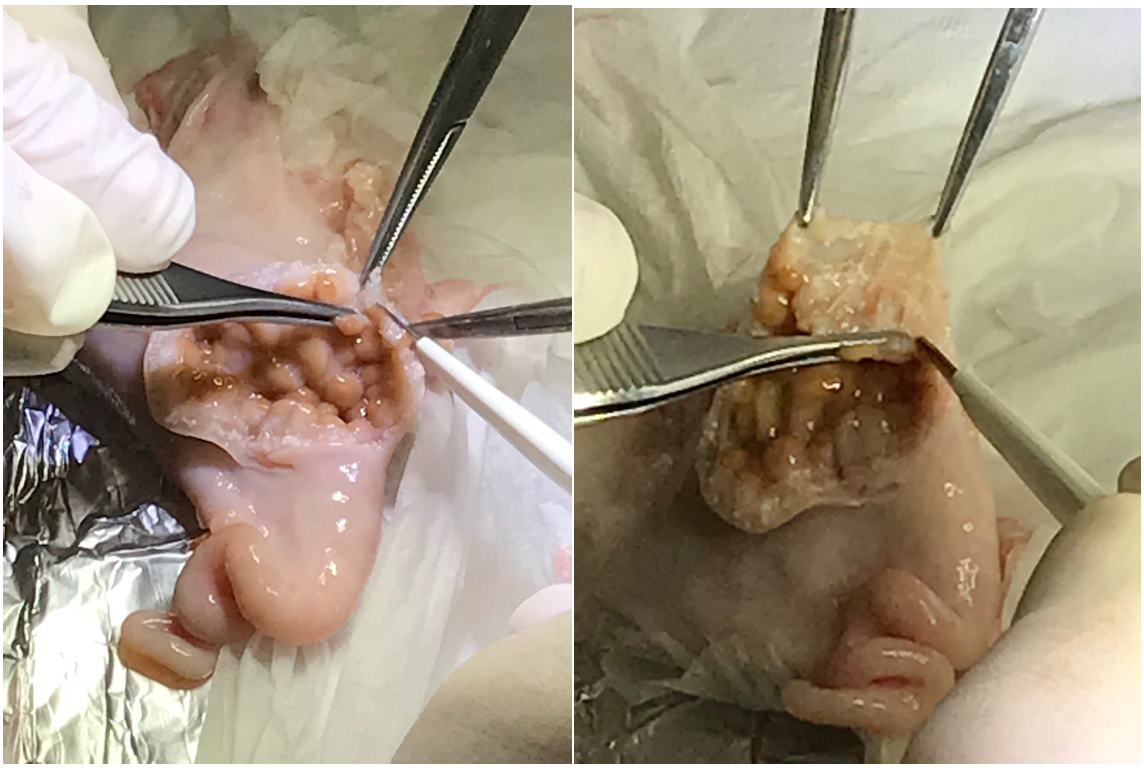


Figure 4a.2: *Identification of endometrium (left) with subsequent resection of the endometrium from the underlying myometrium (right)*



Figure 4a.3: Excision of the endometrium (left) and the *resected specimen post retrieval (right)*

Feasibility study 2 – *Fresh cadaveric rabbit model*

Whilst rabbit pelvic anatomy shares some similarities to that of the goat, there are also some differences. The uterus has separate uterine horns and there is no uterine body. Each uterine horn leads to its own cervix, which open into a single vagina. The uterus is surrounded by mesometrium, which is very vascular, including anastomoses between the uterine and ovarian vasculature.

A uterus was removed from a recently euthanised female rabbit weighing approximately 5kg. One horn of the uterus was opened through the midline and the plane between the endometrium and myometrium was identified, facilitated by the use of fine forceps and 3X magnification surgical loupes (**Figure 4a.4**). Sharp knife dissection was used but given the difficulty identifying the plane, it was evident that endometrium and possibly the superficial layer of the myometrium was possibly being excised. The sample was removed and re-inserted with 6/0 PDS, separated by approximately 1cm gaps.

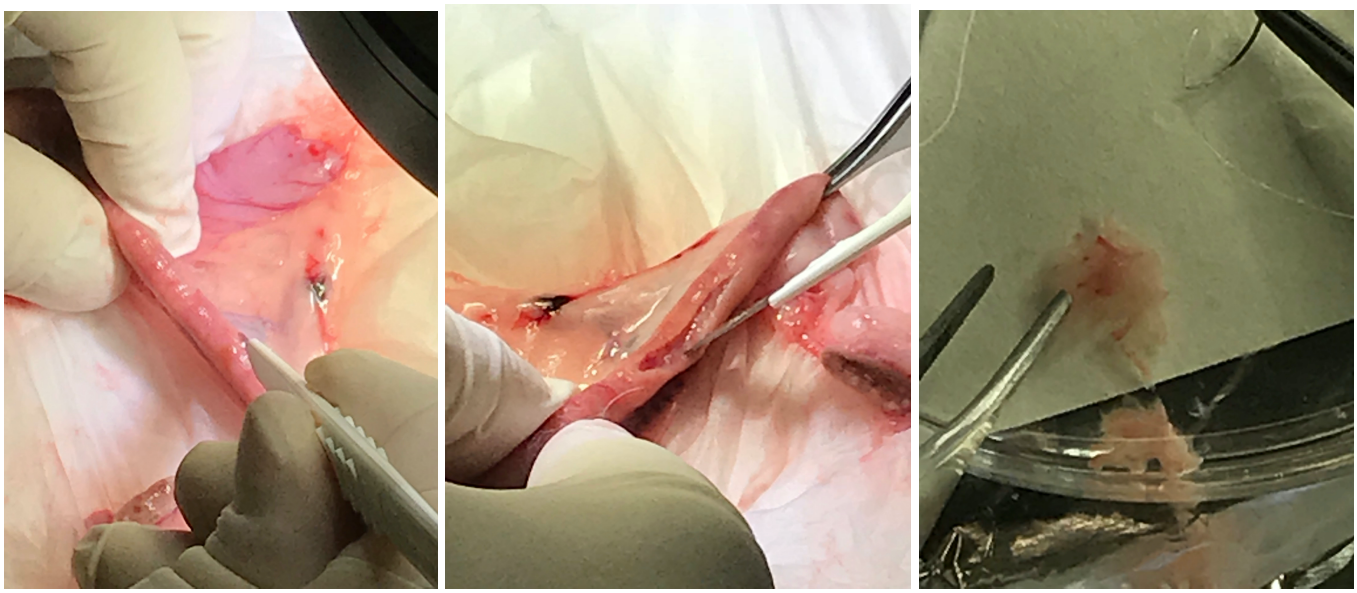


Figure 4a.4: *Midline incision (left), identification of endometrium (centre) and excised endometrium (right)*

Feasibility study 3 – *Living rabbit model*

The final feasibility study was undertaken in a living rabbit model in a terminal experiment, to help determine the potential impact of blood perfusion on the surgical technique. The rabbit was opened though midline laparotomy, entering the midline between the mammary tissue. Two vessels were encountered, which were clipped and ligated, without encountering significant bleeding. Abdominal entry was achieved without blood loss. The uterus was identified with long horns measuring approximately 4cm (**Figure 4a.5**). The uterus was noted to be larger than preceding samples, despite being a similarly sized animal with the same parity. Its reproductive history highlighted that the rabbit had ovulated within the preceding 5 days, which highlighted the potential impact hormonal stimulation may have upon uterine size. Whilst it may also increase the vascularity to the uterus, identification of the endomyometrial interface and subsequent reflection of the endometrium away from the underlying myometrium appeared to be facilitated by this hormonal influence.

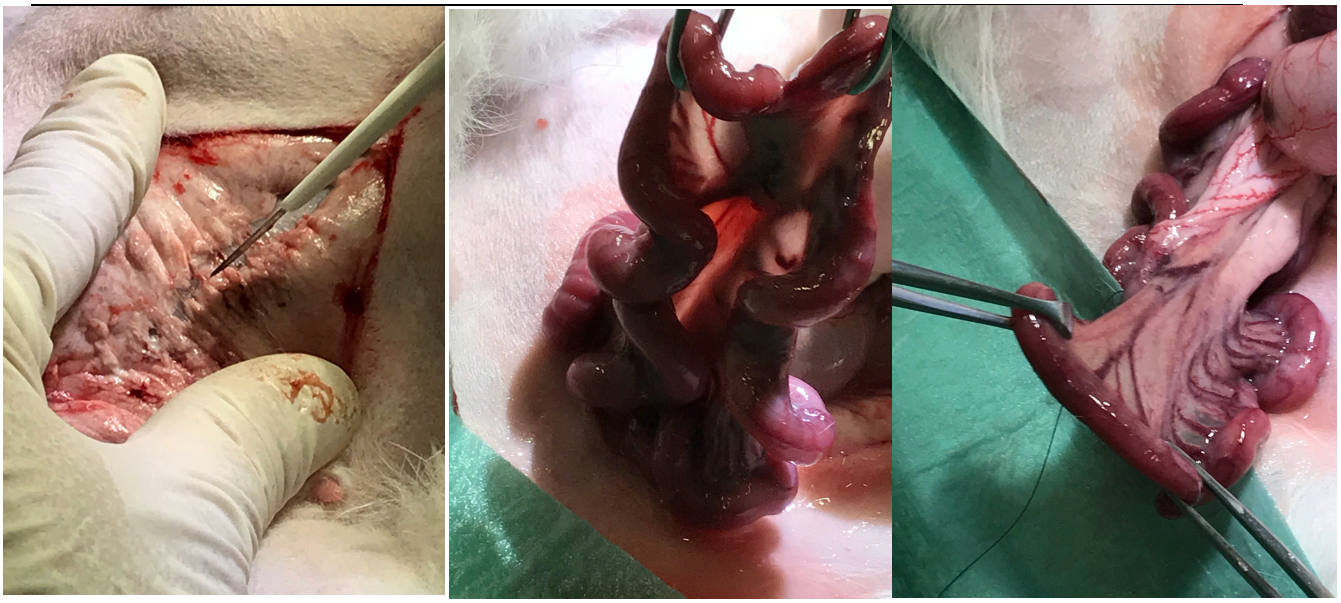


Figure 4a.5: *Midline incision (left), identification of uterus (centre) and use of atraumatic grasper to reveal section of uterus (right)*

In the absence of disposable vascular clips, a 5/0 PDS suture was tied around the base of the uterus at the level of the cervix which resulted in moderate, albeit suboptimal, occlusion of the uterine vessels. An initial incision was made on the left horn, approximately 4 centimetres in length. The

endometrial-myometrial plane was identified, and the endometrium stripped off much more easily than in the cadaveric model (**Figure 4a.6**).

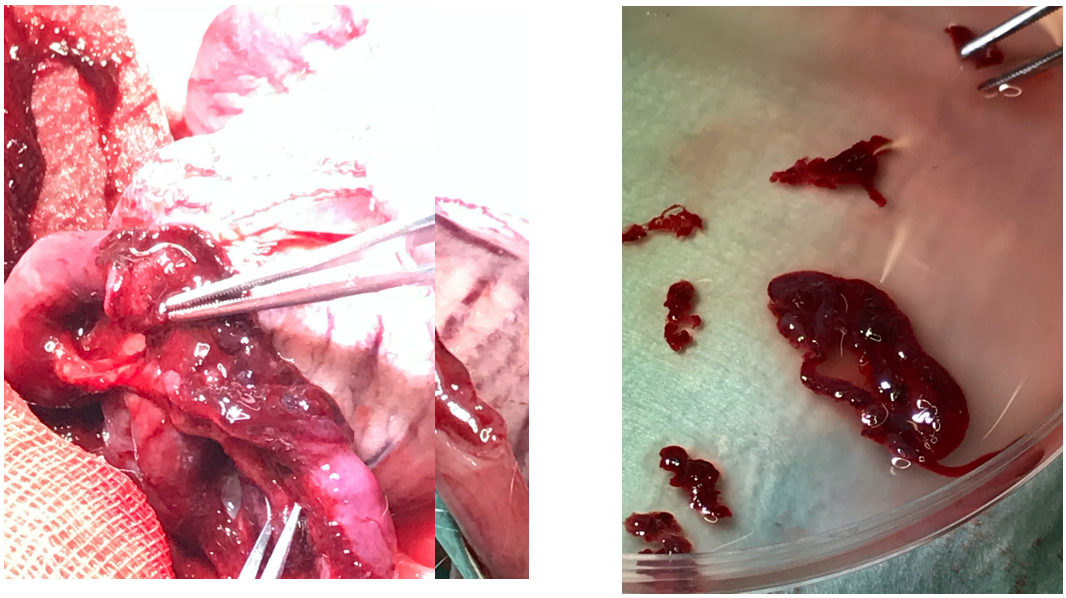


Figure 4a.6: *Midline incision on uterine horn (left), identification and resection of endometrium (centre) and excised endometrium (right)*

Despite progress being hampered by blood loss, which obscured the plane, and in the absence of additional equipment, the surgical technique appeared sound. Various pieces of endometrium were retrieved, one in particular being a very large piece which could have been divided into multiple pieces. The pieces of endometrium were sutured back into the uterus, at 0.5 cm-1cm intervals. A sample of endometrium was fixed in Bouin's solution and embedded in paraffin wax. Sections were stained with hematoxylin and eosin to ensure the full thickness of endometrium had been excised (**Figure 4a.8**).

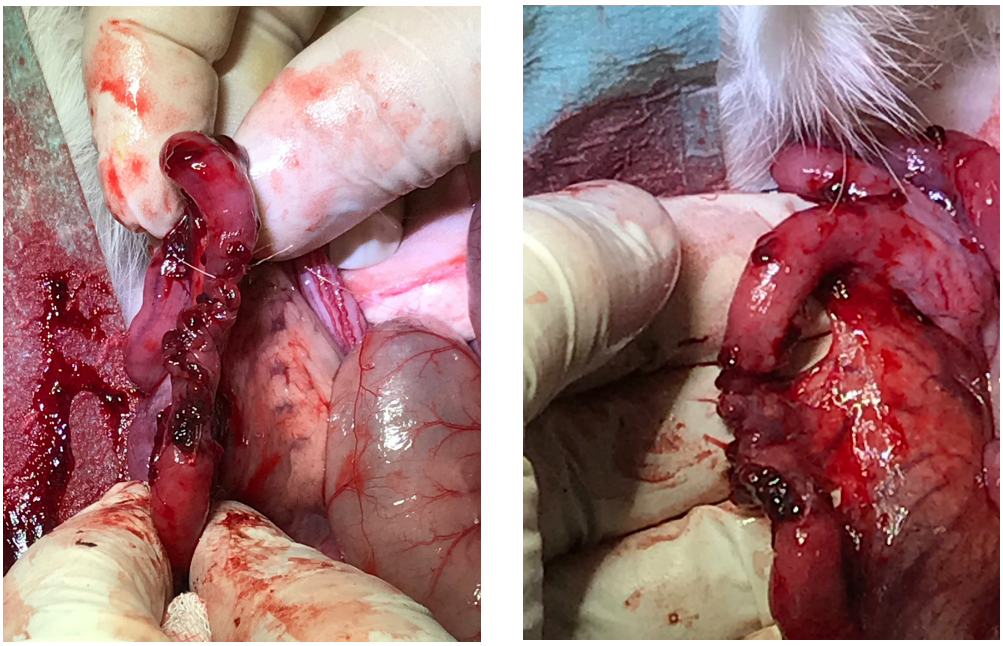


Figure 4a.7: *Horn of uterus closed, and haemostasis achieved*

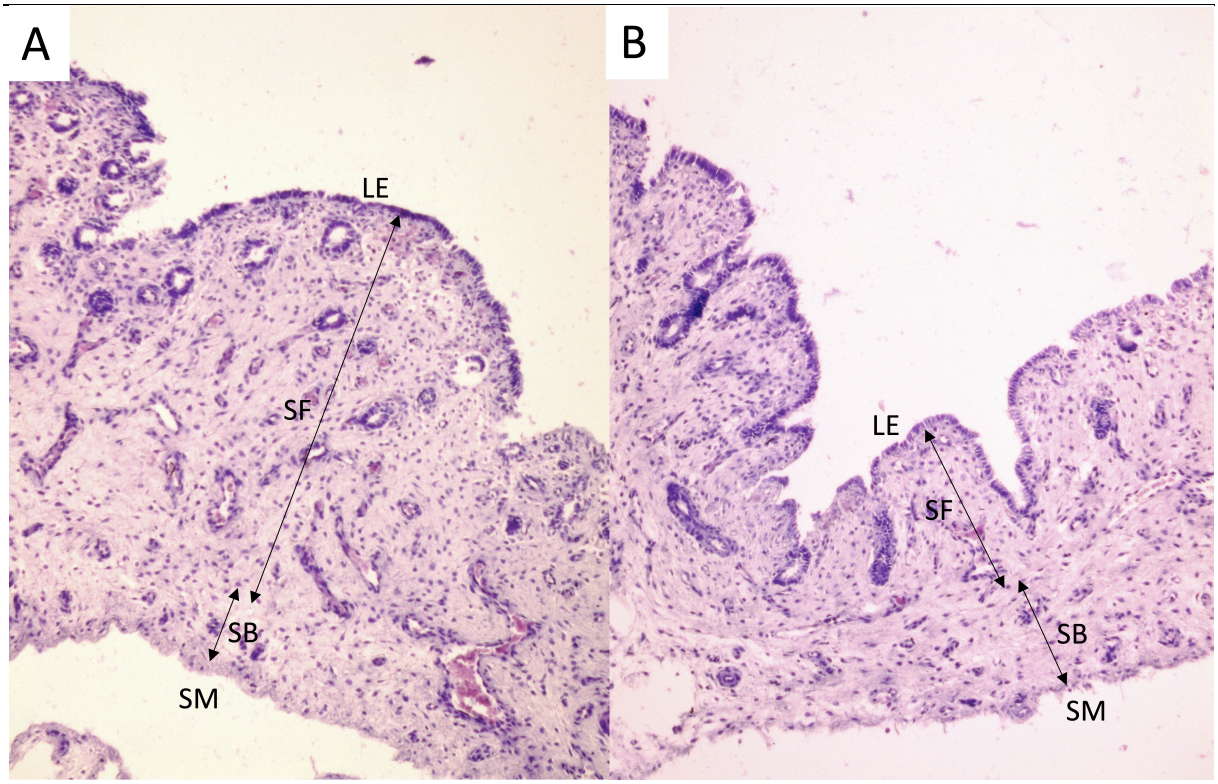


Figure 4a.8: *Histological analysis of endometrial samples excised using surgical technique (LE; Luminar epithelium, SF; Stratum functionalis, SB; Stratum basalis, SM; Submucosa)*

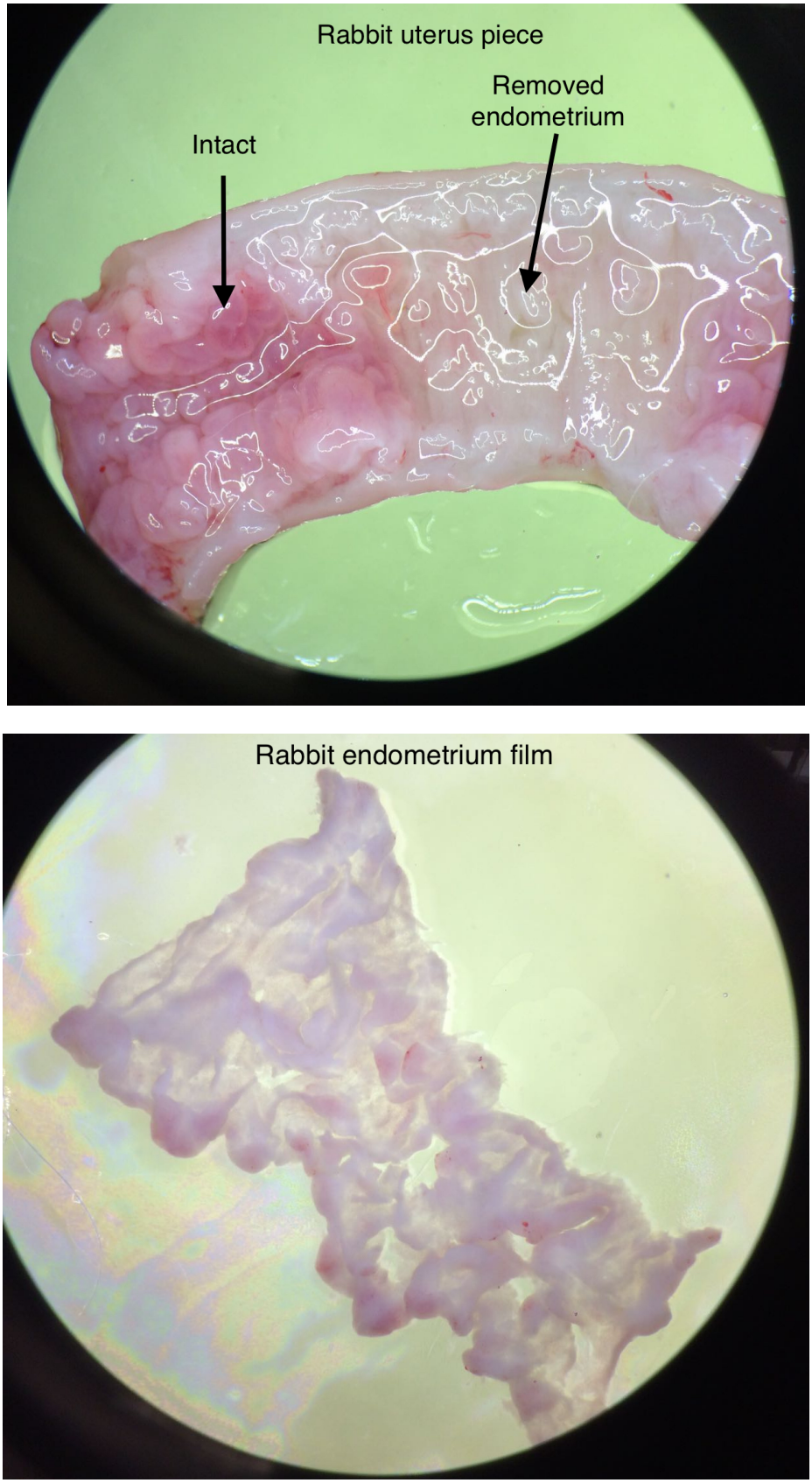


Figure 4a.9: *Macroscopic appearance of endometrium removed from myometrium (top) and laid out ex-vivo (bottom)*

These pilot studies identified that the endometrium is a resectable structure, separate to the underlying myometrium, thereby providing proof of concept. This is further demonstrated in **Figure 4a.9**. It also highlighted that by controlling ovulation, uterine size can be manipulated to facilitate the dissection and tissue planes. Moreover, the living rabbit model identified that haemorrhage may hinder the surgical technique, which may prolong operating and warm ischaemia times as well as lead to anaemia along with its associated morbidity. As such, the need for the use of temporary vascular clips was identified, to reduce blood flow transiently to the uterus during dissection and reimplantation.

Feasibility study 4 – *Impact of ovulation upon uterine size*

Rabbits ovulate following a cascade of neuroendocrine responses,[547] including a surge in luteinising hormone (LH),[548] that is naturally triggered following sensory stimulation associated with mating.[549] However, induction can be controlled in the context of ART in rabbits by utilising medications, the most common and reliable of which being the administration of GnRH, because it allows for multiple doses if necessary, and does not induce antibody formation, which may occur following repeated injections of LH or human chorionic gonadotrophin (HCG).[550] Prior to ovulation, the an-oestrous and pro-oestrous phases are characterised by thin endometrium. However, following the oestrogen driven oestrous phase, and the subsequent progesterone dominant period following ovulation, the endometrium is thickened to facilitate implantation. In rodents, Carter et al (1980) identified that in prairie voles, following ovulation there was an associated increase in uterine weight was observed within two days, which lasted for at least 10 days.[551] Following on from the observation noted from feasibility study 3, an additional preliminary study was undertaken to further determine and quantify the potential impact of ovulation, and post ovulatory hormones upon the uterine dimensions.

Two multiparous 5kg rabbits with similar reproductive histories were euthanised. In one rabbit, ovulation was induced with 1 μg buserelin acetate (Suprefact; Hoechst Marion Roussel, S.A., Madrid, Spain) which was given intramuscularly five days previously. The other rabbit did not have ovulation induced. Following midline laparotomy, isolation and removal of the uterus in both animals, the uteri

were compared as demonstrated in **Figures 4a.10** and **Figure 4a11**. The overall uterus size, including the vascular mesometrium, in the ovulation induced rabbit was 11 X 14cm, which proved much larger than that seen in the uterus which had not been influenced by ovulation (8X10cm) **Figure 4a.10**. Moreover, the endometrium was subjectively much easier to identify and more vascular and thicker in the induced ovulation rabbit (3 vs 1.5mm; **Figure 4a.11**).

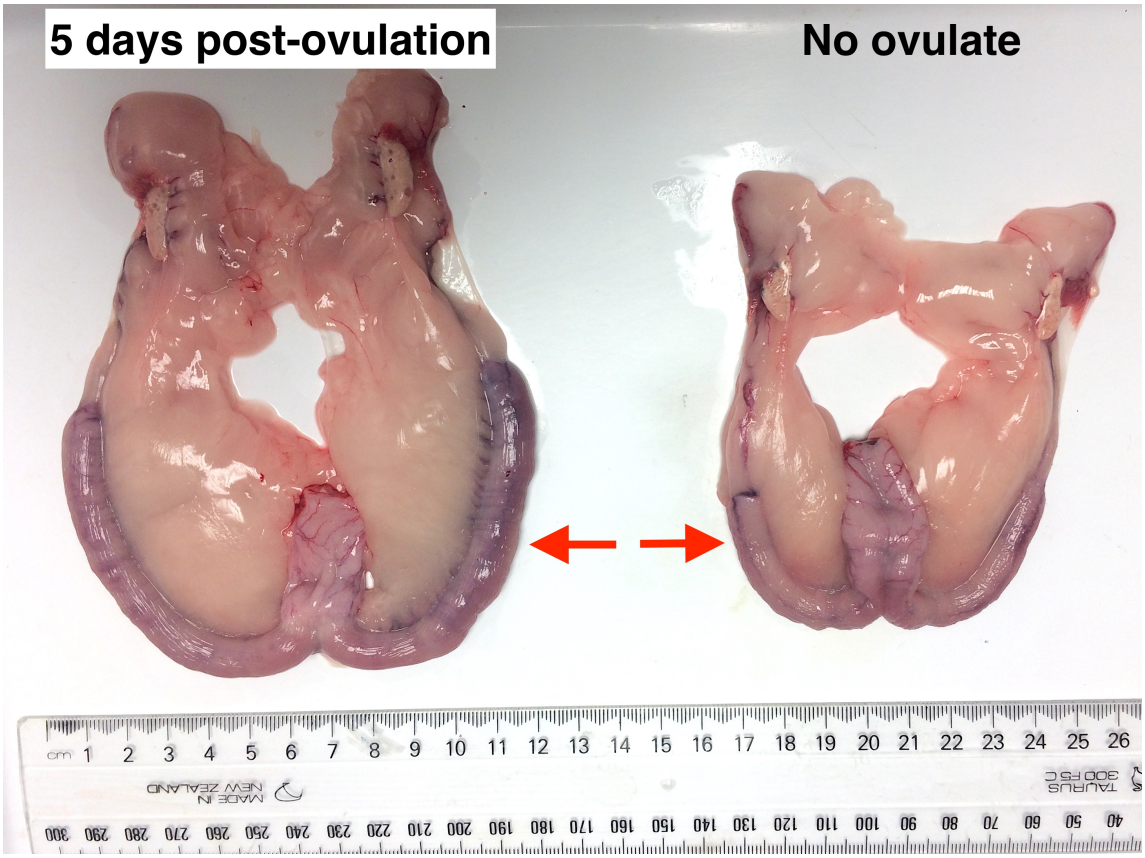


Figure 4a.10: *Demonstration of increased uterine size 5 days post ovulation, compared to no preceding ovulation*

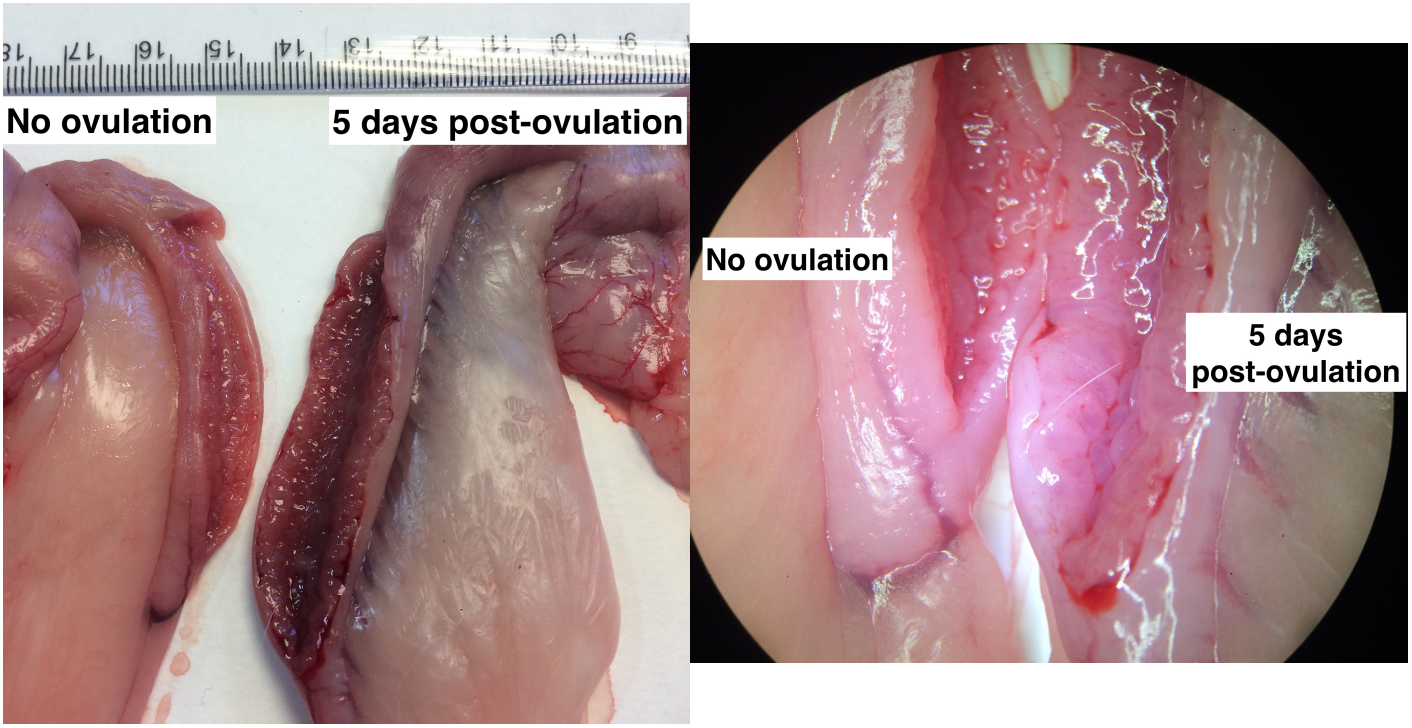


Figure 4a.11 – *Demonstration of increased uterine size and vascularity, in addition to endometrial thickness associated with post-ovulatory appearances*

This final pilot provided important insight into the potential role induction of ovulation may have in ETx. Induction of ovulation increases the size of all uterine dimensions and thereby facilitates the dissection of the intricate endometrium. However, the vascularity is also increased, highlighting the need for transient measures such as the use of temporary vascular clamps, to minimise intraoperative blood loss.

Conclusion

This series of pilot studies provided essential insight into the feasibility of ETx, ultimately demonstrating the endometrium is a distinct resectable structure and as such providing proof of concept. This series also highlighted that by controlling ovulation, uterine size can be manipulated to facilitate the dissection and tissue planes and highlighted the need for the use of temporary vascular clips to reduce haemorrhage.

Study 4b

Endometrial auto-transplantation in a rabbit model

Introduction

Following the successful demonstration of the surgical technique described in the feasibility studies in study 4a, progression was made to the 'development' and 'exploration' phases of the IDEAL framework.[546] As is often the case in innovative surgical techniques, this entails a methodical progression through varying animal models, initially starting in small animals, with progression into large animals, before undertaking work in primates, which show the closest similarity to humans. The use of animal studies allows various aspects of the procedure to be addressed, including clinical, radiological, biochemical and reproductive outcomes. The rabbit model provides an appropriate model to undertake preliminary studies due to a number of reasons. Not only do they have the advantage of being an intermediate size between rodents and larger animals like primates, but they have a longer life span when compared to other animals such as rodents.[552] Furthermore, female rabbits have a duplex uterus with two separate uterine horns and two cervices, which makes them an ideal candidate for reproductive studies. In addition, the gestation period of between 27-30 days presents an ideal opportunity to assess subsequent reproductive outcomes, subject to surgical success. In the innovation of transplant procedures, it is not only the surgical technique that requires assessment, but also the additional immunological complexities associated with transplantation.

Whereas the immunological genes of rabbits have been demonstrated to be closer to those of humans than other small animals such as rodents,[553] an autotransplant model is most appropriate in the first instance, to control for potential damage that may be caused by rejection, and negates the intricacies associated with the necessity to take immunosuppressive medications. This minimalisation of variables therefore isolates the outcomes to be directly related to the surgical technique, and therefore helps ascertain feasibility of the concept.

The aim of this study was to assess the feasibility of ETx by undertaking a short-term viability study on endometrial auto-transplantation in the rabbit model.

Methodology

Subjects

Ten female adult rabbits were attained from the Universitat Politècnica de València in Spain. The rabbits were initiated from a line based on reproductive longevity criteria by selecting females from commercial farms, irrespective of their origin. The initial criteria for selection included a minimum of 25 prior pregnancies with > 7.5 livebirths per partum. They were subsequently preferentially selected by litter size at weaning for 12 generations. The rabbits were maintained on a 12:12-h light: dark cycle at a constant temperature of 20°C (± 1 °C) with relative humidity between 40% and 60%.
The animals were transferred 1-2 weeks pre-operatively to allow appropriate acclimatisation to the new environment. The animals were fed pelleted concentrated feed twice a day and had free access to hay and water as required.

Study design

Rabbits were randomly assigned to two groups; group A and group B. Females in group A underwent endometrial resection of the right uteri with subsequent re-implantation into the same uterus (n=8). Females in group B underwent endometrial resection of the right uteri, without subsequent re-implantation (n=2). In both groups, the endometrium within the left uterus was left intact as a control side. Group A was used to assess the feasibility of ETx, whilst group B was used to control for endometrial auto-regeneration.

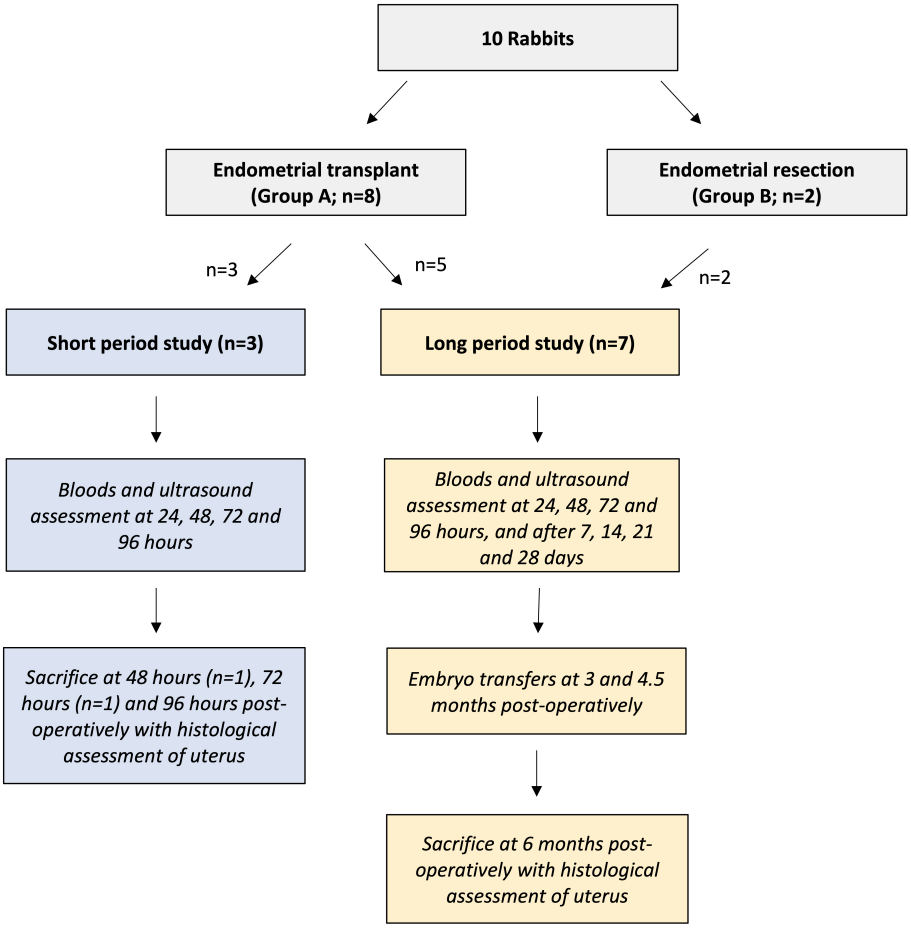


Figure 4b.1: *Study design*

The experiment was divided into short and long periods. The 'short period' experiment (n=3), from 0 to 96 hours post-operatively, was developed to assess the immediate post-operative outcomes and early endometrial response by euthanising a single animal at 48, 72 and 96 hours, with subsequent macroscopic and histological assessment. The 'long period' experiment (n=7) was developed to evaluate long term clinical and reproductive outcomes, up to 6 months post-operatively, through fertility treatment and pregnancy until death or euthanasia. The study design is described in **Figure 4b.1**

Surgical technique

A 4-5cm midline laparotomy was undertaken. Following identification of the uterus, it was exteriorised (**Figure 4b.2; A**), and an avascular window was identified in the broad ligament. Single use vascular clips (Vascu-Statt® II, Scanlan, St. Paul, MN, USA) were used to occlude the uterine arterio-venous circulation (**Figure 4b.2; B**). A midline incision was subsequently made along the length of the right uterus (**Figure 4b.2; C**). Using cold knife dissection, a plane was developed between the endometrio-myometrium interface. A tri-phasic technique was subsequently implemented, dissecting each side laterally from the edge to the mesenteric border, before removing the endometrium in its entirety from the cervix to the Fallopian tube (**Figure 4b.2; D**).

Following retrieval (**Figure 4b.2; E**), the endometrial pieces were stored in cold UW solution (**Figure 4b.2; F**), before being re-implanted in multiple 1-2cm pieces with 5/0 polypropylene (Prolene) (**Figure 4b.2; H+I**). The uterus was subsequently closed with 5/0 Polypropylene (Prolene) before the vascular clamps were removed (**Figure 4b.2; J**). Abdominal closure was performed with 3/0 polydiaxone (PDS) and 3/0 polyglactin 910 (Vicryl) was used to close the skin (**Figure 4b.2; K**). The left uterus was left untouched as a control in all cases. The two endometrial resection procedures included the same surgical retrieval technique but following excision of the endometrium, it was not re-implanted, and the uterus and abdomen were closed in the same fashion.

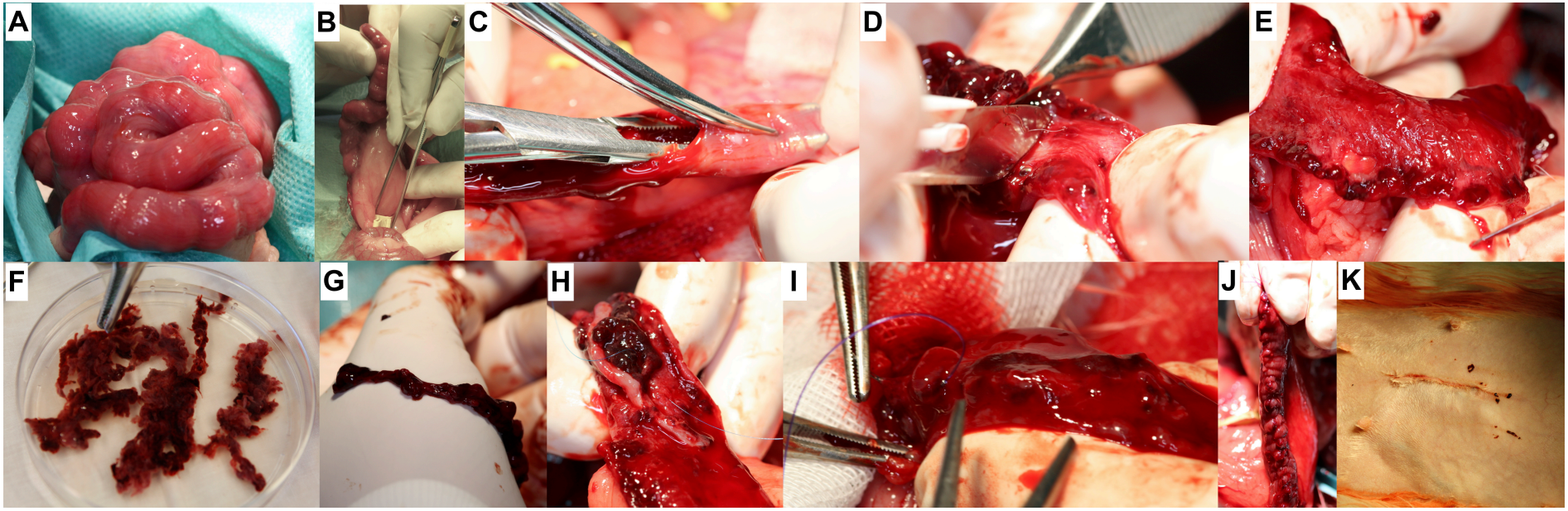


Figure 4b.2: *Endometrial transplantation surgical technique*

Haematology and Biochemistry

Peripheral blood samples were obtained before surgery, after 24, 48, 72 and 96 hours post-operatively (short study) and on the 7th, 14th, 21st and 28th day (long study). Haematological and biochemical analysis was undertaken using FBC, including white blood cells (WBCs), lymphocytes, monocytes and granulocytes in addition to a C-Reactive Protein (CRP). Within 10 minutes of collection, samples were analysed using an automated haematology analyser (MS 4e automated cell counter, Melet Schloesing Laboratories, France), according to manufacturer instructions. CRP concentration was measured using a human immunoturbidimetric test (CRP OSR 6147 Olympus Life and Material Science Europe GmbH, Lismeehan, O'Callaghan Mills, Co. Clare, Ireland).

Ultrasound examination

Transabdominal ultrasound (4–12 MHz linear probe, MyLab 60 machine, Esaote, Spain) was used to monitor uterine dimensions (maximum diameter in midsagittal section; **Figure 4b.3**) at 24, 48, 72 and 96 hours and on the 7th, 14st, 21st and 28st post-operative day. The does were awake and unsedated during these examinations.

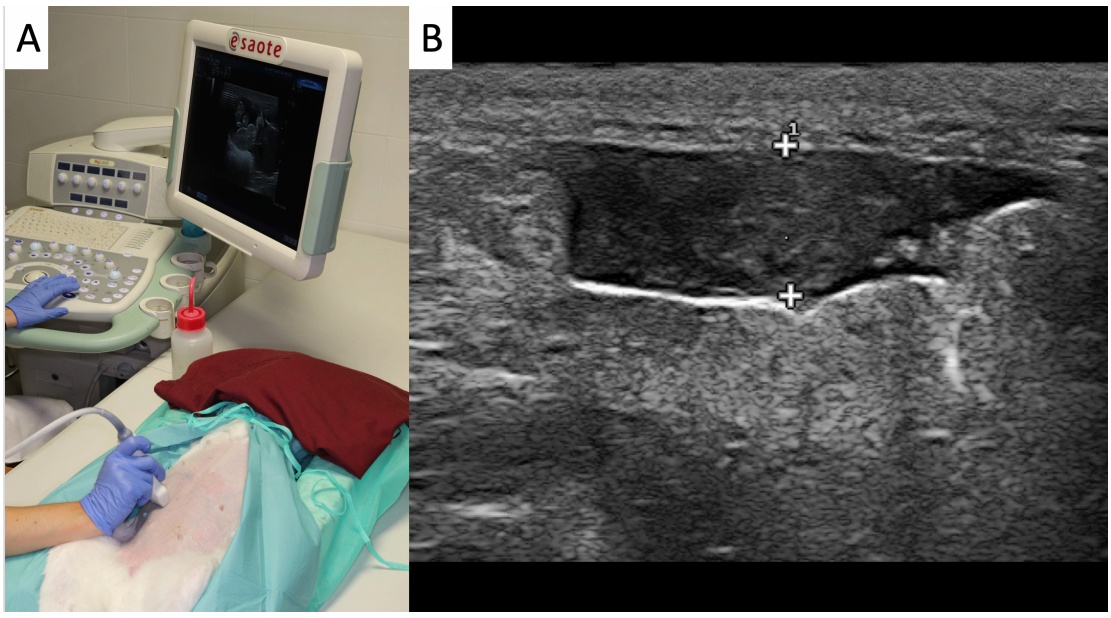


Figure 4b.3: *A) Demonstration of post-operative ultrasound technique and B) Measurement of maximal mid-sagittal uterine depth*

Histopathology

A single rabbit was euthanised at 48, 72 and 96 hours after surgery, for macroscopic and histological assessment, as part of the short study protocol. In the remaining rabbits, similar assessments were undertaken in the event of unexpected death or following euthanasia at 6 months after ETx. Samples were fixed in Bouin's solution and embedded in paraffin wax. Sections were stained with hematoxylin and eosin as per standardised protocols.

Embryo transfer

Four does, separate from the transplant group, were prepared for embryo donation with 3 µg of long-acting follicle stimulating hormone agonist (Corifollitropin alfa; Elonva, Merck Sharp & Dohme S.A, Spain) as described in detail in peer reviewed literature.[554] Ovulation was induced with 1 µg buserelin acetate (Suprefact; Hoechst Marion Roussel, S.A., Madrid, Spain) given intramuscularly. Females were inseminated with 1ml of pooled semen 60 h after gonadotropin injection, and donor females were euthanised at 72 h post-insemination. A total of 84 morulae were recovered by perfusion of each oviduct and uterine horn with 10 mL pre-warmed Dulbecco Phosphate Buffered Saline supplemented with 0.2% of Bovine Serum Albumin. Fresh embryos were transferred into oviducts laparoscopically to 3 does, in two consecutive sessions, utilising the procedure described in detail previously.[555] Ovulation was induced in recipient does (2 ETx females and 1 endometrial resection female), with an intramuscular dose of 1 mg of buserelin acetate 72 h before transfer. Reproductive outcomes were assessed using implantation rate, which was assessed laparoscopically 14 days following embryo transfer, and livebirth rate.

Details of ethics approval

This study was undertaken at the Institute of Science and Animal Technology (ICTA), at the Polytechnic University of Valencia in Spain. All animals were handled in accordance with the principles of animal care published by Spanish Royal Decree 53/2013 (BOE 2013). The experiments

were approved by the Committee of Ethics and Animal Welfare Committee of the Polytechnic University of Valencia (2017/VSC/PEA/00130)

Statistics

Statistical analysis was performed with SPSS 21.0 software package. Descriptive statistical analysis was described as mean +/- SD. A general linear model was fitted for the analysis of FBC (WBCs, lymphocytes, monocytes and granulocytes), CRP and uterine dimensions, including the experimental group (ETx and ER) as fixed effect. Differences of $p<0.05$ were considered significant.

Results

Ten procedures were performed, including 8 endometrial auto-transplants and 2 endometrial resections. The key surgical data is summarised in **Table 4b.1**. Eight procedures were successful, but two rabbits from the ETx group died intra-operatively, one due to anaesthetic related issues, and the other due to haemorrhage which contributed to difficulty in titrating anaesthetic concentrations. The average rabbit weight was 5.3 +/- 0.3kg. The mean retrieval and implantation times were 35.2 +/- 15.9 minutes and 14.8 +/- 3.6 minutes respectively. The number of pieces of endometrium retrieved per case was 10.2 (+/- 5.2), whilst the number re-implanted was 4.9 +/- 1.0. The average vascular clamp duration was 52.1 +/- 15.8 minutes. The mean overall surgery and anaesthetic times were 1h 22hm +/- 20.8m and 1h 43m +/- 25.9m respectively. The mean estimated blood loss was 17.0 +/- 10.6mls.

Serum trends in haematology and biochemistry 0-96 hours post-operatively are displayed in **Figure 4b.4**. Total and differential WBC counts were initially elevated post-operatively, and significantly higher WBC counts were observed at 48 hours after ETx (4.3 +/- 2.79 Vs 15.4 +/- 2.79; p=0.05), with the exception of granulocytes (p=0.143). There was no significant difference in CRP between ETx and ER animals during the first 48 hours post-operatively, but between 72-96 hours the CRP was significantly higher in the ETx group (155.3+/-15.79 Vs 95.5+/-15.79 for 72 hours; p=0.08 and 147.2+/-15.79 Vs 92.7+/-15.79 for 96 hours; p=0.046).

	Rabbit 1	Rabbit 2	Rabbit 3	Rabbit 4	Rabbit 5	Rabbit 6	Rabbit 7	Rabbit 8	Rabbit 9	Rabbit 10	Mean (+/-SD)
Weight (g)	5200	5000	5300	5500	5000	5600	5700	5100	5800	5000	5320 +/- 308.4
Resection time (min)	61	30	19	51	43	47	43	15	24	19	35.2 +/- 15.9
Number of pieces retrieved	<5mm – 6 5-10mm – 4 >10 - 5	40mm – 2 20mm – 2 <20 - 4	10-20mm – 5 5-10mm - 11	50-60-mm - 1 30-40 - 1 10-20mm – 2 5-10mm - 3	20-30mm - 1 10-20mm - 0 5-10mm – 2 <5-mm - 16	10-20mm - 3 5-10mm – 4 <5-mm – 0	10-20mm - 1 5-10mm – 10 <5-mm – 0	10-20mm - 1 5-10mm – 10 <5-mm – 0	70-80mm - 1 30-40mm – 1	10-20mm - 6	10.2 +/- 5.2
Time of clamps (min)	65	68	30	78	52	55	51	28	51	43	52.1 +/- 15.8
Implantation time (min)	n/a	18	20	17	n/a	16	9	12	13	13	14.8 +/- 3.6
Number of pieces re-implanted	n/a	40mm X1 20mm X 2	5 X 10mm	1 X 5-6cm 2 x 2cm 1 X 1cm	n/a	3 X 10-20mm 2 X 5-10mm	1 X 10-20mm 4 X 5-10mm	1 X 10-20mm 5 X 5-10mm	5 X 10-20mm 1 x 5-10mm	10-20mm - 5	4.9 +/- 1.0
Operation time (min)	106	107	68	117	72	72	n/a	65	72	66	82.8 +/- 20.8
Anaesthesia time (min)	130	139	94	142	93	93	n/a	80	88	75	103.8 +/- 25.9
EBL (mls)	10	20	10	30	10	10	40	10	20	10	17 +/- 10.6
IVF attempted	No	2 cycles	No	No	2 cycles	No	No	No	2 cycles	No	
Pregnancies in operated horn	No	Cycle 1 - 2 Cycle 2 - 1	n/a	n/a	No	n/a	n/a	n/a	Cycle 1 - 2 Cycle 2 - 0	n/a	
Pregnancies in control horn	n/a	Cycle 1 - 3 Cycle 2 - 3	n/a	n/a	Cycle 1 - 5 Cycle 2 - 5	n/a	n/a	n/a	Cycle 1 - 4 Cycle 2 - 5	n/a	
Deceased	Died 1-month post op	Euthanised after 2 cycles IVF	Euthanised 96 hours post op	Euthanised 72 hours post op	Yes - sacrificed after 2 cycles IVF	Died intra-operatively due to anaesthesia	Died intra-operatively due to blood loss / anaesthesia	Euthanised 48 hours post-op	Euthanised after 2 cycles IVF	Died 2 months post-op	

Table 4b.1: *Surgical data and post-operative data including reproductive outcomes*

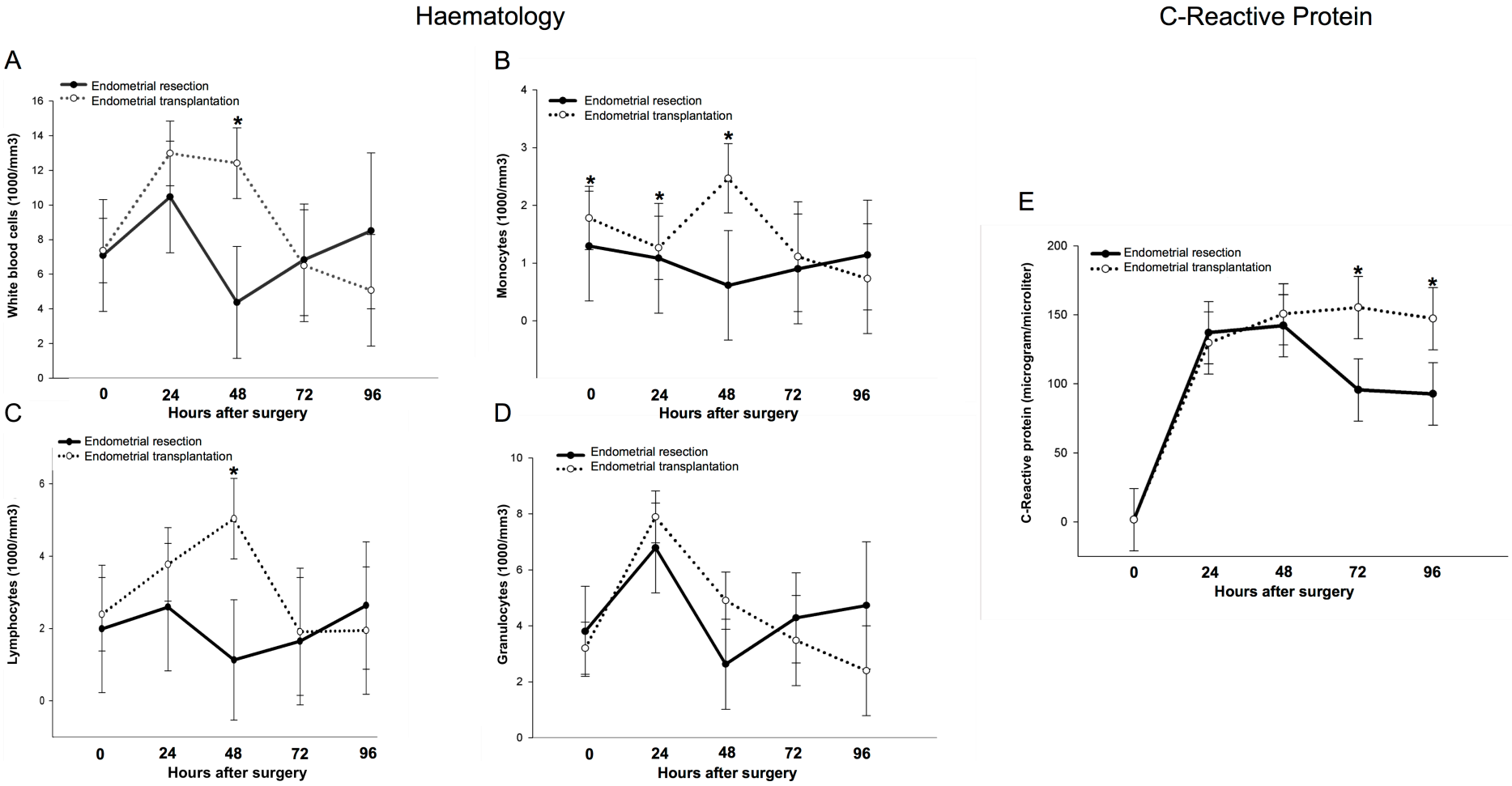


Figure 4b.4: *Comparison of haematological parameters and C-reactive protein (CRP) trends during first 96 hours between endometrial transplantation and endometrial resection groups. *denotes significant differences between experimental groups.*

Ultrasonographically, comparison of uterine depth post-operatively between ETx, ER and the intact uteri is displayed in **Figure 4b.5**. There was a significant difference in uterine depth between ETx animals and intact uterus groups until 22 days (528 hours) post-operatively ($p<0.001$). From 1 month (696 hours) onwards uterus depth was similar between all experimental groups (8.2+/-0.61 Vs 7.2+/-0.61 Vs 6.9+/-0.61 for ETx, ER and the intact uteri at 696 hours, respectively; $p=0.195$).

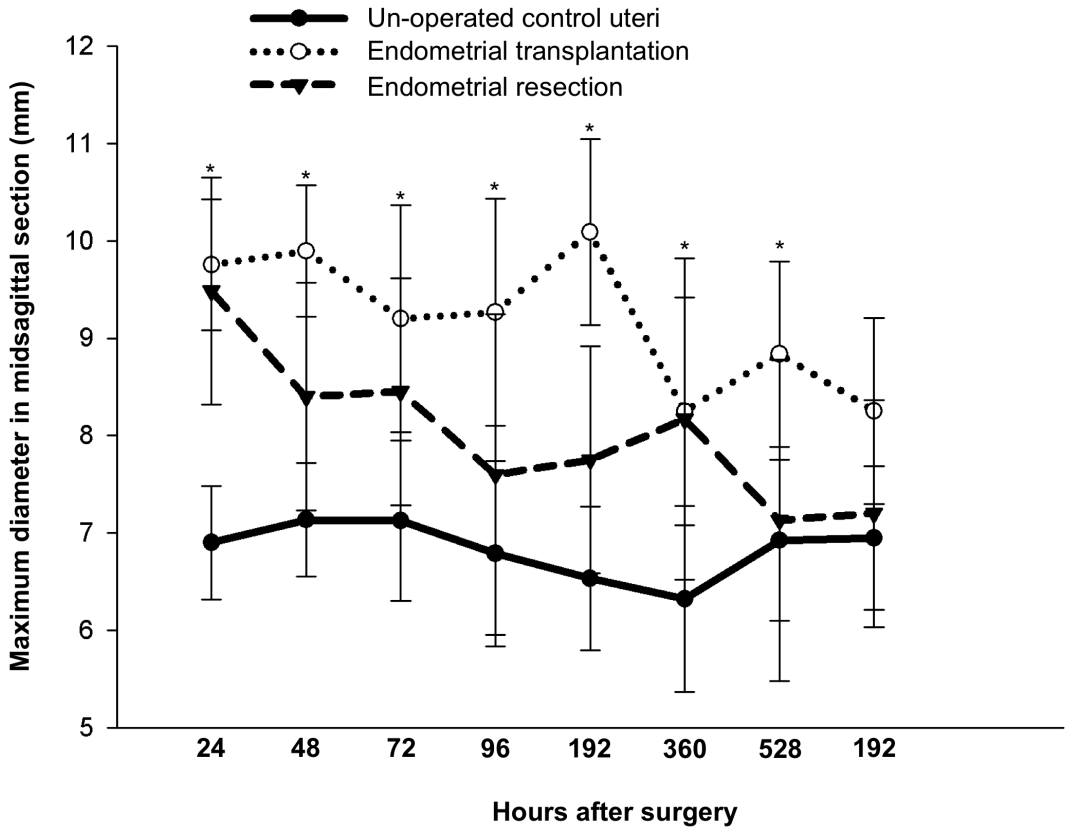


Figure 4b.5: *Comparison of maximum uterine depth between un-operated control uteri, endometrial transplantation and endometrial resection groups throughout study. *denotes significant differences between experimental groups.*

Two rabbits, one from the ETx group and one from the ER group, died four weeks and eight post-operatively of undetermined cause. Three rabbits subsequently underwent embryo transfer using the pooled embryos. Reproductive outcomes are shown in **Table 4b.1**. During the first cycle, undertaken three months post-operatively, seven embryos were transferred into each uterus of the three remaining animals, two of which were following ETx and the other following ER.

All three recipient animals subsequently became pregnant (**Figure 4b.6**). In the un-operated uteri, an implantation rate of 57% (12 implantations) was observed. In the operated uteri of the two ETx rabbits, an implantation rate of 28.5% (4 implantations) was observed. In the operated uterus of the ER female, an implantation rate of 0% was observed. In the second cycle, undertaken 4.5 months post-operatively, a further seven embryos were transferred into each uterus of the three females. As previously, all three recipient animals became pregnant (**Figure 4b.7**). In the un-operated uteri, an implantation rate of 61.9% (13 implantations) was seen. In the two ETx females, an implantation rate of 14.3% (2 implantations) was observed. As previously, no embryo implantation was seen after ER. Despite successful implantations in both cycles in the ETx rabbits, no livebirths were achieved.

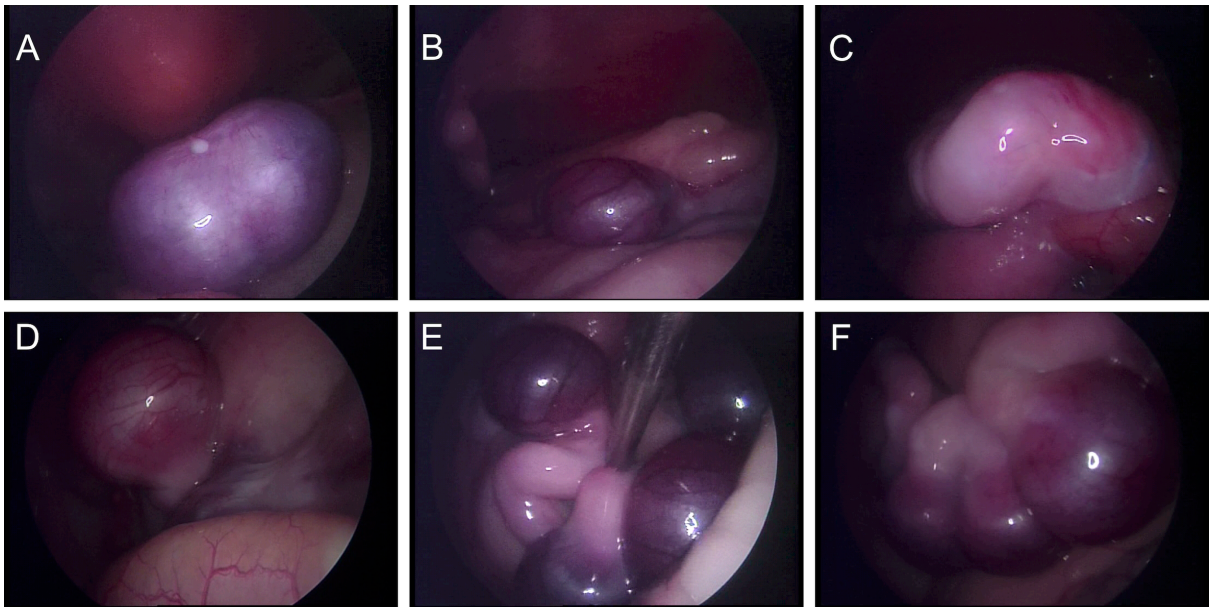


Figure 4b.6: *Laparoscopic identification of implantation during first IVF cycle, with evidence of pregnancies in both ETx uteri (A&B), no evidence of pregnancy in the ER uteri (C), and confirmation of pregnancies in the control uteri (D-F)*

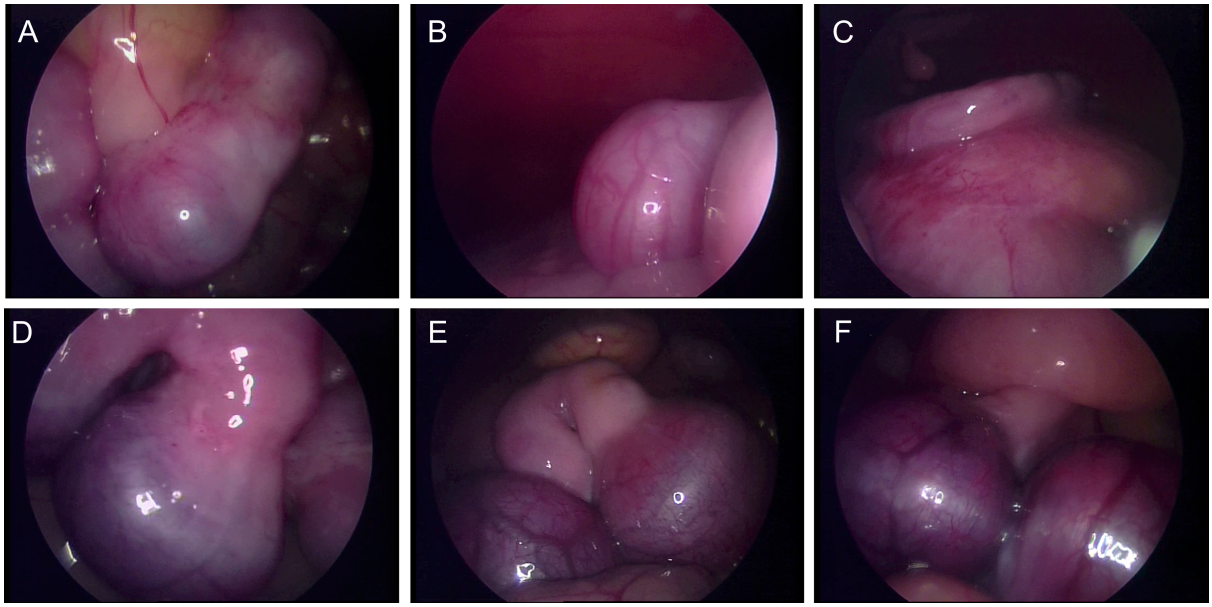


Figure 4b.7: *Laparoscopic identification of implantation during second IVF cycle, with evidence of pregnancies in one ETx uteri (A), no evidence of pregnancy in the other ETx or the ER uteri (B-C), and confirmation of pregnancies in the control uteri (D-F)*

Following euthanasia of the rabbits at 48, 72 and 96 hours, macroscopic evaluation of the endometrium identified healthy-appearing endometrium after ETx, as early as 96 hours post-operatively (**Figure 4b.8**). Subsequent histological assessment demonstrated the fingerlike projections associated with normal endometrium (**Figure 4b.9; A**) but with stromal haemorrhage and scanty surface erosion (**Figure 4b.9; B**). The gross appearance of the endometrium six months following ETx (**Figure 4b.10; B**), was similar to normal endometrium (**Figure 4b.10; A**), and fetal tissue was evident (**Figure 4b.10; C**). The presence of viable appearing convoluted endometrium with no surface erosions was subsequently confirmed histologically (**Figure 4b.9; D**). The uterus of the ER rabbit euthanised six months post-operatively had a macroscopically scarred cavity with no villous endometrium (**Figure 4b.10; D**) and with marked absence of the fingerlike projections associated with normal endometrium on histology (**Figure 4b.9; C**).

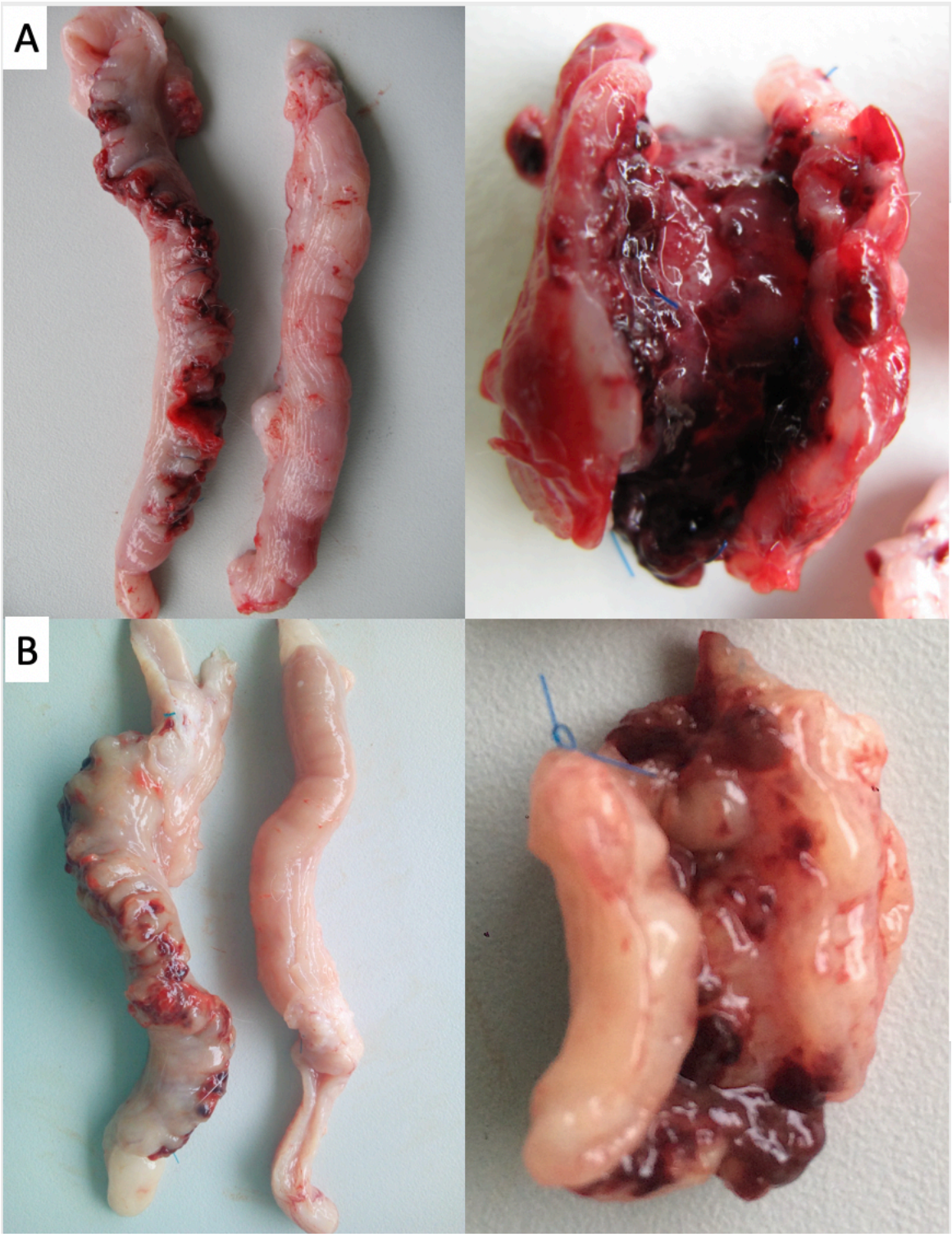


Figure 4b.8: *Comparison of the macroscopic appearance of the uterine cavity between A) 48 hours post endometrial transplantation and B) 96 hours post endometrial transplantation*

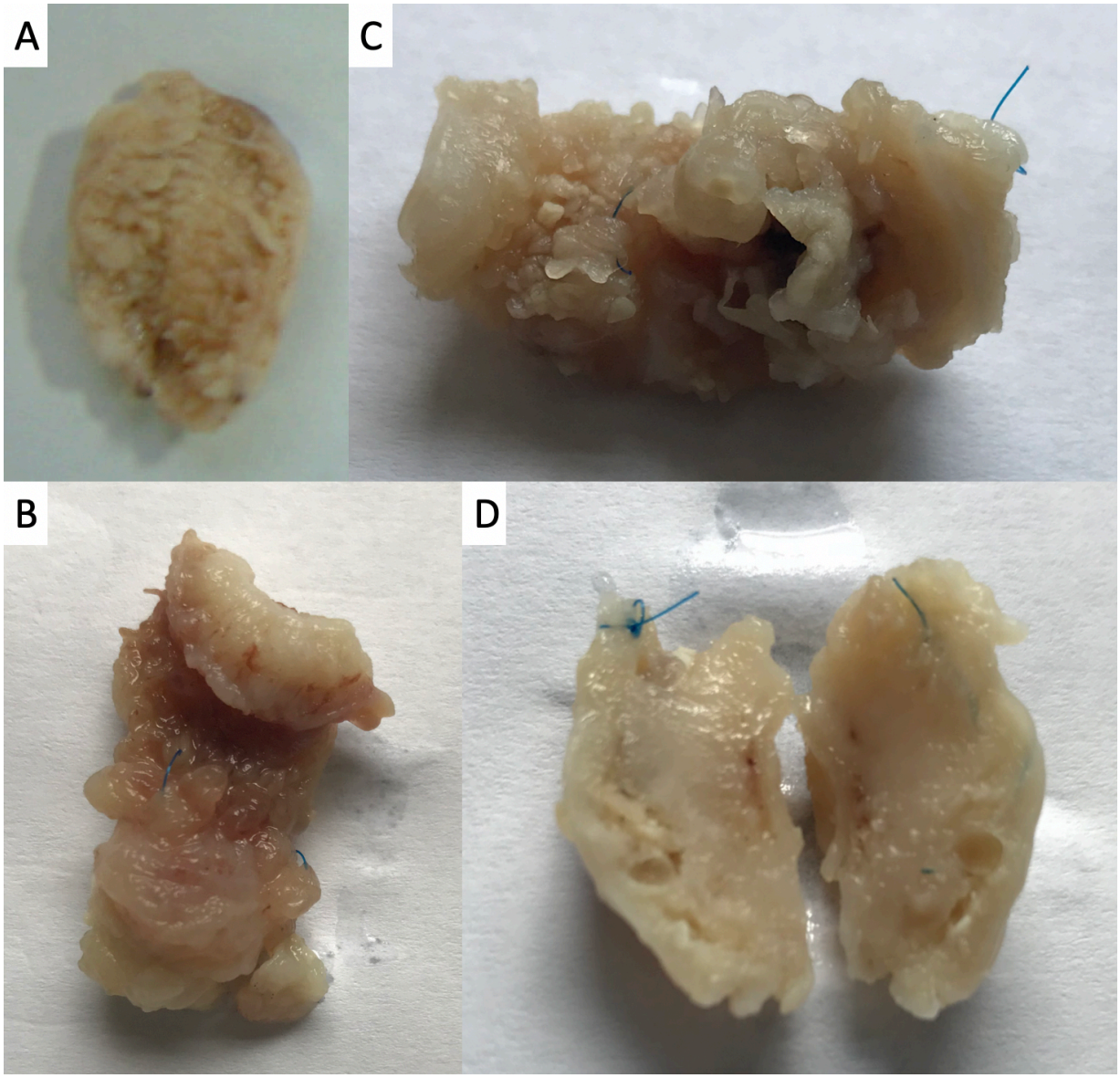


Figure 4b.9: *Comparison of the macroscopic appearance of the uterine cavity between A) an un-operated control uterus, B) 6 months post-operatively after IVF, C) 6 months post-operatively after IVF with presence of fetal tissue and D) 6 months after endometrial resection*

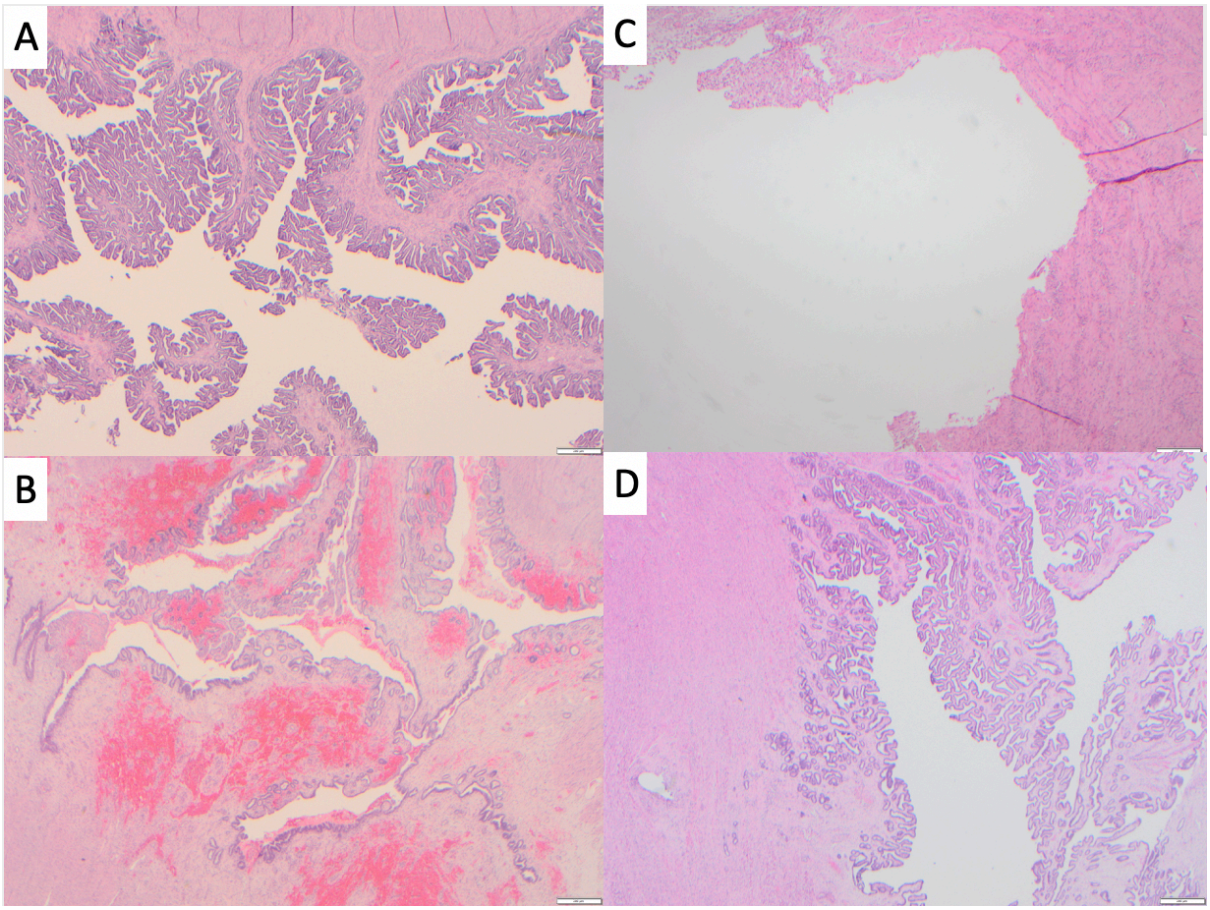


Figure 4b.10: *Comparison of the macroscopic appearance of the uterine cavity between A) an un-operated control uterus, B) 6 months post-operatively after IVF, C) 6 months post-operatively after IVF with presence of fetal tissue and D) 6 months after endometrial resection*

Discussion

The data presented herein demonstrates, for the first time, gross and microscopic evidence of viable endometrium following ETx, along with the achievement of clinical pregnancies, albeit in the absence of achieving successful livebirth. The main limitation of this study was the failure to achieve a livebirth following ETx, and the inferior reproductive outcomes when compared to the contralateral 'control' uteri. Despite evidence of viability on gross and microscopic appearance, it is likely defective tissue vascularisation may have been a contributing factor, leading to problems with implantation of the embryo and pregnancy loss. Restricted endovascular invasion during placentation can also lead to several antenatal complications including intra-uterine growth restriction, preterm

birth and intra-uterine death, as demonstrated following pregnancies achieved in women after endometrial ablation.[556] For ETx to become a feasible treatment for women with AS, future studies will need to consider ways to potential re-vascularisation between the endometrio-myometrial interface, in order to optimise the chances of a livebirth. A further limitation includes the small sample size, although following the proof of concept demonstrated herein, this paves the way for further studies to expand upon.

When compared to UTx, ETx involves the transplant of less tissue, which may result in less immunogenic burden. However, the use of immunosuppression will remain essential to prevent rejection. Whereby cervical biopsies have been shown to be a consistent and achievable method of detecting rejection in the graft in UTx,[56,123,131] ETx would instead demand hysteroscopy and endometrial biopsy, which increases the opportunity for bacteria to be introduced inside the cavity. Whilst endometrial biopsies have been shown in the baboon model to be less consistent in the detection of rejection of the uterus than cervical biopies,[131] it would be the only way to prove the diagnosis of rejection after ETx histologically. Moreover, the use of hysteroscopic guided endometrial biopsies have successfully been used in cases of UTx performed in India,[184,185] although detailed immunological outcomes and correlation between endometrial and cervical biopsies are awaited.

When compared to the application of autologous stem cell treatment however, ETx is associated with greater risk. From a surgical perspective it would likely require a longer operative time, and necessite an open surgical approach during implantation, in addition to further major operations, including Caesarean sections to deliver subsequent offspring, and a hysterectomy after the completion of the recipient's family. In addition, ETx would necessitate the use of immunosuppression, whereas autologous stem cell treatment does not, and as such ETx has the additional complexity of immunosuppression related burden. A recent systematic review and metanalysis of outcomes following autologous stem cell therapy demonstrated it to be highly effective at restoring menstruation, increasing endometrial thickness, and achieving pregnancy in women who were previous refractory to conventional treatments for AS.[557] In addition, no severe adverse reactions were

observed following treatment with stem cell therapy, with just three patients out of 88 (3.57%) cramps, which resolved spontaneously. Whereas stem cell therapy certainly appears promising, with just eight small studies reporting outcomes of 88 women, it remains in its infancy, and longer term follow up of larger numbers of women are required.

Conclusion

This study demonstrates gross and microscopic evidence of viable endometrium following ETx, along with the achievement of clinical pregnancies. Given the inadequate clinical and reproductive outcomes following conservative management of AS and the challenges that novel bioengineering strategies present, this may present a novel avenue for women with AUFI secondary to AS to acquire motherhood. Despite the promising results demonstrated herein, it is essential further studies are undertaken, in particular to assess and optimise pregnancy development after ETx. Moreover, further data is needed to assess the impact of cold and warm ischaemia times, as well as the allotransplant studies, before this is considered in human models.

Study 5a

Elective oocyte cryopreservation; perceptions and regret following social egg freezing

Introduction

Reproductive planning is a dynamic concept, orientated around parenthood aspirations, which are influenced by each individual's core values, in the context of the resources available to that individual. Societal evolution over the last half a century has led to gender equality advancement which has seen greater educational and professional opportunity for women worldwide. This has contributed to the current trend of women delaying their childbearing years, which has led to a rise in age of first-time motherhood. However, given the inevitable physiological decline in ovarian reserve and oocyte quality as age advances, the risky trend of deferring motherhood for 'social' reasons has been associated with increasing rates of involuntary childlessness.[366]

Following the demonstration of comparable pregnancy and livebirth rates between vitrified and fresh oocytes,[369] the possibility of electively cryopreserving oocytes at a younger age for use at a more convenient time in the future has become realistic, a concept which has since been referred to as social egg freezing (SEF). Whilst some women however have no reasonable alternative, such as single women approaching their late thirties,[153] SEF cannot guarantee against involuntary childlessness because of the possibility of unsuccessful future embryo transfers leading to exhaustion of the stored oocytes, usually at an age when there is no possibility of retrieving anymore. Moreover, the risk of age-related obstetric complications remains, and there are significant economic implications which continue to make it a controversial option to extend women's reproductive years.[372]

Despite the upward trend in women electively cryopreserving their oocytes, there is little data about women's perceptions, having undergone the process, particularly in a UK setting. An initial study demonstrated in a sample of 65 women that no women experienced regret following SEF.[558] Conversely, a more recent study identified that almost half (49%) of women experience mild-moderate regret after undergoing the process,[559] highlighting discrepancy. The objective of this study was to investigate the motivations of women who have undergone SEF in a UK setting, identify their

perceptions following treatment, assess the impact upon treatment on their quality of life, and evaluate feelings of regret.

Materials and methods

An electronic questionnaire was sent to all the women who had consented to be contacted for research purposes after undergoing SEF at the Centre for Reproductive and Genetic Health in London between January 2008 and December 2018. It consisted of 73-questions (**Appendix 5a.1**) and was distributed via e-mail through SurveyMonkey between 1st September 2018 and 31st March 2019. A retrospective case note review was concurrently undertaken to ascertain data from the SEF treatment cycles including age at treatment, number of cycles and numbers of oocytes retrieved and cryopreserved.

Clinical Protocols

During the SEF treatment, all women underwent controlled ovarian stimulation with GnRH antagonist suppression. Gonadotrophin was commenced on day 3 with Fostimon (IBSA, Switzerland) and Merional (IBSA, Switzerland) or menotrophin (Menopur®, Ferring Pharmaceuticals Inc., USA). GnRH antagonist (Cetrotide®; Merck-Serono, Germany) was commenced from day 7 and continued daily until ovulation trigger. The ovulation trigger was administered when three or more follicles greater than 17 mm in diameter were observed on ultrasound scan from day 10 of the cycle onwards. The majority of women received a dual trigger, consisting of GnRH antagonist (1ml Suprefact, Hoechest, Denmark) and 1,500 IU of HCG. Those women deemed at high risk of OHSS were instead administered a single bolus of GnRH antagonist (1ml Suprefact, Hoechest, Denmark). Oocyte retrieval was performed under ultrasound guidance 37 hours after the trigger. All mature oocytes retrieved were cryopreserved by vitrification.

Details of ethics approval

The SEF process was explained comprehensively by fertility specialists and written consent was received. Local institutional review board approval was obtained for the use of the electronic

questionnaire. All women consented to be contacted for research purposes at the time of their treatment.

Statistical analyses

SPSS version 24 software (SPSS, Chicago, Illinois, USA) was used for analysis. Descriptive statistical analysis was described as mean +/- SD or median +/- range. Normality was assessed using Shapiro-Wilks test. Comparisons between subgroups were undertaken using the Mann Whitney U test. Statistical significance was set at $P<0.05$. The individualised probability of achieving at least one live birth was made using a previously validated predictive model,[560] which is based upon the age at the time of freezing and the number of oocytes frozen. If multiple cycles were undertaken, then cumulative estimates were calculated to determine overall probability. To quantify the Likert scale responses to ascertain the most influential factor for undergoing SEF, a weighted ranking system was utilised, assigning a score of 0 to 4 for each answer to the various influencing factors (0 = not at all; 4 = definitely).

Results

The survey was sent to 100 women who underwent SEF between 1st January 2008 and 31st December 2018. The response rate was 85% (n=85). 75 (88%) respondents completed it in its entirety whereas it was partially completed by 10 (12%). The mean time between cryopreservation of oocytes and completing the questionnaire was 2 years 9 months (+/- 2 years 3 months) The cohort demographics are summarised in **Table 5a.1**. The median age of participants at their first cycle was 37 years (range 26-44). The majority of women were single (71%; n=60), Caucasian (n=67; 78.8%) and highly educated with 92% (n=78) having a higher education degree. The vast majority were also in full time employment or self-employed (93%; n=79). The mean number of cycles undertaken per woman was 1.46 +/- 0.8. The mean number of oocytes stored per cycle was 7.4 +/- 4.4, whilst the number stored per woman was 10.1+/-6.7.

		Number	%
Age	25-29	2	2
	30-34	8	9
	35-39	61	72
	40-44	14	16
Ethnicity	White	67	79
	Asian	10	12
	Black	1	1
	Mixed	3	4
	Middle Eastern	1	1
	Other	2	2
	Would rather not say	1	1
Relationship status	Single	60	71
	In relationship	10	12
	Living with partner	5	6
	Married	4	4
	Separated	5	6
	Divorced	1	1
Employment	Employed (Full time)	65	76
	Employed (Part time)	1	1
	Self Employed	14	16
	Student	0	0
	Unemployed	5	6
Education	No formal qualifications	0	0
	GCSEs	2	2
	A levels / Diploma	0	0
	Professional qualification	5	6
	Undergraduate degree	30	35
	Postgraduate degree	36	42
	Doctorate	12	14

Table 5a.1: *Demographic data of women undergoing social egg freezing*

Perceptions and attitudes

The vast majority (93%; n=79) of participants 'agreed' or 'strongly agreed' that they were worried about their declining fertility at the time of freezing, whereas 89% (n=76) agreed or strongly agreed having their own biological children was very important to them. The contributing factors that influenced the decision to undergo SEF are outlined in **Figure 5a.1**. 70% (n=56) 'definitely' felt that not having a partner influenced their decision. Conversely, the majority reported that desire to further their education (n=75; 93%) or pursue their career (n=48; 59%), had no influence in their decision to freeze their oocytes. Following quantification of the Likert responses using the aforementioned weighted scoring system, 'not having a partner' was the most important influencing factor, representing 31% of the available points, following by 'not being ready to start a family' on 17%, and

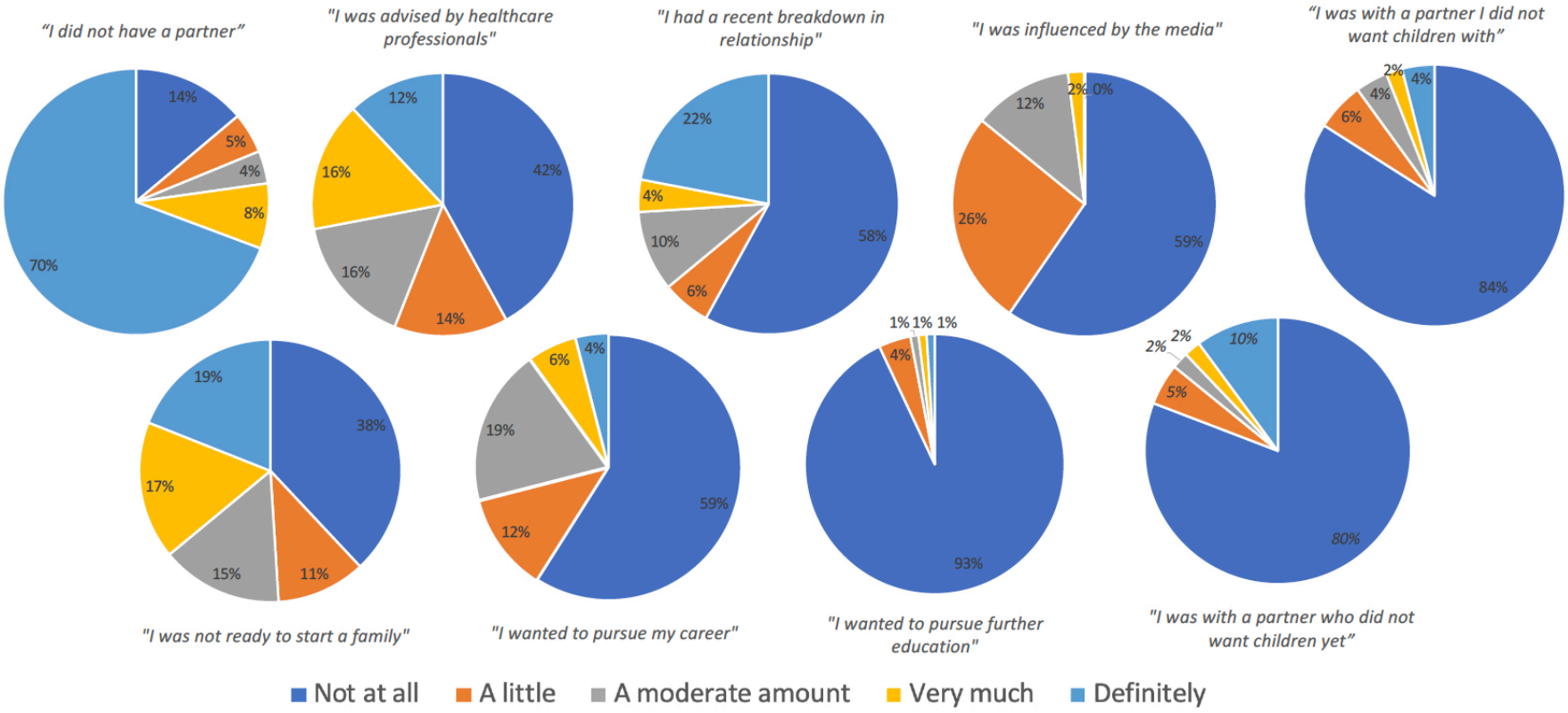


Figure 5a.1: *Contributing factors that influenced decision to undergo social egg freezing*

being advised by healthcare professionals on 14%. Based on the weighted points system, **Figure 5a.2** demonstrates the perceived importance of each factor across participants. The majority (63%, n=52) planned to store their oocytes between 3 and 6 years before using them; whilst around an eighth (12%; n=10) planned to store them for >10 years.

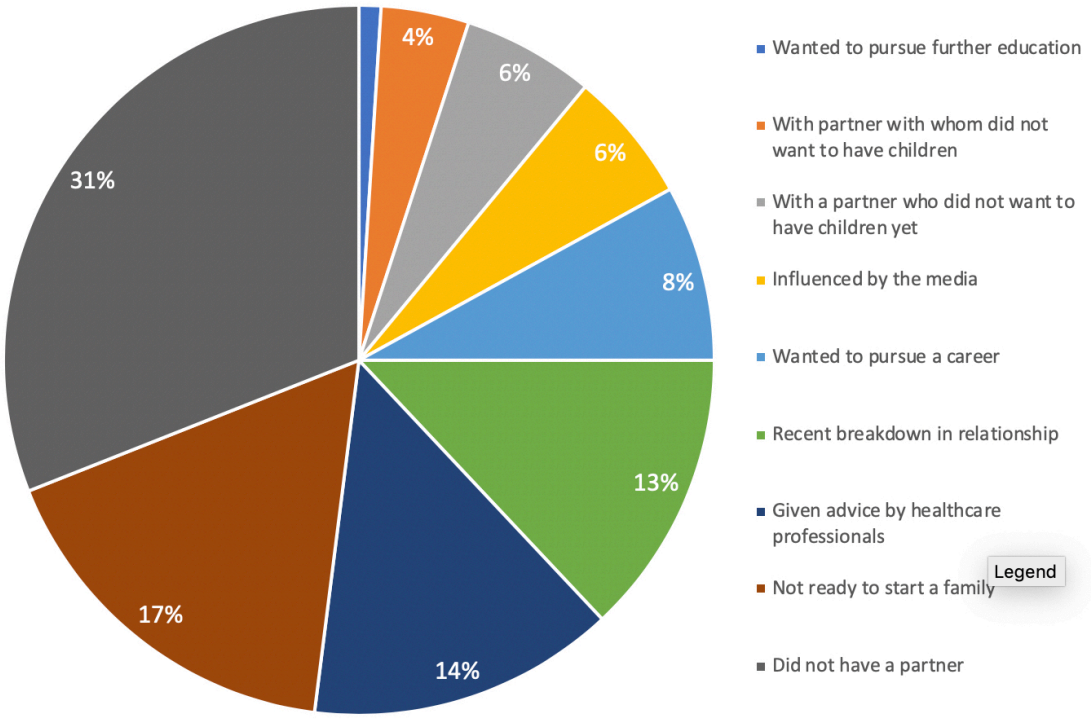


Figure 5a.2: *Perceived importance of each factor influencing decision across the participants*

Information and support

At the time of oocyte cryopreservation, 61% (n=50) of respondents felt the information they received with regards to their fertility was good (49%; n=40) or excellent (12%, n=10), with 28% (n=23) and 11% (n=9) finding it to be average or poor respectively. The majority (83%, n=68) felt that the chance of treatment failure was explained, whereas (13%, n=11) were unsure and 4% (n=3) felt this was not explained adequately. With regard to the individualised chances of achieving a livebirth, 23% (n=19) believed their chances to be <20%, whereas a similar proportion (n=17; 20%) believed their chances to be between 20-40%. Smaller proportions were more optimistic, with 14% (n=12), 5% (n=4) and 3% (n=2) rating their chances between 40-60%, 60-80% and 80-100% respectively. Around a third

(n=29; 35%) of respondents were unsure. Following calculation of each individual probability of achieving at least one livebirth, using the counselling tool described by Goldman et al 2017, which incorporates the number of oocytes stored and the age at the time of treatment,[560] 12% (n=10) correctly estimated their chances of achieving a livebirth. However, 8% (n=10) overestimated their chances and 42% (n=34) underestimated their chances of achieving a livebirth. **Figure 5a.3** demonstrates the participants' perception of their chances of achieving a livebirth.

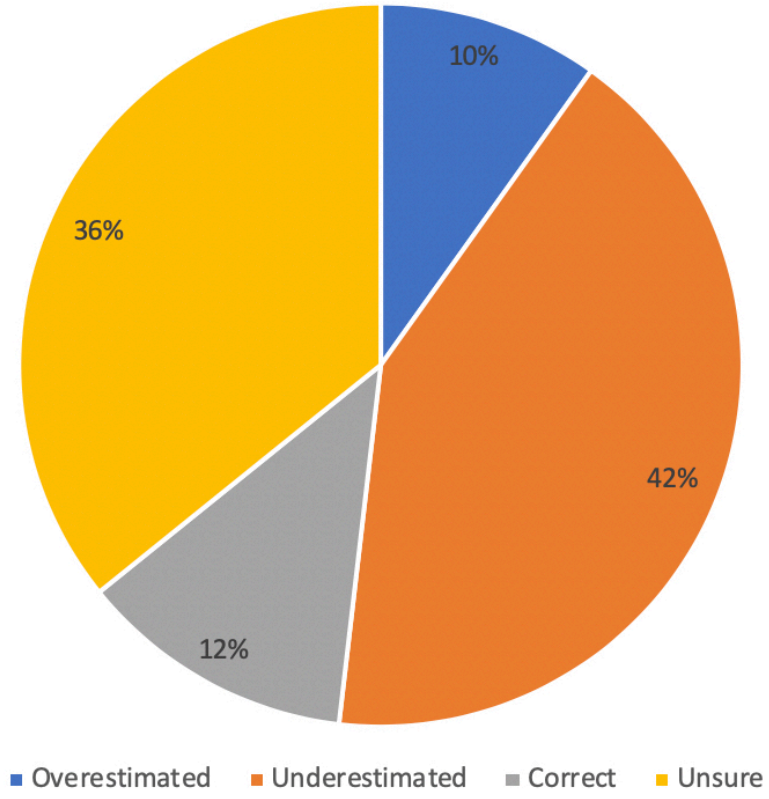


Figure 5a.3: *Participants' perception of their chances of achieving a livebirth*

Implications of getting pregnant at an older age

Almost three quarters of respondents (72%; n=59) were aware that becoming pregnant at advanced maternal age can be associated with greater risk of pre-eclampsia, pulmonary embolism and preterm birth, with the majority (83%; n=68) prepared to accept the risks of to enable them to fall pregnant at a more suitable time.

Women's experience of freezing their oocytes

The vast majority (91%; n=73) did not report any regret having frozen their oocytes. Six of the seven women who experienced regret quantified it as 'a little', whereas one 'definitely' regretted her decision to undergo SEF. The most common reason cited for regret was the associated financial expense, being reported by six out of the seven women (86%). Other reasons included low number of oocytes stored, which was reported by three women (43%) and poor supportive counselling at time of freezing, lack of verbal/written information, excessive side effects/complications, unsuccessful pregnancies and desire to have undergone treatment at an earlier age, each cited by one women (14%). Almost two thirds (64%; n=52) of respondents said they would freeze their oocytes again, whilst 21% (n= 17) would not, and the remainder (15%, n=12) were unsure.

The future

The participants' answers to questions related to the future are summarised in **Table 5a.2**. With regard to reproductive outcomes, 13 (17%) had given birth to one child, and 2 (3%) were pregnant at the time of questionnaire. Around half (53%; n=8) conceived naturally, 13% (n=2) used their stored oocytes with their partner's sperm, 13% (n=2) used their stored oocytes with donor sperm, and 20% (n=3) conceived by other means.

Out of those who had not used their oocytes (n=62), around a third (n=20; 32%) were planning to use them with their current partner, 60% (n=37) were hoping to use them with a future partner, 3 (5%) were going to use a sperm donor and 2 (3%) were not intending to use them at all. More than half (n=39; 51%) reported that in the eventuality that they did not subsequently find a partner, they would consider using a sperm donor, whereas a quarter (n=18; 24%) would not. The median oldest age the respondents would consider using their stored oocytes was 44.5 years +/- 3 years.

		Number	%
"I feel empowered after freezing my eggs"	Strongly disagree	6	8
	Disagree	4	5
	Neither agree, nor disagree	24	33
	Agree	29	40
	Strongly agree	10	14
"I feel less pressured to have children now I have frozen my eggs"	Strongly disagree	9	12
	Disagree	21	29
	Neither agree, nor disagree	19	26
	Agree	20	27
	Strongly agree	4	5
"I feel that freezing my eggs gave me an extended opportunity to find a suitable partner"	Strongly disagree	5	7
	Disagree	9	12
	Neither agree, nor disagree	16	22
	Agree	29	40
	Strongly agree	14	19
What is your plan with regards to using your stored eggs?	Use with current partner	20	32
	Use with a future partner	37	60
	Use sperm donor	3	5
	Not planning to use them	2	3
Would you use a sperm donor as a last resort if you were not in a relationship at the time you needed to use your eggs?	Strongly disagree	14	18
	Disagree	15	20
	Neither agree, nor disagree	17	22
	Agree	21	28
	Strongly agree	9	12
"I would accept my eggs being discarded if they exceeded the maximum storage limit"	Strongly disagree	14	18
	Disagree	15	20
	Neither agree, nor disagree	17	22
	Agree	21	28
	Strongly agree	9	12
Do you agree that the maximum storage period of 10 year is fair?	Strongly disagree	16	21
	Disagree	23	30
	Undecided	22	29
	Agree	13	17
	Strongly agree	2	3
If you do not agree with the 10-year storage period, what time period do you think would be most appropriate?	5 years	0	0
	15 years	12	24
	20 years	6	12
	30 years	1	2
	Indefinite	17	33
	Until aged 50	15	29
If you decided to not use some or all of the eggs you have stored, what would you decide to do with them?	Discard them	4	5
	Donate them to research	31	41
	Donate them to couples in need of an egg donor	19	25
	Unsure	22	29

Table 5a.2: *Participants perceptions to the future following social egg freezing*

Regarding knowledge of the maximum period of time oocytes can be stored for in the UK, 79% (n=60) of respondents correctly identified this as 10 years. However, 7% (n=5) incorrectly answered the question and 14% (n=11) were 'unsure'. A fifth (n=15; 20%) were unaware that their oocytes would be discarded after the maximum storage time. More than half (n=39; 51%) disagreed or strongly disagreed that the 10-year limit is fair, whereas more than a third (n=19; 38%) disagreed or strongly disagreed to their oocytes being discarded if they reached the maximum storage time. One third of respondents (n=17; 33%) felt the storage time should be indefinite whilst 29% (n=15) believed it should be up to the age of 50. In the eventuality that their oocytes were not subsequently used, two thirds (n=50; 66%) of women would either donate them to couples in need of donor oocytes or research, 29% were unsure (n=22) and 5% (n=4) would discard them.

Discussion

In our population, the most frequent reported motivation for undergoing the process was not having a partner. **Table 5a.3** summarises studies that have been undertaken across varying populations that assess perceptions towards SEF. Single status is consistently identified to be a major motivating factor in a number of studies assessing perceptions across varying populations.[561-566] Whilst of interest, it is important to note these studies are not undertaken in women who have undergone SEF, and the demographics surveyed are not reflective of those who undergo the process. Single status however remains the most frequent motivating factor in women who undergo SEF, where it accounts for 79-88% of women.[385,388,567,568] Of note, in one study almost half (47%) of those who subsequently returned to use their oocytes had found a partner, highlighting the potential for SEF to extend the window of opportunity to find a partner.[385]

	N	Age (years) Median (Range)	Awareness of SEF (%)	Positive attitude towards SEF (%)	Single (%)	Higher Education (%)	Employed (%)	Influential motivators for considering SEF				Positive attitude to donating eggs (%)
								Single status	Financial Stability	Career Aspiration	Advancing Age	
Tozzo et al 2019.[569]	930	(18-35)	34.3	19.5	48.7	100	n/r	26.5	50.2	50.2	n/r	35.1
Mahesan et al 2019.[570]	169	24	80.0	n/r	n/r	100	n/r	34.0	n/r	62.0	72.0	n/r
Esfandiari et al 2019.[561]	103	(26-30)	n/r	44.7	n/r	100	100	57.5	n/r	n/r	n/r	46.0
O'Brien et all 2017.[562]	663	(18-44)	89.7	72.2	n/r	n/r	n/r	75.7	n/r	n/r	n/r	n/r
Lallemant et al 2016.[392]	973	31 (18-68)	83.0	19.0	19.0	50.0	58.0	27.0	n/r	20.0	32.0	57.0
Daniluk et al 2016.[563]	500	28.5 (18-38)	67.8	66.0	58.2	73.0	54.0	54.6	n/r	n/r	n/r	57.2
Lewis et al 2016.[564]	1064	18-65	86.3	17.8	n/r	n/r	n/r	63.2	58.0	72.1	n/r	n/r
Wennberg et al 2015.[490]	987	34.4	n/r	47.0	16.0	75.0	n/r	76.0	n/r	n/r	n/r	n/r
Tan et al 2014.[566]	129	23.1 (20-31)	36.4	26.4	n/r	100	n/r	46.5	n/r	45.7	n/r	n/r

Table 5a.3: *Summary of studies that assess perceptions towards SEF across varying populations*

Key: ***N****; Number of cases****, n/r****; not recorded, ****SEF****; social egg freezing*

Concerns regarding age related fertility decline and the limited window of opportunity to find a lifelong partner often coexist. More than half the women (59%) in this cohort perceived that SEF had extended their opportunity to find a suitable partner and 41% felt less pressure to conceive. This is consistent with a recent study that presented findings following thematic analysis from semi-structured interviews which explored the motivations of 31 women who had undergone SEF. It demonstrated the use of SEF was centralised around a fear of running out of time to form a conventional family unit, but also highlighted a keenness to avoid committing to unsuitable relationships because of declining reproductive potential.[571] This was reinforced in our data. Of those who had a partner at the time of freezing their oocytes (n=20), 13 (65%) expressed that being with a partner whom they did not wish to have children with contributed to a degree to their decision to undergo SEF. This shift in attitudes emphasises the evolution of reproductive challenges women now face, and in the context of rising divorce rates in women over the age of 35,[572] SEF perhaps allows women greater autonomy over their relationships.

Other factors that are widely reported to influence motivations to undergo SEF include financial stability and career aspiration. Recent studies amongst student populations have identified career aspiration to be the most prevalent reason to undergo SEF, with 50.2-63% willing to consider it for this reason.[569,570] However these studies were undertaken in women in their early 20's, which is not reflective of the population who undergo SEF. In our study, only 28% felt that pursuing their career influenced their decision definitely, very much or to a moderate extent, with the majority (59%) stating that career aspiration was 'not at all' involved in their decision. Given the median age of 37, and the context that >90% had a higher education degree and were either employed full time or self-employed, it is likely our cohort may have already met their career aspirations sufficiently. This is similar to other published data from women who have undergone SEF, with a mean age of 38 years, which showed similarly that around a quarter (24%) of women attributed professional reasons to influence their decision.[388] This highlights the dynamic nature of reproductive aspirations, and exemplifies the importance of the available resources to women at differing chronological points in

their lives. This is further reinforced by a recent study that assessed perceptions to SEF amongst the general population which identified that almost three quarters (72%) found it acceptable to freeze oocytes for career advancement, but those in the older age group (45-65) were statistically less likely to support SEF for this reason.[564]

Our study highlights that counselling prior to SEF in this cohort was perceived favourably; 61% felt that the information provided with regards to their fertility was 'good' or 'excellent'. Appropriate counselling is essential to help manage expectations, and poor perception of the adequacy of counselling has been shown to increase decision regret following SEF.[559] In our study, the one woman to 'definitely' experience regret cited unsuccessful pregnancies and the financial expense as being the primary reasons. Despite freezing 18 oocytes between the ages of 38-39, which gave her a 75% individualised chance of achieving a livebirth, she was unsuccessful in achieving a livebirth and subsequently exhausted her supply. Whilst her regret is understandable given the context, she correctly predicted her chances of a livebirth between 60-80%, suggesting she was appropriately counselled. This case alone epitomises the reproductive gamble associated with SEF. Our study highlights that only around 1 in 10 (12%) correctly estimated their chances of success based on their age and number of oocytes stored, highlighting that counselling may be suboptimal in this regard. However, surprisingly just 8% overestimated and 42% underestimated their probability, suggesting that counselling may have been overly cautious to avoid overly inflating expectations. It has previously been suggested that a >50% or >80% chance of conceiving a child from a frozen cycle would be justifiable to deem it acceptable.[392] However, estimations of the chance of a successful livebirth based on the age and number of oocytes stored in our data revealed that only a third of the women (n=28; 34%) had a >50% likelihood of success, whilst just 5% (n=4) had >80% chance. Interestingly the mean age in those with >80% of success is 33.5+/-1.3, whereas it was 36+/-1.4 in the 50-80% category and 37.8+/-3 in those whose chance of success was <50%. This reinforces the previous well-known observation that storing oocytes earlier optimises outcomes, with the highest probability of a livebirth seen prior to the age of 34 years old.[573] The average age at SEF was 37 in this study, which compares similarly to studies that have been undertaken in Australia,[568] Belgium,[558]

Spain,[385] the UK,[567] and the United States,[388,559] where the average age ranges from 36.5 to 38. This demonstrates that women undergoing SEF globally appear to be doing it too late, which may subsequently result in future suboptimal outcomes when they return to use their oocytes. One of the main arguments against freezing oocytes for social reasons too early, is the chance of spontaneous conception in the remaining fertile years which would result in unnecessary treatment.[574] However, it should be noted that in this cohort, none of the women who subsequently went on to spontaneously conceive whilst their oocytes were in storage experienced any degree of regret.

The first study to assess regret following SEF identified no regret in a series of 65 women.[558] However a more recent and detailed study in >200 women utilised the validated decision regret scale,[575] and demonstrated 49% had mild-moderate regret following SEF, acknowledging that regret was more likely if low numbers of oocytes were stored.[559] This was not observed in our population, where the mean number of oocytes stored in those who experienced regret was actually slightly higher than those who did not experience regret, albeit not to a significant extent (10.9 vs 10.0 p=0.68). However, the number of oocytes stored per cycle in those who experienced regret was less than in those who did not experience regret (6.2 vs 7.5; p=0.67), although again not to a significant extent. Given that financial expense was the most frequent reason for regret in our cohort, consideration for the cost-effectiveness of each oocyte frozen may be more appropriate than the number of oocytes frozen.

Another risk of undergoing SEF too early is the risk of premature use or disposal of oocytes owing to the HFEA regulations that only permit the storage of gametes for 10 years in the UK. The data presented herein demonstrates that 1 in 5 women were unaware of the storage limit, and the same proportion were unaware their oocytes would subsequently be disposed of if they remained unused. Moreover, when considering that around an eighth (12%) of the women wanted to store their oocytes for longer than 10 years, this clearly identifies are area of counselling that requires improvement. With regards to the perceptions of our cohort of women to the storage limit, more than half questioned its fairness and a third would object to their oocytes being disposed of. This clearly identifies

discontent within this cohort, and reinforces the current drive for the reconsideration of the HFEA to update its legislation.[393]

Limitations of this study include the sample size, particularly when subgroup analysis was undertaken between those who experienced regret and those who did not. Given the retrospective nature of the study and the inclusion of participants from a single centre, there is also potential for bias. Future prospective, multi-centre studies with increased sample size should be considered to minimise such bias.

Conclusion

The data presented herein provides important and novel insight into the motivations and perceptions of women from a UK population who have undergone SEF. Despite potential physical, psychological and financial burden, only a small minority of women experience regret after SEF. Those who subsequently achieved pregnancy naturally, thereby not using their stored oocytes, did not experience regret, potentially negating this as a potential counterargument to storing oocytes earlier. We also clearly highlight discontent with the current HFEA storage limit in the UK and reciprocate the ongoing calls for reconsideration of current legislation. It is only right that as artificial reproductive technologies have advanced, and in the context of progression of societal evolution and gender equality, legislation needs to be updated to remain fit for purpose. This study also highlights the need for women to receive more comprehensive information prior to embarking upon SEF, including age specific individualised counselling regarding success rates, the risks of pregnancy at advantage maternal age, and the current legal storage limits.

Study 5b

Elective oocyte cryopreservation; impact of social egg freezing upon quality of life

Introduction

As described previously in detail, SEF enables women to opportunity to defer their childbearing years if they are not in a position to conceive prior to the inevitable physiological decline in ovarian reserve. Whilst the concept is still in its infancy, and long-term reproductive outcomes remain unpublished, debate continues over the optimum time to undergo SEF. Whilst SEF in a woman's earlier reproductive years optimises future chances of livebirths,[573,576] the likelihood of spontaneous conception in those remaining reproductive years is significant. This potentially renders stored oocytes redundant, and the associated physical risk, emotional impact and financial burden needed to retrieve and store them unnecessary.[574] As the first ethical construct is '*primum non nocere'*, or *'first, do no harm'*, there is a clear need for data to evaluate the impact of treatment upon women who have undergone SEF.

Quality of life (QoL) can be defined as 'an individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns'.[577] FertiQoL is the first internationally validated psychometric tool that objectively measures the quality of life of individuals experiencing issues related to fertility.[436] It consists of two general questions which evaluate the general physical health and quality of life of the individual, and a *core* module which assesses the individual's emotional, mind-body, relational and social impacts of infertility. The *treatment* module can then be used to appraise the individual's environment and tolerability of subsequent fertility treatment. Women undergoing SEF are not physiologically infertile, thereby invalidating many of the questions in the *core* module. However, the *treatment* module remains an effective and reliable method to assess the impact of treatment on women undergoing SEF. The aim of this study is to use the FertiQoL treatment module to evaluate the impact and acceptability of SEF to women who have undergone the process.

Materials and methods

An online questionnaire was distributed to all women who had undergone SEF at the Centre for Reproductive and Genetic Health in London between 1st January 2008 and 31st October 2019, thereby representing an extended study sample from the study group included in study 5a. It consisted of questions assessing demographic information including ethnicity, relationship status, employment status and education level before asking the FertiQoL treatment module questions. The wording was rephrased in the context of SEF as demonstrated in **Figure 5b.1**. The questionnaire was sent via e-mail utilising the SurveyMonkey platform between 1st September 2018 and 1st November 2019. A retrospective review of case notes was simultaneously undertaken for clinical data from the SEF cycles including age at the time of SEF, number of cycles undertaken, numbers of oocytes cryopreserved and complications including OHSS. Clinical classification of OHSS was defined according to the Royal College of Obstetrics and Gynaecology (RCOG) utilising mild, moderate, severe and critical categories.[578]

Treatment Environment Subscale

T2	*Are the fertility medical services you would like available to you?*
T5	*Do you feel the fertility staff understand what you are going through?*
T7	*Are you satisfied with the quality of services available to you to address your emotional needs?*
T8	*How would you rate the surgery and/or medical treatment(s) you have received?*
T9	*How would you rate the quality of information you received about medication, surgery and/or medical treatment?*
T10	*Are you satisfied with your interactions with fertility medical staff?*

Treatment Tolerability Subscale

T1	*Did the social egg freezing treatment negatively affect your mood?*
T3	*How complicated is dealing with the procedure and/ or administration of medication for your infertility treatment(s)?*
T4	*Are you bothered by the effect of treatment on your daily or work-related activities?*
T6	*Are you bothered by the physical side effects of fertility medications and treatment?*

Figure 5b.1. *FertiQoL treatment module in context of social egg freezing*

Clinical Protocols

As described in study 5a, all women underwent controlled ovarian stimulation with GnRH antagonist suppression. The ovulation trigger was given when three or more follicles >17 mm in diameter were seen from day 10 onwards. Most women received a dual trigger of GnRH antagonist (1ml Suprefact, Hoechest, Denmark) and 1,500 IU of HCG, but those deemed high risk of OHSS were given a single bolus of GnRH antagonist (1ml Suprefact, Hoechest, Denmark) instead. Under ultrasound guidance, oocyte retrieval was undertaken 37 hours following ovulation trigger. All mature oocytes retrieved were subsequently cryopreserved by vitrification.

Details of Ethics approval

Local institutional review board approval was obtained for this study. At the time of SEF, all women consented to be contacted in the future for research purposes, as per the HFEA consent for disclosure form.

Statistical analyses

SPSS version 26 software (SPSS, Chicago, Illinois, USA) was used for analysis. Descriptive statistical analysis was undertaken using mean +/- SD or median +/- range. Normality was assessed using Shapiro-Wilk test. Comparisons between subgroups were undertaken using the Independent T test. Statistical significance was set at $P<0.05$.

Results

The survey was sent to 140 women who agreed to be contacted after undergoing SEF between 1st January 2008 and 30th October 2019. The response rate was 67% (n=94). The cohort demographics are summarised in **Figure 5b.2**. The median age of participants was 37 years (range 26-44). The majority of women were single (70%; n=66) and more than three quarters referred to themselves as Caucasian (n=74; 78.7%). The cohort were highly educated with 91% (n=86) having a higher education degree and a similarly high proportion were either self-employed or in full time employment (n=90; 96%).

The majority of women underwent a single cycle (n=68; 72%, whilst 17 (18%) underwent two cycles, seven (8%) had 3 cycles and a single woman each had 4 or 5 cycles respectively, resulting in 132 cycles in total. The mean number of oocytes collected per cycle was 6.9 +/- 4.4, whereas the mean total number of oocytes cryopreserved per woman was 9.8 +/- 6.4. The mean number of days of stimulation was 13 +/- 1.3.

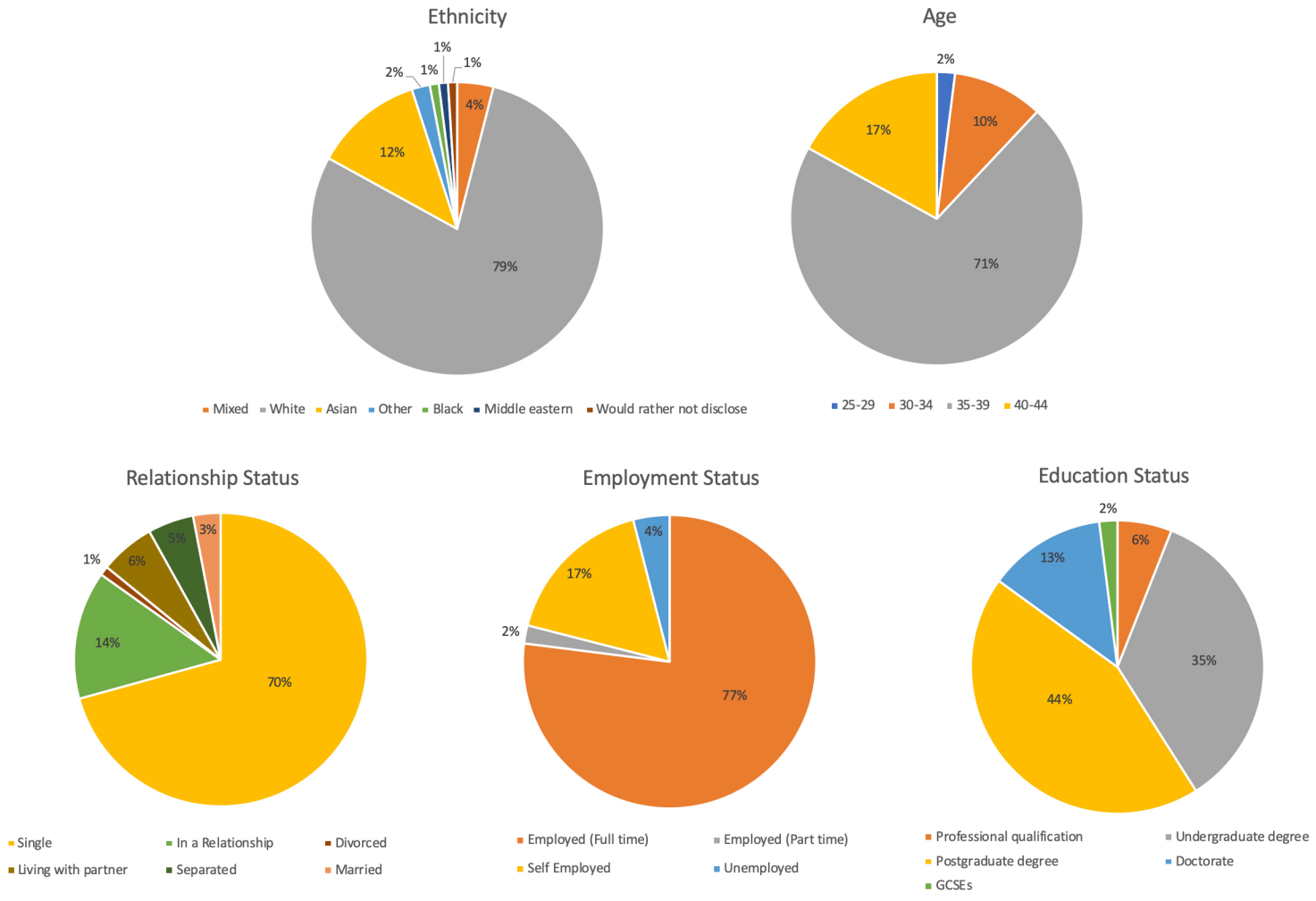


Figure 5b.2. *Demographics of women undergoing social egg freezing*

FertiQol Treatment Module

The mean overall treatment score was 65.5 (+/- 17.7). The average tolerability and environment scores were 62.4 (+/-21.5) and 68 (+/-19.9) respectively. The individual responses to the FertiQol Treatment module from our cohort are summarised in **Table 5b.1** and **Table 5b.2**.

Environment subscale		**Number**	**%**
"Are the fertility medical services you would like available to you"	*Always*	*31*	*33*
	Very Often	*28*	*30*
	Quite Often	*25*	*26*
	Seldom	*9*	*10*
	Never	*1*	*1*
"Do you feel the fertility staff understand what you are going through?"	*An extreme Amount*	*5*	*5*
	Very Much	*48*	*51*
	A moderate Amount	*23*	*25*
	A little	*13*	*14*
	Not at all	*5*	*5*
"Are you satisfied with the quality of services available to you to address your emotional needs?"	*Very Dissatisfied*	*4*	*4*
	Dissatisfied	*8*	*9*
	Neither Satisfied nor Dissatisfied	*37*	*39*
	Satisfied	*35*	*37*
	Very Satisfied	*10*	*11*
"How would you rate the surgery and / or medical treatment(s) you have received?"	*Very Dissatisfied*	*0*	*0*
	Dissatisfied	*6*	*6*
	Neither Satisfied nor Dissatisfied	*16*	*17*
	Satisfied	*47*	*50*
	Very Satisfied	*25*	*27*
"How would you rate the quality of information you received about medication, surgery and/or medical treatment?"	*Very Dissatisfied*	*3*	*3*
	Dissatisfied	*10*	*11*
	Neither Satisfied nor Dissatisfied	*10*	*11*
	Satisfied	*48*	*51*
	Very Satisfied	*23*	*24*
"Are you satisfied with your interactions with fertility medical staff?"	*Very Dissatisfied*	*2*	*2*
	Dissatisfied	*9*	*10*
	Neither Satisfied nor Dissatisfied	*11*	*12*
	Satisfied	*49*	*52*
	Very Satisfied	*23*	*24*

Table 5b.1: *Participants responses to FertiQol treatment module environment subscale*

Tolerability subscale		Number	%
"Did the social egg freezing treatment negatively affect your mood"	*Always* *Very Often* *Quite Often* *Seldom* *Never*	3 15 15 33 28	*3* *16* *16* *35* *30*
"How complicated is dealing with the procedure and/or administration of medication for your infertility treatment (s)?"	*An extreme Amount* *Very Much* *A moderate Amount* *A little* *Not at all*	*7* *15* *31* *20* *21*	*8* *16* *33* *21* *22*
"Are you bothered by the effect of treatment on your daily or work-related activities?"	*An extreme Amount* *Very Much* *A moderate Amount* *A little* *Not at all*	*4* *13* *32* *28* *17*	*4* *14* *34* *30* *18*
"Are you bothered by the physical side effects of fertility medications and treatment?"	*An extreme Amount* *Very Much* *A moderate Amount* *A little* *Not at all*	*3* *14* *31* *30* *16*	*3* *15* *33* *32* *17*

Table 5b.2: *Participants responses to FertiQol treatment module tolerability subscale*

With regards to the tolerability questions, almost two thirds of women felt their SEF treatment 'never' or 'seldom' negatively affected their mood (65%; n= 61). Less than a quarter (24%; n=22) found dealing with the treatment or medications complicated 'very much' or an 'extreme amount'. Almost half (48%; n=45) of the women reported 'a little' or 'not at all' when asked if they were bothered by the effect of treatment on their daily or work-related activities whereas a minority (n=17; 18%) felt it impacted 'very much' or an 'extreme amount'. With regards to physical effects of medication and treatment, around half (n=46; 49%) of the cohort reported they were 'not at all' bothered or bothered 'a little', whereas 17 (18%) felt it did 'very much' or an 'extreme amount'.

When considering the environment questions, the majority felt the fertility medical services were available either 'always' or 'very often' (n=59; 63%). Moreover, more than half (n=53; 56%) felt the fertility staff understood what they were going through an 'extreme amount' or 'very much'. More than three quarters of the women were 'satisfied' or 'very satisfied' with the treatment received (77%; n= 72), the quality of information regarding the procedures (75%; n= 71) and the interactions with the

fertility staff (76%; n=72). Around half (n=45; 48%) of the cohort were 'satisfied' or 'very satisfied' with the quality of services available in respect to emotional needs.

Specific group analysis

Table 5b.3 summarises the comparisons in treatment scores when specific cohorts are considered. Younger women (n=11; ≤=34) had a higher treatment score than women >34 years old (n=83; 71.8+/-10.8 Vs 64.7+/-18.3; p=0.21), although this was not to a statistically significant extent. Being married or in a relationship (n=22) was also associated with higher, albeit not statistically significant, mean scores (69.2+/-17.4), when compared to single women alone (n=66; 66.3+/-17.1; p=0.49). However, being separated or divorced (n=6) was associated with significantly worse scores when compared to married women, women in a relationship and single women (n=88; 43.3+/-11 vs 67.0+/-17.1; p=0.001). When considering number of oocytes stored, those who stored ≤7 had a mean score of 63.1 +/- 19.1 (n=51, whilst those who stored >7 had a mean of 68.0 +/- 16.0 (n=43), although this difference was not statistically significant (p=0.19). Similarly, there was no difference between those with stimulation cycles with a duration ≤ 12 days (n=32) than those with 12 days or more (n=64, 66.6 +/- 14.3 vs 65.0 +/- 19.3; p=0.69), or those who had single treatment cycles (n=66; 65.9 +/- 18.3) versus those who had multiple cycles (n=28; 64.5+/-16.9; p=0.73).

Regarding OHSS, there were six cases in total (6.4%), all of which were classified as mild in severity as per the RCOG classification and were managed conservatively. There were no associated hospital admissions or significant adverse events. The average treatment scores between the OHSS group and non-OHSS group were statistically similar (68.3+/-6.8 vs 65.3+/18.2; p=0.69). All women (n=6) in the OHSS cohort reported 'never' or 'seldom' when asked regarding if the SEF experience negatively affected their mood. They were also all 'very satisfied' or 'satisfied' with the quality of information received and interactions with the fertility medical staff.

	Number of cycles			Number of oocytes frozen			Duration of stimulation (days)			Relationship Status			Age at cryopreservation		
	Single cycle (n=66)	Multiple cycle (n=28)	P value	≤7 (n=51)	>7 (n=43)	P value	≤12 (n=32)	>12 (n=64)	P value	Single (n=66)	Married/ relationship (22)	P value	≤34 (n=11)	>34 (n=83)	P value
FertiQol Treatment Module Score (Mean +/- SD)	65.9 +/- 18.3	64.5 +/- 16.9	0.73	63.1 +/- 19.1	68.0 +/- 16	0.19	66.6 +/- 14.3	65 +/- 19.3	0.69	66.3 +/- 17.1	69.2 +/- 17.4	0.49	71.8 +/- 10.8	64.7 +/- 18.3	0.21

Table 5b.3: Subgroup analysis of FertiQol Treatment scores

Author	Number of women	Mean Age (Years)	University Education (%)	Employed (%)	Infertility duration (Years)	Infertility Cause				Fertility treatment	FertiQoL		
						Female	Male	Both	Unknown		Treatment score	Environment score	Tolerability Score
Wu et al 2019.[579]	686	35.69	NS	NS	4.88	40.0	36.0	11.0	13.0	IVF	67.5	67.4	59.4
Zeren et al 2019.[580]	213	NS	32.4	NS	NS	NS	NS	NS	NS	NS	NS	37.6	67.4
Li et al 2019.[581]	253	31.78	43.9	50.6	4.7	39.5	11.5	25.3	23.7	IVF + ICSI	61.8	NS	NS
Massarotti et al 2019.[582]	89	35.54	NS	NS	3.27	34.8	29.2	36.0	0.0	IVF	NS	84.8	71.1
Hekmatzadeh et al 2018.[583]	300	30.65	NS	NS	NS	NS	44.1	NS	44.1	NS	NS	57.7	59.3
Maroufizadeh et al 2017.[584]	155	31.03	43.2	NS	6.25	18.7	45.8	16.8	18.7	IVF	58.4	61.3	54.0
Zurlo et al 2017.[585]	206	NS	36.9	NS	NS	32.0	28.2	24.8	15.0	IVF + ICSI + IUI	60.5	NS	NS
Haemmerli Keller et al 2017.[586]	44	34	NS	NS	3.7	NS	57.9	NS	NS	NC-IVF	67.9	NS	NS
	45	34.9	NS	NS	2.7	NS	61.3	NS	NS	cIVF	62.9	NS	NS
Desai et al 2017.[587]	156	29.2	93	NS	3.1	NS	4.6	NS	30.7	OI + IUI	66.7	73.3	60.1
Goker et al 2017.[588]	127	27.5	NS	22	3.8	29.1	6.3	5.5	59.1	NS	65.6	61.5	71.8
Porat-Katz et al 2016.[589]	213	35.6	63.8	95.7	NS	NS	NS	NS	NS	IVF with complementary medicine nonusers	NS	78.7	65.6
	110	36.8	82.7	96.3	NS	NS	NS	NS	NS	IVF with complementary medicine users	NS	76.5	62.4
Santulli et al 2016.[590]	50	33.3	82.6	NS	NS	NS	16.7	NS	NS	ICSI + IVF (endometriosis)	61.7	66.4	57.6
	75	35	78.7	NS	NS	NS	54.8	NS	NS	ICSE + IVF	60.4	63.9	59.9
Li et al 2016.[591]	58	30.66	60.3	67.2	NS	55.2	19.0	24.1	1.7	IVF + Mindfulness	NS	66.2	67.0
	50	30.7	42	68	NS	62.0	8.0	28.0	2.0	IVF	NS	58.6	53.4
Kahyaoglu Sut et al 2015.[592]	89	30	21.3	36	5.2	NS	NS	NS	NS	IVF + IUI	65.4	63.3	68.6
Karabulut et al 2013.[593]	212	NS	13.2	32.5	NS	27.4	21.7	17.9	33.0	NS (Primary infertility)	NS	64.0	66.0
	61	NS	3.3	21.3	NS	31.2	19.7	14.7	34.4	NS (Secondary infertility)	NS	63.2	74.5
Heredia et al 2013.[594]	41	33.34	NS	NS	3.74	13.2	65.8	18.4	2.6	IVF + IUI	62.4	56.6	68.2

Table 5b.4: *Summary of studies using FertiQoL treatment module in infertile populations*

Discussion

This is the first study of its kind to utilise the FertiQoL treatment module to determine the impact of SEF on the quality of life of women who have undergone the process. The FertiQoL treatment module has been used previously in the context of infertile women in a number of studies internationally, as displayed in **Table 5b.4.**[579-595] The mean age of women from these studies was 32.7 years; five years younger than our cohort. Education level varied across studies, with a university level of education ranging from 3.3% to 93% (mean 49.8%), with an average of 54.4% women reporting being employed. This is different to our SEF population where >90% had higher education degrees and were either in full time employment or self-employed. The mean treatment module score was 62.9, with women reporting an average *environment* score of 64.8 and *tolerability* score of 63.9. The mean treatment module score in our cohort of women who underwent SEF was 65.5, demonstrating it is comparably favourable, largely tolerable and does not seriously adversely impact women's quality of life who undergo the process. However, the responses also highlight that approximately a fifth of women experience significant physical and psychological side effects.

QoL can be impacted by a number of interrelated factors, including physical health, psychological state, level of independence and social relationships. It is therefore unsurprising that certain characteristics have been shown to impact FertiQoL treatment scores in infertile women. For example, younger women have been shown to have higher treatment module scores than older infertile women (<=34 yr vs >34 yr old 61.06 vs 58.34 $p<0.05$).[585] In our cohort, despite a bigger difference seen between the same age groups (71.8+/-10.8 Vs 64.7+/-18.3) it was not statistically significant ($p=0.21$), likely secondary to small sub-group numbers. Whilst the optimal time for electively cryopreserving oocytes has been suggested to be <34 years,[573] the average age of women herein is 37, which is similar to other published data on women undergoing SEF with ages between 36.5-38,[596] suggesting women are potentially embarking upon the process too late. Whilst it has been shown that the most cost-effective time to undergo SEF is < 38 years,[597] the improvement in QoL in younger populations, along with favourable outcomes, adds to the argument of storing oocytes earlier.[576]

Undergoing IVF has long been recognised as a stressful and emotionally demanding process.[598] Partner support has been identified to be important in the alleviation of infertility associated burden. It is therefore of interest that our data identified that there was no statistical difference between single women and women who were married or in relationships. However, women who were currently separated or divorced from their husbands, had statistically inferior FertiQoL treatment module scores. This identifies this as a sub-group of women who may require additional counselling and support during the process of SEF. Parity has also been demonstrated to impact *tolerability* treatment scores, whereby nulliparous women report lower scores (nulliparous; 58.13 vs multiparous; 64.25, p=0.04).[583] In the context of SEF, where the population is likely predominantly single and nulliparous, it is likely these factors may negatively impact quality of life during the process. This highlights the need for optimised counselling in women who undergo SEF, with active monitoring throughout the process and the availability of emotional support if required.

The characteristics generally associated with women who choose to undergo SEF may also have protective factors. It has previously been shown that a positive correlation exists between education level and FertiQoL treatment score.[585,593] Given that >90% of our cohort had a higher education degree, this higher than average level of education may enable them to have a greater understanding and insight into the process of SEF. Moreover, duration of infertility has also been shown to impact treatment scores, with those experiencing infertility for longer periods of time reporting lower scores.[583,585,593] Therefore in SEF, as they are not physiologically infertile, higher scores would be expected. However, as their social situation renders them unable to conceive at that time, thereby introducing the concept of social infertility, this may also impact quality of life.

When considering controllable factors such as management of ovarian stimulation, it has previously been shown that gonadotropin stimulation results in lower FertiQoL treatment module scores, when compared to natural cycle IVF (62.9 vs 67.9; $p<0.05$).[586] As our cohort all underwent controlled ovarian stimulation with gonadotropins, lower scores may arise due to greater side effects or fear of complications associated with ovarian stimulation. However, in our cohort there was no significant

difference in scores between those who underwent longer periods of stimulation versus those with shorter stimulation cycles. Moreover, the FertiQoL treatment score in those women who suffered from OHSS was slightly higher than in those who didn't (68.3 vs 65.3), although this was not statistically significant. Finally, women who have previously undergone fertility treatment have been demonstrated to have lower FertiQoL treatment scores than those who haven't (58.3 vs 61.3; $p<0.05$),[585,592] although this was not evident in our study.

Whilst this study offers novel insight into the quality of life of women who undergo SEF, it has limitations including the modest sample size, which likely impacted statistical significance in the subgroup analysis. Moreover, as the data collection was retrospective and only included participants from a single centre, there is potential for bias. Future prospective studies with increased sample size across multiple centres should therefore be considered.

Conclusion

This is the first study of its kind to utilise the FertiQoL treatment subscale to measure treatment quality in SEF. The data presented herein suggests SEF is largely tolerable and that women are satisfied following treatment, although almost a fifth of women experience significant side effects to treatment. Certain characteristics of women who undergo SEF, such as education level and lack of physiological infertility, may facilitate tolerability and resilience in what is inherently a stressful and demanding process. However, whilst such women are physiologically fertile, circumstance renders them socially infertile, with no partner support in the majority of cases to help buffer potential stress. Our study highlights that women who are separated from their partners, or are divorced, experience worse QoL during SEF than single women, or those who are married or in relationships. Such women should be identified as a population that requires additional support if embarking upon SEF, who should be offered extensive counselling, active monitoring throughout the process and additional support if required.

Study 5c

Elective oocyte cryopreservation; laboratory, clinical and reproductive outcomes in social egg freezing

Introduction

Despite the theoretical benefit of preserving reproductive potential through SEF, it cannot guarantee against involuntary childlessness. Following SEF, if women subsequently return to use their cryopreserved oocytes, there is risk of loss during thawing, or following unsuccessful embryo transfers, resulting in potential exhaustion of their pool of cryopreserved oocytes prior to achieving livebirth. Replenishment may subsequently be difficult in the same woman at a later age owing to the inevitable physiological depletion in ovarian reserve. Therefore, at the time of SEF, it is important to maximise the number of oocytes retrieved for cryopreservation to improve the probability of eventually achieving a successful livebirth.[599]

Various characteristics have been shown to be associated with oocyte yield such as age at time of oocyte retrieval.[600] This encapsulates the very principle of SEF, and directly relates to the progressive depletion in primordial follicles and the reduced neuroendocrine response to ovarian stimulation. This clinically manifests with poorer oocyte quantity and quality, as demonstrated following previous studies that have utilised multivariate analysis to control for other confounding variables.[601] Contrarily, increased BMI has been shown to be associated with a reduction in yield.[602]

There are also various markers of ovarian reserve which can be used to predict oocyte yield following controlled ovarian stimulation.[601] The two which have been consistently demonstrated to be the most accurate are antral follicle count (AFC) and anti-Mullerian hormone (AMH).[603] Endocrine markers including oestradiol (E2), a measure of granulosa cell function has also been used.[604] Elevated levels of follicle stimulating hormone (FSH) on day 2-3 of a cycle have also been associated with a significant reduction in yield, when compared to women with lower FSH of the same age.[605] Radiologically, a follicular volume between 13-23mm/1-6mL at the time of ovulation trigger has also been associated with optimal oocyte yields.[606]

Oocyte yield is strongly correlated with cumulative livebirth rate in infertile women.[607] However, women undergoing SEF are not comparable to infertile women as if they are undertaking the process at an appropriate age, they will be physiologically fertile. For this reason, data extrapolated from oocyte donor cycles is probably more accurate, as it can be assumed that both cohorts have similar reproductive capacity. Similarly to infertile women, a study assessing cumulative outcomes from 6 years of oocyte donation cycles illustrated that the probability of live birth using vitrified oocytes in donor cycles progressively increases with the number of oocytes retrieved.[608] This reinforces the importance of maximising oocyte yield in SEF cycles to subsequently enhance the ultimate goal of achieving a successful live birth. However, no studies have so far investigated the relationship between patient characteristics and markers of ovarian reserve with subsequent oocyte yields in women undergoing SEF. Moreover, reproductive outcomes following SEF remain scarce, predominantly owing to lack of follow up in cohorts so far.

The primary aim of this study was to assess the relationship oocyte yield with a number of clinical, biochemical and radiological markers including age, BMI, AMH, AFC, E2 and total number of follicles >12mm at trigger. This information is important to not only counsel women undergoing SEF to help manage expectations, but better individualise stimulation protocols to maximise oocyte yields. Moreover, a secondary aim of this study is to report the reproductive outcomes following SEF in this cohort so far.

Materials and Methods

Selection criteria

This retrospective cohort includes all women who approached the Centre for Reproductive and Genetic Health in London, UK for SEF between 2008 and 2018.

Clinical Protocols

A comprehensive fertility assessment was carried out, which included a blood test for AMH (Roche Assay), FSH and E2. An AFC was also performed between days 1-4 of the menstrual cycle. The ovarian stimulation protocol was individualised depending on age, AFC, AMH and BMI. Ovarian stimulation, using an antagonist protocol, was commenced on the third day of the menstrual cycle. From day 6, the gonadotrophin dose was adjusted according to E2 levels and ultrasound evidence of stimulation. On day 7 of the cycle and/or once the leading follicle was ≥14 mm in diameter, Cetrotide 0.25 mg (Merck Serono, Germany) was commenced. Oocyte maturation was triggered once the mean diameter of ≥3 follicles ≥18mm with a combination of Suprefact 1ml (Sanofi-Aventis, Germany) and Pregnyl 1,500 IU (Organon, Netherlands). In the event women were at high risk of OHSS, Suprefact 1ml (Sanofi-Aventis, Germany) was administered and LH was checked 24 hours later. Oocyte retrieval was scheduled 37 hours after ovulation trigger. Denudation was carried out 39 hours post HCG injection and metaphase II oocytes were vitrified immediately after.

Details of Ethics Approval

Ethics approval was not necessary for this retrospective study as vitrification of oocytes is a validated technique that should no longer be considered experimental.

Statistical analyses

SPSS version 24 software (SPSS, Chicago, Illinois, USA) was used for analysis. Descriptive statistical analysis was described as median +/- range. Multivariate Poisson regression was used to evaluate the effect of the aforementioned variables with the number of oocytes retrieved. Mann Whitney U test was used to determine a relationship between the variables and their effect on OHSS. The Kruskal Wallis test and Mann Whitney U tests were used to determine significance between age groups and probability of livebirth based upon their age and number of oocytes retrieved. Chi squared

testing was utilised to determine statistical significance in those who returned to use their oocytes, to assess between age groups and achievement of livebirth. Statistical significance was set at $p<0.05$.

Results

315 oocyte retrievals were undertaken in 235 women. The median number of oocytes retrieved per cycle was 7 (1-37), whilst the number of metaphase II oocytes was 6 (1-23). The median age was 38 (26-45) years and BMI was 22 (17-34) kg/m. The median baseline AMH was 10.1 (0.7-98.3) pmol/l and the AFC was 11 (1-47). At ovulation trigger, the median E2 level was 6744 (557-23924) pmol/l and the total number of follicles > 12mm was 9 (1-69). The background demographics and stimulation data are summarised in **Table 5c.1.**

	Median (range)
Age	*38 (26-45)*
BMI	*21.8 (17.4-34.3)*
FSH	*7.5 (0-21.6)*
AMH	*10.1 (0.7-98.3)*
Baseline antral follicle count	*11 (1-47)*
Number of days of stimulation	*9 (7-14)*
Total dose of stimulation IU	*3600 (900-6000)*
Antral follicles > 12mm on day of trigger	*9 (1-69)*
Oestradiol levels on day of trigger per 1000 (pmol/l)	*6.74 (0.56-23.92)*
Number of oocytes	*7 (1-37)*
Number of Metaphase II oocytes	*6 (1-23)*

Table 5c.1: *Demographics, baseline clinical data and stimulation outcomes*

Univariable Poisson regression was used to assess the effect of each variable on oocyte yield (**Table 5c.2**). This identified that age, baseline AFC, AMH, E2, and follicle count ≥12mm on the day of trigger were all significant predictors of oocytes yield (p<0.001). Multivariable Poisson regression subsequently demonstrated that BMI (Incidence rate ratio; IRR; 1.02; 95% CI 1.00 -1.03; 95% p=0.04), E2 level per 1000 pmol/l increase (IRR 1.050; 95% CI 1.04 -1.07; p<0.001) and the number of follicles >12mm at trigger (IRR 1.02; 95% CI 1.01-1.03; p<0.001) were all significant predictors of oocyte yield. However, there was no significant relationship between AFC (IRR=1.00; 95% CI 0.99-1.02 p=0.43) or AMH (IRR 1.00; 95% CI 0.99 – 1.01; p=0.71) with oocyte yield. An increase in age by one-year resulted in a 4% reduction in yield after adjusting for all other variables (IRR 0.96; 95% CI 0.94-0.98; p<0.001).

	IRR	**95% CI**	**p-value**
Age (years)	0.92	0.91-0.93	**<0.001**
BMI (kg/m2)	0.99	0.97-0.99	**0.019**
AFC	1.39	1.03-1.04	**<0.001**
AMH	1.02	1.03-1.02	**<0.001**
Oestradiol on day of trigger (per 1000 pmol/l)	1.08	1.07-1.09	**<0.001**
Number of follicles >12 mm on day of trigger	1.04	1.04-1.04	**<0.001**

Table 5c.2: *The incidence rate ratio (IRR), confidence interval and p-values to assess the effect of each variable on oocyte yield*

	OHSS mean (95% CI)	Non OHSS mean (95% CI)	p value
BMI	*21.4 (18.6-24.1)*	*22.5 (21.9-23.2)*	0.347
Age	*36 (32.8-39.2)*	*37.7 (37.2-38.2)*	**0.019***
Total AFC	*17.7 (8.8-26.6)*	*13.45 (12.07-14.83)*	**0.027***
AMH	*57.2 (38.8-153.2)*	*16.9 (11.5-22.3)*	0.055
No follicles >12mm	*14.57 (9.9-19.3)*	*11.0 (9.57-12.49)*	**0.001***
FSH	*5.6 (3.3-7.9)*	*10.2 (7.3-13.1)*	**0.006***
Oestradiol	*133.7 (77.2-190.2)*	*181.8 (147.8-215.9)*	0.361

Table 5c.3: *Mann Whitney U and confidence interval and p-values to assess the effect of each variable on oocyte yield on OHSS and Non-OHSS groups*

Regarding OHSS, there were thirteen cases in total (4.2%), twelve of which were classified as mild and one was moderate in severity as per the RCOG classification.[578] There were no associated hospital admissions or significant adverse events. Analysis using the Mann Whitney U test identified that age, total AFC, number of follicles more than 12mm and FSH were all significant predictors of OHSS ($p<0.05$). BMI, AMH and E2 levels however were not significant predictors of OHSS. **(Table 5c.3)**

Following calculation of each individual probability of achieving at least one livebirth, using the counselling tool described by Goldman et al 2017, which incorporates the number of oocytes stored and the age at the time of treatment,[560] **Figure 5c.1** represents the estimated probability of a successful livebirth in this cohort, stratified by age.

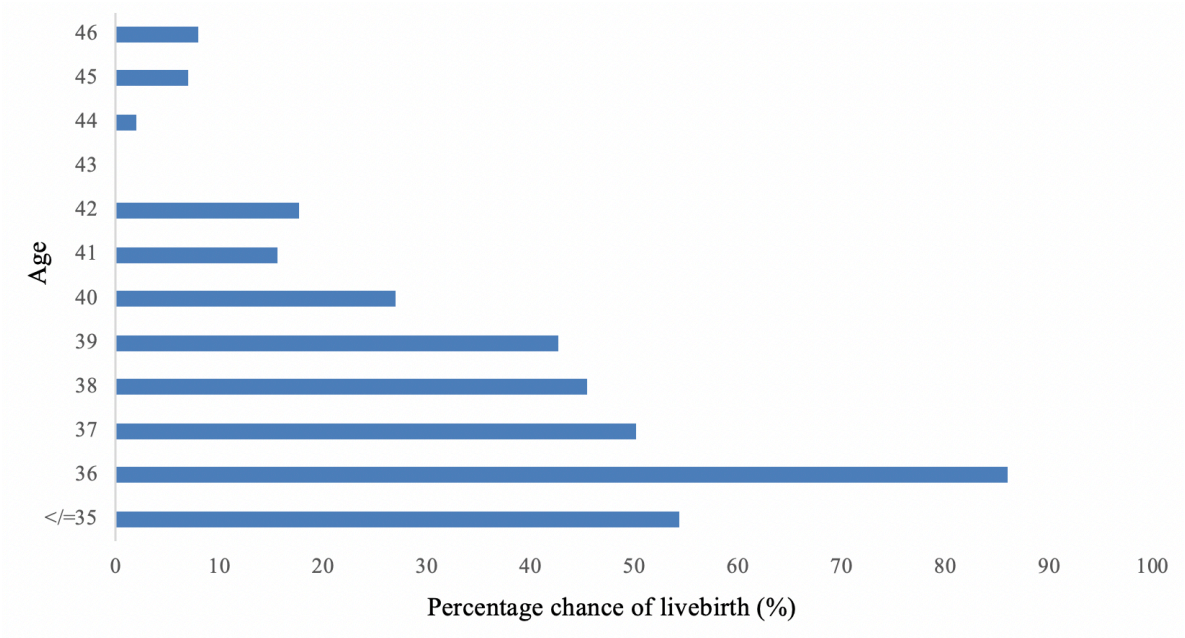


Figure 5c.1: *Estimated chance of livebirth utilising counselling tool by Goldman et al.2017,*[560] *which quantities probability of livebirth based upon age and number of oocytes cryopreserved*

When categorised into age groups, as displayed in **Table 5c.4,** those who underwent SEF ≤35 years (n=62) had a mean estimated probability of achieving a livebirth was 66.5% (+/-18.7%), those who did it between 36-39 years (n=168) had a 41.4% (+/-19.2) chance, and the chance of success in those aged over 40 years was 12.4% (+/- 10.2). The difference in probability of achieving a livebirth between age groups was statistically significant difference both between those aged ≤35 and 36-39 ($p<0.001$), and also between those aged 36-39 and ≥40 ($p<0.001$)**.**

Age group (years)	Number	Probability of livebirth (%)	SD
≤35	63	66.5	18.7
36-39	168	41.4	19.2
≥40	84	12.4	10.2

Table 5c.4: *Predicted average chance of livebirth per age group*

36 women (15.3%) subsequently returned to use their cryopreserved oocytes, with 41 cycles being undertaken in total. The median age at thaw was 43 years old (26-48). A third of the cohort (n=14; 34%) used donor sperm whereas 66% (n=27) created embryos using their partners sperm. The mean time interval between first SEF cycle and thaw was 3.8 years (38.3 vs 42.1). 16 women achieved pregnancies, resulting in a pregnancy rate of 40% per cycle and 45.7% per woman. 13 livebirths were subsequently achieved amongst 11 women resulting in a livebirth rate of 31.4% in those who returned to use their oocytes, and a livebirth rate per cycle of 32.5%. One woman had a biochemical pregnancy loss, whereas two suffered miscarriages, both of whom were >40 years old.

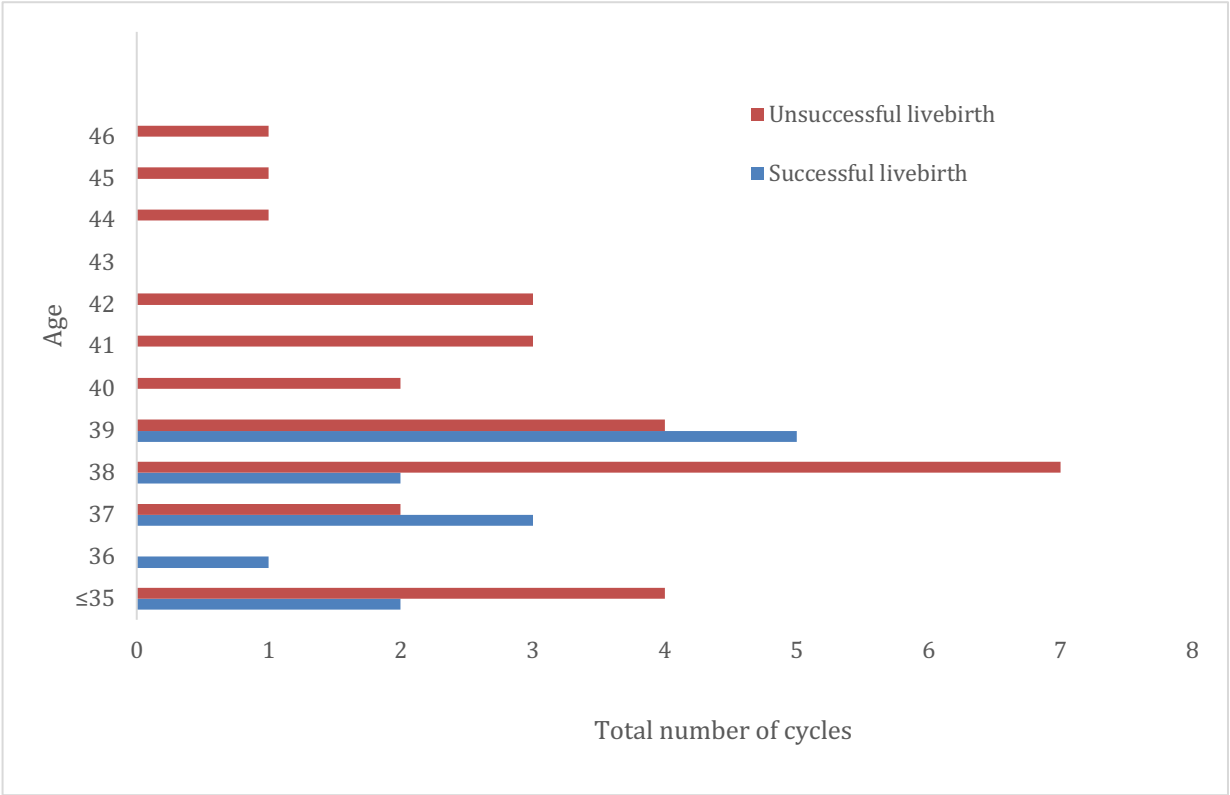


Figure 5c.2: *Number of patients per age group at first thaw cycle (successful livebirths vs unsuccessful cycles)*

In women aged <35 years, out of six cycles, two livebirths were achieved, resulting in a livebirth rate of 33%. In those aged 36-39, 24 cycles were undertaken, which led to 11 livebirths resulting in a livebirth rate of 45.8%. In those aged 40 years and over, all 11 cycles were unsuccessful, resulting in a livebirth rate of 0%. The average age at cryopreservation of those who achieved a livebirth was 37,

whereas those who did not achieve a livebirth, the average age was 40 (p=0.07). Almost half of the cohort (48.6%; n=17) exhausted their supply of stored oocytes without achieving a livebirth. In those aged <35, of the six women who underwent thaw cycles, two women (33%) exhausted their supply of oocytes without achieving a livebirth. In those aged 36-39, of the 24 women, 12 women (50%) exhausted their oocytes without achieving a livebirth. Finally, in those aged 40 and above, of the 11 women who thawed their oocytes, none of whom achieved a livebirth, and almost three quarters (n=8; 72.7%) ran out of oocytes.

Discussion

Predictors of oocyte yield

This study demonstrates herein that markers such as E2, BMI and the number of follicles ≥12mm at trigger were all independent significant predictors of oocyte yield in women undergoing SEF. However, whilst AFC and AMH were significant predictors of oocyte yield utilising univariate analysis, the relationship was not significant when controlling for other potential confounders. Whilst there are no previous studies that have assessed these relationships in the context of SEF, comparisons can be made from data from cycles performed for other indications, with consideration for the differences in population characteristics.

In contrast to the findings demonstrated herein, AMH and AFC have consistently been identified to be two of the best predictors of oocyte yield, and have been shown to have similar predictive value.[609] However, it is well known that AMH levels are strongly correlated with age.[610] Given the relationship between AMH and age, when assessing oocyte yield whilst controlling for age, the interrelationship between age and AMH may nullify the impact of AMH. This was demonstrated herein, as AMH was a statistically significant predictor of yield utilising univariate analysis, but when controlling for other variables using multivariate analysis, no significant relationship was identified. As such, it is likely the impact of the interrelationship between age and AMH was so prominent it negated the influence of AMH on oocyte yield.

It is important to emphasise that women undergoing SEF are young and fertile, unlike the vast majority of other women undergoing IVF. AMH is often used as a surrogate marker of ovarian reserve in women undergoing ART, and therefore much of the evidence is limited to women with pathologically ovarian reserve. Such causes of subfertility include previous ovarian surgery or following chemotherapy or radiotherapy, in which a lower AMH may be present, regardless of age.[610] As such it is unsurprising that data in infertile populations, AMH has been shown to be an independent significant predictor of oocyte yield.[611] However, as demonstrated herein, in women who undergo SEF, age remained an independent significant predictor even when controlling for other variables, highlighting it as a more accurate predictor of oocyte yield than AMH in this population. BMI has previously been identified to be associated with oocyte yield.[612] However, whereas it was previously identified that oocyte quantity was lower in obese women, compared with those with a normal BMI,[612] the findings demonstrated herein contrarily show that oocyte yield increased by 2% per kg/m^2 increase of BMI. The majority of women in our cohort had a normal BMI 20.0-24.9 (n=111), less were categorised as being underweight (BMI <20.0, n=34) and very few were obese (BMI >30.0, n= 9). In contrast to previous literature, the mean number of oocytes collected in the obese group was higher than the underweight group, although not to a significant extent (9.6 vs 8.5). However, this is further evidence that in the context of SEF, patient demographics are dissimilar to the standard IVF population. Whereas obese infertile women may have associated subfertility related to their body habitus, such as those with polycystic ovarian syndrome, overweight fertile women may not have a discernible reduction in oocyte yield, although given the lack of statistical significance, and small subgroup numbers, little can be drawn from this data. This is exemplified by previous studies where obese women contributed to 8.9% of the total cohort, compared to only 2.9% in our own cohort.[613] This reinforces further that much of the evidence on prediction of oocyte yield deduced from IVF cycles in infertile women may not be extrapolatable to SEF populations.

This study does however reaffirm findings from a large study of 5499 women which established that serum E2 is a significant predictor of oocyte yield.[604] However, when considering data in oocyte donation cycles, a population in which demographics and fecundity are more comparable to SEF

cohorts, it is worth noting high E2 levels have been associated with impaired endometrial receptivity.[614] However, given that women undergoing SEF do not subsequently undergo embryo transfer in the same cycle, the risks of unsuccessful implantation are not relevant.

This data also demonstrates that follicular size >12mm diameter is a predictor of oocyte yield. This supports findings that optimal follicular volume for a high oocyte yield is 13-23mm.[606] Furthermore, whereas small follicles (8-12mm) still have capacity for normal blastocyst development and potential to achieve livebirth (64%), a much higher yield and quality of oocyte occurs from size ≥12mm (77%).[606]

Ovarian hyperstimulation syndrome

OHSS is a serious condition with potential, albeit rare, life threatening implications,[4] which are mainly as a consequence to thrombo-embolic sequelae and multi-organ involvement.[5] However, in milder cases symptoms such as nausea, vomiting and pain can significantly reduce quality of life, albeit temporarily. Women undergoing SEF are inherently at higher risk of developing OHSS by virtue of their younger age and higher ovarian reserve.[615] This study is the first of its kind to demonstrate OHSS risk in SEF cycles, showing an incidence of 4.1%. Age, higher AFC, lower FSH, and increasing number of follicles >12mm were all significantly associated with the development of OHSS. It has previously been shown that OHSS cases can be predicted using an AFC cut off value of 8 or more with 78% sensitivity and 65% specificity.[616] Herein, 92% of the women who subsequently developed OHSS had an AFC of >8, whereas overall, around half (52.6%) of women with an AFC > 8 did not develop OHSS, resulting in a specificity of 32% and sensitivity of 92%.

It has previously been identified that women with an AMH >6.95ng/ml are at higher risk of OHSS and therefore a cut off of 7 ng/ml was proposed.[617] Data from this cohort demonstrates similar findings as all but one woman who developed OHSS (92%) had an AMH >7, although this was statistically insignificant, likely due to the small sub-group size. However, almost half (47.3%) of this

cohort had an AMH of 7 or more, but did not develop OHSS, resulting in a specificity of 35% and sensitivity of 92%.

Whilst this is the first study of its kind to assess OHSS rate in women undergoing SEF, comparisons can be made with data recorded in autologous IVF cycles, but with due consideration for the differing demographics of the respective cohorts of women. A previous retrospective study analysing fresh autologous cycles demonstrated a much lower OHSS risk (<1%).[618] However, it should be noted that the classification of OHSS was only made if it was severe enough to warrant ascitic drainage, comparable to severe or critical cases by RCOG standards.[578] If a similar classification was used in this study, no cases would have been reported, which would have underrepresented the potential adverse impact. However, another study in women undergoing either autologous or cryopreservation cycles demonstrated a much higher OHSS rate in women who received a dual trigger (8.6%),[619] which was more accurately categorised according to Golan's classification.[620] However, it should be noted that many women in this study subsequently became pregnant which, owing to the biphasic nature of OHSS, may overestimate the risk compared to women undergoing SEF. However, a recent retrospective study on oocyte donors receiving a dual trigger identified a similar OHSS rate of 8.5%.[621] However, given the selection criteria of being an oocyte donor, including being younger than 34 years old, by virtue of their physiologically higher ovarian reserve and inherent ability to produce multiple follicles, are at greater risk of developing OHSS.[615]

Despite the comparisons made between the reproductive potential of women undergoing SEF with oocyte donors, the underlying ethical principles may be different. Given the altruistic nature of oocyte donation, it is essential to balance the benefits of optimising the numbers of oocytes retrieved with adverse risks of OHSS. Particularly as oocyte donation in the UK is in short supply, it is essential efforts are made to reduce risks, not only to reduce complications, but to promote positive experiences, which may encourage more women to donate.[621] However, for women undergoing SEF, the underlying goal is to optimise oocyte yield in order to improve chances of cumulative successful livebirth. Whilst it has been suggested an oocyte yield of at least 15 is required to ensure good embryo

quality and successful live birth, beyond this, the risks of complications such as OHSS supersede the benefits.[607] However, as it is in the woman's interest to retrieve as many mature oocytes as possible, some women may deem the potential risk of OHSS more acceptable than undergoing multiple cycles, thereby exposing themselves to additional further risk and financial expense.

Reproductive outcomes

At the time of writing 11% of the women who underwent SEF had returned to use their oocytes, a mean of 3.8 years after cryopreservation. A previous study reporting outcomes from 1382 women who underwent SEF at a mean age of 37.7 years showed that 8.7% (n=120) women returned to use their oocytes after a short follow up time of 2.2 years.[385] Another study, using a smaller sample of 254 women in Sweden, reported that 15% (n=38) returned to use their oocytes after a longer follow up period, returning after a mean of 4 years,[622] whilst another survey reported just 6% of 96 women had returned to use their oocytes, 2.8 years after undergoing SEF.[568] As described in chapter 5a, which assessed intentions of women who underwent SEF, almost two thirds (63%) intend to use their oocytes 3-6 years after storing them.[596] Owing to lack of follow up, it is difficult to distinguish if these intentions will be adhered to, although the current utilisation rates appear low. As some women may subsequently spontaneously conceive whilst their oocytes are cryopreserved, a lower utilisation rate is likely to remain.

The clinical pregnancy rate demonstrated in our cohort of women was 40%. This was achieved in women at a median age of 43 years old, which compares to the average pregnancy rates reported by the HFEA in women aged between 35-37 years.[9] This epitomises the reproductive potential of SEF and provides further clinical correlation of the concept. Just under a third (31.4%) of women who returned to use their stored oocytes attained a livebirth. This is favourable compared to a recent study analysing 10 years of data from two UK clinics who had a success rate, pertaining to livebirths or ongoing pregnancies of 17.5%.[623] In that study, which had a similar mean age at cryopreservation of 37 years, successful outcomes including a livebirth or ongoing pregnancy were achieved in 20-23% of women aged <40. In the data presented herein, the success rate in those who cryopreserved oocytes

<40 years was much higher, at 48%. In the Swedish study that published outcomes of 38 women after SEF the cumulative live birth rate was 63%, 26% and 0% in women of ages 36-37, 38-39 and >/=40 years at vitrification respectively. When stratifying using similar age groups in the cohort presented herein, this data presents similar findings, with livebirth rates in women who underwent SEF ≤37 years of 50% (n=12), 39% aged 38-39 (n=18), and 0% ≥40 years (n=10).

In those women who underwent SEF over the age of 40 years old, no women who returned to thaw their oocytes had a successful outcome, and of those almost three quarters (72.7%) had exhausted their oocyte store. This proportion is more than double that reported following assessment of outcomes from the aforementioned Swedish study where in the 11 women who underwent SEF over the age of 40, none achieved a livebirth, and four (36%) had exhausted their oocyte supply. The previously discussed UK study across two centres fared slightly better, with 7% of those aged 40-42 having a successful outcome. Whilst the overall numbers remain small, it is clear undergoing SEF aged 40 or over is unlikely to be successful. Not only are the success rates low, but the risk of miscarriage remains elevated, exemplified by the fact the two women who suffered miscarriages in this study were both aged >40. This is reinforced by data that has identified the risk of miscarriage in women aged 40-44 is 51%.[364] As such, it is essential that there is increased awareness of female ovarian reserve amongst the general public, and if women choose to undergo SEF, that they do so at an age associated with better outcomes. It has previously been identified that the most cost-effective age for women to undergo SEF is prior to the age of 38 years, justified as the age where the greatest improvement in live birth rate is observed when compared to no action.[573] However, the highest chance of attaining a successful live birth has been shown to be when it is undertaken prior to the age of 34.[573] Additionally, oocyte cryopreservation prior to 36 years has been shown to have the highest discriminative capacity to predict livebirth.[370] It has also been identified that following vitrification, women aged 30-36 had a more than two-fold increase in implantation, pregnancy and livebirth rates compared to those aged 37-39.[371]

This reaffirms that if women choose to undergo SEF, it is likely to offer most benefit to do so by the age of 36. However, caution should be taken advising women to cryopreserve 'too' early. Each case should be individually considered, with appropriate contemplation of the pertinent physiological, obstetric, legal and economic considerations.[372] Although storing oocytes earlier optimises oocyte quality and enhances success rates, the likelihood of spontaneous conception in those additional reproductive years is greater, which would potentially have resulted in unnecessary physical risk and substantial financial burden, in addition to the expense of longer oocyte storage. Moreover, in the context of UK legislation, current HFEA regulations permit the storage of oocytes for a maximum period of 10 years. Therefore, storing oocytes earlier, to optimise future success, may be counterproductive, necessitating the need to prematurely use a sperm donor, to prevent the oocytes being discarded. However, following concern regarding the appropriateness of this legal time frame,[393,596] it is possible this legal chronological restriction may be alleviated in the future. Ultimately, timing of SEF cannot be prescribed by healthcare professionals. It should be determined by the fully informed women undergoing oocyte cryopreservation, following extensive counselling on their physiological fertility decline and associated pregnancy risks with becoming pregnant at advanced maternal age, in the context of the economic and legal considerations. However, in light of the findings demonstrated here, and reaffirmed by other studies,[622] undertaking SEF over the age of 40 appears to be associated with very low success rates.

The findings of this study add substantially to published literature to identify factors that impact success following SEF. The associated enhanced ability to counsel women regarding their probability of oocyte yield, and subsequent livebirth is important as some women may not wish to continue with cryopreservation if there is a suboptimal chance of success. Whilst speculative, if women in this cohort were aware they had a very low chance of success, they may have undergone further cycles to optimise their future chances. Other studies have shown that women are willing to undergo two or more cycles in order to retrieve sufficient numbers of eggs for storage.[624] Therefore, as described herein, it is essential for women to have the most accurate individualised estimate of their likelihood of future success, in order to make informed choices regarding number of cycles and facilitate the

management of future expectations. However, it is paramount that no matter how high the predicted percentage chance of achieving a livebirth, women must be made aware that the likelihood of a livebirth cannot be guaranteed. This is particularly important given the findings demonstrated in study 5a that evidence has suggested women who overestimate expectancy of livebirth, or have few eggs to preserve often regret freezing, resulting in subsequent emotional and psychological sequelue.[596]

Limitations of this study include that all participants of the study were from a single centre, and as such, there is potential for bias. Moreover, the decision to give either GnRH antagonist or dual trigger were made on an individual basis and was based on clinical judgment, including consideration of previous response to stimulation along with baseline AMH and AFC and E2 levels on the day of trigger. The lack of strict criteria introduces the potential for bias in this analysis. All of the above could be minimised by the use of a multi-centre randomised powered study.

Conclusion

This study highlights that age, BMI, E2, and number of follicles >12mm at trigger can help predict oocyte yield in SEF cycles. As such, individualising SEF cases with consideration of these markers, should be undertaken in order to optimise oocyte yield. AMH and AFC are however not independently significantly associated with oocyte yield. In addition, this is the first study to establish an incidence of OHSS in SEF cycles and establish a significant relationship between age, total AFC, number of follicles >12mm and FSH as predictors of OHSS. Moreover, the reproductive outcomes presented herein highlight that undertaking SEF over the age of 40 years is suboptimal and associated with a low chance of success. Whilst there was no significant difference in livebirth rates in women <35 years and aged 36-39, it is advisable that SEF should be undertaken at or prior to the age 36, to optimise subsequent outcomes. This data has significant implications for clinical practice, and can be used to individualise care, enhance counselling and control expectations in women undergoing SEF.

The Future

The Future of Ovarian and Uterine Fertility Restoration

At the time of writing, UTx cases performed to date using DBD contributed to just 20% of cases performed (n=12), and only two successful livebirths had been reported. In humans, uterine grafts have been successfully retrieved at the beginning of a multi-organ retrieval,[53,210] and after the retrieval of the other solid organs,[58,150,213,492] and no negative impact upon the other organs retrieved or the retrieval process have been observed. Following the first livebirth following UTx using DBD in 2017,[58] this demonstrated UTx is feasible in this model. However, given the disparity in cases performed in this model, compared to LD, it is difficult to draw accurate conclusions from the small number of cases performed so far, to determine which donor results in better outcomes. Given that other solid organ transplant outcomes are superior in living donors compared to DBD,[218,219] further cases are needed to enable detailed comparisons to be made. However, given the complete removal of donor risk, it is likely that DBD will continue to be undertaken in countries where there is an established organ retrieval network.

In the UK, the multi-organ retrieval process is orchestrated by NHS Blood and Transplant (NHSBT). Between 2018-2019, 1361 deceased donor retrievals were undertaken in England, which resulted in 3313 transplants subsequently being undertaken. 814 of the retrievals were undertaken in the context of DBD whilst 547 were undertaken following DCD. 116 DBD multi-organ retrievals were undertaken in London between 2018-2019.

Over the last five years, following a multitude of meetings with myself and various key stakeholders at NHSBT, including the National Retrieval Group (NRG), the Research, Innovation and Novel Technologies group (RINTAG) and National organ donation committee (NODC), a collaborative strategy was created with the aim to seamlessly fit into what is already a very successful multi-organ retrieval process. NHSBT senior management committee approval was given on 15th January 2019 to commence the INSITU study, initially starting in the London area, with a view to extending to other regions depending on donor availability.

Donor assessment

The process of donor identification was facilitated by the already established pathways of identifying donors used by NHSBT. Following the identification of a potential donor, exclusion criteria will be applied to determine potential suitability to potentially proceed with uterine donation (**Figure 6.1**).

- Donation after brainstem death
- Female
- Aged 18-50 years old
- No absolute or relative contraindications to organ donation
- No known absolute tissue donation contraindications
- Any known specific contraindications to UTx

Figure 6.1: *Initial screening selection criteria for deceased donors*

Potential donors that meet the selection criteria will then be further assessed by a specialist nurse in organ donation (SNOD), prior to approaching the donor's family for consent. Background information will be sought from the patient notes and general practitioner records to determine if the donor meets the full criteria to be considered a suitable donor (**Figure 6.2**). Investigation results such as blood group and any known microbiology/serology will also be required.

- Pre-menopausal
- Family consent for organ donation
- No history of cancer
- No previous multiple / significant uterine surgery – 2 X LSCS acceptable but no myomectomies
- No systemic disease precluding donation
- Multiparous (4 or less deliveries)
- No previous obstetric problems including delivery <37/40
- No previous recurrent miscarriages (3 or more)
- Live birth to miscarriage ratio >1 (maximum of 2 miscarriages)
- No previous significant cervical surgery (cone biopsy or large loop excision of the transformation zone)
- ABO compatible and negative cross match

Figure 6.2: *Selection criteria for deceased donors*

Following confirmation of donor eligibility, an approach would be made toward gaining consent from the donor's family by an appropriately trained SNOD. A donor family information leaflet would accompany the counselling and consent process.

Following donor family consent, a UTx scout team will be deployed to perform the final investigations required to confirm donor suitability. These include a transvaginal ultrasound, a cervical smear/HPV test and a GUM screen, including chlamydia, gonorrhoea and TV. Given the urgent nature for results needed within a short time frame, the Cepheid Xpert (Cepheid, Sunnyvale, CA, USA) HPV, trichomonas vaginalis and Chlamydia/Gonorrhoea tests will be used. The transvaginal ultrasound should be essentially normal, although minor uterine pathology such as small (<1cm) fibroids, which are not submucosal, would be acceptable. After confirmation of donor suitability, the multi-organ retrieval would proceed. Every effort has been made to ensure there is minimal disruption to the multi-organ retrieval process.

Uterine transplantation in context of multi-organ retrieval

The surgery would be undertaken through a midline thoracolaparotomy incision, thereby not requiring any additional incisions. During the initial organ mobilisation phase, prior to cross clamp, an additional 45-60 minutes would be needed to optimise the proposed graft. Initial pelvic side wall dissection will be undertaken, with subsequent lymphadenectomy around the major pelvic vessels including the common iliac, internal external iliac and obturator nodes. Ureteric dissection would subsequently be undertaken, with careful reflection of the ureters away from the iliac and uterine vessels. Subsequent vascular dissection would ensue, including mobilisation and skeletalisation of the internal iliac arteries and veins, starting from the bifurcation and moving distally towards the uterine vessels. All other vessels not included in the graft should be ligated. The ovarian veins should be assessed and included in the graft if of sufficient calibre and quality. The Fallopian tubes can be dissected and tied together to use to manoeuvre the uterus intra-operatively. Antero-laterally, the round ligaments should be divided as laterally as possible, the bladder should be reflected anteriorly,

and the ureteric tunnels should be opened. Posteriorly, the rectum should be mobilised, and the uterosacral ligaments should be divided as close to the sacrum as possible.

Following this initial dissection, the perfusion / decompression phase can commence. In addition to the usual cannulation and perfusion of the aorta, with subsequent venting through the IVC, an additional perfusion circuit should be implemented to ensure the uterus continues to be perfused with cold perfusion solution whilst the other abdominal organs are being retrieved. The second circuit will include cannulation of both external iliac arteries, as close to the inguinal ligaments as possible, with coexistent decompression of the external iliac veins at the same level. Ice should be introduced to the pelvic cavity to ensure the period of ischaemia is as cold as possible, to reduce the risk of more damaging warm ischaemia. The cardiothoracic team should then retrieve the heart +/- lungs if being retrieved, before the abdominal team retrieve the abdominal organs. Following the retrieval of the other organs, the uterine graft should be isolated, including long vascular pedicles to the level of the anterior division of internal iliac vessels, including long lengths of ovarian veins, extensive round and uterosacral ligaments and bladder peritoneum. The vagina can then be transected 30mm caudal to vaginal fornix, before major vessel transection of anterior division of the internal iliac vessels and transection of the ovarian veins. The graft can then be removed, and abdominal closure undertaken.

Back table dissection

The uterine graft will then be removed and submerged in a sterile bowl containing cold preservation solution and ice. The arterial inflow will then be cannulated and flushed with preservation solution. A post-retrieval cervical biopsy will be taken. The bowl will then be placed in a series of three sterile plastic bags following nationally agreed guidelines for the packaging of organs intended for transplantation, before being placed in a kidney storage box with ice.

Implantation

The implantation operation will be undertaken through an infraumbilical midline incision. The operation would only begin once the graft had been appropriately flushed at back table dissection, and agreement within the team was in place to proceed with the implantation. The vaginal vault would initially be mobilised, dissecting it away from the bladder anteriorly and rectum posteriorly. In women with MRKH, if a uterine rudiment was present, it would be excised. With the graft still in cold storage, sutures would be inserted to be used for uterine fixation following introduction of the graft. The sutures would be placed bilaterally through round ligaments, uterosacral ligaments and paravaginal tissue. The vascular preparation in the recipient can then be undertaken. The external iliac vessels will then be skeletonised and prepared for implantation. The external iliac should subsequently be clamped, and the uterine graft be introduced to the pelvis. It should be placed into its normal anatomical position, and the donor vagina should be anastomosed to the recipient vagina and round ligaments.

An end-to-side internal iliac to external iliac anastomosis should then be undertaken, initially unilaterally with the vein, and then the artery, before repeating on the contralateral side. The external iliac vein should then be unclamped, whilst maintaining the systolic blood pressure above 100mmHG, before removing the arterial clamps. If included in the graft, the ovarian veins should then be anastomosed to the external iliac vein.

Following implantation, the perfusion to the graft would then be inspected macroscopically, ICG PINPOINT fluoroscopy, and the novel use of multispectral imaging (MSI). The PINPOINT Endoscopic Fluorescence Imaging system can be used to assess organ perfusion. It has previously been used for perfusion assessment of bowel perfusion after anastomoses in colorectal surgery,[625] and in free flap design in plastic surgery.[626] It has also been used to assess perfusion following UTx in non-human primates. [627] The MSI system is used to detect oxygenation within the uterus, and has been used successfully in animal models to observe uterine oxygenation after uterine transplantation.[83] It is used in conjunction with a rigid endoscope and light source which are both

standard pieces of clinical equipment, made by recognised medical device manufacturers. The MSI system, which is connected to the rigid endoscope, will be used strictly to gather research data. It will not be used to make any clinical decisions related to the health of patients. The graft is then tied to the remaining previously placed ligament sutures, before overlapping the bladder peritoneum over the fundus of the bladder. A post-implantation cervical biopsy should then be undertaken. Following achievement of haemostasis +/- insertion of a drain, routine closure is undertaken.

Induction immunosuppression will include methylprednisolone 500mg IV infused over at least 30 minutes, prior to reperfusion of organ and a single dose of Alemtuzumab (Campath) 30mg SC administered on completion of methylprednisolone infusion. A five-day course of meropenem 500mg TDS IV, and micafungin 100mg once daily (OD) IV will be initiated and extended depending on clinical condition and culture results. CMV prophylaxis with valganciclovir 900mg OD will be commenced, and PCP prophylaxis with co-trimoxazole 480mg OD. VTE prophylaxis will include unfractionated heparin 7500 units twice daily (BD) subcutaneously (SC) whilst an inpatient and switching to dalteparin 5000 units OD SC to complete 6 weeks post-operatively. Post-transplant the recipient will be required to take aspirin and ongoing maintenance immunosuppressive therapy whilst the graft remains in place. Maintenance immunosuppression includes tacrolimus MMF. Tacrolimus will be given at a dose of 0.05mg/Kg BD immediately post-op, with the dose being rounded up to the nearest 0.5mg and a target level between 8-12ng/ml. MMF will be prescribed for the first 3-4 months at a dose of 750mg BD PO, to start immediately post-op.

Post-operative care

Following an inpatient stay of 5-7 days, intensive outpatient follow up will ensue, initially twice weekly during first postoperative month, then once weekly between months 2-3, before decreasing in frequency to fortnightly between months 4-6. Each visit will include weight, review of menstrual diary and an examination including recording of blood pressure (BP), heart rate (HR), temperature, and respiratory rate (RR). Speculum examination should be undertaken to visualise the cervix and an HVS should be sent for microscopy, culture and sensitivities. A cervical biopsy should be taken at

weeks 1, 2 and 4 and monthly thereafter, as well as in the event of symptoms or signs suggestive of rejection. Blood tests including FBC, U&Es, LFTs, CRP, erythrocyte sedimentation rate, glucose, tacrolimus levels and human leukocyte antigen (HLA) donor specific antibodies should be undertaken at each visit. Once CMV prophylaxis is stopped CMV PCR should be measured for further 3 months at each clinic visit and when clinically indicated. Routine urinalysis should be performed, with a sample sent for protein creatinine ratio and BK virus. Ultrasound should be undertaken, trans-abdominally, trans-vaginally, or transrectally depending on the clinical situation, to assess uterine structure and vascularity.

After 6 months, and following cessation of potentially teratogenic medications, embryo transfer will be attempted. A single embryo transfer of a euploid embryo will be undertaken. Following successful conception, high risk antenatal care, with fortnightly follow up, would ensue with delivery by Caesarean Section between 35-37 weeks' gestation, unless clinically indicated sooner. In the absence of complications, the process could be repeated for another child if desired by the recipient. Hysterectomy would be planned for six months following the completion of the woman's family.

Following completion of the INSITU study, in combination with analysis of other multi-centre outcomes following both LD and DBD UTx, determination of outcomes with respect of donor type can be undertaken. It is well established that transplant outcomes are better following LD than DBD. Given that 21 out of 23 livebirths following UTx have been achieved following LD, with just two livebirths after DBD,[6] a similar trend may develop in UTx. However as just 20% of cases (n=9) have been performed using DBD, more cases and longer follow-up are essential before accurate conclusions can be drawn. Furthermore, evolution in surgical technique has greatly enhanced living donor risk profile, with the introduction of minimal access retrieval techniques and an alternative vascular dissection that significantly reduces operative time and surgical risk.[6] The first livebirth following robotic assisted UTx retrieval was recently reported, highlighting the potential for minimally invasive retrieval techniques. However, if similar or superior outcomes are demonstrated in DBD compared to LD following long-term follow up, DBD should be prioritised to minimise risk to

living donors. However, given the finite supply of deceased donors in the context of an exponential rise in UTx procedures being performed, it is likely a combination of LD and DBD will be necessary in the short-term future. In the long-term future, the development of a bioengineered uterus may eventually supersede conventional organ transplantation methods, although it is unlikely this will be a realistic option for at least 10-15 years.

Funding and provision

Further clarification and consensus are also required regarding future provision and funding for UTx in the UK. Criticisms have been deployed specifically against public funding of UTx in both countries with socialised medical care,[73,74] and insurance-based or mixed systems.[75] Moreover, consideration is required as to whether or not a quality of life improving procedure such as UTx may divert scarce resources away from others who need lifesaving treatment. As such, it has been argued that while UTx improves upon adoption and surrogacy by satisfying personal desire for the experience of gestation and childbirth, this is difficult to justify given the economic implications associated with UTx. However, it is not possible to generalise the quantification of how suitable adoption and surrogacy are for women with AUFI. Their availability and appropriateness are inextricably linked to individual circumstances, taking account of personal values, legal context, as well as religious and cultural backgrounds. As aforementioned, in most countries, if it's not prohibited, surrogacy remains socially, psychologically and legally complex. In such circumstances, despite the significant associated risk and financial costs, UTx may be considered a reasonable preference.[70] Moreover, concerns about discriminatory social bias are a general critique of reproductive medicine in general, instead of a specific issue related to UTx. However, UTx is presently more difficult to justify than IVF owing to the comparatively high costs and heightened level of risk.[63,64] Finally, it is difficult to ascertain why the mere existence of alternatives dictates the necessity for cessation of provision. Other interventions such as breast reconstruction after mastectomy, scalp cooling for chemotherapy and pinnaplasty are primarily performed to enhance quality of life, but also facilitate protection from hostility associated with not conforming to prevailing social norms. UTx could be considered similarly, and even if there are alternatives, performing UTx may still be justified if performed in the

woman's best interests.[319] Finally, when considered in a reproductive medicine context, women with *treatable* causes of infertility, such as endometriosis, intrauterine adhesions or submucosal fibroids often undergo surgical intervention with the primary aim of improving conception rates. If UTx creates a situation whereby AUFI is considered *treatable*, it may be difficult to justify not offering it. As part of establishing future NHS funding of UTx in the UK, following application to the Prescribed Specialised Services Advisory Group (PSSAG), it has been agreed that if it were to be funded, it would be commissioned nationally by NHS England.

Tissue Bioengineering

The long-term future of UTx will undoubtedly focus on the development of a bioengineered uterine graft, which would negate surgical risk to the donor as well as the risks associated with immunosuppression in the recipient. Whilst the concept remains in its infancy, the Swedish team have established a technique to repair a rat uterus using a bioengineered uterine patch and have demonstrated functionality by achieving pregnancies.[19] This development has set the benchmark for the future of UTx, and with ongoing innovation and collaboration, the options for fertility restoration in women with AUFI should continue to become more available and acceptable.

Over the last decade, there has been considerable effort in developing bioengineering strategies for fertility restoration. Bioengineering encompasses the fields of tissue engineering and regenerative medicine, which aim to regenerate or replace damaged cells, tissues and organs to restore normal biological function. Bioengineering approaches can overcome or minimise shortfalls often experienced in organ transplantation, such as availability of donors and complications of immunosuppression. Tissue engineering typically involves combining cells, biomaterial scaffolds and biologically active molecules to generate functional constructs that resemble native tissue. The biomaterials used may consist of natural polymers, such as collagen, fibrin and alginate or biodegradable synthetic polymers. Scaffolds can also be generated from decellularized tissue.

Decellularization is the process of removing the cells from tissue and leaving behind the extracellular matrix (ECM), which retains biochemical and structural properties of the tissue.[628] The scaffold can then be recellularized using cells from the host to create an implantable three-dimensional (3D) tissue construct.

In female reproductive organs tissue engineered scaffolds, including decellularized ECM, have been used to reconstruct the cervix,[629-631] vagina,[630-635] and create artificial ovaries. [10-12] In the uterus, tissue engineered constructs have demonstrated the ability to partially repair the uterus in animal studies,[19,636-639] and limited human studies.[640,641] The two main approaches have been the use of biodegradable scaffolds to deliver cells and growth factors for uterine repair and regeneration of decellularized uterine matrices to engineer replacement tissue.

Uterine tissue engineering for in vitro models

Tissue engineering can be used to create 3D models, which can offer superior biomimetic systems compared to two-dimensional cell culture for *in vitro* studies of cell interactions, normal physiology and disease. 3D tissue engineered endometrial models, involving scaffolds made from naturally derived polymers,[642-645] or synthetic polymers, [646] seeded with endometrial cells, can mimic normal endometrial function,[643] and histology.[646] These can be used to study endocrine and paracrine responses,[642] cancer invasion,[647] and implantation.[645] Lu et al. (2009) seeded endometrial epithelial and smooth muscle cells onto a collagen and Matrigel matrix to engineer a three-layered uterine construct which demonstrated the ability to support embryo development *in vitro*.[644]

Tissue engineering for uterine repair and endometrial regeneration

An early example of uterine tissue engineering for an *in vivo* application was described by Campbell et al. (2008), where rat peritoneal cavity was utilised as a bioreactor to generate autologous myofibroblast tissue grafts for uterine reconstruction.[648] Following transplantation into uterine defects,

the grafts developed complex tissue architecture that resembled endometrial and myometrial layers. Embryos were subsequently able to implant and grow in the grafted uterine horn.[648]

Biodegradable scaffolds to deliver stem cells or growth factors

Biodegradable collagen scaffolds can also be used as a vehicle to deliver multipotent stem cells or growth factors for uterine repair and endometrial regeneration.[637,639,640,649-651] Stem cell therapy has shown promising results from *in vivo* studies as a treatment for endometrial damage and scarring.[202,204,652,653] The use of scaffolds allows for better concentration and targeting of stem cells, or other treatment, at the site for repair. In a rat model of uterine damage and scarring, vascular endothelial growth factor,[649] and basic fibroblast growth factor,[650] loaded on collagen membranes have demonstrated ability to stimulate endometrial healing and improve pregnancy outcomes. Ding et al. (2014) incorporated collagen scaffolds seeded with bone marrow-derived mesenchymal stem cells into injured rat uteri.[637] These grafts led to increased endometrial and myometrial proliferation, regeneration of microvasculature and restoration of fertility. Constructs made of human embryonic stem cells combined with collagen scaffolds were transplanted into damaged rat uteri and resulted, similarly, in endometrial regeneration and improved pregnancy rates.[638] Notably, implantation was seen in areas of the uterus that had been damaged. Xu et al. (2017) injected umbilical cord-derived mesenchymal stem cells (UC-MSCs) combined with degradable collagen fibres to treat scarred uterine horns in a rat model.[639] UC-MSCs facilitated collagen degradation in uterine scars, through upregulation of MMP-9, and promoted regeneration of endometrium, myometrium and vasculature in the scarred tissue. The group treated with collagen-UC-MSC demonstrated a higher pregnancy rate compared to control or collagen or UC-MSC alone.[639]

The promising results from these animal studies have led to two human studies involving the application of stem cell enriched collagen scaffolds to treat patients with infertility secondary to intrauterine adhesions.[640,641] In a preliminary study, five patients with severe Asherman's syndrome and secondary infertility received bone marrow derived mononuclear cells loaded on collagen scaffolds as an adjunct to adhesiolysis surgery.[640] These patients had regeneration of their

endometrium, successful pregnancies and all achieved live births after treatment. A subsequent trial involved 26 patients with infertility due to recurrent intrauterine adhesions who received transplants of collagen scaffolds loaded with UC-MSCs following adhesiolysis.[641] Amongst the 25 patients that were followed up, mean endometrial thickness increased and intrauterine adhesion scores decreased. Ten patients became pregnant resulting in 8 livebirths, 1 first trimester miscarriage and 1 ongoing pregnancy at the time of publication.[641] The authors attribute the effect of stem cells on endometrial regeneration to downregulation of ΔNp63, a protein that is associated with endometrial quiescence and more highly expressed in Asherman's syndrome.[640]

Decellularized tissue scaffolds

Decellularized tissue is a valuable biomaterial for scaffold generation because it retains some essential properties of native tissue ECM. ECM gives mechanical and biochemical cues to cells to regulate cell attachment, proliferation, organisation and migration. It also plays a role in stimulating stem cell recruitment and differentiation.[654] The process of decellularization removes immunogenic cellular antigens so that after transplantation, ECM scaffolds are readily incorporated into host tissues without a foreign body immune reaction.[655] Furthermore, decellularized ECM can provoke a beneficial host immune response by polarising macrophages to a M2 phenotype, which facilitates tissue repair and regeneration, [656] and reduces fibrosis.[655]

Tissue can be decellularized by a variety of physical, chemical or biological means [628]. Decellularization has been achieved in rat,[19,636,657-660] pig,[661] and human uterine tissue.[657] Typically, uterine matrix patches have been recellularized with stem cells.[19,636,659,661] *In vitro*, decellularized uterine matrices have shown preservation of the tissue architecture and recapitulation of native tissue organisation after recellularization. *In vivo*, these recellularized uterine matrices have demonstrated the ability to result in tissue regeneration when transplanted into rats that were given uterine defects.[19,636] Moreover, pregnancy was supported in rats that received the implanted recellularized tissue patches.[19,636,658] To date, the largest whole reproductive organ decellularization was reported in

a porcine uterus,[661] which was subseuently partially recellularized with disks of uterine scaffold using human stem cells. The decellularization approach brings promise for an eventual bioengineered whole uterus, though this remains a highly complex objective. Some significant challenges remain with regard to clinical translation, such as the sourcing of organs and cells and determining host responses to small amounts of residual cellular and nuclear material.

Non-uterine tissue ECM has also been used for uterine reconstruction and endometrial repair. In a rat model of intrauterine adhesions, Kong et al. (2017) transplanted porcine small intestine submucosa (SIS) ECM into a mechanically and infectiously injured uterine horn and found that the endometrium was thicker, fibrosis significantly lower and pregnancy rate higher compared to the untreated injured horn.[662] There have been two published case series on the use of SIS ECM to reconstruct the uterine cervix in cases of cervical malformations,[630] or cervical aplasia.[631] In both these papers, the authors reported good functional outcomes in patients who received SIS grafts as defined by maintained cervical patency.

The research findings to date show huge potential for bioengineering methods to regenerate and replace the damaged or absent uterus and represent an exciting future where many more treatment options will become available to women with AUFI.

Ovarian cortex cryopreservation

The long-term survival rates for malignancies affecting women of reproductive age has improved significantly over the last few decades.[663] This has inevitably led to a focus on optimisation of fertility preservation prior to gonadotoxic treatment, as reproductive autonomy is now considered an imperative quality of life component for women. Recent fertility preservation developments have facilitated the method of ovarian tissue cryopreservation (OTC) as an established technique, predominantly undertaken in oncology patients prior to gonadotoxic chemotherapy.[664] OTC involves ovarian tissue extraction of either the ovarian cortex containing primordial follicles, whole ovary or ovarian follicles; followed by cryopreservation and subsequent autotransplantation.[665] The concept was proposed to prevent the risks of secondary POI in women undergoing gonadotoxic chemotherapy, but also to preserve fertility in pre and peri-pubertal girls, in whom oocyte cryopreservation is not possible.[665,666] This is unsurprising given that following six cycles of the commonly used regime of cyclosphomide, methotrexate and fluorouracil, the risks of chemotherapy induced amenorrhoea in women <40 years and >=40 years old are as high as 33% and 81% respectively.[667] Figures from national databases suggest that between 2500-6500 OTC procedures take place in Europe per year.[668] Given the increasing use and acceptance it is no longer considered experimental according to criteria by the European Society of Human Reproduction and Embryology (ESHRE).[669]

OTC offers multiple advantages over other methods of fertility preservation in this context. There is no need for ovarian stimulation, which may cause a delay in lifesaving treatment. In addition, unlike oocyte or embryo vitrification, OTC can preserve hundreds of primordial follicles at once.[670] Recent updates on reproductive outcome suggest that following the first livebirth in 2004,[186] there have been more than 130 livebirths achieved following ovarian cortex re-implantation worldwide.[670] The birth rate is approximately 30-35%, of which it is presumed this figure will continue to rise as the method of cryopreservation is optimised and surgical techniques improve.[663] The majority of such pregnancies achieved have been spontaneous rather than from assisted reproductive techniques.[670] Data

extrapolated from various national databases highlight that the majority of pregnancies following OTC were carried to term without significant additional complication and with positive perinatal outcomes.[671,672] Overall a congenital abnormality rate of 1.2% has been quoted amongst babies born after OTC, which is comparable to rates seen in the general population.[672]

Following implantation, ovarian endocrine function is also restored in 95% of cases, even in cases with low AMH.[188] It takes between 2-6 months for ovarian function to resume, as evidenced by a reduction in FSH and concomitant increase in oestradiol.[673] Following hormonal restoration, the mean duration of ovarian function is 5 years, although some have been demonstrated to function for ten years.[188,673] Restoration of endocrine function is often dependent on the age of the woman and the follicular density at the time of OTC.[673] Recent data from one of the largest national fertility databases, suggests that OTC should be performed on patients up to a maximum age of 40 years.[674] This is based on data assessing 95 orthoptic transplantations which quoted pregnancy rates of 33%, 18% and 0% in women undergoing cryopreservation under 35, over 35 and above 40 years old respectively.[670] However, these rates do not appear related to damage following follicle survival of frozen and thawed tissue, as a recent study has identified similar follicle survival between fresh and frozen tissue, highlighting that the loss of follicles is negligible.[675]

One of the notable disadvantages of OTC for fertility restoration in cancer patients is the potential inadvertent risk of spreading tumour cells following re-implantation.[676] It is believed that if there were malignant cells present in the frozen thawed ovarian tissue, this could subsequently potentiate recurrence of the primary tumour.[677] The highest risk of which has been associated with haematological malignancies such as leukaemia.[670]

Following the success of OTC as a method of FP in women with cancer, it has now also evolved as an established technique for women in whom the preservation of ovarian function is directly or indirectly threatened, not only due to malignancy, but also due to other benign conditions.[670,677] This includes autoimmune, haematological or medical illness treated by cytotoxic agents, presence of bilateral

ovarian tumours, severe recurrent ovarian endometriosis and recurrent ovarian torsion. As such, it is now advised that all women undergoing treatment with a high, intermediate or low risk of POI should be offered counselling regarding FP and the options of OTC, providing future reproductive autonomy to make well informed decisions regarding their fertility.[674]

Whereas SEF allows women the opportunity to preserve and restore their reproductive potential, it is associated with a number of limitations. Ultimately, the storage of a finite number of oocytes does not guarantee future offspring, it merely offers an opportunity, which may be limited by loss during thaw, future unsuccessful cycles or future miscarriage. In such women, for the same indications as SEF, OTC in the context of preserving and restoring reproductive potential may offer a solution. In women with two healthy ovaries, the ovarian cortex from one ovary could be preserved at a time when age and follicular density were optimal. When their social situation determines a time when conception is achievable or desired, if the remaining ovarian reserve has physiologically deteriorated, re-implantation of the cryopreserved ovarian cortex can be undertaken, thereby subject to success, restoring their reproductive potential. This is a concept referred to herein as social ovary freezing (SOF).

Consideration of the potential risks and benefits is essential in such a novel and arguably radical approach. When evaluating the safety of cryopreservation of ovarian tissue, the primary risks are that of the multiple laparoscopic procedures required, including removal and implantation, which are comparable to other laparoscopic procedures.[670] In reality, the complication rate reported amongst 1302 women who have undergone OTC was 0.2%.[674] Similarly, another analysis of 476 women identified no cases of significant adverse events during laparoscopies.[678] In addition, the elective removal of ovarian tissue may have a subsequent impact upon future physiological function, prior to reimplantation. However, a retrospective study of >23,000 women has identified that unilateral oophorectomy only brings forward the age of menopause by approximately one year, which is surprising given the degree of removal of follicular ovarian reserve.[183] SOF however does not necessitate conception by IVF, and thereby reduces the potential risks associated with future IVF

cycles required in women undergoing SEF. As such OTC has been regarded as being less risky compared to ovarian stimulation protocols required for oocyte cryopreservation.[679] The potential for natural conception would almost certainly have significant psychological, emotional and economic advantages, whilst reserving the option of IVF if necessary. The use of SEF in the UK is currently constrained by a 10-year storage limit. The storage of ovarian tissue would be restricted by similar legal constraints, unless it was perceived by the clinician that the elective unilateral oophorectomy contributed to premature infertility, which regardless of the indication for which it was undertaken, almost certainly would. This opens up the opportunity of storing the tissue and using it longer than the timeframe currently allowed in the context of SEF. Clearly, if this hypothesis transcends into clinical practice, legislation should be put in place to ensure tissue is not implanted for fertility restoration purposes in women outside of their natural reproductive years. As such, limiting the age to a maximum of 45 years at reimplantation may be an appropriate compromise, although further ethical reflection and debate is needed.

Conclusion

This thesis adds substantially towards various novel approaches to restore fertility in women with AUFI and OFI.

Following the achievement of 23 livebirths worldwide, UTx appears to be a viable option for women with AUFI to acquire motherhood. Teams undertaking UTx are now established globally, including our own team in the UK, and cases being performed are expected to increase exponentially in the near future. In the UK alone, on behalf of my team, I have helped established the INSITU deceased donor research programme, which is now live, with ten DBD UTx cases expected to be performed over the following 24 months, and a study endpoint within five years. With the use of DBD, despite two successful livebirths now having been reported, it remains a research concept with further cases needed, including longer follow up, before comparisons between the two donor types can be evaluated. With regards to living donors, detailed outcomes are now available for more than 30 cases which have resulted 21 livebirths so far. Over the course of this research, I have also helped establish a clinical programme using living donors, which is also now live in the UK. At the time of writing, both programmes were temporarily suspended on 6th March 2020 because of the Covid-19 pandemic.

Prior to commencing a clinical programme in UTx, experience in animal and human cadaveric models should be undertaken, and initial cases are mentored by teams with significant UTx experience. Membership with the International Society of Uterine Transplantation (ISUTx) is advised, and attendance at their annual meetings recommended, to refine and collaborate on research, as well as reflect and learn from cases performed. All cases should be registered with an international registry to enable performance and safety monitoring, including appropriate follow up of donors, recipients and offspring.

This thesis also details extensive analysis of >200 women with AUFI who are interested in potentially undergoing UTx, which represents the largest study of its kind globally to date. Whilst organisational, political, institutional and now microbiological factors have delayed the undertaking of UTx procedures, I highlight various important insights into pre-operative screening process of women with

AUFI who desire to undergo the process. Moreover, I have included herein detailed pre-operative clinical, biochemical, radiological and psychological evaluation of such women, including their perceptions toward UTx and their options to acquire motherhood.

My research also demonstrates novel insight into the motivation of women who wish to altruistically donate their uterus and have identified high levels of acceptability after consideration of the risks and expected recovery. Despite the physical risk and expected lengthy recovery process, women who donate their uterus expect to gain psychological and emotional benefit by enabling another woman to bear child themselves. In the context of expected shortages in DD availability, reliance solely upon directed LD is not ethical, nor is it sustainable. As such, consideration of the use of non-directed altruistic living donors may offer an alternative solution, following comprehensive medical and psychological assessment and extensive counselling. However, as demonstrated herein, despite desire and motivation to donate, the selection criteria currently being implemented reduce the number of potential donors significantly. Whilst enhancing outcomes remains paramount, further work is needed to ratify and validate selection criteria, to optimise donor availability without impacting graft quality.

This thesis also presents novel data from 186 M2F transgender women that clearly highlights a desire to experience physiological experiences that are unique to women, such as menstruation and gestation, as well as potentially having a physiologically functioning transplanted vagina. The findings suggest that transgender women believe the potential benefits of UTx outweigh the significant risks with which it is associated and may improve quality of life, happiness and dysphoric symptoms while enhancing feelings of femininity. As such, just as the desire to experience gestation and psychological sequelae spurred on UTx research in women assigned female at birth with AUFI, UTx in transgender women should be considered in the same light and research should be undertaken regarding its feasibility. This thesis further includes an initial pilot study, to help ascertain the feasibility of undertaking UTx in M2F transgender women, highlighting the lack of feasibility in a goat model, owing to its narrow pelvis. As described herein, whilst there are various additional complicating factors that require consideration with regard to performing UTx in this model, a

feasible technique has been proposed herein, to undertake UTx in the rabbit model, although due to previous poor outcomes in UTx studies in this model, it may not provide an adequate model to prove viability.

The final chapter on fertility restoration in AUFI introduces the concept of ETx, including initial pilot studies that helped develop the initial surgical technique, as well as a short-term viability study, in endometrial autotransplantation. The short-term viability ETx study represents the first study undertaken globally and demonstrated gross and microscopic evidence of viable endometrium following endometrial autotransplantation, along with the achievement of clinical pregnancies. The major limitation is the lack of achievement of a livebirth, which was attributed to suboptimal neovascularisation between the endo-myometrial interface, causing inability of pregnancy to develop beyond two weeks.

Finally, this thesis presents a detailed account of the prospect of elective oocyte cryopreservation, with a main focus on the restoration of OFI secondary to the physiological deterioration of ovarian reserve associated with increasing age. I demonstrate novel insight into the perceptions of such women, having undergone the process, including novel quantitative analysis of how it impacts their quality of life. I also present detailed outcomes of stimulation cycles including new perspectives on markers of oocyte yield in such populations, and present reproductive outcomes including the attainment of livebirths, which highlights poor outcomes in women undertaking it over the age of 40 years old. The creation of official guidelines and recommendations is necessary to help regulate SEF and guide management. The development of impartial and widely available patient information and support groups is essential to encourage appropriate education, to facilitate autonomous decision making, and prevent commercial exploitation. Whilst SEF shows promise in a select group of fully informed women in certain scenarios, it should not be advertised as a commercialised reproductive insurance policy, that exploits young women to freeze their oocytes on the off chance they need to use them in the future. Moreover, extensive fertility and preconception counselling is essential, including the possibility of future unsuccessful treatment and the risk of age-related obstetric complications. It is

essential that good clinical practice for IVF treatment continues to be adopted to ensure women achieve pregnancy in their natural reproductive years, to avoid potential negative social aspects and economic implications of raising children near retirement age. This is reinforced by HFEA regulations that only allow the storage of oocytes for any period up to 10 years, where further extensions are only granted if the woman or partner are or likely to become prematurely infertile. Women should no longer be punished with childlessness for not finding a partner, nor should they feel pressured into a relationship because of their declining ovarian reserve. Whilst it does not guarantee against involuntary childlessness, SEF extends the window of opportunity for single women to find a partner and offers them hope where their "*biological clock"* would otherwise run out of time.

Finally, this thesis provides insight to the future of fertility uterine and ovarian restoration. With regards to restoration of fertility in women with AUFI, whilst the immediate future focuses on the development of UTx in LD and DBD contexts, the long-term future almost certainly lies in tissue bioengineering. Such a feat would negate the need for immunosuppression, reducing significantly the cumulative risk involved, and most likely allow the uterus to remain in situ for the patient's lifetime, which may have further benefits in some groups of women. With regard to OFI, ovarian cortex cryopreservation is becoming established as a treatment allowing subsequent fertility restoration in women with cancer who undergo gonadotoxic therapy. However, it's usage in the context of physiologically prolonging a women's reproductive years, introducing the concept of social ovary freezing, may offer additional benefits over SEF.

Appendices

Appendix 1a.1: Pre-screen consent form (1/08/2017)

Study title:	**Investigational Study into Transplantation of the Uterus**
Short title:	**INSITU**
REC reference:	**18/LO/0217**
IRAS project ID:	**235711**

2. /I confirm that I have read the participant general information leaflet and I have had the opportunity to consider the information, ask questions and had these answered satisfactorily.

3. /I understand that to partake in the pre-screening process, I will complete the 'pre-screening questionnaire (V1 01-08-17) which will be used to determine if I am suitable to undergo uterine transplantation. If so it will subsequently be used to identify the candidates most suitable to partake in the process, utilising a scoring system.

4. I understand that relevant sections of my medical notes and data collected relating to the pre-screening may be looked at by individuals from the sponsor, regulatory authorities or Oxford University Hospitals NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

5. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

6. I understand that the information collected about me may be used to support other research in the future and may be shared anonymously with other researchers. ☐

Name of Participant	Date	Signature

Name of Person Taking informed consent	Date	Signature

Appendix 1a.2: Participant general information & invitation leaflet

Study title:	**Investigational Study into Transplantation of the Uterus**
Short title:	**INSITU**
REC reference:	**18/LO/0217**
IRAS project ID:	**235711**

This is an invitation to take part in a research study

Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and feel free to discuss this with your partner, your family and with your doctor. Ask us if there is anything that is not clear, or you would like more information. Take time to decide whether or not you wish to take part.

Why have I been invited to participate?

You have been invited to participate because you have been diagnosed with absolute uterine factor infertility (AUFI), a condition which affects one in 500 women of childbearing age. Women with this condition have infertility due to either the absence of their uterus (womb) or they have an anatomically or physiologically non-functioning uterus. This may be congenital (from birth) or acquired (for example following hysterectomy to treat gynaecological cancer, benign gynaecological disease or following severe haemorrhage after childbirth).

What is this study about?

This study will seek to offer up to 10 women with AUFI the opportunity to receive a womb transplant from a compatible donor. The donor will be deceased, having been declared brain dead after suffering permanent and irreversible brain injury. Prior to undergoing the transplant, each participant will have her fertilised eggs preserved by freezing (cryopreservation) in readiness for transfer into the transplanted womb from 6 months onwards following the operation. Following conception each woman will be closely monitored and looked after as a ‘high risk’ pregnancy. The subsequent birth of the baby will be by elective Caesarean Section at approximately 37 weeks’ gestation.

How have patients/public been involved in the development of this study?

You will initially undergo a pre-screening selection process which involves using a questionnaire to determine if you meet the main selection criteria. The questionnaire was developed and validated with the assistance of a patient group, some of whom, have undergone organ transplant themselves.

Do I have to take part?

No, it is up to you to decide whether or not to take part. If you decide to participate you will be given this leaflet to keep and be asked to sign a consent form when you are satisfied that all your queries have been answered fully.

Pre-screening Process

To determine which women are most suitable to undergo a womb transplant a pre-screening process will be carried out. A questionnaire has been created that will be used to elicit previous medical, surgical and psychological history, along with various lifestyle and social factors. Following the return of the questionnaire, selection criteria will be used to establish which women would be appropriate to partake in the process. A scoring system will then be used to identify those most suitable to undergo the process.

What will happen to me if I decide to take part?

Those selected will undergo an investigation process to confirm that you meet the selection criteria to take part in the trial. More detailed information will be provided in the corresponding information leaflets for each stage of the study process:

- Fertility treatment
- Transplant & immunotherapy Antenatal care
- Caesarean section/postnatal care
- Hysterectomy

As part of the selection process you will undergo a number of pre-operative investigations including various blood tests, an electrocardiogram (ECG), a magnetic resonance imaging (MRI) scan of the abdomen and pelvis along with swabs to check for genito-urinary infections. If the MRI scan indicates an abnormality that requires further investigation of intervention, then you may need to undergo a keyhole operation in your abdomen (laparoscopy), but this is unlikely. Your partner will also need to undergo blood tests to exclude infection and also undergo a check for genito-urinary infections. If you are both found to be physically and psychologically suitable you will be enrolled in the clinical trial.

Prior to the womb transplant, if you do not have embryos stored already, it will be necessary to undergo fertility treatment to create ten embryos that will subsequently be transferred following transplantation. The process of creating embryos is the same as what you would need to undergo if you were undergoing surrogacy. This treatment will take place at the Lister Fertility Clinic at the Lister Hospital in London.

You will meet the transplant team at the Churchill Hospital in Oxford and the team at Queen Charlottes Hospital who will look after you whilst you are pregnant. You will have the opportunity to ask questions about every aspect of the trial. You will also meet with Dr Maria Jalmbrant, the clinical psychologist for the trial, who will undertake an interview of both you and your partner and undertake a number of questionnaires to assess your psychological wellbeing. You will continue to see Maria throughout the process, and complete various questionnaires to continually assess your wellbeing. Support will also be given as needed, including the option of enrolment in a stress reduction programme.

From this point, you will be placed on a waiting list until a suitable donor is identified. It is planned that the transplants will be performed over a two-year period so you may be waiting for an organ to become available for up to two years. Initial funding has been secured for the first three transplants

and further procedures will not be performed until further funding has been raised. The first three women who proceed to transplantation will be chosen based on blood group, to optimise the chances of finding a compatible donor. When a donor becomes available and it has been confirmed that you are a suitable match for the organ, you will receive a call from the transplant coordinator to come to the Churchill Hospital. It is likely this will be at short notice (12-24hours).

Once the donor womb has been retrieved they will be ready to proceed with your operation. The transplant is a major operation that will be performed by the consultant surgeon. The operation usually takes between 6-8 or longer. You will be in the Churchill Hospital for approximately 7 days after the transplant. While you are living with your transplanted uterus (womb) you will need to remain on medication called immunosuppression, which works by reducing the body's natural defence system (immune system), to prevent your womb being rejected.

If all remains well you will undergo embryo transfer from 6 months following the transplant. This allows sufficient time to ensure that the uterus (womb) is working properly, to monitor for rejection and ensure that you are on a stable dose of immunosuppression that will not cause any problems to the pregnancy or a growing fetus. For the embryo transfer to have the best chance of success you will be treated with various hormones to help prepare the womb for the embryo. Only one embryo will be used at a time to reduce the risk of multiple pregnancy.

If your pregnancy test is positive your care will be transferred to the obstetric team at Queen Charlotte and Chelsea Hospital for pregnancy care. During this time you will continue with hormone therapy until 12 weeks' gestation. If the pregnancy test is negative then the process will be repeated.

Following an uncomplicated pregnancy your baby will be delivered by caesarean section at 36–37 weeks' gestation under a spinal anaesthesia. This will be undertaken by the obstetric team at the Queen Charlotte's and Chelsea Hospital. The baby needs to be delivered by Caesarean section due to the risks of vaginal birth on the transplanted uterus.

If you experience complications during the pregnancy this will be carefully monitored and any decision to deliver your baby earlier will be made by the obstetric team in conjunction with the neonatologist and the surgical team.

Following your Caesarean section, you will be transferred to the postnatal ward for a few days while you recover and establish infant feeding. You should be able to breastfeed on immunosuppressive therapy if you want to. If your progress is normal, you will be discharged into the care of your community midwife who will visit you at home.

Subject to an uncomplicated course, it may be possible to repeat this process and try for another child. Once you have completed your family, approximately 6 months following the birth of your baby you will undergo removal of the transplanted womb at the Hammersmith Hospital. Following hysterectomy, you will be able to stop the immunosuppressive medications and all other medicines that were started as part of the transplant process.
Six weeks after hysterectomy you will be seen at the clinic and debriefed by the surgeons.

If no pregnancies are achieved within five years following the transplant then, to reduce the risk of long-term immunosuppressive medications, the transplant will have been deemed unsuccessful and hysterectomy will be advised.

Will taking part be kept confidential?

Yes, please be assured that as an NHS patient your data will be protected under the data protection act. Data collected throughout the study will be stored on a password protected database, on a secure password protected NHS computer in a locked NHS office, and identifiable details will be amended under a study number. Manual files, including consent forms, will be stored within the study file. This is kept safely within the locked research filing area located in a secure research team office at Hammersmith Hospital. This office will be locked, and the research file will only be accessible to research team members. Video recording may be used during the operation if you consent to it. The area filmed will only be the operative site. Any video recordings containing patient sensitive information will be treated securely on a secure password protected NHS computer in a locked NHS office. The recordings, which do not contain sensitive information, or identify any participants, may be used in future for teaching, presentation or in journals. The video recordings, along with all other research data, will be stored for 30 years. In the event if data being transferred between sites, data will be anonymised, compressed and encrypted prior to transfer, to ensure it remains secure.

What will happen to my information & samples afterwards?

The data collected from the study will be used to contribute towards a PhD research degree for Dr Benjamin Jones. Research information obtained during the study will be retained for 30 years. We will store certain tissue samples so that they may be used for future ethically approved research.

What will happen if I decide to withdraw from the study after I have given my consent?

If you decide to take part you are still free to withdraw **at any time** without giving an explanation, or, a decision not to take part, will not affect the care you receive in any way at present or in the future. Data and samples already taken will be retained for research purposes, but no future data will be collected or stored.

Who is funding the study?

This study has been funded by the registered charity Womb Transplant UK (Charity no 1138559).

What are the possible benefits to me if I take part?

This study will offer women with absolute uterine factor infertility, the opportunity to conceive and grow a pregnancy to term and give birth to a biologically related infant.

What are the risks or side-effects to me if I take part?

This is a complex study with numerous interventions and various stages over a long period of time. Each stage of the transplant process carries different and significant risks, which are explained in detail in the corresponding information leaflets. There are multiple major surgeries planned, including the transplant itself, up to two Caesarean sections and a hysterectomy. Each operation has associated anaesthetic risk, as well as risk of bleeding, infection, damage to internal organs and blood vessels and

blood clots in veins or the lungs. One of the biggest risks is rejection of the transplanted uterus which may lead to it being removed. Conversely there are also risks with the immunosuppressive medications given to reduce the risk of organ rejection, including infection, diarrhoea, diabetes and cancer. This increased risk is largely transient as these medications will be stopped after hysterectomy. There are risks with the fertility treatment, including bleeding and infection related to the egg retrieval, and the ovaries can also be overstimulated which causes a condition known as ovarian hyperstimulation syndrome (OHSS). There is also a risk that the fertility treatment is unsuccessful, leads to miscarriage or results in a poor pregnancy outcome. These risks are explained further in the individual patient information leaflets addressing the fertility care, the transplant and immunotherapy, the Caesarean sections and the hysterectomy.

What if something goes wrong?

Imperial College Healthcare NHS Trust holds standard NHS Hospital Indemnity and insurance cover with NHS Litigation Authority for NHS Trusts in England, which apply to this study. This does not affect your legal rights to seek compensation.

If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the Investigator. The normal National Health Service complaints mechanisms are also available to you and you will be able to liaise with the Patient Advice and Liaison Service (PALS) at the hospital who can provide confidential advice, support and information. If you are still not satisfied with the response, you may contact the Imperial AHSC Joint Research Compliance Office.

Who do I contact if I want to know more about the study?

If you have any further questions please do not hesitate to contact the study coordinator who will direct your queries to the appropriate member of the study team.

Mr J Richard Smith – Chief Investigator **Telephone: 0207 730 0431**
Email:
admin@jrsmithgynaecology.com

Thank you for reading this leaflet and considering whether to take part in the study

The approach, consent and assessment process prior to uterine transplant will take place ***at Imperial College Healthcare NHS Trust, Hammersmith Hospital***

Appendix 1a.3: Pre-screen questionnaire

Study title:	**Investigational Study into Transplantation of the Uterus**
Short title:	**INSITU**
REC reference:	**18/LO/0217**
IRAS project ID:	**235711**

1) Information about yourself

Title:

First Name:

Surname:

Date of Birth:

Email address:

Telephone number: Home: Mobile:

Address:

GP name and address:

Country of Birth:

Do you currently live in the UK? Yes ☐ No ☐

If born outside the UK, how long have you lived in the UK?

Ethnicity:

2) Reason for infertility

1) **MRKH or other womb abnormality** Yes ☐ No ☐

If 'yes', please answer the questions in this subsection (1) and then proceed to section '3) Previous medical history'

If 'no', please proceed to subsection 2 'Previous Hysterectomy'

a) Have you had any operations to lengthen the vagina? Yes ☐ No ☐

If yes, please provide details including method and please include whether any other tissue such as intestine was used:

b) Have you used dilator therapy to attain adequate vaginal length?

Yes ☐ No ☐

c) Do you have any problems with your kidneys (Single kidney/horseshoe kidney etc)?

Yes ☐ No ☐

If yes, please provide details and any subsequent treatment:

d) Do you have any problems with your ovaries? Yes ☐ No ☐

If yes, please provide details and any subsequent treatment:

e) Does MRKH affect any other organ (Skeleton/Ears/Heart/Fingers etc)?

Yes ☐ No ☐

If yes, please provide details and any subsequent treatment:

2) Previous hysterectomy

Yes ☐ No ☐

If 'yes', please answer the questions in this section and then proceed to section '3) Previous medical history'.

If 'no', please proceed straight to section 3) Previous medical history'

a) What is the reason for the previous hysterectomy? Please give details below:

Year	Reason	Complications	Additional comments

b) If the hysterectomy was for cancer what was the type of cancer?

c) What year was it diagnosed?

d) Please describe any further treatment since the operation (e.g. chemotherapy / radiotherapy):

e) Have you been in remission (told the cancer has gone) for longer than 5 years?

Yes ☐ No ☐

f) Do you still have both ovaries? Yes ☐ No ☐

g) Was the cervix removed during the operation (Total hysterectomy) or was it left behind (Sub-total hysterectomy)?

Total hysterectomy ☐ Sub-total hysterectomy ☐

3) Previous medical History

Height (cm):

Weight (kg):

Do you have any medical problems we should be aware of?

Yes ☐ No ☐

If yes, please detail below:

Do you have any psychiatric problems we should be aware of?

Yes ☐ No ☐

If yes, please detail below:

4) Previous Surgical History

Have you had any previous operations? Yes ☐ No ☐

If yes, please provide details:

Year	Operation	Complications	Additional comments

5) Medications

Do you take any medications regularly? Yes ☐ No ☐

If yes, please list the name and dose of the medication:

Do you have any allergies including to any medications?

6) Family History

Are there any significant medical conditions that affect your family?
Yes ☐ No ☐

If yes please list below:

7) Fertility questions

Have you previously considered adoption? Yes ☐ No ☐

Have you previously attempted adoption? Yes ☐ No ☐

Have you successfully adopted a child? Yes ☐ No ☐

If so, how many?

Please explain the main reason you would rather have a uterine transplant over adoption:

Have you previously considered surrogacy? Yes ☐ No ☐

Have you previously attempted surrogacy? Yes ☐ No ☐

Have you successfully had a child born through surrogacy?
Yes ☐ No ☐

If so, how many?

Please describe the main reason why you would prefer to have a uterine transplant over using a surrogate:

Do you have any of your own eggs or embryos stored? Yes ☐ No ☐

If yes,

How many are stored?

How old were you when they were retrieved?

If you have previously had a hysterectomy:

Had you given birth previously? Yes ☐ No ☐

Did you have any previous miscarriages? Yes ☐ No ☐

Did you have any previous terminations? Yes ☐ No ☐

How many children did you have? Yes ☐ No ☐

8) Social questions

Relationship status:

Single ☐ Married ☐ In relationship ☐ Divorced ☐

Length of current relationship:

Occupation:

What is your main language?

If your main language is not English, are you fluent in English? Yes ☐ No ☐

Do you smoke? Yes ☐ No ☐

If yes, how many cigarettes do you smoke a day?

Have you smoked previously? Yes ☐ No ☐

If yes,

How many years did you smoke for?

On average how many cigarettes did you smoke per day?

When did you give up smoking?

Do you drink alcohol? Yes ☐ No ☐

If yes, how many units of alcohol so you consume a week?

Do you use recreational drugs? Yes ☐ No ☐

If yes, please give details below:

Have you been involved in fundraising or raising awareness for Womb Transplant UK?

If yes, please give details: Yes ☐ No ☐

How many years have you been in contact with the researchers at Womb Transplant UK?

<1 ☐ 1-2 ☐ 3-4 ☐ >5 ☐

End of questionnaire – *Thank you for your time*

Appendix 1b.1: Transplant and immunotherapy participant information leaflet

Study title:	**Investigational Study into Transplantation of the Uterus**
Short title:	**INSITU**
REC reference:	**18/LO/0217**
IRAS project ID:	**235711**

The Assessment

As you are undergoing an organ transplant, you will have a full assessment that involves a medical, physical, psychological and social evaluation. You will be asked about your health and social situation and undergo a number of blood tests and investigations. This is to ensure that the womb transplant is the best option for you. However we understand that as part of careful pre-screening of patients much of this will have already been discussed with you up to 6 – 12 months during regular consultations with your NHS consultant, and a psychologist.

People you will meet as part of our team

You will meet many people during your transplant assessment and as part of this project.

Consultant Surgical Team

As you are part of a research study you will meet teams from both Imperial College Healthcare NHS Trust and Oxford University Hospitals NHS Trust. You are primarily under the care of the Imperial team where the study Chief Investigator Mr Richard Smith and co-investigator Mr Benjamin Jones work. The Oxford team will be undertaking the transplant as part of our research ethics approved protocol and this team will be led by Professor Peter Friend.

These experienced teams are accredited by NHS Blood and Transplant who represent governance and oversight for transplants carried out within the NHS.

Anaesthetic Team

You will be met by the Consultant Anaesthetist for assessment and other investigations. The anaesthetic team will be with you while you are in theatre and until you wake up. They will also be responsible for pain management to ensure your maximum comfort.

Transplant Co-ordinators

A team of specialist nurses who will guide you through the transplant journey; it will be one of them who contacts you by telephone to tell you that a suitable organ has been found. Along with your Imperial Consultant team they will support you throughout the process and following surgery.

Research Nurse/Midwife

We will have a dedicated research Nurse/Midwife working as part of our Imperial team. Nuala Dixon will, along with your consultant team, support you throughout the process before, during and after transplant and of course then throughout your pregnancy and delivery.

Pharmacist

Following the transplant you will be taking new tablets, until the womb is eventually removed after the completion of your family. The surgical team including Oxford, including a pharmacist, will explain the importance of taking the medication and any side effects.

Investigations that will be undertaken

Blood Tests

You will have several routine blood tests and these will be taken by your nurse or doctor, usually from your arm. The blood tests are performed as a baseline to ensure that you are a suitable candidate to undergo the process. They will check your general health and exclude anaemia, check your kidney and liver function, evaluate your tissue and blood typing, check how your blood clots and look for a variety of infections. Bloods will also be performed on your partner to check for infections and assess tissue typing.

- Bloods – Full blood count (FBC), Urea & Electrolytes (U&Es), Liver Function Tests (LFTs), Clotting, Group & Save (G&S), Tissue typing, Thrombophilia Screen, Infection screen (HIV, Hepatitis B & C, Epstein Barr Virus (EBV), Toxoplasmosis, Rubella, Cytomegalovirus, Herpes (TORCH), Syphilis and Tuberculosis
- Partner - Tissue typing, Infection screen (HIV, Hepatitis B & C, Epstein Barr Virus (EBV), Toxoplasmosis, Rubella, Cytomegalovirus, Herpes (TORCH), Syphilis

Other Investigations

We will perform a routine check of your heart using an electrocardiograph (ECG). We will also perform a genito-urinary medicine (GUM) screen to exclude infections such as chlamydia, gonorrhoea and trichomonas vaginalis. We will also perform an ultrasound of your pelvis and an MRI of your abdomen and pelvis to gain further detail of the internal anatomy.

Donation of the transplant organs – Where will they come from?

The womb used for transplantation will be donated from deceased donors. These are from women who have been recently declared brain dead and are currently being kept alive on 'life support' machines. A number of investigations will be performed to ensure the organ is safe and of sufficient quality to be donated. After receiving consent from the family of the donors an operation will be performed to remove the organs that are being donated, including the womb and its blood supply. The womb will be appropriately matched to you individually to reduce the risk of rejection.

Being Prepared for 'The Call'

Once a suitable donor has been identified, you will then receive a call from the transplant co-ordinator to come to hospital. It is likely this will be at very short notice, within a few hours. We advise that you keep a bag ready and that you have made necessary travel plan arrangements. You may also want to

keep a pen and a piece of paper by your telephone to note details. A checklist can also be very helpful.

On arrival at the Churchill Hospital in Oxford you should go straight to the ward as instructed over the telephone. You will be provided with a map of where to go and trip options should be planned in advance to avoid any undue stress.

After your arrival at the Churchill Hospital you may need to wait for many hours before the transplant operation can go ahead. You may want to bring reading material, your laptop, a tablet or music to listen to. The nursing and medical staff will keep you informed of the results of tests and expected timing of the operation.

Transplant – the day of your surgery

Once the donor womb has been retrieved then we will be ready to proceed with the operation. You will be joined by the surgical team and have the chance to answer any last minute questions. The anaesthetist will subsequently discuss the proposed anaesthesia and pain management plan during and after the operation.

You will then be taken to the operating theatre where the anaesthetic team will meet you and attach monitoring such as a blood pressure cuff, ECG leads and a pulse oximeter on your finger. This is where you will be put to sleep under general anaesthesia.

The anaesthetist will place a cannula in the back of your hand in order to deliver the anaesthetic. You will also have other lines as well to include but not all those below;

- Peripheral IV or Central Line (for fluid delivery)
- Arterial Line (a line in your wrist)
- Urinary Catheter
- Epidural (for pain relief)

This is the point at which you will drift off to sleep under general anaesthesia.

The transplant is a major operation that will be performed by a team of surgeons including consultants from both gynaecology and transplant teams. The operation will take between 6-8 hours or possibly longer. Video recording may be used during the operation if you consent for its usage. The area filmed will only be the operative site. The recordings will not contain information that would lead to your identification, may be used in future for teaching, presentation or in journals.

Step by Step

- The surgeon will take a midline incision from your umbilicus (belly button) to your pubic bone
- A space in your pelvis will be created for the donor womb
- The donor womb will be placed in the pelvic cavity
- The supporting ligaments will be attached at either side
- The blood vessels of the transplant womb will be attached to your blood vessels
- The vagina will be attached to the womb
- You may have a drain in place to assist with any temporary leakage of blood or other fluids.

Potential Surgical Complications

There are risks involved in this surgery, some of which may result in a longer stay in the high dependency unit or ward. These include;

- Anaesthetic risks
- Bleeding (including the possibility that you may have to have a blood transfusion)
- Infection
- Damage to internal organs such as bowel, bladder or the tubes that connect the kidneys to the bladder (ureters)
- Venous thromboembolism (blood clot)
- Immediate graft failure with subsequent need to remove the womb.

After you Surgery – When you Wake Up

After the operation you will wake up and be transferred from the recovery room to the transplant ward for close monitoring by nursing staff. Your surgical team will be with you to monitor your progress.

You will have medications including antibiotics, pain relief, anti-sickness to prevent vomiting, laxatives to help your bowels, aspirin to prevent blood clots. You will also have a daily ultrasound and blood tests. You will be wearing stockings to reduce the risk of blood clots in the legs, and the nurses will encourage you to walk as soon as possible.

During this time you will start treatment with anti-rejection medication to prevent your body's immune system rejecting the womb transplant. Your immune system protects you against infection and cancer and without treatment it would recognise the transplant as "foreign" and would attack and destroy the transplant. The surgical team and pharmacist will discuss all aspects of this treatment with you. The medication must be taken daily while you have the transplant. It is essential that you take all your medication as prescribed.

Later Complications

Acute rejection can happen in about 20% of patients and at around 6 days after surgery but can happen at any time. Rejection can be detected on the regular biopsies and investigation that will be performed. There may be other symptoms but your care team will be monitoring you closely for all complications. Rejection can be treated successfully with steroids and other drugs.

Higher risk of infection following the transplant because your anti-rejection treatment reduces the capacity of your immune system to fight infections. The transplant team will monitor you closely to reduce the risk of infection and promptly treat you if there are signs of infection.

Higher risk of cancer: A normal immune system detects and kills cancerous cells. The anti-rejection treatment reduces the ability of your immune system to recognise and destroy cancer cells. Most cancers associated with this type of drugs are skin cancers and you will be advised to avoid direct sun exposure and use high factor sun blockers. Other cancers such as lymph glands cancers have been associated with anti-rejection treatment. You will only need to take anti-rejection treatment whilst the transplanted womb is in place (expected to be 2-3 years) and therefore the risk of cancer will be much lower than other transplant recipients who need the treatment for decades.

Anti-rejection drugs (Immunosuppression Medication)

While you are living with your transplanted womb you will need to remain on medication. The anti-rejection medication (immunosuppression) will be needed until your womb is removed following the birth of your baby.

Side-effects:

- Increase risk of infection
- Increase risk of cancer

You will be prescribed the following anti-rejection medication:

- Tacrolimus
- Mycophenolate mofetil
- Azathioprine

Tacrolimus: This medications prevents rejection of the womb. You will need to take it twice a day. It is important that you don't take your tacrolimus dose in the morning when you come to clinic until you have your blood taken. We measure the level of tacrolimus in your blood and the dose of the drug will be very carefully monitored and adjusted.

Grapefruit, some herbal remedies and other medication can affect the tacrolimus levels. It is essential that you consult with a member of the transplant team before starting any new medication.

Tacrolimus side-effects:

- Increase risk of developing diabetes
- Hand and arm shakes when the dose of tacrolimus is too high
- Diarrhoea

Mycophenolate Mofetil (MMF): This medication also prevents rejection. It is normally taken twice a day.

MMF side-effects:

- Bone marrow suppression causing anaemia, low white cell count and/or low platelets. The dose of MMF will need to be reduced or stopped. Low white cell count may increase the risk of infection.
- Diarrhoea or sickness. The dose of MMF or the frequency of administration may need to be changed.
- MMF may be harmful to the fetus and therefore this drug will be discontinued 3-4 months after your transplant to allow three months before IVF treatment.

Azathioprine: This also prevents rejection in a similar way to MMF but it is not associated with the same problems during pregnancy. Azathioprine will be started when MMF is stopped 3-4 months after the transplant.

Azathioprine side-effects:

- Bone marrow suppression
- Diarrhoea and sickness

Other Medications

Aspirin: This helps reduce blood clots

Heparin injections: You will be trained to administer the heparin injections yourself. Heparin thins your blood and reduces the risk of blood clots.

Ranitidine: Reduces the risk of stomach ulcers

Co-trimoxazole: This is an antibiotic that reduces the risk of chest infections caused by a fungus (Pneumocystis). This common fungus causes no harm to healthy people but can cause a serious chest infection on patients on anti-rejection medication. You will have to have this antibiotic for 6-12 months following the transplant.

Vanganciclovir: This drug reduces the risk of infection caused by a common virus (cytomegalovirus CMV). This virus can cause severe chest, intestinal and other organ infections. This anti-viral drug will be prescribed to you if you are considered at risk of developing CMV.

Going Home

You will be in the Churchill hospital in Oxford for approximately 7 days after the transplant. Your transplant co-ordinator will spend time with you and your family to make sure you are supported when discharged from hospital. The transplant nurses and pharmacists will provide training to help you become familiar with your new medication.

You will have regular appointments with your Imperial and Oxford consultant team for follow-up care. These will initially be twice weekly for the first month, then weekly for the following two months and then fortnightly between months 4-6. You will then be seen monthly from six months onwards by the transplant team if all remains uncomplicated. During this time you will keep a menstrual diary, to help monitor the function of the womb. Fertility treatment, resulting in transfer of one of the previously stored embryos, will commence from six months onwards. You will also be able to contact Dr Benjamin Jones, the research coordinator for the trial, should you feel the need for information, or you are at all concerned.

Your transplant will be undertaken at ***The Churchill Hospital,*** *part of* ***Oxford University Hospitals NHS Trust***

Appendix 1b.2: Fertility treatment information leaflet

Study title:	**Investigational Study into Transplantation of the Uterus**
Short title:	**INSITU**
REC reference:	**18/LO/0217**
IRAS project ID:	**235711**

Fertility Treatment

Prior to uterine transplantation it will be necessary to undergo fertility treatment to ensure that your ovaries have enough embryos available for fertility treatment following transplantation. This treatment will take place at the Lister Fertility Centre, at the Lister Hospital in London. You will initially see a specialist nurse and receive counselling about the treatment you will receive. You will undergo blood tests and an ultrasound scan to ensure you are suitable to undergo this treatment. A sample of your partner's sperm will also be analysed to ensure it is satisfactory.

Egg Collection

Blood or urine tests will initially be used to monitor your hormonal cycle and determine when you ovulate. Two weeks after you ovulate you will be commenced on injections of follicle stimulating hormone (FSH) for 10-16 days to increase the number of eggs you produce. If your Anti-Mullerian Hormone (AMH) level was low then you may need injections of a medication called Buserelin to switch off your cycle prior to starting the FSH injections. Another medication, called Cetrotide or Orgaultran, will then be started 6 days after the start of the FSH injection to prevent ovulation. Over this time, you will be monitored with ultrasound and blood tests every 2-3 days. When there is a satisfactory ovarian response, you will be booked for the egg collection. Approximately 33-39 hours prior to egg collection you will have a different injection to help your eggs mature.

The egg collection will be carried out under ultrasound guidance under general anaesthetic using a thin, hollow needle. If it is not possible to do this vaginally, then it will be performed through the abdomen or during laparoscopy. After the procedure, you may experience some abdominal cramping and have a little vaginal bleeding.

Whilst complications rarely occur, they include the following:

- Adverse drug reactions
- Pelvic infection
- Damage to bowel, bladder and blood vessels
- Ovarian hyperstimulation syndrome (OHSS) – This occurs in women who are sensitive to the fertility drugs used to increase egg production. This can cause the ovaries to increase in size and cause bloating and abdominal pain. Severe cases are rare but can cause damage to organs such as the kidneys or lungs and increases the risk of blood clots in legs or lungs.

IVF (ICSI) and embryo freezing

On the same day as the egg collection a sample of your partner's sperm will be collected. Your partner should abstain from intercourse for 3 days prior to this. Some men may choose to have a sample frozen previously as a backup. A single sperm will then be injected directly into each egg. Following fertilisation genetic testing (PGT) will be undertaken to ensure there is no genetic problem that may result in miscarriage or a genetic disorder. Five to six days after this, once the embryos have developed into blastocysts, they will be frozen for future use after the transplant.

The egg collection process may need to be repeated to ensure we have a satisfactory number of embryos stored before you receive the uterine transplant.

Embryo Transfer

If all remains well, the plan is to start the fertility treatment from 6 months onwards following the transplant. This gives us time to ensure the uterus is working correctly, to monitor for rejection and to ensure you are on a stable dose of immunosuppression that will not cause a significant problem to the pregnancy of growing fetus.

For the embryo transfer to have the best chances of success we will treat you with various hormones to help prepare your womb for the embryo. We will initially give you an injection called Syneral or Suprecur from the 1st day of your period (day 1), which will switch off your regular cycle, preventing your ovaries from producing further hormones. When you start this medication, you need to call the nurse at the Lister Fertility Centre who will arrange an ultrasound scan between day 3-5 of your cycle.

The ultrasound will measure the lining of the womb (endometrium) and, provided it is thin and no abnormalities are detected, you will be prescribed oestrogen medication (Prodynova or Oestraderm) and continue the Syneral or Suprecur injections. The oestrogen is used to thicken the endometrium by replacing the hormone usually produced by the developing follicles within the ovary. You will have a repeat ultrasound scan 10-12 days after you start the oestrogen, which will check the endometrial thickness. Scanning will continue on alternate days until the lining of womb thickness is greater than 7mm. The oestrogen dose may need to be increased to ensure the endometrium reaches >7mm. Once this is achieved, the injections are stopped and we will give you progesterone pessaries (e.g. Cyclogest), twice daily, which will maintain the endometrial thickness.

Once the endometrium is greater than 7mm, the embryo transfer can be planned. A single embryo will be thawed on the day of the transfer and implanted into the uterus, through the cervix, under ultrasound guidance. Only one embryo will be used at a time to reduce the risk of multiple pregnancy (twins or triplets). You will continue the oestrogen and progesterone therapy for 2 weeks further, at which you will perform a pregnancy test.

If the pregnancy test is positive you will be referred to Queen Charlottes & Chelsea Hospital for high risk pregnancy care. You will continue the hormone therapy until 12 weeks' gestation. If the pregnancy test is negative then this process will be repeated.

*This treatment will be conducted at **The Lister Hospital, London**. Please refer to their IVF patient information leaflet for further information.*

Appendix 1b.3: Antenatal treatment information leaflet

Study title:	**Investigational Study into Transplantation of the Uterus**
Short title:	**INSITU**
REC reference:	**18/LO/0217**
IRAS project ID:	**235711**

Ante-natal care

Following successful conception, you will be closely monitored by a specialist multi-disciplinary team of doctors, nurses and midwives to ensure your pregnancy runs as smoothly as possible. This team will work closely together to optimise the health of both yourself and your developing baby. You will continue to take immunosuppressive medication as guided by the transplant team who will tailor your medication accordingly throughout your pregnancy. You will be looked after at the Centre for Fetal Care (CFC) at Queen Charlottes & Chelsea Hospital where you will be closely monitored for signs of complications related to the womb transplant and immunosuppressive therapy. The normal risks associated with pregnancy also still apply. You will be routinely seen every two weeks throughout your pregnancy. If you have any other queries or concerns at any other time then you can contact the research team 24 hours a day.

1st Trimester (<12 weeks)

Once pregnancy has been confirmed, you will attend a clinic at the CFC. You will have your blood pressure taken and a sample of your urine will be checked. Blood tests will be taken and swabs will be sent to exclude MRSA which is a type of bacteria that is resistant to common antibiotic. You will have an ultrasound scan in the early pregnancy unit to ensure the pregnancy is progressing normally and undergo a speculum examination to ensure the cervix appears normal.

You will then be seen again at 10 weeks' gestation where you will have your blood pressure and urine checked again and undergo a further speculum examination.

At 12 weeks' gestation you will undergo a routine ultrasound to date the pregnancy and undergo further blood tests including drug levels.

2nd Trimester (13-27 weeks)

You will be seen every two weeks throughout the second trimester. At each appointment you will:

- Have the opportunity to talk with the medical team and discuss any symptoms or concerns.
- Have your blood pressure check and urine sample tested
- Undergo an examination including speculum assessment of the cervix.
- Undergo a detailed ultrasound scan (from 16 weeks onwards) that will assess:
    1. Fetal growth
    2. The blood supply to the fetus
    3. The blood supply to the pelvis

4. The length of your cervix.
5. Note - During the scans at 16 and 18 and 22 weeks you will also have a detailed scan that looks for any structural abnormalities in your growing baby. There is no increased risk of structural abnormality associated with taking the immunosuppressive medications.

At alternate appointments (once a month) you will have routine blood tests as well as tests to check drug levels and for antibodies. The blood tests will also check for an infection called cytomegalovirus (CMV) which is of importance during pregnancy as it can cause fetal abnormalities if left untreated.

A biopsy of the cervix will also be taken at 16 weeks to look for evidence of rejection.

If your blood group is rhesus negative then you will require an injection to prevent potential antibodies being formed in the future at 26 weeks' gestation.

3rd Trimester (28-40 weeks)

You will continue to be seen fortnightly throughout the third trimester following the same format as the bullet points listed above. In addition you will need to have the following:

- A special blood test called an oral glucose tolerance test at 28 weeks' gestation which will determine if you have developed diabetes in pregnancy.
- A further biopsy from the cervix at 30 weeks' gestation.
- At 34-35 weeks' gestation the timing of delivery will be discussed and you will be given a date for the Caesarean at 37 weeks' gestation unless needed sooner. See the patient information leaflet on Caesarean Section for further information about the mode of delivery.

*This treatment will be conducted at **Queen Charlottes & Chelsea Hospital**, part of **Imperial College NHS Trust***

Appendix 1b.4: Caesarean section information leaflet

Study title:	**Investigational Study into Transplantation of the Uterus**
Short title:	**INSITU**
REC reference:	**18/LO/0217**
IRAS project ID:	**235711**

Following an uncomplicated pregnancy, you will deliver by Caesarean Section at 37 weeks' gestation. If you experience complications during the pregnancy and the benefit of delivering your baby sooner outweighs the potential risks, then that decision will be made between yourself, the obstetric team and the surgical team.

Why do I need this operation?

The baby needs to be delivered by Caesarean Section due to the risks of vaginal delivery on the transplanted uterus. Uncertainties regarding the blood supply and ligamentous support of the transplanted uterus during labour make Caesarean Section the safest mode of delivery at this stage.

Pre-operative care

You will be admitted to Queen Charlottes & Chelsea Hospital on the day of your operation. You will be seen by a nurse and be given stockings to wear to reduce the risk of blood clots in your legs. You will then be seen by the obstetric team who will explain the operation in further detail and go through a consent form which you will complete once you are satisfied.

How will the operation be performed?

The operation will be performed under spinal (regional) anaesthesia so you will be awake during the procedure but will not feel any pain. The surgeons will aim to use the same midline incision as used previously, although they may use a bikini line (transverse) incision instead. Once the regional anaesthesia is effective you will have a catheter inserted in your bladder. The surgeons will clean your abdomen and put a screen up so you and your partner won't see the operation, but it will be possible for this to be lowered for you to see your baby once it is born. You will be given antibiotics as a precaution to reduce the chance of infection after the operation. Your baby will be born after a few minutes, dried, wrapped in a blanket and then be brought over to you so you can have 'skin to skin' contact. The operation will take approximately one hour.

What are the risks of the operation?

The risks of a Caesarean include anaesthetic risks, bleeding (including the possibility of you needing a blood transfusion), infection, damage to internal organs such as bowel, bladder, ureters and blood vessels, fetal injury and venous thromboembolism (blood clot).

What will happen after the operation?

After the operation, you will be transferred back to the ward where you will be monitored closely with frequent observations of your vital signs. You will be offered painkillers and kept comfortable whilst you recover. You will have a catheter in your bladder that will be removed the following day. You may also have a drain in place which reduces the risk of internal bruising. It is likely this will also be removed the day after the operation. You will once again receive heparin injections and wear the compression stockings to help prevent a blood clot.

You will remain in hospital for a few days whilst recovering and establishing feeding. You should be able to breastfeed on immunosuppressive therapy without any problems.

Follow up

You will continue to have access to the research team if you have any questions of queries at any point. You will be seen in clinic a week following the operation to ensure your recovery is progressing adequately. Following this you will continue to be seen monthly as per the usual post-operative follow up protocol. If the transplant remains stable, without significant complication, and you desire a further pregnancy, then embryo transfer can be undertaken from 3-6 months post-delivery.

*This treatment will be conducted at **Queen Charlottes & Chelsea Hospital**. The Royal College & Obstetricians Caesarean Section patient information leaflet should also be consulted for further information and advice.*

Appendix 1b.5: Hysterectomy participant information leaflet

Study title:	**Investigational Study into Transplantation of the Uterus**
Short title:	**INSITU**
REC reference:	**18/LO/0217**
IRAS project ID:	**235711**

Once you have completed your family, then approximately 6 months following the birth of your baby you would undergo removal of the transplanted womb (hysterectomy). If you are keen to have another child then it may be possible to delay hysterectomy, undergo further IVF treatment, continue the immunosuppressive treatment, deliver again by Caesarean Section before undergoing hysterectomy 6 months later.

What will be removed?

The surgery will include the removal of all the tissue that was transplanted previously including the uterus, cervix, surrounding ligamentous tissues and blood vessels. Your ovaries will not be removed.

Why do I need this operation?

This operation needs to be performed to remove the tissue that was previously transplanted. This allows you to stop the immunosuppressive therapy and all other medications. This is very important as using immunosuppressive medications for long term can have serious complications including infection and cancer.

Pre-operative care

You will be admitted to Hammersmith Hospital on the day of your operation. You will be seen by a nurse and be given stockings to wear to reduce the risk of blood clots in your legs. You will then be seen by the surgical team who will explain the operation in further detail and go through a consent form which you will complete once you are satisfied.

How will the operation be performed?

In a similar fashion to your first operation you will be put to sleep under general anaesthesia. The surgeons will aim to use the same midline incision as used previously. You will be given antibiotics as a precaution to reduce the chances of infection after the operation. You will also be given painkillers to ensure you do not wake up in pain.

What are the risks of the operation?

The risks of hysterectomy include anaesthetic risks, bleeding (including the possibility that you may have to have a blood transfusion), infection, venous thromboembolism (blood clot in veins or arteries) and damage to internal organs such as bowel, bladder, the tubes that connect the kidneys to the bladder (ureters) and blood vessels.

What will happen after the operation?

You will wake up in recovery with a catheter in place and a drip in your arm. You will be transferred back to the ward where you will be monitored closely with frequent observations of your vital signs. You will be offered painkillers and kept comfortable whilst you recover. You will have a catheter in your bladder that will be removed the following day. You may also have a drain in place which reduces the risk of internal bruising. It is likely this will also be removed the day after the operation. You will once again receive heparin injections and wear the compression stockings to help prevent a blood clot.

Following hysterectomy you can safely stop the immunosuppressive medications as well as all other medications that have been started as part of the transplant process.

Follow up

You will continue to have access to the research team if you have any questions of queries. You will be seen in clinic 6 weeks following the operation to be debriefed by the surgical team.

This treatment will be conducted at Hammersmith Hospital, part of Imperial College NHS Trust. The Royal College & Obstetricians hysterectomy patient information leaflet should also be consulted for further information and advice

Appendix 1b.6: Partner general information and invitation leaflet

Study title:	**Investigational Study into Transplantation of the Uterus**
Short title:	**INSITU**
REC reference:	**18/LO/0217**
IRAS project ID:	**235711**

This is an invitation to take part in a research study

Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and feel free to discuss this with your partner, your family and with your doctor. Ask us if there is anything that is not clear or you would like more information. Take time to decide whether or not you wish to take part.

Why have I been invited to participate?

You have been invited to participate because your partner has been diagnosed with absolute uterine factor infertility (AUFI), a condition which affects one in 500 women of childbearing age. Women with this condition have infertility due to either the absence of their uterus (womb) or they have an anatomically or physiologically non-functioning uterus. This may be congenital (from birth) or acquired (for example following hysterectomy to treat gynaecological cancer, benign gynaecological disease or following severe haemorrhage after childbirth). Your partner has met the initial screening criteria set out by the research team to be considered as part of the first research trial in womb transplantation in the UK.

What is this study about?

This study will offer up to 10 women with AUFI the opportunity to receive a womb transplant from a compatible donor. The donor will be deceased, having been declared brain dead after suffering permanent and irreversible brain injury. Prior to undergoing the transplant, each participant will have her fertilised eggs preserved by freezing (cryopreservation) in readiness for transfer into the transplanted womb from 6 months onwards following the operation. Following conception each woman will be closely monitored and looked after as a ‘high risk’ pregnancy. The subsequent birth of the baby will be by elective Caesarean Section at approximately 37 weeks’ gestation. There will be the opportunity to repeat the fertility treatment and have a further baby if desired and clinically appropriate. The study will end six weeks following the removal of the donor uterus.

What will happen to me if I decide to take part?

To have got to this point you and your partner will have undergone a pre-screening process to ensure you meet the main selection criteria. If you both agree to participate then you and your partner will undergo an investigation process to confirm that you both meet all the selection criteria. More detailed information is provided about this in the corresponding information leaflets for each stage of the study process:

- Fertility treatment
- Transplant & immunotherapy Antenatal care

- Caesarean section/postnatal care
- Hysterectomy

As part of the selection process you will need to undergo blood tests to exclude infection and also undergo a check for genito-urinary infections. This is to eliminate possible future complication resulting from the transplant. If you are both found to be physically and psychologically suitable you will continue in the study.

Prior to the womb transplant, if you and your partner do not have embryos stored, it will be necessary to undergo fertility treatment to create ten embryos that will subsequently be transferred following transplantation. The process of creating embryos is the same process that you would experience if you opted for surrogacy. Samples of your sperm will be used to fertilise your partners eggs, and then they will be frozen for future use. This treatment will take place at the Lister Fertility Clinic at the Lister Hospital in London.

Your partner will meet the transplant team at the Churchill Hospital in Oxford and the Obstetric team at Queen Charlottes Hospital who will look after your partner whilst you are pregnant. It is advised that you attend these meetings with your partner so you have a full understanding of what the trial entails. You will also have the opportunity to ask questions about every aspect of the trial. You will also meet with Dr Maria Jalmbrant, the clinical psychologist for the trial, who will undertake an interview of both you and your partner and undertake a number of questionnaires to assess your psychological wellbeing. You and your partner will continue to see Maria throughout the process, and complete further questionnaires to continually assess your wellbeing. Support will also be given as needed, including the option of enrolment in a stress reduction programme.

From this point, your partner will be placed on a waiting list until a suitable donor is identified. It is planned that the transplants will be performed over a two-year period so she may be waiting for an organ to become available for up to two years. When a donor becomes available and it has been confirmed that your partner is a suitable match for the organ, your partner will receive a call from the transplant coordinator to come to the Churchill Hospital. It is likely this will be at short notice (12-24hours).

The transplant is a major operation that will be performed by the consultant surgeon. The operation usually takes between 6-8 hours or longer. Your partner will be in the Churchill Hospital for approximately 7 days after the transplant. While your partner is living with her transplanted uterus (womb) she will need to remain on medication called immunosuppression, which works by reducing the body's natural defence system (immune system), to prevent the womb being rejected.

If all remains well your partner will undergo embryo transfer from 6 months following the transplant. Following conception your partner will be cared for by the obstetric team at Queen Charlotte's and Chelsea Hospital. Following an uncomplicated pregnancy your baby will be delivered by caesarean section at 36–37 weeks' gestation. This will be undertaken by the obstetric team at the Queen Charlotte's and Chelsea Hospital.

Subject to an uncomplicated transplant journey, it may be possible to repeat this process with the same donor uterus and try for another child. Once you and your partner have completed your family, approximately 6 months following the birth of your baby your partner will undergo removal of the transplanted womb at the Hammersmith Hospital. Following hysterectomy, your partner will be able to stop the immunosuppressive medications and all other medicines that were started as part of the transplant process.

Do I have to take part?

No, it is up to you to decide whether or not to take part. If you decide to participate you will be given this leaflet to keep and be asked to sign a consent form when you are satisfied that all your queries have been answered fully.

Will taking part be kept confidential?

Yes, please be assured that as an NHS patient your data will be protected under the data protection act. Data collected throughout the study will be stored on a password protected database, on a secure password protected NHS computer in a locked NHS office, and identifiable details will be amended under a study number. Manual files, including consent forms, will be stored within the study file. This is kept safely within the locked research filing area located in a secure research team office at Hammersmith Hospital. This office will be locked and the research file will only be accessible to research team members. Video recording may be used during the operation if you consent to it. The area filmed will only be the operative site. Any video recordings containing patient sensitive information will be treated securely on a secure password protected NHS computer in a locked NHS office. The recordings, which do not contain sensitive information, or identify any participants, may be used in future for teaching, presentation or in journals. The video recordings, along with all other research data, will be stored for 30 years. In the event if data being transferred between sites, data will be anonymised, compressed and encrypted prior to transfer, to ensure it remains secure.

What will happen to my information & samples afterwards?

The data collected from the study will be used to contribute towards a PhD research degree for Dr Benjamin Jones. Research information obtained during the study will be retained for 30 years. We will ask for your consent to store any spare samples so that they may be used for future ethically approved research.

What will happen if I decide to withdraw from the study after I have given my consent?

If you decide to take part you are still free to withdraw **at any time** without giving an explanation, or, a decision not to take part, will not affect the care you receive in any way at present or in the future.

Who is funding the study?

This study has been funded by the registered charity Womb Transplant UK (Charity no 1138559).

What are the possible benefits to me if I take part?

This study will offer your partner the opportunity to conceive and grow a pregnancy to term and give birth to an infant that is biologically related to both yourself and your partner.

What are the risks or side-effects to me if I take part?

This is a complex study with numerous interventions and various stages over a long period of time. Each stage of the transplant process carries different and significant risks to your partner. These have been discussed in some detail in the corresponding information leaflets.

Ultimately the main risks to your partner include rejection of the transplanted uterus, unsuccessful conception and pregnancy or poor obstetric outcome. The physical risks to you, of the pre-operative investigations and use of your sperm are minimal.

What if something goes wrong?

Imperial College Healthcare NHS Trust holds standard NHS Hospital Indemnity and insurance cover with NHS Litigation Authority for NHS Trusts in England, which apply to this study. This does not affect your legal rights to seek compensation.

If you or your partner are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you or your partner have been treated during the course of this study then you should immediately inform the Investigator. The normal National Health Service complaints mechanisms are also available to you. If you are still not satisfied with the response, you may contact the Imperial AHSC Joint Research Compliance Office.

Who do I contact if I want to know more about the study?

If you have any further questions please do not hesitate to contact the study coordinator who will direct your queries to the appropriate member of the study team.

Mr J Richard Smith – Chief Investigator

Telephone: 0207 730 0431
Email: admin@jrsmithgynaecology.com

Thank you for reading this leaflet and considering whether to take part in the study

The approach, consent and assessment process prior to uterine transplant will take place ***at Imperial College Healthcare NHS Trust, Hammersmith Hospital***

Appendix 1b.7: Partner consent form

Study title:	**Investigational Study into Transplantation of the Uterus**
Short title:	**INSITU**
REC reference:	**18/LO/0217**
IRAS project ID:	**235711**

1. I confirm that I have read the following study information sheets and I have had the opportunity to consider the information, ask questions and had these answered satisfactorily.
    a. Partner General Information & Invitation (01/08/2017) – Version 1)
    b. Transplant and Immunotherapy (01/08/2017 – Version 1)
    c. Fertility Treatment (01/08/2017 – Version 1)
    d. Ante-natal Care (01/08/2017 – Version 1)
    e. Caesarean Section (01/08/2017 – Version 1)
    f. Hysterectomy (01/08/2017 – Version 1)

☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.

☐

3. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from the sponsor, regulatory authorities or Oxford University Hospitals NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

☐

4. I understand that the information collected about me will be used to support other research in the future and may be shared anonymously with other researchers.

☐

5. I agree to my General Practitioner being informed of my participation in the study.

☐

6. I agree to take part in the above study.

☐

Name of Participant	Date	Signature

Name of Person Taking informed consent	Date	Signature

Appendix 2.1: Womb Transplant donor information

Background information

Uterine transplantation is a potential treatment for women who cannot become pregnant or maintain pregnancy due to either not having a womb or having a womb that is damaged or not functional. This affects one in 500 women of childbearing age. The current options to acquire motherhood for these women include adoption or surrogacy. However, not only are these options associated with complex legal, financial, cultural, ethical and religious factors, but they do not offer the women to experience gestation. Uterine transplantation provides an opportunity to overcome these issues whilst also giving these women the opportunity to conceive and carry pregnancy themselves.

More than 60 procedures have now been performed worldwide, and at least 15 babies have been born as a result, 14 of which were from using living donors. This demonstrates that uterine transplantation, using living donors, is viable option for women with uterine factor infertility.

The advantage of living uterus donation is that living donor organs are in better condition, and the uterus will only be without blood for a very short time after it is removed from the donor, which increases the chances of a successful transplant. Although there is no guarantee that any uterine transplant will work, the fact that 14 of the 15 successful livebirths following uterine transplant so far have been from living donors, exemplifies the potential advantages to using living donors. The success rates of other solid organs are also better from using living donors. Living uterus donation has the benefit of allowing the transplant operation to be scheduled at a time that is convenient for the recipient and the donor.

One of the most frequent concerns of potential living uterus donors is whether the loss of one uterus will impact on their health in later life. A healthy person can live a completely normal life without a uterus. Lifestyle is not affected, and normal work can continue. Therefore, it is possible to remove a uterus from a healthy living person and transplant it into someone who needs it, with no ill effects on the donor other than the operation itself.

Pre-operative assessment

There is a sequence of tests that are necessary to thoroughly examine the health of the potential donor as well as the wellbeing and anatomy of the uterus. These tests will be performed as an outpatient. Throughout the period of assessment, potential donors should bear in mind that these tests may reveal a reason for the uterus donation being unacceptable.

Blood tests

Blood samples will be taken for routine analyses. Haematology tests can show anaemia or signs of infection, and blood chemistry tests can determine uterus and liver function, or the suggestion of diabetes. Samples are also tested for hepatitis B and C, HIV, CMV, EBV and syphilis. Consent will be obtained before testing for HIV and counselling can be provided before and after the test. Another blood sample will be taken from both the donor and recipient to check that the recipient does not have ‘antibodies’ that may react against the donor. This is called cross matching.

Urine analysis

A routine urine test will be undertaken to assess for infection and any potential undiagnosed problems which may impact ability to donate.

Electrocardiogram (ECG)

This test is used to check that the heart is healthy and functioning properly. An ECG involves having several small electrosensitive pads placed at different points on your chest, arms and legs for a few minutes. You may wear your clothes or a hospital gown for the procedure. The pads monitor the electrical activity of your heart to produce a tracing. The pads will not cause any pain or give you an electric shock. If heart disease is present, an abnormal tracing may be seen, and this could increase the risks associated with anaesthesia during the hysterectomy.

X-rays

A chest X-ray may be taken to ensure there is no undiagnosed medical condition that may affect the lungs or heart.

Genito-urinary infection (GUM) screen / High vaginal swab

A routine genito-urinary screen including gonorrhoea, chlamydia and trichomonas vaginalis will be undertaken to ensure there is no infection. A swab will also be taken to look for other infections, or organisms, which may require treatment, such as thrush.

Cervical Smear

A cervical smear/HPV test is required to ensure there are no abnormal cells on the cervix, prior to transplantation.

Pelvic ultrasound

This is an ultrasound scan that checks the size and shape of the uterus, and can exclude any anatomical abnormalities. It is performed vaginally to ensure the best views are available.

CT and MR Angiography

A combination of both CT and MRI angiography will be used to reveal the number and size of blood vessels taking blood to and from the uterus, as well as further detail of the uterus itself. This is very important to ensure the surgeon can have detailed knowledge of the vessels that will be used to connect the uterus to the recipient. CT angiography is a special kind of X-ray taken of the abdomen. Iodine containing “dye” is then injected into a vein in the arm and the scan is repeated. A computer is then used to build a 3-dimensional view of the uterus as well as it’s supplying and draining blood vessels. The whole procedure takes about 30-60 minutes. There is a risk of radiation exposure with the CT, which will be kept to a minimum, but may present a small increased risk of cancer in the future. MR angiography is a similar technique using a powerful magnet rather than X-rays.

The operation

The hysterectomy (removal of a uterus)

You will be admitted to hospital on the day of your operation. You will be seen by a nurse and be given stockings to wear to reduce the risk of blood clots in your legs. You will then be seen by the surgical team who will explain the operation in further detail and go through a consent form which you will complete once you are satisfied. A repeat blood test will be undertaken to ensure that, in the unlikely event of excessive bleeding, that we have matched blood available to give a blood transfusion if need be.

You will then be put to sleep under general anaesthesia. The surgeons will use a midline incision beneath your umbilicus (belly button), or a bikini line incision across the bottom of your abdomen. You will be given antibiotics as a precaution to reduce the chance of infection. You will also be given painkillers to ensure you do not wake up in pain. The operation includes the removal of the womb and surrounding tissues from the donor. This includes the cervix (neck of the womb), a small cuff of vagina, the surrounding supporting ligaments, as well as the major blood vessels supplying and draining the uterus. The fallopian tubes will also be removed, as they are connected to the womb, but not implanted into the recipient, to reduce future risk of ectopic pregnancy. The ovaries will not routinely be removed but if you have gone through the menopause, this will be an option that is discussed with you depending on your wishes. The graft is lifted out of the wound, flushed with a cold solution to wash out blood and slow the metabolism, after which it is carried into the adjacent operating theatre where the recipient is waiting.

The incision is then sewn up in layers and she returns, via the recovery room, to the ward. Whilst the initial surgical technique resulted in an operation which took 12 hours, evolution of the surgical technique has resulted in an operation which takes 4-6 hours. You will have several temporary tubes or lines inserted during the operation. These may include a tube inserted into the bladder (called a catheter) and a drainage tube from the wound. Fluids can be administered through a drip and, because the incision can be painful afterwards, injections or infusions of painkilling drugs can be given, as required. Tubes are usually removed after the first one to three days when the donor is encouraged to get out of bed and sit in a chair. That way, the risk of complications can be minimised.

What are the risks of the operation?

The risks associated with this operation include anaesthetic risks, bleeding (including the possibility of receiving a blood transfusion), infection, venous thromboembolism (blood clot in veins in your legs or lungs) and damage to internal organs such as bowel, bladder, the tubes that connect the uterus to the bladder (ureters), blood vessels and nerves. There is also a small risk of the stitches coming away at the top of the vagina. There is a small risk of requiring a second operation in the event of complication.

What are the benefits to donating your womb?

The biggest advantage donors can enjoy is that they have given the gift of parenthood, which in turn creates new life. The feeling of satisfaction, which comes from donating a uterus to a someone, is immense and cannot be overestimated. Seeing someone enjoy a better quality of life because of your gift is very rewarding. This very positive aspect of living donation often outweighs the associated physical challenges.

What will happen after the operation?

You will wake up in recovery with a catheter in place and a drip in your arm. You will be transferred back to the ward where you will be monitored closely with frequent observations of your vital signs. You will be offered painkillers and kept comfortable whilst you recover. You will have a catheter in your bladder that will be removed the following day. You may also have a drain in place which reduces the risk of internal bruising. It is likely this will also be removed the day after the operation. You will also receive blood thinning injections and wear the compression stockings to help prevent a blood clot.

After your operation you will stay in hospital for 3-4 days. During this time, you will continue to receive pain killers, and symptomatic relief to facilitate your recovery. You will be helped to mobilise and discharged when you feel comfortable and safe.

How will you feel afterwards?

You will be asked to return to the hospital within the first few weeks after her operation to ensure that she has made a good recovery from the operation and that the wound has healed well.

Psychological effects

After donating a uterus, some people can feel quite emotional. There can be a sense of anticlimax; so much time has been spent thinking about the operation that life may seem a little empty afterwards. The donor may also feel sad and have an unconscious resentment towards the recipient if she feels unsupported by relatives and hospital staff after the operation, as attention is shifted to the recipient. This kind of feeling can be more pronounced if the recipient does not make good progress or the transplant is unsuccessful. In some cases, donors may need additional help and support, including counselling, which can be arranged. Counselling facilities are provided for the donor at some centres. The relationship between donor and recipient and the impact of donation will be individual to each pair. People who receive a uterus are always grateful, but they are unable to repay the gift. So, it is important to avoid reminding them of their ‘debt’. The donor may be able to help by maintaining a normal relaxed attitude towards the recipient.

Getting back to a routine

The first three months after a transplant is the ‘settling down’ period and when most problems tend to occur. Once these three months have passed, both the donor and the recipient can start to resume a normal routine. Depending on their work or lifestyle commitments and the type of surgery, donors can expect to be at home recuperating after the operation for up to 8 weeks. Sometimes this can be a frustrating time, wanting to return to a normal life, but without the energy and overall health. Patience is required, as is support from other family members. The donor should allow between 6-8 weeks to get back to full activity If the donor regularly sees the recipient, this can be an added source of satisfaction – watching the recipient return to good health can ease some of the possible negative feelings.

Driving

The Driver and Vehicle Licensing Agency (DVLA) has no hard and fast rules with regard to starting to drive again. Generally, if the donor feels well and capable, she can usually return to driving after four to six weeks. Car insurance should be checked, as the length of time after an operation that you are not insured to drive varies depending on your policy.

Exercise

Maintaining a healthy lifestyle is as important after donation as beforehand. A post-donation exercise programme should begin slowly, with the length of time spent exercising and the effort involved being increased over a period of time.

Sexual relationships

Donors should be able to resume their usual sexual relationships as soon as they feel comfortable. To allow healing of the vaginal vault, a period of 4-6 weeks abstinence should be adopted. It may take a few months before normal activities can be undertaken, but this depends on the particular individual's recuperation.

Appendix 2.2: Questionnaire

Full title: Motivations and attitudes amongst women offering to donate their womb for womb transplantation

Please complete the following questionnaire after reading the participant information sheet (V2 30-4-19).

Consent

1. I confirm that I have read and understand the participant information leaflet (V1 21-04-19) and have had the opportunity to consider the information, ask questions and had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my legal rights being affected. ☐

3. I understand that the information collected may be used to support other research in the future and may be shared anonymously with other researchers. ☐

4. I agree to take part in the above study. ☐

1. Demographic information

a) Age (years)

16-19 ☐
20-29 ☐
30-39 ☐
40-49 ☐
50-59 ☐
60+ ☐
Would rather not say ☐

b) Ethnicity

White ☐
Asian ☐
Black ☐
Mixed ☐

Other ☐
Would rather not say ☐

c) Employment status

Employed (Full time) ☐
Employed (Part time) ☐
Self Employed ☐
Student ☐
Housewife ☐
Unemployed ☐
Would rather not say ☐

a) Religion

Christian ☐
Muslim ☐
Hindu ☐
Other ☐
Atheist ☐
Would rather not say ☐

b) Relationship status

Single ☐
Living with partner ☐
Married ☐
Divorced ☐
Separated ☐
Widowed ☐
Would rather not say ☐

2. Medical history

Height (cm):

Weight (kg):

Do you have any medical problems we should be aware of?

Yes ☐ No ☐

If yes, please detail below:

Have you previously had cancer?

Yes ☐ No ☐

If yes, please detail below:

Do you have HIV, hepatitis or syphilis?

Yes ☐ No ☐

If yes, please detail below:

Have you been diagnosed with endometriosis or adenomyosis?

Yes ☐ No ☐

If yes, please detail below:

Do you have any psychiatric problems we should be aware of?

Yes ☐ No ☐

If yes, please detail below:

3. Surgical History

Have you had any previous operations inside your abdomen, in particular on your womb or cervix?

Yes ☐ No ☐

If yes, please provide details:

Year	Operation	Complications	Additional comments

4. Childbearing history

Have you had children before?

Yes ☐ No ☐

If yes: How many children have you had?

0☐ 1 ☐ 2☐ 3 ☐ 4+☐

Were any of your babies delivered by Caesarean section?

Yes ☐ No ☐

If yes: How many?

0☐ 1 ☐ 2☐ 3 ☐ 4+☐

Did you have any of these problems in any of the pregnancies?

Pre-term delivery (<37 weeks) ☐ Pre-eclampsia ☐
Heavy bleeding after delivery☐ Gestational diabetes ☐

Obstetric cholestasis □ Not applicable □

If so, please explain further…

Have you completed your family?

Yes □ No □

5. Gynaecological history

Have you had any previous miscarriages?

Yes □ No □

If yes: how many miscarriages have you had?

1 □ 2 □ 3+ □

Have you previously had an abnormal smear?

Yes □ No □

If yes: did you have any surgical treatment to remove the abnormal cells?

Yes □ No □

If yes: have you had a normal smear since?

Yes □ No □

Are you still having regular periods?

Yes □ No □

6. Social history

Occupation:

What is your main language?

If your main language is not English, are you fluent in English?

Yes ☐ No ☐

Do you smoke? Yes ☐ No ☐

If yes, how many cigarettes do you smoke a day?

Have you smoked previously? Yes ☐ No ☐

If yes,

How many years did you smoke for?

On average how many cigarettes did you smoke per day?

When did you give up smoking?

Do you drink alcohol? Yes ☐ No ☐

If yes, how many units of alcohol so you consume a week?

7. Knowledge about womb transplantation

I believe that adoption and surrogacy are suitable methods to have children for women who do not have a functional womb

Strongly agree ☐ Agree ☐ Undecided ☐ Disagree ☐ Strongly Disagree ☐

If disagree/strongly disagree, why? ……..……

How much do you know about womb transplantation?

A lot ☐ A fair amount ☐ Heard it discussed only a few times ☐ Nothing ☐

I understand the benefits of womb transplantation.

Strongly agree ☐ Agree ☐ Undecided ☐ Disagree ☐ Strongly Disagree ☐

If disagree/strongly disagree, why? ……..……

I understand the risks of donating my womb

Strongly agree ☐ Agree ☐ Undecided ☐ Disagree ☐ Strongly Disagree ☐

If disagree/strongly disagree, why? ……..……

I am aware that if I donate my womb, I will be unable to have further children.

Yes ☐ No ☐

I am aware that if I donate my womb, my ovaries will not be removed, so I will continue to have normal hormones

Yes ☐ No ☐

I understand that if I donate my womb, my ovaries will not be removed, so any future babies born in the womb after donation will not be genetically related to me.

Yes ☐ No ☐

I understand that after I donate my womb, I would no longer have periods

Strongly agree ☐ Agree ☐ Undecided ☐ Disagree ☐ Strongly Disagree ☐

If disagree/strongly disagree, why? ……..……

I believe that not having periods would improve my quality of life

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

If disagree/strongly disagree, why? ……..……

8. Motivations for womb donation

Please indicate if you agree/disagree with the following statements:

I want to donate my womb to help someone else carry and give birth to their own baby

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

I know someone personally who is infertile who I would like to donate my womb to

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

I want to donate my womb to help others

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

I want to donate my womb to help contribute to science

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

I want to donate my womb because I am already an organ donor and want to donate another organ

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

I want to donate my womb because I previously was or considered being a surrogate

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

I want to donate my womb because I no longer need it

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

I believe that the potential benefits of donating my womb to help an infertile woman give birth outweigh the potential risks of the surgery

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

If disagree/strongly disagree, why? ……..……

I believe that women should be freely able to voluntarily donate their womb for womb transplant, as long as they are fully informed

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

If disagree/strongly disagree, why? ……..……

I would be happy to undergo the necessary pre-operative investigations to ensure my womb is safe for transplantation

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

If disagree/strongly disagree, why? ……..……

If you are menopausal, would you be accepting of taking hormone replacement therapy for 3-6 months pre-operatively. This will give you monthly withdrawal bleeds to help demonstrate that the lining of your womb is still functional.

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □
Not applicable □

If disagree/strongly disagree, why? ……..……

I understand and accept the expected recovery period of 3-4 days in hospital and up to 8 weeks before being fully back to normal

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

If disagree/strongly disagree, why? ……..……

I understand that I would not receive any payment for donating my womb

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

If disagree/strongly disagree, why? ……..……

If you donated your womb for transplantation, would you want to be informed of subsequent progress of recipient, for example to find out that they have become pregnant or had a baby?

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

If disagree/strongly disagree, why? ……..……

Are you aware that the woman who receives a womb transplant is at greater risk of cancer and infection because of the need to take immunosuppressive medications after the transplant?

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

If disagree/strongly disagree, why? ……..……

Does knowing about the additional risks of cancer and infection on the woman who receives a womb impact your perceptions on womb transplantation?

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

If strongly agree or disagree, why? ……..……

Given the novelty of the operation, and the media interest in the process, I understand there is a small risk of unwanted media intrusion if I went ahead with donating my womb

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

If disagree/strongly disagree, why? ……..……

Having read the patient information sheet, and considered the risks, I am still keen to donate my womb for transplantation.

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

If disagree/strongly disagree, why? ……..……

Any other comments to add?

Thank you for completing this questionnaire and taking part in this study

Appendix 3a.1: Consent form and participant information leaflet

Full title: Male to female transgender women's perceptions to womb transplantation

Name of Principal Investigator: Mr J Richard Smith

Please initial box

1. I confirm that I have read and understand the participant information leaflet (V1 01-08-18) and have had the opportunity to consider the information, ask questions and had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my legal rights being affected. ☐

3. I understand that the information collected may be used to support other research in the future and may be shared anonymously with other researchers. ☐

4. I agree to take part in the above study. ☐

Name of Patient/Participant	Signature	Date

Name of Person taking consent (if different from Principal Investigator)	Signature	Date

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Part 1 tells you the purpose of this study and what will happen to you if you take part. Part 2 gives you more detailed information about the conduct of the study. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of this study?

This study will seek to gain a greater understanding of the desire, and therefore need, to perform womb transplantation as part of gender reassignment surgery. It includes a questionnaire that aims to assess demographic information, details of your previous history and treatment and details of previous fertility preservation opportunities. It also assesses feelings of your current options to acquire motherhood, your present knowledge of womb transplantation and ultimately your perceptions towards womb transplantation in M2F transgender women as part of gender reassignment. This research study ultimately aims to assess the perceptions of M2F transgender women to womb transplantation, to determine if there is a need to research this further. Further research studies are essential to prove that this is a feasible operation, before it could be considered being performed as a clinical procedure in transgender women.

Background information

The concept of womb transplantation has been investigated for the last 20 years and has recently made the transition into clinical practice in natal women. At least 44 womb transplants have now been performed, and at least 12 livebirths have been achieved. The fertility rates in the Swedish cohort of seven women with successful womb transplants was 100%. It therefore appears that womb transplantation is a viable therapeutic option for natal women who do not have a functional womb. Following these developments, speculation has escalated regarding the possibility of performing womb transplantation in male to female (M2F) transgender women, as part of gender reassignment surgery. The UK womb transplant research team hypothesises that it is an achievable operation, with modification to the surgical technique to the operation that has been performed so far in natal women.

The surgical technique so far includes the transplantation of the womb, including cervix (neck of the womb), a cuff of vagina, the surrounding connective tissues, as well as the major blood vessels that supply the womb. In the M2F transgender model, the vagina would also require transplanting, to ensure the womb had a suitable environment to achieve and maintain pregnancy. This should also mean that the vagina functions normally. The ovaries would not be transplanted. The prostate shouldn’t need to be removed but could be depending on each case. The surgical technique is demonstrated in figures 2 and 3:

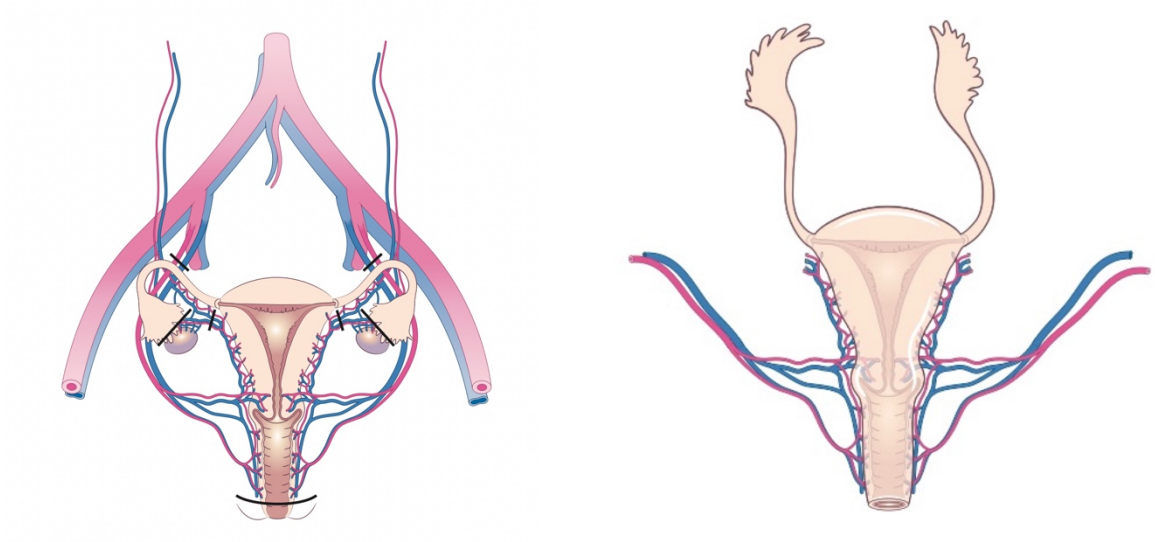


Figure 2 – *Removal of the womb, the neck of the womb (cervix), the vagina and connecting blood vessels from the donor (left) and the graft that would then be implanted into a M2F transgender woman (right)*

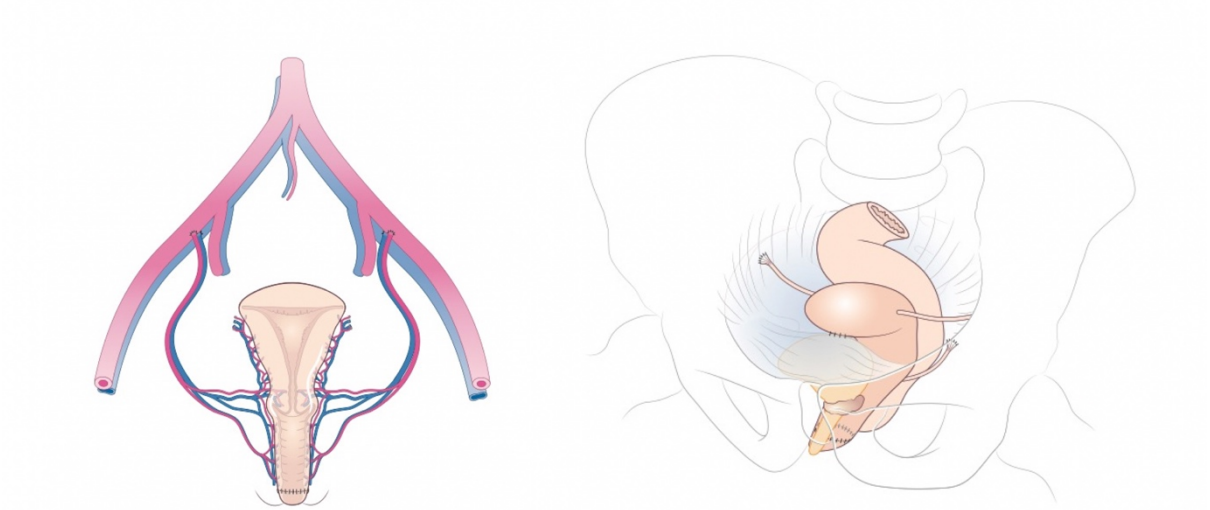


Figure 3 – *Transplantation of the womb, the neck of the womb (cervix), vagina and connecting blood vessels into the recipient (left) and the connective tissue surrounding the womb after transplantation (right)*

After the operation, the recipient would continue to take hormonal therapy, but in addition to oestrogen, progesterone would also be required to reduce the risk of abnormal cells growing in the lining of the womb. Instead of being given the same doses each day, a cyclical regime would be given instead, to enable the recipient to experience periods. This helps to determine if the womb is functional. After a period of at least 6 months, fertility treatment can be undertaken to achieve pregnancy. Embryos will have previously been created, using either the recipients stored sperm, their partners sperm or donor sperm, along with donor eggs. The pregnancy will need to be closely monitored and will need delivery by Caesarean section. Immunosuppressive medications would need to be taken as long as the graft was in place, to reduce the risk of the body rejecting the transplanted womb. These would continue through pregnancy. These medications are deemed safe to take in pregnancy, with no increased risk of structural abnormality to the growing fetus. The long-term risks to this medication include infection and cancer, particularly if taken for longer than 5 years. As such, after the recipient has completed her family, the recipient will be advised to have the womb removed to minimise these risks.

Why have I been chosen?

You have been invited to participate because you are a male to female (M2F) transgender woman. You therefore have absolute uterine factor infertility (AUFI), a condition which affects one in 500 women of childbearing age. Women with this condition have infertility due to either the absence of their uterus (womb) or they have an anatomically or physiologically non-functioning uterus. This may be congenital, such as in M2F transgender women, or can be acquired (for example in natal women following hysterectomy to treat cancer or following severe haemorrhage after childbirth). The causes of AUFI are shown in figure 1:

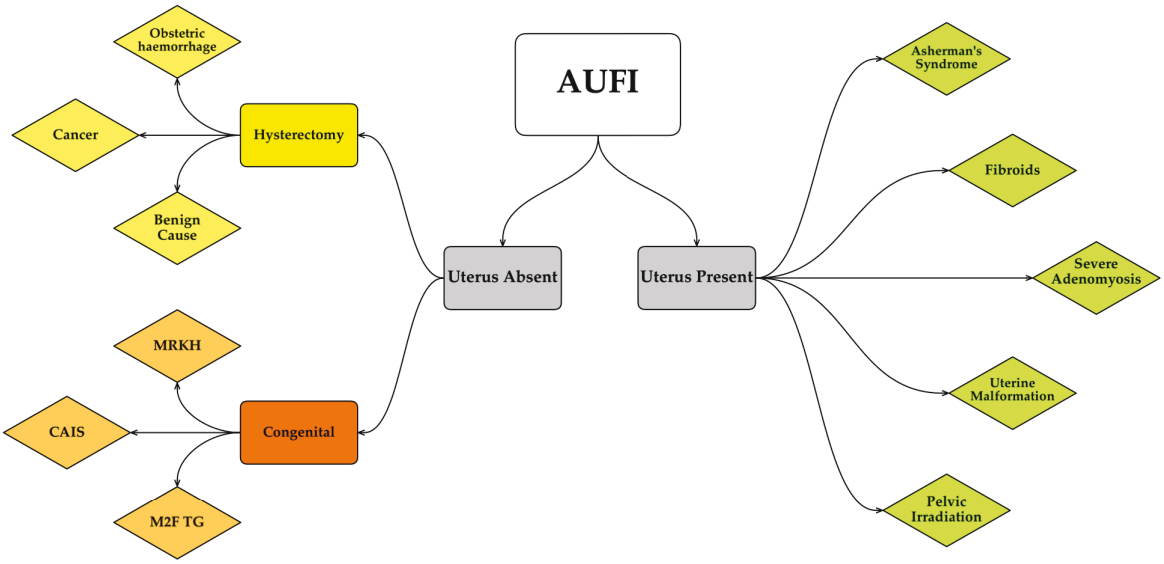


Figure 1 – *Causes of absolute uterine factor infertility (Key: AUFI; Absolute Uterine Factor Infertility, CAIS; complete androgen insensitivity syndrome, M2F TG; Male to female transgender women, MRKH; Mayer Rokitansky Küster Hauser Syndrome)*

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason.

What do I have to do?

After reading this patient information leaflet, you will complete and sign the consent form to agree to be part of this study. Once you have scanned and returned it to Dr Benjamin Jones at Benjamin.jones@nhs.net he will send you a link to the questionnaire on www.surveymonkey.com. You will then complete the questionnaire online which contains 27 questions. It should take approximately 10 minutes to complete. Following the completion of the questionnaire, no further action will be required.

What are the possible benefits to me if I take part?

This study will give you the opportunity to express your views on womb transplantation in transgender women. Whilst the results may not directly influence you, your responses, along with the other responses received, will determine the need for our research team to pursue this research concept further.

What are the possible disadvantages and risks of taking part?

There are no disadvantages or risks to your partaking in this questionnaire.

What if something goes wrong?

Imperial College holds a Public Liability ("negligent harm") insurance policy which applies to this trial. If you can demonstrate that you experienced serious and enduring harm as a result of your participation in this trial, you may be eligible to claim compensation without having to prove that Imperial College is at fault. If the injury resulted from any procedure which is not part of the trial, Imperial College will not be required to compensate you in this way. Your legal rights to claim compensation for injury where you can prove negligence are not affected. Please contact the Principal Investigator if you would like further information about the insurance arrangements which apply to the trial.

Will taking part be kept confidential?

This information will not identify you and will not be combined with other information in a way that could identify you. The information will only be used for the purpose of research and cannot be used to contact you or to affect you in the future. It will not be used to make decisions about future services available to you, such as insurance.

Imperial College London is the sponsor for this study based in the United Kingdom. We will be using information from you in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly.

Information on Imperial College London's retention periods may be found at https://www.imperial.ac.uk/media/imperial-college/administration-and-support-services/records-and-archives/public/RetentionSchedule.pdf.

Legal Basis

As a university we use personally-identifiable information to conduct research to improve health, care and services. As a publicly-funded organisation, we have to ensure that it is in the public interest when we use personally-identifiable information from people who have agreed to take part in research. This means that when you agree to take part in a research study, we will use your data in the ways needed to conduct and analyse the research study. Health and care research should serve the public interest, which means that we have to demonstrate that our research serves the interests of

society as a whole. We do this by following the UK Policy Framework for Health and Social Care Research.

International Transfers

There may be a requirement to transfer information to countries outside the European Economic Area (for example, to a research partner). Where this information contains your personal data, Imperial College London will ensure that it is transferred in accordance with data protection legislation. If the data is transferred to a country which is not subject to a European Commission (**EC**) adequacy decision in respect of its data protection standards, Imperial College London will enter into a data sharing agreement with the recipient organisation that incorporates EC approved standard contractual clauses that safeguard how your personal data is processed.

Contact us

If you wish to raise a complaint on how we have handled your personal data or if you want to find out more about how we use your information, please contact Imperial College London's Data Protection Officer via email at dpo@imperial.ac.uk, via telephone on 020 7594 3502 and via post at Imperial College London, Data Protection Officer, Faculty Building Level 4, London SW7 2AZ. If you are not satisfied with our response or believe we are processing your personal data in a way that is not lawful you can complain to the Information Commissioner's Office (ICO). The ICO does recommend that you seek to resolve matters with the data controller (us) first before involving the regulator.

Imperial College London will collect information from you and for this research study in accordance with our instructions. Imperial College London may use your name and contact details to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study. Individuals from Imperial College London may look at your research records to check the accuracy of the research study. The only people at Imperial College London who will have access to information that identifies you will be people who need to contact you to audit the data collection process. The people who analyse the information will not be able to identify you and will not be able to find out your name or contact details. Imperial College London will keep identifiable information about you from this study for 10 years after the study has finished.

What will happen to the results of the research study?

The data collected from the study will be used to determine the need to investigate this concept further, with the final aim to perform a sustainable and achievable womb transplant operation in M2F transgender women. The information gained from the questionnaires will contribute towards a PhD research degree for Dr Benjamin Jones. The data may also be used for publication in peer reviewed journals and included in oral and poster presentations at national and international conferences. You will not be able to be identified in any publications or presentations.

Who is organising and funding the study?

This study has been organised by the registered charity Womb Transplant UK (Charity no 1138559). No healthcare professional will receive payment for conducting this research.

Contact for further information

If you have any further questions please do not hesitate to contact the study coordinator, Dr Benjamin Jones, at Benjamin.jones@nhs.net who will be able to answer any queries.

Thank you for reading this leaflet and considering whether to take part in the study

Mr J Richard Smith – Principal Investigator **Telephone: 07740358900**

Email: info@wombtransplantuk.org

Appendix 3a.2: Questionnaire (V1 - 01/08/2018)

Full title: Male to Female Transgender women's perceptions to womb transplantation

1. Demographic information

a) Age (years)

16-19 ☐
20-29 ☐
30-39 ☐
40-49 ☐
50+ ☐
Would rather not say ☐

b) Assigned sex at birth

Male ☐
Female ☐

c) Currently assigned sex

Male ☐
Female ☐
In process of transitioning ☐

d) Religion

Christianity ☐
Islam ☐
Hinduism ☐
Other ☐
Atheist ☐
Would rather not say ☐

e) Relationship status

Single ☐
Living with partner ☐
Married ☐
Divorced ☐
Separated ☐
Widowed ☐
Would rather not say ☐

f) Sexual orientation

Attracted to men ☐
Attracted to women ☐
Attracted to both men and women ☐
Not attracted to either men or women ☐
Would rather not say ☐

2. How many years ago did you develop symptoms of discomfort with the sex you were assigned at birth?

Less than 1 year □
Between 1 and 5 years □
Between 6 and 10 years □
Between 11 and 15 years □
Between 16 and 20 years □
>20 years □

3. If relevant, how long have you been taking hormonal therapy?

Never taken □
Less than 1 year □
Between 1 and 5 years □
Between 6 and 10 years □
Between 11 and 15 years □
Between 16 and 20 years □
>20 years □

4. Please indicate, if relevant, how long ago you underwent gender reassignment surgery

Never had GRS □
Less than 1 year □
Between 1 and 5 years □
Between 6 and 10 years □
Between 11 and 15 years □
Between 16 and 20 years □
>20 years □

5. Prior to undergoing hormonal or surgical treatment, were you offered the chance to freeze your sperm for use in the future?

Yes □ No □

6. Prior to undergoing hormonal or surgical treatment, did you freeze your sperm for future use?

Yes □ No □

If No: What was the main reason for not preserving your sperm?

Do not want children □
Do not want biologically related children □
Too expensive □
Sperm already not healthy after hormone therapy □
Already had children and completed family □
Too young and sperm not developed □
Did not want to delay treatment □
Other (please state)……

If Yes: Have you used the stored sperm yet?

Yes, in surrogate □ Yes, in partner □ No □

7. Did you have children prior to transitioning?

Yes □ No □

If yes: How many children did you have prior to transitioning?

0□ 1 □ 2□ 3 □ 4+□

8. Have you had children since transitioning?

Yes □ No □

If yes: How many

0□ 1 □ 2□ 3 □ 4+□

9. Do you want children in the future?

Yes □ No □

10. Do you believe that the current fertility options for women with absolute womb factor infertility (adoption and surrogacy), are suitable methods for M2F transgender women to have children.

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

If disagree/strongly disagree, why? ……..……

11. Do you feel that M2F transgender women are hindered, more than natal women, by discrimination in the process of adoption?

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

If disagree/strongly disagree, why? ……..……

12. Do you feel that M2F transgender women are hindered, more than natal women, by discrimination in the process of surrogacy?

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

If disagree/strongly disagree, why? ……..……

13. How much do you know about Womb Transplantation?

A lot □ A fair amount □ Heard it discussed only a few times □ Nothing □

14. I understand the benefits of Womb Transplantation.

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

If disagree/strongly disagree, why? ……..……

15. I understand the risks of Womb Transplantation.

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

If disagree/strongly disagree, why? ……..……

16. I believe the potential benefits of Womb Transplantation outweigh the potential risks in M2F transgender women

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

If disagree/strongly disagree, why? ……..……

17. I believe that having periods would make me feel like more of a woman

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

If disagree/strongly disagree, why? ……..……

18. I believe that having the ability to become pregnant, carry pregnancy and give birth to my own child would make me feel like more of a woman

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

If disagree/strongly disagree, why? ……..……

19. I believe that I would feel more satisfied with my assigned sex after having a womb transplant

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

If disagree/strongly disagree, why? ……..……

20. I believe that the development of womb transplantation will lead to greater patient happiness in M2F transgender women.

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

If disagree/strongly disagree, why? ……..……

21. I believe that if womb transplantation becomes an established treatment for women assigned female at birth who do not have a womb, it should be offered to M2F transgender women once it is proven to be feasible.

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

If disagree/strongly disagree, why? ……..……

22. I believe that if womb transplantation becomes an established treatment for M2F transgender women, I would have been more inclined to freeze my sperm to use to create embryos to be used in my womb in the future?

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

If disagree/strongly disagree, why? ……..……

23. As part of womb transplantation, having a transplanted, functioning vagina as part of the procedure would most likely improve my sex life

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

If disagree/strongly disagree, why? ……..……

24. As part of womb transplantation, having a transplanted, functioning vagina as part of the procedure would make me feel like more of a woman.

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

If disagree/strongly disagree, why? ……..……

25. As part of womb transplantation, having a transplanted, functioning vagina as part of the procedure would improve my quality of life.

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

If disagree/strongly disagree, why? ……..……

26. If you received a womb transplant, would you be accepting of removal of the womb after you have completed your family, to avoid the long-term risks associated with the medications needed to prevent rejection?

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □

If disagree/strongly disagree, why? ……..……

27. If, when you are ready to have children, and your partner was a female and had a functioning womb, would you still want a womb transplant yourself for the purpose of having children?

Strongly agree □ Agree □ Undecided □ Disagree □ Strongly Disagree □
n/a - I am sexually attracted to men □

If disagree/strongly disagree, why? ……..……

Thank you for completing this questionnaire and taking part in this study

Appendix 5a.1: Social egg freezing perceptions questionnaire

1. Demographic information

a) What was your relationship status at the time of egg freezing?

Single □
In relationship □
Living with partner □
Married □
Divorced □
Separated □
Widowed □

b) What is your ethnicity?

White □
Asian □
Black □
Mixed □
Would rather not say □
Other (please specify) □

c) What is the highest degree or level of school you completed?

No formal qualifications □
GCSEs □
A levels / Diploma □
Professional qualification □
Undergraduate degree □
Postgraduate degree □
Doctorate □

d) What is your employment status?

Employed (Full time) □
Employed (Part time) □
Self Employed □
Student □
Unemployed □

2. Perceptions and feelings regarding social egg freezing

Please indicate if you agree/disagree with the following statements:

a) **At the time of my egg freezing, I was worried about my declining fertility**

Strongly disagree □ Disagree □ Neither agree, nor disagree □ Agree □ Strongly Agree □

b) **At the time of egg freezing, having my own biological children was very important to me**

Strongly disagree □ Disagree □ Neither agree, nor disagree □ Agree □ Strongly Agree □

c) **At the time of my egg freezing, I wanted to become a parent at any cost**

Strongly disagree □ Disagree □ Neither agree, nor disagree □ Agree □ Strongly Agree □

d) **At the time of my egg freezing, finding a lifelong partner was more important to me than having children**

Strongly disagree □ Disagree □ Neither agree, nor disagree □ Agree □ Strongly Agree □

3. Egg Freezing Questions

a) To what extent, did the following factors influence your decision to freeze your eggs:

i. I was not ready to start a family
Definitely □ Very much □ A moderate amount□ A little □ Not at all□

ii. I wanted to pursue my career
Definitely □ Very much □ A moderate amount□ A little □ Not at all □

iii. I wanted to pursue further education
Definitely □ Very much □ A moderate amount□ A little □ Not at all □

iv. I did not have a partner
Definitely □ Very much □ A moderate amount□ A little □ Not at all □

v. I was with a partner who I did not want to have children with
Definitely □ Very much □ A moderate amount□ A little □ Not at all □

vi. I was with a partner who was not yet ready for children
Definitely □ Very much □ A moderate amount□ A little □ Not at all □

vii. I had a recent breakdown in a relationship
Definitely □ Very much □ A moderate amount□ A little □ Not at all □

viii. I was influenced by the media
Definitely □ Very much □ A moderate amount□ A little □ Not at all □

ix. I was given advice from healthcare professionals
Definitely □ Very much □ A moderate amount□ A little □ Not at all □

b) When you froze your eggs, how likely did you think it was going to be that you would eventually use them?

No plans to use them □
Unlikely □
50:50 □
Likely □
Very likely □
Definitely □

c) When you froze your eggs, how long did you plan for them to be stored for, before you intended to use them?

Less than 1 year □
Between 1 and 2 years □
Between 3 and 4 years □
Between 5 and 6 years □
Between 7 and 8 years □
Between 8 and 9 years □
10 years or more □

4. Information and Support

a) At the time of freezing your eggs, how comprehensive did you feel that the information you received with regards to your fertility was?

Very Poor □ Poor □ Average □ Good □ Excellent □

b) At the time of freezing your eggs, how clear was the information you received regarding your individualised chance of having a baby, per frozen egg?

Very Poor □ Poor □ Average □ Good □ Excellent □

c) At the time of freezing your eggs, was it explained to you that there could be chance of treatment failure in the future and that a future livebirth could not be guaranteed?

Yes □ No □ Unsure □

d) With the number of eggs that you had collected, what was your understanding about your chances of achieving a livebirth?

0-20% □ 20-40% □ 40-60% □ 60-80% □ 80-100% □ Unsure □

5. Implications of getting pregnant at an older age

a) Are you aware that getting pregnant at advanced maternal age can be associated with greater risk of high blood pressure in pregnancy, blood clots in legs and lungs and your baby being born prematurely?

Yes □ No □

b) I am prepared to accept the risks of delaying my pregnancy using egg freezing to enable me to fall pregnant at a better time for me

Yes □ No □

c) Do you think you could have received better counselling regarding the risks of getting pregnant at an older age?

Yes □ No □ Unsure □

6. Your experience of freezing your eggs

a) Did the social egg freezing experience negatively affect your mood?

Always □ Very often □ Quite often □ Seldom □ Never □

b) Were the fertility medical services you wanted available to you?

Always □ Very often □ Quite often □ Seldom □ Never

c) How complicated was dealing with the procedure and/or administration of medication during the egg freezing process?

An extreme amount□ Very much □ A moderate amount□ A little □ Not at all □

d) Were you bothered by the effect of treatment on your daily or work-related activities?

An extreme amount□ Very much □ A moderate amount□ A little □ Not at all □

e) Did you feel the fertility staff understood what you were going through?

An extreme amount□ Very much □ A moderate amount□ A little □ Not at all □

f) Were you bothered by the physical side effects of fertility medications and treatment?

An extreme amount□ Very much □ A moderate amount□ A little □ Not at all □

g) Were you satisfied with the quality of services available to you to address your emotional needs?

Very dissatisfied □ Dissatisfied □ Neither satisfied, nor dissatisfied □ Satisfied □ Very Satisfied □

h) How would you rate the medical treatment you received?

Very dissatisfied □ Dissatisfied □ Neither satisfied, nor dissatisfied □ Satisfied □ Very Satisfied □

i) How would you rate the quality of information you received about medication, surgery and the overall process?

Very dissatisfied □ Dissatisfied □ Neither satisfied, nor dissatisfied □ Satisfied □ Very Satisfied □

j) Were you satisfied with your interactions with fertility medical staff?

Very dissatisfied □ Dissatisfied □ Neither satisfied, nor dissatisfied □ Satisfied □ Very Satisfied □

k) Would you freeze your eggs again?

Yes □ No □ Unsure □

l) Do you regret freezing your eggs?

Yes definitely □ Very much □ A moderate amount□ A little □ Not at all □

If yes, please choose the most appropriate reason(s) for your regret:

Poor supportive counselling at time of freezing □
Lack of verbal/written information □
Low number of eggs stored □
Excessive side effects / complications □
Unsuccessful pregnancies using frozen eggs □
Complications during subsequent pregnancy □
Financial expense □
Other (please state) □

7. The future

Please indicate if you agree/disagree with the following statements:

a) I feel empowered after freezing my eggs

Strongly disagree □ Disagree □ Neither agree, nor disagree □ Agree □ Strongly Agree □

b) I feel less pressured to have children now I have frozen my eggs

Strongly disagree □ Disagree □ Neither agree, nor disagree □ Agree □ Strongly Agree □

c) I feel that freezing my eggs gave me an extended opportunity to find a suitable partner

Strongly disagree □ Disagree □ Neither agree, nor disagree □ Agree □ Strongly Agree □

d) Have you had children since freezing your eggs?

Yes □ No □ Currently pregnant □

If yes: How many children?

1 □
2 □
3 □
Four or more □

If yes, how did you conceive?

Naturally □
Using stored eggs with partners sperm □
Using stored eggs with donor sperm □
Other □

If you have not used your eggs already:

a) What is your plan with regards to using your stored eggs?

Use with current partner □ Use with a future partner □ Use sperm donor □

Not planning to use them □

b) What is the oldest age you would consider using your eggs / becoming pregnant?

c) Would you use a sperm donor as a last resort if you were not in a relationship at the time you needed to use your eggs?

Yes □ No □ Unsure □

d) Do you know how long the maximum period that your eggs can be stored for?

2 years □ 4 years □ 6 years □ 8 years □ 10 years□ 15 years □ 20 years□

e) Are you aware that after the maximum period of storage, your eggs will be discarded, so you will not be able to use them?

Yes □ No □

f) I would accept my eggs being discarded if they exceeded the maximum storage limit

Strongly disagree □ Disagree □ Neither agree, nor disagree □ Agree □ Strongly Agree □

g) Do you agree that the maximum storage period of 10 year is fair?

Strongly disagree □ Disagree □ Undecided □ Agree □ Strongly agree □

h) If you do not agree with the 10-year storage period, what time period do you think would be most appropriate?

5 years □ 15 years □ 20 years □ 30 years □ Indefinite □ Until aged 50 □

i) If you decided to not use some or all of the eggs you have stored, what would you decide to do with them?

Discard them □ Donate them to research □
Donate them to couples in need of an egg donor □
Unsure □

Do you have any additional comments that you would like to make about your experience of egg freezing?

Thank you for completing this questionnaire and taking part in this study

Dr Benjamin Jones – Principal Investigator
Telephone: 07740358900
Email: Benjamin.jones@nhs.net

References

1. National Institute for Health and Care Excellence (NICE). Fertility: assessment and treatment for people with fertility problems. London. 2013: NICE. (Date accessed 2nd February 2020).
2. Zegers-Hochschild F, Adamson GD, de Mouzon J, et al. International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology, 2009. Fertil Steril 2009;92:1520-4.
3. Legro RS, Dodson WC, Kris-Etherton PM, et al. Randomized Controlled Trial of Preconception Interventions in Infertile Women With Polycystic Ovary Syndrome. The Journal of clinical endocrinology and metabolism 2015;100:4048-58.
4. Brown J, Farquhar C. Clomiphene and other antioestrogens for ovulation induction in polycystic ovarian syndrome. Cochrane Database of Systematic Reviews 2016.
5. Berger JJ, Bates GW, Jr. Optimal management of subfertility in polycystic ovary syndrome. Int J Womens Health 2014;6:613-21.
6. Sharpe A, Morley LC, Tang T, Norman RJ, Balen AH. Metformin for ovulation induction (excluding gonadotrophins) in women with polycystic ovary syndrome. Cochrane Database Syst Rev 2019;12:Cd013505.
7. Campo S. Ovulatory cycles, pregnancy outcome and complications after surgical treatment of polycystic ovary syndrome. Obstetrical & gynecological survey 1998;53:297-308.
8. Weiss NS, Kostova E, Nahuis M, Mol BWJ, van der Veen F, van Wely M. Gonadotrophins for ovulation induction in women with polycystic ovary syndrome. Cochrane Database Syst Rev 2019;1:Cd010290.
9. Human Fertilisation and Embryology Authority. Fertility treatment in 2017: Trends and Figures.2017.
10. Laronda MM, Jakus AE, Whelan KA, Wertheim JA, Shah RN, Woodruff TK. Initiation of puberty in mice following decellularized ovary transplant. Biomaterials 2015;50:20-9.
11. Laronda MM, Rutz AL, Xiao S, et al. A bioprosthetic ovary created using 3D printed microporous scaffolds restores ovarian function in sterilized mice. Nature communications 2017;8:15261.
12. Liu WY, Lin SG, Zhuo RY, et al. Xenogeneic Decellularized Scaffold: A Novel Platform for Ovary Regeneration. Tissue engineering Part C, Methods 2017;23:61-71.
13. Sheikhansari G, Aghebati-Maleki L, Nouri M, Jadidi-Niaragh F, Yousefi M. Current approaches for the treatment of premature ovarian failure with stem cell therapy. Biomed Pharmacother 2018;102:254-62.
14. Winston RM, Margara RA. Microsurgical salpingostomy is not an obsolete procedure. British journal of obstetrics and gynaecology 1991;98:637-42.
15. Marana R, Quagliarello J. Proximal tubal occlusion: microsurgery versus IVF--a review. Int J Fertil 1988;33:338-40.
16. Varasteh NN, Neuwirth RS, Levin B, Keltz MD. Pregnancy rates after hysteroscopic polypectomy and myomectomy in infertile women. Obstetrics and gynecology 1999;94:168-71.
17. Pritts EA. Fibroids and infertility: a systematic review of the evidence. Obstetrical & gynecological survey 2001;56:483-91.
18. Pabuccu R, Atay V, Orhon E, Urman B, Ergun A. Hysteroscopic treatment of intrauterine adhesions is safe and effective in the restoration of normal menstruation and fertility. Fertil Steril 1997;68:1141-3.
19. Hellstrom M, Moreno-Moya JM, Bandstein S, et al. Bioengineered uterine tissue supports pregnancy in a rat model. Fertil Steril 2016;106:487-96.e1.
20. Heller-Boersma JG, Schmidt UH, Edmonds DK. Psychological distress in women with uterovaginal agenesis (Mayer-Rokitansky-Kuster-Hauser Syndrome, MRKH). Psychosomatics 2009;50:277-81.
21. Holt R, Slade P. Living with an incomplete vagina and womb: an interpretative phenomenological analysis of the experience of vaginal agenesis. Psychology, health & medicine 2003;8:19-33.
22. Saso S, Clarke A, Bracewell-Milnes T, et al. Psychological Issues Associated With Absolute Uterine Factor Infertility and Attitudes of Patients Toward Uterine Transplantation. Progress in transplantation (Aliso Viejo, Calif) 2016;26:28-39.
23. Reichman DE, Laufer MR. Mayer-Rokitansky-Kuster-Hauser syndrome: fertility counseling and treatment. Fertil Steril 2010;94:1941-3.
24. Park K. Choosing Childlessness: Weber's Typology of Action and Motives of the Voluntarily Childless. Sociological Inquiry 2005;75:372-402.
25. Lechner L, Bolman C, van Dalen A. Definite involuntary childlessness: associations between coping, social support and psychological distress. Human reproduction (Oxford, England) 2007;22:288-94.
26. Verhaak CM, Smeenk JM, Evers AW, Kremer JA, Kraaimaat FW, Braat DD. Women's emotional adjustment to IVF: a systematic review of 25 years of research. Human reproduction update 2007;13:27-36.

27. Bryson CA, Sykes DH, Traub AI. In vitro fertilization: a long-term follow-up after treatment failure. Human fertility (Cambridge, England) 2000;3:214-20.
28. Laggari V, Diareme S, Christogiorgos S, et al. Anxiety and depression in adolescents with polycystic ovary syndrome and Mayer-Rokitansky-Kuster-Hauser syndrome. Journal of psychosomatic obstetrics and gynaecology 2009;30:83-8.
29. Gameiro S, Finnigan A. Long-term adjustment to unmet parenthood goals following ART: a systematic review and meta-analysis. Human reproduction update 2017;23:322-37.
30. van Balen F, Bos HMW. The social and cultural consequences of being childless in poor-resource areas. Facts Views Vis Obgyn 2009;1:106-21.
31. Brodzinsky DM. Children's understanding of adoption: Developmental and clinical implications. Professional Psychology: Research and Practice 2011;42:200-7.
32. Department for Education. Children looked after in England (including adoption), year ending 31 March 2019. Department for Education, London 2019.
33. Juffer F, van Ijzendoorn MH. Behavior problems and mental health referrals of international adoptees: a meta-analysis. Jama 2005;293:2501-15.
34. Miller BC, Fan X, Christensen M, Grotevant HD, van Dulmen M. Comparisons of adopted and nonadopted adolescents in a large, nationally representative sample. Child development 2000;71:1458-73.
35. Crea TM, Barth RP, Guo S, Brooks D. Behavioral outcomes for substance-exposed adopted children: Fourteen years postadoption. American Journal of Orthopsychiatry 2008;78:11-9.
36. Gunnar MR, Van Dulmen MHM. Behavior problems in postinstitutionalized internationally adopted children. Development and Psychopathology 2007;19:129-48.
37. Hjern A, Palacios J, Vinnerljung B. Can adoption at an early age protect children at risk from depression in adulthood? A Swedish national cohort study. BMJ Paediatrics Open 2018;2:e000353.
38. van Ijzendoorn MH, Juffer F, Poelhuis CW. Adoption and cognitive development: a meta-analytic comparison of adopted and nonadopted children's IQ and school performance. Psychological bulletin 2005;131:301-16.
39. Shenfield F, Pennings G, Cohen J, Devroey P, de Wert G, Tarlatzis B. ESHRE Task Force on Ethics and Law 10: surrogacy. Human reproduction (Oxford, England) 2005;20:2705-7.
40. International Federation of Fertility Societies (IFFS). IFFS Surveillance 2016. Global Reproductive Health 2016;1:1-143.
41. Crawshaw M, Blyth E, van den Akker O. The changing profile of surrogacy in the UK – Implications for national and international policy and practice. Journal of Social Welfare and Family Law 2012;34:267-77.
42. Soderstrom-Anttila V, Wennerholm UB, Loft A, et al. Surrogacy: outcomes for surrogate mothers, children and the resulting families-a systematic review. Human reproduction update 2016;22:260-76.
43. Jadva V, Prosser H, Gamble N. Cross-border and domestic surrogacy in the UK context: an exploration of practical and legal decision-making. Human Fertility 2018:1-12.
44. Palattiyil G, Blyth E, Sidhva D, Balakrishnan G. Globalization and cross-border reproductive services: Ethical implications of surrogacy in India for social work. International Social Work - INT SOC WORK 2010;53:686-700.
45. Jadva V, Blake L, Casey P, Golombok S. Surrogacy families 10 years on: Relationship with the surrogate, decisions over disclosure and children's understanding of their surrogacy origins. Human reproduction (Oxford, England) 2012;27:3008-14.
46. Jadva V, Murray C, Lycett E, MacCallum F, Golombok S. Surrogacy: the experiences of surrogate mothers. Human reproduction (Oxford, England) 2003;18:2196-204.
47. Eraslan S, Hamernik RJ, Hardy JD. Replantation of uterus and ovaries in dogs, with successful pregnancy. Archives of surgery (Chicago, Ill : 1960) 1966;92:9-12.
48. Smith JR, Boyle DC, Corless DJ, et al. Abdominal radical trachelectomy: a new surgical technique for the conservative management of cervical carcinoma. British journal of obstetrics and gynaecology 1997;104:1196-200.
49. Kim CH, Abu-Rustum NR, Chi DS, et al. Reproductive outcomes of patients undergoing radical trachelectomy for early-stage cervical cancer. Gynecologic oncology 2012;125:585-8.
50. Ebisawa K, Takano M, Fukuda M, et al. Obstetric outcomes of patients undergoing total laparoscopic radical trachelectomy for early stage cervical cancer. Gynecologic oncology 2013;131:83-6.
51. Brannstrom M, Wranning CA, Altchek A. Experimental uterus transplantation. Human reproduction update 2010;16:329-45.
52. Fageeh W, Raffa H, Jabbad H, Marzouki A. Transplantation of the human uterus. Int J Gynaecol Obstet 2002;76:245-51.

53. Ozkan O, Akar ME, Ozkan O, et al. Preliminary results of the first human uterus transplantation from a multiorgan donor. Fertil Steril 2013;99:470-6.
54. Erman Akar M, Ozkan O, Aydinuraz B, et al. Clinical pregnancy after uterus transplantation. Fertil Steril 2013;100:1358-63.
55. Brännström M, Johannesson L, Bokström H, et al. Livebirth after uterus transplantation. The Lancet 2015;385:607-16.
56. Brannstrom M, Johannesson L, Dahm-Kahler P, et al. First clinical uterus transplantation trial: a six-month report. Fertil Steril 2014;101:1228-36.
57. Brannstrom M, Dahm Kahler P, Greite R, Molne J, Diaz-Garcia C, Tullius SG. Uterus Transplantation: A Rapidly Expanding Field. Transplantation 2018;102:569-77.
58. Ejzenberg D, Andraus W, Baratelli Carelli Mendes LR, et al. Livebirth after uterus transplantation from a deceased donor in a recipient with uterine infertility. The Lancet 2018.
59. Jones BP, Saso S, Bracewell-Milnes T, et al. Human uterine transplantation: a review of outcomes from the first 45 cases. BJOG: An International Journal of Obstetrics & Gynaecology 2019;126:1310-9.
60. Johannesson L, Wall A, Putman JM, Zhang L, Testa G, Diaz-Garcia C. Rethinking the time interval to embryo transfer after uterus transplantation – DUETS (Dallas UtErus Transplant Study). BJOG: An International Journal of Obstetrics & Gynaecology 2019;126:1305-9.
61. London NJ, Farmery SM, Will EJ, Davison AM, Lodge JP. Risk of neoplasia in renal transplant patients. Lancet (London, England) 1995;346:403-6.
62. Kasiske BL, Snyder JJ, Gilbertson DT, Wang C. Cancer after kidney transplantation in the United States. Am J Transplant 2004;4:905-13.
63. Catsanos R, Rogers W, Lotz M. The Ethics Of Uterus Transplantation. Bioethics 2013;27.
64. Arora KS, Blake V. Uterus transplantation: ethical and regulatory challenges. Journal of Medical Ethics 2014;40:396-400.
65. Daar J, Klipstein S. Refocusing the ethical choices in womb transplantation. Journal of Law and the Biosciences 2016;3:383-8.
66. Robertson JA. Impact of uterus transplant on fetuses and resulting children: a response to Daar and Klipstein. Journal of Law and the Biosciences 2016;3:710-7.
67. McTernan E. Uterus transplants and the insufficient value of gestation. Bioethics 2018;32:481-8.
68. Alghrani A. Uterus Transplantation in and beyond cisgender women: revisiting procreative liberty in light of emerging reproductive technologies. Journal of Law and the Biosciences 2018;5:301-28.
69. Guntram L, Williams NJ. Positioning uterus transplantation as a 'more ethical' alternative to surrogacy: Exploring symmetries between uterus transplantation and surrogacy through analysis of a Swedish government white paper. Bioethics 2018;32:509-18.
70. Robertson JA. Other women's wombs: uterus transplants and gestational surrogacy. Journal of Law and the Biosciences 2016;3:68-86.
71. Lotz M. Uterus transplantation as radical reproduction: Taking the adoption alternative more seriously. Bioethics 2018;32:499-508.
72. Petropanagos A. PRONATALISM, GENETICISM, AND ART. International Journal of Feminist Approaches to Bioethics 2017;10:119-47.
73. Wilkinson S, Williams NJ. Should uterus transplants be publicly funded? J Med Ethics 2016;42:559-65.
74. Sandman L. The importance of being pregnant: On the healthcare need for uterus transplantation. Bioethics 2018;32:519-26.
75. Blake VK. Financing uterus transplants: The United States context. Bioethics 2018;32:527-33.
76. Hashmi AA, Naz S, Edhi MM, et al. Accuracy of intraoperative frozen section for the evaluation of ovarian neoplasms: an institutional experience. World J Surg Oncol 2016;14:91-.
77. Sukumaran R, Somanathan T, Mathews A, Kattor J, Sambasivan S, Nair RP. Role of frozen section in intraoperative assessment of ovarian masses: a tertiary oncology center experience. Indian J Surg Oncol 2014;5:99-103.
78. Brucker SY, Brannstrom M, Taran FA, et al. Selecting living donors for uterus transplantation: lessons learned from two transplantations resulting in menstrual functionality and another attempt, aborted after organ retrieval. Arch Gynecol Obstet 2018;297:675-84.
79. Bessede T, Droupy S, Hammoudi Y, et al. Surgical prevention and management of vascular complications of kidney transplantation. Transplant international : official journal of the European Society for Organ Transplantation 2012;25:994-1001.
80. Testa G, Koon EC, Johannesson L, et al. Living Donor Uterus Transplantation: A Single Center's Observations and Lessons Learned From Early Setbacks to Technical Success. Am J Transplant 2017;17:2901-10.

81. Johannesson L, Enskog A, Dahm-Kahler P, et al. Uterus transplantation in a non-human primate: long-term follow-up after autologous transplantation. Human reproduction (Oxford, England) 2012;27:1640-8.
82. Sieunarine K, Hakim NS, Corless DJ, et al. Is it feasible to use a large vessel patch with a uterine allograft en bloc for uterine transplantation? International surgery 2005;90:257-61.
83. Clancy NT, Saso S, Stoyanov D, et al. Multispectral imaging of organ viability during uterine transplantation surgery in rabbits and sheep. J Biomed Opt 2016;21:106006.
84. Saso S, Petts G, David AL, et al. Achieving an early pregnancy following allogeneic uterine transplantation in a rabbit model. Eur J Obstet Gynecol Reprod Biol 2015;185:164-9.
85. Racho El-Akouri R, Kurlberg G, Brannstrom M. Successful uterine transplantation in the mouse: pregnancy and post-natal development of offspring. Human reproduction (Oxford, England) 2003;18:2018-23.
86. Mihara M, Kisu I, Hara H, et al. Uterine autotransplantation in cynomolgus macaques: the first case of pregnancy and delivery. Human reproduction (Oxford, England) 2012;27:2332-40.
87. Ramirez ER, Ramirez Nessetti DK, Nessetti MB, et al. Pregnancy and outcome of uterine allotransplantation and assisted reproduction in sheep. J Minim Invasive Gynecol 2011;18:238-45.
88. Patton DL, Thwin SS, Meier A, Hooton TM, Stapleton AE, Eschenbach DA. Epithelial cell layer thickness and immune cell populations in the normal human vagina at different stages of the menstrual cycle. Am J Obstet Gynecol 2000;183:967-73.
89. Blaskewicz CD, Pudney J, Anderson DJ. Structure and function of intercellular junctions in human cervical and vaginal mucosal epithelia. Biol Reprod 2011;85:97-104.
90. Yu L, Wang L, Chen S. Toll-like receptors, inflammation and tumor in the human female reproductive tract. American journal of reproductive immunology (New York, NY : 1989) 2009;62:1-8.
91. Yarbrough VL, Winkle S, Herbst-Kralovetz MM. Antimicrobial peptides in the female reproductive tract: a critical component of the mucosal immune barrier with physiological and clinical implications. Human reproduction update 2015;21:353-77.
92. Anderson DJ, Marathe J, Pudney J. The structure of the human vaginal stratum corneum and its role in immune defense. American journal of reproductive immunology (New York, NY : 1989) 2014;71:618-23.
93. Lee SK, Kim CJ, Kim D-J, Kang J-H. Immune cells in the female reproductive tract. Immune Netw 2015;15:16-26.
94. Ming L, Xiaoling P, Yan L, et al. Purification of antimicrobial factors from human cervical mucus. Human reproduction (Oxford, England) 2007;22:1810-5.
95. Gipson IK, Ho SB, Spurr-Michaud SJ, et al. Mucin genes expressed by human female reproductive tract epithelia. Biol Reprod 1997;56:999-1011.
96. Ravel J, Gajer P, Abdo Z, et al. Vaginal microbiome of reproductive-age women. Proceedings of the National Academy of Sciences of the United States of America 2011;108 Suppl 1:4680-7.
97. Alakomi HL, Skytta E, Saarela M, Mattila-Sandholm T, Latva-Kala K, Helander IM. Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. Applied and environmental microbiology 2000;66:2001-5.
98. Lamont RF, Sobel JD, Akins RA, et al. The vaginal microbiome: new information about genital tract flora using molecular based techniques. BJOG : an international journal of obstetrics and gynaecology 2011;118:533-49.
99. Caillouette JC, Sharp CF, Jr., Zimmerman GJ, Roy S. Vaginal pH as a marker for bacterial pathogens and menopausal status. Am J Obstet Gynecol 1997;176:1270-5; discussion 5-7.
100. Weyers S, Verstraelen H, Gerris J, et al. Microflora of the penile skin-lined neovagina of transsexual women. BMC microbiology 2009;9:102.
101. Petricevic L, Kaufmann U, Domig KJ, et al. Molecular detection of Lactobacillus species in the neovagina of male-to-female transsexual women. Scientific reports 2014;4:3746-.
102. Toolenaar TA, Freundt I, Wagenvoort JH, et al. Bacterial flora of the sigmoid neovagina. Journal of clinical microbiology 1993;31:3314-6.
103. Bracewell-Milnes T, Saso S, Nikolaou D, Norman-Taylor J, Johnson M, Thum MY. Investigating the effect of an abnormal cervico-vaginal and endometrial microbiome on assisted reproductive technologies: A systematic review. American journal of reproductive immunology (New York, NY : 1989) 2018;80:e13037.
104. Haahr T, Zacho J, Brauner M, Shathmigha K, Skov Jensen J, Humaidan P. Reproductive outcome of patients undergoing in vitro fertilisation treatment and diagnosed with bacterial vaginosis or abnormal vaginal microbiota: a systematic PRISMA review and meta-analysis. Bjog 2019;126:200-7.
105. Haahr T, Jensen JS, Thomsen L, Duus L, Rygaard K, Humaidan P. Abnormal vaginal microbiota may be associated with poor reproductive outcomes: a prospective study in IVF patients. Human reproduction (Oxford, England) 2016;31:795-803.

106. Moreno I, Codoner FM, Vilella F, et al. Evidence that the endometrial microbiota has an effect on implantation success or failure. Am J Obstet Gynecol 2016;215:684-703.
107. Hay PE, Lamont RF, Taylor-Robinson D, Morgan DJ, Ison C, Pearson J. Abnormal bacterial colonisation of the genital tract and subsequent preterm delivery and late miscarriage. BMJ (Clinical research ed) 1994;308:295-8.
108. Donders GG, Van Bulck B, Caudron J, Londers L, Vereecken A, Spitz B. Relationship of bacterial vaginosis and mycoplasmas to the risk of spontaneous abortion. Am J Obstet Gynecol 2000;183:431-7.
109. Hillier SL, Nugent RP, Eschenbach DA, et al. Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. The Vaginal Infections and Prematurity Study Group. The New England journal of medicine 1995;333:1737-42.
110. Tabatabaei N, Eren AM, Barreiro LB, et al. Vaginal microbiome in early pregnancy and subsequent risk of spontaneous preterm birth: a case-control study. Bjog 2019;126:349-58.
111. Brown RG, Al-Memar M, Marchesi JR, et al. Establishment of vaginal microbiota composition in early pregnancy and its association with subsequent preterm prelabor rupture of the fetal membranes. Translational research : the journal of laboratory and clinical medicine 2018.
112. DiGiulio DB, Callahan BJ, McMurdie PJ, et al. Temporal and spatial variation of the human microbiota during pregnancy. Proceedings of the National Academy of Sciences of the United States of America 2015;112:11060-5.
113. Yang B, Wang N, Zhang S, Wang M. Vaginal reconstruction with sigmoid colon in patients with congenital absence of vagina and menses retention: a report of treatment experience in 22 young women. International urogynecology journal 2013;24:155-60.
114. Kisku S, Varghese L, Kekre A, et al. Bowel vaginoplasty in children and young women: an institutional experience with 55 patients. International urogynecology journal 2015;26:1441-8.
115. Sato Y, Murakami T, Kadowaki M, Konno R, Yoshida S, Okamura K. A remnant tubal pregnancy after cloacal malformation repair. Fertil Steril 2001;75:440-1.
116. Champer M, Wong AM, Champer J, et al. The role of the vaginal microbiome in gynaecological cancer. Bjog 2018;125:309-15.
117. Lee JE, Lee S, Lee H, et al. Association of the vaginal microbiota with human papillomavirus infection in a Korean twin cohort. PLoS One 2013;8:e63514.
118. Mitra A, MacIntyre DA, Marchesi JR, Lee YS, Bennett PR, Kyrgiou M. The vaginal microbiota, human papillomavirus infection and cervical intraepithelial neoplasia: what do we know and where are we going next? Microbiome 2016;4:58.
119. Wiik J, Sengpiel V, Kyrgiou M, et al. Cervical microbiota in women with cervical intra-epithelial neoplasia, prior to and after local excisional treatment, a Norwegian cohort study. BMC women's health 2019;19:30.
120. Brusselaers N, Shrestha S, van de Wijgert J, Verstraelen H. Vaginal dysbiosis and the risk of human papillomavirus and cervical cancer: systematic review and meta-analysis. Am J Obstet Gynecol 2018.
121. Engels EA, Pfeiffer RM, Fraumeni JF, Jr., et al. Spectrum of cancer risk among US solid organ transplant recipients. Jama 2011;306:1891-901.
122. Madeleine MM, Finch JL, Lynch CF, Goodman MT, Engels EA. HPV-related cancers after solid organ transplantation in the United States. Am J Transplant 2013;13:3202-9.
123. Mölne J, Broecker V, Ekberg J, Nilsson O, Dahm-Kähler P, Brännström M. Monitoring of Human Uterus Transplantation With Cervical Biopsies: A Provisional Scoring System for Rejection. Am J Transplant 2017;17:1628-36.
124. Farren J, Jalmbrant M, Falconieri N, et al. Posttraumatic stress, anxiety and depression following miscarriage and ectopic pregnancy: a multicenter, prospective, cohort study. American Journal of Obstetrics & Gynecology.
125. Blume C, Pischke S, von Versen-Hoynck F, Gunter HH, Gross MM. Pregnancies in liver and kidney transplant recipients: a review of the current literature and recommendation. Best Pract Res Clin Obstet Gynaecol 2014;28:1123-36.
126. Brannstrom M, Enskog A, Kvarnstrom N, Ayoubi JM, Dahm-Kahler P. Global results of human uterus transplantation and strategies for pre-transplantation screening of donors. Fertil Steril 2019;112:3-10.
127. Brännström M. 1st Congress of International Society of Uterine Transplantation (ISUTx). 2017.
128. Azizi R, Aghebati-Maleki L, Nouri M, Marofi F, Negargar S, Yousefi M. Stem cell therapy in Asherman syndrome and thin endometrium: Stem cell- based therapy. Biomed Pharmacother 2018;102:333-43.

129. von Wolff M, Rohner S, Santi A, Stute P, Popovici R, Weiss B. Modified natural cycle in vitro fertilization an alternative in vitro fertilization treatment with lower costs per achieved pregnancy but longer treatment time. J Reprod Med 2014;59:553-9.
130. Sunkara SK, LaMarca A, Polyzos NP, Seed PT, Khalaf Y. Live birth and perinatal outcomes following stimulated and unstimulated IVF: analysis of over two decades of a nationwide data. Human Reproduction 2016;31:2261-7.
131. Johannesson L, Enskog A, Molne J, et al. Preclinical report on allogeneic uterus transplantation in non-human primates. Human reproduction (Oxford, England) 2013;28:189-98.
132. Kvarnström N, Järvholm S, Johannesson L, Dahm-Kähler P, Olausson M, Brännström M. Live donors of the initial observational study of uterus transplantation-Psychological and medical follow up until 1 year after surgery in the 9 cases. Transplantation 2016.
133. Jarvholm S, Johannesson L, Clarke A, Brannstrom M. Uterus transplantation trial: Psychological evaluation of recipients and partners during the post-transplantation year. Fertil Steril 2015;104:1010-5.
134. Wei L, Xue T, Tao KS, et al. Modified human uterus transplantation using ovarian veins for venous drainage: the first report of surgically successful robotic-assisted uterus procurement and follow-up for 12 months. Fertil Steril 2017;108:346-56 e1.
135. Flyckt RL, Farrell RM, Perni UC, Tzakis AG, Falcone T. Deceased Donor Uterine Transplantation: Innovation and Adaptation. Obstetrics and gynecology 2016;128:837-42.
136. Flyckt R, Kotlyar A, Arian S, Eghtesad B, Falcone T, Tzakis A. Deceased donor uterine transplantation. Fertil Steril 2017;107:e13.
137. Schulze J, Sonnenborn U. Yeasts in the gut: from commensals to infectious agents. Dtsch Arztebl Int 2009;106:837-42.
138. Silveira FP, Kusne S. Candida infections in solid organ transplantation. Am J Transplant 2013;13 Suppl 4:220-7.
139. Pappas PG, Alexander BD, Andes DR, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2010;50:1101-11.
140. Kusne S, Furukawa H, Abu-Elmagd K, et al. Infectious complications after small bowel transplantation in adults: an update. Transplantation proceedings 1996;28:2761-2.
141. Vincent JL, Rello J, Marshall J, et al. International study of the prevalence and outcomes of infection in intensive care units. Jama 2009;302:2323-9.
142. Seelig MS. Mechanisms by which antibiotics increase the incidence and severity of candidiasis and alter the immunological defenses. Bacteriol Rev 1966;30:442-59.
143. Safety of Blood TaO. Microbiological Safety Guidelines. London.2017.
144. Renoux C, Dell'Aniello S, Suissa S. Hormone replacement therapy and the risk of venous thromboembolism: a population-based study. Journal of thrombosis and haemostasis : JTH 2010;8:979-86.
145. Daly E, Vessey MP, Hawkins MM, Carson JL, Gough P, Marsh S. Risk of venous thromboembolism in users of hormone replacement therapy. Lancet (London, England) 1996;348:977-80.
146. Anderson FA, Jr., Spencer FA. Risk factors for venous thromboembolism. Circulation 2003;107:I9-16.
147. Sweetland S, Green J, Liu B, et al. Duration and magnitude of the postoperative risk of venous thromboembolism in middle aged women: prospective cohort study. BMJ (Clinical research ed) 2009;339:b4583.
148. Fedele L, Bianchi S, Dorta M, Brioschi D, Zanotti F, Vercellini P. Transvaginal ultrasonography versus hysteroscopy in the diagnosis of uterine submucous myomas. Obstetrics and gynecology 1991;77:745-8.
149. Dewan K, Hefeda M, Elkholy D. Septate or bicornuate uterus: Accuracy of three-dimensional trans-vaginal ultrasonography and pelvic magnetic resonance imaging. The Egyptian Journal of Radiology and Nuclear Medicine 2014;45.
150. Fronek J, Janousek L, Chmel R. Deceased donor uterus retrieval - The first Czech experience. Rozhledy v chirurgii : mesicnik Ceskoslovenske chirurgicke spolecnosti 2016;95:312-6.
151. Li X, Li J, Wu X. Incidence, risk factors and treatment of cervical stenosis after radical trachelectomy: A systematic review. Eur J Cancer 2015;51:1751-9.
152. Johannesson L, Wallis K, Koon EC, et al. Living uterus donation and transplantation: experience of interest and screening in a single center in the United States. Am J Obstet Gynecol 2018;218:331 e1- e7.
153. Jones BP, Serhal P, Ben-Nagi J. Social egg freezing should be offered to single women approaching their late thirties: FOR: Women should not suffer involuntary childlessness because they have not yet found a partner. Bjog 2018.
154. Testa G, McKenna GJ, Gunby RT, Jr., et al. First live birth after uterus transplantation in the United States. Am J Transplant 2018;18:1270-4.

155. Willows K, Lennox G, Covens A. Fertility-sparing management in cervical cancer: balancing oncologic outcomes with reproductive success. Gynecologic oncology research and practice 2016;3:9.
156. Sieunarine K, Moxey P, Boyle DC, et al. Selective vessel ligation in the pelvis: an invaluable tool in certain surgical procedures. International journal of gynecological cancer : official journal of the International Gynecological Cancer Society 2005;15:967-73.
157. Jeve YB, Potdar N, Opoku A, Khare M. Donor oocyte conception and pregnancy complications: a systematic review and meta-analysis. BJOG: An International Journal of Obstetrics & Gynaecology 2016;123:1471-80.
158. Margolis M, Austin J, Wu L, et al. Effects of Social Support Source and Effectiveness on Stress Buffering After Stem Cell Transplant. International journal of behavioral medicine 2019.
159. Meier-Kriesche HU, Cibrik DM, Ojo AO, et al. Interaction between donor and recipient age in determining the risk of chronic renal allograft failure. Journal of the American Geriatrics Society 2002;50:14-7.
160. Nagji AS, Hranjec T, Swenson BR, et al. Donor age is associated with chronic allograft vasculopathy after adult heart transplantation: implications for donor allocation. The Annals of thoracic surgery 2010;90:168-75.
161. Keith DS, Demattos A, Golconda M, Prather J, Norman D. Effect of donor recipient age match on survival after first deceased donor renal transplantation. Journal of the American Society of Nephrology : JASN 2004;15:1086-91.
162. Hendriks EJ, Beulens JW, de Jong PA, et al. Calcification of the splenic, iliac, and breast arteries and risk of all-cause and cardiovascular mortality. Atherosclerosis 2017;259:120-7.
163. Vaitkevicius PV, Fleg JL, Engel JH, et al. Effects of age and aerobic capacity on arterial stiffness in healthy adults. Circulation 1993;88:1456-62.
164. Alexander JW, Vaughn WK. The use of "marginal" donors for organ transplantation. The influence of donor age on outcome. Transplantation 1991;51:135-41.
165. Soares SR, Troncoso C, Bosch E, et al. Age and uterine receptiveness: predicting the outcome of oocyte donation cycles. The Journal of clinical endocrinology and metabolism 2005;90:4399-404.
166. Chmel R, Novackova M, Janousek L, et al. Revaluation and lessons learned from the first nine cases of a Czech uterus transplantation trial: four deceased donor and five living donor uterus transplantations. Am J Transplant 2018;19:855-64.
167. Testa G, Koon EC, Johannesson L, et al. Living Donor Uterus Transplantation: A Single Center's Observations and Lessons Learned from Early Setbacks to Technical Success. Am J Transplant 2017.
168. Papanikolaou EG, Camus M, Kolibianakis EM, Van Landuyt L, Van Steirteghem A, Devroey P. In vitro fertilization with single blastocyst-stage versus single cleavage-stage embryos. The New England journal of medicine 2006;354:1139-46.
169. Fernandez-Shaw S, Cercas R, Brana C, Villas C, Pons I. Ongoing and cumulative pregnancy rate after cleavage-stage versus blastocyst-stage embryo transfer using vitrification for cryopreservation: impact of age on the results. Journal of assisted reproduction and genetics 2015;32:177-84.
170. Kashani BN, Holden EC, Morelli S, Alderson D, Jindal SK, McGovern PG. Transferring frozen-thawed embryos at the blastocyst stage significantly improves pregnancy outcomes compared to cleavage stage: a sartcors analysis of over 250,000 FET cycles over a decade. Fertility and Sterility 2017;108:e165.
171. Maheshwari A, Hamilton M, Bhattacharya S. Should we be promoting embryo transfer at blastocyst stage? Reprod Biomed Online 2016;32:142-6.
172. Scott KL, Hong KH, Scott RT, Jr. Selecting the optimal time to perform biopsy for preimplantation genetic testing. Fertil Steril 2013;100:608-14.
173. Levy B, Sigurjonsson S, Pettersen B, et al. Genomic imbalance in products of conception: single-nucleotide polymorphism chromosomal microarray analysis. Obstetrics and gynecology 2014;124:202-9.
174. Forman EJ, Tao X, Ferry KM, Taylor D, Treff NR, Scott RT, Jr. Single embryo transfer with comprehensive chromosome screening results in improved ongoing pregnancy rates and decreased miscarriage rates. Human reproduction (Oxford, England) 2012;27:1217-22.
175. Dahdouh EM, Balayla J, Garcia-Velasco JA. Comprehensive chromosome screening improves embryo selection: a meta-analysis. Fertil Steril 2015;104:1503-12.
176. Lee E, Illingworth P, Wilton L, Chambers GM. The clinical effectiveness of preimplantation genetic diagnosis for aneuploidy in all 24 chromosomes (PGD-A): systematic review. Human reproduction (Oxford, England) 2015;30:473-83.
177. Forman EJ, Hong KH, Ferry KM, et al. In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial. Fertil Steril 2013;100:100-7.e1.
178. Conde-Agudelo A, Belizan JM, Lindmark G. Maternal morbidity and mortality associated with multiple gestations. Obstetrics and gynecology 2000;95:899-904.

179. Helmerhorst FM, Perquin DA, Donker D, Keirse MJ. Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. BMJ (Clinical research ed) 2004;328:261.
180. Kamath MS, Antonisamy B, Sunkara SK. Zygotic splitting following embryo biopsy: a cohort study of 207 697 single-embryo transfers following IVF treatment. BJOG: An International Journal of Obstetrics & Gynaecology 2020;127:562-9.
181. Rienzi L, Gracia C, Maggiulli R, et al. Oocyte, embryo and blastocyst cryopreservation in ART: systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance. Human reproduction update 2017;23:139-55.
182. Brucker S. State of the Art (SOTA) Meeting of International Society of Uterine Transplantation. Ghent2018.
183. Bjelland EK, Wilkosz P, Tanbo TG, Eskild A. Is unilateral oophorectomy associated with age at menopause? A population study (the HUNT2 Survey). Human Reproduction 2014;29:835-41.
184. Puntambekar S, Telang M, Kulkarni P, et al. Laparoscopic-Assisted Uterus Retrieval From Live Organ Donors for Uterine Transplant: Our Experience of Two Patients. J Minim Invasive Gynecol 2018;25:622-31.
185. Puntambekar S, Puntambekar S, Telang M, et al. A Novel Anastomotic Technique for Uterine Transplant Using Utero-ovarian Veins for Venous Drainage and Internal Iliac Arteries for Perfusion in Two Laparoscopically Harvested Uteri. J Minim Invasive Gynecol 2019;26:628-35.
186. Donnez J, Dolmans MM, Demylle D, et al. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet (London, England) 2004;364:1405-10.
187. Donnez J, Dolmans MM. Fertility Preservation in Women. The New England journal of medicine 2017;377:1657-65.
188. Donnez J, Dolmans M-M, Diaz C, Pellicer A. Ovarian cortex transplantation: time to move on from experimental studies to open clinical application. Fertility and Sterility 2015;104:1097-8.
189. Donnez J, Dolmans MM. Ovarian cortex transplantation: 60 reported live births brings the success and worldwide expansion of the technique towards routine clinical practice. Journal of assisted reproduction and genetics 2015;32:1167-70.
190. Herlin M, Bjorn AM, Rasmussen M, Trolle B, Petersen MB. Prevalence and patient characteristics of Mayer-Rokitansky-Kuster-Hauser syndrome: a nationwide registry-based study. Human reproduction (Oxford, England) 2016;31:2384-90.
191. Timmreck LS, Reindollar RH. Contemporary issues in primary amenorrhea. Obstetrics and gynecology clinics of North America 2003;30:287-302.
192. Petrozza JC, Gray MR, Davis AJ, Reindollar RH. Congenital absence of the uterus and vagina is not commonly transmitted as a dominant genetic trait: outcomes of surrogate pregnancies. Fertil Steril 1997;67:387-9.
193. Friedler S, Grin L, Liberti G, Saar-Ryss B, Rabinson Y, Meltzer S. The reproductive potential of patients with Mayer-Rokitansky-Kuster-Hauser syndrome using gestational surrogacy: a systematic review. Reprod Biomed Online 2016;32:54-61.
194. Fontana L, Gentilin B, Fedele L, Gervasini C, Miozzo M. Genetics of Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome. Clin Genet 2017;91:233-46.
195. Ma X, Yao B, Pan Q, Xu W, Xu K, Ma F. Familial occurrence of Mayer-Rokitansky-Kuster-Hauser syndrome. Journal of obstetrics and gynaecology : the journal of the Institute of Obstetrics and Gynaecology 2016;36:817-8.
196. Herlin M, Hojland AT, Petersen MB. Familial occurrence of Mayer-Rokitansky-Kuster-Hauser syndrome: a case report and review of the literature. American journal of medical genetics Part A 2014;164a:2276-86.
197. Takahashi K, Hayano T, Sugimoto R, et al. Exome and copy number variation analyses of Mayer-Rokitansky-Kuster- Hauser syndrome. Human genome variation 2018;5:27.
198. Ledig S, Wieacker P. Clinical and genetic aspects of Mayer-Rokitansky-Kuster-Hauser syndrome. Medizinische Genetik : Mitteilungsblatt des Berufsverbandes Medizinische Genetik eV 2018;30:3-11.
199. Williams LS, Demir Eksi D, Shen Y, et al. Genetic analysis of Mayer-Rokitansky-Kuster-Hauser syndrome in a large cohort of families. Fertility and sterility 2017;108:145-51.e2.
200. Al-Inany H. Intrauterine adhesions. An update. Acta Obstet Gynecol Scand 2001;80:986-93.
201. Deans R, Abbott J. Review of intrauterine adhesions. J Minim Invasive Gynecol 2010;17:555-69.
202. Singh N, Mohanty S, Seth T, Shankar M, Bhaskaran S, Dharmendra S. Autologous stem cell transplantation in refractory Asherman's syndrome: A novel cell based therapy. Journal of human reproductive sciences 2014;7:93-8.
203. Tan J, Li P, Wang Q, et al. Autologous menstrual blood-derived stromal cells transplantation for severe Asherman's syndrome. Human reproduction (Oxford, England) 2016;31:2723-9.

204. Santamaria X, Cabanillas S, Cervello I, et al. Autologous cell therapy with CD133+ bone marrow-derived stem cells for refractory Asherman's syndrome and endometrial atrophy: a pilot cohort study. Human reproduction (Oxford, England) 2016;31:1087-96.
205. Lavoue V, Vigneau C, Duros S, et al. Which Donor for Uterus Transplants: Brain-Dead Donor or Living Donor? A Systematic Review. Transplantation 2017;101:267-73.
206. Bruno B, Arora KS. Uterus Transplantation: The Ethics of Using Deceased Versus Living Donors. The American journal of bioethics : AJOB 2018;18:6-15.
207. Williams N. Should Deceased Donation be Morally Preferred in Uterine Transplantation Trials? Bioethics 2016;30:415-24.
208. Dion L, Tardieu A, Garbin O, et al. Should brain-dead or living donors be used for uterus transplantation? A statement by the CNGOF French Uterus Transplantation Committee (CETUF). J Gynecol Obstet Hum Reprod 2018.
209. Fujioka H, Kato T, Sone M, Horita T, Sugawara S, Tsukagoshi S. Study of Preoperative 3D-CT Angiography of Uterine Artery in Patients with Uterine Cervical Cancer. Nihon Hoshasen Gijutsu Gakkai zasshi 2017;73:112-9.
210. Testa G, Anthony T, McKenna GJ, et al. Deceased donor uterus retrieval: A novel technique and workflow. Am J Transplant 2018;18:679-83.
211. Totsuka E, Fung JJ, Hakamada K, et al. Synergistic effect of cold and warm ischemia time on postoperative graft function and outcome in human liver transplantation. Transplant Proc 2004;36:1955-8.
212. Schwarz A, Mengel M, Gwinner W, et al. Risk factors for chronic allograft nephropathy after renal transplantation: a protocol biopsy study. Kidney international 2005;67:341-8.
213. Gauthier T, Piver P, Pichon N, et al. Uterus retrieval process from brain dead donors. Fertil Steril 2014;102:476-82.
214. Tricard J, Ponsonnard S, Tholance Y, et al. Uterus tolerance to extended cold ischemic storage after auto-transplantation in ewes. Eur J Obstet Gynecol Reprod Biol 2017;214:162-7.
215. Wranning CA, Molne J, El-Akouri RR, Kurlberg G, Brannstrom M. Short-term ischaemic storage of human uterine myometrium--basic studies towards uterine transplantation. Human reproduction (Oxford, England) 2005;20:2736-44.
216. Ramani A, Testa G, Ghouri Y, et al. DUETS (Dallas UtErus Transplant Study): Complete report of 6-month and initial 2-year outcomes following open donor hysterectomy. Clin Transplant 2019:e13757.
217. Matter YE, Nagib AM, Lotfy OE, et al. Impact of Donor Source on the Outcome of Live Donor Kidney Transplantation: A Single Center Experience. Nephro-urology monthly 2016;8:e34770.
218. Terasaki PI, Cecka JM, Gjertson DW, Takemoto S. High survival rates of kidney transplants from spousal and living unrelated donors. The New England journal of medicine 1995;333:333-6.
219. Roodnat JI, van Riemsdijk IC, Mulder PG, et al. The superior results of living-donor renal transplantation are not completely caused by selection or short cold ischemia time: a single-center, multivariate analysis. Transplantation 2003;75:2014-8.
220. Pratschke J, Neuhaus P, Tullius SG. What can be learned from brain-death models? Transplant international : official journal of the European Society for Organ Transplantation 2005;18:15-21.
221. Brannstrom M, Dahm-Kahler P, Kvarnstrom N. Robotic-assisted surgery in live-donor uterus transplantation. Fertil Steril 2018;109:256-7.
222. Dahm Kahler P. Place of minimal invasive surgery in uterus transplantation. State of the Art (SOTA) Meeting of International Society of Uterine Transplantation Ghent2018.
223. Kim JY, Khavanin N, Rambachan A, et al. Surgical duration and risk of venous thromboembolism. JAMA Surg 2015;150:110-7.
224. Donohoe JF, Venkatachalam MA, Bernard DB, Levinsky NG. Tubular leakage and obstruction after renal ischemia: structural-functional correlations. Kidney international 1978;13:208-22.
225. Mathur AK, Heimbach J, Steffick DE, Sonnenday CJ, Goodrich NP, Merion RM. Donation after cardiac death liver transplantation: predictors of outcome. Am J Transplant 2010;10:2512-9.
226. Kisu I, Umene K, Adachi M, et al. Allowable warm ischemic time and morphological and biochemical changes in uterine ischemia/reperfusion injury in cynomolgus macaque: a basic study for uterus transplantation. Human reproduction (Oxford, England) 2017;32:2026-35.
227. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Annals of surgery 2004;240:205-13.
228. Erman Akar M, Ozkan O, Ozkan O, et al. Short Term Follow Up Results of the First Human Uterus Transplantation from Cadaver. Journal of Minimally Invasive Gynecology 2012;19.

229. Haynes R, Harden P, Judge P, et al. Alemtuzumab-based induction treatment versus basiliximab-based induction treatment in kidney transplantation (the 3C Study): a randomised trial. Lancet (London, England) 2014;384:1684-90.
230. Hanaway MJ, Woodle ES, Mulgaonkar S, et al. Alemtuzumab induction in renal transplantation. The New England journal of medicine 2011;364:1909-19.
231. Diaz-Garcia C, Akhi SN, Wallin A, Pellicer A, Brannstrom M. First report on fertility after allogeneic uterus transplantation. Acta Obstet Gynecol Scand 2010;89:1491-4.
232. Perez-Aytes A, Marin-Reina P, Boso V, Ledo A, Carey JC, Vento M. Mycophenolate mofetil embryopathy: A newly recognized teratogenic syndrome. European journal of medical genetics 2017;60:16-21.
233. Fishman JA, Rubin RH. Infection in organ-transplant recipients. The New England journal of medicine 1998;338:1741-51.
234. Akouri R. The Lebanon Experience. State of the Art (SOTA) Meeting of International Society of Uterine Transplantation. Ghent2018.
235. Dantal J, Hourmant M, Cantarovich D, et al. Effect of long-term immunosuppression in kidney-graft recipients on cancer incidence: randomised comparison of two cyclosporin regimens. Lancet (London, England) 1998;351:623-8.
236. NHS Blood and Transplant (NHSBT). Organ Donation and Transplantation Activity Report 2018/2019. Watford: NHSBT 2019.
237. Murray JE, Reid DE, Harrison JH, Merrill JP. Successful pregnancies after human renal transplantation. The New England journal of medicine 1963;269:341-3.
238. McKay DB, Josephson MA. Pregnancy in recipients of solid organs--effects on mother and child. The New England journal of medicine 2006;354:1281-93.
239. Wielgos M, Pietrzak B, Bobrowska K, Bablok L, Kaminski P. Pregnancy after organ transplantation. Neuro Endocrinol Lett 2009;30:6-10.
240. Durst JK, Rampersad RM. Pregnancy in Women With Solid-Organ Transplants: A Review. Obstetrical & gynecological survey 2015;70:408-18.
241. Mohammadi FA, Borg M, Gulyani A, McDonald SP, Jesudason S. Pregnancy outcomes and impact of pregnancy on graft function in women after kidney transplantation. Clinical Transplantation 2017;31:e13089.
242. Brannstrom M, Bokstrom H, Dahm-Kahler P, et al. One uterus bridging three generations: first live birth after mother-to-daughter uterus transplantation. Fertil Steril 2016;106:261-6.
243. Castellon LAR, Amador MIG, Gonzalez RED, et al. The history behind successful uterine transplantation in humans. JBRA assisted reproduction 2017;21:126-34.
244. Akouri R. The Swedish uterus transplantation trial - past, present and future. British Transplantation Society (BTS) Annual Congress 2017. Harrogate2017.
245. Brännström M, Dahm-Kähler P, Kvarnström N, et al. Livebirth after robotic-assisted live donor uterus transplantation. Acta Obstetricia et Gynecologica Scandinavica 2020;99:1222-9.
246. Kristek J, Kudla M, Chlupac J, et al. Acute appendicitis in a patient after a uterus transplant: A case report. World J Clin Cases 2019;7:4270-6.
247. Flyckt R, Falcone T, Quintini C, et al. First birth from a deceased donor uterus in the United States: from severe graft rejection to successful cesarean delivery. American Journal of Obstetrics and Gynecology 2020;223:143-51.
248. Brucker SY, Taran FA, Rall K, et al. Experiences of a multistep process with medical and psychological interventions for patients with congenital uterine aplasia to achieve motherhood: the Gothenburg-Tubingen collaboration. Facts Views Vis Obgyn 2019;11:121-6.
249. Barzilai A, Paldi E, Gal D, Hampel N. Autotransplantation of the uterus and ovaries in dogs. Israel journal of medical sciences 1973;9:49-52.
250. Farag S, Frazzini Padilla P, Smith KA, Flyckt R, Sprague ML, Zimberg SE. Fallopian tube perfusion in ex-vivo and in-vivo laparoscopic hysterectomy specimens: potential application for uterine transplantation. Human reproduction (Oxford, England) 2018;33:2232-40.
251. Beran B, Arnolds K, Shockley M, et al. Livebirth and utero-placental insufficiency in Papio hamadryas baboons with uterus angiosome perfused by bilateral utero-ovarian microsurgical anastomoses alone. Human reproduction (Oxford, England) 2017;32:1819-26.
252. Brannstrom M. Womb transplants with live births: an update and the future. Expert Opin Biol Ther 2017;17:1105-12.
253. Merlob P, Stahl B, Klinger G. Tetrada of the possible mycophenolate mofetil embryopathy: a review. Reprod Toxicol 2009;28:105-8.

254. Kim M, Rostas S, Gabardi S. Mycophenolate fetal toxicity and risk evaluation and mitigation strategies. Am J Transplant 2013;13:1383-9.
255. Congenital Cytomegalovirus Infection: Update on Treatment. BJOG: An International Journal of Obstetrics & Gynaecology 2018;125:e1-e11.
256. Grotz W, Siebig S, Olschewski M, Strey CW, Peter K. Low-dose aspirin therapy is associated with improved allograft function and prolonged allograft survival after kidney transplantation. Transplantation 2004;77:1848-53.
257. CLASP: a randomised trial of low-dose aspirin for the prevention and treatment of pre-eclampsia among 9364 pregnant women. CLASP (Collaborative Low-dose Aspirin Study in Pregnancy) Collaborative Group. Lancet (London, England) 1994;343:619-29.
258. Roberge S, Nicolaides KH, Demers S, Villa P, Bujold E. Prevention of perinatal death and adverse perinatal outcome using low-dose aspirin: a meta-analysis. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 2013;41:491-9.
259. Davison JM, Bailey DJ. Pregnancy following renal transplantation. J Obstet Gynaecol Res 2003;29:227-33.
260. Deshpande NA, Coscia LA, Gomez-Lobo V, Moritz MJ, Armenti VT. Pregnancy after solid organ transplantation: a guide for obstetric management. Rev Obstet Gynecol 2013;6:116-25.
261. Christopher V, Al-Chalabi T, Richardson PD, et al. Pregnancy outcome after liver transplantation: a single-center experience of 71 pregnancies in 45 recipients. Liver Transpl 2006;12:1138-43.
262. Deshpande NA, James NT, Kucirka LM, et al. Pregnancy outcomes in kidney transplant recipients: a systematic review and meta-analysis. Am J Transplant 2011;11:2388-404.
263. Dagher O, Alami Laroussi N, Carrier M, et al. Pregnancy after heart transplantation: a well-thought-out decision? The Quebec provincial experience - a multi-centre cohort study. Transplant international : official journal of the European Society for Organ Transplantation 2018.
264. Mattila M, Kemppainen H, Isoniemi H, Polo-Kantola P. Pregnancy outcomes after liver transplantation in Finland. Acta Obstetricia et Gynecologica Scandinavica 2017;96:1106-11.
265. Johannesson L, Kvarnstrom N, Molne J, et al. Uterus transplantation trial: 1-year outcome. Fertil Steril 2015;103:199-204.
266. Deshpande NA, James NT, Kucirka LM, et al. Pregnancy outcomes of liver transplant recipients: a systematic review and meta-analysis. Liver Transpl 2012;18:621-9.
267. Bramham K, Parnell B, Nelson-Piercy C, Seed PT, Poston L, Chappell LC. Chronic hypertension and pregnancy outcomes: systematic review and meta-analysis. BMJ : British Medical Journal 2014;348:g2301.
268. Kallen B, Westgren M, Aberg A, Olausson PO. Pregnancy outcome after maternal organ transplantation in Sweden. Bjog 2005;112:904-9.
269. Brannstrom M, Dahm-Kahler P. State of the Art (SOTA) Meeting of International Society of Uterine Transplantation. Ghent2018.
270. Jackson RA, Gibson KA, Wu YW, Croughan MS. Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. Obstetrics and gynecology 2004;103:551-63.
271. Roque M, Haahr T, Geber S, Esteves SC, Humaidan P. Fresh versus elective frozen embryo transfer in IVF/ICSI cycles: a systematic review and meta-analysis of reproductive outcomes. Human reproduction update 2019;25:2-14.
272. Casele HL, Laifer SA. Association of pregnancy complications and choice of immunosuppressant in liver transplant patients. Transplantation 1998;65:581-3.
273. Balayla J, Edwards M, Lefkowitz A. Uterine artery as an arterial conduit for coronary artery bypass graft (CABG) surgery in women: A role for estrogen-receptor alpha (ER-alpha) in the prevention of post-CABG accelerated atherosclerosis and graft disease. Medical hypotheses 2013;80:162-6.
274. Heinonen PK. Gestational hypertension and preeclampsia associated with unilateral renal agenesis in women with uterine malformations. Eur J Obstet Gynecol Reprod Biol 2004;114:39-43.
275. Steele SEJ, Terry JE, Page LM, Girling JC. Pregnancy in women known to be living with a single kidney. Obstetric Medicine 2018;12:22-6.
276. Williams D, Davison J. Chronic kidney disease in pregnancy. BMJ (Clinical research ed) 2008;336:211-5.
277. de Mattos AM, Olyaei AJ, Bennett WM. Nephrotoxicity of immunosuppressive drugs: long-term consequences and challenges for the future. Am J Kidney Dis 2000;35:333-46.
278. Imbasciati E, Gregorini G, Cabiddu G, et al. Pregnancy in CKD stages 3 to 5: fetal and maternal outcomes. Am J Kidney Dis 2007;49:753-62.
279. Sibanda N, Briggs J, Davison J, Johnson R, Rudge C. Pregnancy After Organ Transplantation: A Report From the U.K. Transplant Pregnancy Registry. Transplantation 2007;83:1301-7.

280. Kampmann U, Madsen LR, Skajaa GO, Iversen DS, Moeller N, Ovesen P. Gestational diabetes: A clinical update. World J Diabetes 2015;6:1065-72.
281. Joss N, Staatz CE, Thomson AH, Jardine AG. Predictors of new onset diabetes after renal transplantation. Clin Transplant 2007;21:136-43.
282. Lammert F, Marschall HU, Glantz A, Matern S. Intrahepatic cholestasis of pregnancy: molecular pathogenesis, diagnosis and management. Journal of hepatology 2000;33:1012-21.
283. Day C, Hewins P, Sheikh L, Kilby M, McPake D, Lipkin G. Cholestasis in pregnancy associated with ciclosporin therapy in renal transplant recipients. Transplant international : official journal of the European Society for Organ Transplantation 2006;19:1026-9.
284. Jabiry-Zieniewicz Z, Szpotanska-Sikorska M, Pietrzak B, et al. Pregnancy outcomes among female recipients after liver transplantation: further experience. Transplant Proc 2011;43:3043-7.
285. Roman ID, Fernandez-Moreno MD, Fueyo JA, Roma MG, Coleman R. Cyclosporin A induced internalization of the bile salt export pump in isolated rat hepatocyte couplets. Toxicological sciences : an official journal of the Society of Toxicology 2003;71:276-81.
286. Shah N, Al-Khoury S, Afzali B, et al. Posttransplantation anemia in adult renal allograft recipients: prevalence and predictors. Transplantation 2006;81:1112-8.
287. Yabu JM, Winkelmayer WC. Posttransplantation Anemia: Mechanisms and Management. Clinical Journal of the American Society of Nephrology 2011;6:1794.
288. Winkelmayer WC, Kewalramani R, Rutstein M, Gabardi S, Vonvisger T, Chandraker A. Pharmacoepidemiology of Anemia in Kidney Transplant Recipients. Journal of the American Society of Nephrology 2004;15:1347.
289. Kozuki N, Lee AC, Katz J. Moderate to severe, but not mild, maternal anemia is associated with increased risk of small-for-gestational-age outcomes. J Nutr 2012;142:358-62.
290. Hoeltzenbein M, Weber-Schoendorfer C, Borisch C, Allignol A, Meister R, Schaefer C. Pregnancy outcome after paternal exposure to azathioprine/6-mercaptopurine. Reprod Toxicol 2012;34:364-9.
291. Wiles KS, Tillett AL, Harding KR. Solid organ transplantation in pregnancy. The Obstetrician & Gynaecologist 2016;18:189-97.
292. Kainz A, Harabacz I, Cowlrick IS, Gadgil SD, Hagiwara D. Review of the course and outcome of 100 pregnancies in 84 women treated with tacrolimus. Transplantation 2000;70:1718-21.
293. Nevers W, Pupco A, Koren G, Bozzo P. Safety of tacrolimus in pregnancy. Canadian Family Physician 2014;60:905-6.
294. Langagergaard V, Pedersen L, Gislum M, Norgard B, Sorensen HT. Birth outcome in women treated with azathioprine or mercaptopurine during pregnancy: A Danish nationwide cohort study. Aliment Pharmacol Ther 2007;25:73-81.
295. Akturk S, Celebi ZK, Erdogmus S, et al. Pregnancy After Kidney Transplantation: Outcomes, Tacrolimus Doses, and Trough Levels. Transplant Proc 2015;47:1442-4.
296. Rahamimov R, Ben-Haroush A, Wittenberg C, et al. Pregnancy in renal transplant recipients: long-term effect on patient and graft survival. A single-center experience. Transplantation 2006;81:660-4.
297. Shah S, Venkatesan RL, Gupta A, et al. Pregnancy outcomes in women with kidney transplant: Metaanalysis and systematic review. BMC Nephrol 2019;20:24.
298. Gielchinsky Y, Rojansky N, Fasouliotis SJ, Ezra Y. Placenta Accreta—Summary of 10 Years: A Survey of 310 Cases. Placenta 2002;23:210-4.
299. Fitzpatrick KE, Sellers S, Spark P, Kurinczuk JJ, Brocklehurst P, Knight M. Incidence and risk factors for placenta accreta/increta/percreta in the UK: a national case-control study. PloS one 2012;7:e52893-e.
300. Hansen AK, Wisborg K, Uldbjerg N, Henriksen TB. Risk of respiratory morbidity in term infants delivered by elective caesarean section: cohort study. BMJ (Clinical research ed) 2008;336:85-7.
301. Murray SR, Shenkin SD, McIntosh K, et al. Long term cognitive outcomes of early term (37-38 weeks) and late preterm (34-36 weeks) births: A systematic review. Wellcome Open Res 2017;2:101-.
302. Aliyu MH, Salihu HM, Keith LG, Ehiri JE, Islam MA, Jolly PE. High parity and fetal morbidity outcomes. Obstetrics and gynecology 2005;105:1045-51.
303. Al-Shaikh GK, Ibrahim GH, Fayed AA, Al-Mandeel H. Grand multiparity and the possible risk of adverse maternal and neonatal outcomes: a dilemma to be deciphered. BMC Pregnancy Childbirth 2017;17:310.
304. Fenton TR, Kim JH. A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants. BMC Pediatrics 2013;13:59.
305. Gardiner SJ, Begg EJ. Breastfeeding during tacrolimus therapy. Obstetrics and gynecology 2006;107:453-5.

306. Chusney GD BK, Nelson-Piercy C, et al. Tacrolimus monitoring during breastfeeding in neonates of transplant recipients. Ther Drug Monit 2011;33:476.
307. Zucker KJ, Lawrence AA, Kreukels BP. Gender Dysphoria in Adults. Annu Rev Clin Psychol 2016;12:217-47.
308. Coleman E, Bockting W, Botzer M, et al. Standards of Care for the Health of Transsexual, Transgender, and Gender-Nonconforming People, Version 7. International Journal of Transgenderism 2012;13:165-232.
309. Access to fertility services by transgender persons: an Ethics Committee opinion. Fertil Steril 2015;104:1111-5.
310. Alghrani A. Uterus transplantation: does procreative liberty encompass a right to gestate? J Law Biosci 2016;3:636-41.
311. Lefkowitz A, Edwards M, Balayla J. Ethical considerations in the era of the uterine transplant: an update of the Montreal Criteria for the Ethical Feasibility of Uterine Transplantation. Fertility and Sterility 2013;100.
312. Murphy TF. Assisted Gestation and Transgender Women. Bioethics 2015;29:389-97.
313. Veevers JE. Voluntary Childlessness. Marriage & Family Review 1979;2:1-26.
314. Houseknecht SK. Timing of the Decision To Remain Voluntarily Childless: Evidence for Continuous Socialization. Psychology of Women Quarterly 1979;4:81-96.
315. Lotz M. Commentary on Nicola Williams and Stephen Wilkinson: "Should Uterus Transplants Be Publicly Funded?'. Journal of Medical Ethics 2016;42:570-1.
316. Hamada A, Kingsberg S, Wierckx K, et al. Semen characteristics of transwomen referred for sperm banking before sex transition: a case series. Andrologia 2015;47:832-8.
317. De Sutter P, Verschoor, A., Hotimsky, A., & Kira, K. The desire to have children and the preservation of fertility in transsexual women: A survey. The International Journal of Transgenderism 2002;6.
318. Wierckx K, Stuyver I, Weyers S, et al. Sperm freezing in transsexual women. Arch Sex Behav 2012;41:1069-71.
319. Wilkinson S, Williams NJ. Public funding, social change and uterus transplants: a response to commentaries. Journal of Medical Ethics 2016;42:572-3.
320. Hammond-Browning N. Uterine Transplantation in Transgender Women: Medical, Legal and Ethical Considerations. Bjog 2018.
321. Jones BP, Alghrani A, Smith JR. Re: Uterine Transplantation in Transgender Women: Medical, Legal and Ethical Considerations. BJOG: An International Journal of Obstetrics & Gynaecology 2018.
322. Anderson RA, Mitchell RT, Kelsey TW, Spears N, Telfer EE, Wallace WH. Cancer treatment and gonadal function: experimental and established strategies for fertility preservation in children and young adults. Lancet Diabetes Endocrinol 2015;3:556-67.
323. Williams NJ. Should Deceased Donation be Morally Preferred in Uterine Transplantation Trials? Bioethics 2016;30:415-24.
324. Murphy TF. The ethics of fertility preservation in transgender body modifications. J Bioeth Inq 2012;9:311-6.
325. Green R. Transsexuals' children. 1998;2.
326. Chiland C, Clouet AM, Golse B, Guinot M, Wolf JP. A new type of family: Transmen as fathers thanks to donor sperm insemination. A 12-year follow-up exploratory study of their children. Neuropsychiatrie de l'Enfance et de l'Adolescence 2013;61:365-70.
327. Stotzer R, Herman J, Hasenbush A. Transgender Parenting: A Review of Existing Research2014.
328. White T, Ettner R. Adaptation and adjustment in children of transsexual parents. European Child & Adolescent Psychiatry 2007;16:215-21.
329. Fatu C, Puisoru M, Fatu IC. Morphometry of the internal iliac artery in different ethnic groups. Ann Anat 2006;188:541-6.
330. Timaran CH, Stevens SL, Freeman MB, Goldman MH. External iliac and common iliac artery angioplasty and stenting in men and women. J Vasc Surg 2001;34:440-6.
331. Jones BP, Williams NJ, Saso S, et al. Uterine Transplantation in Transgender Women. Bjog 2018.
332. Jin B, Turner L, Walters WA, Handelsman DJ. The effects of chronic high dose androgen or estrogen treatment on the human prostate [corrected]. The Journal of clinical endocrinology and metabolism 1996;81:4290-5.
333. Wesp LM, Deutsch MB. Hormonal and Surgical Treatment Options for Transgender Women and Transfeminine Spectrum Persons. Psychiatr Clin North Am 2017;40:99-111.
334. Furness S, Roberts H, Marjoribanks J, Lethaby A. Hormone therapy in postmenopausal women and risk of endometrial hyperplasia. Cochrane Database Syst Rev 2012:Cd000402.

335. Smith DC, Prentice R, Thompson DJ, Herrmann WL. Association of exogenous estrogen and endometrial carcinoma. The New England journal of medicine 1975;293:1164-7.
336. Penn I. Evaluation of transplant candidates with pre-existing malignancies. Annals of transplantation 1997;2:14-7.
337. Bobrowska K, Kaminski P, Cyganek A, et al. High rate of endometrial hyperplasia in renal transplanted women. Transplant Proc 2006;38:177-9.
338. Brantelid IE, Nilver H, Alehagen S. Menstruation during a lifespan: A qualitative study of women's experiences. Health care for women international 2014;35:600-16.
339. van Kesteren PJ, Asscheman H, Megens JA, Gooren LJ. Mortality and morbidity in transsexual subjects treated with cross-sex hormones. Clin Endocrinol (Oxf) 1997;47:337-42.
340. Gooren LJ, Giltay EJ, Bunck MC. Long-term treatment of transsexuals with cross-sex hormones: extensive personal experience. The Journal of clinical endocrinology and metabolism 2008;93:19-25.
341. Arnold JD, Sarkodie EP, Coleman ME, Goldstein DA. Incidence of Venous Thromboembolism in Transgender Women Receiving Oral Estradiol. J Sex Med 2016;13:1773-7.
342. Ott J, Kaufmann U, Bentz EK, Huber JC, Tempfer CB. Incidence of thrombophilia and venous thrombosis in transsexuals under cross-sex hormone therapy. Fertil Steril 2010;93:1267-72.
343. Sweetland S, Beral V, Balkwill A, et al. Venous thromboembolism risk in relation to use of different types of postmenopausal hormone therapy in a large prospective study. Journal of thrombosis and haemostasis : JTH 2012;10:2277-86.
344. Canonico M, Oger E, Plu-Bureau G, et al. Hormone therapy and venous thromboembolism among postmenopausal women: impact of the route of estrogen administration and progestogens: the ESTHER study. Circulation 2007;115:840-5.
345. Schulze C. Response of the human testis to long-term estrogen treatment: morphology of Sertoli cells, Leydig cells and spermatogonial stem cells. Cell Tissue Res 1988;251:31-43.
346. Kaneko H, Kikuchi K, Nakai M, et al. Generation of Live Piglets for the First Time Using Sperm Retrieved from Immature Testicular Tissue Cryopreserved and Grafted into Nude Mice. PLOS ONE 2013;8:e70989.
347. Frydman R, Grynberg M. Introduction: Male fertility preservation: innovations and questions. Fertil Steril 2016;105:247-8.
348. Chen D, Simons L, Johnson EK, Lockart BA, Finlayson C. Fertility Preservation for Transgender Adolescents. The Journal of adolescent health : official publication of the Society for Adolescent Medicine 2017;61:120-3.
349. Foreman M, Hare L, York K, et al. Genetic Link Between Gender Dysphoria and Sex Hormone Signaling. The Journal of clinical endocrinology and metabolism 2019;104:390-6.
350. Diamond M. Transsexuality Among Twins: Identity Concordance, Transition, Rearing, and Orientation. International Journal of Transgenderism 2013;14:24-38.
351. Jones BP, Saso S, Yazbek J, Smith JR. Uterine transplantation: past, present and future. Bjog 2016;123:1434-8.
352. Lawrence AA. Sexuality before and after male-to-female sex reassignment surgery. Arch Sex Behav 2005;34:147-66.
353. Keller DL. Risks related to the use of finasteride (propecia) in pregnant women. Am Fam Physician 2010;82:102; author reply
354. Rathnayake D, Sinclair R. Use of spironolactone in dermatology. Skinmed 2010;8:328-32; quiz 33.
355. Dragojevic Dikic S, Vasiljevic M, Jovanovic A, et al. Premature ovarian insufficiency - novel hormonal approaches in optimizing fertility. Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology 2020;36:162-5.
356. Iscan MY, Steyn M. The Human Skeleton in Forensic Medicine2013.
357. Correia H, Balseiro S, De Areia M. Sexual dimorphism in the human pelvis: testing a new hypothesis. Homo 2005;56:153-60.
358. Williams TM, Carroll SB. Genetic and molecular insights into the development and evolution of sexual dimorphism. Nat Rev Genet 2009;10:797-804.
359. Sitek A, Fijalkowska M, Zadzinska E, Antoszewski B. Biometric characteristics of the pelvis in female-to-male transsexuals. Arch Sex Behav 2012;41:1303-13.
360. Moore FD. Ethical Problems Special to Surgery: Surgical Teaching, Surgical Innovation, and the Surgeon in Managed Care. Archives of Surgery 2000;135:14-6.
361. Office for National Statistics. Statistical bulletin: Births in England and Wales: 2016. Live births, stillbirths, and the intensity of childbearing measured by the total fertility rate.

(https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/livebirths/bulletins/birthsummarytablesenglandandwales/2016). Accessed 2nd November 2017
362. Crawford NM, Steiner AZ. Age-related infertility. Obstetrics and gynecology clinics of North America 2015;42:15-25.
363. Faddy MJ, Gosden RG, Gougeon A, Richardson SJ, Nelson JF. Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause. Human reproduction (Oxford, England) 1992;7:1342-6.
364. Nybo Andersen AM, Wohlfahrt J, Christens P, Olsen J, Melbye M. Maternal age and fetal loss: population based register linkage study. BMJ (Clinical research ed) 2000;320:1708-12.
365. Morris JK, Mutton DE, Alberman E. Revised estimates of the maternal age specific live birth prevalence of Down's syndrome. Journal of medical screening 2002;9:2-6.
366. Nicoletti C, Tanturri ML. Differences in Delaying Motherhood Across European Countries: Empirical Evidence from the ECHP. European Journal of Population / Revue européenne de Démographie 2008;24:157-83.
367. Chen C. Pregnancy after human oocyte cryopreservation. Lancet (London, England) 1986;1:884-6.
368. Smith GD, Serafini PC, Fioravanti J, et al. Prospective randomized comparison of human oocyte cryopreservation with slow-rate freezing or vitrification. Fertil Steril 2010;94:2088-95.
369. Crawford S, Boulet SL, Kawwass JF, Jamieson DJ, Kissin DM. Cryopreserved oocyte versus fresh oocyte assisted reproductive technology cycles, United States, 2013. Fertility and sterility 2017;107:110-8.
370. Cil AP, Bang H, Oktay K. Age-specific probability of live birth with oocyte cryopreservation: an individual patient data meta-analysis. Fertil Steril 2013;100:492-9.e3.
371. Chang C-C, Elliott TA, Wright G, Shapiro DB, Toledo AA, Nagy ZP. Prospective controlled study to evaluate laboratory and clinical outcomes of oocyte vitrification obtained in in vitro fertilization patients aged 30 to 39 years. Fertility and Sterility 2013;99:1891-7.
372. Jones BP, Saso S, Mania A, Smith JR, Serhal P, Ben Nagi J. The dawn of a new ice age: social egg freezing. Acta Obstet Gynecol Scand 2018;97:641-7.
373. Knight M NM, Tuffnell D, Kenyon S, Shakespeare J, Brocklehurst P, Kurinczuk JJ (Eds). Saving Lives, Improving Mothers' Care - Surveillance of maternal deaths in the UK 2012-14 and lessons learned to inform maternity care from the UK and Ireland Con dential Enquiries into Maternal Deaths and Morbidity 2009-14. In: Oxford: National Perinatal Epidemiology Unit UoO, ed.2016.
374. Fitzpatrick KE, Tuffnell D, Kurinczuk JJ, Knight M. Pregnancy at very advanced maternal age: a UK population-based cohort study. BJOG: An International Journal of Obstetrics & Gynaecology 2017;124:1097-106.
375. Bianco A, Stone J, Lynch L, Lapinski R, Berkowitz G, Berkowitz RL. Pregnancy outcome at age 40 and older. Obstetrics and gynecology 1996;87:917-22.
376. Levron Y, Dviri M, Segol I, et al. The 'immunologic theory' of preeclampsia revisited: a lesson from donor oocyte gestations. Am J Obstet Gynecol 2014;211:383.e1-5.
377. Odibo AO, Nelson D, Stamilio DM, Sehdev HM, Macones GA. Advanced maternal age is an independent risk factor for intrauterine growth restriction. American journal of perinatology 2006;23:325-8.
378. Waldenstrom U, Cnattingius S, Norman M, Schytt E. Advanced Maternal Age and Stillbirth Risk in Nulliparous and Parous Women. Obstetrics and gynecology 2015;126:355-62.
379. Porreco RP, Harden L, Gambotto M, Shapiro H. Expectation of pregnancy outcome among mature women. Am J Obstet Gynecol 2005;192:38-41.
380. Paulson RJ, Boostanfar R, Saadat P, et al. Pregnancy in the sixth decade of life: obstetric outcomes in women of advanced reproductive age. Jama 2002;288:2320-3.
381. Noyes N, Porcu E, Borini A. Over 900 oocyte cryopreservation babies born with no apparent increase in congenital anomalies. Reprod Biomed Online 2009;18:769-76.
382. Tur-Kaspa I, Gal M, Horwitz A. Genetics and health of children born from cryopreserved oocytes. Fertility and Sterility;88:S14.
383. Harwood K. Egg freezing: a breakthrough for reproductive autonomy? Bioethics 2009;23:39-46.
384. Lockwood GM. Social egg freezing: the prospect of reproductive 'immortality' or a dangerous delusion? Reprod Biomed Online 2011;23:334-40.
385. Cobo A, Meseguer M, Remohi J, Pellicer A. Use of cryo-banked oocytes in an ovum donation programme: a prospective, randomized, controlled, clinical trial. Human reproduction (Oxford, England) 2010;25:2239-46.
386. Brown S. Europe's demographic crisis is over-unofficial. Focus Reprod May, 28–31.
387. HFEA. Human Fertility and Embryology Authority Code of Practice 8.0 ed. London2017.
388. Hodes-Wertz B, Druckenmiller S, Smith M, Noyes N. What do reproductive-age women who undergo oocyte cryopreservation think about the process as a means to preserve fertility? Fertil Steril 2013;100:1343-9.

389. Schmidt L, Sobotka T, Bentzen JG, Nyboe Andersen A. Demographic and medical consequences of the postponement of parenthood. Human reproduction update 2012;18:29-43.
390. Fergusson DM, Woodward LJ. Maternal Age and Educational and Psychosocial Outcomes in Early Adulthood. Journal of Child Psychology and Psychiatry 1999;40:479-89.
391. Hardy JB, Astone NM, Brooks-Gunn J, Shapiro S, Miller TL. Like mother, like child: intergenerational patterns of age at first birth and associations with childhood and adolescent characteristics and adult outcomes in the second generation. Dev Psychol 1998;34:1220-32.
392. Lallemant C, Vassard D, Nyboe Andersen A, Schmidt L, Macklon N. Medical and social egg freezing: internet-based survey of knowledge and attitudes among women in Denmark and the UK. Acta Obstet Gynecol Scand 2016;95:1402-10.
393. Bowen-Simpkins P, Wang JJ, Ahuja KK. The UK s anomalous 10-year limit on oocyte storage: time to change the law. Reprod Biomed Online 2018;37:387-9.
394. Chmel R, Novackova M, Pastor Z, Fronek J. The Interest of Women with Mayer-Rokitansky-Kuster-Hauser Syndrome and Laparoscopic Vecchietti Neovagina in Uterus Transplantation. J Pediatr Adolesc Gynecol 2018.
395. Gauthier T, Garnault D, Therme JF, et al. Transplantation utérine : une demande réelle ? Gynécologie Obstétrique & Fertilité 2015;43:133-8.
396. Taran FA, Schöller D, Rall K, et al. Screening and evaluation of potential recipients and donors for living donor uterus transplantation: results from a single-center observational study. Fertil Steril 2019;111:186-93.
397. Pompili G, Munari A, Franceschelli G, et al. Magnetic resonance imaging in the preoperative assessment of Mayer-Rokitansky-Kuster-Hauser syndrome. La Radiologia medica 2009;114:811-26.
398. Lermann J, Mueller A, Wiesinger E, et al. Comparison of different diagnostic procedures for the staging of malformations associated with Mayer-Rokitansky-Kuster-Hauser syndrome. Fertil Steril 2011;96:156-9.
399. Goldfarb JM, Austin C, Peskin B, Lisbona H, Desai N, de Mola JR. Fifteen years experience with an in-vitro fertilization surrogate gestational pregnancy programme. Human reproduction (Oxford, England) 2000;15:1075-8.
400. Strubbe EH, Cremers CW, Willemsen WN, Rolland R, Thijn CJ. The Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome without and with associated features: two separate entities? Clin Dysmorphol 1994;3:192-9.
401. Raziel A, Friedler S, Gidoni Y, Ben Ami I, Strassburger D, Ron-El R. Surrogate in vitro fertilization outcome in typical and atypical forms of Mayer-Rokitansky-Kuster-Hauser syndrome. Human reproduction (Oxford, England) 2012;27:126-30.
402. Kapczuk K, Iwaniec K, Friebe Z, Kedzia W. Congenital malformations and other comorbidities in 125 women with Mayer-Rokitansky-Kuster-Hauser syndrome. Eur J Obstet Gynecol Reprod Biol 2016;207:45-9.
403. Oppelt P, Renner SP, Kellermann A, et al. Clinical aspects of Mayer-Rokitansky-Kuester-Hauser syndrome: recommendations for clinical diagnosis and staging. Human reproduction (Oxford, England) 2006;21:792-7.
404. Vajdic CM, van Leeuwen MT. Cancer incidence and risk factors after solid organ transplantation. International journal of cancer 2009;125:1747-54.
405. Brattstrom C, Granath F, Edgren G, Smedby KE, Wilczek HE. Overall and cause-specific mortality in transplant recipients with a pretransplantation cancer history. Transplantation 2013;96:297-305.
406. Acuna SA, Huang JW, Daly C, Shah PS, Kim SJ, Baxter NN. Outcomes of Solid Organ Transplant Recipients With Preexisting Malignancies in Remission: A Systematic Review and Meta-Analysis. Transplantation 2017;101:471-81.
407. Michala L, Cutner A, Creighton SM. Surgical approaches to treating vaginal agenesis. Bjog 2007;114:1455-9.
408. ACOG. Committee opinion: no. 562: mullerian agenesis: diagnosis, management, and treatment. Obstetrics and gynecology 2013;121:1134-7.
409. Callens N, De Cuypere G, De Sutter P, et al. An update on surgical and non-surgical treatments for vaginal hypoplasia. Human reproduction update 2014;20:775-801.
410. Jones BP, Saso S, L'Heveder A, et al. The vaginal microbiome in uterine transplantation. BJOG: An International Journal of Obstetrics & Gynaecology 2019;127:230-8.
411. Hampton HL, Meeks GR, Bates GW, Wiser WL. Pregnancy after successful vaginoplasty and cervical stenting for partial atresia of the cervix. Obstetrics and gynecology 1990;76:900-1.
412. Nargund G, Parsons J. A successful in-vitro fertilization and embryo transfer treatment in a woman with previous vaginoplasty for congenital absence of vagina. Human reproduction (Oxford, England) 1996;11:1654.

413. Acien P, Acien MI, Quereda F, Santoyo T. Cervicovaginal agenesis: spontaneous gestation at term after previous reimplantation of the uterine corpus in a neovagina: Case Report. Human reproduction (Oxford, England) 2008;23:548-53.
414. Bastu E, Akhan SE, Mutlu MF, et al. Treatment of vaginal agenesis using a modified McIndoe technique: Long-term follow-up of 23 patients and a literature review. The Canadian journal of plastic surgery = Journal canadien de chirurgie plastique 2012;20:241-4.
415. Rezaei Z, Omidvar A, Niroumanesh S, Omidvar A. Cervicovaginal anastomosis by Gore-Tex in Mullerian agenesis. Arch Gynecol Obstet 2015;291:467-72.
416. Kriplani A, Kachhawa G, Awasthi D, Kulshrestha V. Laparoscopic-assisted uterovaginal anastomosis in congenital atresia of uterine cervix: follow-up study. J Minim Invasive Gynecol 2012;19:477-84.
417. Chakravarty B, Konar H, Chowdhury NN. Pregnancies after reconstructive surgery for congenital cervicovaginal atresia. Am J Obstet Gynecol 2000;183:421-3.
418. Zafar M, Saeed S, Kant B, Murtaza B, Dar MF, Khan NA. Use of amnion in vaginoplasty for vaginal atresia. Journal of the College of Physicians and Surgeons--Pakistan : JCPSP 2007;17:107-9.
419. Del Priore G, Saso S, Meslin EM, et al. Uterine transplantation--a real possibility? The Indianapolis consensus. Human reproduction (Oxford, England) 2013;28:288-91.
420. Hammond-Browning N. UK Criteria for Uterus Transplantation: A review. BJOG: An International Journal of Obstetrics & Gynaecology 2019;126:1320-6.
421. Bayefsky MJ, Berkman BE. Toward the Ethical Allocation of Uterine Transplants. American Journal of Bioethics 2018;18:16-7.
422. Hammond-Browning N. UK Criteria For Uterus Transplantation: Looking Ahead. 2018.
423. Bayefsky MJ, Berkman BE. The Ethics of Allocating Uterine Transplants. Cambridge Quarterly of Healthcare Ethics 2016;25:350-65.
424. Banu az-Zubair MK. Who is a parent? Parenthood in Islamic ethics. Journal of medical ethics 2007;33:605-9.
425. Pollack D, Bleich M, Jr C, Fadel M. Classical Religious Perspectives of Adoption Law. The Notre Dame law review 2008;79.
426. Serour GI. Islamic perspectives in human reproduction. Reproductive BioMedicine Online 2008;17:34-8.
427. Sallam HN, Sallam NH. Religious aspects of assisted reproduction. Facts Views Vis Obgyn 2016;8:33-48.
428. Wolowelsky JB, Grazi RV. Current Jewish perspectives on maternal identity. Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology 2014;30:929-30.
429. Akouri R, Maalouf G, Abboud J, et al. Uterus transplantation: An update and the Middle East perspective. Middle East Fertility Society Journal 2017;22:163-9.
430. Saso S, Bracewell-Milnes T, Ismail L, et al. Psychological assessment tool for patients diagnosed with absolute uterine factor infertility and planning to undergo uterine transplantation. Journal of obstetrics and gynaecology : the journal of the Institute of Obstetrics and Gynaecology 2014;34:504-7.
431. Clarke A, Butler PE. The psychological management of facial transplantation. Expert review of neurotherapeutics 2009;9:1087-100.
432. Jarvholm S, Johannesson L, Brannstrom M. Psychological aspects in pre-transplantation assessments of patients prior to entering the first uterus transplantation trial. Acta Obstet Gynecol Scand 2015;94:1035-8.
433. Baker C. Mental health statistics for England: prevalence, services and funding. London: House of Commons library; 2018.
434. Baranyi A, Krauseneck T, Rothenhäusler H-B. Overall mental distress and health-related quality of life after solid-organ transplantation: results from a retrospective follow-up study. Health And Quality Of Life Outcomes 2013;11:15-.
435. Development of the World Health Organization WHOQOL-BREF quality of life assessment. The WHOQOL Group. Psychol Med 1998;28:551-8.
436. Boivin J, Takefman J, Braverman A. The fertility quality of life (FertiQoL) tool: development and general psychometric properties†. Human Reproduction 2011;26:2084-91.
437. Snaith RP. The Hospital Anxiety And Depression Scale. Health and quality of life outcomes 2003;1:29-.
438. Sharpley CF, Cross DG. A Psychometric Evaluation of the Spanier Dyadic Adjustment Scale. Journal of Marriage and Family 1982;44:739-41.
439. Lins L, Carvalho FM. SF-36 total score as a single measure of health-related quality of life: Scoping review. SAGE Open Med 2016;4:2050312116671725-.
440. Whisman MA, Snyder DK, Beach SR. Screening for marital and relationship discord. J Fam Psychol 2009;23:247-54.

441. Moser A, Stuck AE, Silliman RA, Ganz PA, Clough-Gorr KM. The eight-item modified Medical Outcomes Study Social Support Survey: psychometric evaluation showed excellent performance. J Clin Epidemiol 2012;65:1107-16.
442. Wei L, Champman S, Li X, et al. Beliefs about medicines and non-adherence in patients with stroke, diabetes mellitus and rheumatoid arthritis: a cross-sectional study in China. BMJ Open 2017;7:e017293.
443. Lim Y, Lyall H. Congenital cytomegalovirus - who, when, what-with and why to treat? J Infect 2017;74 Suppl 1:S89-s94.
444. Koval CE. Prevention and Treatment of Cytomegalovirus Infections in Solid Organ Transplant Recipients. Infect Dis Clin North Am 2018;32:581-97.
445. Rubin RH. The pathogenesis and clinical management of cytomegalovirus infection in the organ transplant recipient: the end of the 'silo hypothesis'. Curr Opin Infect Dis 2007;20:399-407.
446. Kranz B, Vester U, Wingen A-M, et al. Acute rejection episodes in pediatric renal transplant recipients with cytomegalovirus infection. Pediatric Transplantation 2008;12:474-8.
447. Hjelmesaeth J, Müller F, Jenssen T, Rollag H, Sagedal S, Hartmann A. Is there a link between cytomegalovirus infection and new-onset posttransplantation diabetes mellitus? Potential mechanisms of virus induced beta-cell damage. Nephrol Dial Transplant 2005;20:2311-5.
448. Hodson EM, Jones CA, Webster AC, et al. Antiviral medications to prevent cytomegalovirus disease and early death in recipients of solid-organ transplants: a systematic review of randomised controlled trials. Lancet (London, England) 2005;365:2105-15.
449. Kanj SS, Sharara AI, Clavien PA, Hamilton JD. Cytomegalovirus infection following liver transplantation: review of the literature. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 1996;22:537-49.
450. Fowler KB, Stagno S, Pass RF, Britt WJ, Boll TJ, Alford CA. The outcome of congenital cytomegalovirus infection in relation to maternal antibody status. The New England journal of medicine 1992;326:663-7.
451. Kenneson A, Cannon MJ. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. Rev Med Virol 2007;17:253-76.
452. Pass RF, Fowler KB, Boppana SB, Britt WJ, Stagno S. Congenital cytomegalovirus infection following first trimester maternal infection: symptoms at birth and outcome. J Clin Virol 2006;35:216-20.
453. Dollard SC, Grosse SD, Ross DS. New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection. Rev Med Virol 2007;17:355-63.
454. Hauck FR, Neese BH, Panchal AS, El-Amin W. Identification and management of latent tuberculosis infection. Am Fam Physician 2009;79:879-86.
455. King TC, Upfal M, Gottlieb A, et al. T-SPOT.TB Interferon-γ Release Assay Performance in Healthcare Worker Screening at Nineteen U.S. Hospitals. American Journal of Respiratory and Critical Care Medicine 2015;192:367-73.
456. Achille MA, Ouellette A, Fournier S, Vachon M, Hebert MJ. Impact of stress, distress and feelings of indebtedness on adherence to immunosuppressants following kidney transplantation. Clin Transplant 2006;20:301-6.
457. Hawthorne G, Herrman H, Murphy B. Interpreting the WHOQOL-BREF: Preliminary population norms and effect sizes. Social Indicators Research 2006;77:37-59.
458. Garratt AM, Stavem K. Measurement properties and normative data for the Norwegian SF-36: results from a general population survey. Health and Quality of Life Outcomes 2017;15:51.
459. Jenkinson C, Stewart-Brown S, Petersen S, Paice C. Assessment of the SF-36 version 2 in the United Kingdom. Journal of epidemiology and community health 1999;53:46-50.
460. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand 1983;67:361-70.
461. Chen TH, Chang SP, Tsai CF, Juang KD. Prevalence of depressive and anxiety disorders in an assisted reproductive technique clinic. Human reproduction (Oxford, England) 2004;19:2313-8.
462. Pasch LA, Holley SR, Bleil ME, Shehab D, Katz PP, Adler NE. Addressing the needs of fertility treatment patients and their partners: are they informed of and do they receive mental health services? Fertil Steril 2016;106:209-15.e2.
463. Chisholm MA. Issues of adherence to immunosuppressant therapy after solid-organ transplantation. Drugs 2002;62:567-75.
464. Shabany Hamedan M, Mohamad Aliha J. Relationship between immunosuppressive medications adherence and quality of life and some patient factors in renal transplant patients in Iran. Glob J Health Sci 2014;6:205-12.

465. De Geest S, Burkhalter H, Bogert L, et al. Describing the evolution of medication nonadherence from pretransplant until 3 years post-transplant and determining pretransplant medication nonadherence as risk factor for post-transplant nonadherence to immunosuppressives: the Swiss Transplant Cohort Study. Transplant international : official journal of the European Society for Organ Transplantation 2014;27:657-66.
466. Dobbels F, Vanhaecke J, Dupont L, et al. Pretransplant predictors of posttransplant adherence and clinical outcome: an evidence base for pretransplant psychosocial screening. Transplantation 2009;87:1497-504.
467. Silva AN, Moratelli L, Tavares PL, et al. Self-efficacy beliefs, locus of control, religiosity and non-adherence to immunosuppressive medications in kidney transplant patients. Nephrology (Carlton) 2016;21:938-43.
468. Weng LC, Yang YC, Huang HL, Chiang YJ, Tsai YH. Factors that determine self-reported immunosuppressant adherence in kidney transplant recipients: a correlational study. J Adv Nurs 2017;73:228-39.
469. Horne R, Weinman J. Patients' beliefs about prescribed medicines and their role in adherence to treatment in chronic physical illness. J Psychosom Res 1999;47:555-67.
470. Berben L, Dobbels F, Kugler C, Russell CL, De Geest S. Interventions used by health care professionals to enhance medication adherence in transplant patients: a survey of current clinical practice. Progress in transplantation (Aliso Viejo, Calif) 2011;21:322-31.
471. Telles-Correia D, Barbosa A, Mega I, Monteiro E. Adherence correlates in liver transplant candidates. Transplant Proc 2009;41:1731-4.
472. Porteous T, Francis J, Bond C, Hannaford P. Temporal stability of beliefs about medicines: implications for optimising adherence. Patient Educ Couns 2010;79:225-30.
473. Andersson Sundell K, Jönsson AK. Beliefs about medicines are strongly associated with medicine-use patterns among the general population. International Journal of Clinical Practice 2016;70:277-85.
474. White-Williams C, Grady KL, Naftel DC, Myers S, Wang E, Rybarczyk B. The relationship of socio-demographic factors and satisfaction with social support at five and 10 yr after heart transplantation. Clin Transplant 2013;27:267-73.
475. Leahy-Warren P, McCArthy G, Corcoran P. First-time mothers: social support, maternal parental self-efficacy and postnatal depression. Journal of Clinical Nursing 2012;21:388-97.
476. Cooper P, Murray L. Postanatal depression. British medical journal 1998;20:1884-6.
477. Lindström B, Eriksson M. Salutogenesis. Journal of Epidemiology and community health 2005;59:440-2.
478. Ahnquist J, Wamala SP, Lindstrom M. Social determinants of health – A question of social or economic capital? Interaction effects of socioeconomic factors on health outcomes. Social Science & Medicine 2012;74:930-9.
479. Gómez-Campelo P, Pérez-Moreno EM, de Burgos-Lunar C, Bragado-Álvarez C, Jiménez-García R, Salinero-Fort MÁ. Psychometric properties of the eight-item modified Medical Outcomes Study Social Support Survey based on Spanish outpatients. Quality of Life Research 2014;23:2073-8.
480. Jarvholm S, Warren AM, Jalmbrant M, Kvarnstrom N, Testa G, Johannesson L. Preoperative psychological evaluation of uterus transplant recipients, partners, and living donors: Suggested framework. Americal Journal of Transplantation 2018;18:1-6.
481. Denhaerynck K, Dobbels, F., Cleemput, I., et al. Prevalence, consequences, and determinants of nonadherence in adult renal transplant patients: a literature review. Transplant international : official journal of the European Society for Organ Transplantation 2005;18:1121-33.
482. Cano-Prous A, Martín-Lanas R, Moyá-Querejeta J, Beunza-Nuin MI, Lahortiga-Ramos F, García-Granero M. Psychometric properties of a Spanish version of the Dyadic Adjustment Scale. International Journal of Clinical and Health Psychology 2014;14:137-44.
483. Milliez J. Uterine transplantation FIGO Committee for the Ethical Aspects of Human Reproduction and Women's Health. International Journal of Gynecology and Obstetrics 2009;106.
484. Olausson M, Johannesson L, Brattgard D, et al. Ethics of uterus transplantation with live donors. Fertility and Sterility 2014;102.
485. Caplan AL. Moving The Womb. The Hastings Centre Report 2007;37:18-20.
486. Mumtaz Z, Levay A. Ethics Criteria for Uterine Transplants: Relevance for Low-Income, Pronatalistic Societies? Clinical Research & Bioethics 2012.
487. Kristek J, Johannesson L, Testa G, et al. Limited Availability of Deceased Uterus Donors: A Transatlantic Perspective. Transplantation 2019;103:2449-52.
488. Jones BP, Saso S, Quiroga I, Yazbek J, Smith JR. Re: UK criteria for uterus transplantation: a review. Bjog 2019;126:1507-8.

489. Hariton E, Bortoletto P, Goldman RH, Farland LV, Ginsburg ES, Gargiulo AR. A Survey of Public Opinion in the United States Regarding Uterine Transplantation. J Minim Invasive Gynecol 2018.
490. Wennberg A-L, Rodriguez-Wallberg KA, Milsom I, Brännström M. Attitudes towards new assisted reproductive technologies in Sweden: a survey in women 30–39 years of age. Acta Obstetricia et Gynecologica Scandinavica 2015;95:38-44.
491. Kisu I, Banno K, Soeda E, et al. Survey of Attitudes toward Uterus Transplantation among Japanese Women of Reproductive Age: A Cross-Sectional Study. PloS one 2016;11:e0156179-e.
492. Del Priore G, Stega J, Sieunarine K, Ungar L, Smith JR. Human uterus retrieval from a multi-organ donor. Obstetrics and gynecology 2007;109:101-4.
493. Rodrigue JR, Tomich D, Fleishman A, Glazier AK. Vascularized Composite Allograft Donation and Transplantation: A Survey of Public Attitudes in the United States. Am J Transplant 2017;17:2687-95.
494. Warren AM, Testa G, Anthony T, et al. Live nondirected uterus donors: Psychological characteristics and motivation for donation. Am J Transplant 2018.
495. Abouna GM. Ethical Issues in Organ Transplantation. Medical Principles and Practice 2003;12:54-69.
496. Messersmith EE, Gross CR, Beil CA, et al. Satisfaction With Life Among Living Kidney Donors: A RELIVE Study of Long-Term Donor Outcomes. Transplantation 2014;98:1294-300.
497. Yang LS, Shan LL, Saxena A, Morris DL. Liver transplantation: a systematic review of long-term quality of life. Liver international : official journal of the International Association for the Study of the Liver 2014;34:1298-313.
498. Goddijn M, Leschot NJ. Genetic aspects of miscarriage. Baillieres Best Pract Res Clin Obstet Gynaecol 2000;14:855-65.
499. Rai R, Regan L. Recurrent miscarriage. Lancet (London, England) 2006;368:601-11.
500. American Psychiatric Association. Diagnostic and statistical manual of mental disorders (5th edition). 2013.
501. Lubbert H, Leo-Rossberg I, Hammerstein J. Effects of ethinyl estradiol on semen quality and various hormonal parameters in a eugonadal male. Fertil Steril 1992;58:603-8.
502. Liu W, Schulster ML, Alukal JP, Najari BB. Fertility Preservation in Male to Female Transgender Patients. Urol Clin North Am 2019;46:487-93.
503. Nahata L, Tishelman AC, Caltabellotta NM, Quinn GP. Low Fertility Preservation Utilization Among Transgender Youth. J Adolesc Health 2017;61:40-4.
504. UN Office of the High Commissioner for Human Rights (OHCHR). Reproductive Rights are Human Rights: A Handbook for National Human Rights Institutions Published jointly with UNFPA and the Danish Institute for Human Rights 2014;HR/PUB/14/6.
505. Factor R, Rothblum E. Exploring gender identity and community among three groups of transgender individuals in the United States: MTFs, FTMs, and genderqueers. Health Sociology Review 2008;17:235-53.
506. Kenagy GP, Hsieh C-M. Gender Differences in Social Service Needs of Transgender People. Journal of Social Service Research 2005;31:1-21.
507. Lawrence AA. Factors Associated with Satisfaction or Regret Following Male-to-Female Sex Reassignment Surgery. Archives of Sexual Behavior 2003;32:299-315.
508. Wood H, Sasaki S, Bradley SJ, et al. Patterns of referral to a gender identity service for children and adolescents (1976-2011): age, sex ratio, and sexual orientation. J Sex Marital Ther 2013;39:1-6.
509. Aitken M, Steensma TD, Blanchard R, et al. Evidence for an Altered Sex Ratio in Clinic-Referred Adolescents with Gender Dysphoria. The Journal of Sexual Medicine 2015;12:756-63.
510. Brik T, Vrouenraets L, Schagen SEE, Meissner A, de Vries MC, Hannema SE. Use of Fertility Preservation Among a Cohort of Transgirls in the Netherlands. J Adolesc Health 2019;64:589-93.
511. Jones CA, Reiter L, Greenblatt E. Fertility preservation in transgender patients. International Journal of Transgenderism 2016;17:76-82.
512. Fishman JA. Infection in solid-organ transplant recipients. The New England journal of medicine 2007;357:2601-14.
513. Yonemoto RH, du Sold WD, Deliman RM. Homotransplantation of uterus and ovaries in dogs: A preliminary report. American Journal of Obstetrics and Gynecology 1969;104:1143-51.
514. Lee S, Mao L, Wang Y, et al. Transplantation of reproductive organs. Microsurgery 1995;16:191-8.
515. Wranning CA, Akhi SN, Kurlberg G, Brannstrom M. Uterus transplantation in the rat: model development, surgical learning and morphological evaluation of healing. Acta Obstet Gynecol Scand 2008;87:1239-47.
516. Racho El-Akouri R, Kurlberg G, Dindelegan G, Molne J, Wallin A, Brannstrom M. Heterotopic uterine transplantation by vascular anastomosis in the mouse. J Endocrinol 2002;174:157-66.

517. Wranning CA, Akhi SN, Diaz-Garcia C, Brannstrom M. Pregnancy after syngeneic uterus transplantation and spontaneous mating in the rat. Hum Reprod 2011;26:553-8.
518. Sieunarine K, Zakaria FB, Boyle DC, et al. Possibilities for fertility restoration: a new surgical technique. International surgery 2005;90:249-56.
519. Wranning CA, El-Akouri RR, Lundmark C, et al. Auto-transplantation of the uterus in the domestic pig (Sus scrofa): Surgical technique and early reperfusion events. Journal of Obstetrics and Gynaecology Research 2006;32:358-67.
520. Avison DL, DeFaria W, Tryphonopoulos P, et al. Heterotopic uterus transplantation in a swine model. Transplantation 2009;88:465-9.
521. Johannesson L, Enskog A. Experimental uterus transplantation. Best Pract Res Clin Obstet Gynaecol 2014;28:1198-210.
522. Dahm-Kähler P, Wranning C, Lundmark C, et al. Transplantation of the uterus in sheep: Methodology and early reperfusion events. Journal of Obstetrics and Gynaecology Research 2008;34:784-93.
523. Wranning CA, Marcickiewicz J, Enskog A, Dahm-Kähler P, Hanafy A, Brännström M. Fertility after autologous ovine uterine-tubal-ovarian transplantation by vascular anastomosis to the external iliac vessels. Human Reproduction 2010;25:1973-9.
524. Ramirez ER, Ramirez DK, Pillari VT, Vasquez H, Ramirez HA. Modified uterine transplant procedure in the sheep model. J Minim Invasive Gynecol 2008;15:311-4.
525. Enskog A, Johannesson L, Chai DC, et al. Uterus transplantation in the baboon: methodology and long-term function after auto-transplantation. Human Reproduction 2010;25:1980-7.
526. Saso S, Petts G, Thum M-Y, et al. Achieving uterine auto-transplantation in a sheep model using iliac vessel anastomosis: a short-term viability study. Acta Obstetricia et Gynecologica Scandinavica 2015;94:245-52.
527. Saso S, Petts G, Chatterjee J, et al. Uterine allotransplantation in a rabbit model using aorto-caval anastomosis: a long-term viability study. European Journal of Obstetrics and Gynecology and Reproductive Biology 2014;182:185-93.
528. Liu Y, Zhang Y, Ding Y, et al. Successful orthotopic uterine allotransplantation in a rabbit model using aorta and cava anastomoses: a short-term viability study. Archives of Gynecology and Obstetrics 2019.
529. Lowrance EW. Linear growth and appearance of sex difference in the rabbit pelvis. The Anatomical Record 1968;161:413-7.
530. Saso S, Hurst S, Chatterjee J, et al. Test of long-term uterine survival after allogeneic transplantation in rabbits. Journal of Obstetrics and Gynaecology Research 2014;40:754-62.
531. Fritsch H. Ein Fall von volligen schwund der Gebaumutterhohle nach Auskratzung. Zentralbl gynaekol 1894;18:1337-42.
532. Asherman JG. Traumatic intra-uterine adhesions. The Journal of obstetrics and gynaecology of the British Empire 1950;57:892-6.
533. Hooker AB, de Leeuw R, van de Ven PM, et al. Prevalence of intrauterine adhesions after the application of hyaluronic acid gel after dilatation and curettage in women with at least one previous curettage: short-term outcomes of a multicenter, prospective randomized controlled trial. Fertil Steril 2017;107:1223-31.e3.
534. Schenker JG, Margalioth EJ. Intrauterine adhesions: an updated appraisal. Fertil Steril 1982;37:593-610.
535. Westendorp IC, Ankum WM, Mol BW, Vonk J. Prevalence of Asherman's syndrome after secondary removal of placental remnants or a repeat curettage for incomplete abortion. Human reproduction (Oxford, England) 1998;13:3347-50.
536. Rabau E, David A. INTRAUTERINE ADHESIONS: ETIOLOGY, PREVENTION, AND TREATMENT. Obstetrics and gynecology 1963;22:626-9.
537. Czernobilsky B. Endometritis and infertility. Fertil Steril 1978;30:119-30.
538. Baradwan S, Baradwan A, Al-Jaroudi D. The association between menstrual cycle pattern and hysteroscopic march classification with endometrial thickness among infertile women with Asherman syndrome. Medicine (Baltimore) 2018;97:e11314-e.
539. Hooker AB, Lemmers M, Thurkow AL, et al. Systematic review and meta-analysis of intrauterine adhesions after miscarriage: prevalence, risk factors and long-term reproductive outcome. Human reproduction update 2014;20:262-78.
540. Yu D, Wong YM, Cheong Y, Xia E, Li TC. Asherman syndrome--one century later. Fertil Steril 2008;89:759-79.
541. Yamamoto N, Takeuchi R, Izuchi D, et al. Hysteroscopic adhesiolysis for patients with Asherman's syndrome: menstrual and fertility outcomes. Reprod Med Biol 2013;12:159-66.
542. Thomson AJ, Abbott JA, Kingston A, Lenart M, Vancaillie TG. Fluoroscopically guided synechiolysis for patients with Asherman's syndrome: menstrual and fertility outcomes. Fertil Steril 2007;87:405-10.

543. Song D, Liu Y, Xiao Y, Li TC, Zhou F, Xia E. A matched cohort study comparing the outcome of intrauterine adhesiolysis for Asherman's syndrome after uterine artery embolization or surgical trauma. J Minim Invasive Gynecol 2014;21:1022-8.
544. Nagori CB, Panchal SY, Patel H. Endometrial regeneration using autologous adult stem cells followed by conception by in vitro fertilization in a patient of severe Asherman's syndrome. Journal of human reproductive sciences 2011;4:43-8.
545. Saso S, Haddad J, Ellis P, et al. Placental site trophoblastic tumours and the concept of fertility preservation. Bjog 2012;119:369-74; discussion 74.
546. McCulloch P, Cook JA, Altman DG, Heneghan C, Diener MK. IDEAL framework for surgical innovation 1: the idea and development stages. BMJ (Clinical research ed) 2013;346:f3012.
547. Bakker J, Baum MJ. Neuroendocrine regulation of GnRH release in induced ovulators. Front Neuroendocrinol 2000;21:220-62.
548. Dufy-Barbe L, Franchimont P, Faure JMA. Time-Courses of LH and FSH Release After Mating in the Female Rabbit. Endocrinology 1973;92:1318-21.
549. Jones EE, Bain JB, Odell WD. Postcoital luteinizing hormone release in male and female rabbits as determined by radioimmunoassay. Fertil Steril 1976;27:848-52.
550. Morrell JM. Artificial insemination in rabbits. British Veterinary Journal 1995;151:477-88.
551. Carter CS, Getz LL, Gavish L, Mc Dermott JL, Arnold P. Male-related Pheromones and the Activation of Female Reproduction in the Prairie Vole (Microtus ochrogaster). Biology of Reproduction 1980;23:1038-45.
552. Esteves PJ, Abrantes J, Baldauf H-M, et al. The wide utility of rabbits as models of human diseases. Experimental & Molecular Medicine 2018;50:66.
553. Graur D, Duret L, Gouy M. Phylogenetic position of the order Lagomorpha (rabbits, hares and allies). Nature 1996;379:333-5.
554. Viudes-de-Castro MP, Marco-Jimenez F, Mas Pellicer A, Garcia-Dominguez X, Talavan AM, Vicente JS. A single injection of corifollitropin alfa supplemented with human chorionic gonadotropin increases follicular recruitment and transferable embryos in the rabbit. Reproduction in domestic animals = Zuchthygiene 2019;54:696-701.
555. Garcia-Dominguez X, Marco-Jimenez F, Viudes-de-Castro MP, Vicente JS. Minimally Invasive Embryo Transfer and Embryo Vitrification at the Optimal Embryo Stage in Rabbit Model. Journal of visualized experiments : JoVE 2019.
556. Hare AA, Olah KS. Pregnancy following endometrial ablation: a review article. Journal of obstetrics and gynaecology : the journal of the Institute of Obstetrics and Gynaecology 2005;25:108-14.
557. Zhao Y, Luo Q, Zhang X, et al. Clinical Efficacy and Safety of Stem Cell-Based Therapy in Treating Asherman Syndrome: A System Review and Meta-Analysis. Stem Cells Int 2020;2020:8820538.
558. Stoop D, Nekkebroeck J, Devroey P. A survey on the intentions and attitudes towards oocyte cryopreservation for non-medical reasons among women of reproductive age. Human reproduction (Oxford, England) 2011;26:655-61.
559. Greenwood EA, Pasch LA, Hastie J, Cedars MI, Huddleston HG. To freeze or not to freeze: decision regret and satisfaction following elective oocyte cryopreservation. Fertil Steril 2018;109:1097-104.e1.
560. Goldman RH, Racowsky C, Farland LV, Munné S, Ribustello L, Fox JH. Predicting the likelihood of live birth for elective oocyte cryopreservation: a counseling tool for physicians and patients. Human Reproduction 2017;32:853-9.
561. Esfandiari N, Litzky J, Sayler J, Zagadailov P, George K, DeMars L. Egg freezing for fertility preservation and family planning: a nationwide survey of US Obstetrics and Gynecology residents. Reproductive biology and endocrinology : RB&E 2019;17:16.
562. O'Brien Y, Martyn F, Glover LE, Wingfield MB. What women want? A scoping survey on women's knowledge, attitudes and behaviours towards ovarian reserve testing and egg freezing. Eur J Obstet Gynecol Reprod Biol 2017;217:71-6.
563. Daniluk JC, Koert E. Childless women's beliefs and knowledge about oocyte freezing for social and medical reasons. Human reproduction (Oxford, England) 2016;31:2313-20.
564. Lewis EI, Missmer SA, Farland LV, Ginsburg ES. Public support in the United States for elective oocyte cryopreservation. Fertil Steril 2016;106:1183-9.
565. Wennberg AL, Rodriguez-Wallberg KA, Milsom I, Brannstrom M. Attitudes towards new assisted reproductive technologies in Sweden: a survey in women 30-39 years of age. Acta Obstet Gynecol Scand 2016;95:38-44.
566. Tan SQ, Tan AW, Lau MS, Tan HH, Nadarajah S. Social oocyte freezing: a survey among Singaporean female medical students. J Obstet Gynaecol Res 2014;40:1345-52.

567. Baldwin K, Culley L, Hudson N, Mitchell H, Lavery S. Oocyte cryopreservation for social reasons: demographic profile and disposal intentions of UK users. Reprod Biomed Online 2015;31:239-45.
568. Hammarberg K, Kirkman M, Pritchard N, et al. Reproductive experiences of women who cryopreserved oocytes for non-medical reasons. Human reproduction (Oxford, England) 2017;32:575-81.
569. Tozzo P, Fassina A, Nespeca P, Spigarolo G, Caenazzo L. Understanding social oocyte freezing in Italy: a scoping survey on university female students' awareness and attitudes. Life sciences, society and policy 2019;15:3.
570. Mahesan AM, Sadek S, Ramadan H, Bocca S, Paul ABM, Stadtmauer L. Knowledge and attitudes regarding elective oocyte cryopreservation in undergraduate and medical students. Fertility research and practice 2019;5:5.
571. Baldwin K, Culley L, Hudson N, Mitchell H. Running out of time: exploring women's motivations for social egg freezing. Journal of psychosomatic obstetrics and gynaecology 2019;40:166-73.
572. Kennedy S, Ruggles S. Breaking up is hard to count: the rise of divorce in the United States, 1980-2010. Demography 2014;51:587-98.
573. Mesen TB, Mersereau JE, Kane JB, Steiner AZ. Optimal timing for elective egg freezing. Fertil Steril 2015;103:1551-6.e1-4.
574. Jones BP, Serhal P, Ben Nagi J. Social egg freezing: Early is not always best. Acta Obstet Gynecol Scand 2018;97:1531.
575. Brehaut JC, O'Connor AM, Wood TJ, et al. Validation of a decision regret scale. Medical decision making : an international journal of the Society for Medical Decision Making 2003;23:281-92.
576. Bracewell-Milnes T, Norman-Taylor J, Nikolaou D. Social egg freezing should be offered to single women approaching their late thirties: AGAINST: Women should be freezing their eggs earlier. BJOG: An International Journal of Obstetrics & Gynaecology 2018.
577. Study protocol for the World Health Organization project to develop a Quality of Life assessment instrument (WHOQOL). Qual Life Res 1993;2:153-9.
578. The Management of Ovarian Hyperstimulation Syndrome Green-top Guideline No. 5 2016.
579. Wu M-H, Su P-F, Chu W-Y, et al. Quality of life and pregnancy outcomes among women undergoing in vitro fertilization treatment: A longitudinal cohort study. Journal of the Formosan Medical Association 2019.
580. Zeren F, Gursoy E, Colak E. The Quality of Life and Dyadic Adjustment of Couples Receiving Infertility Treatment. Afr J Reprod Health 2019;23:117-27.
581. Li J, Luo H, Long L. Mindfulness and fertility quality of life in Chinese women with infertility: assessing the mediating roles of acceptance, autonomy and self-regulation. J Reprod Infant Psychol 2019;37:455-67.
582. Massarotti C, Gentile G, Ferreccio C, Scaruffi P, Remorgida V, Anserini P. Impact of infertility and infertility treatments on quality of life and levels of anxiety and depression in women undergoing in vitro fertilization. Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology 2019;35:485-9.
583. Hekmatzadeh S-F, Bazarganipour F, Hosseini N, et al. Psychometrics properties of the Iranian version of fertility quality of life tool: A cross- sectional study. Int J Reprod Biomed (Yazd) 2018;16:191-8.
584. Maroufizadeh S, Ghaheri A, Amini P, Samani RO. Psychometric Properties of The Fertility Quality of Life Instrument in Infertile Iranian Women. Int J Fertil Steril 2017;10:371-9.
585. Zurlo MC, Cattaneo Della Volta MF, Vallone F. Predictors of quality of life and psychological health in infertile couples: the moderating role of duration of infertility. Qual Life Res 2018;27:945-54.
586. Haemmerli Keller K, Alder G, Loewer L, Faeh M, Rohner S, von Wolff M. Treatment-related psychological stress in different in vitro fertilization therapies with and without gonadotropin stimulation. Acta Obstetricia et Gynecologica Scandinavica 2018;97:269-76.
587. Desai HJ, Gundabattula SR. Quality of life in Indian women with fertility problems as assessed by the FertiQoL questionnaire: a single center cross sectional study. Journal of psychosomatic obstetrics and gynaecology 2019;40:82-7.
588. Goker A, Yanikkerem E, Birge O, Kuscu NK. Quality of life in Turkish infertile couples and related factors. Human fertility (Cambridge, England) 2018;21:195-203.
589. Porat-Katz A, Paltiel O, Kahane A, Eldar-Geva T. The effect of using complementary medicine on the infertility-specific quality of life of women undergoing in vitro fertilization. Int J Gynaecol Obstet 2016;135:163-7.
590. Santulli P, Bourdon M, Presse M, et al. Endometriosis-related infertility: assisted reproductive technology has no adverse impact on pain or quality-of-life scores. Fertil Steril 2016;105:978-87.e4.

591. Li J, Long L, Liu Y, He W, Li M. Effects of a mindfulness-based intervention on fertility quality of life and pregnancy rates among women subjected to first in vitro fertilization treatment. Behav Res Ther 2016;77:96-104.
592. Kahyaoglu Sut H, Balkanli Kaplan P. Quality of life in women with infertility via the FertiQoL and the Hospital Anxiety and Depression Scales. Nursing & Health Sciences 2015;17:84-9.
593. Karabulut A, Özkan S, O uz N. Predictors of fertility quality of life (FertiQoL) in infertile women: analysis of confounding factors. European Journal of Obstetrics and Gynecology and Reproductive Biology 2013;170:193-7.
594. Heredia M, Tenias JM, Rocio R, Amparo F, Calleja MA, Valenzuela JC. Quality of life and predictive factors in patients undergoing assisted reproduction techniques. Eur J Obstet Gynecol Reprod Biol 2013;167:176-80.
595. Hsu PY, Lin MW, Hwang JL, Lee MS, Wu MH. The fertility quality of life (FertiQoL) questionnaire in Taiwanese infertile couples. Taiwan J Obstet Gynecol 2013;52:204-9.
596. Jones BP, Kasaven L, L'Heveder A, et al. Perceptions, outcomes and regret following social egg freezing in the UK; a cross-sectional survey. Acta Obstet Gynecol Scand 2019.
597. Devine K, Mumford SL, Goldman KN, et al. Baby budgeting: oocyte cryopreservation in women delaying reproduction can reduce cost per live birth. Fertility and sterility 2015;103:1446-53.e532.
598. Dennerstein L, Morse C. A review of psychological and social aspects of in vitro fertilisation. Journal of Psychosomatic Obstetrics & Gynecology 1988;9:159-70.
599. Cobo A, García-Velasco JA, Coello A, Domingo J, Pellicer A, Remohí J. Oocyte vitrification as an efficient option for elective fertility preservation. Fertil Steril 2016;105:755-64.e8.
600. Devesa M, Tur R, Rodríguez I, Coroleu B, Martínez F, Polyzos NP. Cumulative live birth rates and number of oocytes retrieved in women of advanced age. A single centre analysis including 4500 women ≥38 years old. Human reproduction (Oxford, England) 2018;33:2010-7.
601. Al-Azemi M, Killick SR, Duffy S, et al. Multi-marker assessment of ovarian reserve predicts oocyte yield after ovulation induction. Human reproduction (Oxford, England) 2011;26:414-22.
602. Fedorcsák P, Dale PO, Storeng R, et al. Impact of overweight and underweight on assisted reproduction treatment. Human reproduction (Oxford, England) 2004;19:2523-8.
603. Nelson SM, Klein BM, Arce JC. Comparison of antimüllerian hormone levels and antral follicle count as predictor of ovarian response to controlled ovarian stimulation in good-prognosis patients at individual fertility clinics in two multicenter trials. Fertil Steril 2015;103:923-30.e1.
604. Vaughan DA, Harrity C, Sills ES, Mocanu EV. Serum estradiol:oocyte ratio as a predictor of reproductive outcome: an analysis of data from >9000 IVF cycles in the Republic of Ireland. Journal of assisted reproduction and genetics 2016;33:481-8.
605. El-Shawarby SA, Khalaf Y. Age-specific serum FSH concentrations and their correlation with the outcome of ovarian stimulation for IVF. Reproductive BioMedicine Online 2009;18:750-5.
606. Wirleitner B, Okhowat J, Vištejnová L, et al. Relationship between follicular volume and oocyte competence, blastocyst development and live-birth rate: optimal follicle size for oocyte retrieval. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 2018;51:118-25.
607. Steward RG, Lan L, Shah AA, et al. Oocyte number as a predictor for ovarian hyperstimulation syndrome and live birth: an analysis of 256,381 in vitro fertilization cycles. Fertil Steril 2014;101:967-73.
608. Cobo A, Garrido N, Pellicer A, Remohí J. Six years' experience in ovum donation using vitrified oocytes: report of cumulative outcomes, impact of storage time, and development of a predictive model for oocyte survival rate. Fertility and Sterility 2015;104:1426-34.e8.
609. Majumder K, Gelbaya TA, Laing I, Nardo LG. The use of anti-Müllerian hormone and antral follicle count to predict the potential of oocytes and embryos. Eur J Obstet Gynecol Reprod Biol 2010;150:166-70.
610. Meczekalski B, Czyzyk A, Kunicki M, et al. Fertility in women of late reproductive age: the role of serum anti-Müllerian hormone (AMH) levels in its assessment. J Endocrinol Invest 2016;39:1259-65.
611. Barad DH, Weghofer A, Goyal A, Gleicher N. Age specific anti-müllerian hormone (AMH) levels discriminate at each age between poorer and better oocyte yields. Fertility and Sterility 2009;92:S101.
612. Zhang JJ, Feret M, Chang L, Yang M, Merhi Z. Obesity adversely impacts the number and maturity of oocytes in conventional IVF not in minimal stimulation IVF. Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology 2015;31:409-13.
613. Christensen MW, Ingerslev HJ, Degn B, Kesmodel US. Effect of Female Body Mass Index on Oocyte Quantity in Fertility Treatments (IVF): Treatment Cycle Number Is a Possible Effect Modifier. A Register-Based Cohort Study. PLOS ONE 2016;11:e0163393.

614. Simón C, Cano F, Valbuena D, Remohí J, Pellicer A. Clinical evidence for a detrimental effect on uterine receptivity of high serum oestradiol concentrations in high and normal responder patients. Human reproduction (Oxford, England) 1995;10:2432-7.
615. Delvigne A, Rozenberg S. Epidemiology and prevention of ovarian hyperstimulation syndrome (OHSS): a review. Human reproduction update 2002;8:559-77.
616. Ocal P, Sahmay S, Cetin M, Irez T, Guralp O, Cepni I. Serum anti-Müllerian hormone and antral follicle count as predictive markers of OHSS in ART cycles. Journal of assisted reproduction and genetics 2011;28:1197-203.
617. Aghssa MM, Tarafdari AM, Tehraninejad ES, et al. Optimal cutoff value of basal anti-mullerian hormone in iranian infertile women for prediction of ovarian hyper-stimulation syndrome and poor response to stimulation. Reprod Health 2015;12:85.
618. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C. Comparison of “triggers” using leuprolide acetate alone or in combination with low-dose human chorionic gonadotropin. Fertility and Sterility 2011;95:2715-7.
619. O'Neill KE, Senapati S, Maina I, Gracia C, Dokras A. GnRH agonist with low-dose hCG (dual trigger) is associated with higher risk of severe ovarian hyperstimulation syndrome compared to GnRH agonist alone. Journal of assisted reproduction and genetics 2016;33:1175-84.
620. Golan A, Ron-el R, Herman A, Soffer Y, Weinraub Z, Caspi E. Ovarian hyperstimulation syndrome: an update review. Obstetrical & gynecological survey 1989;44:430-40.
621. Jones BP, Al-Chami A, Gonzalez X, et al. Is oocyte maturity influenced by ovulation trigger type in oocyte donation cycles? Human fertility (Cambridge, England) 2019:1-7.
622. Wennberg AL, Schildauer K, Brännström M. Elective oocyte freezing for nonmedical reasons: a 6-year report on utilization and in vitro fertilization results from a Swedish center. Acta Obstet Gynecol Scand 2019;98:1429-34.
623. Gürtin ZB, Morgan L, O'Rourke D, Wang J, Ahuja K. For whom the egg thaws: insights from an analysis of 10 years of frozen egg thaw data from two UK clinics, 2008–2017. Journal of assisted reproduction and genetics 2019;36:1069-80.
624. Nekkebroeck J, Stoop D, Devroey P. A preliminary profile of women opting for oocyte cryopreservation for non-medical reasons. Human reproduction (Oxford, England) 2010;25:i15-i6.
625. Wada T, Kawada K, Takahashi R, et al. ICG fluorescence imaging for quantitative evaluation of colonic perfusion in laparoscopic colorectal surgery. Surg Endosc 2017;31:4184-93.
626. Liu Z, Li Z, Peng X, et al. [Multivariable analysis for flap-related complications in autologous breast reconstruction and economic analysis of intraoperative indocyanine green angiography]. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi 2020;34:92-7.
627. Kisu I, Banno K, Mihara M, et al. Indocyanine green fluorescence imaging for evaluation of uterine blood flow in cynomolgus macaque. PLoS One 2012;7:e35124.
628. Keane TJ, Swinehart IT, Badylak SF. Methods of tissue decellularization used for preparation of biologic scaffolds and in vivo relevance. Methods (San Diego, Calif) 2015;84:25-34.
629. House M, Sanchez CC, Rice WL, Socrate S, Kaplan DL. Cervical tissue engineering using silk scaffolds and human cervical cells. Tissue engineering Part A 2010;16:2101-12.
630. Ding JX, Chen XJ, Zhang XY, Zhang Y, Hua KQ. Acellular porcine small intestinal submucosa graft for cervicovaginal reconstruction in eight patients with malformation of the uterine cervix. Human reproduction (Oxford, England) 2014;29:677-82.
631. Shen F, Zhang XY, Yin CY, Ding JX, Hua KQ. Comparison of small intestinal submucosa graft with split-thickness skin graft for cervicovaginal reconstruction of congenital vaginal and cervical aplasia. Human reproduction (Oxford, England) 2016;31:2499-505.
632. De Filippo RE, Bishop CE, Filho LF, Yoo JJ, Atala A. Tissue engineering a complete vaginal replacement from a small biopsy of autologous tissue. Transplantation 2008;86:208-14.
633. Ding JX, Chen LM, Zhang XY, Zhang Y, Hua KQ. Sexual and functional outcomes of vaginoplasty using acellular porcine small intestinal submucosa graft or laparoscopic peritoneal vaginoplasty: a comparative study. Human reproduction (Oxford, England) 2015;30:581-9.
634. Ho MH, Heydarkhan S, Vernet D, et al. Stimulating vaginal repair in rats through skeletal muscle-derived stem cells seeded on small intestinal submucosal scaffolds. Obstetrics and gynecology 2009;114:300-9.
635. Zhang JK, Du RX, Zhang L, et al. A new material for tissue engineered vagina reconstruction: Acellular porcine vagina matrix. Journal of biomedical materials research Part A 2017;105:1949-59.
636. Miyazaki K, Maruyama T. Partial regeneration and reconstruction of the rat uterus through recellularization of a decellularized uterine matrix. Biomaterials 2014;35:8791-800.

637. Ding L, Li X, Sun H, et al. Transplantation of bone marrow mesenchymal stem cells on collagen scaffolds for the functional regeneration of injured rat uterus. Biomaterials 2014;35:4888-900.
638. Song T, Zhao X, Sun H, et al. Regeneration of uterine horns in rats using collagen scaffolds loaded with human embryonic stem cell-derived endometrium-like cells. Tissue engineering Part A 2015;21:353-61.
639. Xu L, Ding L, Wang L, et al. Umbilical cord-derived mesenchymal stem cells on scaffolds facilitate collagen degradation via upregulation of MMP-9 in rat uterine scars. Stem cell research & therapy 2017;8:84.
640. Zhao G, Cao Y, Zhu X, et al. Transplantation of collagen scaffold with autologous bone marrow mononuclear cells promotes functional endometrium reconstruction via downregulating ΔNp63 expression in Asherman's syndrome. Sci China Life Sci 2016.
641. Cao Y, Sun H, Zhu H, et al. Allogeneic cell therapy using umbilical cord MSCs on collagen scaffolds for patients with recurrent uterine adhesion: a phase I clinical trial. Stem cell research & therapy 2018;9:192.
642. Schutte SC, James CO, Sidell N, Taylor RN. Tissue-engineered endometrial model for the study of cell-cell interactions. Reproductive sciences (Thousand Oaks, Calif) 2015;22:308-15.
643. Schutte SC, Taylor RN. A tissue-engineered human endometrial stroma that responds to cues for secretory differentiation, decidualization, and menstruation. Fertil Steril 2012;97:997-1003.
644. Lu SH, Wang HB, Liu H, et al. Reconstruction of engineered uterine tissues containing smooth muscle layer in collagen/matrigel scaffold in vitro. Tissue engineering Part A 2009;15:1611-8.
645. Wang H, Pilla F, Anderson S, et al. A novel model of human implantation: 3D endometrium-like culture system to study attachment of human trophoblast (Jar) cell spheroids. Mol Hum Reprod 2012;18:33-43.
646. MacKintosh SB, Serino LP, Iddon PD, et al. A three-dimensional model of primary bovine endometrium using an electrospun scaffold. Biofabrication 2015;7:025010.
647. Park DW, Choi DS, Ryu HS, Kwon HC, Joo H, Min CK. A well-defined in vitro three-dimensional culture of human endometrium and its applicability to endometrial cancer invasion. Cancer letters 2003;195:185-92.
648. Campbell GR, Turnbull G, Xiang L, et al. The peritoneal cavity as a bioreactor for tissue engineering visceral organs: bladder, uterus and vas deferens. Journal of tissue engineering and regenerative medicine 2008;2:50-60.
649. Lin N, Li X, Song T, et al. The effect of collagen-binding vascular endothelial growth factor on the remodeling of scarred rat uterus following full-thickness injury. Biomaterials 2012;33:1801-7.
650. Li X, Sun H, Lin N, et al. Regeneration of uterine horns in rats by collagen scaffolds loaded with collagen-binding human basic fibroblast growth factor. Biomaterials 2011;32:8172-81.
651. Su J, Ding L, Cheng J, et al. Transplantation of adipose-derived stem cells combined with collagen scaffolds restores ovarian function in a rat model of premature ovarian insufficiency. Human reproduction (Oxford, England) 2016;31:1075-86.
652. Alawadhi F, Du H, Cakmak H, Taylor HS. Bone Marrow-Derived Stem Cell (BMDSC) transplantation improves fertility in a murine model of Asherman's syndrome. PLoS One 2014;9:e96662.
653. Cervello I, Gil-Sanchis C, Santamaria X, et al. Human CD133(+) bone marrow-derived stem cells promote endometrial proliferation in a murine model of Asherman syndrome. Fertil Steril 2015;104:1552-60.e1-3.
654. Watt FM, Huck WT. Role of the extracellular matrix in regulating stem cell fate. Nature reviews Molecular cell biology 2013;14:467-73.
655. Keane TJ, Badylak SF. The host response to allogeneic and xenogeneic biological scaffold materials. Journal of tissue engineering and regenerative medicine 2015;9:504-11.
656. Brown BN, Valentin JE, Stewart-Akers AM, McCabe GP, Badylak SF. Macrophage phenotype and remodeling outcomes in response to biologic scaffolds with and without a cellular component. Biomaterials 2009;30:1482-91.
657. Young RC, Goloman G. Allo- and xeno-reassembly of human and rat myometrium from cells and scaffolds. Tissue engineering Part A 2013;19:2112-9.
658. Santoso EG, Yoshida K, Hirota Y, et al. Application of detergents or high hydrostatic pressure as decellularization processes in uterine tissues and their subsequent effects on in vivo uterine regeneration in murine models. PLoS One 2014;9:e103201.
659. Hellstrom M, El-Akouri RR, Sihlbom C, et al. Towards the development of a bioengineered uterus: comparison of different protocols for rat uterus decellularization. Acta biomaterialia 2014;10:5034-42.
660. Padma AM, Tiemann TT, Alshaikh AB, Akouri R, Song MJ, Hellstrom M. Protocols for Rat Uterus Isolation and Decellularization: Applications for Uterus Tissue Engineering and 3D Cell Culturing. Methods Mol Biol 2017.
661. Campo H, Baptista PM, Lopez-Perez N, Faus A, Cervello I, Simon C. De- and recellularization of the pig uterus: a bioengineering pilot study. Biol Reprod 2017;96:34-45.
662. Kong D, Zhang L, Xu X, Zhang J, Li Y, Huang X. Small Intestine Submucosa Is a Potential Material for Intrauterine Adhesions Treatment in a Rat Model. Gynecologic and obstetric investigation 2018;83:499-507.

663. Beckmann MW, Lotz L, Toth B, et al. Concept Paper on the Technique of Cryopreservation, Removal and Transplantation of Ovarian Tissue for Fertility Preservation. Geburtshilfe Frauenheilkd 2019;79:53-62.
664. Martinez F. Update on fertility preservation from the Barcelona International Society for Fertility Preservation-ESHRE-ASRM 2015 expert meeting: indications, results and future perspectives. Human reproduction (Oxford, England) 2017;32:1802-11.
665. Salama M, Woodruff TK. New advances in ovarian autotransplantation to restore fertility in cancer patients. Cancer Metastasis Rev 2015;34:807-22.
666. Jensen AK, Rechnitzer C, Macklon KT, et al. Cryopreservation of ovarian tissue for fertility preservation in a large cohort of young girls: focus on pubertal development. Human reproduction (Oxford, England) 2017;32:154-64.
667. Goldhirsch A, Gelber RD, Castiglione M. The magnitude of endocrine effects of adjuvant chemotherapy for premenopausal breast cancer patients. Annals of Oncology 1990;1:183-8.
668. Van der Ven H, Liebenthron J, Beckmann M, et al. Ninety-five orthotopic transplantations in 74 women of ovarian tissue after cytotoxic treatment in a fertility preservation network: tissue activity, pregnancy and delivery rates. Human reproduction (Oxford, England) 2016;31:2031-41.
669. Provoost V, Tilleman K, D'Angelo A, et al. Beyond the dichotomy: a tool for distinguishing between experimental, innovative and established treatment. Human reproduction (Oxford, England) 2014;29:413-7.
670. Lotz L, Dittrich R, Hoffmann I, Beckmann MW. Ovarian Tissue Transplantation: Experience From Germany and Worldwide Efficacy. Clin Med Insights Reprod Health 2019;13:1179558119867357-.
671. Jensen AK, Macklon KT, Fedder J, Ernst E, Humaidan P, Andersen CY. 86 successful births and 9 ongoing pregnancies worldwide in women transplanted with frozen-thawed ovarian tissue: focus on birth and perinatal outcome in 40 of these children. Journal of assisted reproduction and genetics 2017;34:325-36.
672. Pacheco F, Oktay K. Current Success and Efficiency of Autologous Ovarian Transplantation: A Meta-Analysis. Reproductive sciences (Thousand Oaks, Calif) 2017;24:1111-20.
673. Takae S, Suzuki N. Current state and future possibilities of ovarian tissue transplantation. Reprod Med Biol 2019;18:217-24.
674. Beckmann MW, Dittrich R, Lotz L, et al. Fertility protection: complications of surgery and results of removal and transplantation of ovarian tissue. Reprod Biomed Online 2018;36:188-96.
675. Kristensen SG, Andersen CY. Cryopreservation of ovarian tissue: opportunities beyond fertility preservation and a positive view into future. Frontiers in Endocrinology 2018;9:347.
676. Dolmans MM, Masciangelo R. Risk of transplanting malignant cells in cryopreserved ovarian tissue. Minerva Ginecol 2018;70:436-43.
677. Jadoul P, Guilmain A, Squifflet J, et al. Efficacy of ovarian tissue cryopreservation for fertility preservation: lessons learned from 545 cases. Human reproduction (Oxford, England) 2017;32:1046-54.
678. Dolmans MM, Jadoul P, Gilliaux S, et al. A review of 15 years of ovarian tissue bank activities. Journal of assisted reproduction and genetics 2013;30:305-14.
679. Silber S. Ovarian tissue cryopreservation and transplantation: scientific implications. Journal of assisted reproduction and genetics 2016;33:1595-603.