**Prevalence of abnormal semen analysis and levels of adherence with fertility preservation in men undergoing therapy for newly diagnosed cancer: A retrospective study in 2906 patients**

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<td>Ramsay, Jonathan; Hammersmith Hospital, Department of Andrology</td>
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<td>Dhillo, Waljit; Imperial College Faculty of Medicine, Department of Metabolic Medicine</td>
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Title: Prevalence of abnormal sperm function and levels of adherence with fertility preservation in men undergoing therapy for newly diagnosed cancer: A retrospective study in 2906 patients

Running title: Sperm freezing in male cancer

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Keywords: Sperm / cryopreservation / infertility / cancer
Abstract

**Background:** Sperm cryopreservation (freezing) should be offered to all men with cancer due to risk of infertility. However, many men with cancer already have impaired spermatogenesis prior to sperm cryopreservation. Furthermore, physical ill-health may hinder attendance of freeze visits. Investigating both the distribution of sperm function and freeze attendance rates in men with newly diagnosed cancer, may identify patients benefiting from targeted reproductive fertility support.

**Methods:** We performed a retrospective study of 2906 male patients undergoing sperm cryopreservation prior to cancer therapy at a single UK tertiary centre between 1989 and 2013; all patients were asked to attend three hospital semen collection visits prior to cancer therapy.

**Results:** Fifteen percent (433/2906) of men with newly diagnosed cancer had had severely impaired semen quality (i.e. sperm total motile count, TMC<1 million) during the first semen collection visit. However, patients with severely impaired semen quality had the poorest attendance of subsequent semen collection visits despite being requested to do so (non-attendance in TMC < 1 million: 43.4%; TMC<1-30 million; 35.7%, P<0.05 vs. <1million; TMC>30 million: 33.2%, P<0.01 vs. <1million).

**Conclusions:** This study is the first to investigate the ability of men with cancer to adhere to fertility preservation recommendations. Our data suggest that patients with the poorest semen quality paradoxically suffer the poorest attendance rates of sperm cryopreservation appointments prior to commencing cancer therapy. We suggest that additional support may be of clinical benefit to men with newly diagnosed cancer and TMC<1million sperm.
Introduction

Over 12,000 men aged 16 to 49 are diagnosed with cancer in the United Kingdom (UK) annually. Infertility is one of the most devastating long-term consequences of cancer treatment, which may be permanent in 15-30% of cases.

Sperm cryopreservation is recommended for all men of reproductive age prior to undergoing treatment for cancer that may compromise fertility. The Royal College of Physicians and NICE recommend at least two semen samples be collected for banking and that is widely available across the NHS. Similarly, the Ethics Committee of American Society for Reproductive Medicine do not specify the number of samples required but the Department of Urology in Houston, Texas recommends banking of at least three semen samples for optimal semen quality to be cryopreserved. As several insurance companies do not cover sperm cryopreservation, patients in North America may experience financial limitations in sperm banking. Nevertheless in the UK, fertility preservation is regulated by a public healthcare system, and although it would be expected that men would utilize all opportunities to preserve their fertility, that is not actually observed. The underlying reasons remain unclear.

Cryopreserved sperm may be later used during assisted reproductive technology (ART) to allow infertile survivors of cancer to father children with their partners. While most men with newly diagnosed cancer have reduced sperm function, approximately a third of patients have a normal semen profile as defined by the World Health Organization (WHO). Men with newly diagnosed cancer therefore represent a heterogeneous group consisting of men with either normal or impaired fertility. It is plausible that some men with newly diagnosed cancer would particularly benefit from additional support with decisions around sperm cryopreservation.

We performed a retrospective analysis of 2906 patients with complete records undergoing sperm cryopreservation between 1989 and 2013 at a single tertiary centre. Our study aimed to investigate distribution of semen quality, cumulative yields of sperm collected during cryopreservation appointments, and attendance...
rates of semen collection visits in post-pubertal men with a new diagnosis of cancer undergoing cryopreservation.
Materials and methods

Approvals and subjects: The Department of Andrology, Hammersmith Hospital, UK is regulated by the Human Fertilisation and Embryology Authority (HFEA). A database held by the Department of Andrology was used in this audit study. This revealed 2932 patients undergoing sperm cryopreservation for cancer treatment between 1989 and 2013, with 2906 patients having complete records of seminal parameters. The mean age of the population was 31.4 years (range 13.4-73.8).

Semen collection and analysis: In North West London, each patient is referred by his Oncologist and invited to walk in to our Department for sperm cryopreservation before cancer treatment and more precisely before orchidectomy in cases of testicular cancer. Most semen samples are collected pre-treatment, although a minority are collected within four weeks of treatment before sperm quality substantively reduces following chemotherapy or radiotherapy. Three visits for sperm banking are offered to cryopreserve a maximum of three samples of semen. There is flexibility in booking appointments according to personal preferences and departmental availability. All semen samples received complete WHO semen analysis prior to cryopreservation using standard methods.

Data collection: Sperm parameters from each visit were recorded including sperm concentration, percentage motility and semen volume. Total motile sperm count (TMC) was calculated for each patient per sample [sperm conc. (million/ml) × percentage total motility (%) × semen volume (ml)]. Sperm TMC is an established marker of male reproductive function, which is commonly used for clinical decision making in fertility units performing ART. Furthermore, a recent study suggested that TMC is a better predictor of spontaneous pregnancy than WHO parameters.

Data analysis: Analysis of variance (ANOVA) with Tukey’s post-hoc analysis was used to compare mean numeric variables. Fisher’s exact test or Chi squared was used to compare proportions between two and more than two groups, respectively. P<0.05 was considered significant. Cumulative motile sperm cryopreserved was
calculated for each patient by summing the TMC for all semen collection visits attended. Data are means ± SD unless stated otherwise.
Results

Characteristics of men with newly diagnosed cancer:

Of 2906 men with newly diagnosed cancer undergoing sperm cryopreservation, 34% had testicular cancer, 24% had lymphoma, 15% had leukaemia and 27% had other types of cancer (Supplemental Table 1).

Sperm characteristics in men with newly diagnosed cancer:

Visit 1: Histogram analysis revealed a large variation in sperm quality among in men with newly diagnosed cancer attending their initial semen collection visit (sperm TMC, 74.6 ± 119.1 million) (Figure 1A). The most frequently observed TMC range (regardless of tumor type) during Visit 1 was 30-100 million (Supplemental Figure 1), which was observed in 856/2906 (29.5%) patients. However, 14.9% (433/2906) men with newly diagnosed cancer had sperm TMC < 1 million during Visit 1, suggesting severely impaired fertility. TMC < 1 million was the second most prevalent category of sperm quality in the leukaemia group; the 3rd most prevalent category in testicular tumors, and the 4th most prevalent category in lymphoma and other forms of cancer. Patient age did not correlate with sperm TMC during Visit 1 (Supplemental Figure 2A).

Visits 2 and 3: Histogram analysis suggested that sperm quality during semen collections Visits 2 and 3 was similar when compared with Visit 1 (Figure 1B & 1C). Like Visit 1, the most frequently observed TMC range was 30-100 million during Visit 2 and Visit 3. TMC < 1 million was observed in 14.0% of patients during Visit 2 and 12.3% during Visit 3 (P=ns vs. Visit 2). Seventy-six percent of men with TMC < 1 million during Visit 1, also had a TMC < 1 million during Visit 2 (Supplemental Table 2); however, the remaining 24% had TMC > 1 million TMC during Visit 2. A minority of men with a TMC < 1 million during Visit 1 (4.1% ), achieved a TMC over 30 million during the second visit.
Attendance of semen collection visits:

All patients were asked to attend three semen collection visits prior to treatment for cancer. However, only 1099 of the 2906 (37.8%) attended all three visits; 733 (26.6%) patients attended two visits and 1034 (35.6%) attended only the first visit (Figure 2). Patients with the poorest semen quality should benefit more from attending scheduled multiple freeze visits when compared with less severely affected patients. However, non-attendance during Visit 2 was significantly higher in patients with TMC < 1 million (43.4%) when compared with TMC 1-30 million (35.7%, P<0.05) and TMC >30 million (33.2%, P<0.01) using chi-squared test (Figure 3). Histogram analysis was performed to investigate attendance rates in men with testicular cancer, lymphoma, leukaemia and other tumour types (Supplemental Figure 3). A TMC of 30-100 million sperm was the most frequently observed outcome from men with newly diagnosed, regardless of tumour type. Attendance was observe to reduce by 40-60% between visits 1-2 and visits 2-3, regardless of tumour type.

The effects of multiple semen collection visits on cumulative sperm yield in men with newly diagnosed cancer:

The purpose of multiple semen collection visits is for cumulative sperm yield which increases the likelihood of preserving high quality sperm for future use in ART. A greater cumulative sperm quantity also allows for flexibility in the choice of ART and use in multiple cycles if needed. Published studies suggests that 1 million motile sperm are required for optimal outcomes during intracytoplasmic sperm injection (ICSI) \(^{17-19}\). It is therefore highly likely that a minimum of 30 million sperm is adequate for multiple cycles of subsequent ART. Following the first semen collection visit (Visit 1), 1527 of all 2906 (52.5%) patients managed to cryopreserve > 30 million motile sperm. Following the second semen collections visit, 1805 of all 2906 (62.1%) patients had cryopreserved > 30 million motile sperm in total when visits 1 and 2 were combined. Following the third semen collections visit, 1881 of all 2906 (64.7%) patients had cryopreserved > 30 million motile sperm in total during when visits 1 to 3 were combined.
The effects of multiple semen collection visits on cumulative sperm yield in patients with cancer and severely impaired semen quality (TMC<1 million):

We investigated to what extent patients with the poorest sperm function during Visit 1, benefited from attending subsequent semen collection visits. Four hundred and thirty-three patients cryopreserved fewer than 1 million motile sperm during Visit 1.

Visit 2: Nearly half of patients (44.1%; 188/433) cryopreserving < 1 million motile sperm during Visit 1, did not attend any further semen collection visits despite being informed to do so. Fifteen percent (66/433) of patients cryopreserving < 1 million motile sperm during Visit 1, benefited from Visit 2 by cryopreserving > 1 million motile sperm when Visits 1 and 2 were combined; the remaining 41.3% (179/433) of patients had still cryopreserved fewer than 1 million motile sperm when Visits 1 and 2 were combined.

Visit 3: Over half of patients (50.8%; 91/179) cryopreserving cumulatively < 1 million motile sperm during Visits 1 and 2, did not attend semen collection Visit 3 despite being asked to do so; Only a small proportion (7.8%; 14/179) of patients cryopreserving cumulatively less than 1 million motile sperm during Visit 1 and 2, managed to cryopreserve > 1 million motile sperm when Visits 1 to 3 were combined. The remaining 41.3% of these patients had cryopreserved fewer than 1 million motile sperm when Visits 1 to 3 were combined.
Discussion

Current guidelines recommend that men with newly diagnosed cancer are offered multiple semen collection visits, to maximize the future chances of conception should they suffer long-term infertility\(^4,6,7\). However, our study suggests that many with newly diagnosed cancer do not attend more than one semen collection visit, despite recommendations to do so. Additionally, our study suggests that 12-15% of men with newly diagnosed cancer have a sperm TMC <1 million; but these men have a paradoxically worse attendance rate of repeat sperm banking visits when compared with men whose semen quality is less severely affected. We suggest that a subgroup of men with newly diagnosed cancer and the poorest semen quality may benefit from targeted management by clinics offering fertility cryopreservation.

A range of factors are known to affect the semen quality of men with newly diagnosed cancer\(^11,20,21\). Testicular tumours are associated with the lowest mean sperm count when compared with other major tumour types\(^12,22,23\) due to negative effects on spermatogenesis\(^24\). The severity of physical and systemic illness experienced by patients may also affect sperm quality; accordingly, leukaemia has the highest risk of azoospermia especially when compared with other cancers\(^25\). Furthermore, a host of environmental, lifestyle and socioeconomic factors have now been implicated in human semen quality\(^26–28\). Specific lifestyle factors include body mass index, smoking status, and recreational drug use\(^29–31\). Consistent with this previous data, we observed that a considerable proportion of men with newly diagnosed cancer have less than 1 million sperm TMC.

Clinical guidelines differ in recommendation for frequency of semen collection from male cancer patients prior to cancer treatment. A joint guideline by the Royal College of Physicians published in 2014 recommends two or three samples for storage\(^4\), whereas the Ethics Committee of American Society for Reproductive Medicine do not specify the number of samples required\(^6\). Remarkably, unlimited sperm cryopreservation visits do not increase attendance rates and further supportive mechanisms are required for men with impaired semen quality associated with cancer. Men may be traumatized by their diagnosis and irrespective of financial cover for sperm banking, they may simply require timely information and decisive appointments booking. In the cases of
oligospermia and poor semen quality every effort should be made to bank sperm with less common and even
investigative methods if locally available. Infertile men without cancer presenting with sperm TMC less than 1 million would routinely require intracytoplasmic sperm injection (ICSI), since the chances of natural conception would be extremely low. Unlike intrauterine insemination and in-vitro fertilization, intracytoplasmic sperm injection (ICSI) ultimately requires only a single sperm to fertilise each mature oocyte harvested following superovulation. Unsurprisingly, studies have suggested that there is no lower quantifiable lower limit of sperm TMC for successful ICSI. However, sperm concentrations exceeding 1 million have been associated with optimal fertilization rates for ICSI. In our study population during their first sample collection visit, 70% of patients exceeded 10 million motile sperm per ejaculate, which is 10-fold higher than the TMC threshold required for ICSI. Results from this study therefore suggest that most patients undergoing cancer treatment were successful in collecting a sufficient motile sperm count in their first visit. Another 7.9% of patients adding to a total of 77.9%, were likely to achieve satisfactory TMC levels after multiple visits. Though not assessed in the current study, post-thaw sperm viability has been reported as high as 30-50% in men with cancer. Unfortunately, there is little agreement on the effect of specific cancers on post-thaw quality. This disparity has been noted in testicular cancer and Hodgkin's lymphoma patients. However, studies generally agree; those patient groups with poor recoveries are the same groups who present with the poorest prefreeze semen quality.
Collectively, these published data suggest that patients cryopreserving less than 1 million motile sperm may be storing insufficient sperm for ICSI with optimum outcomes. Further work is needed to determine if live birth outcomes (naturally or through ART) would be improved by offering enhanced fertility preservation support for men with newly diagnosed cancer and less than 1 million motile sperm.
We observed that approximately 35.6% of men with newly diagnosed cancer did not attend more than one semen collection visit. It is important to consider these results in the context of the type of semen collection service offered to participants during the current study; patients were offered next day, cost-free sperm freezing
on a walk-in basis. It is therefore unlikely that the results are explained by limitation of access to sperm freezing. We observed that in men with newly diagnosed cancer, those with the poorest sperm quality (sperm TMC <1 million) had significantly lower attendance rates for further sperm freezing visits when compared with other patients. Sperm concentrations < 1 million are associated with reduced fertilization rates during ICSI \(^1\). It is therefore important to consider that one quarter of men with newly diagnosed cancer and TMC < 1 million during Visit 1, achieved TMC > 1 million sperm during Visit 2; furthermore, 4.1% of these patients with severely reduced sperm function during Visit 1, achieved a TMC > 30 million sperm during visit 2. Our data therefore suggest that a minority of patients with severely reduced sperm function will benefit from attending a second sperm collection visit. Men should be counselled by clinicians about the likely benefits of attending a second sperm collection visit, which should be weighed against other factors such as the distress of a new diagnosis and the stress of being asked to return to produce semen samples on multiple occasions.

Spermatogenesis may be viewed as a biomarker of men’s health, so it is plausible that men with the poorest sperm quality had more aggressive cancer or needed more urgent treatment limiting the time available for cryopreservation. Previous research has additionally demonstrated that a patient’s decision to bank or not to bank sperm is complex \(^39,40\) and is usually positive in younger patients, with better health-related quality of life and more likely to desire a child in the future \(^41\). Advice given by oncologists is essential and it appears that patients are more likely to bank sperm when they hold optimistic clinic consultations before banking \(^39\). Essentially, adherence to male fertility preservation in oncology lies on the same values, beliefs and concerns as any other treatment for chronic illness \(^42\). Clinicians should therefore explain the benefits of sperm banking to maintain fertility so that men can weigh these against their concerns and be able to make shared, informed decisions. Additional work is needed to determine if patients with severe cancer disease burden and very poor sperm function patients with TMC <1 million would benefit from targeted communications to improve attendance of multiple semen collection visits and increase total sperm yield.

The usage rate of semen samples stored in our bank \(^43\) is low at 6%, similar to those reported from other centres.
ICS! is preferred over other ART for frozen/thawed sperm and requires a minimum TMC of 1 million for successful outcome. Even though it is beyond the scope of our study to investigate correlations between sperm counts on initial sperm banking and ART utilisation rates, we highlight an important group of men with poor attendance at sperm collection visits. We also noted that some men with newly diagnosed cancer produced far in excess of the 1 million TMC, a greater proportion of men store high quality samples at their first visit and they do not need to come back for more visits. Further consultations are needed for men with low sperm count to decide on best management approach, take emotional cost into consideration and possibly refer to fertility clinic counselling.

**Strengths and Limitations:** This study benefited from a large sample size and was performed at a single centre. However, the population of cancer patients at this centre may not reflect the population of cancer patients in other cryopreservation centres. Although it is best practice to refer all men with newly diagnosed cancer for sperm banking, several clinicians may not refer all suitable patients and similarly not all men offered the option to bank sperm are able to attend their first visit making their assessment in the context of this study impossible. The study was performed in a department serving a large number of different IVF units, so we lack reliable data on confirmed ART cycles and fertility outcomes; it would be interesting to consider whether men with newly diagnosed cancer and a TMC < 1million are more or less likely to use their samples for ART, or have successful ART, when compared with other men with cancer. The study was conducted retrospectively within a cost-free National Health Service in the UK. Therefore, prospective validation in private health systems could be insightful. Finally, it would be interesting to extend upon the findings of this study to account for clinical pregnancy rates in cancer patients. Our study focuses on the treatment of new patients with cancer, the amount of sperm stored, and the number of sperm cryopreservation visits attended. Finally, our study does not investigate the reasons and motivations of patients attending versus non-attending semen collection visits, which warrant future qualitative studies. However, it is reasonable to speculate that poor hospital attendance is likely to indicate significant physical ill-health related to cancer.
Conclusions: In summary, we have conducted a large study investigating the characteristic of patients attending multiple semen collection visits prior to cancer therapy. Our data suggest that many patients can only attend a single semen collection visit. Furthermore, men with newly diagnosed cancer commonly present with severely reduced semen quality, but these patients may face particular challenges attending semen collection visits. Further work is needed to determine if men with newly diagnosed cancer and the poorest sperm quality, would benefit from targeted fertility preservation management. A universal policy of multiple semen collection visits in association with the ability to comply in a cost-free healthcare setting was thoroughly presented for the first time in the literature. More than half of men (52.5%) produce sufficient numbers of sperm during their first semen collection visit. However, attending multiple visits can be a challenge for a considerable number of patients with a new diagnosis of cancer. Results of this study may have potential therapeutic implications as they suggest that men with newly diagnosed cancer would benefit from a more individualized approach to fertility preservation.
Acknowledgements and Funding

The Section of Endocrinology and Investigative Medicine is funded by grants from the MRC, BBSRC, NIHR and is supported by the NIHR Biomedical Research Centre Funding Scheme. The views expressed are those of the author(s) and not necessarily those of NHS, the NIHR or the Department of Health. CNJ is funded by an NIHR Post-Doctoral Fellowship. WSD is funded by an NIHR Research Professorship. The authors are indebted to all staff within Department of Andrology for their assistance and support.

Conflict of interest

None declared
References


Figure Legends.

Figure 1. Sperm yields during each semen collection visit in men with cancer undergoing sperm cryopreservation. Histogram classifying patients by the total motile sperm count (TMC) observed during Visit 1 (A), Visit 2 (B), Visit 3 (C). Bars in green, grey and red denote TMC > 30, 1-30 and < 1 million sperm, respectively.

Figure 2. Attendance of semen collection visits in men with cancer undergoing sperm cryopreservation. The flow chart summarizes the number of patients who attended each visit (attendance) and the number of patients who failed to attend each visit (non-attendance).

Figure 3. Attendance for sperm freezing when stratified by sperm count, in men with cancer undergoing sperm cryopreservation. Attendance of semen collection Visit 2 was analysed for all patients when stratified by their total motile sperm count during their previous visit (Visit 1). Proportions were compared using Chi Squared test. * P<0.05. ** P<0.01.
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**Supplemental Table 1: Levels of total motile sperm count (TMC) in patients with different forms of cancer.** Total motile sperm count (TMC) = sperm conc. (million/ml)

*percentage total motility (%) * semen volume (ml).

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Supplemental Table 2: Sperm yield during second sperm collection visit, in patients with severely reduced sperm quality during visit 1. Results are shown during visit 2, in men who had a total motile sperm count (TMC) < 1 million during Visit 1.

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Supplemental Figure Legends.

Supplemental Figure 1. Sperm yield during the first semen collection visit in men with cancer undergoing sperm cryopreservation. Histogram classifying patients by the total motile sperm count (TMC) observed according to tumour types: testicular cancer (A), lymphoma (B), leukaemia (C) and other cancers (D).

Supplemental Figure 2. Age is not a determinant for sperm yield or attendance in men with cancer undergoing sperm cryopreservation. (A) Scatterplot showing the correlation between the patient’s age with the observed total motile sperm count (TMC) during sperm cryopreservation. (B) Bar graph showing the mean age of men with cancer who attended only one visit, two visits or all three sperm cryopreservation visits. The error bars denote standard deviation of mean.

Supplemental Figure 3. Attendance of semen collection visits in men with cancer undergoing sperm cryopreservation classified by tumour types. The bar graph shows the number of men who attended only one visit, two visits or three visits by tumour type: testicular cancer (A), lymphoma (B), leukaemia (C) and other cancers (D).
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We are grateful to the reviewers for the positive and constructive comments. We have addressed the reviewers’ comments as follows:

**Response to Reviewer 1:**

**Comments:**

**REVIEWER 1.** Title still refers to infertility, which is incorrect. A more suitable title would be 'Prevalence of abnormal semen analysis and levels of ....'

**AUTHORS 1:** We have modified the title as follows:

‘Prevalence of abnormal sperm function and levels of adherence with fertility preservation in men undergoing therapy for newly diagnosed cancer: A retrospective study in 2906 patients.’

Lines 1-2

**REVIEWER 2.** Conclusion of abstract: this is NOT the first study reporting semen quality in men with cancer (it has been studied for decades). It is irritating to read such claims and makes the reader think that the authors do not know how much has been done in this field before.

**AUTHORS 2:** We apologise for the confusion. The conclusion was not intended to imply there had been no previous studies on semen quality in men with cancer; instead, we intended to highlight the novelty of studying semen quality in combination with compliance with fertility preservation recommendations. We have therefore revised the sentence as follows:

Original version: ‘This is the first study reporting semen quality of men with newly diagnosed cancer, and their ability to comply with fertility preservation recommendations’.

Revised version: ‘This study is the first to investigate the ability of men with cancer to adhere to fertility preservation recommendations.’ Page 3, Lines 42-43.

**Response to Reviewer 3:**

**REVIEWER 1.** Minor comment: typo in conclusions page 15, line 16, "2 of men produce sufficient numbers of sperm during their first semen collection visit." Please correct.

**AUTHORS 1:** We have modified the conclusions as follows:
‘More than half of men (52.5%) produce sufficient numbers of sperm during their first semen collection visit.’ Line 261 – 262