MICROVASCULAR OVARIAN TRANSPLANTATION AND STUDY OF FUNCTIONAL MORPHOLOGY OF THE TRANSPLANT IN THE RABBIT

(ANIMAL MODEL)

by

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A Thesis submitted for the Degree of Doctor of Philosophy

(in the Faculty of Medicine)
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October 1983

Dedicated

to

Hamid and the Children

STATEMENT OF ORIGINALITY

1. The technique of microsurgical transplantation of the ovary alone was developed by the author herself.

2. The author personally performed all the experimental operations, including the auto and allotransplantations, and the anaesthesia.

3. The collection and preparation of blood samples for radioimmunoassay, preparation and fixation of the tissue specimens for light and electron microscopical examination, and the study of the tissue sections, except when acknowledged, were carried out by the author.

4. The author prepared all the graphs and illustrations from the experimental data.

5. Mr. R. Baldy, of the Institute of Psychiatry, University of London, advised on the statistical analysis, which was carried out by the author herself.

ACKNOWLEDGEMENTS

I wish to thank Mr. R.M.L. Winston, in whose microsurgery laboratory the surgical experimental studies were carried out, and for his advice during the writing.

I owe a great deal to Profesor Sir John Dewhurst, Dean of the Institute of Obstetrics and Gynaecology, University of London, for his advice, encouragement and support.

I am grateful to Dr. K. Fotherby, Steroid Chemistry Department, Royal Postgraduate Medical School, for the progesterone assays. I also wish to thank A. Abramovei, Professor of Developmental Pathology, Department of Histochemistry, Royal Postgraduate Medical School, for his valuable comments on the histological studies.

I should also like to offer thanks to Mr. P. Moor, Institute of Obstetrics and Gynaecology, to Miss S. Melrose, of the Electron Microscopy Laboratory, for technical help in the microscopic studies, to Mr. W. Hinkes of the Microphotographic Department, and Mr. D. Simmonds, Chief Medical Artist, for allowing me to use the facilities of the Medical Art Unit. I would also like to thank Mr. R.E. Rickwood, Zoology Department, Royal Postgraduate Medical School, for help in the care of the experimental animals and to Mrs. Weller for her efforts in typing this Thesis.

This work was supported by studentship from the University of Baghdad, Iraq.

ABSTRACT

This Thesis reports on the assessment of the functional morphology of ovarian auto- and allografts and on the definition of the factors which influenced the evolution and fate of the grafts. The study also described a microsurgical method for the transplantation of the ovary in the rabbit (animal model).

The preliminary part of this Thesis was concerned with the separate assessment of the effects, on the ovary and animal fertility, of the major steps of the transplantation procedure; it also included experiments on the effect of the immunosuppressant Cyclosporin A on the normal ovary. The latter experiments were dictated by observations which emerged during the study of immunosuppressed allografted ovaries.

The second part of the study dealt with ovarian autotransplantation, and the factors which determine the outcome of the graft. It also dealt with allotransplantation of the ovary, where the role of the immunological processes, and the effect of Cyclosporin A on the survival and function of the allograft were investigated.

The results of the study included:

(a) 91.7% success rate in one group of the autograft, 66.7% of which achieved pregnancy. Second pregnancies were also achieved by the five animals which were followed for over a year.

(b) Successful transplantation of ovaries, across the major histo-compatibility antigens without immunosuppression, and viable functioning allografts with a successful pregnancy, were achieved. This totally

unexpected observation is believed to be probably related to the incorporation, in the transplantation procedure, of an immuno-manipulative step which was described and discussed in the Thesis.

(c) The gonadotoxic effects of Cyclosporin A, hitherto unrecognised, were described and the implications of that to the clinical use of the drug indicated. Moreover, the study showed that, in contrast to previous reports, Cyclosporin A neither produced graft acceptance nor provided extended immunosuppression after withdrawal of the drug.

In addition, many physiological and experimental observations found confirmation in the results of the transplantation experiments. These were indicated, where appropriate, throughout the Thesis.

Finally, a conclusive discussion was presented in which the potential application of ovarian transplantation in the treatment of human infertility was discussed, the relevance of many of the findings to certain clinical problems indicated and some proposals for future research suggested.

CONTENTS

		Page
TITLE	.....	1
DEDICATION	.....	2
STATEMENT OF ORIGINALITY	.....	3
ACKNOWLEDGEMENTS	.....	4
ABSTRACT	.....	5
CONTENTS	.....	7
ABBREVIATIONS	.....	10
LIST OF TABLES	.....	12
LIST OF FIGURES	.....	15
CHAPTER 1: INTRODUCTION		
Organ Transplantation (overview)	.....	21
Ovarian Transplantation	.....	36
Morphology of the ovary	.....	42
Physiology of the ovary	.....	56
Microsurgery, microvascular anastomosis and transplantation	.....	66
CHAPTER 2: GENERAL METHODOLOGY	.....	69
PRELIMINARY STUDY	.....	86
CHAPTER 3: EXPERIMENT I		
Effect of transection and microsurgical reconstruction of the accessory ovarian ligament (mesotubarium superius, uterovarian ligaments), and fimbria ovarica on ovarian functions in the rabbit		
Introduction	.....	87
Materials and Methods	.....	89
Results	.....	92
Discussion	.....	95

			Page
CHAPTER 4:	EXPERIMENT II		
	Effect of ligation and transection of the ovarian vessels and uter-ovarian branches on ovarian function and animal fertility, in the rabbit		
	Introduction	.....	97
	Materials and Methods	.....	99
	Results	.....	103
	Discussion	.....	115
CHAPTER 5:	EXPERIMENT III		
	Study of the effects of transection and microvascular re-anastomosis of the ovarian vessels, on the structure and function of the ovary, in the rabbit "Sham Autografting"		
	Introduction	.....	118
	Materials and Methods	.....	120
	Results	.....	125
	Discussion	.....	134
CHAPTER 6:	EXPERIMENT IV		
	Study of the effects of Cyclosporin A on the morphology and function of the ovary, in the rabbit		
	Introduction	.....	138
	Materials and Methods	.....	140
	Results	.....	142
	Discussion	.....	154
OVARIAN TRANSPLANTATION		.....	159
CHAPTER 7:	EXPERIMENT V		
	Study of ovarian autotransplantation by microvascular technique and assessment of the structure and function of the graft, in the rabbit		
	Introduction	.....	160
	Materials and Methods	.....	162
	Results	.....	170
	Discussion	.....	201

			Page
CHAPTER 8:	EXPERIMENT VI		
	Study of ovarian allotransplantation by microvascular technique and assessment of the function and structure of the transplant, in the rabbit		
	Introduction	.....	207
	Materials and Methods	.....	209
	Results	.....	215
	Discussion	.....	249
CHAPTER 9:	CONCLUSION	.....	257
REFERENCES		.....	262

LIST OF ABBREVIATIONS

Ao	Aorta
B-lymphocyte	Bursa-dependent lymphocyte
^{o}C	Degree Centigrade
C.L.	Corpus luteum
Cm	Centimetre
CyA	Cyclosporin A
ed.	Editor(s)
ed.	Edition
Fig.	Figure
F S H	Follicular Stimulating Hormone
g	Gram
G.S.	Gestational sac(s)
HCG	Human Chorionic gonadotrophin
h.	Hour(s)
I.M.	Intramuscular
I.U.	International Unit
I.V.C.	Inferior vena cava
Kg	Kilogram
L	Litre
L.H.	Luteinizing Hormone
L.M.	Light microscopy
min.	Minute
ml.	Millilitre
mm.	Millimetre
µ	Microgram
ng	Nanogram
p.	Page

pg.	Picogram
RIA	Radioimmunoassay
rpm	Rotatation per minute
S.A.	Specific activity
S.E.M.	Scanning electron microscopy
T.E.M.	Transmission electron microscopy
T-lymphocyte	Thymus-dependent lymphocyte
W/V	Weight/volume

LIST OF TABLES

TABLE		Page
	CHAPTER 3	
3.1	Fertility following transection and reconstruction of the accessory ovarian ligaments and fimbria ovarica	93
3.2	Comparison of the number of corpora lutea and gestational sacs after transection and reconstruction of the accessory ovarian ligaments and fimbria ovarica	94
	CHAPTER 4	
4.1	Comparison of corpora lutea and gestational sacs values within (operated vz. control) and between Group A and B at mid-pregnancy following ligation and transection of the main ovarian vessels and the uterovarian branch	104
4.2	Comparison of ovarian dimensions between the operated and control sides, and between Groups A and B - I: Mid-pregnancy	107
4.3	Grades of adhesions in Groups A and B following ligation and transection of the ovarian and uterovarian vessels	109
4.4	Comparison of ovarian dimensions between the operated and control sides, and between Groups A and B - II: Non-pregnant state	111
	CHAPTER 5	
5.1	Comparison of the external diameter of the ovarian vessels in mm., and the anoxic period in minutes, between Group A and Group B, during transection and microvascular re-anastomosis of the ovarian vessels	126
5.2	Comparison of corpora lutea numbers within (operated vz. control) and between Group A and Group B at 24 h. post-HCG stimulation following transection and re-anastomosis of the ovarian vessels	127
5.3	Comparison of corpora lutea and gestational sacs values within (operated vz. control), and between Group A and Group B, at mid-pregnancy, following transection and re-anastomosis of the ovarian vessels	130

TABLE		Page
	CHAPTER 5 (continued)	
5.4	Distribution, grades and % of adhesions in Group A and Group B, following microvascular transection and re-anastomosis of ovarian vessels	132
	CHAPTER 6	
6.1	Comparison of the mean value of corpora lutea number, gestational sacs number and Nidation Index between treatment group with CyA and control group	143
6.2	Mean peripheral plasma progesterone value in ng/ml ± S.E.M. during treatment with CyA and during pregnancy compared with the controul group	144
	CHAPTER 7	
7.1	Comparison of external diameter of ovarian vessels in mm., anoxic period in minutes, between Group A and Group B during autotransplantation of the ovary	171
7.2	Autografts assessment two weeks post-transplantation	173
7.3	Incidence of adhesions at two weeks post-operative assessment of autografts	174
7.4	Ovulatory function and pregnancy rate of autotransplanted ovaries	176
7.5	Comparison of corpora lutea and gestational sacs value in Group A vz. control, Group B vz. control, and between Groups A and B following autotransplantation of the ovary	177
7.6	Comparison of the mean corpora lutea and mean gestational sacs between autotransplanted recipient groups and control group (c)	178
7.7	Mean peripheral plasma progesterone level in ng/ml ± S.D. following ovarian autografts compared with the control group	179
7.8	Comparison of methods used for autotransplantation	199

TABLE		Page
	CHAPTER 8	
8.1	Comparison of external diameter of ovarian vessels in mm. and anoxic period in minutes between Group A (non-immunosuppressed) and Group B (immunosuppressed) during allografting the ovary	216
8.2	Incidence and grade of adhesion at two week inspection following allografting the ovary	217
8.3	Results of post-transplantation assessments of ovarian allografts	218
8.4	Ovulatory function and pregnancy rate of allografted ovaries	220
8.5	Mean peripheral plasma progesterone level ng/ml $\pm$ S.D. following allografting the ovary in both non-immunosuppressed and immuno-suppressed groups	223
8.6	Mean body weight in kilograms $\pm$ S.D. in non-immunosuppressed compared with immuno-suppressed allografts	225

LIST OF FIGURES

	FIGURES		Page
	CHAPTER 1		
1.1	"Xenograft!" Colossal (16 tons) HUMAN-HEADED WINGED BULL from the City of Nineveh, Mesopotamia (Iraq), 710 B.C. (Nineveh Gallery, British Museum).	.....	22
	CHAPTER 2		
2.1	A. Carl Zeiss OPM 16 operating microscope with lighting and a stand	.....	70
	B. Operating table with hand rest		
2.2	A. Microbipolar coagulation with jeweller's forceps	.....	72
	B. Unipolar diathermy with fine cutting/coagulating needle	.....	72
2.3	Basic microvascular set	.....	72
2.4	Diagramatic representation showing the major steps of the vascular anastomosis procedure	.....	77-78
	CHAPTER 3		
3.1	Diagramatic representation of the upper reproductive tract of the rabbit	.....	90
	CHAPTER 4		
4.1	Diagramatic representation of the vascular supply of the ovary of the rabbit	.....	100
4.2	A. Group A x Group B interaction on difference scores of corpora lutea mean	.....	105
	B. Group A x Group B interaction on difference of gestational sacs mean	.....	105
4.3	Mean ovarian plasma progesterone (ng/ml) in mid-pregnancy	.....	108
4.4	Mean ovarian venous plasma progesterone (ng/ml) in the resting state	.....	112
4.5	Photomicrograph of a section of the ovary of which main ovarian vessels were ligated and transected	.....	114

FIGURES

		Page
	CHAPTER 4 (continued)	
4.6	Photomicrograph of a section of the ovary of which the uterovarian vessels were ligated and transected	114
	CHAPTER 5	
5.1	Diagramatic representation of the reproductive tract in the rabbit, to show the site of anastomosis of the ovarian vessels	
	A. Near their origin	121
	B. Distal to the origin of the uterovarian branch	
5.2	Mean ovarian venous plasma progesterone (ng/ml) 24 h. following HCG injection	129
5.3	Photomicrograph of a section obtained from a 'sham' grafted ovary	133
	CHAPTER 6	
6.1	Graphic representation showing the mean value of peripheral plasma progesterone during CyA treatment and during pregnancy, in comparison with the control group	145
6.2	Graphic representation showing the mean changes in the body weight during CyA treatment and during pregnancy in comparison with the control group	146
6.3	Photomicrograph of rabbit ovarian tissue removed at the end of 14 days course of CyA treatment, showing thickening of the tunica albuginea	149
6.4	Photomicrograph of rabbit ovarian tissue removed at the end of 14 days course of CyA treatment, showing thickening of the theca	150
6.5	Photomicrograph of rabbit ovarian tissue removed at the end of 14 days course of CyA treatment, showing vessel thickening	151
6.6	Photomicrograph of ovarian tissue removed 46 days after the course of CyA treatment, showing thickened theca	152
6.7	Photomicrograph of rabbit ovarian tissue removed 46 days after the course of CyA treatment, showing vascular thickening	153

FIGURES — Page

CHAPTER 7

7.1 Diagramatic representation showing ovarian grafting procedure

A. Preparation of the ovary of the donor side (D), preparation of the vessels of the recipient side (R) and flushing of the donor ovarian vessels 164

B. Grafting of the donor ovary and peritonization of the donor and recipient raw surfaces 166

7.2 Graphic representation showing the mean value of peripheral plasma progesterone in Groups A and B in the immediate post-operative period and during pregnancy, compared with the control 180

7.3 Ovarian autograft: photomicrograph showing extensive ischaemic necrosis and fibrosis 185

7.4 Ovarian autograft: photomicrograph showing

A. Moderate degree of ischaemic changes 186

B. Mild degree of ischaemic changes 187

7.5 Ovarian autograft: Photomicrograph showing bands of fibrous tissue adhesions covering the ovarian surface 188

7.6 Ovarian autograft: Photomicrograph showing completely normal histological structure 189

7.7 Ovarian autograft: S.E.M. micrograph showing normal surface epithelium with a papilla 190

7.8 Ovarian autograft: S.E.M. micrograph showing detailed view of a surface papilloma 191

7.9 Ovarian autograft: S.E.M. micrograph showing a normal pre-ovulatory follicle 192

7.10 Ovarian autograft: S.E.M. micrograph showing the surface of the ovary directly overlying a corpus luteum 193

7.11 Ovarian autograft: T.E.M. micrograph of the surface epithelium. 194

7.12 Ovarian autograft: T.E.M. micrograph showing a mature follicle 195

FIGURES — Page

CHAPTER 7 (continued)

7.13 Ovarian autograft: T.E.M. micrograph showing granulosa cells with evidence of luteinization 196

7.14 Ovarian autograft: T.E.M. micrograph showing theca interna and granulosa cells 197

7.15 Ovarian autograft: T.E.M. micrograph showing a corpus luteal cell 198

7.16 Illustrating ovarian autotransplantation techniques 200

CHAPTER 8

8.1 Photograph of microvascular anastomosis (in process) 211

8.2 Photograph of microvascular anastomosis (completed) 211

8.3 Combined graphic representation, showing peripheral plasma progesterone values in non-immunosuppressed and in CyA immunosuppressed ovarian allografts 224

8.4 Graphic representation of the weight loss in Kg in the immunosuppressed and in non-immunosuppressed groups 226

8.5 Photomicrograph of a biopsy specimen of non-immunosuppressed ovarian allograft one week post-transplantation showing lymphocytic infiltration 231

8.6 Photomicrograph of two weeks biopsy of non-immunosuppressed ovarian allograft showing advanced rejection 232

8.7 Photomicrograph of immunosuppressed allograft showing normal appearance 233

8.8 Photomicrograph of immunosuppressed ovarian allograft, 4 weeks after stopping CyA treatment, showing rejection 233

8.9 Photomicrograph of immunosuppressed ovarian allograft biopsy one week post-transplantation, showing destruction of tissue elements and fibrosis 234

FIGURES

CHAPTER 8 (continued)

		Page
8.10	Photomicrograph of undelivered products of conception	235
8.11	Photomicrograph of non-immunosuppressed ovarian allograft showing normal corpus luteum	235
8.12	Photomicrograph of non-immunosuppressed ovarian allograft (which achieved pregnancy) showing normal corpus luteum and part of fimbria ovarica	236
8.13	Photomicrograph of a section of immunosuppressed ovarian allograft (which achieved pregnancy) showing normal corpus luteum	236
8.14	Photomicrograph of a section of immunosuppressed ovarian allograft 4 weeks after cessation of CyA treatment, showing degenerative changes and lymphocytic infiltration	237
8.15	Photomicrograph of a section of immunosuppressed ovarian allograft 4 weeks after cessation of CyA treatment, showing vessel wall thickening	238
8.16	Photomicrograph of immunosuppressed ovarian allograft 10 weeks after stopping CyA treatment showing thick tunica albuginea and follicular cysts	239
8.17	Photomicrograph of immunosuppressed ovarian allograft showing excessive thickening of the blood vessels' wall	239
8.18	Photomicrograph of a section from ovarian allograft (10 weeks after stopping CyA treatment) showing dilated cystic follicles and normal follicle	240
8.19	Photomicrograph of immunosuppressed ovarian allograft showing excessive adhesion formation and a normal mature follicle	241
8.20	Transmission electron micrograph of non-immunosuppressed ovarian allograft, one week post-transplantation, showing a circulating lymphocyte	242
8.21	Transmission electron micrograph of non-immunosuppressed ovarian allograft 2 weeks post-transplantation showing lymphoblast, lymphocyte and plasma cell, ...	243

FIGURES — Page

CHAPTER 8 (continued)

8.22	Transmission electron micrograph of non-immunosuppressed rejected ovarian allograft 6 weeks after transplantation, showing a plasma cell and neutrophil	.....	244
8.23	Transmission electron micrograph of immunosuppressed ovarian allograft under CyA treatment, showing normal primary follicle	.....	245
8.24	Transmission electron micrograph of immunosuppressed rejected allograft 4 weeks after stopping CyA treatment, showing lymphocytes	.....	246
8.25	Transmission electron micrograph of the surviving immunosuppressed ovarian allograft showing evidence of fibrosis	.....	247
8.26	Graft survival percentage in weeks in the immunosuppressed and the non-immunosuppressed allografted groups	.....	248

CHAPTER 1

INTRODUCTION

ORGAN TRANSPLANTATION

Overview

Although it is usually assumed that the art of grafting is a modern one, it is interesting to note that it has been performed for over two thousand years. Local skin flaps were used by Indian surgeons for the operation of rhinoplasty and the techniques were described by Celsus (Woodruff, 1960). The idea of transplantation, however, seems to have been conceived from time far back in the dawn of history (Fig. 1.1).

Grafting has now become an established procedure and many tissues and organs have now been grafted; some are now clinically accepted methods of therapy, while others are still in the experimental stage (for full review of the present status of organ transplantation - see Transplant.Proc., 15: 1033-1431 (1983).

1. Types of grafts. Grafts, depending on their locations in the host's body and on the genetic dissimilarities between donors and recipients, are classified into (Brent and Bachelor, 1982; Bach, 1982a):

(a) Autografts: Graft of an individual's own tissue to another site;

(b) Isografts: Graft betwen individuals identical for histocompatibility antigens.

(c) Allografts or allogenic grafts (formerly called homograft or homologous graft): Graft between genetically dissimilar members of the

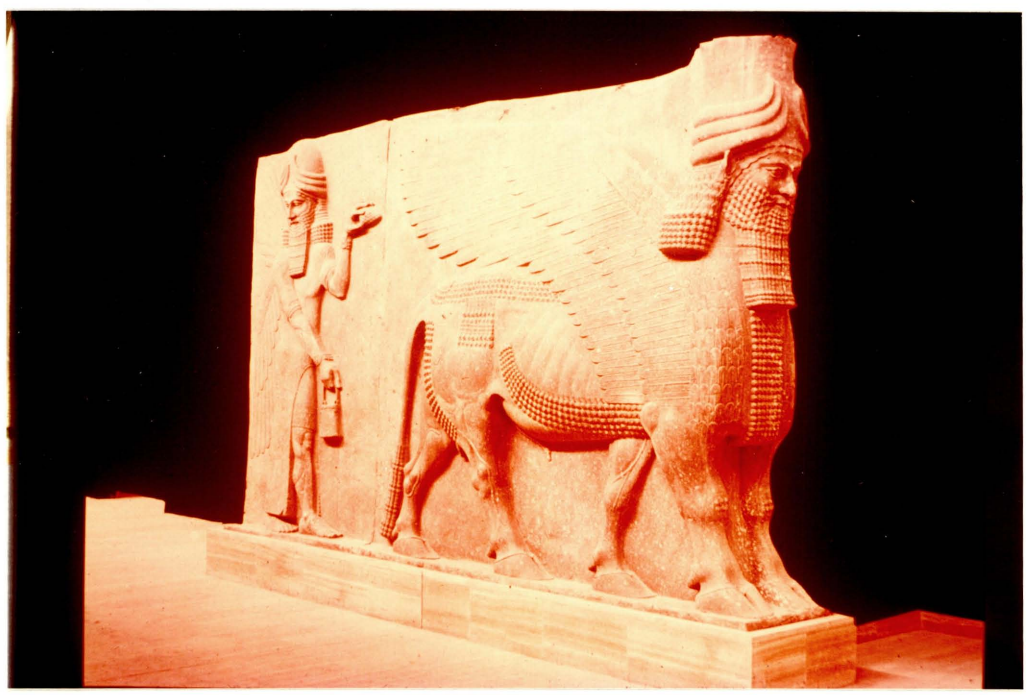


Fig. 1.1 'Xenograft'. Colossal (16 tons) HUMAN-HEADED WINGED BULL from the City of Nineveh, Mesopotamia (Iraq), 710 B.C. (Nineveh Gallery, British Museum)

same species;

(d) Xenografts or xenogenic grafts (formerly called hetero graft or heterologous graft): Graft between individuals belonging to different species.

2. Transplantation immunology. A large percentage of organ grafts are unsuccessful. These failures are related almost entirely to the reaction of the immune system of the recipient to the foreign antigens in the allograft. The rejection reaction by the recipient immune system can lead to death of the graft; attempts to suppress the recipient with immunosuppressives may result in death of the recipient, caused by complications due to these agents.

2.1 The immune system: Consists of the organized lymphoid tissue, including spleen, lymph nodes, recirculating pool of lymphocytes, in the blood and lymph, and the central lymphoid elements, the thymus and bone marrow (Irvine, 1979).

The lymphocytes may be divided into two broad families of cells: (a) T- (thymus-dependent) lymphocytes which constitute about 60% of the circulating lymphocytes. While in the lymph nodes, most of these cells reside in the deep cortex and in the areas between the germinal centres. T-lymphocytes are active in a wide variety of immunological reactions, including delayed hypersensitivity, allograft rejection and tumour surveillance (Irvine, 1979). (b) B- (bone marrow or bursa-dependent) lymphocytes. These are found mostly in the germinal centres of the lymph nodes, and are responsible for the production of immunoglobulins (Ig) G, A, D, M and E. Other cells important in the functioning of the immune system include the monocyte-

macrophage group, the natural killer cells (N.K.), and the K cells. The co-operative and integrated action of all the elements of the immune system is responsible for the various immunological phenomena.

2.2 Graft rejection (Rejection response): The process encompasses all these immunological reactions that lead to the destruction of a graft by a recipient who is genetically non-identical (Robbin et al, 1981a).

2.2.1 Mechanism of rejection: For the rejection response to be evoked, the lymphoid system of the recipient must "recognize" foreign" (non-self) antigens in the donor's tissue cell surface. These surface antigens are called histocompatibility (or transplantation) antigens; the genes that code for these antigens are called histocompatibility genes. The histocompatibility antigens were originally designated in the mouse, the most studied species, into "major" and "minor" on the basis of strength of the rejection response induced by grafts incompatible at these genetic loci (Fabre and Ting, 1979). The gene coding for the major histocompatibility antigen system or complex (MHS or MHC) is located on chromosome 6; the corresponding area in the mouse is called H2-complex.

The genes of the MHS and the antigens they code for are classified on functional grounds into three classes (Brent and Batchelor, 1982):

Class I: Of this class, three loci (A, B and C) were identified on the short arm of chromosome 6 in the human and are called HLA (human leuococyte antigen). Those in the rabbit are called RLA, those in the dog are called DLA, and so on. They are found on the surface of all

nucleated cells (with the exception of placental trophoblasts).

Class II: These are less widely spread, less pleomorphic and, therefore, easier to type. They occupy the so-called D region of the HLA complex; hence the term HLA-D and DR (D-related). They were described in the cell surface of a number of organs and in the endothelial cells of blood vessels (see Morris and Ting, 1982, for a full review), but not in the cells of the ovary. The greater importance of this class of antigens in determining the fate of an allograft is being increasingly recognized (Calne, 1982; Cameron, 1982; Morris and Ting, 1982).

Class III: These genes code for some components of the compliment system of the immune reaction.

The presumed sequence of immunological events leading to rejection consist of several phases (Bach, 1982a): the recognition of the transplant by the recipient lymphocytes, the blastic transformation, proliferation and differentiation of these cells into cytotoxic and into antibody-forming cells, and finally infiltration of the graft by a mixture of cells of the T and B-lymphocytes, macrophages and polymorphs series. Contact and subsequent recognition of the allograft antigens occurs, according to Brent and Batchelor (1982) and Bach (1982a) by way of

(a) Donor's leucocytes and macrophages residing in the graft before transplantation, and flushed out of the graft into the host circulation after anastomosis, "passenger leucocytes";

(b) Graft antigens and cellular debris liberated and released from

the graft into the host circulation;

(c) Host lymphocytes circulating through the graft and contacting the donor's antigens and thus become directly activated.

The actual rejection of an allograft is probably the result of a combined action of several arms of the immune response on various target tissues within the graft, aided by secondary processes, such as vasospasm, oedema and thrombosis.

Both cell-mediated and antibody (humoral) mediated immune processes are involved: the former, damages the graft by direct cytotoxicity through cell-to-cell contact and by the secretion of lymphokines which mediate and amplify the damage, and the latter damages the graft by antibody-dependent cellular cytotoxicity and by the deposition of immune complexes on and the consequent damage to the vascular endothelium and impairment of the circulation (Robbins et al, 1981a; Carpenter, 1979).

2.2.2 Histopathology of rejection: Rejection has been classified as (a) hyperacute, (b) acute and (c) chronic (Walter and Israel, 1979 ; Dunnil, 1979; Robbins et al, 1981a).

(a) Hyperacute rejection: Is rare nowadays, occurs within minutes or hours. It is more likely when there is ABO incompatibility between donor and recipient or when the recipient has been sensitized by previous pregnancies, blood transfusion, or similar allograft. The graft appears congested and blue instead of pink and normal. On light microscopy (L.M.) there is intense engorgment of blood vessels with perivascular

infiltration by polymorphs and (within 24 hours) thrombosis and evidence of fibrinoid necrosis of the vessel wall. With E.M., there is platelet aggregation and degranulation, swelling of the endothelium and disruption of the basement membrane.

(b) Acute rejection: Often occurs within 2-10 days, but may occur much later and is often heralded by sudden sharp deterioration of graft function. The graft generally appears swollen, oedematous and altered in colour. With light microscopy (L.M.), infiltration with lymphocytes, lymphoblasts, plasma cells and eosinophils are seen, aggregation of platelets and fibrin thrombi, and vascular wall damage, may all be seen with variable degree of parenchymatous cell damage. With electron microscopy (E.M.), subendothelial deposition of electron-lucent material, platelet aggregates within the capillary loops, dense focal mononuclear cells, collections with plentiful free ribosomes in their cytoplasm but little rough endoplasmic reticulum, can be seen.

(c) Chronic rejection: Transition of the acute to the chronic phase of rejection is not sharply defined and features of both may be seen in the same histological specimen. The gross appearance is that of pallor and shrinkage. With L.M., the striking features are those of occlusive vascular changes, intimal proliferation and thickening, and medial thickening with necrotic and degenerative changes. The capillary basement membrane is also thickened and foci of mononuclear cells and plasma cells are seen. Immunofluorescent studies show deposits of immunoglobulins; compliment component and fibrinogen are found in varying patterns and quantities. With the E.M., subepithelial and subendothelial deposits of electron-dense and electron-lucent granular

material is seen. They consist of immunoglobulins and compliment.

2.2.3 Monitoring rejection: The immune response to an allograft and the consequent impairment which the latter may sustain, depends upon the degree of histo-incompatibility and the effectiveness of the immunosuppressives. A certain degree of rejection almost always occurs and may, to some extent, be recognized by

(a) Clinical assessment. Rejection may be associated with early depression of function of the grafted organ, e.g. in renal transplant - rise in serum creatinine and fall in creatinine clearance; liver - elevation of transaminases; heart : ECG changes and fall in cardiac output; pancreas : hyperglycaemia; and lung : fall in oxygen saturation of haemoglobin (Bach, 1982a) and a fall in ovarian steroids output would be expected when an ovarian allograft is rejected.

(b) Immunological monitoring: Numerous tests were developed but the results were not always specific or showed close correlation with immunological damage (Stiller and Sinclair, 1979). It is doubtful, at the present, if such tests give earlier warning of a rejection crisis than clinical tests (Brent and Batchelor, 1982).

(c) Graft biopsy: Biopsy of renal allograft shortly after completion of the vascular anastomosis (intra-operative) is now usual practice (Williams, 1979) to detect hyperacute rejection, differentiate rejection from non-immunological causes of graft damage and to provide a histological base line, should other biopsies have to be performed in possible cases of rejection. Needle biopsies have also been found very helpful in the evaluation of renal graft (Banfi et al, 1981).

(d) Ultrasound: Ultrasound proved of definite value in the differential diagnosis of renal allograft rejection episodes (Weil, 1981). Ultrasound also proved useful in the visualization and assessment of the ovary (Kidd et al, 1980; Mastrianni, 1980), and it may turn out to be equally helpful in the evaluation of ovarian allograft too.

2.2.4 Graft survival prolongation: Three approaches can be used to achieve graft acceptance (Robbins et al, 1981a): (a) Tissue typing to choose the most compatible donor; (b) Immunosuppression of the recipient; (c) induction of tolerance.

(a) Tissue typing: The histocompatibility gap can be narrowed using serological and cellular immunological techniques for the determination of the antigens coded for by the HLA, A, B and C, and the HLA - D/DR of the major histocompatibility system (MHS). It appears that it is far more important for the success of the graft and technically more feasible to type for the HLA, D/DR (Morris and Ting, 1982).

It is also important to test for the presence of cytotoxic antibodies in the serum of the recipient against the donor's lymphocytes in order to eliminate the possibility of hyperacute rejection (Calne, 1982). Donors who are incompatible with the recipient at the ABO blood group should not be accepted for success of the graft is impossible under such circumstances (Walter and Isreal, 1979).

(b) Immunosuppression: The objective of immunosuppression is to obtain a complete acceptance of the grafted organ without causing general depression of immunity; such an ideal is yet to be realised. Immunosuppression may be :

(1) Donor specific, that is only lymphocytes specifically active against the allograft, are inhibited. It can be antigen-induced (active enhancement) or antibody-induced (passive enhancement) (Fabre and Morris, 1979). This type of immunosuppression is still in the animal experimental stage (Morris, 1982).

(2) Non-specific immunosuppression: It is non-specific in the sense that its effects are broadly directed at all immune respones including those against the graft. There are many types of non-specific immunosuppression among which are - x-ray irradiation, which is receiving more attention (Calne, 1982); antilymphocyte globulin (ALG) (Morris, 1982) and pharmacological immunosuppression.

The pharmacological immunosuppressive regimen received by transplant recipients nowadays essentially include Azothioprim (Imuran) and steroids, sometimes complimented with antilymphocytic globulin (ALG) (Bach, 1982b).

(a) Azothioprim is an orally active derivative of 6-mercaplopurine to which it is metabolised in vivo in the blood and in the liver. It reduces DNA and RNA synthesis in dividing cells. In man the effect is mainly on IgG-producing B-cells. It also has some anti-inflammatory activity which probably contributes to its therapeutic efficiency. Side effects include bone marrow depression, facilitation of infection,

hepatotoxicity and rarely, induction of tumours.

(b) Corticosteroids: Corticosteroids exert a greater or lesser degree of inhibitory action on virtually every stage of the inflammatory and immunological response in man and in animals (Currie, 1982). In organ transplantation, corticosteroids have been used extensively for the prophylaxis and treatment of rejection. Side effects are frequent, sometimes serious, and, with the exception of avascular necrosis of the bone, are dose-related.

(c) Cyclosporin A (CyA) is a fungal antibiotic, obtained from the soil fungi Trichoderma polysporum Refai and Cylindro-carpon lucidum. It is a hydrophobic cyclic neutral endocapeptide comprising 11 amino acids, one of which is unique to CyA, and has a molecular weight of 1203 daltons. CyA was discovered in 1972 in the laboratories of Sandoz in Switzerland during the search for a biologically produced anti-fungal antibiotic. Its antibiotic activity, however, proved to be very restricted (Borel, 1976). CyA is insoluble in water but soluble in olive oil and organic solvent. It now exists in three forms - one for oral, one for intramuscular and one for intravenous use. The most tested one is the oral preparation, although the absorption from the gastrointestinal tract varies greatly between individuals. The intramuscular route was found to give satisfactory immunosuppressive levels in the rabbit (Dunn et al, 1980). The drug is metabolised in the liver and excreted via the bile; only a small fraction is excreted unchanged in the urine (Britton and Palacios, 1982). The immunosuppressive dose used in various animals to prolong graft survival varied between 25 - 300 mg/Kg body weight/day when the oral route is used, 5 - 50 mg/Kg body weight/day when the intra-

muscular route is used (Thomson et al, 1982).

2.2.5 Immunologically privileged tissues. Different tissue may be subject to varying degrees of rejection for a number of reasons, including difference in expression of transplantation antigen and difference in resistance to immune attack (Russel and Cosmi, 1979). Porcine liver allografts, for example, are frequently accepted without immunosuppression (Brent and Batchelor, 1982), while the ovary, contrary to some previous reports, is readily rejected (Krohn, 1977).

2.3 The immune system in the rabbit: Insight into the complexity of the rabbit histocompatability system was provided by the early experiment of Medawar (1945) on skin cell grafts and those of Klassen and Miligrom (1969) on renal allografts. The complexity of the control of the histocompatibility (transplantation) antigens was also evident from the residual graft rejection between highly inbred rabbits and the early consistent rejection of grafts exchanged between unrelated rabbits (Cohen and Tissot, 1974). Direct histocompatibility testing was attempted to predict more compatible donor/recipient pairs by estimating the rejection time of a second set skin graft (Matsukara et al, 1963). The first attempt at actual histocompatibility typing and identification of the histocompatibility locus in the rabbit was reported by Black (1967). Using cytotoxic antisera, he concluded that all the histocompatibility antigen his antisera detected were controlled by a single locus. Seven complex alleles of the histocompatibility locus of the rabbit (RLA) have been isolated and appear to be similar in complexity to those of the corresponding locus (H2-locus) in the mouse (Cohen and Tissot, 1974). The RLA, however, appears to be the only one which influences graft survival. As the system is not expressed on the antigens of blood groups, blood

compatibility does not produce significant graft prolongation. Recent significant developments in the rabbit model is the ability to produce by genetic manipulation RLA-identical unrelated rabbit which can be used for the study of some of the problems of human transplantation.

3. Transplantation and cancer: Recipients of allografts are prone to higher tumour incidence (Britton and Palacios, 1982). The oncogenic aetilogy is probably multifactorial (Birkenland, 1982). A complex interplay of chronic antigenic stimulation by the graft, disturbance of immunity, immunosuppressive drugs, and oncogenic viruses, is probably the underlying causative mechanism (Penn, 1981; Kinlen et al, 1979).

The common types of malignancies encountered in transplant recipients are lymphoma, carcinoma of skin and carcinoma of the uterine cervix (Sheil, 1979). For further views on this problem, see Penn, (1983) and Walter and Israel (1979).

4. Transplantation and pregnancy. Acute rejection crises are quite rare during any stage of pregnancy, but may occur in the post-partum period. The dose of immunosuppressive agents may be reduced during pregnancy without precipitating a rejection episode (Williams, 1979). It seems that during pregnancy there is some degree of immuno-suppression which may be correlated with the presence of four immuno-suppressive factors in the maternal plasma (Hulka and Mohr, 1969; Turcotte et al, 1971; Walter and Israel, 1979): gluco-corticoids, placental chorionic gonadotrophin, human somatomamotrophin (human placental lactogen) and α faetoprotein.

Risk of teratogenicity. Animal studies have shown that high

doses of prednisolone are rarely associated with increased incidence of cleft palate in humans (Sutherland and Light, 1965). Azothioprim has been associated with higher incidence of limb defect in the rabbit and abnormal bone formation in the rat, and cyclophosphamide was reported to have teratogenic effects in the rabbit (Fritz and Hess, 1971).

To-date the human experience in post transplant mothers has not borne this out, although there have been few congenital malformations in the reported series. These include steroid cataract, immunosuppression in the neonates and, rarely, serious neonatal infection (Cunningham et al, 1983).

5. Ethics of transplantation. The logical source of organs for transplantation are the hopelessly-ill patients on respirators. If it is allowed to salvage organs from such patients, then it is in the interest of the recipient that the transplanted organ functions "efficiently". This would entail "early" salvaging of organs before a significant decline in the general state of the donor becomes evident. This approach, though scientifically desirable, is not immediately morally and spiritually acceptable (Dempster, 1970).

With living donors, the problem is, so far, only related to renal transplantation but may well relate to ovarian transplantation in the future. The operation of nephrectomy (and ovariectomy) is a major procedure and exposes the donor to the hazard of surgery for the benefit of another person. The two main reasons for continued use of living donors are the shortage of cadaveric donors and the more favourable outcome with transplant of living, blood-related donors (Thomson, 1977).

In addition to the risk of operation and the possibility of complications, there is also the negative psychological reaction to donation, especially in the case of unsuccessful transplants (Eklund et al, 1982). However, several studies of donors (Kamastra-Hennen et al, 1981) have noted immediate positive psychological reaction on the part of the majority of related donors. On longterm follow-up, the majority of longterm donors, both successful and unsuccessful, reported positive attitude and little regret, although regret tended to be slightly more common with the unsuccesful donors, who were also more likely to report difficulty in their relations with the recipient.

OVARIAN TRANSPLANTATION

Transplantation of the ovary seems to have been first carried out by Knauer in the rabbit in 1896 (Krohn, 1977). Since then ovaries were transplanted to various sites in the body, by direct implantation and by vascular anastomosis, in a wide range of animals and in man, for investigative and for occasional clinical purposes.

1. Sites of transplantation. Generally ovaries were either grafted istopically (that is, into anatomical positions similar to that from which they came) or heterotopically (that is, into situations which differ from that of the donor site).

1.1 Istopic transplantation: This was used by Krohn (1958, 1965) and by Jones and Krohn (1960) to distinguish between the contribution which age and environment make to the decline in fertility in the mice; successful pregnancy was achieved in these experiments. Other workers attempted isotopic transplantation with variable success - (dogs : Eraslan et al, 1966; O'Leary et al, 1969; Paldi et al, 1975; Yonemoto et al, 1969; pigs : Binns et al, 1969; Camilleri et al, 1976; mice : Ichinoe et al, 1976; rats : Scott et al, 1972; monkeys : Scott et al, 1981; rabbits : Jaszczak and Hafez, 1971; Winston and Brown, 1974; Grimaldi et al, 1982; Green et al, 1982); Betteridge, 1970.

1.2 Heterotopic transplantation: Various heterotopic sites were utilised:

(a) Sites which allow repeated direct observations of the graft. Such sites included the anterior chamber of the eye (rabbit : Ward et al, 1953; cat : Jacobowitz and Laties, 1970), neck (ewe : Goding et al, 1967), ear (rabbit : Shaffer and Hulka, 1969), vagina (ewe : Dierschke and Wallace Williams, 1970).

(b) Portal area : various transplantation sites were used within this area in a number of animal species; rabbit (Jaszczak and Hafez, 1971, 1973), dog (Ber, 1973).

2. Types of ovarian grafts. Ovaries may be transplanted from one site to another in the same animal (autografts) or from one animal to another of the same species (allograft) or from an animal of one species to another of a different species (xenografts).

3. Transplantation by vascular anastomosis. Ovarian grafts cannot be expected to survive unless adequate blood flow is provided by proper vascular anastomosis. It is only with the recent advances in vascular surgery that vascular ovarian transplantation in animals and in man became a possibility. Goding et al (1967) described a technique in the sheep for the transplantation of the ovary and its vascular pedicle from its pelvic position to a subcutaneous site in the neck. Factors affecting steroid secretion from the transplanted ovary were studied using this technique (McCracken et al, 1971). Harrison et al (1968) transplanted the ovary and the uterus together to the neck of the sheep and normal oestrus cycle length and levels of progesterone in the draining blood were found. Successful uterovarian autotransplantation in the dog by direct vascular anastomosis was achieved by Eraslan et al (1966) with successful pregnancy in three out of 18 dogs studied.

4. The histological changes in the graft.

(a) In the non-vascular ovarian autograft. Harris and Eakin (1949) reported that 24 hours after grafting, the graft showed large areas of central necrosis surrounded by a thin rim of viable tissue and, within three days, the demarcation between the central necrosis and the peripheral surviving rim became well defined. Ingrowing vessels of the surrounding tissue were seen linking with the existing vessels in the graft. The central area of necrosis was completely removed within a few weeks by the activity of the macrophages. Many oocytes were lost from ischaemia (Jones and Krohn, 1960). Most of the oocytes were found in the peripheral rim of the graft and therefore were more likely to survive. Follicular growth and formation of corpora lutea took place from this fringe. Autografts generally began hormone secretion 6-12 days after grafting.

(b) In the vascularised graft. Sections obtained at varying intervals after grafting showed well defined follicles and normal corpora lutea (Goding et al, 1967). Histologically normal and some necrotic autografts were reported by Grimaldi et al (1982); failure was probably the result of unsatisfactory anastomosis.

(c) In the allograft. The first sign of reaction, according to Harris and Eakin (1949), occur about 6-7 days after grafting when lymphocytes began to invade the graft; all organised structures soon disappeared,the last being the luteal elements. Lymphocytes were then replaced by connective tissue cells and the dead tissue removed by fatty phagocytic cells. The tissue infiltrate consisted of a mixed population of mononuclear cells,polymorphs and occasional plasma cells. Generally allografts

take longer to get established than autografts (Parkes, 1956). Green et al (1982) have reported that survival of ovarian allograft can be prolonged by the use of the immunosuppressive agent, CyA.

5. Ovarian transplantation in the human. Morris, R.T., in 1895, reported successful transplantation of human ovaries in two patients (Simmer, 1970). The first patient was aged 20, who was suffering from primary amenorrhoea, received an ovarian allograft in the fundus of the uterus. Two months later menstruation occurred for the first time and lasted for ten days. The second patient had suffered from longstanding pelvic infection, which had obliterated much of the ovaries and the tubes; most of the ovaries and tubes were removed, but small piece of her own diseased ovary was autografted into the short stump of the Fallopian tube. The patient became pregnant a month later, but lost a well developed foetus by abortion at the third month.

In 1903, Frank and Martin devised an ingenious operation for a woman from whom both tubes and ovaries had been removed; she received an ovarian allograft into the remains of the broad ligament. A plastic operation to create a new peritoneal opening into the uterus was then performed (Wood, 1981). The patient menstruated regularly for at least two years, but no pregnancy was achieved. Bainbridge (1923) reported one healthy child subsequent to an operation in which a remnant of the patient's own non-vascularised ovary was implanted into the cornua of the uterus.

Foster (1930) reported on 18 patients in whom he autografted the ovaries to the fundus of the uterus. Three women gave birth to

healthy children. The ovarian blood supply was left intact in some of these patients. In 1969, Papanicolaou et al. described a maternal daughter allograft for Turner's syndrome in which clinical evidence of ovarian activity was obtained within several weeks of surgery; there was no mention as to the length of the survival of the graft.

Blanco (1976) reported on ovarian vascular heterotopic allograft in which the rectus muscle was the site of the allograft and the hypogastric artery and vein were anastomosed to the ovarian pedicle. This technique, which resulted in ovarian endocrine function, was attempted in a case of precocious menopause, two cases of testicular feminisation and one case with Turner's syndrome. He used the procedure to allograft the ovary of a woman with primary sterility; the ovary was fixed and orientated towards the infundibulum of the Fallopian tube. The allograft exhibited endocrine function and pregnancy was achieved but terminated by spontaneous abortion.

Gryaznova et al in 1977 (Wood, 1981) reported 30 non-vascular allografts in patients with amenorrhoea; the free ovarian graft was covered by amniotic membrane. Clinical evidence of oestrogen activity was observed in all patients but the survival of the graft was limited.

Clinical reports on ovarian transplantation do not always differentiate between those cases where the ovary is severed from its blood supply and those in which the ovary is mobilised from its anatomical site to another, with its blood vessels remaining intact. The report by Cantor (1983) on "Transplantation" of the Fallopian tube and ovaries from the field of pelvic irradiation is an example of transposition rather than transplantation.

6. Use of ovarian grafts: Apart from the occasional use of ovarian grafts for their endocrine and reproductive functions, they were also used for (Krohn, 1977):

(a) Study of the factors controlling puberty and menopause;

(b) Study of the change in the normal pattern of ovarian secretion induced by change in the anatomical site and local environmental conditions;

(c) Study of the pituitary ovarian relationship;

(d) Study of the sex differences in the function of the pituitary.

OVARIAN MORPHOLOGY

1. General Morphology

The mammalian ovaries are paired organs and are, with few exceptions, approximately equal in size. They are found in the abdominal or pelvic cavity, attached by a mesentery (mesovarium) to the dorsal aspect of the broad ligament.

The ovaries may be enclosed in a peritoneal capsule or ovarian bursa with a space, the periovarian space, between the ovary and the peritoneal lining of the bursa. The ovarian bursa is wide and communicates freely with the peritoneal cavity in the rabbit (Harrison and Weir, 1977), but is poorly developed in man (Eckstein and Zuckerman, 1956).

The quiescent ovary of most mammals is small, flattened, ovoid or spherical, with a relatively smooth surface. In the rabbit the ovary is roughly oval in shape and triangular in cross-section, being reduced in thickness where it is attached to the mesovarium (Burr and Davis, 1951; Craigie, 1966). At the onset of the breeding season, the ovary begins to swell, owing to growth of the follicles. The mature follicles may protrude at the surface or may be just visible as translucent bodies within the substance of the ovary. After ovulation, the follicles are converted into corpora lutea which are usually pigmented in a range of colours from pink to yellow, orange or brown. In the rabbit, where multiple ovulation is the rule, the protruding follicles and the pedunculate corpora lutea give the ovary a grape like appearance.

The exposed surface of the mammalian ovary is covered by a continuous layer of mesothelial cells, commonly referred to as the "germinal" superficial epithelium, as it was once mistakenly believed that these cells were capable of generating new germ cells.

The cut section of the ovary, even to the unaided eye, consists of an outer zone, the cortex and an inner zone, the medulla. The cortex contains the evoluting and involuting follicles and functioning and involuting corpora lutea. The medulla represents the core of the ovary and is formed by a loose connective tissue, elastic fibres and strands of muscle derived from the mesovarium and blood vessels and perivascular nervous network which are cholinergic and adrenergic in nature (Balboni, 1976).

2. The "germinal" superficial epithelium

The superficial epithelium is usually composed of a single layer of polyhedral (columnar or flattened) cells which are tenuously attached to the underlying tunica albuginea. The free surfaces of these cells are covered with a large number of microvilli, the density of which varies in different regions of the same ovary, of ovaries of different mammals, of different ages and in different phases of the oestrus cycle (Van Blerkom and Motta, 1979). The surface of the ovary is frequently evaginated into a series of villous-like projections or papillae which vary in number, size and distribution in different species (Harrison and Mathews, 1951) and are quite numerous in the rabbit (Motta and Van Blerkom, 1975) but comparatively smaller and reduced in number in the human (Jensen and Norris, 1972).

In several species, including the rabbit, areas of superficial epithelium are often invaginated into the adjacent cortical layers, forming crypts and cords which on transmission electron microscopy (TEM) and Scanning electron microscopy (SEM) appear as hollow tubular invaginations in which the lumen opens directly on the surface of the ovary (Motta, 1974). The number, size and the degree of development of papillae, crypts and cords are influenced by the level of circulating pituitary gonadotrophins and/or ovarian hormones (Jensen and Norris, 1972).

The function of the surface epithelium is probably manifold (Peters and McNatty, 1980); its cells are involved in the transport of fluid and ions by surface pinocytosis (Ferenzy and Richart, 1974). During infancy atretic oocytes are transported through the surface epithelium and so are ovulated oocytes during adult life. The demonstration that the germinal epithelial cells covering pre-ovulatory follicles contain numerous lysosome-like bodies suggest that these lytic substances emanating from the overlying epithelium play an important role in effecting weakening of the follicular wall and rupture of the follicle (Bjersing and Cajander, 1974a; Epsey, 1971).

It has also been suggested, on strictly fine structural criteria, that the germinal epithelium may be a source of granulosa cells (Motta, 1974).

3. Stroma and interstitial tissue

The stroma consists of all ovarian tissue lying between follicles, corpora lutea and the vascular system; the interstitial tissue refers the hormone-producing tissues other than follicles and corpora lutea (Peters and McNatty, 1980).

In the adult ovary, the stroma of the outermost cortex is composed of compact fibrous connective tissue (tunica albuginea) which merge with the more cellular stroma containing the follicles, corpora lutea and interstitial tissue (Balboni, 1976). Much of the tissue lying between the growing follicles is derived from regressing follicles (Peters and Levy, 1966). However, not all cells of the regressing follicles are transformed into stroma or interstitial tissue; only a small percentage remain and become hormone-producing (Davies and Broadus, 1968).

The interstitial tissue is particularly well developed in the rabbit and its histochemical and ultrastructural features have been studied in the oestrus, post-ovulatory and pregnant animal (Guraya, 1973). The most common type of stromal cell is represented by the fibrocytes and fibroblasts. Histiocytes and macrophages are more frequently observed near the blood vessels; macrophages are also numerous inside recent corpora lutea (Balboni, 1976). Smooth muscle cells exist in the theca externa of some mammals (Bjersing and Cajander, 1974 b). The ultrastructure of interstitial cells, whether in the cycling or pregnant animal, is typical of steroid-secreting cells, i.e. abundant smooth endoplasmic reticulum (SER), large mitochondria and lipid droplets. In the adult human ovary groups of large polyhedral or round cells, with eosinophilic cytoplasm and lipid vacuolation, are found in the stroma near the hilum and produce androgens.

4. The follicle

These are scattered throughout the cortical zone and may be seen in the various stages of their maturation and regression.

4.1 Follicular development. Various terms and classifications have been used to describe the different stages of the development of the follicle (Peters and McNatty, 1980): terms like - primordial or primary follicle with a single layer of granulosa cells; secondary growing or pre-antral follicles with membrana granulosa of varying thickness and tertiary, vesicular or Graafian follicles for antral follicles at different stages of development. Another classification which has proved helpful in evaluating and comparing changes in the oocyte and follicular growth was developed by Pederson and Peters (1968), based on the size of the oocyte, size of the follicle and the morphology of the follicle.

Primordial follicle: This consists of central germ cell (primary oocyte blocked in the first prophase of meiosis) encircled by a flattened layer of follicular epithelium. With electron microscopy (E.M.), the oocyte presents a spherical nucleus and, in suitable sections, a nucleolus. The cytoplasmic organelles, consisting of closely spaced mitochondria, Golgi vesicles, annulate lamellae, etc., form a complex which is located near the centre and called Balbiani's vitelline bodies. The follicular cells are separated from the surrounding stroma by a distinct basement membrane.

Primary follicle: This consists of a primary oocyte increasing in size and is surrounded by a layer of cuboidal or low columnar follicular epithelial cells. With the E.M., the mitochondria appear to increase in number and to be located in the outer cytoplasm. The plasma membrane of the oocyte gives rise to microvilli that interdigitate with the cytoplasmic projections of the follicular cells

(Gondos, 1970). Between the follicular epithelial cells and the oocyte some P A S material is secreted to form a layer called zona pellucida.

Secondary follicle: The oocyte continues to grow and the follicular epithelium proliferate to form a thick stratified columnar layer called "granulosa". The zona pellucida is now more prominent and, with L.M. (light microscopy) appears, as a translucent intensely P A S positive structureless membrane. With E.M., it presents a granular or filamentous appearance. It is secreted by the follicular (granulosa) cells and the oocyte (Balboni, 1976). Cytoplasmic processes from the innermost layer of the granulosa cells extend through the zona pellucida and may either come into close relations with the plasma membrane of the oocyte or invaginate it. On the other side microvilli from the oocyte penetrate the zona pellucida. These microvilli are generally believed to increase the absorptive capacity of the oocyte surface, which the cytoplasmic projections of the granulosa cells may facilitate the transport of the nutritive material to the egg (Motta et al, 1971; Balboni, 1976). Between the follicular cells small spaces are now developing in which some fluid accumulates (Call-Exner bodies). The accumulated fluid contains filamentous, P A S positive material. At this stage, cells, indistinguishable from fibroblasts, become aligned concentrically around the follicle, marking the beginning of the theca layer formation.

Tertiary (Graafian) follicle: The fluid-filled spaces amongst the granulosa cells tend to coalesce to form a single large follicular cavity or "antrum" in which the follicular fluid is contained. The increasing amount of follicular fluid expands the follicle which

becomes vesicular. The granulosa cells are displaced peripherally, where they form 4-6 layers thick cells and accumulate round the oocyte to form the "cumulus oophorous". The cells that encircle the egg directly with its zona pellucida form the so-called "corona radiata". The inter-granulosa cell junctions are now represented mainly by the gap junctions which are important for the transport of ions and low molecular weight substances (Albertini and Anderson, 1974).

The theca begins to differentiate into theca externa and theca interna. When fully developed the theca interna cells appear as epitheloid-like cells which, with the E.M., show the characteristics of steroidogenic cells (highly developed smooth endoplasmic reticulum (SER), vesicular mitochondria, lipid droplets, etc). The theca externa is fibrous in character.

Mature follicle: The number of follicles that mature and ovulate varies widely in different species. In the human, only one follicle in the two ovaries is destined to rupture and ovulate (Balboni, 1976), while in the rabbit multiple ovulation is the rule.

The fully developed follicle is a spherical structure almost totally embedded within the connective tissue matrix of the ovary, with a bulge on the surface. The wall of the mature follicle is composed of a fibroelastic theca externa, highly vascularised theca interna consisting of spindle cells and polyhedral vacuolated cells, and a layer of granulosa cells sharply demarcated from the theca by a basement membrane (Lipner, 1973). The theca cells form cytoplasmic projections which extend into the granulosa layer (Byskov, 1969). The cumulus

oophorous detaches from the mural granulosa and the cells surrounding the oocytes begin to disperse and the intimate contact between the granulosa cells and the oocytes is lost (Hertig and Barton, 1973). The fluid increases in viscosity in some species (Zachariae and Jensen, 1958) and may become haemorrhagic in other species (Koering, 1969). The oocytes which were in the late prophase of the first meiotic division, complete this division, giving rise to the first polar body and to the secondary oocyte, shortly before ovulation. The second meiotic division then begins, but it may be accomplished only after ovulation and fertilization has occurred (Balboni, 1976).

4.2 Ovulation is the process by which the egg is released from a follicle. Many changes occur in the mature follicle in preparation for follicular rupture. These changes involve the surface epithelium, the follicle envelope and the oocyte (Peters and McNatty, 1980). The cells of the surface epithelium overlying the apex of the follicle (the portion of the follicle related to the ovarian surface) have been shown with SEM to lose their microvilli, become flattened and ultimately slough off from the apex of the follicle (Bejersing and Cajander, 1974 a and b; Motta et al, 1971; Blaundau, 1967). Progressive disintigration of the cellular layers composing the apex follows and is probably affected by the action of proteolytic enzymes (E spey, 1974; Espey and Coons, 1976). It is suggested that the lysosomal-like bodies which appear in the cytoplasm of the overlying surface epithelial cells contribute to digestion and disintegration of the connective tissues of the apex of the follicle (Bejersing and Cajander, 1975). The pronounced increase in fluid (oedema) in the cellular component of

the apical wall also contributes to the weakening and final rupture of the follicle (Van Blerkom and Motta, 1979).

Once the gap appears in the follicle wall, the viscous mass of follicular fluid oozes out of the gap, taking with it the oocyte-cumulus cell mass which became free, floating in antral fluid shortly before follicle rupture. Whether the extrusion of the oocyte involves active contraction of the follicle is not clear. However, smooth muscle cells are present in the theca externa which may have a contractile function that may play a role in the oocyte-cumulous cell mass extrusion (O'Shea, 1973).

Ovulation is not a random event, but occurs following increased concentrations of L.H. and the ovary has been exposed to elevated levels of hormone for a certain period of time (Lipner, 1973). The factors responsible for the initiation of the pre-ovulatory L.H. surge vary in different species. In some, specific increase in the secretion rate of estradiol by the ovary is responsible for L.H. surge (e.g. primates), whereas in other species stimulation of the uterine cervix is needed (e.g. in rabbit) (Peters and McNatty, 1980).

4.3 Follicular (involution) atresia: In the period between birth and puberty a large number of primordial follicles degenerate and disappear. During sexual life only a very small proportion of follicles run through the entire course of maturation.

It is not possible at the present time to distinguish morphologically between "healthy" follicles which are committed to undergo atrasia and "healthy" follicles which will complete their growth phase

(Byskov, 1979). Degenerative changes can become manifested in the different compartments of the follicle and may consist of degeneration of the germ cells, degeneration of the granulosa and its replacement by fibrous tissue, thickening of the basement membrane that encircles the follicle (atresia membrana), hypertrophy of the theca interna and the formation of the so-called interstitial glands (Byskov, 1974).

The atretic process may present different morphologic patterns (Byskov, 1979), in relation to the stage of follicular development; one may distinguish primordial or primary follicles that may degenerate without leaving traces, haemorrhagic, in which intrafollicular haemorrhage occurs, leaving corpus nigrum and cystic atretic follicles.

4.4 Endocrine activity of the growing follicle. The developing follicle produces estrogen, the cell involved in this endocrine activity arethe theca interna cells (Younglai, 1973). The granulosa cells do not seem to be directly capable of estrogen production, although they may collaborate with the theca interna cells in the production of estrogen (Smith and Zuckerman, 1973). The granulosa cells of the growing follicles are probably capable of secreting progesterone and are only waiting for a blood vessel to penetrate the layers after follicular rupture, to luteinize and produce progesterone (Balboni, 1976).

5. Corpus luteum.

After rupture, the collapsed follicle begins to transform into corpus luteum. If fertilization occurs, a corpus luteum of pregnancy develops, that lasts and functions for a period which varies greatly

with the species (for about three months in the human and for 28 days in the rabbit); if fertilization does not occur, a corpus luteum (or corpora lutea in the rabbit) of the cycle (menstrual corpus luteum) develops and remains functional for about 14 days in the human and in the rabbit.

Several stages may be distinguished in the life of the corpus luteum (Balboni, 1976):

(1) Stage of proliferation and hyperaemia. Soon after follicular rupture, granulosa cells undergo some proliferation and the theca interna layer appears prominent and hyperaemic.

(2) Stage of vascularization. Very early, thin-walled blood vessels arise from the theca interna, penetrate through the granulosa into the lumen and branch to form a fine network of capillaries.

(3) Stage of transformation. Simultaneously with the vascular penetration, or immediately after, the granulosa cells undergo rapid hypertrophy and complete their transformation into granulosa lutein cells. The theca cells also participate into the formation of the corpus luteum. They arrange themselves into strands of theca lutein cells at the periphery of the corpus luteum.

(4) Stage of maturation. By the fourth day after ovulation, the granulosa lutein cells have reached their full development and present the cytological and physiological properties of progesterone-producing cells. A wide network of sinusoidal capillaries penetrate them.

(5) Stage of regression. This consists of degeneration of lutein

cells of proliferation of connective tissue, which give rise to a "corpus fibrosum". If hyaline transformation occurs in the fibrous tissue, a "corpus albicans" forms.

5.1 Menstrual corpus luteum (corpus luteum of the cycle). The corpus luteum of the cycle reaches full maturity between the seventh and tenth day from rupture of the follicle and this period corresponds to the peak of the secretory activity of the menstrual corpus luteum (Balboni, 1976).

With light microscopy (L.M.), the main cell in the luteal tissue is the granulosa luteal cell, which is a large cell with a granular cytoplasm, vesicular nucleus and a prominent nucleolus (Peters and McNatty, 1980).

With E.M., the granulosa lutein cells are found to have abundant smooth endoplasmic reticulum (Koering, 1969). The mitochondria change to a highly pleomorphic body and the Golgi complexes increase in number and size and are scattered throughout the cytoplasm (Koering et al, 1973 a, b).

The luteinized granulosa cells develop microvilli on their surface that project into intercellular clefts and pericapillary spaces; these are thought to play a role in the transport of steroid hormones into adjacent capillaries (Adams and Hertig, 1969 a and b). The theca cells also show abundant SER, rod-shaped mitochondria with tubular cristae and large lipid droplets in the cytoplasm (Crisp et al, 1970).

5.2 Corpus luteum of pregnancy. This grows more than the corpus luteum of the cycle and, in the human, attains about 4-5 cms in the third month of pregnancy and is believed to function only in the first tri-

mester of gestation and involute slowly.

With E.M., a distinct feature exhibited by the granulosa lutein cells is the presence of a well developed SER that is well organised in manifold membrane and system concentric membrane whorls (Crisp et al, 1970). These whorls are not observed in the corpus luteum of the cycle.

In the human and rabbit corpora lutea, gap junctions exist in the interdigitations between the luteal cells (Balboni, 1976). The extensive gap junctions found in the luteal cells are probably responsible for spreading the impulse induced by gonadotrophin, first along the surface of the target cells and subsequently on to adjacent cells (Abel et al, 1975).

Whether fertilization and implantation occurs or not, (i.e. whether a corpus luteum of cycle or pregnancy forms) the developmental changes leading to the formation of the corpus luteum are the same as are the subsequent regressive events (Van Blerkom and Motta, 1979).

Ovarian vascular and nervous supply: The main arterial supply to the ovary is from the ovarian artery which arises from the aorta below the level of the renal arteries. About midway between its origin and its entrance into the ovary, the ovarian artery communicates with the uterine artery through a long slender branch, the utero-ovarian branch, which in the rabbit is not significant (Delcampo and Ginther, 1972). Before entering the ovary, the ovarian artery is often thrown into one or more loose coils (Harrison and Weir, 1977) which are more prominent in the rabbit than in the human (Delson et al, 1949). The secondary

and tertiary branches inside the ovary also display spiralling in the rabbit; the spirals open out to accommodate the increased ovarian size during phases of increased activity (Reynolds, 1973). The small coiled vessels give rise to straight vessels which penetrate the thecae of the follicles and give rise to the precapillary vessels which immediately form a rich network of capillaries that completely envelope the follicle (Burr and Davies, 1951).

The veins of the ovary are thin-walled; even the largest intra-ovarian vein in the rabbit is without a muscle coat and only thickened by a layer of connective tissue after they leave the ovary. The course of the veins outside the ovary follows that of the corresponding arteries. The utero-ovarian communication is not significant as it carries only a small portion of the blood supply to the ovary and is not involved in a local utero-ovarian pathway of luteolysin as such a pathway is systemic and not local in the rabbit (Ginther, 1974).

The ovarian nerves are mainly adrenergic (Burden, 1972). They are mainly found accompanying the blood vessels and smooth muscle, and are prominent near follicles; however, muscle elements in the follicle wall bear little relation to ovulation in the rabbit (Lipner, 1973). In the adult ovary the stroma may be randomly innervated. According to Neilson et al (1970) and Fink and Schofield, (1971), the stromal nerves can inhibit the secretory activity of the interstitial tissue and in this way can influence the rate of atresia, the selection of follicles for maturation and the output of steroid.

OVARIAN PHYSIOLOGY

The ovary is capable of the following major functions: the production of female gametes (oocytes), the secretion of specific steroid hormones (e.g. estrogens and progesterones) essential for the sexual events occurring within the genital tract (oestrus cycle, menstrual cycle, pregnancy) and the hormonal regulation of the post-natal growth of the reproductive organs and the development of secondary sexual characteristics.

1. The Production of Oocytes

The primordial germ cells "oogonia" arise outside the uro-genital ridge in the dorsal endoderm of the yolk sac before the primordia of the gonad s form. They migrate by amoeboid movement (Blandau et al, 1963). via the hind gut endoderm, the dorsal mesentery and the coelomic lining to the gonadal ridge (Everett, 1943; Peters and McNatty, 1980). While on their migratory path, the germ cells are usually seen as single cells often in mitosis. After their arrival in the gonad, they tend to cluster and continue to multiply for a certain period. They then enter a meiotic prophase, which lasts longer than a prophase in any other cell, and are subsequently called primary oocytes.

The oocyte then undergoes two meiotic divisions which do not lead to increase in the germ cell number as each meiotic division gives rise to a very much smaller daughter cell, the polar body, which is discarded. The first meiotic division results in the halving of the chromosome number while the second meiotic division does not further reduce it.

The various stages of meiotic prophase can be divided into transitory phase and stationary phase (deplotene); in the latter the oocytes remain until shortly before ovulation (Kennelly et al, 1970). Not all germ cells enter meiotic prophase at the same time, but some germ cells continue to divide mitotically, while others have already entered the meiotic prophase. The duration of oogenesis, i.e. the formation of oocytes from oogonia (germ cells) varies considerably in different species and it is about five months in the human.

Degeneration of the germ cell (germ cell atresia): The highest number of germ cells is present in the female gonads during the time when the oogonia are being transformed into oocytes. Many, however, are lost during the transitory stage of the meiotic prophase; only 5% of the germ cells survive in the gonads in the human at birth.

2. Ovarian Hormone Synthesis

The main steroids produced by the ovary are progestins, androgens and estrogens. The amounts secreted vary during infancy, puberty and adult life. These variations are causally related to the continuously changing proportion of the endocrine tissue within the ovary (Peters and McNatty, 1980).

The common steroid precursor for the biosynthesis of progestins, androgens and estrogens is cholesterol, which in turn can be synthesized from acetate. The intermediate precursor for the synthesis of the ovarian steroids is pregnenolone, which is derived from cholesterol.

Progestin: The major progestins synthesized by the ovary is progesterone. The enzymes required for the conversion of pregnenolone to progesterone are localised in the luteal cells but not in the stroma in women (Friedrich et al, 1974), while in the rabbit these enzymes are present in the stroma as well (Leavitt and Blaha, 1970).

Androgens: The ovary is an important source of androgen and all the three endocrine tissues of the ovary - the stroma, the follicle and the corpus luteum - have the capacity to produce androgens; the stroma and the theca seem to be the major sites for the production of androgen (Seamark et al, 1974).

Estrogens: All ovarian estrogens are derived from the androgens. The theca and granulosa cells of the follicles and in some species, the corpus luteum, are the principal sites for estrogen biosynthesis (Young Lai , 1973).

Steroid Secretion by the Ovary

It may be useful to consider the three main ovarian tissue compartments or subunits as separate steroid-forming glands (Eckstein, 1977). They are: the Graafian follicle (granulosa and theca interna cells), the corpus luteum (theca lutein and granulosa lutein cells) and the stroma (interstitial cells).

Hormone production and physiological role of the special cell aggregates near the ovarian hilus (hilus cells) is still uncertain.

Follicle: Follicles can secrete several steroids, amongst which are: progesterone, oestrone and testosterone (Baird, 1973; McNatty

et al, 1976). The small follicles contribute only a small amount to the total concentration of steroids in ovarian venous plasma. As the follicles enlarge, their contribution of steroid secretion to the amount entering ovarian venous plasma increases significantly (Peters and McNatty, 1980), Progesterone is secreted by granulosa cells of large pre-ovulatory follicles of some species (primates, pigs, etc.) (Baird et al, 1975; Hunter et al, 1976). Androgens are also secreted by the follicles; the follicles become significant sources of plasma androstenedione during their final stages of growth (Baird et al, 1974; McNatty et al, 1976). The large antral follicle is a major source of estrogen (Baird and Frazer, 1974). The major estrogens produced are oestradiol 17β and to a lesser extent oestrone. Rising levels of oestradiol indicate a progressively differentiating large follicle (Baird et al, 1976).

Corpus luteum: The corpus luteum mainly secrete progestins in most species. The secretion of progesterone increases after ovulation and until the mid-luteal phase and correlate closely with the increasing vascularity of the corpus luteum (Corner, 1956; Peters and McNatty, 1980). Recent E.M. observations suggest that the cells of regressing corpus luteum at the end of pregnancy in women and rabbits may be involved in the synthesis and storage of the polypeptide hormone relaxin (Crisp et al, 1970).

Stroma: The stroma seems to function in different ways in different species. In some species (rabbit) it is the major source of progestins (Hilliard et al, 1969; Leavitt et al, 1971); in other species (man) the stroma secretes an appreciable amount of androgens

(Mikhail, 1967; de Jong et al, 1974; Swanston et al, 1977). Only small amounts of estrogens and traces of progesterone were identified in the stromal cells of human ovary (Eckstein, 1977).

The pattern of steroid synthesis and secretion by the ovary is controlled by gonadotrophin released from the anterior pituitary; in some animals the uterus plays a major role in controlling ovarian function (Ginther, 1974).

Steroid Hormones in Ovarian and Peripheral Blood

Direct measurements of the steroid content of the ovarian venous plasma has been carried out with the organ in-situ and/or ovaries autotransplanted to a more acceptable site (for instance, in the sheep neck (Goding et al, 1967; Baird et al, 1968)). As a rule steroid concentrations are considerably higher in ovarian effluent than in peripheral blood.

In man, the nature and concentration of steroids have been reported in the ovarian venous and peripheral plasma (Mikhail, 1967). The wide range of steroid concentrations observed was stressed and related to the cyclic growth and regression of the follicles and corpus luteum (Mikhail, 1970).

In the rabbit, progesterone has been identified as the secretory product of the ovary before ovulation and during pseudopregnancy (Hilliard et al, 1963) and pregnancy (Mikhail et al, 1961). The corpus luteum is the main source of progesterone (Hilliard et al, 1973). This hormone and 20α dihydroprogesterone are produced in addition by the

interstitial cells (stroma) between copulation and ovulation. The synthesis and secretion of progesterone is further stimulated by the pre-ovulatory surge of L H, which occurs in response to coitus in the rabbit. The rabbit's ovary also secretes oestradiol 17β as the main estrogen (Hilliard and Eaton, 1971; Eaton and Hilliard, 1971). The secretion of oestradiol 17β rises at oestrus and reaches a maximum four hours after coitus.

The rabbit ovary also secretes large amounts of androgens in addition to estrogens and progesterone (Mills and Savard, 1973); secretion of testosterone is ten times that of oestradiol and the secretion pattern after coitus or after the HCG administration is similar for both steroids (Hilliard et al, 1974a).

The secretion rates from both ovaries were found to be similar by Hilliard et al (1974b) whereas Shaikh and Harper (1972) found marked differences in the secretion rate between the two ovaries. The technique used by Shaikh and Harper involved ligation of the main ovarian artery and must, therefore, be considered not physiological (Baird, 1977).

The mechanism of action of L H in increasing steroid synthesis in vivo has been investigated in the rabbit by Solod et al (1966) and it was found that following intravenous injection of L H, a marked secretion rate of progesterone occurred due to increased synthesis of the steroid from acetate.

Hypothalamic-Pituitary-Ovarian Relationship

The ordered growth and differentiation of the follicle is dependent on functional inter-relationship between the hypothalamus, pituitary and the ovary. The hypothalamus and the pituitary form a functional unit that secretes a continuous tonic "low" pulsatile as well as cyclic "high" flow of gonadotrophic hormones. The blood levels of these hormones is maintained by tonic release to support follicular growth while the cyclic high secretion bring about the final differentiation of the follicle and ovulation (Peters and McNatty, 1980). The pulsatile release of gonadotrophic hormones is regulated through a pulsatile neuro-secretion of gonadotrophic releasing hormone (GnRH) (Carmel et al, 1976).

The effect of oestradiol on F S H and L H release depends on the concentration of the steroid in the plasma. When the plasma level of oestradiol is low (as in the late luteal or menstruating phase of the cycle in women), the hypothalamic-pituitary secretion of gonadotrophin is high. When the plasma concentration of oestradiol increases (as in the mid follicular phase in women), there is a concomittant reduction of gonadotrophin output because of reduced pituitary sensitivity to GnRH. In addition to an inhibitory influence on F S H output (negative feedback) estrogens are capable, under the right circumstances, of stimulating gonadotrophin release (positive feedback) (Knobil, 1974). The postive feedback action of estrogen leads to pre-ovulatory release of gonadotrophin (Yen et al, 1975). In women, the pre-ovulatory release of F S H and L H under the estrogen secretion rate (200 µg/day) is achieved by the 20 mm antral follicle (Peters and McNatty, 1980).

Progesterone has little effect on the secretion of FSH or LH (Knobil, 1974; Franchimont, 1971), but it modulates the inhibitory and stimulatory action of estrogen and gonadotrophin secretion (Rothchild, 1965). During the luteal phase when there is a high level of progesterone or during lactation when there is a high level of prolactin, the sensitivity of the hypothalamus and the pituitary to the requirement of the developing follicle is impaired. Under these conditions, follicular growth cannot be sustained.

Ovarian activity during gestation

The ovary plays a vital role in the initiation, maintenance and termination of pregnancy. This involves the formation and discharge of the ovum, transport of the ovum through the oviduct, ovum implantation, maintenance of pregnancy and parturition (Amoroso and Finn, 1962; Amoroso and Perry, 1977).

Induced or Reflex Ovulation

In contrast to spontaneous ovulators in which ovulation can occur in the absence of males, there are many mammalian species in which ovulation nearly always fails in the absence of the male. These are called "reflex ovulators". Examples of reflex ovulators are the cat, camel and rabbit, of which the best studied is the domestic rabbit.

Laboratory rabbits, kept under a 16 hour daylight regime, will mate readily throughout the year although even under "constant conditions" there is often a drop in receptivity in November-December (Cohen, 1977). The rabbit will breed at any time of the year and there is no oestrus cycle in the strict sense. The ovarian follicles develop in overlapping waves so that as some follicles begin to degenerate, others have begun to mature. This arrangement favours a rather constant level of estrogen production and sexual receptivity (Feder, 1981).

Although coitus provokes pre-ovulatory growth of the follicle and ovulation, this stimulus is not an absolute requirement. Sometimes ovulation occurs when one female mounts another, or even when females are kept singly in cages (Brooks, 1937).

Endocrinological studies in the rabbit during the oestrus and before coitus showed that peripheral oestradiol levels are of the order of 50 pg/ml and peripheral plasma values for progesterone and L H are low (Feder, 1981). Within 30 minutes after coitus, L H begins to be released in increased quantities and by 90 minutes shows increase by as much as 100 times base line values (Dufy-Barbe et al, 1973). L H levels remain elevated for 6 hours after coitus. The most dramatic effect of this L H surge is the induction of substantial increase in progestin level in ovarian vein plasma; this increase consists mainly of 20α-DHP, the reduced form of progesterone, (with only a modest increase in progesterone) which lasts until about 8 hours after coitus (Wu et al, 1977; Hilliard et al, 1963). The source of this newly synthesized progestin is the ovarian interstitium (Hilliard et al, 1967). The suggestion that the pre-ovulatory 20α-DHP help to maintain the L H surge (Hilliard et al, 1967) is not supported by the more recent studies of Younglai (1977), which show that no ovarian steroids are required within 15 minutes after mating for the normal L H surge to occur. L H release also results in an increased ovarian concentration of oestradiol for about 3 hours (Hilliard and Eaton, 1971). This transient elevation of oestradiol may serve to enhance muscular activity of the oviduct during the ovulatory process. Ovulation occurs about 10-12 hours after coitus at which time steroid and L H levels have returned to basal levels.

Pseudopregnancy

Mating the rabbit with vasoectomized male or induction of ovulation by injection of H C G or L H, or certain metal salts (e.g. copper salt) results in pseudopregnancy which lasts 16-17 days. The size of the corpora lutea of pseudopregnancy increase in 8 days and become maximal betwen 8 and 14 days of pseudopregnancy. These corpora lutea regress late in pseudopregnancy and vanish during the next oestrus (Yo shinaga, 1973). The rabbit is seen plucking at its breast fur and carting hay, as in normal pregnancy (Cohen, 1977); mammary growth is also similar to normal pregnancy. Progestin secretion was determined throughout pseudopregnancy by Hilliard et al (1968). Soon after the administration of an ovulatory dose of L H, an extremely high amount of progestin (20α -DHP) was secreted. The progestin secretion returns to the basal level by the time of ovulation. In the post ovulatory period progestin secretion increased again to a maximal at about 8-14 days and decreased again. The progestin secretin fluctuated in parallel with the average weight of the corpus luteum.

Owman et al (1979) studied plasma oestradiol in HCG-induced pseudopregnancy. They found that plasma oestradiol did not change except for slight rise on Day 18. However, they observed a marked decline in tissue oestradiol on Day 3. The lowest concentration was found at Day 6 but Day 18 values were comparable to those of untreated controls.

Microsurgery, microvascular anastomosis and transplantation

Microsurgery was born in 1921 when Professor Nylen used a simple operating microscope to treat patients with chronic otitis and labyrinthine fistulas (Nylen, 1954). He also performed experimental fenestration in rabbits. For the next 30 years the operating microscope was used almost exclusively by ear, nose and throat surgeons. Ophthalmologists later saw the value of the instrument (Perritt, 1950) and it was recommended for corneal surgery.

The early slow progress of microsurgery was followed in the Fifties by rapid progress which led to the highly sophisticated level of today and influenced every surgical specialty.

Before 1960, results of arterial repair in vessels smaller than 4 mm in diameter had been uniformly poor (Shumacher and Lowenberg, 1948; Hurwitt et al, 1953). However, by using the operating microscope with up to 25 magnification, 100% patency was achieved experimentally in the anastomosis of 1.4 mm diameter arteries (Jacobson and Suarez, 1960). This heralded the clinical use of microvascular surgery and stimulated many surgeons to investigate further in the field.

It soon became obvious that the available microscopes were inadequate for microsurgery because magnification was fixed with manual control and vision was restricted to a single surgeon. To overcome this, two, and later three, microscopes were joined together to view the same site. These were termed the diploscope and triplo-

scope respectively (Littman, 1962; O'Brien, 1970). Later, control of magnification and focusing was replaced with an automatic foot operated mechanism producing the O P M I2 (Littman, 1970). The use of fibroptic light source improved vision and allowed heat to be dissipated away from the operation field; a beam splitter and double zoom mechanism with dual control gave surgeon and assistant independent control of magnification with a common focus.

Little advantage could be gained from operative microscopy without adequate precision and miniaturization of instruments and sutures. Some fine instruments were already available from surgical instrument makers and jewellers, but others have been developed during research on vascular, lymphatic and neural microsurgery.

With the improved microsutures and with accumulating experience in microvascular surgery, an 81% patency rate on rabbit arteries (averaging 1 mm in diameter) was reported (O'Brien et al, 1970). With more refined techniques and further improved sutures, Hayhurst and O'Brien (1975) achieved 98% long patency rates on femoral arteries in rabbit (averaging 0.9 mm diameter) and 85% patency following repair of 0.5 mm arteries was obtained in the rat (O'Brien, 1977). A technique using interrupted nylon sutures (Cobbett, 1967) is now acceptable or preferable. The various aspects of microanastomosis which contribute towards success, have been analysed in great detail and refined, so that vessels of 0.5 mm diameter may now be reliably joined by a practised surgeon (Acland, 1980).

Despite many investigations into the efficiency of various anticoagulants and antispasmodics (Koler et al, 1973; Sinclair, 1980),

their place remains uncertain. However, there is no doubt that excellence of micro-vascular technique is the single most important factor affecting patency rate (Hayhurst and O'Brien, 1975).

Factors which are thought to predispose to occlusion of a micro-vascular anastomosis are generally not very different from those causing thrombosis in other vessels. They include, in addition to factors related to faulty technique: (O'Brien, 1977)

(a) increased clotting tendency of blood during operation and in the immediate post-operative period;

(b) damage to the vessel and exposure of the subendothelial thrombogenic collagen as a result of trauma or chemicals, including contrast media, and the presence of sutures which may act as a nidus for platelet aggregation;

(c) slowing of blood flow.

The outstanding merits of microsurgery (Winston, 1982) stimulated surgeons to perform transplantation of genital organs in the hope that improvement of the technique and more effective control of the rejection process will enable the procedure to be utilised for the treatment of infertility by replacement of irreversibly damaged or lacking genital organs. Microsurgical techniques were used for the allo-transplantation of the oviduct in the human by Wood et al (1978), but the graft was rejected, and by De Cherney and Naftolin (1980), with achievement of unsuccessful pregnancy. Microvascular ovarian transplantation in humans has, so far, not been reported.

CHAPTER 2

GENERAL METHODOLOGY

EQUIPMENT

Operating Microscope: (Fig. 2.1 A)

A Zeiss OPMI6 operating microscope was used for all surgical procedures; it gives binocular vision and it can be fitted with objective lens of varying focal lengths. 12.5 X eye pieces were normally used with a 250 objective lens. Focussing and magnification are controlled remotely by foot pedal. The microscope was provided with a still camera; for these experiments a standard universal microscope stand was used, which is floor-mounted.

Operating Table (Winston, 1975) (Fig. 2.1 B)

The metal table, which can be adjusted in height or tilted in any direction, is 50 cm in length; it can be fitted with four metal stanchions to bind the limbs of the animal; it can be electrically heated to preserve the body heat of animals undergoing longterm anaesthesia.

Hand rest (Acland, 1972a)

Two metal hand rests to reduce tremor were used; these support the whole of the forearm and wrist of the operator, and are adjustable in height.

Bipolar Diathermy: (Fig. 2.2)

This was fitted with insulated jeweller's forceps for controlling

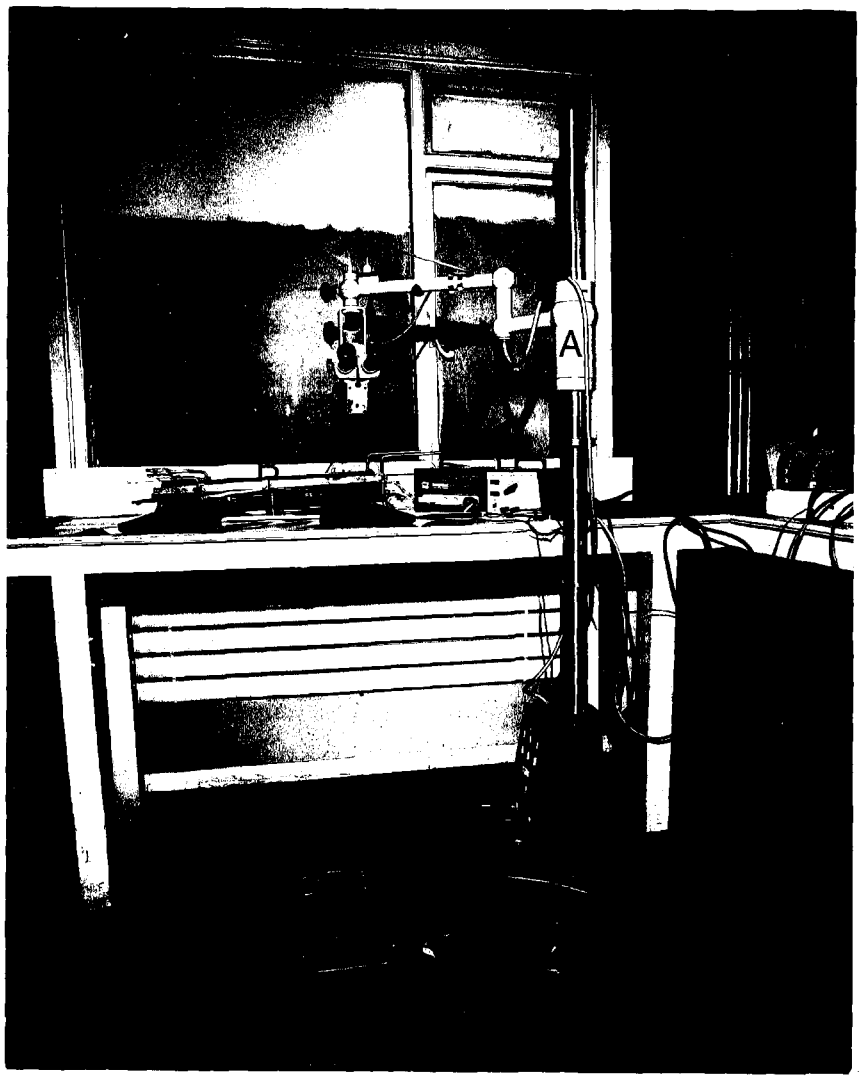


Fig. 2.1 A. Carl Zeiss OPM I6 operating microscope, with fibro-optic lighting and stand.

B. Operating table with hand rest.

bleeding; the diathermy is capable of low power output and is made by (Codman and Shurtleff, U.S.A.).

Microsurgical Instruments (Fig. 2.3)

Jeweller's forceps: Straight No. 5 Dumont forceps were used for tissue handling and sometimes for tying sutures. The tips of these forceps are aligned very precisely (Acland, 1975).

Vessel Dilator (Acland, 1972b): Is a modified jeweller's forceps and has electrically etched tips which are smoothly polished and very fine. When the tips of these forceps are opened inside the cut end of the vessel, they stretch the vessel without causing intimal damage.

Clamp applying forceps: These forceps are used to apply vascular clamps.

Microsurgical needle holder: This has a round handle, 18 cm long without lock. The configuration of the handle facilitates rotation of the needle point. The tips of the needle holder are slightly angulated.

Microscissors: Straight with pointed tips and a flat handle were used to facilitate dissections of the tissues surrounding the vessels and their sheaths.

Two single Acland microvascular clamps: 8 mm in length, were used to occlude the vessel's end.

Double Acland vascular clamps with cleated frame: The frame is used to hold stay sutures and to maintain the vessel in place during suturing (Acland, 1974).



Fig. 2.2 A. Microbipolar coagulator with jeweller's forceps.
B. Unipolar diathermy with fine cutting/coagulating needle.

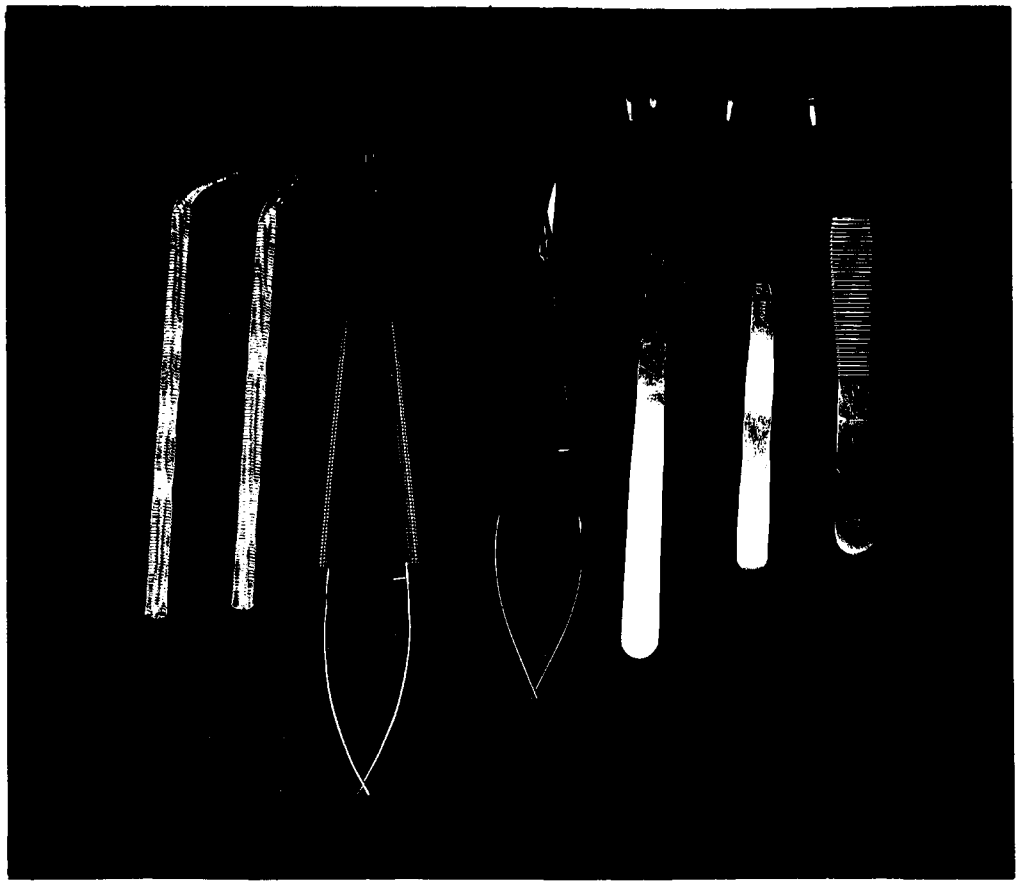


Fig. 2.3 Basic microvascular set: Top row - plastic backgrounds, double Acland microvascular calmp with cleat, two single microvascular clamps. Bottom row - right to left - clamp holding forceps, jeweller's forceps and vessel dilator, No. 5 and,2 microscissors, micro needle holder, two glass rods.

Sutures: Various microsutures (Spingler Tritt GmbH) were used,

10/0 monofilament nylon, 22 micron diameter, with a round bodied 100 μ diameter needle

11/0 monofilament nylon, 18 micron diameter, with flat bodied 75 μ diameter needle

8/0 monofilament nylon, 35 micron diameter, with a round bodied 140 μ diameter needle

Glass rods (Winston, 1981): Two fine tipped right-angled flame-polished glass rods were used for microsurgical manipulations.

Drugs and Solutions

Heparin inj. B.P. (1000 I U/ml) was used for subcutaneous injection in the transplanted animals and for mixing with the irrigating Ringer's solution, to be used topically in microvascular anastomosis (Sinclair, 1980).

Procaine hydrochloride B.P. 2% W/V in ampoules containing 40 mg/2 ml were used to be applied on the vessels at the end of anastomotic procedure as an antispasmodic.

Kemicetine, succinate (1 g vial chloramphenical sodium succinate) Farmitalia Carlo Erba Ltd., Italy, was given by intramuscular injection immediately after surgery and in the post-operative period.

Cyclosporin A: 20 g was kindly supplied by Sandoz Ltd. (Order No. 59615) Pharmaceutical Division Research and Development, Basel, Switzerland. It was prepared for intramuscular injection as follows: 5 g of cyclosporin A powder was weighed and dissolved in 200 ml olive

oil, pre-heated in a dark container to a temperature of 60-80°C (average 70°C). The temperature was kept constant and was monitored using a thermometer which was also utilized for stirring the mixture. Heating and stirring continued until the drug completely dissolved; this process usually took approximately two hours. The solution was decanted into a dark bottle and kept in the dark for three days before use. The solution was made up with a drug concentration of 25 mg/ml.

Compound sodium lactate B.P. (Ringer's solution): Was used for irrigating the tissue, and small blood vessels (Acland, 1980a). Heparin was added to a concentration of 10 I U/ml.

Animals

Healthy mature virgin New Zealand White rabbits (Froxfield, U.K.) weighing between 3-5 Kg, were used throughout this study. Two New Zealand white male rabbits of proven fertility were maintained for breeding purposes.

All the animals were caged individually with a light/dark cycle of 16/8 hours. The ambient temperature was maintained at 20-25°C; standard rabbit chaw and water was provided ad libitum. Food and water were withheld for 24 hours before surgery. Animals were transferred to the laboratory on the day of the surgery.

Anaesthesia

Anaesthesia was induced with an intramuscular injection of Hypnorm (Fentanylcitrate 0.31 mg/ml and fluanisone 10 mg/ml), Crown Chemical Company Ltd., Kent, England) supplied in 5 ml vials. A dose

of 0.5 ml/Kg body weight was given. Anaesthesia was maintained with 1% Fluothane given in 3 litres/minute of oxygen by a plastic face mask; shortly before termination of surgery, Fluothane was discontinued and the rabbit given 2.5 litres/minute oxygen only until recovery.

Laparotomy

The abdomen was shaved with the animal bound to the operating table; the site of incision was then prepared with 0.5% chlorohexidine in alcohol. The abdomen was draped with clean but not sterile towels. Laparotomy was done with a midline incision using unipolar cutting diathermy and/or a sharp scalpel, for initial exposure. Excessive subcutaneous bleeding was controlled with bipolar diathermy. The fascia was incised, the muscle separated and the peritoneum opened with Mayo scissors. The bowel was displaced with clean abdominal packs soaked in warm Ringer's solution. Before abdominal closure, the packs were removed and blood clot and debris washed from the peritoneal cavity using heparinized Ringer's solution. 100 ml of this solution was left in the peritoneal cavity. The peritoneum was closed with the edge everted so that no raw areas were left facing the viscera. The abdominal wall was closed in layers with continuous 4/0 polyglycolic acid sutures. The animals were kept warm until they recovered from anaesthesia, when they were transferred to their cages. 300 mg Chloramphenical were given by intramuscular injection after operation, and was continued daily for five days.

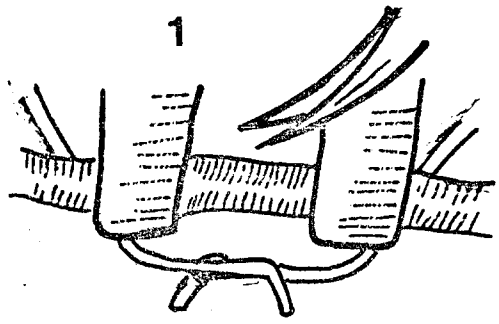
Microvascular anastomosis (Fig. 2.4)

Vascular anastomosis was performed using a modification of the

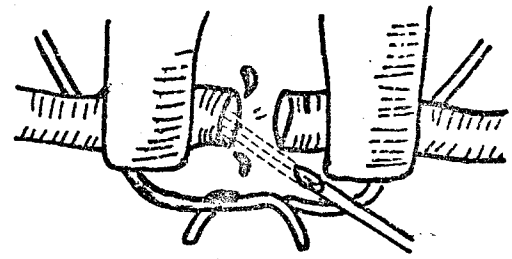
basic technique described by Acland (1980, 1972b) Meticulous attention to gentle handling of the tissue, particularly during the isolation of the vessels, was exercised throughout. The following points were carefully observed: Vessel ends were opposed without twisting and kinking; interrupted sutures were spaced evenly; the operative field was kept free of blood and in accurate focus. This is absolutely essential if damage to the vessel is to be minimised. The tissues were continuously irrigated with warm heparinized Ringer's solution.

Procedure: Vessels were identified in their perivascular sheath and isolated from the surrounding tissues using jeweller's forceps. Arteries could be distinguished from veins by observing pulsation. The artery was separated from the vein after incising the sheath with microscissors. It could then be elevated in isolation and subsidiary branches coagulated if necessary. Dark coloured plastic material was placed behind the artery to increase visualisation of the cut end. An Acland double clamp was applied across the artery before division. The cut end was washed clean of blood with a fine jet of Ringer's solution; when it was completely clean of blood, the vessel wall could be seen as translucent. The adventitia were trimmed to ease identification of the media during suturing. Next, the cut ends of the vessel were dilated using the vessel dilator. This increases luminal size and prevents spasm, thus facilitating suturing. Two 11/0 stay sutures were inserted across the anastompsis 120° apart; this allows the posterior vessel wall to fall away and reduces the risk of placing a stitch through it inadvertently (Cobbett, 1967). After the anterior row of sutures was placed, the clamps with the vessel were rotated 180° and

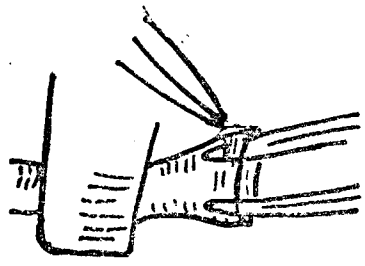
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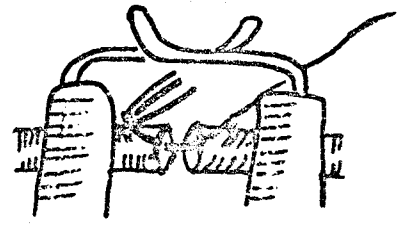
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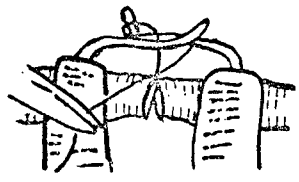
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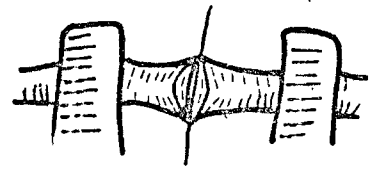
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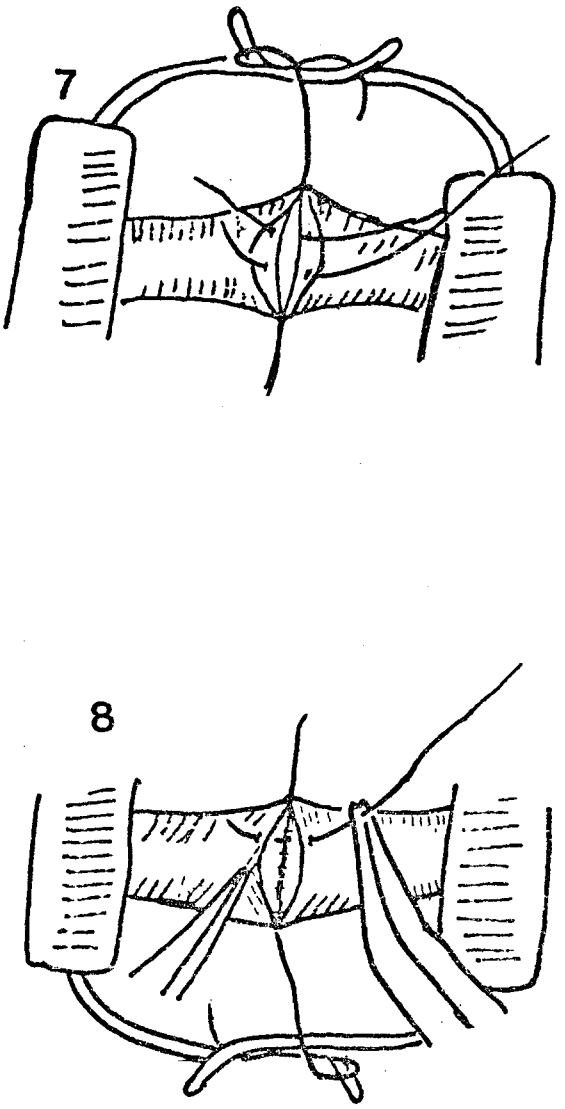


Fig.2.4

Diagramatic representation showing the major steps in the procedure of vascular anastomosis.
1. Isolation of the vessel.
2. Transection and flushing of the cut ends.
3. Dilatation of the cut ends of the vessel.
4, 5, 6. Placement of two stay sutures.
7. Suturing of the anterior row.
8. Suturing the posterior row.

the other side of the vessel was sutured. Before releasing the clamps, Procaine was applied locally to the vessel wall. Some initial oozing of blood from the anastomotic site was not uncommon and was dealt with using temporary gentle pressure.

Anastomosis of veins was done in a similar fashion to arteries but particular attention was paid to the following points:

(1) Extra care was taken in handling the vein as its walls were thinner and could be easily punctured during dissection from the vascular sheath.

(2) Sutures can be placed further apart in the vein as the pressure is lower in it than that in the artery.

Patency of the anastomosis was checked in the following way:

Arteries: Pulsation was confirmed beyond the anastomosis. If there was doubt about patency, the vessel was displaced over a glass hook until the vessel was almost occluded. When the vessel could be seen to fill and collapse beyond the join, patency was confirmed (Acland, 1972c; Hayhurst and O'Brien, 1975).

Veins: A blocked anastomosis results in tension and distortion above the join, whilst below the join the vein remains collapsed. Good dilatation of vein equally on either side of the anastomosis suggests patency. Confirmation of patency can usually be achieved using a glass hook as described for the artery; however, these tests all tend to be inconclusive occasionally (Krag and Holck, 1981). When there is serious doubt, it is best to resect and revise the anastomosis.

Ovarian stimulation tests

1. Ovarian stimulation by mating:

This test depends on the reflex release of luteotrophic hormone (LH) from the anterior pituitary in response to copulation, and the action of the released hormone on the ovary to induce ovulation within 12 hours (Schwartz, 1973) and stimulate a marked increase in ovarian blood flow (McCracken et al, 1969; Wurtman, 1964). The test was used in this study to:

(a) Test the integrity of the hypothalamic-pituitary-ovarian axis.

(b) Assess, quantitatively, the capacity of the ovary to ovulate, form corpora lutea and produce hormones.

(c) To evaluate the ability of the anastomosis of transplantation, to accommodate the augmented ovarian blood flow rates occasioned by the LH surge and the ensuing pregnant state.

Procedure:

Mature virgin New Zealand White female rabbits were used. The vulva was inspected; engorgement suggested that the rabbit was fully receptive (Worden and Leahy, 1962), although receptivity appears to be continuous in the rabbit. The female is then caged with a New Zealand White male rabbit of proven fertility for a period of about 30 minutes. The assumption by the female of the correct lordotic posture with the hindquarters raised, its mounting by the male which

lifts its hind legs (ejaculation reflex) and falls back or sideways as a terminal event, was considered a successful copulation (Beyer and McDonald, 1973; Worden and Leahy, 1962). The animals are then separated. Two weeks after mating, the female was laparotomized (p.75), the ovaries inspected and the number of corpora lutea and gestational sacs recorded. Blood samples for peripheral plasma progesterone estimation were collected (p.82) before mating and at suitable intervals afterwards, as dictated by the conditions of the particular experiment.

2. Ovarian stimulation by human chorionic gonadotrophin (HCG):

The test depends on the similarities of action of HCG and LH (Franchimont, 1970; Morgan et al, 1973) both in inducing ovulation and in stimulating increased blood flow to the ovary (Blasco et al, 1973; Yoshinaga,1973). In this study, the HCG stimulation test rather than stimulation by mating was selected, when the occurrence of pregnancy was not an immediate concern of the experiment, or when the result was required early in the post-operative period. The test was used in this experiment for:

(a) The quantitative assessment of the capacity of the ovary to ovulate and form corpora lutea and produce hormones;

(b) The ability of the vascular anastomosis of transplantation to sustain the increased blood flows required to meet the increased metabolic demands of the HCG-induced pseudopregnant state.

Procedure:

Mature virgin New Zealand White female rabbits were used. Each

animal received 100 I.U. of HCG intramuscularly. Twenty-four hours later animals were laparotomised, the ovaries inspected, the number of ovulated follicles identified under the surgical microscope and their number recorded. 2 ml of blood were withdrawn directly from ovarian vein on either side for plasma progesterone estimation..

Blood sampling:

Blood for plasma progesterone estimation was either collected, depending on the experiment, from:

(a) Peripheral venous blood: collected from the marginal ear vein. After swabbing the tip of the ear with xyline to dilate the vein, blood was collected in a plastic calcium heparin tube, containing 5 mg sodium metasulphite.

(b) Ovarian venous blood: collected from an ovarian vein. Blood was aspirated at laparotomy with a 5 ml syringe and 19 gauge needle. The vein was always punctured with the needle directed towards the ovary. Aspirated blood was transferred immediately into plastic calcium heparin tubes. The blood samples (approximately 3 ml) were centrifuged at 2000 rpm for 5 minutes and the plasma decanted into a second labelled plastic tube which was stored at -20°C.

Radioimmunoassay of plasma progesterone:

The extraction procedure was originally described by Abraham, G.E. et al (1971); however, modificationswas done in this laboratory by dissolving the radioactive tracer and standard progesterone in 10% methanolic buffer instead of plain buffer, because the latter aids

solubility of steroids.

The volume of plasma samples used for extraction varied from 0.1 ml to 0.5 ml. Samples were run in duplicate; the plasma samples were extracted with 10 ml Cold diethylether (BDH) in freezing mixture of methanol and dry ice to separate the lower plasma phase from the upper ether phase which then evaporated to dryness in a vacuum oven at 30-40°C. The residue was dissolved in 2 ml PBS. The labelled 1, 2, 6, 7 ^{3}H Progesterone (S.A. 89 Ci/mmol) was obtained from (Amersham International Ltd., Amersham, U.K.), the total activity which was added to each tube at the time of the assay was adjusted to 8000 CPM/ 100 µl. The overall recovery from progesterone was 98.2 ± 5.1%. Highly specific antisera (rabbit anti-progesterone 11∝ BSA serum) was obtained from (Miles Laboratories Ltd., Slough, England), and used in this assay.

To separate the bound and free steroids, 0.5 ml dextran-charcoal was added to the assay tubes. Duplicates of standard progesterone were run in the assay and were obtained from Miles Laboratories. The sensitivity of the standard curve was 5 pg when applying 95% confidence limit; the minimum detectable level of steroid was 100 pg (0.1 ng/ml) at 1:100 dilution. The intra-assay coefficient of variation was 6.9% and the inter-assay coefficient of variation was 9.4%. Progesterone concentration was expressed in nanograms/millilitre (ng/ml).

Histological examination

Light microscopy:

Tissues were fixed 24 hours in 10% formal saline containing a

phosphate buffer and were progressively dehydrated in ethanol, before clearing in xylol. Tissues were then embedded in paraffin wax before sectioning in 5 µm slices. Sections were stained with Ehrlich's haematoxylin and eosin (Bancroft and Stevens, 1982); they were examined using an Olympus inverted microscope and representative photographs made.

Electron microscopy:

Scanning electron microscopy: Tissues were fixed as for light microscopy, taking special care in handling the tissues to keep the surface undisturbed. Specimens were dehydrated in ascending ethyl-alcohol and dried in a critical point dryer in carbon dioxide (Porter et al, 1972). Specimens were mounted on aluminium stubs and coated with gold in a sputter coater. They were examined in a Cambridge stereoscan 600 and representative photographs made.

Transmission electron microscopy: Tissues were fixed in 3% gluteraldehyde in phosphate buffer in situ. Small pieces of the ovary (1-4 mm) thick were then transferred into 10 mls of fixative, kept at 4°C and fixed for 2 hours before being washed and stored in phosphate buffer. Specimens were subsequently post-fixed in Osmium tetroxide (Millonig, 1970) for one hour and washed in distilled water. They were dehydrated in ethanol and immersed in a propylene oxide/resin before embedding in resin. Semi-thin sections were cut 1 µ thick and stained with Toluidine Blue in 1% borax in order to select representative areas for cutting in an ultramicrotome; ultra-thin sections were placed on uncoated grids, stained with uranyl acetate, followed by lead citrate (Reynolds, 1963) and then examined in an AEI 801 transmission electron microscope.

PRELIMINARY STUDIES

Preliminary Study

This part of the study assessed, separately, the effects of each of the major steps in the planned transplantation procedure, on the structure and function of the ovary and on the animal's fertility.

The first series of experiments studied the effect of division and re-construction of the accessory ovarian ligaments and the fimbria ovarica. In the second series, the relative contribution of the two sources of ovarian blood supply and the effects of altering that contribution by transplantation was assessed. The third series of experiments evaluated the merits of two surgically feasible anastomotic sites of the ovarian vessels, the adequacy of the circulation provided by the anastomosis and the effect of the period of warm ischaemia (normothermic anoxia) which the ovary sustained (at normal body temperature) during the vascular anastomosis.

The preliminary study also included a fourth series in which the direct effect of the immunosuppressant Cyclosporin A, on the normal ovarian tissue, as distinct from its immuno-therapeutic action, was evaluated.

CHAPTER 3

EFFECT OF TRANSECTION AND MICROSURGICAL RECONSTRUCTION OF THE ACCESSORY OVARIAN LIGAMENT (MESOTUBARIUM SUPERIUS, UTEROVARIAN LIGAMENTS) AND FIMBRIA OVARICA ON OVARIAN FUNCTION IN THE RABBIT

EXPERIMENT NO. I

Introduction

It is generally believed that intimate contact of the fimbria with the surface of the ovary is essential for efficient ovum pick up.

In the rabbit not in an oestrus, the fimbria are shrunken and partially cover the ovary. In contrast, at the time of ovulation, the fimbria expand and almost completely surround and enclose the ovary, forming a distinct ovarian bursa and making it less likely that ovulated eggs escape into the peritoneal cavity (Blandau, 1969). Close anatomical contact of the fimbria with the surface of the ovary is achieved in the rabbit and in man by integrated contractions of the oviduct, its accessory membranes, the fimbria and the ovary, at ovulation (Pauerstein, 1974). The mucosal folds of the fimbria are densely covered with cilia which beat actively in the direction of the ostium. The cilia are involved in ovum pick up and in the transport of ova into the abdominal ostium and to the ampullary isthmic junction in the rabbit (Pauerstein and Eddy, 1982; Eddy et al, 1978).

The exact role of the individual structures which collectively take part in the transport of the ovum from the ovary down the tube is

difficult to assess because of the complexity of this process. Moreover, there is considerable difference in the morphology and function of these structures in different species. Surgical reconstruction was used by Kuo and Lim (1928) for the experimental study of the mechanism of ovum transport and the elucidation of the role of the various structures involved. Interest in this type of experimental approach to the study of this problem has been renewed with the advent of microsurgery.

Experiments in the rabbit involving resection of different segments of the oviduct suggest that there is no particular region of the oviduct, except perhaps the fimbriated end, that is specifically required for fertility (Halbert and Patton, 1979). Fertility was reduced only when the ampulla or isthmus were substantially shortened (Winston et al, 1977; Winston, 1978).

Pharmacological manipulation of the adrenergic system does not disrupt normal ovum transport (Eddy and Black, 1974); neither does complete denervation of the appendage after autotransplantation (Winston and Browne, 1974).

The procedure of ovarian transplantation involves division of the mesotubarium superius, uterovarian ligament and fimbria ovarica before their reconstruction with "donor" ovary. These structures are important for maintaining fimbrio-ovarian contact; the fimbria ovarica does, in addition, take part in the ovum pick up and initial transport.

The purpose of this study was to evaluate the effect of division and reconstruction of these structures on animal fertility, before attempting ovarian transplantation.

Materials and Methods

Ten mature virgin female New Zealand White rabbits were used. Under general anaesthesia, the upper reproductive tract was displayed through a 6 cm midline incision. One oviduct and ovary were exposed and placed on green gauze soaked in sterile Ringers solution. A Zeiss operating microscope (magnification between x4 - x10) was used to transect and repair the fimbria ovarica and the associated ligaments. The mesotubarium superius was divided approximately 3 mm from the fimbria at a point adjacent to the medial border of the fimbria. Transection was done in an avascular area using microscissors. The utero-ovarian ligament was transected 1.5 mm from the ovary. The fimbria ovarica was carefully dissected from the cranial pole of the ovary and the fimbrio-ovarian ligament divided after diathermy of its vasculature. Consequently, the fimbria was freely mobilised (Fig. 3.1). The meso-tubarium superius was then reconstructed with four interrupted 10/0 nylon sutures, care being taken to maintain the normal anatomical position of the ligament. The divided ends of the uterovarian liga-ment were brought together and re-sutured with two stitches of 10/0 nylon. The fimbria ovarica was rejoined to the cranial pole of the ovary using one stitch of 10/0 nylon. Handling of tissues was kept to a minimum and the tissues were kept moist with frequent irrigation using sterile heparinized Ringers solution.

Upon completion of the repair of the ligaments, blood clot was removed and the ovary and oviduct were returned to their normal anatomical position. 100 ml of heparinized Ringers solution were instilled in the peritoneal cavity before closing the abdomen.

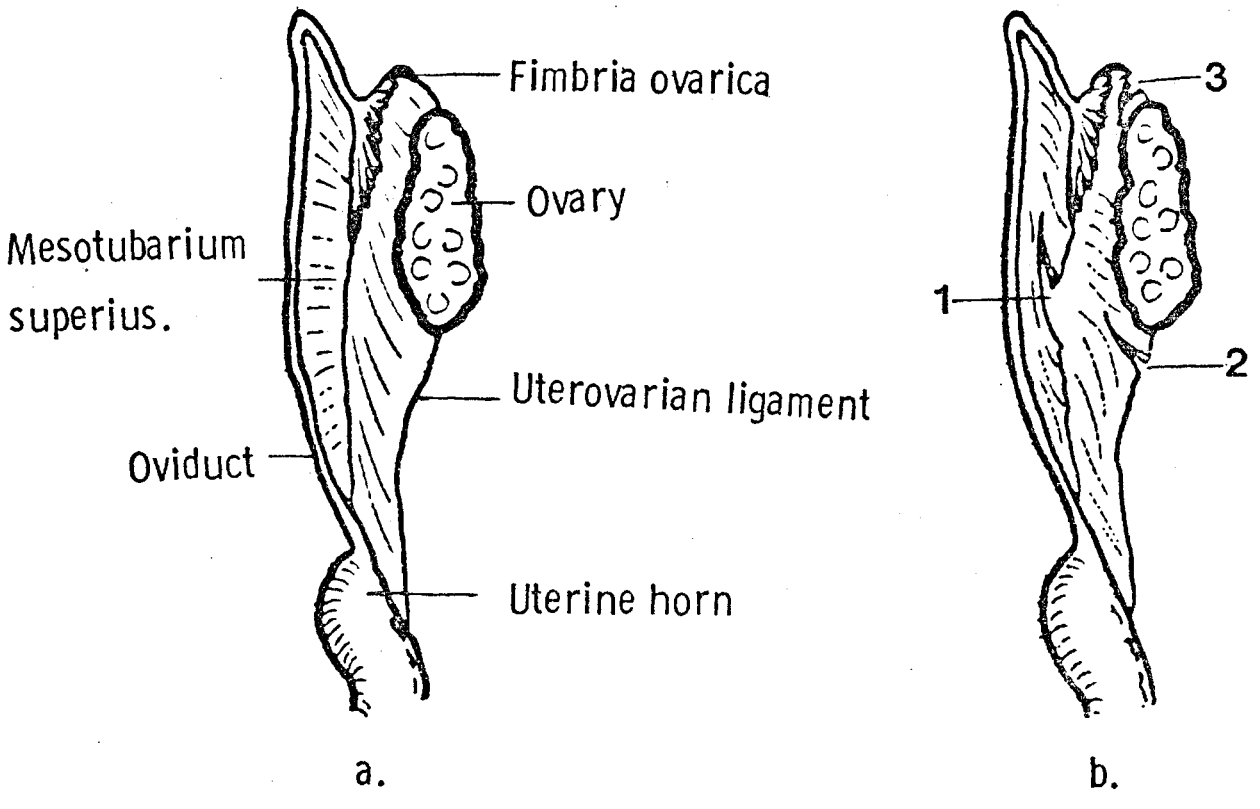


Fig. 3.1

Diagramatic representation of the upper reproductive tract in the rabbit. a. normal b. tract with surgical transection of the 1. mesotubarium superius 2.uterovarian ligament. 3. fimbria ovarica.

Animals were kept under observation until recovery from anaesthesia. They were then given antibiotics and returned to their cages.

Two weeks following surgery, the animals were mated, mating being confirmed by direct observation.

Two weeks after mating, a second laparotomy was performed. The ovaries were inspected, the number of corpora lutea on each side recorded and the number of gestational sacs in each uterine horn recorded. The proportion of ovulated follicles associated with the number of sacs (nidation index) was calculated and adhesions were also graded and recorded. These data were assessed by analysis of variance.

Results

All animals recovered from surgery and all conceived.

Findings at post-mating laparotomy, recorded in Table 3.1 and Table 3.2, showed that the number of corpora lutea on operated side (6.4 ± SEM 0.6) was not significantly different from the mean number of corpora lutea on the control side (7.2 ± SEM 0.53), ($P<0.05$). The mean number of gestational sacs on the operated side (4.9 ± SEM 0.6) was not significantly different from the mean number of gestational sacs on the control side (5.9 ± SEM 0.4) ($P<0.05$).

There were no adhesions on either side after surgery, and the anatomical integrity of the reproductive tract was equally preserved on both sides.

TABLE 3.1

Fertility following transection and reconstruction of the accessory ovarian ligaments and fimbria ovarica

	Operated side			Control side		
Rabbit No.	CL	GS	NI	CL	GS	NI
1	3	3	3/3	5	4	4/5
2	5	5	5/5	9	8	8/9
3	8	8	8/8	5	5	5/5
4	10	4	4/10	7	4	4/7
5	8	6	6/8	5	5	5/5
6	4	3	3/4	9	7	7/9
7	8	8	8/8	8	7	7/8
8	5	3	3/5	7	6	6/7
9	7	4	4/7	8	6	6/8
10	6	5	5/6	9	7	7/9
Total	64	49	49/64	72	59	59/72
Mean	6.4	4.9	0.79	7.2	5.9	0.83
SD ±	2.1	1.9	0.21	1.68	1.37	0.12

Legend: CL = Corpora lutea

GS = Gestational sacs

NI = Nidation Index

TABLE 3.2

Comparison of the number of corpora lutea and gestational sacs after transection and reconstruction of the accessory ovarian ligaments and fimbria ovarica

Variable	Mean ± SEM	F	df	P
CLo	6.4 ± 0.6	0.72	1-9	N.S.
CLc	7.2 ± 0.53			0.05
GSo	4.9 ± 0.6	2.05	1-9	N.S.
GSc	5.9 ± 0.4			0.05

Legend: CLO = Corpora lutea, operated side

CLc = Corpora lutea, control side

GSo = Gestational sacs, operated side

GSc = Gestational sacs, control side

F = The variance ratio

df = Degree of freedom

N.S. = Not significant

Discussion

Division and subsequent reconstruction of the mesotubarium superius, uterovarian ligament and fimbria ovarica did not significantly reduce fertility. The lack of adhesions and avoidance of disruption of the tubal/ovarian anatomy was presumably due to the use of meticulous microsurgical techniques. This may be of considerable importance, in view of the adverse consequences of adhesion formation on ovulation and ovum transport (Winston, 1981). It seems that the minor amount of disturbance of the relationship between ovary and fimbria which occurred as a result of this surgery did not affect fertility; perhaps the maintenance of the spacial orientation of the fimbria to the ovary was a crucial factor. The importance of proper orientation of the fimbria to the ovary for normal ovum capture was suggested by Eddy and Laufe (1983) in the rhesus monkey.

The results of this study agree with the findings of Fredericks and Anderson (1979). Their experiments were limited to transection and reconstruction of the mesotubarium superius and uterovarian ligament; the fimbria ovarica was left undamaged.

Recent experimental work tends, on the whole, to question the indispensibility of the fimbria themselves and also the value of the accessory membranes and ligament in helping to ensure fimbrial contact with the ovary. In an experiment in which the fimbria and the fimbria ovarica were removed and the mesotubarium superius divided, Beyth and Winston (1981) found no effect on conception. They concluded that the fimbria themselves are not essential for reproduction in the rabbit.

This part of the study confirms the lack of the effect on fertility of this aspect of the transplantation procedure (namely division and reconstruction of the accessory ovarian ligaments and the fimbria ovarica) and enabledus to move to the next stage of the project.

CHAPTER 4

EFFECT OF LIGATION AND TRANSECTION OF THE OVARIAN VESSELS AND THE UTEROVARIAN BRANCHES ON OVARIAN FUNCTION AND ANIMAL FERTILITY IN THE RABBIT

EXPERIMENT NO. II

Introduction

In the rabbit, the ovaries possess a dual vascular blood supply; the ovarian arteries and the uterovarian anastomotic branches (Fig.4.1).

The right and left ovarian arteries arise from the abdominal (dorsal) aorta, approximately 5 cm caudal to the origin of the renal arteries and 3 cm cranial to the termination of the aorta. Each artery passes laterally towards the corresponding ovary. Within 3 cm from the ovary, the ovarian artery gives off a branch, which anastomoses with the uterine artery. It then proceeds to supply the ovary and the infundibulum of the oviduct.

The uterovarian anastomotic branch is a long, slender vessel which connects the cranial terminal part of the uterine artery with the ovarian artery. It sends small branches to supply the oviduct and the tip of the uterine horn.

The veins drain the areas supplied by the corresponding arteries; the right ovarian vein empties directly into the inferior vena cava near the origin of the ovarian artery, the left ovarian vein empties into the left iliolumbar vein. The anastomosis of the uterine vein to the ovarian vein is similar to the corresponding arterial anastomosis.

In this study, the contributions which the main ovarian and utero-varian vessels make in supplying blood to the ovary were assessed using selective ligation technique. The understanding of the relative contributions of the two vessels was necessary for the evaluation of the transplantation procedure which altered these contributions.

Materials and methods

Twenty mature, virgin New Zealand White rabbits were used in this study. They were divided into two groups, each of ten animals - Group A, in which the main ovarian vessels were selectively ligated and transected, and Group B, in which the uterovarian anastomotic vessels were selectively ligated and transected.

In both groups, a midline incision was made under general anaesthesia, and the reproductive tract was exposed. One ovary, together with its mesentery, was displayed on clean green gauze soaked in Ringer's solution. The operating microscope was used to identify the ovarian vessels , which were then traced from their origin at the aorta and inferior vena cava (IVC), (Fig. 4.1)

In Group A: The ovarian vessels were isolated from the surrounding tissue by incising the overlying peritoneum, 1 cm from their origin. Jeweller's forceps and straight microscissors were used for dissection. The vessels were separated from the perivascular sheath and the artery and the vein were ligated. The vessels, separately tied with two 8/0 nylon sutures spaced 3 mm apart, were cauterised under a fine jet of heparinized Ringer's solution, and transected. The cut ends of each vessel were sutured to the adjacent peritoneum to prevent re-union. The peritoneum over the vessels was then repaired with continuous 8/0 nylon sutures so that the peritoneal surface remained completely smooth.

In Group B: The uterovarian anastomotic artery and vein of one side were identified where they joined the main ovarian vessels. They were then traced to the region near the utero-tubal junction. The peritoneum

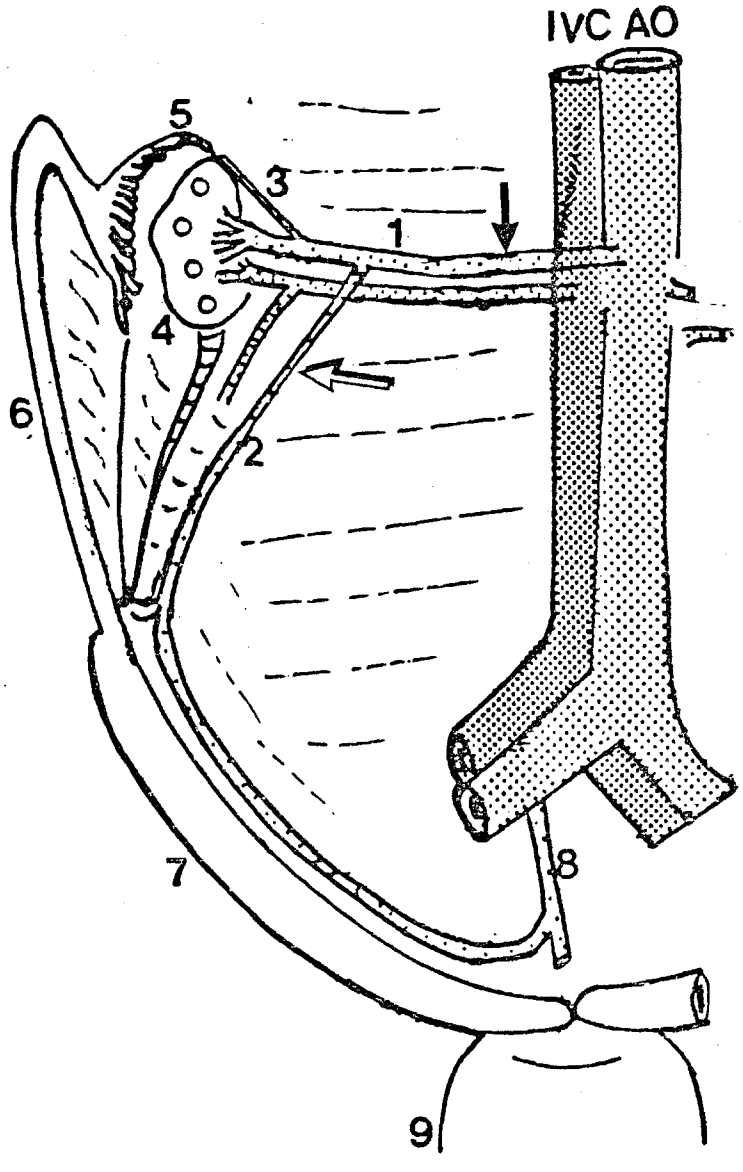


Fig.4.1

Diagramatic representation of the vascular supply of the ovary in the rabbit.

IVC = Inferior Vena Cava.
AO = Abdominal aorta.
1. = Ovarian artery.
2. = Uterovarian anastamotic branch.
3. = Branch to the fimbria ovarica.
4. = Ovary.
5. = Fimbria.
6. = Uterine tube.
7. = Uterine horn.
8. = Uterine vessels.
9. = Vagina.

Ligation Sites
GroupA →
GroupB ⇒

overlying the vessels was incised longitudinally over the vessels, approximately 1 cm from their junction with the main ovarian supply. They were then isolated from the surrounding tissues and from each other. The artery and the vein were ligated separately by two ligatures 3 mm apart, cauterised and transected. The cut end was sutured to the adjacent peritoneum to prevent spontaneous re-anastomosis, and the peritoneum repaired like the animals in Group A.

In both groups, the abdominal wound was closed in layers leaving 100 ml of warm heparinized Ringer's solution in the peritoneal cavity. Animals were kept in the laboratory until recovery from anaesthesia and then re-caged.

Two weeks after surgery, animals were mated with a fertile male. Evidence of mating was confirmed visually.

Two weeks following mating, a second laparotomy was done; the uterus and ovary were carefully inspected on both sides and the number of corpora lutea and gestational sacs recorded. The ovarian dimensions (length and width) were also measured using a metal ruler, and adhesions recorded using Winston's Classification (Winston, 1975). 2 ml of blood were aspirated directly from the ovarian vein on both sides with the needle point towards the ovary, on the side where the vessel had been transected venepuncture was distal to the ligation site. Progesterone was assayed in the ovarian effluent using a modification of Abraham's method (p. 82). The abdomen was then repaired and the animals allowed to recover.

A further laparotomy was made one week following delivery; on this occasion the dimensions of the ovary were again recorded and 2 ml of

blood withdrawn from the ovarian vein on each side for progesterone estimation. The animals were then sacrificed and the ovaries were removed for histological studies. (p 83).

Data collected from these animals were compared using analysis of variance and the difference between the experimental and control sides in each group, and between the two groups, were measured.

Results

All animals made a full recovery from surgery. All the animals mated at the first exposure and became pregnant.

I: Assessment of the response of the ovary to stimulation by mating

1. Corpora lutea and gestational sacs (Table 4.1). In Group A; (ligation of the main ovarian vessel) the mean number of corpora lutea on the operated side (3 ± SEM 0.25) was significantly low ($P<0.01$) compared with the mean number of corpora lutea on the control sides (7.3 ± SEM 0.94). The mean number of gestational sacs on the operated side (1 ± SEM 0.3) was significantly low ($P<0.001$) compared with the mean number of gestational sacs (6.7 ± SEM 0.7)on the control side. In Group B; (ligation of uterovarian vessels) the mean number of corpora lutea on the operated side (5.2 ± SEM 0.5)was not significantly different ($P<0.05$) from the mean number of corpora lutea on the control side (5.6 ± SEM 0.6). The mean number of gestational sacs on the operated side (4.3 ± SEM 0.6) was not significantly different from the mean number of gestational sacs on the control side (4.6 ± SEM 0.5). When the results on the operated sides in Group A and B were compared, there was a significant difference ($P<0.01$) in the mean ± SEM of corpora lutea and gestational sacs, whereas there was no significant difference in the mean ± SEM number of corpora lutea and gestational sacs of the control side in both groups ($P<0.05$) This suggests that in both groups the control sides were homogenous. There was interaction between the mean values of corpora lutea and gestational sacs in operated and control sides of both Group A and B (Fig. 4.2).

TABLE 4.1

Comparison of corpora lutea and gestational sacs values within (operated vz. control) and between Group A and B at mid-pregnancy following ligation and transection of the main ovarian vessels and the uterovarian branch

Variable	Mean ± SEM	F	df	P
CLoA CLcA	3 ± 0.25 7.3 ± 0.94	14.84	1,9	S 0.01
CLoB CLcB	5.2 ± 0.5 5.6 ± 0.6	0.05	1,9	N.S. 0.05
CLoA CLoB	3 ± 0.25 5.2 ± 0.5	12.23	1,18	S 0.01
CLcA CLcB	7.3 ± 0.94 5.6 ± 0.6	3.49	1,18	N.S. 0.05
GSoA GScA	1 ± 0.3 6.7 ± 0.7	32.45	1,9	H.S. 0.001
GSoB GScB	4.3 ± 0.6 4.6 ± 0.5	0.15	1,9	N.S. 0.5
GSoA GSoB	1 ± 0.3 4.3 ± 0.6	9.5	1,18	S 0.01
GScA GScB	6.7 ± 0.7 4.6 ± 0.5	4.81	1,18	N.S. 0.05

Legend:
CLoA = corpora lutea, operated side, Group A
CLcA = corpora lutea, control side, Group A
CLoB = corpora lutea, operated side, Group B
CLcB = corpora lutea, control side, Group B
GSoA = gestational sacs, operated side, Group A
GScA = gestational sacs, control side, Group A
GSoB = gestational sacs, operated side, Group B
GScB = gestational sacs, control side, Group B
df = degree of freedom
S = significant
N.S. = not significant
H.S. = highly significant
n1 = Number of rabbits in Group A =10
n2 = Number of rabbits in Group B =10

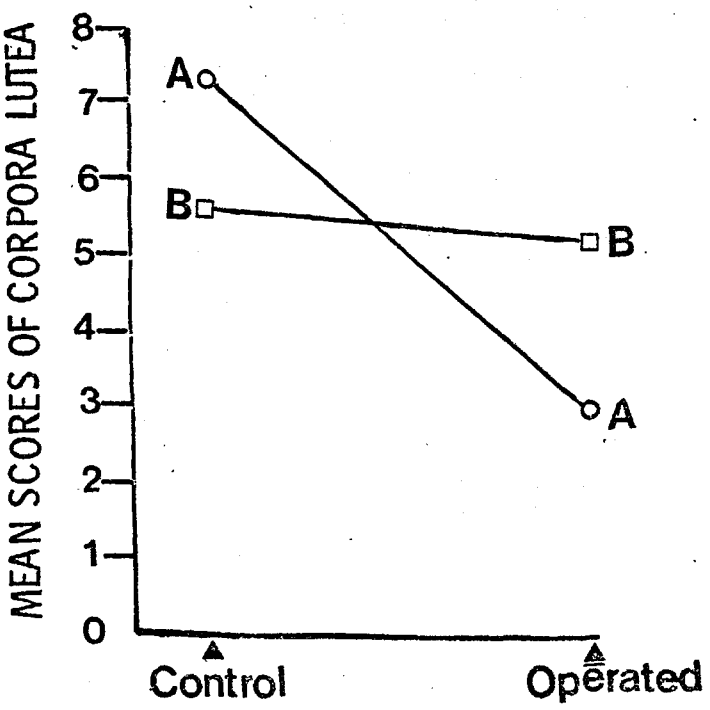


Fig.4.2 A

Group A X group B interaction on difference scores of corpora lutea mean.

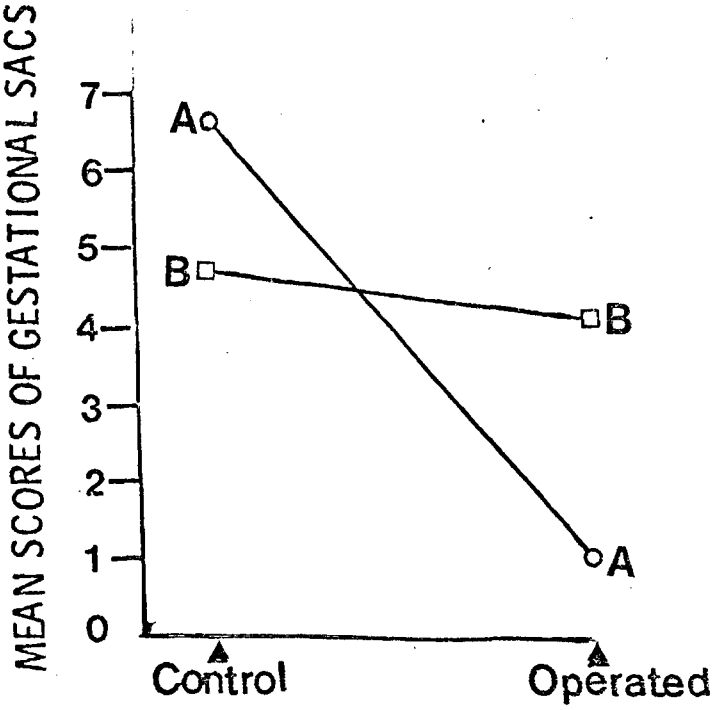


Fig.4.2 B

Group A X group B interaction on difference scores of gestational sacs mean.

2. Ovarian dimensions. The product of the length and width of the ovaries was utilised for comparison (Table 4.2).

In Group A, the mean of the products of ovarian dimensions on the operated side (0.68 ± S.D. 0.2) was significantly less ($P < 0.01$) than the mean of the control (1.4 ± S.D. 0.19). In Group B, the mean of the products of the ovarian dimension on the operated side (1.3 ± S.D. 0.18) was not significantly different from the mean of the control side (1.32 ± S.D. 0.2). ($P < 0.05$).

When the results on the operated side in Groups A and B were compared, there was a significant difference ($P < 0.01$) in the mean of ovarian dimension, whereas there was no significant difference ($P < 0.05$) in the control sides of both groups.

3. Ovarian vein plasma progesterone (Fig. 4.3). In Group A, the mean ovarian plasma progesterone level (ng/ml) from the operated side (149.8 ± SEM 8.57) was significantly low ($P < 0.01$) compared with the mean from the control side (348.5 ± SEM 19.8). In Group B, the mean ovarian plasma progesterone level from the operated side (309 ± SEM 15.5) was not significantly different ($P < 0.05$) from the controls (352 ± SEM 16.5). When the results on the operated sides in Groups A and B were compared, there was a significant difference ($P < 0.01$) in the mean values of the ovarian plasma progesterone, whereas there was no significant difference ($P < 0.05$) between the control sides of both groups.

4. Adhesions (Table 4.3). Adhesions were minimal in both groups and confined to the operation sites. In Group A, 70% of the animals were free of adhesions, while in Group B, 80% of animals were free of adhesions.

TABLE 4.2

Comparison of ovarian dimensions between the operated and control sides, and between Group A and B.

I. Mid-pregnancy

Variable	Mean ± SD Cm^2	P
Ov.di oA	0.68 ± 0.2	S
Ov.di cA	1.4 ± 0.19	0.01
Ov.di oB	1.3 ± 0.18	N.S.
Ov.di cB	1.32 ± 0.2	0.05
Ov.di oA	0.68 ± 0.2	S
Ov.di oB	1.3 ± 0.18	0.01
Ov.di cA	1.4 ± 0.19	N.S.
Ov.di cB	1.32 ± 0.2	0.05

Legend: Ov.di oA = ovarian dimensions operated side, Group A
Ov.di cA = ovarian dimensions, control side, Group A
Ov.di oB = ovarian dimensions, operated side, Group B
Ov.di cB = ovarian dimensions, control side, Group B
S = significant
N.S. = not significant

OA = Progesterone value in operated side group A

CA = Progesterone value in control side group A

OB = Progesterone value in operated side group B

CB = Progesterone value in control side group B

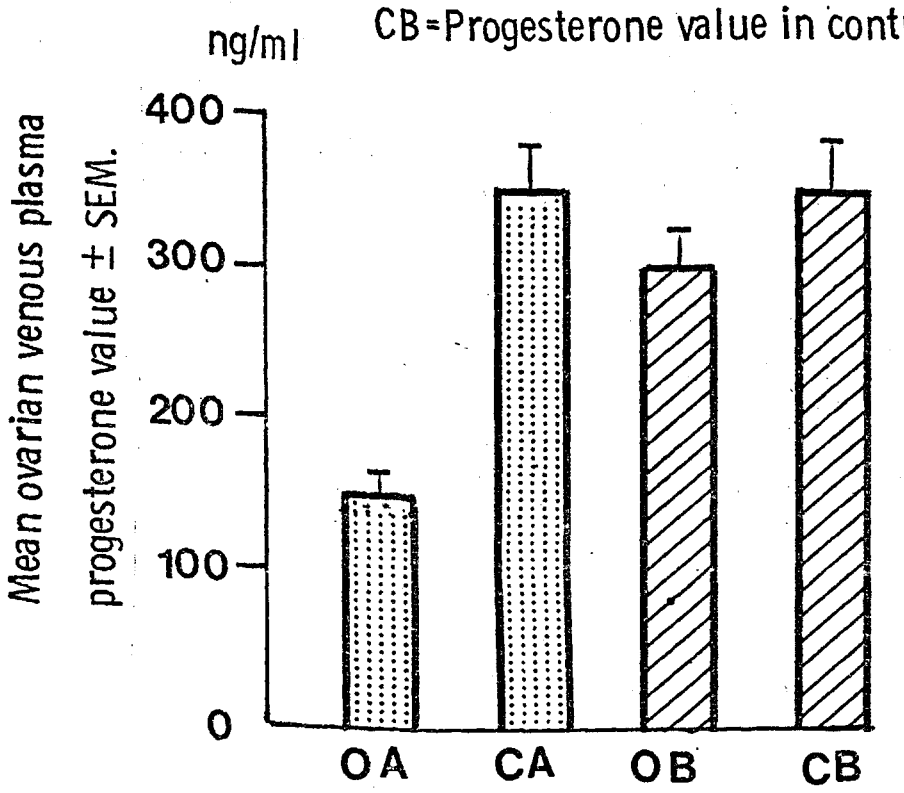


Fig.4.3

Mean ovarian venous plasma progesterone (ng/ml) in the operated and control sides of group A and group B in midpregnancy.

TABLE 4.3

Grades of adhesions in Group A and B following ligation and transection of the ovarian and uterovarian vessels

Adhesions Grades	Group A	%	Group B	%
0	7	70	8	80
1	1	10	1	10
2	2	20	1	10
3	0	0	0	0

n1 = 10 (number of animals in Group A)

n2 = 10 (number of animals in Group B)

II: Assessment of the ovary in the resting state (at the end of the experiment)

1. Ovarian dimensions (Table 4.4). In Group A, mean ovarian dimensions on the operated sides (0.4 ± S.D. 0.1) tended to be smaller ($P < 0.01$) than the controls (0.7 ± S.D. 0.2). In Group B, the mean ovarian dimensions on the operated side (0.64 ± S.D. 0.2) was not significantly different ($P < 0.05$) from that of the control sides (0.71 ± S.D. 0.2). When the ovarian results of the operated sides in Groups A and B were compared, there was significant difference ($P < 0.01$) in the products of the ovarian dimensions, whereas there was no significiant difference ($P < 0.05$) between the control sides of both Groups.

2. Ovarian vein plasma progesterone (Fig. 4.4). In Group A, the mean plasma progesterone levels (ng/ml) from the operated sides (4.8 ± SEM 0.3) was significantly lower ($P < 0.01$) than from the control sides (8.89 ± SEM 0.2). In Group B, there was no significant difference ($P < 0.05$) between plasma progesterone level from the operated sides (8.4 ± SEM 0.2) and that of the control sides (8.9 ± SEM 0.2). When the results between the operated sides in Groups A and B were compared, there was significant difference ($P < 0.01$). The controls of the two groups did not differ ($P < 0.05$).

III: Morphological observations

The general appearances of the ovaries removed at the termination of the experiment were within normal limits except those from the operated

TABLE 4.4

Comparison of ovarian dimensions between the operated and control sides, and between Group A and B

II. Non-pregnant state

Variable	Mean ± SD cm^2	P
Ov.di oA	0.4 ± 0.1	S
Ov.di cA	0.7 ± 0.2	0.01
Ov.di oB	0.64 ± 0.2	N.S.
Ov.di cB	0.71 ± 0.2	0.05
Ov.di oA	0.4 ± 0.1	S
Ov.di oB	0.64 ± 0.2	0.01
Ov.di cA	0.7 ± 0.2	N.S.
Ov.di cB	0.71 ± 0.2	0.05

Legend: Ov.di oA = ovarian dimensions, operated side, Group A
Ov.di cA = ovarian dimensions, control side, Group A
Ov.di oB = ovarian dimensions, operated side, Group B
Ov.di cB = ovarian dimensions, control side, Group B
S = significant
N.S. = not significant

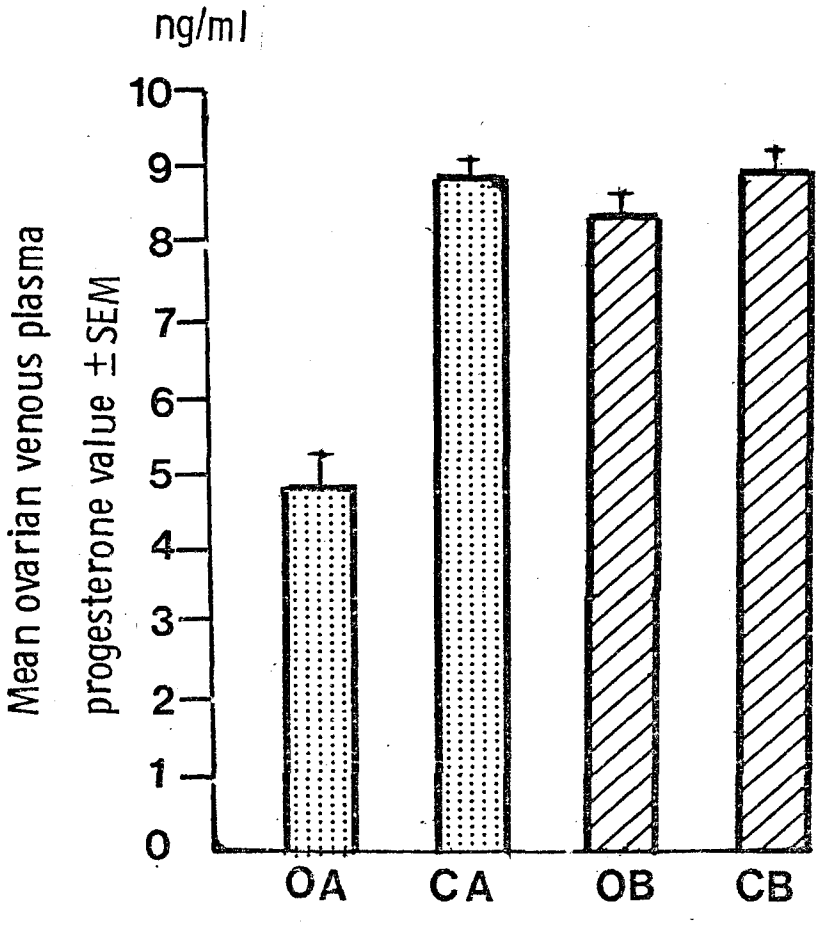


Fig.4.4

Mean ovarian venous plasma progesterone(ng/ml)in the operated and the control sides of group A and group B in the resting state.

side in Group A, which were shrunken.

Light microscopical examination revealed:

In Group A (Fig. 4.5), the germinal epithelium was normal; there was degeneration and necrosis in some of the secondary follicles, while primary follicles were not affected. There were haemorrhages in some of the tertiary follicles and increased numbers of atretic follicles were noted in most of the sections. There were some normal corpora lutea and follicles at different stages of development.

In Group B, no macroscopical or microscopical structure changes could be detected by light microscopy (Fig. 4.6).

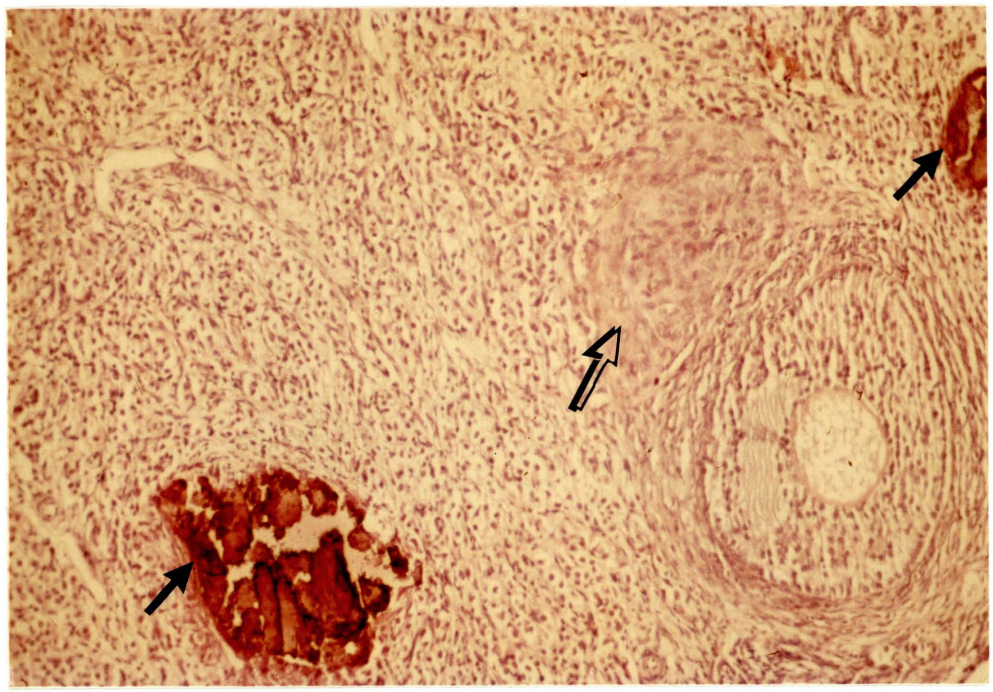


Fig. 4.5 Photomicrograph of a section of rabbit ovary in which the main ovarian vessels were ligated and transected. Note necrotic follicles (black arrows) and areas of hyalinization (hollow arrow)

X 10

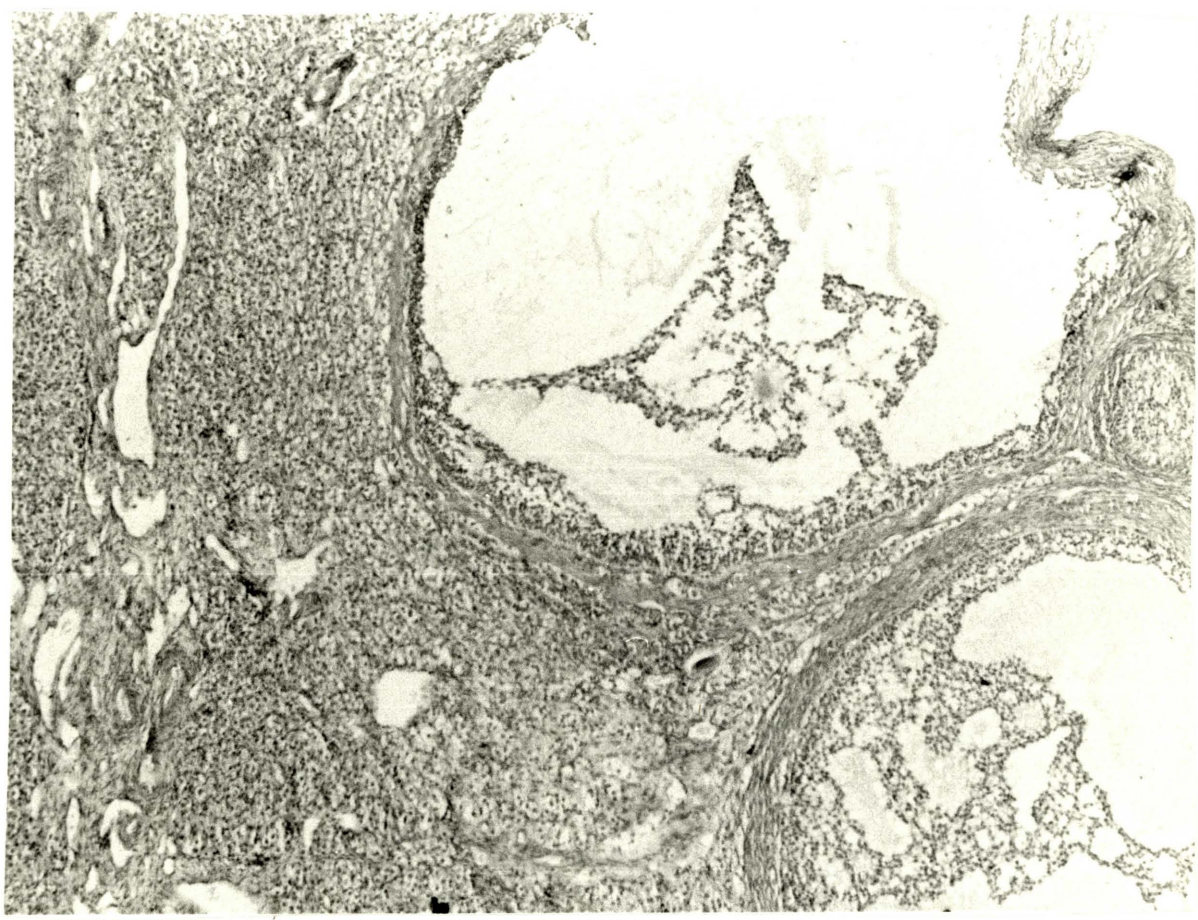


Fig. 4.6 Photomicrograph of a section of rabbit ovary in which the uterovarian vessels were ligated and transected, showing completely normal structure

X 10

Discussion

The results of the present study showed that ligation of the main ovarian artery produced a decline in function of the ovary, with a significant ($P<0.01$) lowering of the ovarian plasma progesterone, and reduction in the number of corpora lutea and gestational sacs. Ligation of the uterovarian branch produced no significant change in ovarian function on the ligated side. This suggests that the main ovarian artery is the major source of blood supply to the ovary in the rabbit. These results are in agreement with the findings of Blasco et al (1973), who showed that, in the rabbit, the ovarian artery carried 80-90% of the blood supply to the ovary. Our results, however, disagree with their suggestion that the effect of ligation of the ovarian artery on ovulation, fertilization and ovum implantation were only noticeable in the immediate post-ligation interval and that, within a week or so, no significant difference was detectable between the ligated and the normal sides of the reproductive tract. This study demonstrated that the morphological changes and functional impairment of the ovary which followed ligation of the main ovarian artery persisted during the few weeks of the experimental observation.

Del Campo and Ginther (1972), in studies of the vascular anatomy of the uterus and ovary of a number of species, found that while the uterovarian branch is prominent in certain species (e.g. rat), it is long and slender in the rabbit and is likely to carry only a minor portion of the blood supply to the ovary. The data from this study confirm this anatomical finding from the functional standpoint in that ligation of the uterovarian branch did not reduce corpora lutea formation, ovum implantation or progesterone secretion.

The effect of ligation and transection of the ovarian veins is difficult to discern from those of the simultaneously ligated ovarian arteries. It is likely, however, that the congestion and mild degree of extravasation of red cells seen in some of the histological sections were due to venous obstruction - for this is the usual picture in organs, like the ovary, in which venous outflow is limited to the obstructed vessel (Robbins et al, 1981b).

The uterovarian vein appears to be draining the uterine tubes and not the ovary (Del Campo and Ginther,1972) and, in contrast to comparable vessels in other species such as the rat, guinea pig and sheep which have a local utero-luteal pathway (Baird and Land, 1973; Ginther, 1974), in the rabbit this vein is not involved in the transfer of the uterine luteolytic factor to the ovary, a systemic route is implicated instead. The function and life span of the corpus luteum is therefore not affected by ligation of the uterovarian branch.

Morphologically, ovaries with the main ovarian artery ligated showed general atrophy and histological examination revealed that there were areas of necrosis, degeneration and occasionally haemorrhage. These changes were on the whole less severe than would be expected from sudden interruption of 80-90% of the blood supply to an organ. However, the effects of ischaemia depend on the capacity of the cells to survive until the blood flow is resumed or adequate collateral established. These, in turn, depend on the type of cells and their metabolic demands at the time of the ischaemic episode, and also on the effectiveness of the collateral circulation (Anderson, 1980;

It is known that ovarian tissue is relatively tolerant to ischaemia (Goding et al, 1972); the effectiveness of the collateral, however, remains to be studied. It is just possible, however, that under the haemodynamic settings which prevailed locally following ligation of the main ovarian artery, the uterovarian branch assumed a greater functional role and carried a larger share of ovarian blood supply, and this might have reduced the extent of the ischaemic changes in the ovary. The potential role of this vessel in the blood supply to the ovary, under certain conditions, will be further considered in the subsequent experiments.

Ovaries of the operated sides of Group B animals, where only the uterovarian vessels were ligated, were normal on light microscopy.

CHAPTER 5

STUDY OF THE EFFECT OF TRANSECTION AND MICRO-VASCULAR RE-ANASTOMOSIS OF THE OVARIAN VESSELS ON THE STRUCTURE AND FUNCTION OF THE OVARY IN THE RABBIT

"SHAM AUTOGRAFTING"

EXPERIMENT NO. III

Introduction

The ovary is a highly vascular organ; ovarian blood flow is high relative to other organs (Reynolds, 1973; Goding et al, 1972) and varies with the different phases of the reproductive and endocrine activities of the organ (Bindon, 1969 ; Yoshinaga et al, 1969).

In the resting state, of all the organs studied in the rabbit, the ovary had the highest blood flow (460 ml/min/100 g tissue) compared with 270 ml/min/100 g of the kidney (Reynolds, 1973). High rates of ovarian blood flow were also demonstrated by Blasco et al (1973) in the oestrus rabbit.

In pseudopregnancy, Navoy and Cook (1973), found that ovarian blood flow in the rabbit increased from 1.7 ml/min/g tissue before ovulation to 9.4 ml/min/g tissue at Day 9 of pseudopregnancy; a blood flow of this magnitude would amount to nearly 2% of the cardiac output, yet the ovaries together constitute less than 0.1% of the total body weight.

During gestation, the blood flow to the ovaries in the rabbit

increases ten-fold from Day 0 to Day 16, but decreases after Day 20; the increased blood flow is mainly distributed to the corpus luteum (Abdul-Karim and Bruce, 1973).

The implication of these remarkably high blood flows to ovarian grafting is that anastomoses between the recipient and donor vessels must be capable of sustaining flows within these ranges if the structure and function of the organ are not to be undermined by circulatory inadequacy.

In the present study, a sham-grafting procedure was used to assess the ability of the vascular anastomosis to provide the blood flow rates required to meet the increased metabolic demands of the ovary during pregnancy and pseudopregnancy, and to evaluate the effect, on the graft, of the normothermic anoxia, which occur while anastomosing the vessels.

Materials and methods

Twenty sexually mature New Zealand White virgin female rabbits were used in this study and were divided into two groups, Group A and Group B, each consisting of ten animals.

Anaesthesia was induced by hypnorm and maintained by open inhalation of halothane/oxygen(p 74). 120 I.U./Kg body weight heparin was injected subcutaneously prior to laparotomy.

The reproductive tracts were exposed through a midline laparotomy incision (p. 75). On the experimental side, the ovary, together with its mesentery, was placed on green gauze soaked in Ringer's lactate solution. The operating microscope was set with properly adjusted eye pieces and objective lenses to give the right focus.

In Group A: Under the operating microscope and at magnification of x 25, the ovarian vessels were identified in the retroperitoneal region as they arise from their origin and they were traced laterally toward the ovary (Fig. 5.1A).

The peritoneum which covers the vessels was incised along the direction of the vessels 1 cm from the aorta and IVC (inferior vena cava). The ovarian vessels were exposed and isolated from the structures beneath them and from each other; any branch from the vessels to the neighbouring tissue was cauterized using fine bipolar diathermy jeweller's forceps under a fine jet of warm irrigating heparinized Ringer's solution. The ovarian artery was freed completely from the vein and plastic contrast material was placed underneath it to

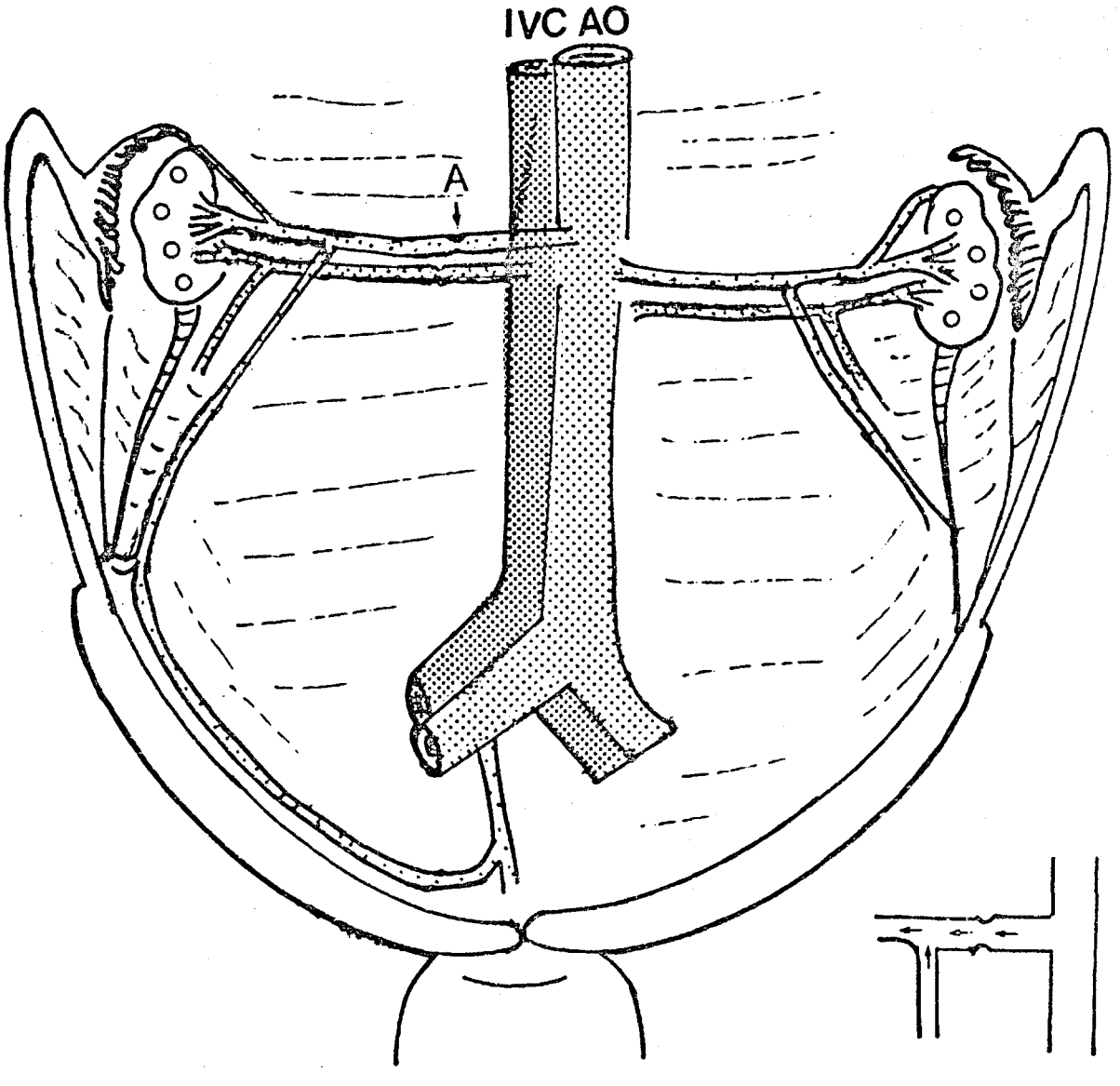


Fig. 5.1 A

Diagramatic representation of the reproductive tracts of the rabbit showing the anastomotic sites of the ovarian vessels

A. Near the origin of the vessels.

facilitate visualisation. Two Acland vascular double clamps were applied to occlude the artery and the vein to facilitate the anastomotic procedure (Acland, 1972). The artery and the vein were then transected midway between the two clamps, and the blood removed from the cut ends of the vessels by a fine jet of warm heparinized solution. The cut ends of the vein were then re-anastomosed by end-to-end anastomosis using 11/0 nylon suture (p. 75).

The procedure for arterial anastomosis was followed in a similar way to that of the vein. The patency of the vessels was then checked. The cut edges of the peritoneum over the vessels were then repaired with continuous 8/0 nylon sutures so that the peritoneal surface facing the abdominal viscera remained completely smooth.

In Group B: The ovarian vessels were traced from their origin laterally until they entered the ovarian mesentery. 1 cm from the junction of the uterovarian branch with the main ovarian artery (Fig. 5.1B) the overlying peritoneum was incised and transection and re-anastomosis of the vessels, at that site, performed as in Group A. At the end of the anastomosis, in both Groups A and B, clots were removed and the viscera irrigated with heparinized Ringer's solution, of which 100 mls were left in the peritoneal cavity prior to closure of the abdomen. In both groups, the diameter of the artery and vein and the duration of the anastomosis and the total duration of the operation were measured and recorded.

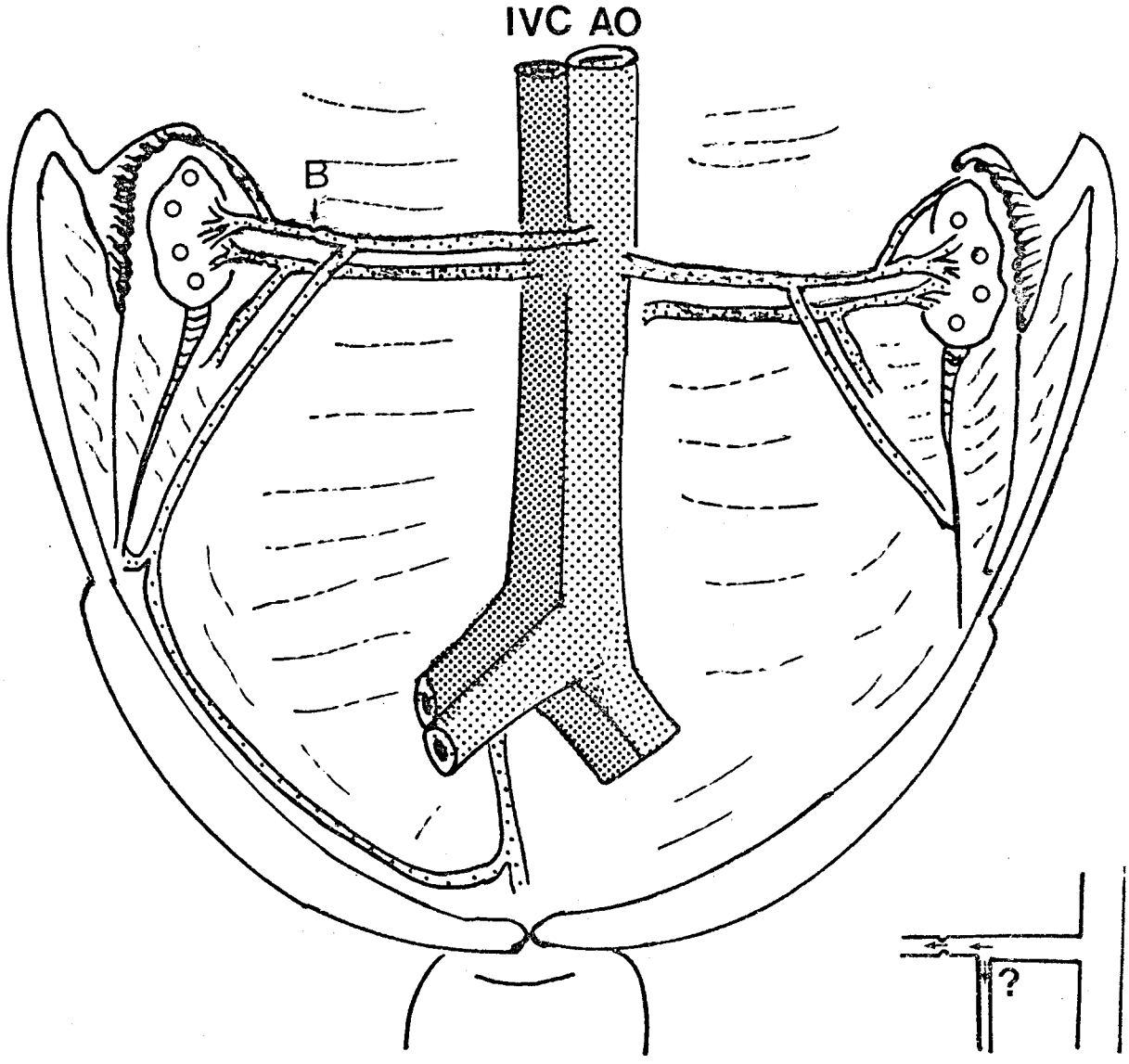


Fig. 5.1 B.

B. distal to the origin of the uterovarian branch.

The animals were kept in the laboratory until recovery from anaesthesia, and then re-caged.

Two weeks after the operation, all animals received 100 i.u. HCG I.M and 24 h later, the animals were laparotomized, reproductive tracts exposed, number of corpora lutea counted, adhesions graded and recorded, and 2 mls of blood withdrawn directly from the ovarian vein, in the experimental and the control sides, for plasma progesterone estimation (p. 82).

Two weeks later (i.e. four weeks after the anastomotic procedure), animals were mated and the mating behaviour observed and recorded.

Two weeks following mating, a third laparotomy was performed and the condition of the ovary observed, number of corpora lutea, and the number of gestational sacs in the experimental and the control sides of the reproductive tracts, were recorded.

At the end of the experiment, the animals were sacrificed, the anastomosis examined and the ovaries removed for histological studies.

Data collected from these animals were compared using analysis of variance, and differences between experimental and control sides in each group and between the two groups were measured.

Results

1. Operative findings (anastomotic procedure):

(a) All animals made a full recovery from the operations performed during the experiment.

(b) External diameter of the anastomosed vessels (Table 5.1). The mean external diameter of the ovarian artery (Fig. 5.1A) at anastomotic site A (0.71 ± S.D. 0.06 mm) was significantly greater than the mean external diameter of the ovarian artery at anastomotic site B (0.36 ± S.D. 0.03 mm), $P < 0.001$. The mean external diameter of the ovarian vein at anastomotic site A (0.9 ± S.D. 0.05 mm) was significantly greater than the mean external diameter of the ovarian vein at anastomotic site B (0.6 ± S.D. 0.04 mm), $P < 0.001$.

(c) The longterm patency rate was 100% for both anastomotic sites A and B.

(d) Total duration of warm ischaemia (normothermic anoxia) to the ovary (Table 5.1): The mean of the total duration of warm ischaemia to the ovary in the anastomotic procedure at site A (53 ± S.D. 6.6 min) was significantly longer than that at site B (41 ± S.D. 5.7 min), $P < 0.01$.

2. Results of HCG stimulation test (Table 5.2):

(a) Corpora lutea: In Group A (where the ovarian vessels were anastomosed near their origin): the mean value of the corpora lutea in the operated side (6.5 ± S.D. 3.2) was not significantly different

TABLE 5.1

Comparison of the external diameter of the ovarian vessels in mm., and the anoxic period in minutes, between Group A and Group B during transection and micro-vascular re-anastomosis of the ovarian vessels

Variable	Group	Mean ± S.D.	P
Ext. diameter o.a.	A	0.71 ± 0.06	H.S.
Ext. diameter o.a.	B	0.36 ± 0.03	0.001
Ext. diameter o.v.	A	0.9 ± 0.05	H.S.
Ext. diameter o.v.	B	0.6 ± 0.04	0.001
Anoxic period	A	53 ± 6.6	S. 0.01
Anoxic period	B	41 ± 5.7	

Legend: Ext. diameter o.a. = External diameter of ovarian artery in mm.

Ext. diameter o.v. = External diameter of ovarian vein in mm.

H.S. = Highly significant

S. = Significant

n1 = 10 (number of rabbits in Group A)

n2 = 10 (number of rabbits in Group B)

TABLE 5.2

Comparison of corpora lutea numbers within (operated vz. control) and between Group A and Group B at 24 h. post-HCG stimulation following transection and re-anastomosis of the ovarian vessels

Variable	Mean ± S D	F	df	P
Clo A	6.5 ± 3.2	1.58	1-9	N.S.
Clc A	7.8 ± 2.8			0.05
Clo B	4.7 ± 1.9	10.33	1-9	S
Clc B	7.3 ± 2.4			0.05
Clo A	6.5 ± 3.2	2.30	1-18	N.S.
Clo B	4.7 ± 2.8			0.05
Clc A	7.8 ± 2.8	0.18	1-18	N.S.
Clc B	7.3 ± 2.4			0.05

Legend: Clo A = Corpora lutea, operated side, Group A
Clc A = Corpora lutea, control side, Group A
Clo B = Corpora lutea, operated side, Group B
Clc B = Corpora lutea, control side, Group B
S = Significant
N.S. = Not significant
$n_1 = 10$
$n_2 = 10$

from their mean value in the control side (7.8 ± S.D. 2.8), $P<0.05$. In Group B (where the ovarian vessels were anastomosed distal to the origin of the uterovarian branch): the mean number of the corpora lutea in the operated side (4.7 ± S.D. 1.9) was significantly less than their mean number on the control side (7.3 ± S.D. 2.4), $P<0.05$. There was no significant difference in the mean number of corpora lutea between the operated sides of Group A and Group B, nor was there significant difference in the mean number of corpora lutea between the control sides of the two groups, $P < 0.05$.

(b) Ovarian plasma progesterone levels (Fig. 52): In Group A: the mean ovarian plasma progesterone level in the operated side (63.7 ± SEM 8.5 ng/ml) was not significantly different from the mean ovarian plasma progesterone level in the control side (70.97 ± SEM 7.5 ng/ml), $P<0.05$. In Group B: the mean ovarian plasma progesterone level in the operated side (58.7 ± SEM 7.0 ng/ml) was significantly lower than the control (78.23 ± SEM 8.80 ng/ml), $P<0.05$. There was no significant difference in the mean ovarian plasma progesterone levels between the operated sides of Group A and Group B, nor was there significant difference between the mean progesterone levels of the control sides of the two groups, $P<0.05$.

3. Results of ovarian stimulation test by mating (Table 53):

(a) Corpora lutea: In Group A: the mean number of corpora lutea in the operated side (6.4 ± SEM 0.49) was not significantly different from the mean value of the corpora lutea in the control side (6.8 ± SEM 0.69), $P<0.05$. In Group B: the mean number of corpora lutea in the operated side (4.2 ± SEM 0.37) was significantly lower than the mean

OA=Progesterone value in operated side group A

CA=Progesterone value in control side group A

OB=Progesterone value in operated side group B

CB=Progesterone value in control side group B

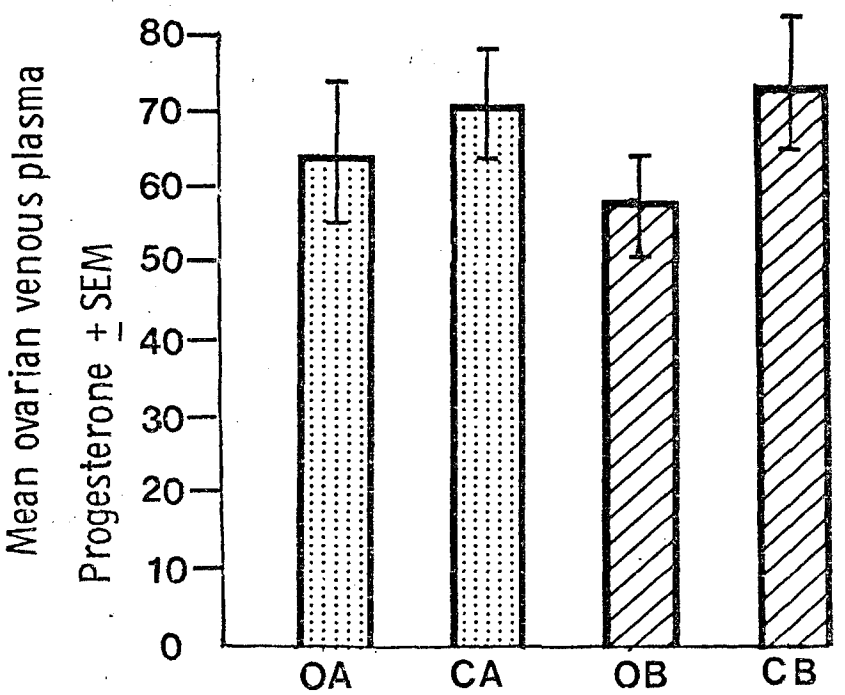


Fig.5.2

Mean ovarian venous plasma progesterone (ng/ml) in the operated and control sides of group A and group B, 24h following HCG injection.

TABLE 5.3

Comparison of corpora lutea and gestational sacs values within (operated vz. control) and between Group A and Group B at mid-pregnancy following transection and re-anastomosis of the ovarian vessels

Variable	Mean ± SEM	F	df	P
CLo A	6.4 ± 0.49	0.22	1,9	N.S.
CLc A	6.8 ± 0.69			0.05
CLo B	4.2 ± 0.37	22.74	1,9	S
CLc B	7.5 ± 0.58			0.01
CLo A	6.4 ± 0.49	10.6	1,18	S
CLo B	4.2 ± 0.58			0.01
CLc A	6.8 ± 0.69	2.06	1,18	N.S.
CLc B	7.5 ± 0.58			0.05
GSo A	4.8 ± 0.69	1.5	1,9	N.S.
GSc A	5.8 ± 0.5			0.05
GSo B	2.7 ± 0.36	23.39	1,9	S
GSc B	6.3 ± 0.44			0.001
GSo A	4.8 ± 0.69	7.13	1,18	S
GSo B	2.7 ± 0.36			0.05
GSc A	5.8 ± 0.5	0.49	1,18	N.S.
GSc B	6.3 ± 0.44			0.05

Legend:
CLo A = corpora lutea, operated side, Group A
CLc A = corpora lutea, control side, Group A
CLo B = corpora lutea, operated side, Group B
CLc B = corpora lutea, control side, Group B
GSo A = gestational sacs, operated side, Group A
GSc A = gestational sacs, control side, Group A
GSo B = gestational sacs, operated side, Group B
GSc B = gestational sacs, control side, Group B
df = degree of freedom
S = significant
N.S. = not significant

n1 = 10 (Group A)
n2 = 10 (Group B)

number of the corpora lutea in the control side (7.5 ± SEM 0.58), $P<0.01$. There was a significant difference ($P<0.01$) in the mean number of corpora lutea between the experimental sides of the two groups but there was no significant difference between the control sides of the two groups.

(b) Gestational sacs (Table 5.3): In Group A: the mean number of gestational sacs in the operated sides (4.8 ± SEM 0.69) was not significantly different from the mean number of gestational sacs in the control sides (5.8 ± SEM 0.5), $P<0.05$. In Group B: the mean number of the gestational sacs in the operated side (2.7 ± SEM 0.36) was significantly lower than the mean number of the gestational sacs in the control side (6.3 ± SEM 0.4), $P<0.001$. There was significant difference in the mean number of gestational sacs in the operated sides of Group A and Group B ($P<0.05$); but there was no significant difference in the number of gestational sacs between the control sides of Group A and Group B ($P<0.05$).

4. Adhesions formation (Table 5.4):

In Group A: the operated sides were free from adhesions in 40% of the animals.

In Group B: the operated sides were free from adhesions in 50% of animals.

The control sides of all the animals in both groups were free from adhesions.

5. Light microscopical examination of the ovaries removed from experimental and control sides at the end of the experiment showed no morphological changes (Fig. 5.3).

TABLE 5.4

Distribution, grades and % of adhesions in Group A and Group B following micro-vascular transection and re-anastomosis of ovarian vessels

Grades of adhesions	Group A	%	Group B	%
0	4	40	5	50
1	3	30	3	30
2	2	20	2	20
3	1	10	0	0

n_1 = 10 (number of animals in Group A)

n_2 = 10 (number of animals in Group B)

Control side in both Groups was free of adhesions

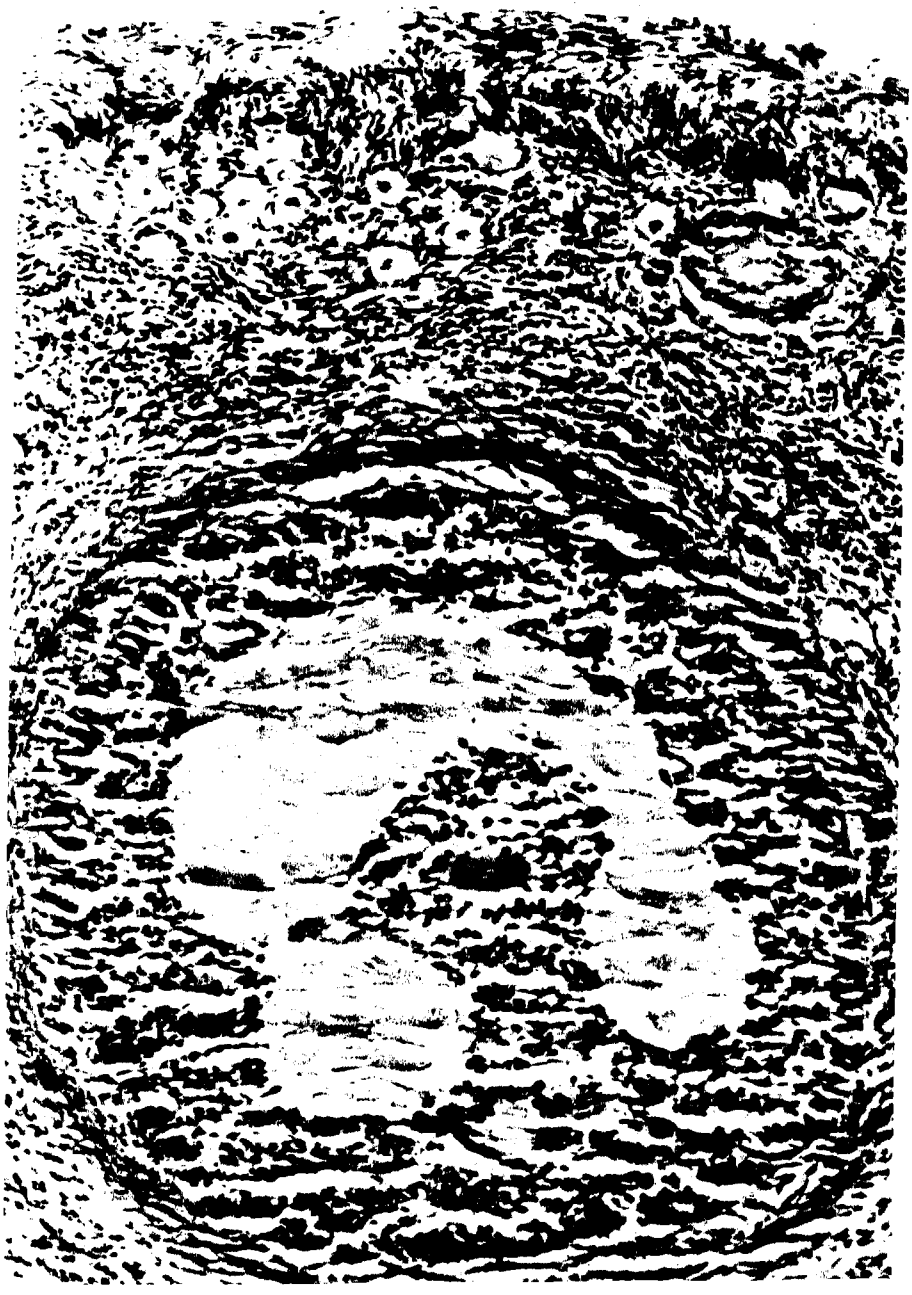


Fig. 5.3 Photomicrograph of a section obtained from a "sham" grafted ovary (transection and microvascular re-anastomosis) showing normal structure.

X 10

Discussion

The results of this study showed that a 100% longterm patency rate was achieved both when the ovarian vessels were anastomosed at site A, near their origin (average vessel diameter of 0.7 mm for the artery and 0.9 mm for the vein), and at site B, distal to the origin of the uterovarian branch (average vessel diameter of 0.36 mm for the artery and 0.6 mm for the vein).

However, the success of a microvascular anastomotic procedure depends not only on the establishment and maintenance of patent arterial and venous anastomoses but also on the capacity of the anastomosis to accommodate the blood flow rates required during various levels of tissue activities.

This physiologically "ideal" anastomosis appeared to have been achieved when the ovarian vessels were anastomosed near their origin (Group A). In this group, the result of ovarian stimulation either by HCG or by mating, each of which is known to increase the blood flow to the ovary several folds, showed no significant difference in the functional response of the ovary on the sides of the anastomosis compared with the control side, in terms of number of corpora lutea, gestational sacs and ovarian plasma progesterone levels. In order to fully appreciate the significance of these findings, it is necessary to consider two main peculiarities of the ovarian vascular and blood supply; (1) Ovarian function is closely regulated by blood flow (Niswender et al, 1976); follicular growth, ovulation and development of corpora lutea depend on adequate blood flow (Reynolds, 1973; Baird, 1977; Moor et al, 1975; Burr and Davis, 1951; Westman and Jacobson,

1936), and progesterone output and plasma progesterone level are highly correlated with ovarian blood flow (Niswender, 1976; McCracken et al, 1969). It follows, therefore, that alteration of ovarian blood would be reflected in a similar change in the endocrine and reproductive features of the ovary. The absence of significant difference in the variables measured in Group A between the anastomosis side, and the control, allow us to conclude that the anastomosis was capable of providing blood flow rates comparable to those on the normal control sides.

(2) In the rabbit, the ovarian vessels appear to be without auto-regulatory control, hence blood flow is almost totally related to perfusion pressure, so that a small drop in pressure (consequent upon even slight luminal narrowing at the anastomotic site), can cause a considerable decrease in the blood flow to the ovary (Janson and Albricht, 1975).

Therefore, if the blood flow (and function) of the graft was not to be limited, the anastomosis should cause practically no narrowing of the ovarian vessels. In other words, in an ovarian transplantation procedure in the rabbit, a physiologically ideal anastomosis should be a technically perfect one as well. This appeared to have been the case in Group A.

When the vessels were anastomosed distal to the origin of the uterovarian branch, Group B, the results showed that on the side of the anastomosis, the number of corpora lutea were less ($P < 0.05$) and the level of ovarian plasma progesterone was lower ($P < 0.05$) in pseudo-pregnancy, and the number of corpora lutea and gestational sacs

were less ($P < 0.01$ and $P < 0.001$ respectively) during pregnancy, compared with the control sides. This means that anastomosis at this site could not provide the augmented flow rate required during pregnancy and pseudopregnancy.

A plausable explanation of the failure of anastomosis at site B to provide effective blood flow, is that ovarian vessels are narrower at this site ($P < 0.001$) than at their origin, and that further, even very slight, narrowing at the site of anastomosis, could have been critical to the flow in such a small vessel.

It is, however, possible to speculate further and bring the uterovarian branch into the picture; in Group A, slight fall in perfusion pressure distal to the anastomosis, consequent upon slight luminal narrowing at the anastomotic site, might have encouraged more flow from the uterovarian branch to compensate for any possible reduction of ovarian blood flow along the main ovarian artery (insert A Fig. 5.1). On the other hand, narrowing at anastomotic site B might have caused a rise in pressure proximal to the anastomosis and this rise in pressure might have impeded or even reversed the flow in the uterovarian branch, depriving the ovary of some of its blood supply (insert B Fig. 5.1). The practical implication of this possibility is that efforts to preserve a collateral anastomotic branch must be made in the light of anatomic as well as haemodynamic considerations. Angiographic and ultrasound/Doppler studies would help to identify the direction of flow under the different haemodynamic settings.

Histological examination of the ovaries in both groups showed normal structure on light microscopy, indicating that the ovary could

tolerate periods of warm ischaemia (normothermic anoxia) of some 53 ± S.D. 6.6 min. duration without the development of changes in structure. It is, however, possible that changes which occurred during the acute ischaemic episode resolved after restoration of the circulation, so that by the time the ovaries were examined at the termination of the experiment (average of 8 weeks), no changes were detectable by light microscopy. Although anastomosis at site B did not provide as adequate blood flow as site A, yet the duration of anastomosis (period of normothermic anoxia) was shorter (41 ± 5.7 min.) than at site A (53 ± S.D. 6.6 min) and the incidence of adhesion (50%) lower than in Group A (60%). The true significance of these differences might become obvious in the subsequent full transplantation experiments.

In conclusion, the experiment demonstrated that a 100% longterm patency rate could be achieved in anastomosing ovarian vessels of 0.36-0.7 mm diameter. It also demonstrated that anastomosis of the ovarian vessels near their origin provided blood flows within the normal requirement ranges, while anastomosis of these vessels at a somewhat narrower vessel site imposed significant limitations on the function of the ovary, thus highlighting the critical role of the circulatory factor to the outcome of the graft. A period of normothermic anoxia of 41-53 min. did not cause structural change in the ovary, suggesting that, unless excessively delayed, cutting off the circulation during anastomosis is not likely to injure the ovary.

CHAPTER 6

STUDY OF THE EFFECTS OF CYCLOSPORIN A ON THE MORPHOLOGY AND FUNCTION OF THE OVARY IN THE RABBIT

EXPERIMENT NO. IV

Introduction

Cyclosporin A (CyA) is a fungal metabolite produced by the soil fungi Trichoderma polysporum rifai and Cylindrocarpum Lucidum. It was isolated in 1972 and was soon shown to have powerful immunosuppressive properties (Borel et al, 1976) but very restricted antibiotic activity (Morris, 1981). It is insoluble in water but soluble in olive oil and other organic solvents and is available for oral, intramuscular and intravenous administration. Although the intramuscular route gives very low blood levels, nonetheless, it was found effective in the rabbit (Dunn et al, 1980).

The mechanism of action of CyA is still largely unknown but it appears that the drug acts selectively on the T-lymphocytes, inhibiting their early activation and proliferation, and in this way removes that part of the immune response which relies on the T-cells.(Morris, 1981; White et al, 1979; Britton and Palacios, 1982; Hess et al, 1982).

CyA has been successfully used for immunosuppression in experimental and clinical transplantation (Morris, 1981; Starz l et al, 1981; White and Calne, 1982; Green et al , 1982; DuToit et al, 1982)

and in some conditions of presumed immunological aetiology (Rothier et al, 1980; Herramann and Muller, 1979).

Side effects were reported from the use of immunosuppressive doses of CyA in animals and in man. With the exception of the occasional development of lymphomas, these side effects were generally of a mild nature and usually reversible on reducing the dose (Morris, 1981; Bieber et al, 1980; White and Calne, 1982; Thomson et al, 1982, 1981). However, in none of the series so far reported on the side effects of CyA was there any mention of gonadotoxicities.

It is worth noting that most of the reports on CyA toxic effects were drawn from observations on transplant recipient. Under such conditions, the effects of the transplant surgery, the impaired immunity and possibly some other complication, render it difficult to evaluate and discern the pathogenic effect of the drug from those due to simultaneously operating pathogenic mechanisms.

In this experiment, the effect of short course immunosuppressive doses of CyA on the ovary was studied in the normal surgically intact rabbit.

Materials and methods

Thirteen mature virgin New Zealand White rabbits were used (p. 74). They were divided into two groups:

1. Treatment group (8 rabbits): Each rabbit in this group received 15 mg CyA in olive oil (p.73)/Kg body weight by deep intramuscular injection daily for fourteen days.

2. Control group (5 rabbits): Each rabbit in this group received 2 ml of olive oil (the equivalent volume of the average dose of CyA received by the animals in the treatment group) intramuscularly daily for fourteen days.

On Day 15 of the experiment, in both groups, a midline incision was made under general anaesthesia (p. 74) and, the reproductive tract was exposed and unilateral ovariectomy performed. The removed ovaries were then immediately fixed for light microscopic examination (p.83) after their dimensions (length and width) were measured, using a metal ruler. The abdomen was then closed in layers leaving 100 mls of warm heparinized Ringer's solution in the peritoneal cavity. Animals were kept in the laboratory until recovery from anaesthesia and then re-caged. Each animal received 300 mg of chloramphenical intramuscularly immediately after operation and daily for the four subsequent days.

Two weeks after ovariectomy, animals were mated with a fertile male; evidence of mating was observed (p. 80).

Two weeks following mating, a second laparotomy was performed on

all animals of both groups. The number of corpora lutea and gestational sacs were recorded. The abdominal incision was then closed and the animals returned to their cages after recovery from the anaesthesia.

The animals were followed until delivery. Litters were weighed and examined for gross evidence of congenital abnormalities. The animals were then sacrificed and their ovaries immediately removed and fixed.

Blood sampling: In both groups, blood samples for peripheral plasma progesterone estimation were collected from the marginal ear veins on the day before the beginning of the experiment, on Day 6 and Day 12 of the experiment, on mating day and at six-day intervals afterwards until delivery. Blood samples were handled and assayed according to Abraham's modified method (p. 82).

All animals were weighed before the beginning of the experiment and at weekly intervals afterwards until the end of the experiment.

Data collected from the experimental and control group on ovarian dimensions, corpora lutea and gestational sacs were analysed by analysis of variance, while data for peripheral plasma progesterone and body weight were analysed by repeated measure analysis of variance.

Results

Three animals in the treatment group died during the course of treatment (Day 9, 12 and 13). They showed evidence of severe anorexia and marked loss of weight before death. Exact cause of death was not identified. These three animals were dropped from the series, though their ovaries were removed for histological examination.

All the remaining animals made a full recovery from the first and second laparotomies.

All animals in the control group and three animals in the treatment group mated on first exposure, while two animals in the treatment group mated at a second attempt, and all animals became pregnant.

Findings at post-mating laparotomy: Table 6.1 showed that: the mean number of corpora lutea in the treatment group (2.4 $\pm$ S.D. 1.1) was significantly lower than the mean number of corpora lutea in the control group (8.8 $\pm$ S.D. 1.3), $P<0.001$. The mean number of gestational sac in the treatment group (1.8 $\pm$ S.D. 0.8) was significantly lower than that in the control group (8 $\pm$ S.D. 0.7), $P<0.001$.

All animals delivered at full term, 31 $\pm$ 1 day post-mating. Animals in the control group delivered normal mature litters, with average weight 65 $\pm$ 5 g, while in the treatment group two animals delivered normal mature litters, average weight 60 $\pm$ 5 g, two delivered small dead litters, 30 $\pm$ 5 g, and one delivered small macerated mass.

There was a significant decline in the peripheral plasma progesterone level ($P<0.001$) in the treatment group during treatment and

TABLE 6.1

Comparison of the mean value of corpora lutea number, gestational sacs number, and Nidation Index, between treatment group with CyA and control group.

Variable	Mean ± S D	F	df	P
CLt	2.4 ± 1.1	38.27	1,8	H.S.
CLc	8.8 ± 1.3			0.001
Gst	1.8 ± 0.8	60.27	1,8	H.S.
Gsc	8 ± 0.7			0.001
NIt	0.8 ± 0.2	1.69	1,8	N.S.
NIc	0.9 ± 0.08			0.05

Legend:
CLt = Corpora lutea, treated group
CLc = Corpora lutea, control group
Gst = Gestational sacs, treated group
Gsc = Gestational sacs, control group
NIt = Nidation Index, treated group
NIc = Nidation Index, control group
H.S. = Highly significant
N.S. = Not significant
n_1 = 5 "Number of animals in treated group"
n_2 = 5 "Number of animals in control group"

TABLE 6.2

Mean peripheral plasma progesterone value in ng/ml ± SEM during treatment with CyA and during pregnancy compared with the control group

Group	Base Value	Post treatment days		Mating	Post mating days (pregnancy)				
		6	12		6	12	18	24	30
Treated	1.1±0.1	0.7±0.07	0.37±0	1.0±0.08	8.3±0.8	8.5±0.7	5.6±0.2	2.7±0.4	1.8±0.6
Control	1.0±0.1	1.01±0.1	1±0.2	1.3±0.2	8.7±1.0	11.2±1.7	8.7±1.3	3.8±0.6	2.6±0.5

No. of treated group = 5

No. of control group = 5

F = 32.8

df = 1,8

S = Significant at
$P < 0.001$

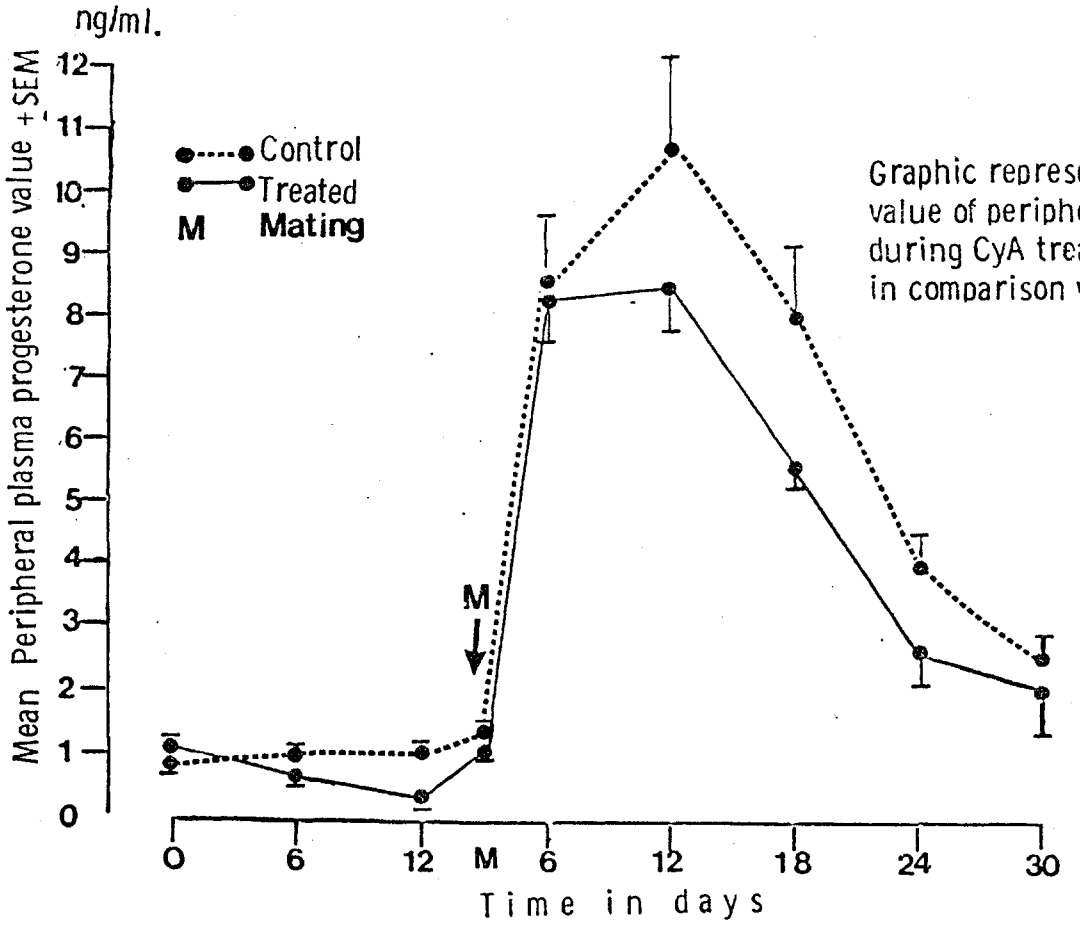


Fig.6.1

Graphic representation showing the mean value of peripheral plasma Progesterone during CyA treatment and during pregnancy in comparison with the control group.

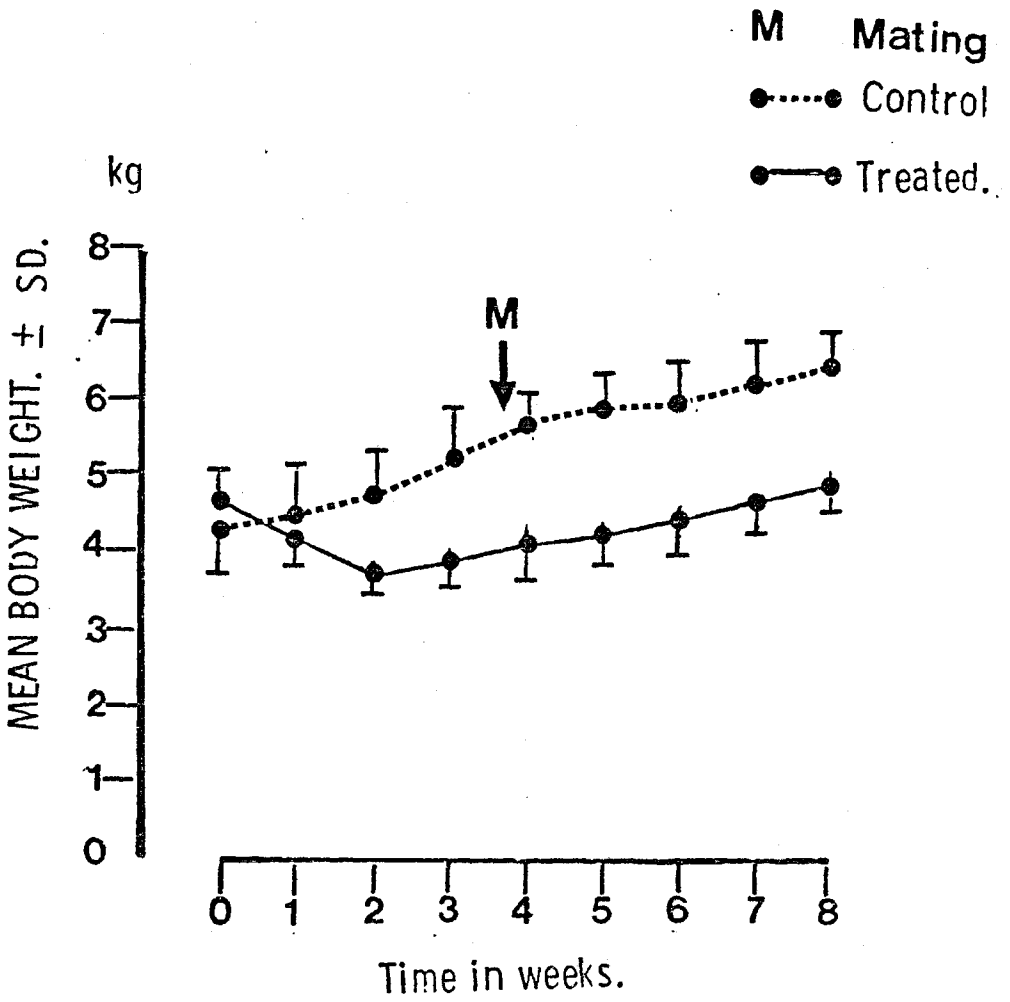


Fig. 6.2

Graphic representation showing the mean changes in the body weight during CyA treament and during pregnancy in comparison with the control group.

during gestation compared with the control group, Table 6.2, Fig. 6.1. The plasma progesterone level tended to rise after cessation of CyA treatment. There was a significant loss of weight in the treatment group, $P < 0.01$, during treatment compared with the control group, However, as can be seen in Fig. 6.2 animals started to gain weight after cessation of treatment, although the weight remained below the control.

There was a significant difference in the mean dimension of the ovary in the treatment group (0.5 ± 0.06 cm) and the control (0.7 ± 0.09 cm) $P < 0.01$.

Histological observations:

Light microscopical examination of the ovaries of the treatment group revealed

1. At the end of the 14 day CyA treatment, Figs. 6.3, 6.4, 6.5:

   (a) Hyperplasia of the theca externa cells of the secondary and tertiary follicles;

   (b) Areas of follicular necrosis and fibrosis and thickening of the theca in most of the sections examined;

   (c) Areas of hyalinized homogenous material between and inside the follicles;

   (d) Degenerative changes in the oocytes in most of the primary and secondary follicles;

   (e) Thickening of the vessel wall (the media and the adventitia).

   (f) Some normal follicles.

2. At the end of the experiment, average 46 days after the termination of CyA treatment, revealed the following (Figs. 6.7, 6.8):

   (a) Thickening and fibrosis of the interstitial tissue beneath the germinal epithelium;

   (b) Primary follicles fewer in number and show degeneration of the follicular cells and of the oocytes;

   (c) Secondary follicles also fewer in number and showed thickening of the theca. Some of the secondary follicles were necrotic, some fibrotic and some have become converted into hyaline mass surrounded by a number of follicular cells. Necrotic oocytes were seen in the secondary follicles. Few normal secondary follicles were present.

   (d) Some Graafian follicles showed thickening of the theca, others showed loss of oocytes and in some follicles oocytes showed loss of turgescence and folding of the plasma membrane. In some slides no Graafian follicles could be seen.

   (e) Normal corpora lutea were seen in some sections and there were many corpora albicans.

   (f) Arteriolar wall thickening, especially affecting the media. Normal blood vessels with little congestion were seen in some sections.

Histological examination of the control ovaries showed normal structure.

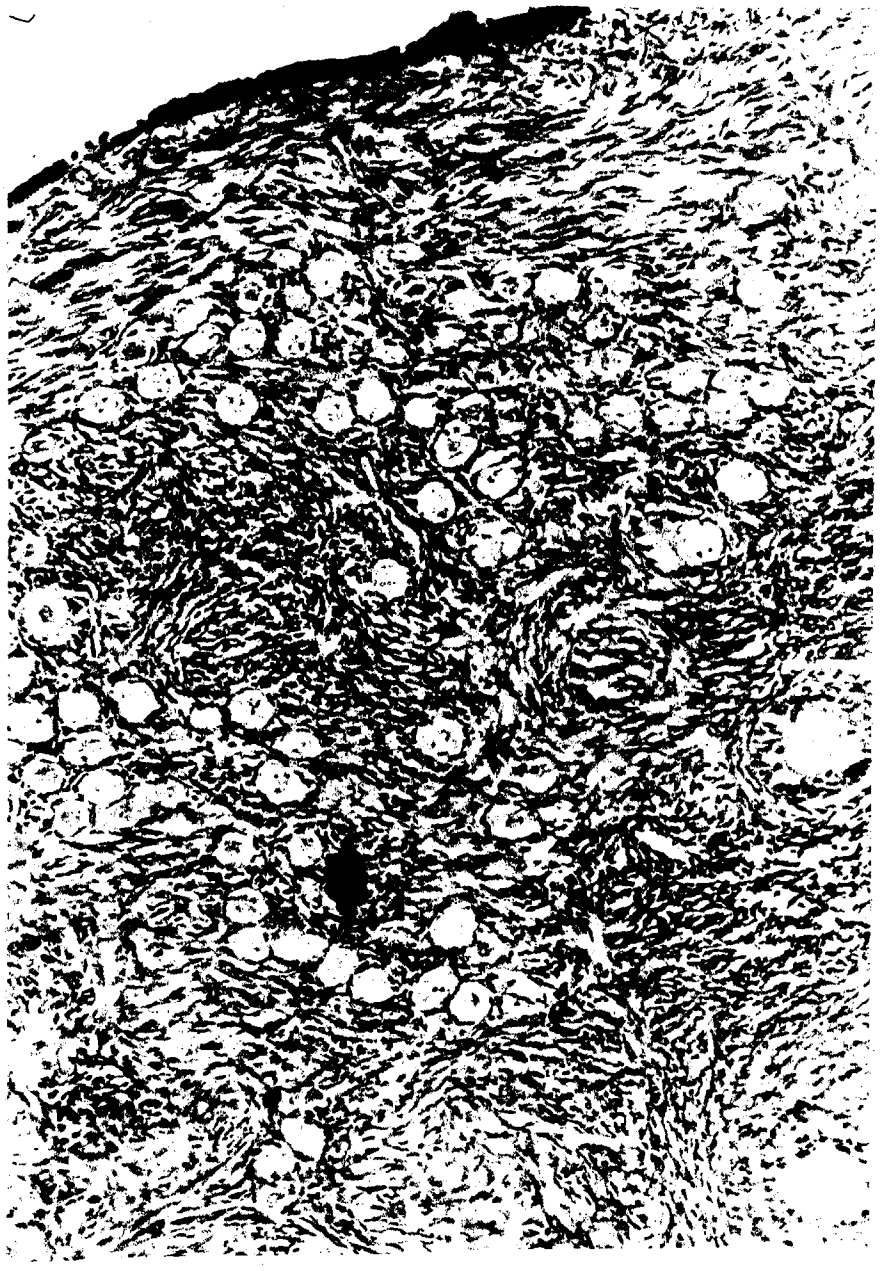


Fig. 6.3 Photomicrograph of rabbit ovarian tissue removed at the end of 14 days' course of CyA, showing thickening of subgerminal epithelial layer (tunica albuginea), oocyte degeneration in the primary follicles and follicular fibrosis; degeneration of secondary follicles; increase of fibrous elements of the ovary.

X 10

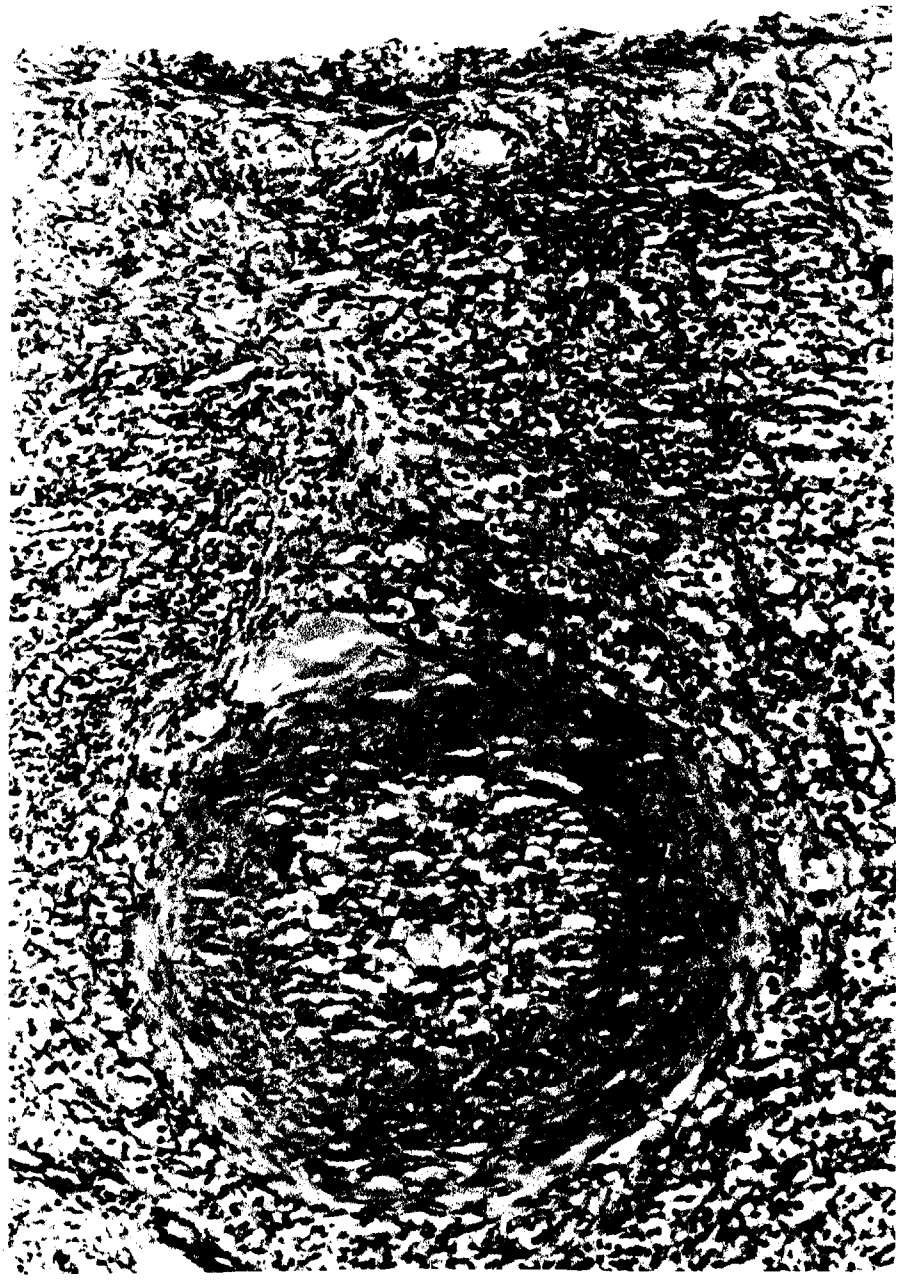


Fig. 6.4 Photomicrograph of rabbit ovarian tissue removed at the end of 14 days' course of CyA, showing thickening of subgerminal epithelial layer, increase of fibrotic trabeculae from the cortex to the medulla, destroyed primary follicles and early destruction of the oocyte, with pyknotic nucleus (arrow); degeneration of secondary follicles and thickened theca (T)

X 10



Fig. 6.5 Photomicrograph of rabbit ovarian tissue removed at the end of 14 days' course of CyA, showing excessive vascular thickening which affects mainly the media and the adventitia

X 10



Fig. 6.6 Photomicrograph of ovarian tissue removed 46 days after CyA course, showing thickening of the thecal layer of secondary and mature follicle (black arrows), increased fibrous element of the ovary (hollow arrows)

X 10



Fig. 6.7 Photomicrograph of rabbit ovarian tissue removed 46 days after CyA course, showing vascular thickening - thickening mainly affecting the media and the vessel's wall.

X 10

Discussion

Injury to the ovary by cytotoxic and immunosuppressive agents has long been demonstrated in animals (Bollag, 1953; Miller and Cole, 1970) and is being increasingly recognised as a complication of the use of these agents in the treatment of non-gonadal disorders (Shalet, 1980; Sieber and Adamson, 1975).

The results of this study showed that CyA, which has not been reported to affect the ovary before, can, in immunotherapeutic doses, damage the structure of the ovary and impair its function.

In this study, ovarian dysfunction was shown by the significant decline ($P<0.001$) in the peripheral plasma progesterone levels, both during treatment and gestation, in the animals which received CyA. Ovarian dysfunction is also demonstrated by the impaired response of the ovary to mating. This was evidenced from the lower number of corpora lutea and gestational sacs ($P<0.001$).

The tendency of the progesterone levels to rise after cessation of treatment probably indicates that the effect of CyA on the ovary was partly reversible. Experience with some cytotoxic/immunosuppressive drugs, in the human, showed a certain degree of reversibility of their effects on the ovary (Kumar et al, 1972; Uldall, 1972; Rose and Davis, 1977).

The small dead and macerated litters might have possibly been the result of the declining ovarian function, rather than an embryotoxic effect, for it is known that ovariectomy during pregnancy in the rabbit invariably leads to abortion or resorption of the foetus,

although evacuation of the uterus is not always immediate (Amoroso and Perry, 1977). Also no specific embryopathic or teratogenic effect occurred in rabbits given CyA doses of 10-300 mg/Kg/day between 6-18 days of gestation (Sandoz Internal Report, 1980).

The ovaries of the treatment group were atrophic and structural changes revealed by light microscopy were severe, widespread, and involved the different elements of the ovarian structure. They included general atrophy of the ovary, excessive thickening and fibrosis of the tunica albuginea, decrease in the number of follicles, particularly the primordial follicles, follicular necrosis, fibrosis and hyalinization, oocyte degeneration and loss, and thickening of the theca.

These changes are similar to those described in biopsy and autopsy specimens of ovaries of patients who received cytotoxic/immunosuppressive drug therapy. Among the histological changes reported were: inhibition of follicular maturation (Siris et al, 1976; Himelstein-Braw et al,1978), reduction in the number of primordial follicles (Chapman et al, 1979; Chapman, 1983), reduction-to-total absence of ova and follicles (Miller et al, 1971; Warne et al, 1973), atrophy and extensive fibrosis (Belo horsky et al, 1960), and thickening of the tunica albuginea, stromal hyalinization, intra-ovarian haemorrhages and perioophoritis (Morgenfeld et al, 1972).

An interesting finding in the ovaries examined in the present study was the thickening of the wall of the blood vessels, particularly involving the media.

Mild vascular wall thickening was reported by Keown et al (1981) in renal biopsies of some of their CyA-treated transplant recipients who showed unexplained deterioration of graft function. They, however, did not relate the change to CyA or the impairment of the function of the graft to the vascular change. Veith et al (1982) described early and prominent perivascular cuffing and reduction of blood flow in lung allograft of dogs immunosuppressed with CyA and not in those receiving other immunosuppressants or in those without immunosuppression. Veith et al. considered this finding as a form of pulmonary allograft rejection in animals immunosuppressed with CyA and that the perfusion/ventilation mismatch produced as a result, offers an earlier and easier diagnostic clue to rejection.

It appears from this study that the vessel wall thickening observed in the ovaries of the CyA-treated rabbits was the result of the action of CyA itself. The relevance of this observation to the function and fate of the graft remains to be seen. If the circulation to the organ is compromised as a result of the vascular changes, and the experiments of Veith et al., referred to above, suggests that it could be, then to organs like the ovary whose function is regulated by blood flow (Niswender et al, 1976), this change could be critical. This may explain, in part, the deterioration of the function of the ovary observed in the treatment group of rabbits in this study.

Although caution is needed in extrapolating results of animal experiments to human, the possibility still remains that the ovaries of CyA-treated patients may be adversely affected by CyA. This possibility is strengthened by the similarity of pattern of changes

in the ovary observed in the present study and those described in the human with other immunosuppressive and cytotoxic drugs.

These effects of CyA on the ovary should not be altogether unexpected, in view of the fact that some fungal antibiotics (e.g. bleomycin) are among the most potent gonadotoxic agents (Dobson and Felton, 1983).

One of the interesting findings in the histology of the ovaries in the treatment group was the excessive deposition of fibrous tissue. This may be clinically relevant in view of the reported development of benign breast lumps (fibromata) in patients on CyA therapy, and suggests the need to look for the possibility of development of fibrosis in other areas of the body.

Another interesting feature in this study, which is illustrated in Fig.6.2, is the significant loss ($P < 0.01$) of weight in the treated group. The cause of this loss of weight may be related to the anorexia, which was a prominent feature in the treated group, and to the failure to digest food (Gratwohl et al, 1981). Weight loss in association with anorexia and diarrhoea was also reported in CyA-treated patients (Hamilton et al, 1982).

In conclusion: This study originated from the observation of certain peculiarities of the rejection process of allografted ovaries in rabbits immunosuppressed with CyA, and, although it forms a part of the preliminary work, it was in fact the last experiment in this Thesis. It demonstrated an hitherto unrecognised serious side-effect of Cy A namely ovarian toxicity. If the results of this experiment can be extrapolated to humans, the implication would be a serious one, as a

large number of female patients of various age groups, who are on this drug for immunosuppression of transplant or other disease processes, are probably at risk of developing premature ovarian failure with all the physical and emotional sequelae. The experiment also drew attention to the fact that although systemic effects of graft immunosuppressants are almost always watched for, local toxic effects on the graft are either missed, as in the report on CyA-immunosuppressed ovarian allograft (Green et al, 1982) or dismissed, as in the reports on CyA-immunosuppressed renal allograft (Keown et al, 1981) and lung allograft (Veith et al, 1982), referred to earlier in the discussion. A plea is made here to attempt to discern the toxic effect of immunosuppressants, on the graft, from their immunotherapeutic action.

It is suggested, as a result of the findings in the present study, that the ovarian status of females on CyA immunotherapy be periodically evaluated and that symptoms of flushes, dyspareunia, irritability and emotional difficulties, etc., in such patients be investigated with the possibility of ovarian failure in mind, regardless of the age of the patient. It is also suggested that a study be organised to investigate the immediate and longterm effects of CyA on the gonads in the human.

OVARIAN TRANSPLANTATION

CHAPTER 7

STUDY OF OVARIAN AUTOTRANSPLANTATION BY MICRO-VASCULAR TECHNIQUE AND ASSESSMENT OF THE STRUCTURE AND FUNCTION OF THE GRAFT IN THE RABBIT

EXPERIMENT NO. V.

Many techniques of ovarian transplantation were used since Knauer first transplanted the ovary in 1896 (Krohn, 1977). The most common procedure was implantation in the peritoneum or omentum. Although few pregnancies were achieved in the rabbit (Jaszcak and Hafez, 1971) and rat (Ichinoe et al, 1976), using implantation techniques, the invariable occurrence of extensive ischaemic necrosis (Harris and Eakins, 1949), periovarian adhesions and the irregular ovarian function, rendered the procedure unsatisfactory. It was evident that an ovarian graft would not be expected to survive adequately unless proper vascular anastomoses were prepared. Betteridge (1970) performed vascular transplantation of the ovary in the rabbit with limited success. He excised segments of the aorta and inferior vena cava to facilitate anastomosis and avoid working with the small ovarian vessels. Blanco (1976) transplanted vascularised ovaries in the human by anastomosing the uterine artery to the hypogastric artery, and achieved unsuccessful pregnancy. Technical difficulties and the uniformly poor results of arterial surgery in vessels below 4 mm in diameter (Hurwitt, 1953) prevented transplantation by anastomosis of vessels of diameters within that range.

The advent of microvascular surgery, however, and the achievement

by Jacobson and Swarez (1960) of 100% patency rate in the anastomoses of 1.4 mm diameter arteries, stimulated surgeons to tackle the problem of vascular transplantation again. As microsurgical techniques became sufficiently reliable, Winston and McClure Browne (1974) transplanted en bloc vascularised ovaries with adnexa in rabbit. Scott et al (1981) reported "microsurgical transplantation of the ovary in the monkey"; what in fact they had done was a "sham" operation in which the ovarian vessels were dislocated from the aorta and inferior vena cava and were then either re-implanted in their original site or in the common iliac artery and vein. The ovary was not disturbed, except on one failed attempt.

In this study, microvascular technique for the isotopic auto-transplantation of the ovary alone was used, the structure and function of the graft assessed and the factors which influence the outcome defined. The functional reserve of the graft, in particular, was evaluated by studying the response of the graft to proper stimulation.

Materials and Methods

Animals: 35 New Zealand White sexually mature virgin female rabbits were used in this experiment. They were divided into three groups.

Group A consisted of 15 animals. In this group, the donor's vascular pedicle was anastomosed to the recipient vascular stump, close to the origin of the ovarian vessels (Fig. 5.1 A).

Group B consisted of 15 animals. In this group, the donor's vascular pedicle was anastomosed to the recipient stump, close to the ovary (Fig. 5.1 B), the uterovarian branch preserved.

Group C consisted of 5 animals; in these only unilateral ovariectomy was performed. This group served as control.

Anaesthesia: was induced by hypnorm and maintained by fluathane and oxygen (p. 74).

In both Group A and Group B, each animal received 120 I.U. heparin/Kg body weight subcutaneously prior to laparotomy.

Group A (Figs. 7.1 A and B)

(1) Laparotomy: Laparotomy was performed through a midline incision and the reproductive tract exposed (p. 75).

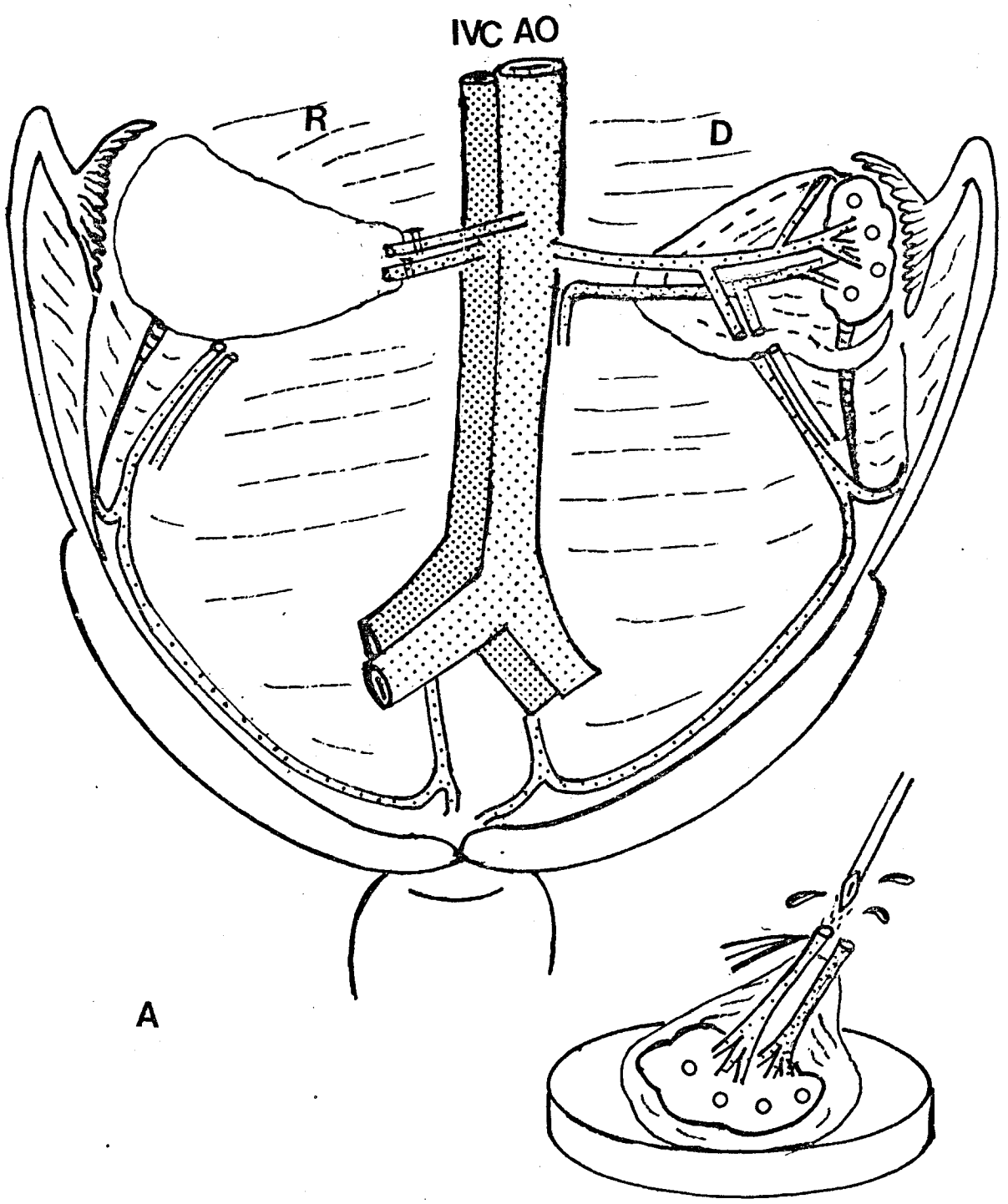
(2) Preparation of the donor side. Under the operating micro-

scope and at magnification x 25, the ovarian artery and veins were identified near their origin from the dorsal aorta and the I.V.C. in the retroperitoneum. The peritoneum covering the vessels was incised at a distance of 1 cm from the origin of the vessels. The vessels were exposed and separated from their surrounding structures; twigs arising from these vessels at these sites were cauterized using fine diathermy forceps under a fine jet of warm irrigating heparinized Ringer's solution. The perivascular sheath was then incised and the artery separated from the vein, and their external diameter measured. Handling of the vessels was kept to a minimum to avoid spasm. The uterovarian branch was cauterized using fine diathermy forceps under a fine jet of warm irrigating heparinized Ringer's solution. The fimbria ovarica was then dissected free from the cranial pole of the ovary, after cauterizing its vascular connection with it. The uterovarian ligament was cut at a distance of 2 mm from the ovary. The mesotubarium superius and the mesovarium between the cut ends of the uterovarian ligament and the fimbrica ovarica was incised, leaving 3 mm free peritoneum hanging along the margin of the ovary. The periovarian fatty peritoneal tissue was incised through a V-shaped incision with the apex of the V towards the already exposed part of the ovarian vessels, so that the donor ovary lay free, attached only by the prepared ovarian vascular pedicle. The graft, thus prepared, was covered by moist gauze which was kept constantly moist by frequent irrigation with warm Ringer's solution, pending transplantation.

(3) Preparation of the recipient side. Similar steps were followed as in the preparation of the donor side, so that the ovary lay free, being attached only by its vascular pedicle. Two single

Fig. 7.1

Diagramatic representation showing ovarian autografting procedure.
A. Preperation of the ovary of the donor side(D).
Preperation of the vessels of the recipient side(R).

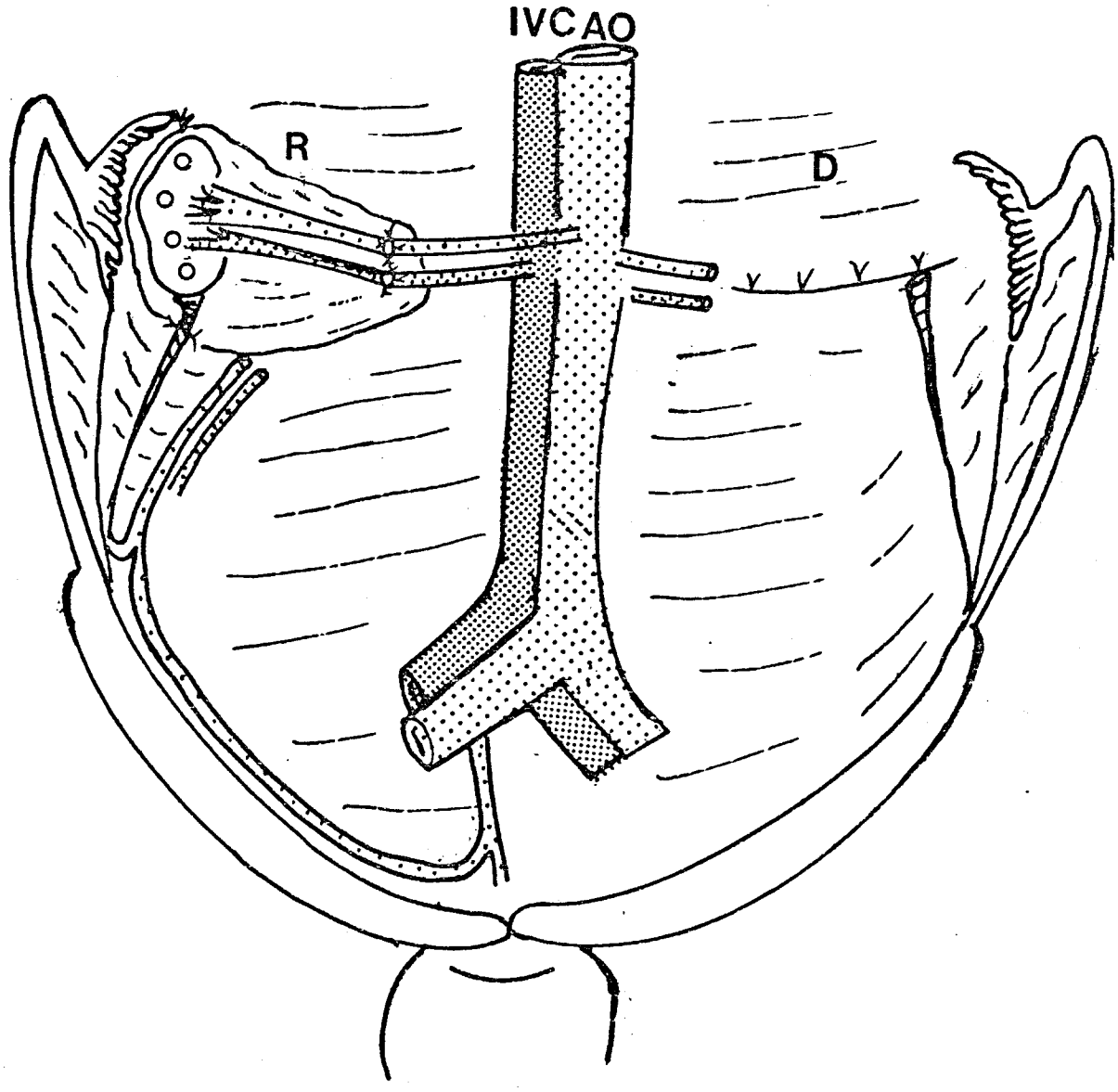


Flushing of the donor vessels.

Acland clamps were applied to the ovarian artery and vein, which were then severed, leaving a stump of 1 cm on the recipient side. The ovary was then removed.

(4) Transfer and grafting of the donor ovary to the recipient side. The donor ovarian vessels were clamped and divided, so that the graft was completely free. The graft was then transferred to a Petri dish and the ovarian artery and vein flushed with heparinized Ringer's solution (to avoid clotting of stagnant blood) with a fine needle of irrigating syringe, until the vessels were free from blood and appeared translucent. The graft was then transferred to the donor side and laid in its normal anatomical position with the vascular pedicle closely facing the recipient vascular stump. One double vascular clamp was applied across the stump of the recipient artery and the donor artery, and a second double vascular clamp was applied similarly across the recipient and the donor veins; the single clamps were then removed from the recipient's vascular stump. The cut ends of the recipient vessels were irrigated with warm heparinized Ringer's solution. Contrast plastic sheet was put beneath the donor and recipient vessels. End-to-end venous anastomosis (p. 75) was done first, to avoid congestion of the graft which could occur if arterial anastomosis was completed while the veins were still occluded. Arterial anastomosis followed. 11/0 nylon sutures on 7 μ needle was used for both the venous and arterial anastomosis. On completion of the anastomosis, 2% solution of procain was applied to the vessels, vascular patency checked, clots removed from the area and the tissues were irrigated with heparinized Ringer's solution.

B. Grafting of the donor ovary and peritonization of donor and recipient raw surfaces.



B

For details of anatomical structures see Fig. 4.1

The next step was the suturing of the stump of uterovarian ligament and the remnant of mesotubarum superius of the donor side to the corresponding structures in the recipient side using continuous 10/0 nylone suture. The fimbria ovarica of the recipient side was then joined to the cranial pole of the ovarian graft using 10/0 nylon sutures also. The freeedges of the grafted fatty peritoneum were sutured to the corresponding recipient peritoneum, leaving smooth surface facing the viscera.

On the donor side, the cut edges of the peritoneum were sutured together leaving no raw area.

The abdomen was then irrigated with warm heparinized Ringer's solution, of which 100 ml were left in the abdominal cavity prior to closure of the abdomen (p. 75).

Group B

The same procedure was followed as in Group A, except:

(a) the uterovarian vessels were preserved, and

(b) the anastomosis of the vascular pedicle of the graft to the vascular stump of the recipient side was performed distal to the origin of the uterovarian branch.

Group C (the control group)

The upper reproductive tract on one side was exposed through a midline abdominal incision and unilateral ovariectomy performed.

After completion of the operation, all animals were kept warm and dry until they recovered from anaesthesia before they were returned to their cages (p. 75).

The following intra-operative measurements were made:

(a) External diameter of the vessels anastomosed (arteries and veins;

(b) Duration of the total period during which the ovarian circulation was cut off (period of warm ischaemia or normothermic ischaemia);

(c) Immediate patency rates of the vascular anastomosis.

Post-transplantation graft assessment: All animals in Group A, B and C were laparotomized two weeks post-transplantation. The gross appearance of the graft (colour and size) and the presence of follicles were noted. Patency of the anastomosis, evidence of atrophy of the genitalia and the presence and grade of adhesions were also noted.

Assessment of the response of the ovary to mating: Two weeks after the second laparotomy, all the animals in Groups A, B and C were caged with male rabbits of proven fertility (p. 80) and mating behaviour noted.

Two weeks after mating, the animals in the three groups were laparotomised and the number of corpora lutea and gestational sacs recorded. Animals which did not achieve pregnancy were sacrificed and the ovaries removed for histological examination by light microscopy.

Pregnant animals were followed until delivery and the litters weighed and examined for evidence of congenital abnormalities.

All the rabbits (with the exception of five that were followed) were sacrificed and their ovaries removed for histological examination by light microscopy and electron microscopy. The five rabbits that were followed (3 from Group A and 2 from Group B) were mated a year later. The litters were weighed and examined for evidence of congenital abnormalities. The rabbits were sacrificed and the ovaries removed for histological examination by light microscopy and electron microscopy.

Blood sampling: Blood samples for peripheral plasma progesterone estimation (p. 82) were collected before induction of anaesthesia, Day 6 and 12 post-transplantation, on mating day, and at six day intervals afterwards until delivery.

Data collected from Groups A, B and C following mating were analysed by one-way analysis of variance, and data collected of peripheral plasma progesterone from the three groups were analysed by repeated measure analysis of variance.

Results

1. Operative (grafting procedure)

(a) Three rabbits from Group A died; one died 16 hours post-operatively because of prolonged anaesthesia, the second died on the sixth post-operative day during blood sampling, and the third died on the ninth post-operative day during laparoscopic examination, most probably from an air embolism. These three rabbits were dropped from the series. Their ovaries were, however, examined and showed no evidence of infarction, and the anastomoses were patent. In Group B, two animals died; one died on the third post-operative day and the other on the sixth post-operative day. Their ovaries showed ischaemic necrosis. The two animals were also dropped from the series.

The rest of the animals in both groups made a full recovery.

(b) External diameter of the anastomosed vessels (Table 7.1): Comparing the external diameter of the vessels at the anastomotic sites in Group A and Group B animals, it was found that:

The mean external diameter of the artery at anastomotic site A (0.65 $\pm$ S.D. 0.05 mm) was significantly larger than that at anastomotic site B (0.4 $\pm$ S.D. 0.03 mm), $P < 0.001$;

The mean external diameter of the vein at anastomotic site A (0.9 $\pm$ S.D. 0.07 mm) was significantly larger than that at anastomotic site B (0.6 $\pm$ S.D. 0.05 mm), $P < 0.001$.

(c) Patency rates: In Group A (site A) the immediate patency rate was 100% and the patency assessed 14 days post-transplantation was

TABLE 7.1

Comparison of external diameter of ovarian vessels in mm., and anoxic period in minutes, between Group A and Group B during autotransplantation of the ovary

Variable	Group	Mean ± S D	P
Ext.diameter o.a.	A	0.65 ± 0.05	H.S.
Ext.diameter o.a.	B	0.4 ± 0.03	0.001
Ext.diameter o.v.	A	0.9 ± 0.07	H.S.
Ext.diameter o.v.	B	0.6 ± 0.05	0.001
Anoxic period	A	56 ± 7.5	N.S.
Anoxic period	B	55 ± 7.8	0.05

For Legend see Table 5.1

n1 = 15 (number of rabbits in Group A)
n2 = 15 (number of rabbits in Group B)

91.7%. In Group B (site B) the immediate patency rate was also 100%, but patency rates assessed 14 days post-transplantation were 61.5%. (Table 7.2)

(d) Total duration of normothermic anoxia (warm ischaemia) (Table 7.1): There was no significant difference in the mean duration of normothermic anoxia in Group A (56 $\pm$ S.D. 7.5 min.) and that of Group B (55 $\pm$ 7.8 min.), $P < 0.05$.

2. Assessment of the graft at two weeks post-transplantation laparotomy (Table 7.2).

In Group A, 11 out of 12 animals (91.7%) the grafts were of normal appearance (colour and size) and contained follicles; the genitalia were normal. One graft was pale but of normal size and its follicles were not clearly defined; the genitalia were normal.

In Group B, 8 out of 13 animals (61.5%) showed that the grafts were of normal appearance and contained follicles. The genitalia were normal. In the remaining five animals, the grafts were pale and somewhat atrophic, and the genitalia were atrophic too.

In Group C (control group), the findings were all normal.

Adhesion formation: The incidence and grade of adhesions complicating the grafting procedure are shown in Table 7.3. 41.67% of the animals in Group A were free from adhesions, while 53. 8% of Group B were free from adhesions. All animals in Group C were free from adhesions.

TABLE 7.2

Autograft assessment two weeks post transplantation

Group	Trans-planted	Post-operative living animals	Normal colour of graft	Normal size of graft	Presence of follicles	Normal genitalia	Patent anastomosis	% of viable grafts	Total % of viable grafts
A	15	12	11	12	11	12	11	91.7	80
B	15	13	8	8	8	8	8	61.5	

3 animals died between 1 - 9 days post-operative period in Group A

2 animals died between 3 - 6 days post-operative period in Group B

TABLE 7.3

Incidence of adhesions at 2 weeks post-operative assessment of the autografts

Adhesions Grade	Group A	%	Group B	%
0	5	41.67	7	53.8
1	3	25	4	30.77
2	3	25	2	15.38
3	1	8.3	0	0

n1 = 12 "Number of recipients in Group A"

n2 = 13 "Number of recipients in Group B"

Control group had no adhesions

All grafts without adhesions achieved pregnancy.

No grafts with Grade 2 and 3 adhesions became pregnant.

3. Results of ovarian stimulation by mating: (Table 7.4).

In Group A, all animals accepted the male. Eleven out of 12 animals ovulated and developed corpora lutea (ovulation rate 91.7%). Of those which ovulated (11 rabbits) only 8 achieved pregnancy (pregnancy rate 66.7%). In Group B, only 8 out of 13 accepted the male. All of those which accepted the male (8 rabbits) ovulated and developed corpora lutea (ovulation rate 61.5%). Of the 8 animals which ovulated, only 7 achieved pregnancy (pregnancy rate 53.8%). In Group C, all the animals achieved pregnancy (pregnancy rate 100%).

The pregnant animals in the three groups were compared by corpora lutea and gestational sacs (Tables 7.5, 7.6) and the results were

(a) Corpora lutea: In Group A, the mean number of corpora lutea (8.37 $\pm$ S.D. 2.2) was not significantly different from that in the control group (9.2 $\pm$ S.D. 0.84), $P<0.05$, while in Group B, the mean number of corpora lutea (5.42 $\pm$ S.D. 0.8) was significantly lower than that in the control group (9.2 $\pm$ S.D. 0.84), $P<0.001$. There was a significant difference in the mean number of corpora lutea between Group A and Group B, $P<0.01$.

(b) Gestational sacs: In Group A, the mean number of gestational sacs (5.5 $\pm$ S.D. 1.9) was significantly lower than that in the control group (9.2 $\pm$ S.D. 0.8), $P<0.01$. In Group B, the mean number of gestational sacs (3.6 $\pm$ S.D. 0.6) was significantly lower than that in the control group (9.2 $\pm$ S.D. 0.8), $P<0.001$. There was a significant difference in the mean number of gestational sacs between Group A and Group B, $P<0.05$.

TABLE 7.4

Ovulatory function and pregnancy rate of auto-transplated ovaries

Group	Transplanted	Mated	Ovulation %	Pregnancy	%
A	12	11	11 (91.7%)	8	66.7
B	13	8	8 (61.5%)	7	53.8
(C) Control	5	5	5 (100%)	5	100

Group A = Autotransplantation near origin

Group B = Autotransplantation near the ovary

Group C = Ovarectomy "Control Group"

Total pregnancy rate in (Autotransplanted animals, Group A and B) = 60%

Note: Mating was attempted in all the animals of both groups.

TABLE 7.5

Comparison of corpora lutea and gestational sacs value in Group A vz. control, Group B vz. control and between Group A and B following autotransplantation of the ovary

Variable	Mean ± S D	F	df	P
CLA	8.37 ± 2.2	0.63	1,11	N.S.
CLC	9.2 ± 0.84			0.05
CLB	5.42 ± 0.8	23.68	1,10	S
CLC	9.2 ± 0.84			0.001
CLA	8.37 ± 2.2	11.21	1,13	S
CLB	5.42 ± 0.8			0.01
GSA	5.5 ± 1.9	16.09	1,11	S
GSC	9.2 ± 0.8			0.01
GSB	3.6 ± 0.6	51.48	1,10	S
GSC	9.2 ± 0.8			0.001
GSA	5.5 ± 1.9	6.5	1,13	S
GSB	3.6 ± 0.6			0.05

Legend: CLA = Corpora lutea in Group A
CLB = Corpora lutea in Group B
CLC = Corpora lutea in Group C
GSA = Gestational sacs in Group A
GSB = Gestational sacs in Group B
GSC = Gestational sacs in Group C
n1 = 8 "Number of animals in Group A
n2 = 7 "Number of animals in Group B
n3 = 5 "Number of animals in Group C

TABLE 7.6

Comparison of the mean corpora lutea and mean gestational sacs between autotransplanted recipient groups and control group (C)

Variable	Group	Mean ± SD	F	df	P
Corpora lutea	A	8.37 ± 2.2			
Corpora lutea	B	5.42 ± 0.8	10.6	2,17	0.01
Corpora lutea	C	9.2 ± 0.84			
Gestational sacs	A	5.5 ± 1.9			
Gestational sacs	B	3.6 ± 0.6	25.9	2,17	0.001
Gestational sacs	C	9.2 ± 0.8			

n1 = 8 (Number of rabbits in Group A)
n2 = 7 (Number of rabbits in Group B)
n3 = 5 (Number of rabbits in Group C)

TABLE 7.7

Mean peripheral plasma progesterone level in ng/ml ± S.D. following ovarian autograft compared with the control group

Group	Base Value	Post-operative days		Mating	Post mating days					df	F	P
		6	12		6	12	18	24	30			
A	1.45±0.6	0.3±0.2	1.1±0.5	1.9±0.6	7±1.9	8.1±1.4	8.4±0.9	4.6±0.6	2.5±0.6			S
B	1.4 ±0.5	0.28±0.2	0.8±0.45	1.3±0.2	6.1±0.9	7.7±1.6	7.8±1.3	3.7±0.4	2.3±0.4	2,17	6.04	0.05
C	1 ±0.3	0.9±0.15	1±0.2	1.6±0.3	7.7±1.4	9.2±2.5	8.5±1.4	5.2±0.3	2.9±0.3			

n1 = 8 (number of viable pregnant recipients in Group A
n2 = 7 (number of viable pregnant recipients in Group B
n3 = 5 (number of pregnant rabbits in control group, C

	F	df	P_S
Group A vz. Group C =	8.2	1,11	0.05 S
Group B vz. Group C =	10.4	1,10	0.01 S
Group A vz. Group B =	0.01	1,13	0.05 N.S.

S = Significant

N.S. = Not significant

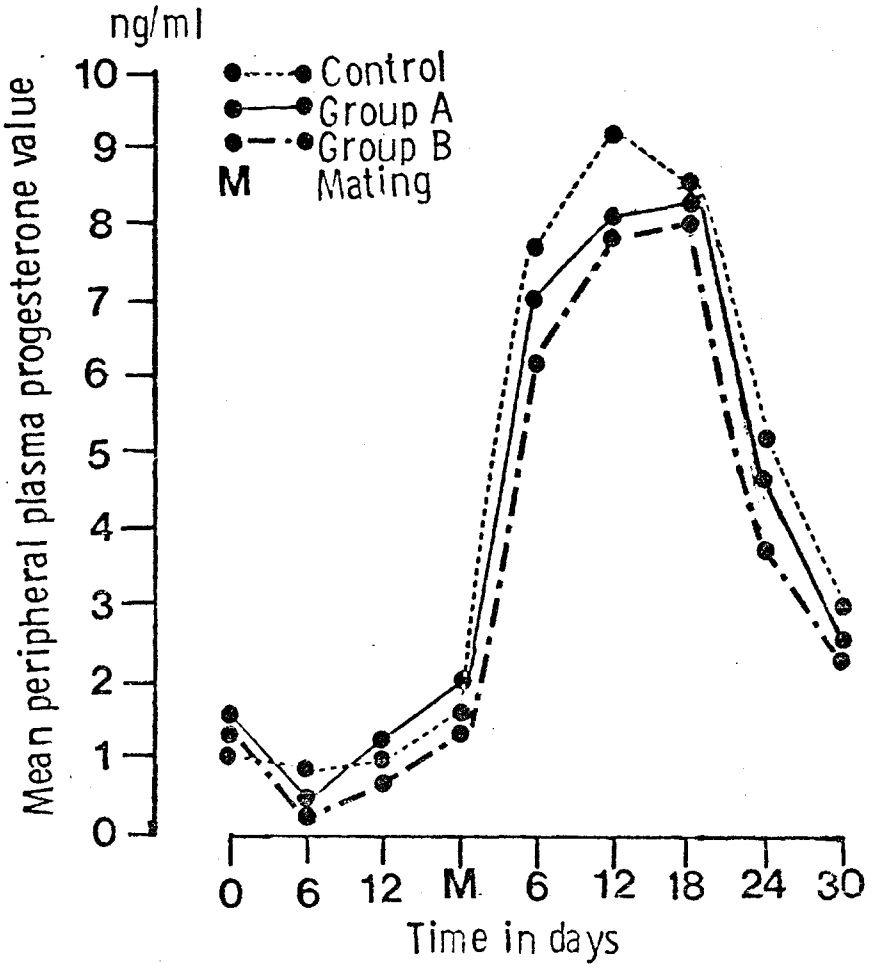


Fig. 7.2

Graphic representation showing the mean peripheral plasma progesterone value in group A and B in the immediate post operative period and during pregnancy compared with the control group.

4. Plasma progesterone levels (Table 7.7), (Fig. 7.2):

There was a significant decline in peripheral plasma progesterone levels during the immediate post-operative period and mid-pregnancy in:

(a) Group A and B compared with the control ($P < 0.05$);

(b) Group A compared with the control ($P < 0.05$);

(c) Group B compared with the control ($P < 0.01$),

while there was no significant difference between Group A and Group B ($P < 0.05$).

Results of the histological studies

A. Light microscopical examination

1. Ovaries which, on the second laparotomy, were found to be shrunken and atrophic with failure of the anastomosis, and did not ovulate, showed ischaemic necrosis and fibrosis with haemosiderin deposits. In some places few atretic follicles could be seen but no primary or secondary follicles could be identified. Some islands of luteal cells were seen. New blood vessels formed near the periphery. The germinal epithelium was not affected and there were adhesions to the adjacent peritoneal surfaces (Fig 7.3).

2. Ovaries which failed to ovulate or form corpora lutea; in these ovaries the changes were milder than in the former group and consisted of necrotic changes in the follicle, mainly the secondary and tertiary, affecting the follicular cells and the oocytes. The primary follicles were not badly affected; in some of them the

changes involved the oocyte. Tertiary follicles were also affected but appeared to be relatively resistant. Most of the blood vessels appeared empty and distended, and the endothelial lining was not continuous but denuded in some areas (Fig. 7.4, A and B).

3. Ovaries which responded to mating by ovulation and corpora lutea formation, but without achieving pregnancy (Fig. 7.5). These showed normal primary and secondary follicles. There were some normal tertiary follicles. There were several regressing corpora lutea and blood vessels were normal. Bands of fibrous tissue adhesions surrounded the surface epithelium.

4. Ovaries which responded to mating by ovulation and corpora lutea formation and achieved pregnancy (Fig. 7.6): These showed no adhesions, normal surface epithelium and normal internal structure.

B. Electron microscopical examination

Electron microscopical examination of the ovaries which appeared normal on light microscopy included

Scanning, electron microscopy: This showed a normal surface epithelium which appeared as a continuous unicellular layer of hemispherical cells covered with micro villi. In some areas the surface epithelium was evaginated into villus-like projections (papillae); some were single, finger-like (Fig.7.7), while others were branched (Fig.7.8). Pre-ovulatory follicles were seen slightly bulging on the surface (Fig.7.9). Corpora lutea were also seen. They were covered by a network of flattened papillae radiating outwards of the centre (Fig.7.10).

Transmission electron microscopy; showed a normal surface epithelium consisting of cuboidal cells with numerous tall microvilli on the free peritoneal surface. The cytoplasm contains numerous dense bodies, which were seen in the subnuclear regions and sometimes above the nucleous (Fig. 7.11). Beneath the basement of the surface epithelium were the connective cells of the tunica albuginea overlying the rest of the stroma. The cells of the stroma were poorly differentiated. Golgi complexes were most often seen located peripherally. Polysomes and ribosomes and smooth endoplasmic reticulum were abundant. Mitochondria with tubular cristae which characterise their organelles with steroidogenic cells were frequently seen. Follicles at different stages of development were also seen. The secondary and tertiary follicles (Fig. 7.12) demonstrated the characteristic features of spherical oocytes with prominent nucleous and nucleolus. The cytoplasm of the oocytes contained abundance of grouped and sometimes scattered organelles. A granular, sometimes filamentous zona pellucida, surrounded the oocytes. Microvilli from the oocyte were seen penetrating the zona. Granulosa cells form multilayer envelope around the oocyte with variable accumulations of follicular fluid, and were seen sending cytoplasmic processes from their innermost layer, extending through the zona pellucida (Fig. 7.12). The cytoplasm of the granulosa cell was found rich in mitochondria, with well developed endoplasmic reticulum and contained lipid droplets. In the pre-ovulatory follicles, the granulosa cells showed signs of luteinization which included enlargement of the cell, granular appearance of the cytoplasm, increase of the smooth endoplasmic reticulum, spherical mitochondria with tubular cristae and lipid droplets (Fig. 7.13). The various stages of differentiation of

the perifollicular stroma into theca layers, interna and externa, were clearly demonstrated. The fully developed theca interna cells appeared as epithelioid-like cells and the characteristics of steroid secreting cells with well developed smooth endoplasmic reticulum, round vesicular mitochondria and lipid droplets (Fig. 7.14). The theca externa consisted mainly of fibrous tissue cells (Fig. 7.11). The cells of the corpus were seen containing abundant endoplasmic reticulum with pleomorphic mitochondria and increased numbers of Golgi complexes and lipid droplets, features which are characteristic of steroid secreting cells (Fig. 7.15).

Both the scanning and transmission electron microscopical examinations did not appear to show any abnormal finding, thus confirming the result of the light microscopical examination.

Assessment of the rabbits which were followed a year after transplantation showed a normal response to mating, normal pregnancies and normal litters. Histological studies of the ovaries, removed at the end of the experiment, were also normal.

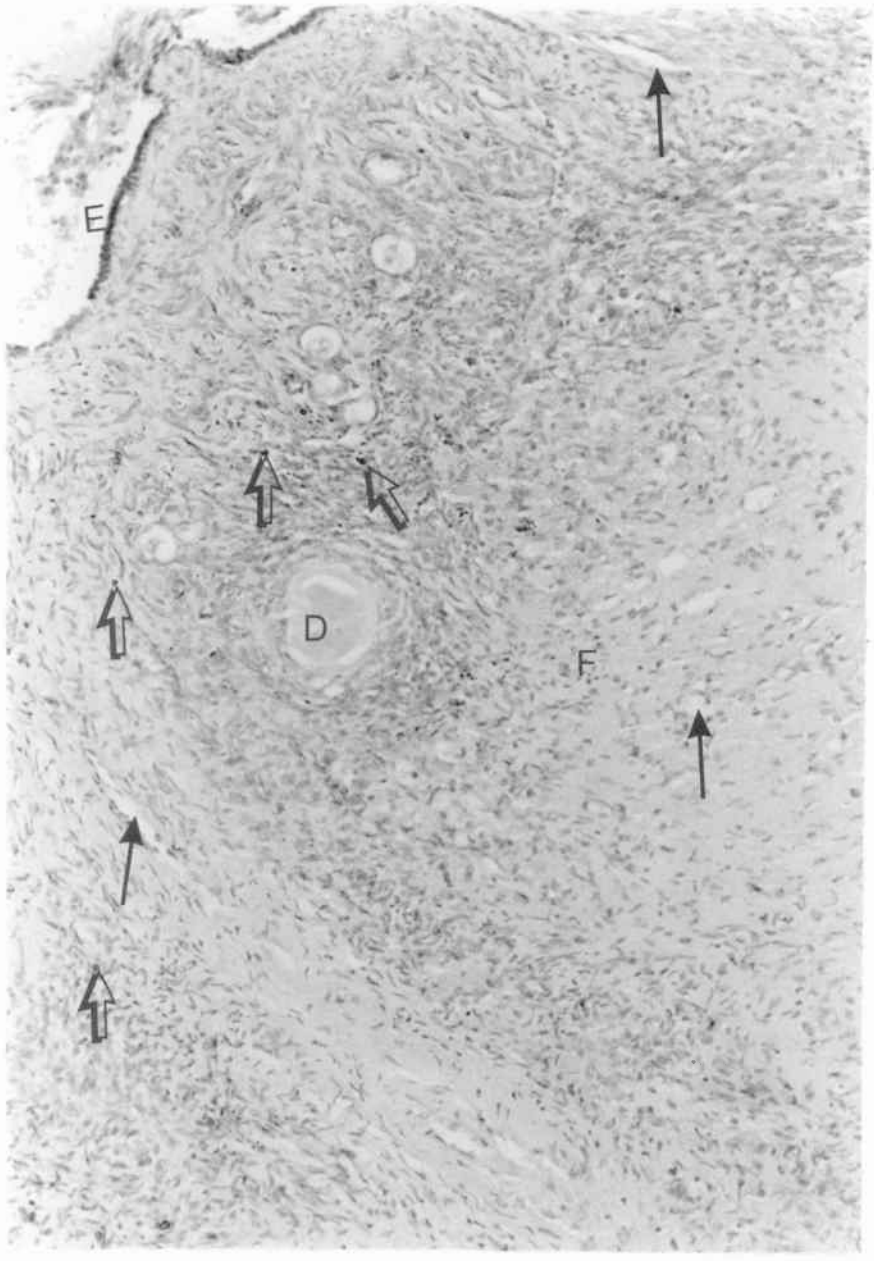


Fig. 7.3 Ovarian autograft.

Photo micrograph showing normal surface epithelium (E), extensive fibrosis (F), degenerated and fibrosed primary and secondary follicles (D), new blood vessels (black arrows), haemosiderin deposits (hollow arrows).

X 10

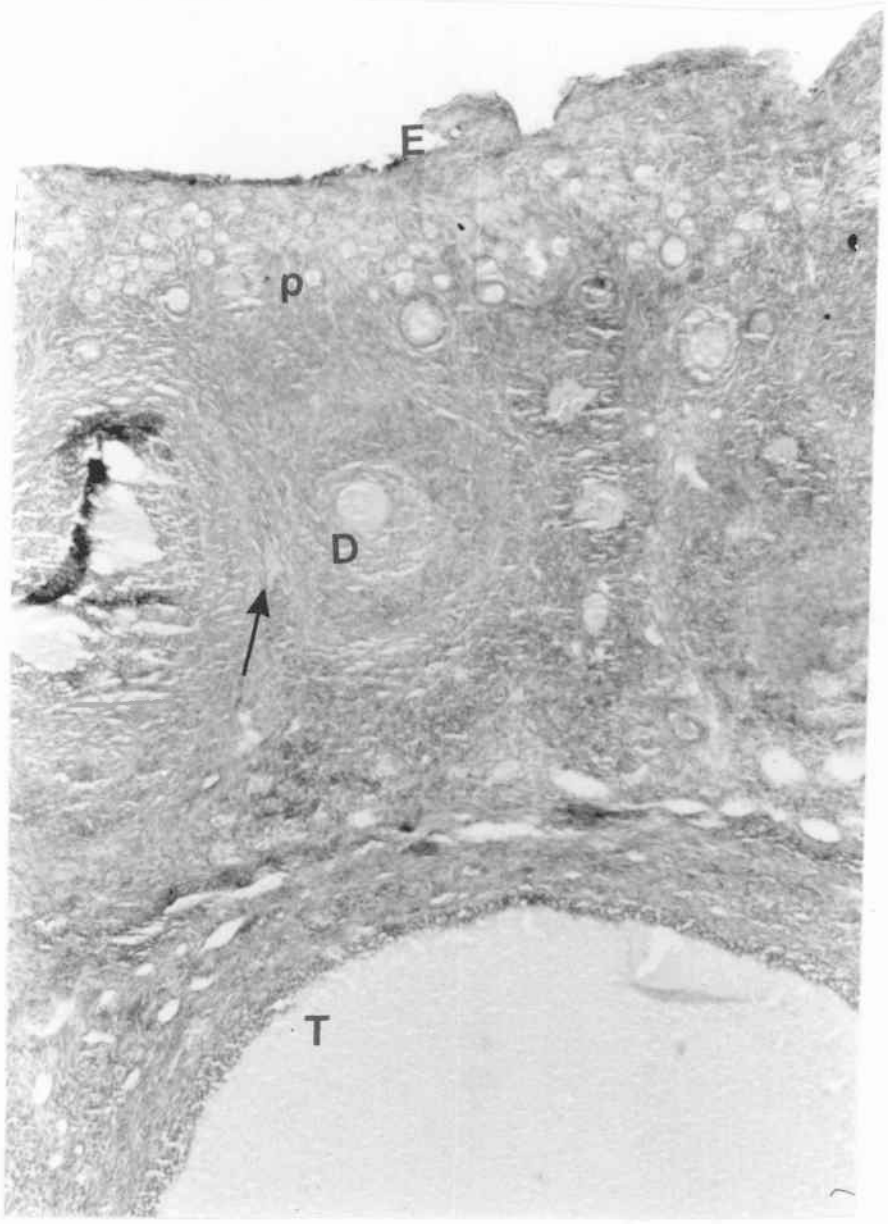


Fig. 7.4A Ovarian autograft.

Photo micrograph showing normal surface epithelium (E), mildly affected primary follicle (P), necrosed and degenerated secondary follicle (D), hyalinization and fibrosis of stroma (arrows), normal tertiary follicle (F).

X 10

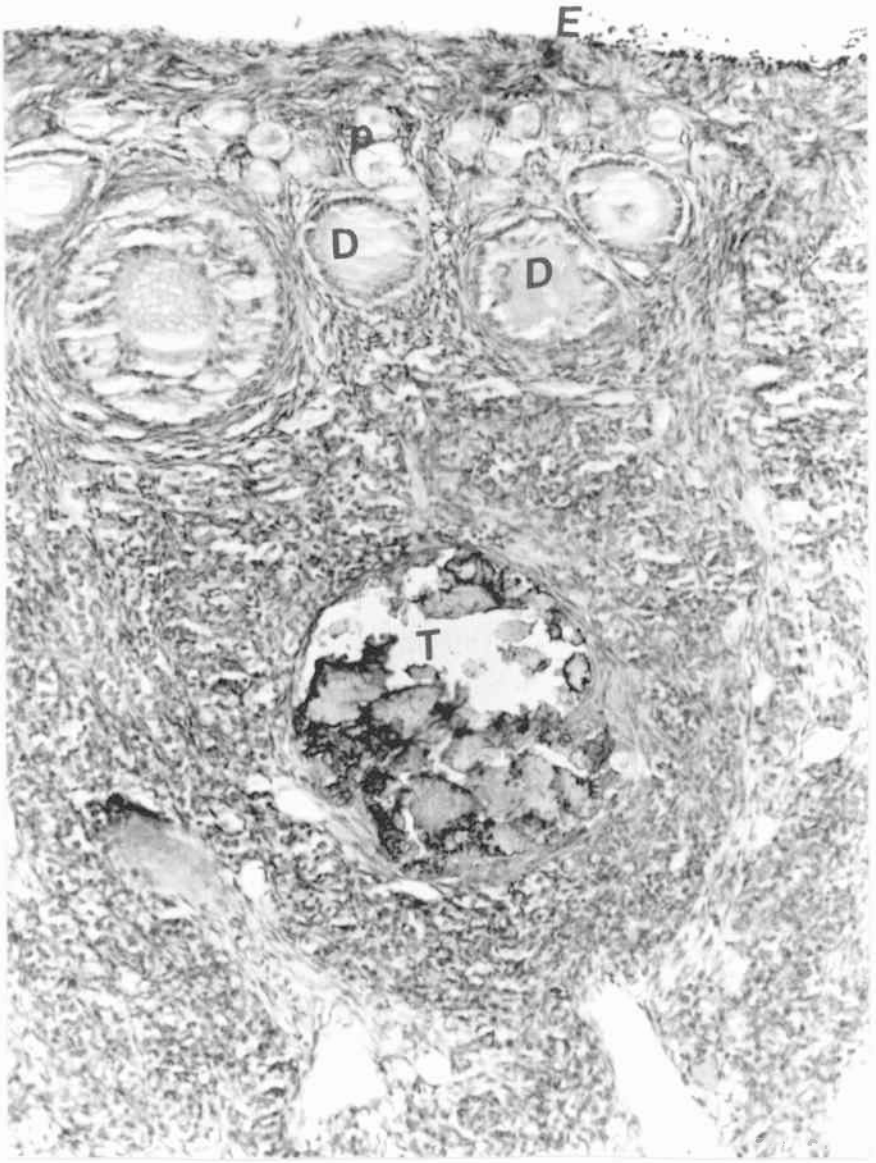


Fig. 7.4B Ovarian autograft.

Photo micrograph showing normal surface epithelium (E), normal primary follicles (P), degenerated secondary follicle (D), necrosis and fibrosis of tertiary follicle (T).

X 10

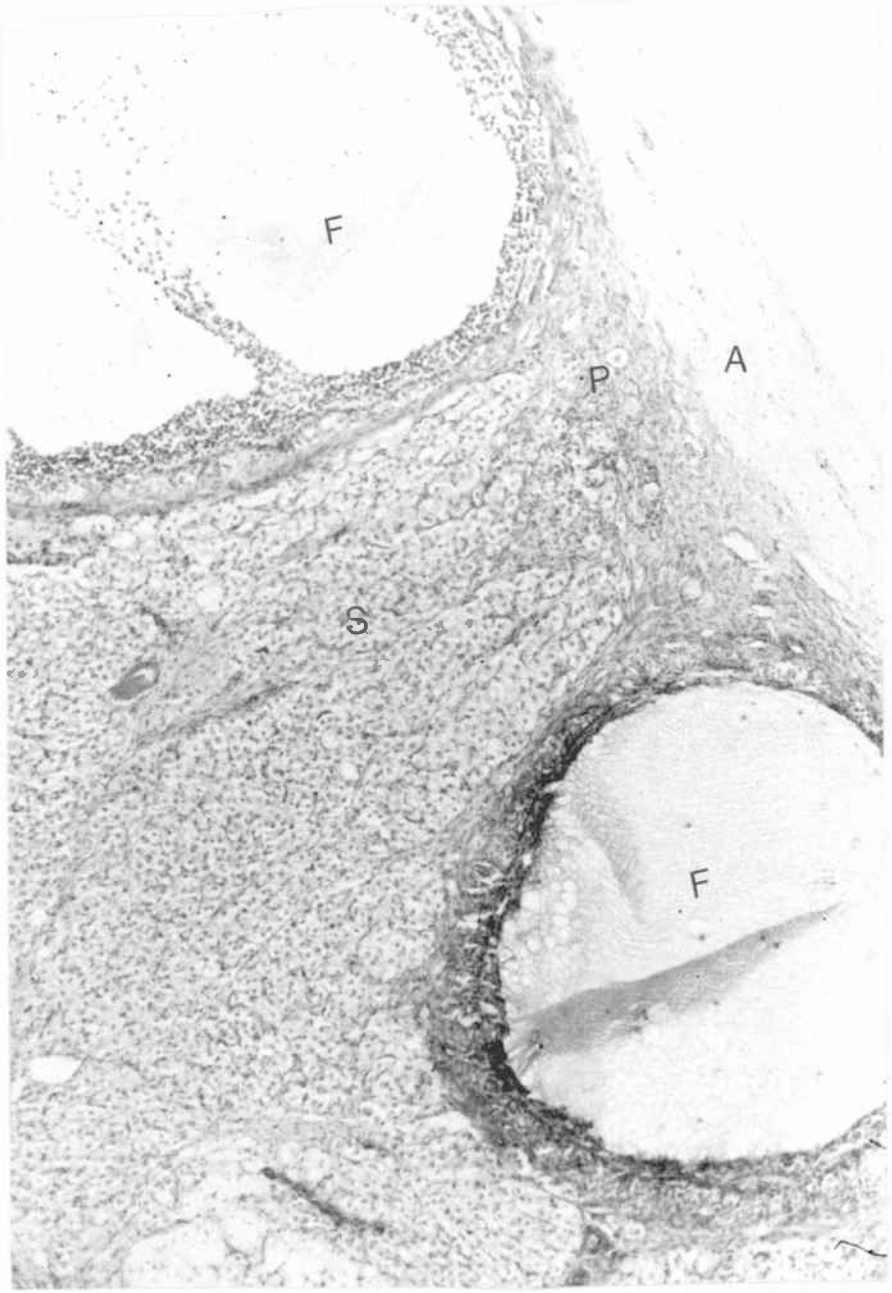


Fig. 7.5 Ovarian autograft.

Photo micrograph showing bands of fibrous tissue adhesion covering the ovarian surface (A), normal mature follicle (F), normal stroma (S).

X 10

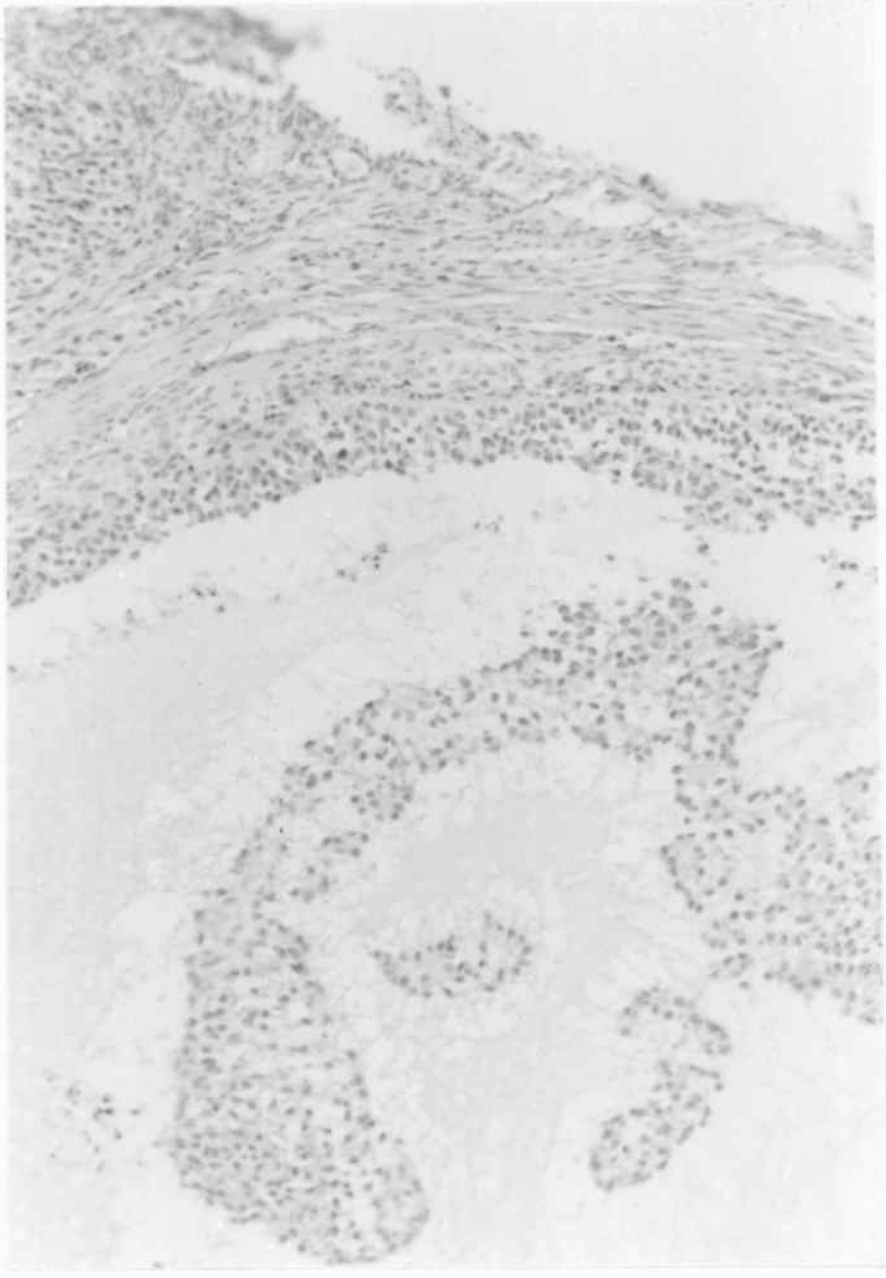


Fig. 7.6 Ovarian autograft.

Photo micrograph showing completely normal histological structures.

X 10

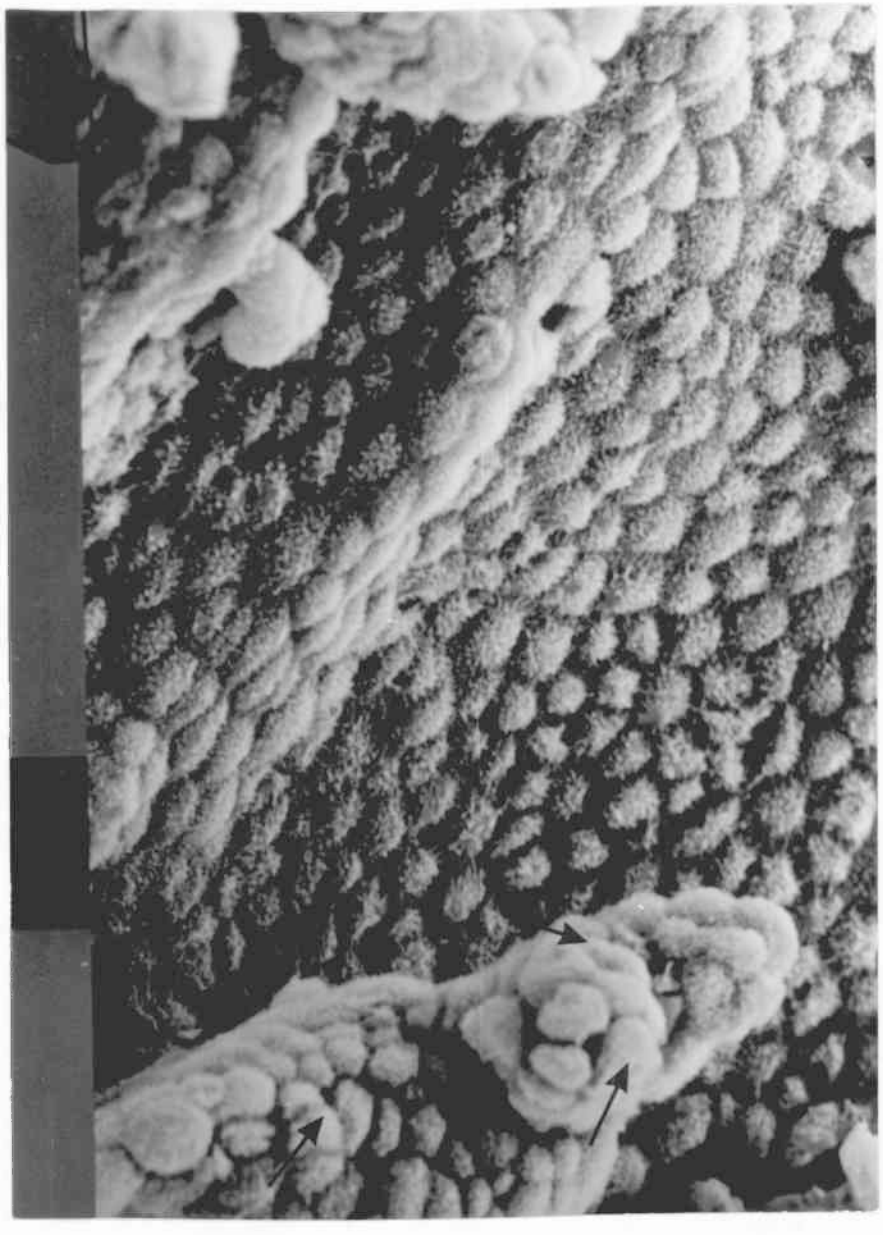


Fig. 7.7 Ovarian autograft.

Scanning electron micrograph showing normal surface epithelium and finger-like villus projections (papillae), covered by rounded epithelial cells (black arrows).

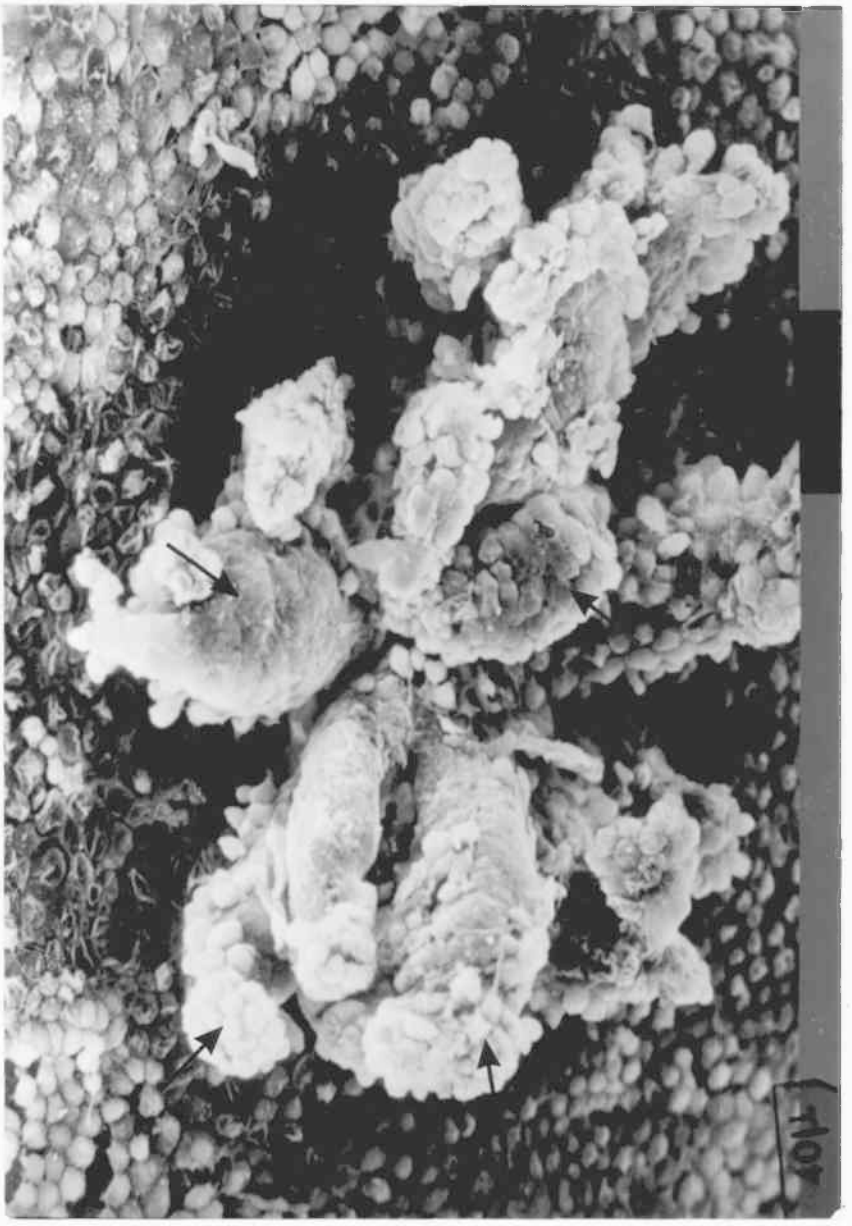


Fig. 7.8 Ovarian autograft

Scanning electron micrograph showing detailed view of surface papilloma (arrows).

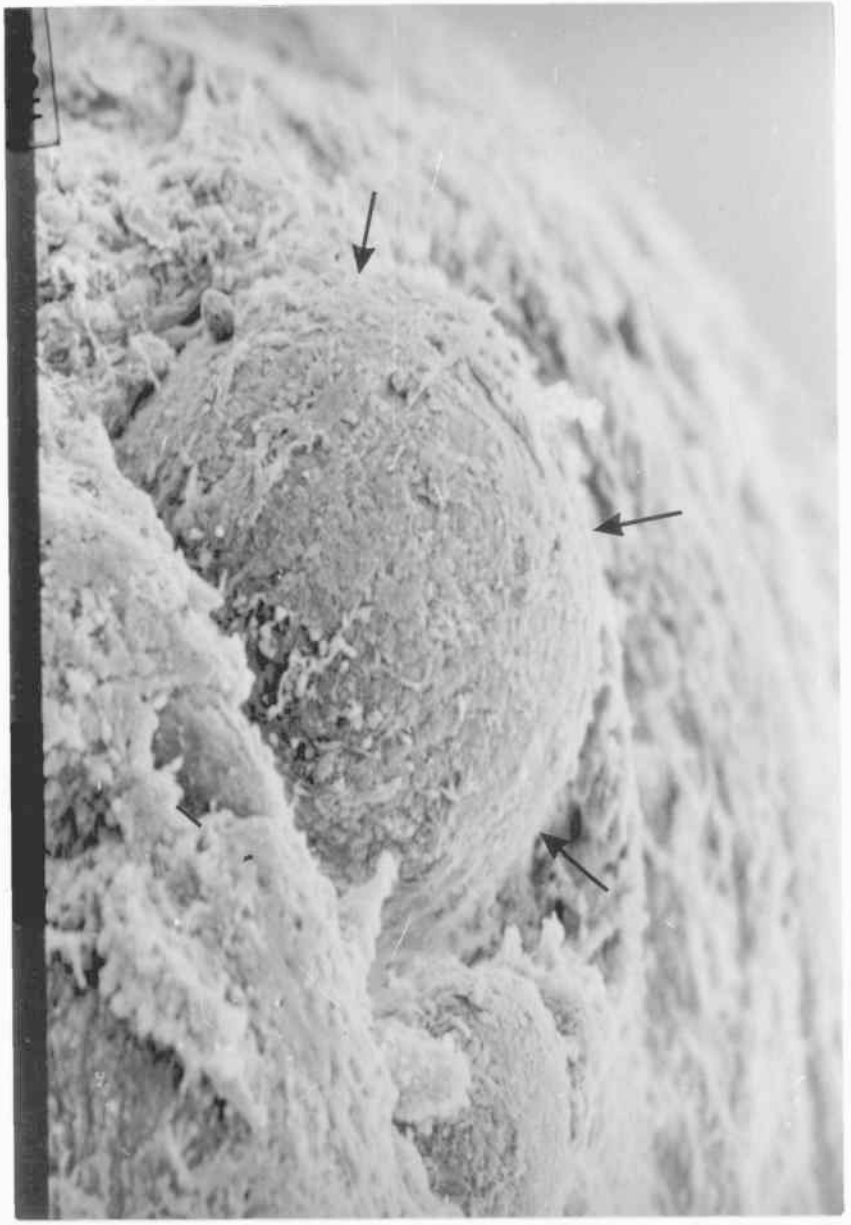


Fig. 7.9 Ovarian autograft.

Scanning electron micrograph showing normal pre-ovulatory follicle (arrows) covered by surface epithelium.

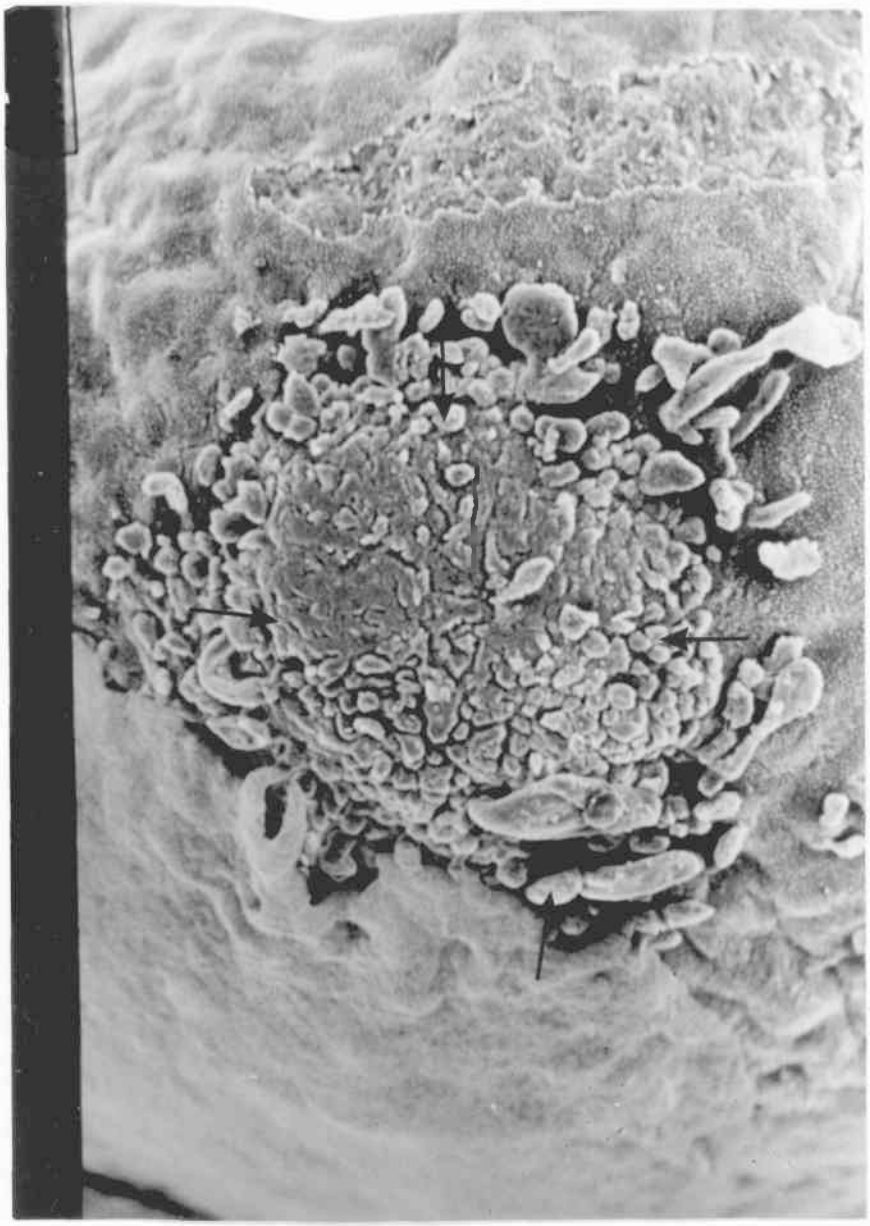


Fig. 7.10 Ovarian autograft.

Scanning electron micrograph illustrating the surface of the ovary directly overlying a corpus luteum which looks like a network of comparatively flattened papillae radiating outwards (arrows).

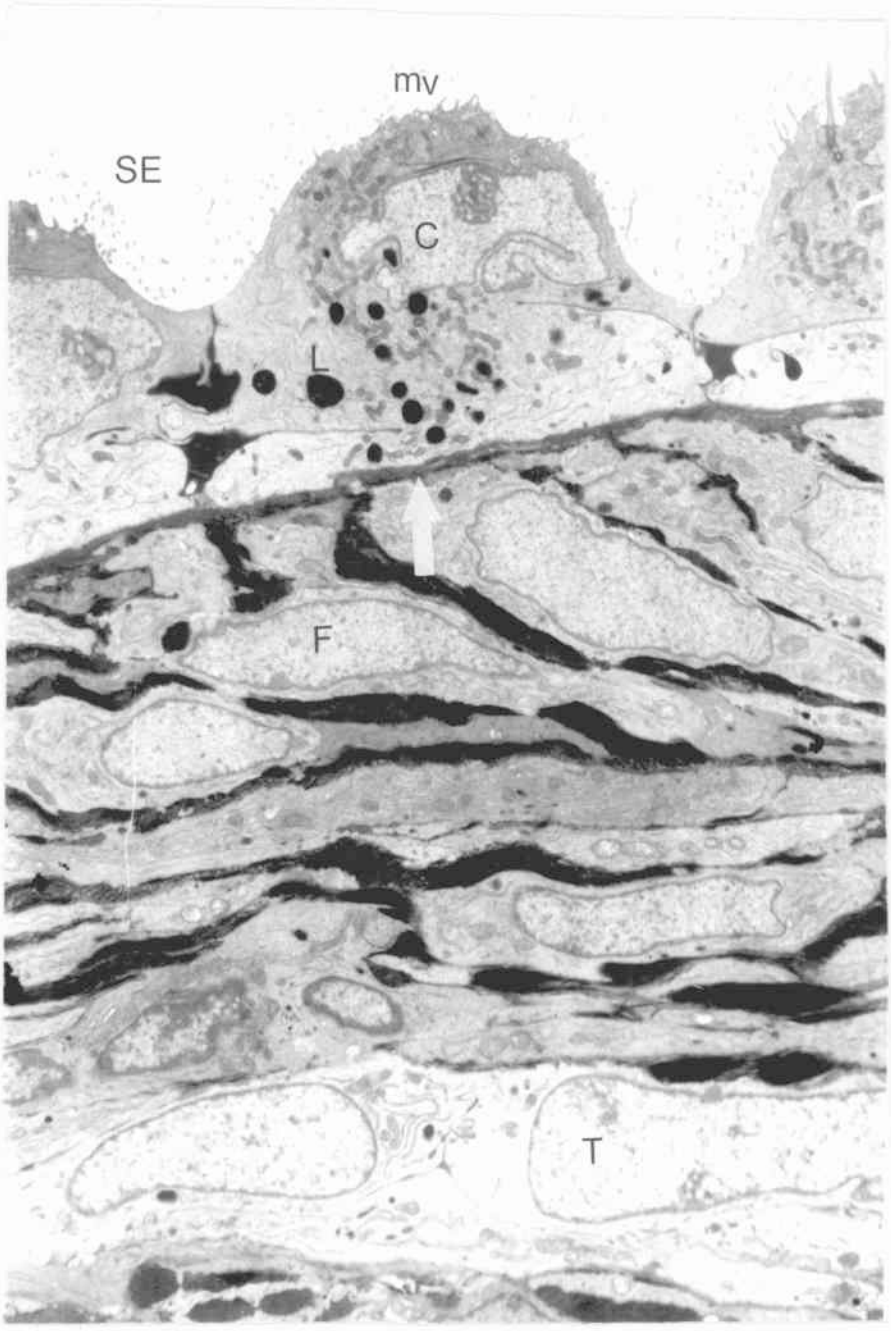


Fig. 7.11 Ovarian autograft.

Transmission electron micrograph showing surface epithelium (SE), microvilli (mv), cuboidal epithelial cells (C), intracellular electron-dense granules (L)(lysosomal-like bodies), basement membranes (white arrow), fibroblasts of tunica albuginea (F), theca externa cells (T)

X 5,250

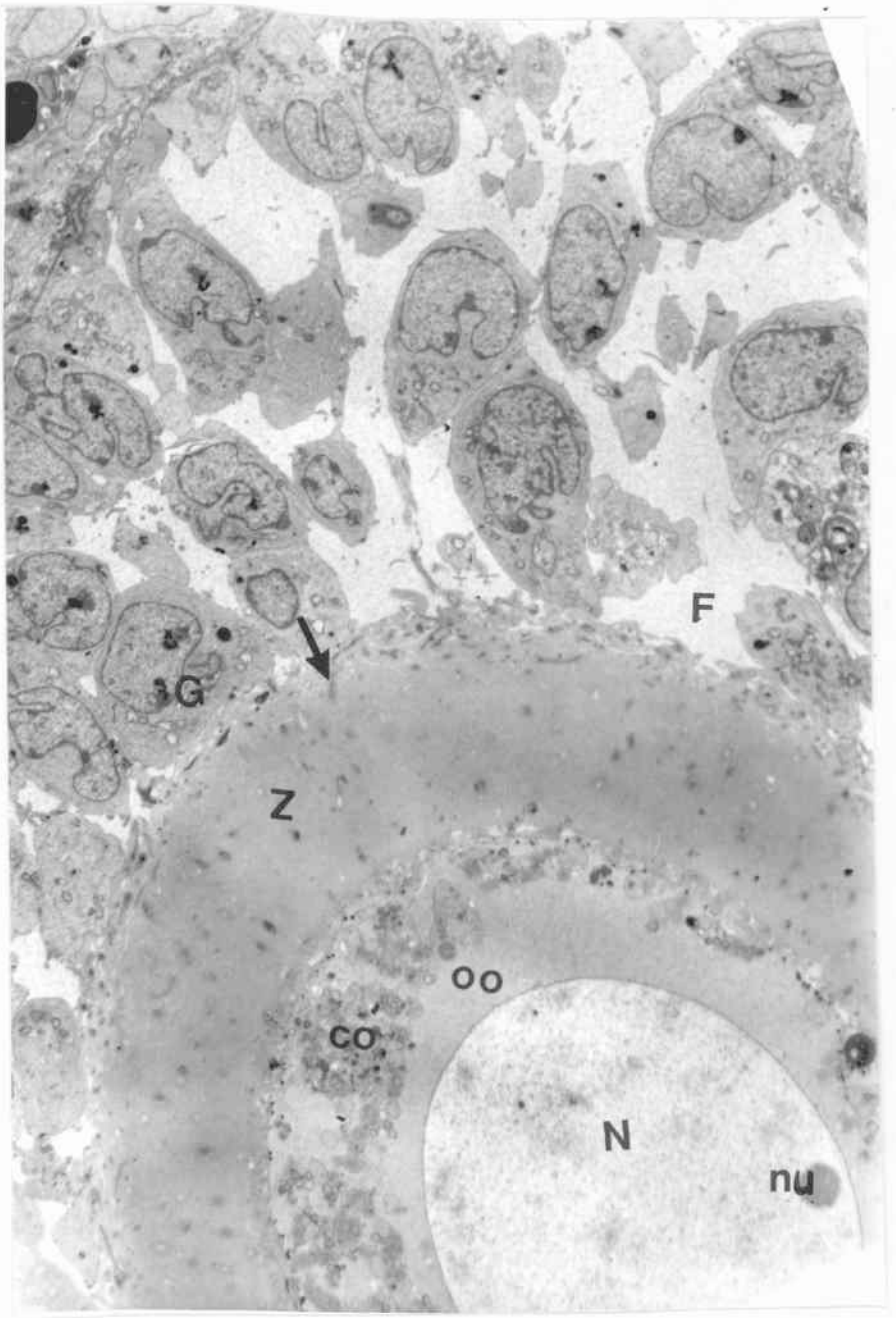


Fig. 7.12 Ovarian autograft.

Transmission electron micrograph showing mature follicle granulosa cells (G) (showing cytoplasmic projections to the zona, black arrow), granular zona pellucida (Z), oocyte (oo), nucleus (N), nucleolus (nu), cytoplasm of oocyte showing cytoplasmic organelles (co).

X 2,100

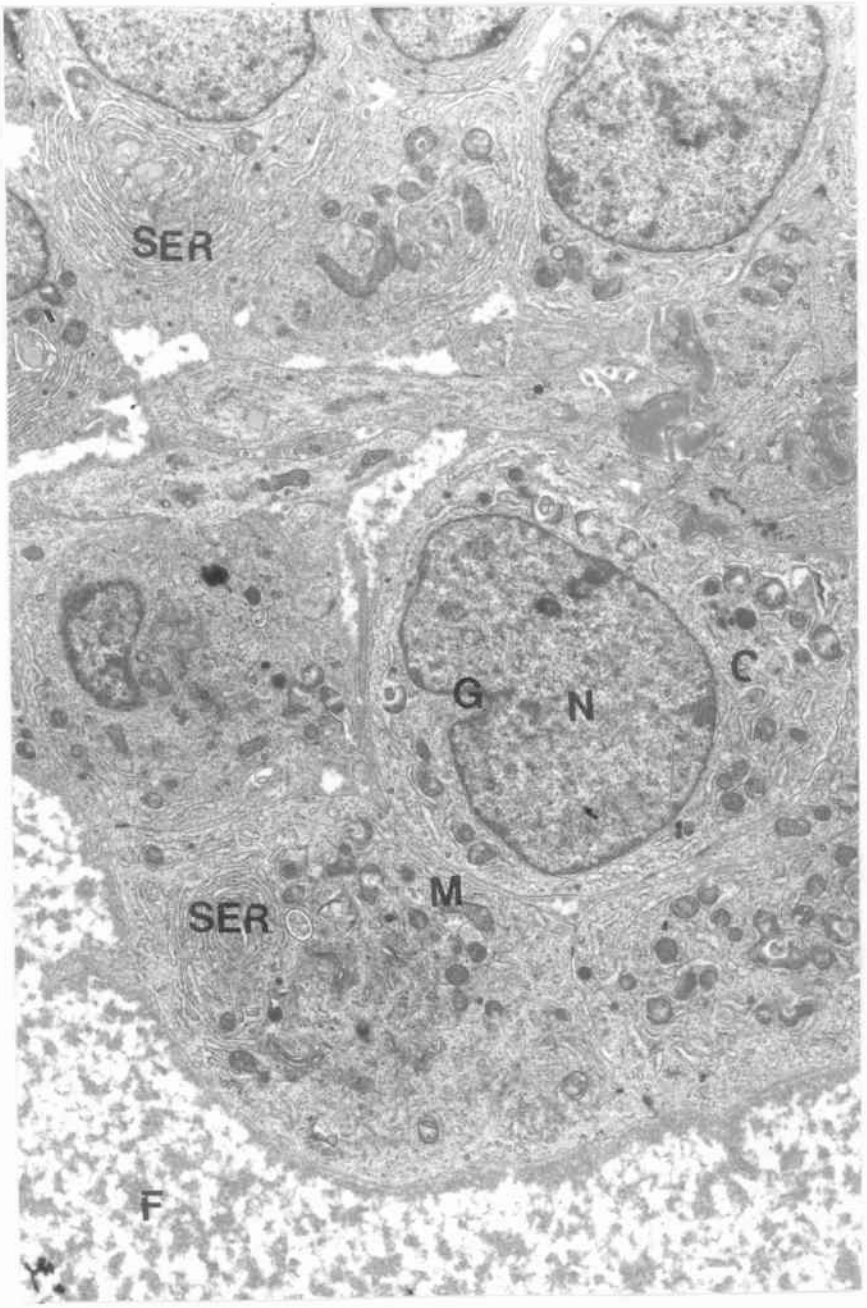


Fig. 7.13 Ovarian autograft.

Transmission electron micrograph showing granulosa cells (G), with evidence of luteinization and steroidogenic activity, granular cytoplasm (C), smooth endoplasmic reticulum (SER), spherical mitochondria (M), nucleus (N), follicular fluid (F).

X 5,250

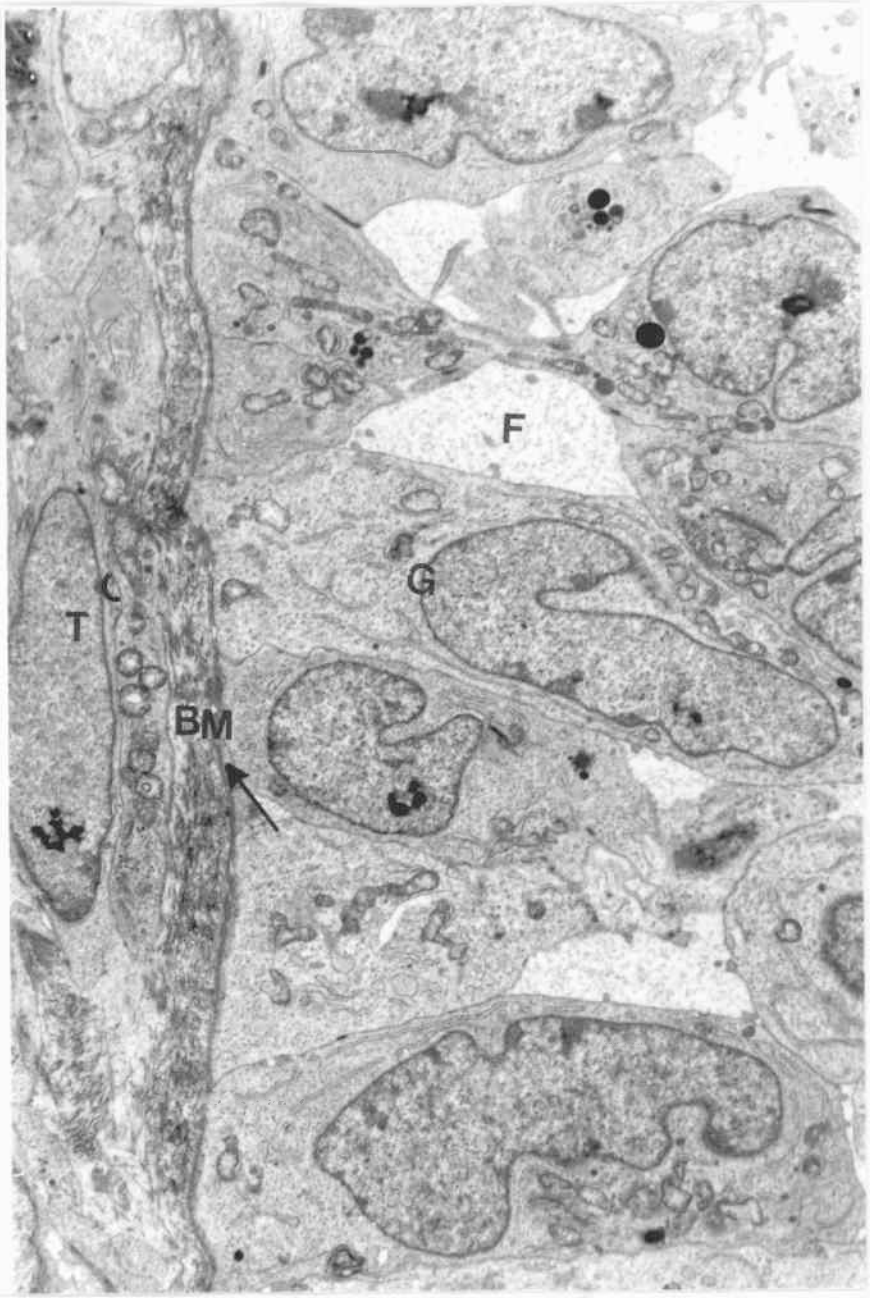


Fig. 7.14 Ovarian autograft.

Transmission electron micrograph showing theca interna cells (T), basement membrane (BM), granulosa cells (G) and follicular fluid (F).

X 2,250

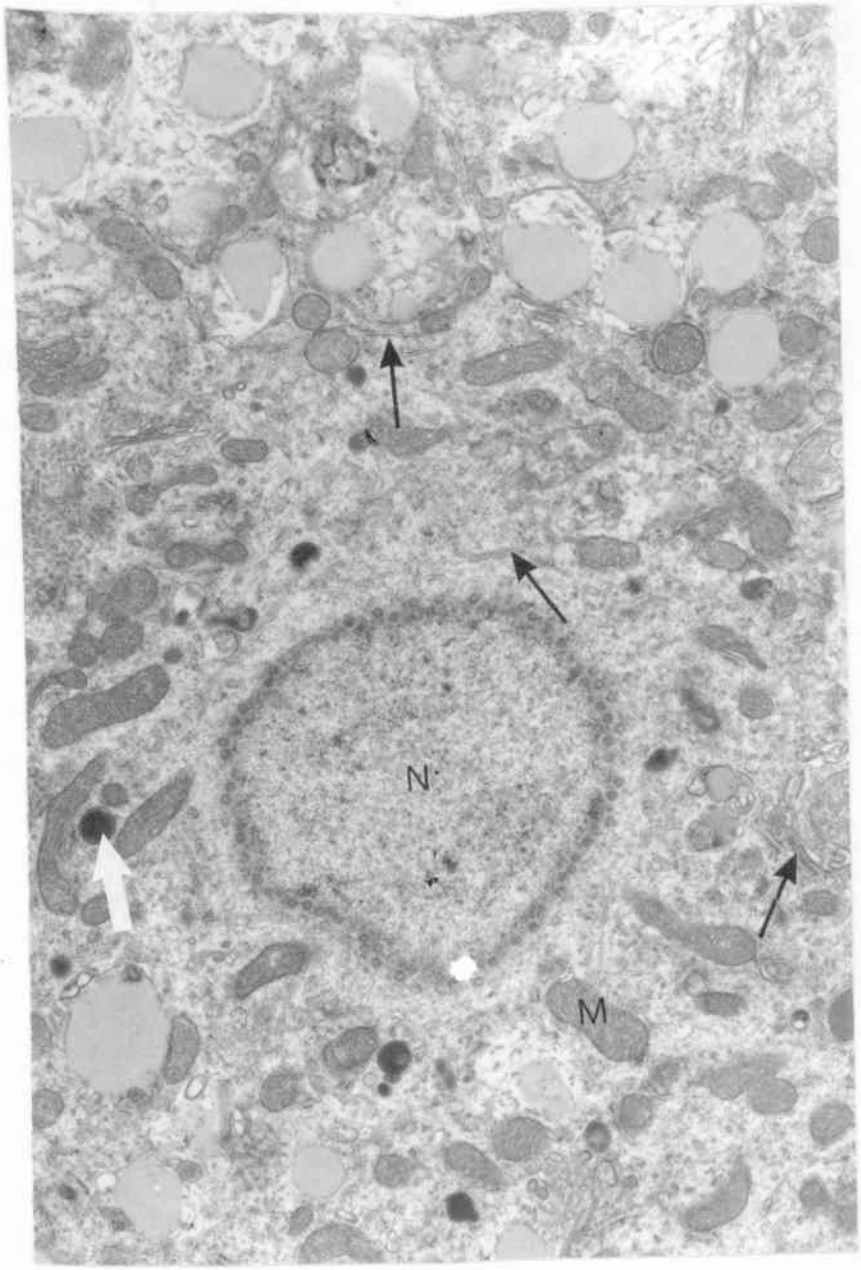


Fig. 7.15 Ovarian autograft.

Transmission electron micrograph showing corpus luteal cell with abundant pleomorphic mitochondria (M), fat droplets (F), Golgi apparatus (G), densely stained granules (believed to be associated with progesterone secretion (white arrow), smooth endoplasmic reticulum (black arrow), nucleus (N)

X 6,300

TABLE 7.8

Comparison of methods used for autotransplantation

Author	Group	Patency				Ovulation	%	Pregnancy	%
		Immediate	%	Late	%				
Winston & Browne, 1974	-	-		3/4	75	-		2/4	50
Al-Chalabi, 1981	A	15/15	100	11/12	91.7	11/12	91.7	8/12	66.7
	B	15/15	100	8/13	61.5	8/13	61.5	7/13	53.8
Denjean et al, 1982	II	-	-	10/19	52.6	5/11	45.5	-	-
	III	-	-	6/13	46	5/8	62.5	-	-
Grimaldi et al, 1982	(2)	-	-	5/10	50	-	-	3/10	30
	(3)	-	-	5/10	50	-	-	1/10	10

Fig. 7.16 Ovarian autotransplantation techniques - (see Fig. for identification of anatomical structures).

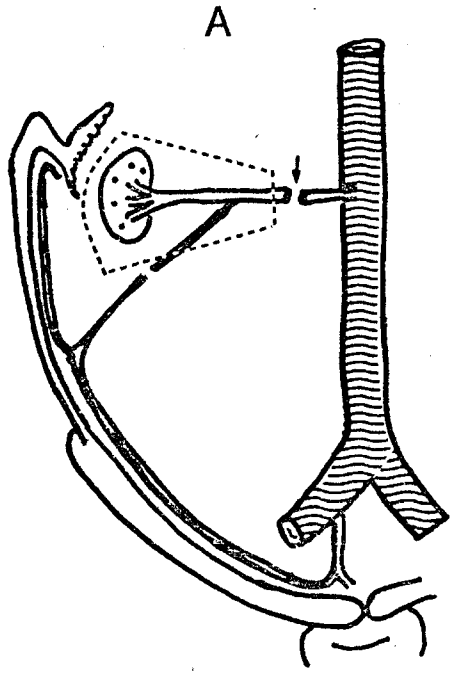
A. Al-Chalabi (1981). Transplantation by anastomosing the ovarian vessels close to their origin (Group A);

B. Winston and Browne (1974), and Grimaldi *et al* (1982). *En bloc* transplantation of ovary and tube, ovarian vessels anastomosed near their origin.

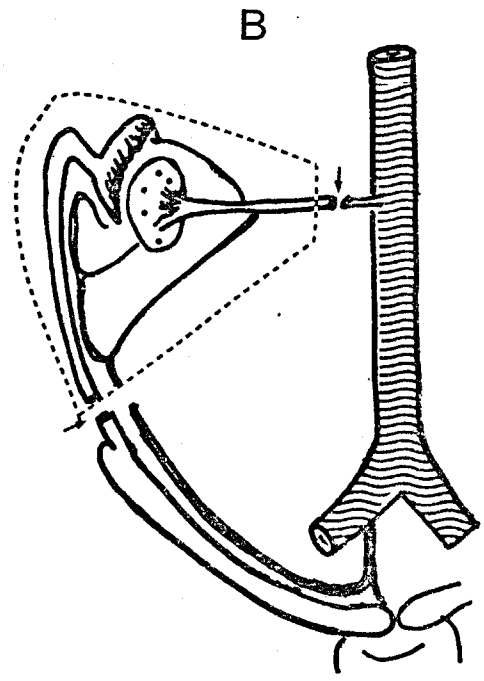
C. Al-Chalabi (Group B) (1981) and Grimaldi *et al* (1982). Transplantation by anastomosing ovarian vessels close to the ovary.

D. Denjean *et al* (1982). Heterotopic transplantation of the ovary. Ovarian vessels were anastomosed to the inferior epigastric vessels.

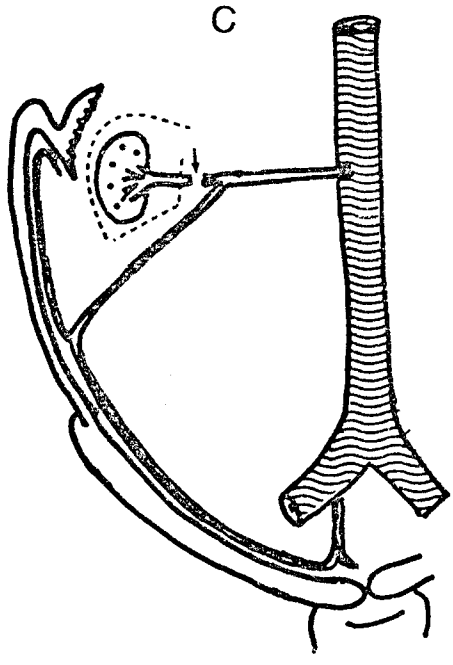
Fig. 7.16



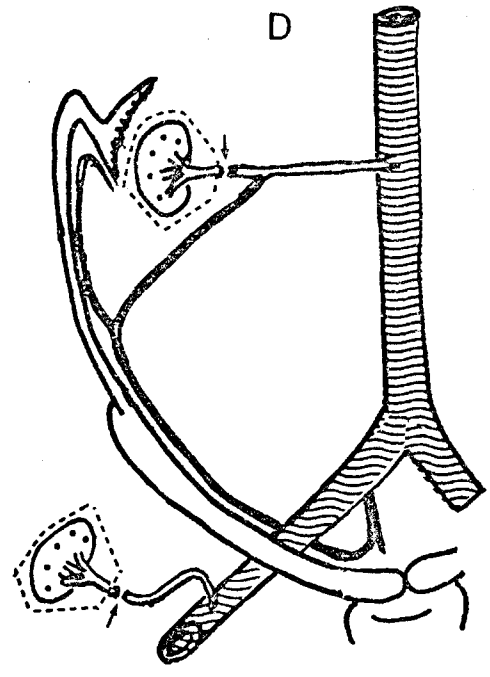
AL-CHALABI/1981.A



WINSTON AND BROWNE / 1974.
GRIMALDI et al/1982



AL-CHALABI / 1981.B
GRIMALDI et al/1982.



DENJEAN et al /1982

Discussion

The present study showed that successful ovarian autografts sustained a severe depression of their function during the first few post-operative days (Fig. 7.2). Function was then gradually recovered, so that by the end of the second post-transplantation week, the grafts regained their full functional potentials and responded normally to mating and achieved successful pregnancies. Light and electron microscopical studies of the grafts at the end of the experiments, demonstrated normal structure. Follow-up assessment of these grafts, after one year, showed that they were capable of repeated pregnancies and indefinite survival.

This study also defined a number of factors which decisively influenced the evolution and fate of the grafts. These factors will be discussed separately.

(1) Transplantation Procedure: The microvascular ovarian autotransplantation technique adopted for this study (1981) proved highly satisfactory in terms of vascular patency, ovulation and pregnancy rates (Tables 7.2 and 7.4). Our technique is compared in Fig. (7.16) and Table (7.8) with other methods of microvascular autotransplantation of the ovary reported so far. Winston and Browne (1974) transplanted en bloc ovaries and adnexa in the rabbit by anastomosing the ovarian vessels near their origin, while Denjean et al (1982) performed heterotopic transplantation (in the groin) in two groups of rabbit by anastomosing the ovarian vessels to the inferior epigastric vessels. In one group, the ovary was enclosed in a peritoneal sac. The aim was

to study the effect of that on adhesion formation. Enclosure of the ovary in a peritoneal sac significantly reduced adhesions formation. Grimaldi et al (1982) autotransplanted ovaries in two groups of rabbit. In one group, the ovarian vessels were anastomosed close to the ovary and in the other group en bloc ovaries and adnexa were transplanted. In the former group (which was similar to Group B of our study) out of ten, longterm vascular patency was achieved in five and pregnancy in three. In the other group, five out of ten achieved longterm patency and pregnancy was achieved in only one rabbit.

(2) The period of warm ischaemia (normothermic anoxia). The graft sustained a state of warm ischaemia during the interval between the ligation of the ovarian vessels and the restoration of the circulation on completion of the anastomosis of the donor's and recipient's vessels. The mean duration of that interval was 56 $\pm$ 7.5 min. The immediate effect of the period of warm ischaemia was on graft function and was manifested (Fig. 7.2) by significant decline in peripheral plasma progesterone concentration compared with the control ($P<0.05$ and $P<0.01$ for Group A and Group B respectively). The mechanism underlying the functional deficit is believed to be a complex one, involving every organelle and subcellular system, and occasioned by the acute deprivation of oxygen and consequent arrest of mitochondrial function and adenosine triphosphate (ATP) synthesis (Trump et al, 1976).

With the restoration of the blood flow, the graft gradually recovered its function and the progesterone level began to rise.

In all the grafts which achieved longterm patency, the effect of the episode of warm ischaemia did not appear to extend beyond a trans-

ient impairment of graft function. Light and electron microscopical examination of these grafts later in the experiment did not reveal any structural alteration. This would not necessarily rule out morphological changes which resolved after re-establishment of the circulation and were not detectable at the time of histological examination. In fact, the development of functional deficit suggests the occurrence of some degree of reversible fatty degeneration (Robbins et al, 1981c).

(3) Vascular patency and blood flow. This was singled out in this study as the most important factor determining the fate of the graft. All the grafts which failed to ovulate or produce corpora (one out of 12 in Group A and five out of 13 in Group B) were those which showed partial or total occlusion of the anastomosis. In fact, histological examination of these grafts beautifully demonstrated the graded effects of ischaemia on the structure of the ovary; for, on the one hand, there was the complete occlusion of the anastomosis with total necrosis and fibrosis of the ovarian tissues (Fig. 7.3), sparing only the surface epithelium which seemed to derive nourishment from adjacent sources, and, on the other hand, there was the partial occlusion and impairment of blood flow, causing varying degrees of ischaemic damage to the elements of the structure of the ovary. The secondary and tertiary follicles were most affected, and the primordial quiescent follicles were least affected. This is not surprising in view of the known relationship between the level of metabolic activity of the cells and their susceptibility to hypoxic injury (French and McFarlane, 1970). In one graft, although histological examination showed many developed follicles (Fig. 7.4B), yet it failed to ovulate or form corpora lutea, thus demonstrating, in a grafting experiment,

the critical role of blood supply in the normal functioning of the ovary, a role which old and recent researches repeatedly pointed to (Westman and Jacobson, 1936; Burr and Davis, 1951; Niswender, 1976).

Only one failure of anastomosis occurred (one out of 12) in Group A (where the ovarian vessels were anastomosed near their origin) while five (five out of 13) occurred in Group B, where the vessels were anastomosed near the ovary, and distal to the origin of the uterovarian branch. The explanation of this finding must lie in the smaller calibre of these vessels in which, as was explained in Experiment III (p. 137), even a slight luminal narrowing can cause drastic reduction in blood flow. This, coupled with surgical manipulation, may precipitate thrombosis (O'Brien, 1977).

(4) Adhesion formation. The incidence and grades of adhesion formation (Table 7.4) were low relative to the extensiveness of the surgery. We relate that mainly to the use of the periovarium fatty peritoneum to cover the raw area at the recipient site and the technique of inverting the adjacent edges of the donor's and recipient's peritoneum during suturing. The importance of raw areas in the formation of post-operative adhesion has already been pointed to by other workers (Winston, 1980). Other factors such as minimization of tissue handling or damage, particularly to the peritoneum (Nisell and Larsson, 1978), the use of anticoagulant (Ellis, 1971) and constant intra-operative irrigation of the tissue with Ringer's solution (Winston, 1981) probably contributed, to some extent, to the lowering of the incidence and severity of the adhesion formation in this study.

Fewer and milder adhesions were encountered on grafts in Group B

(46.2%) compared with (58.3%) in those of Group A, a difference which may be related, in part at least, to the lesser tissue damage and lesser handling in Group B. However, the most interesting feature of these results was that all the grafts without adhesion achieved pregnancy and that the rest of the grafts which achieved pregnancy had only mild (Grade 1) adhesions. Grafts with Grade 2 or 3 adhesions ovulated but did not achieve pregnancy. Furthermore, grafts with adhesions produced less ovulations; the one with the least number of ovulations was almost completely surrounded by adhesions (Fig. 7.5). These results demonstrate the adverse effect of adhesion on the capacity of the graft to ovulate and achieve pregnancy. The findings in this study concerning the effects of adhesion agree with the conclusion of Winston (1980) that post-operative pelvic adhesions involving the ovary can cause significant reduction of ovulation and ovum pick up.

In conclusion: The study showed that functionally and structurally normal ovarian autograft could be achieved in the rabbit using micro-vascular transplantation technique. The grafts were capable of repeated pregnancies and indefinite survival. The experiments highlighted the importance of certain influences to the outcome of the graft.

In the human, there are a number of situations where ovarian auto-grafting may be considered. One such situation is when there is a single tube on one side and a single ovary on the other side; if neither the ovary nor the tube can be mobilised to the opposite side without interfering with the vessels, then transplantation would be indicated (Wood, 1982). In patients who require radiation treatment

for pelvic neoplasm, the ovaries may be relocated away from the radiation field, outside the true pelvis or in the abdominal cavity (Cantor, 1983). In this way, it may be possible to protect the ovaries from the known injurious effects of radiation (Dobson and Felton, 1983).

CHAPTER 8

STUDY OF OVARIAN ALLOTRANSPLANTATION BY MICROVASCULAR TECHNIQUE AND ASSESSMENT OF THE FUNCTION AND THE STRUCTURE OF THE TRANSPLANT, IN THE RABBIT

EXPERIMENT NO. VI

Introduction

Transplant surgeons are presently concerned with those organs essential to maintaining life. With the present advance in surgical techniques and the better understanding of transplant immunology, attention may now be directed to transplantation of organs, the absence or malfunction of which interferes with the fulfilment of normal life. Within this frame of thinking, ovarian transplantation may be approached as a potential method of treating intractable clinical problems such as Turner syndrome, premature ovarian failure, surgical castration and irreparable damage to the ovary by inflammatory or other pelvic diseases.

In recent decades, great strides have been made in the transplantation of reproductive organs; ovaries were either transplanted alone or with other genital organs, using omentopexy or vascular anastomosis, in animals (p.36) or in man (p.39). Ischaemic necrosis, failure of anastomosis and immunological rejection were the main causes of failure in the majority of cases. Improved surgical techniques and use of immunosuppressants improved the results (Green et al, 1982).

The primary objective of this study was to perform allotransplantation

of the ovary by microvascular technique, and assess the structure and function of the graft. The influences of the immunosuppressed and non-immunosuppressed rejection reaction on the survival and function of the graft were also evaluated. For immunosuppression in this study Cyclosporin A was used on the basis of its reported potency, freedom from serious side effects and prolongation of graft survival long after withdrawal of the drug (Morris, 1981; Green et al, 1982).

Materials and methods

(1) Animals: 25 mature virgin New Zealand White female rabbits were used; 10 rabbits were used as donors (in some donors both ovaries had to be used for grafting) and 15 as recipients. The recipients were divided into two groups, A and B:

Group A (non-immunosuppressed group): This group consisted of five recipients which did not receive immunosuppressants.

Group B (immunosuppressed group): This consisted of 10 recipients which received 15 mg of Cyclosporin A (CyA) in olive oil/Kg body weight, by deep intramuscular injection daily for 14 days.

(2) Anaesthesia: Anaesthesia was induced by Hypnorm and maintained by fluothane and oxygen (p. 74). Each of the animals (donors and recipients) received 120 I.U. of Heparin/Kg body weight, subcutaneously prior to laparotomy.

(3) Procedure:

(a) Laparotomy: In both recipients and donors, laparotomy was performed through a midline incision and the reproductive tract exposed (p.75).

(b) Preparation of the graft in the donor: Under the operating microscope, at magnification x 25, the ovarian vessels were identified 1 cm from their origin from the dorsal aorta and inferior vena cava, exposed, isolated from surrounding structures and their subsidiary

branches coagulated. The ovarian artery was then separated from the vein after incising the perivascular sheath. The uterovarian branches were diathermi ed, and the fimbria ovarica was dissociated from the cranial pole of the ovary after cauterising its vascular connections with it. The uterovarian ligament was then divided 2 mm from the ovary. The ovarian mesentery between the cut ends of the fimbria ovarica and the uterovarian ligament was also incised, leaving 3 mm free peritoneum attached along the margin of the ovary. The peri-ovarian fatty peritoneum was incised through a V-shaped incision with the apex of the V towards the already exposed portion of the ovarian vessels, so that the donor ovary lay free, being only attached by the prepared ovarian vascular pedicle. The prepared graft was then covered with moist gauze which was kept constantly soaked by frequent irrigation with Ringer's solution pending transplantation.

(c) Preparation of the recipient. Steps similar to those in the donor were followed in the preparation of the ovarian vascular pedicle of the recipient, so that the ovary lay free, being attached only by the vascular pedicle. Two single Acland clamps were applied to the ovarian artery and vein, which were then severed, leaving a stump of 1 cm. The ovary was then removed and discarded.

(d) Transfer and grafting of the ovary: The donor ovarian vessels were clamped and divided, so that the graft was completely freed. The graft was then transferred to a petri dish where its vasculature was gently but repeatedly flushed with heparinized Ringer's solution, until the effluent became clear, the ovary pale, and the ovarian vessels transparent (Fig. 7.1A). The aim of this step was not only

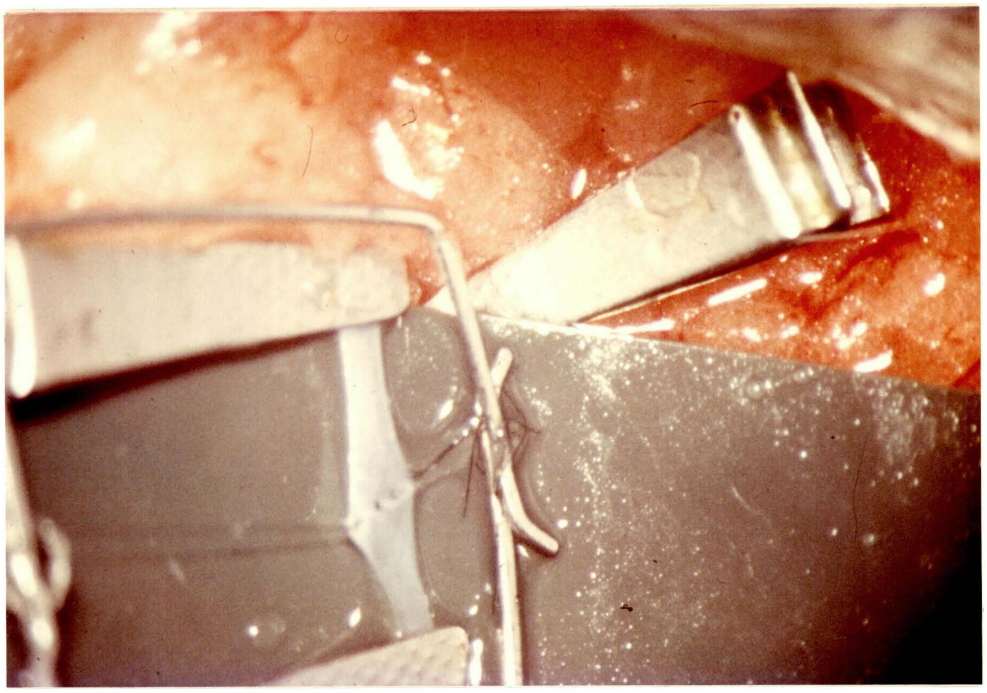


Fig. 8.1 Photograph of microvascular anastomosis, in process (under surgical microscope

X 25

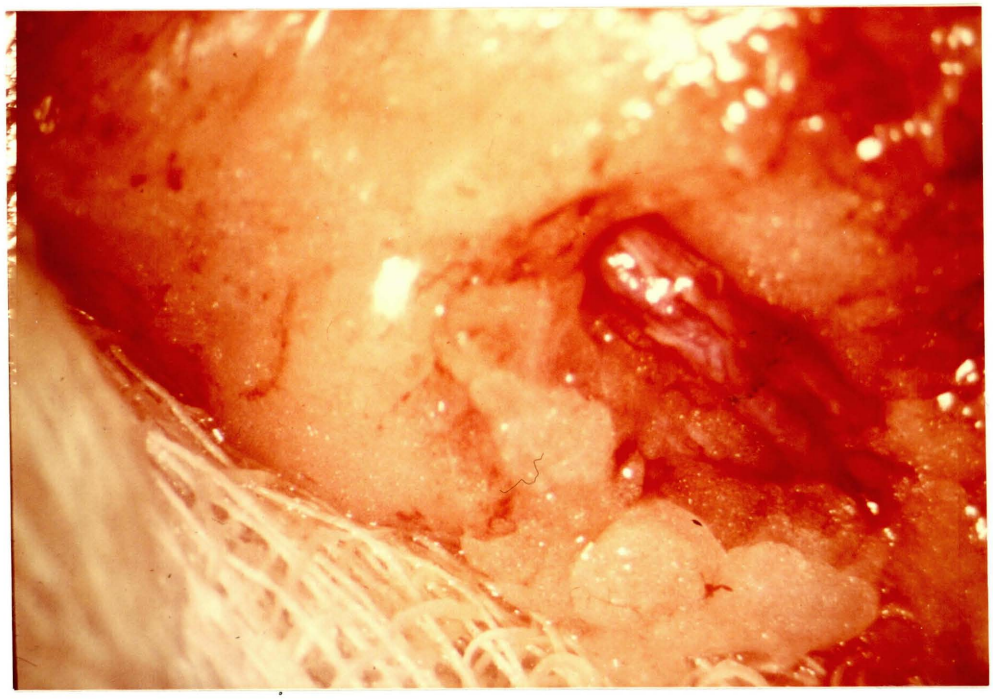


Fig. 8.2 Photograph of microvascular anastomosis, complete (under surgical microscope

X 25

to remove stagnant blood and avoid clotting, but also, and more importantly, to flush out "passenger leucocytes" (leucocytes residing in the graft at the time of transfer) as these leucocytes, particularly lymphocytes, are believed to be an important part of the antigenic stimulus of the graft (Lafferty and Woolnough, 1977). The graft was then transferred to the corresponding side of the recipient and laid in the normal anatomical position previously occupied by the recipient's own ovary, the graft's vascular pedicle facing the recipient vascular stump. One Acland double vascular clamp was applied across the stump of the recipient's and donor's artery, and a second clamp was similarly applied across the recipient's and donor's veins. End-to-end venous anastomosis was then performed, followed by arterial anastomosis (Figs. 8.1,8.2)(p. 75). Patency was checked, clots removed and tissue irrigated. The part of the uterovarian ligament and the part of the mesotuborium superius attached to the graft were sutured to the remnant of the corresponding structures in the recipient using 10/0 nylon sutures. The fimbria ovarica of the recipient was joined to cranial pole of grafted ovary and the free edge of the periovarian fatty peritoneum sutured to the corresponding peritoneum of the recipient, leaving smooth surface facing the viscera.

(e) Removal of remaining ovary of the recipient: The remaining ovary of the recipient was carefully dissected from its surroundings and then removed after its vessels were ligated and divided. Haemostatis was achieved by bipolar coagulation. Clots and debris were washed out from the peritoneal cavity using Ringer's solution, of which 100 mls were left in the peritoneal cavity before the abdomen was

closed (p.75). Animals were kept warm until they recovered; they were then recaged.

(4) The following intra-operative measurements were made:

(a) The external diameter of the ovarian vessels, at the site of the anastomosis, using metal ruler for measurement.

(b) The total duration of the period during which the ovarian circulation was cut off, the period of normothermic anoxia (warm ischaemia).

(5) Post-transplantation graft assessment: (a) All animals in Group A and B were laparotomised one week and two weeks post-transplantation; the gross appearance of the graft (colour and size), presence of follicles and the condition of the genitalia were noted. Evidence of rejection was looked for (colour change, swelling, haemorrhage and necrosis) and the presence and grade of adhesions were also noted. A 1 mm^3 biopsy specimen was taken from each graft by wedge resection for histological examination by light and electron microscopy (p. 83, 84). The site of the biopsy was repaired in each graft using 10/0 nylon sutures, leaving no raw area on the surface of the grafted ovary. (b) At the end of the fourth post-transplantation week, an attempt to mate the animals was made (p.80) and the mating behaviour noted. Two weeks after mating, all the animals (in both groups) were laparotomised to assess the condition of the graft and its response to mating. The appearance of the graft was noted, genitalia inspected for evidence of atrophy (pallor and shrinkage), the number of corpora lutea, gestational sacs, and presence and grade of adhesions recorded. Animals with

rejected ovaries were sacrificed, and their ovaries removed for histological examination. (c) Animals with non-rejected viable ovaries (2 from Group A and 4 from Group B) were re-mated again. Two weeks following the second mating, laparotomy was performed; condition of the graft and genitalia observed, number of corpora lutea and gestational sacs, and the presence and extent of adhesions recorded. (d) Pregnant animals were followed until delivery and their litters weighed and examined for gross congenital anomalies. (e) At the termination of the experiments, animals were sacrificed and their ovaries removed for histological examination.

(6) Hormonal assessment. 2 ml of peripheral blood was collected from the marginal ear vein for peripheral plasma progesterone estimation (p.82). Samples were collected before transplantation, at Day 6 and 12 post-transplantation, on mating day and at 6 days intervals until delivery, in those animals which achieved pregnancy.

(7) Assessment of animal weight. Animal weights were recorded before the beginning of the experiment and at weekly intervals following transplantation for four weeks (which usually corresponded with the mating date).

(8) Statistical analysis. Data for peripheral plasma progesterone levels collected from Group A and B were analysed by repeated measure analysis of variance, differences between pre- and post-operative progesterone level in both groups were analysed by paired t-test. Data for animals' weight collected from Group A and B were analysed by repeated measure analysis of variance.

Results

(1) Operative

(a) All recipients made a full recovery from the operation.

(b) The external diameter of the anastomosed vessels (Table 8.1): There was no significant difference between the mean external diameter of the artery in Group A (0.74 ± S.D. 0.06 mm) and that in Group B (0.7 ± S.D. 0.05 mm), $P < 0.05$, nor was there a difference between the mean external diameter of the vein in Group A (0.93 ± S.D. 0.07 mm) and that of Group B (0.95 ± S.D. 0.04 mm), $P < 0.05$:

(c) Vascular patency rates: The immediate and the one week post-transplantation vascular patency rate was 100% for the two groups.

(d) Total duration of graft normothermic anoxia (warm ischaemia): There was no difference between the mean duration of normothermic anoxia sustained by grafts of Group A (65.7 ± S.D. 8 min) and that of Group B (69 ± S.D. 10.8 min), $P < 0.05$. (Table 8.1).

(2) Post-transplantation graft assessment (Table 8.3)

(a) One week post-transplantation: In Group A (non-immunosuppressed), two grafts showed evidence of early rejection (swelling and change of colour from shiny pink to dull dark red) and three grafts were of normal appearance. Condition of the genitalia of all the animals was normal. In Group B (immunosuppressed), all grafts appeared normal (Fig. 8.7) as was the condition of the genitalia.

Adhesion formation (Table 8.2): In Group A, only two

TABLE 8.1

Comparison of the external diameter of ovarian vessels in mm. and anoxic period in minutes, between Group A (non-immunosuppressed) and Group B (immunosuppressed) during allografting the ovary

Variable	Group	Mean ± S D	P
Ext. diameter o.a.	A	0.74 ± 0.06	N.S.
Ext. diameter o.a.	B	0.7 ± 0.05	0.05
Ext. diameter o.v.	A	0.93 ± 0.07	N.S.
Ext. diameter o.v.	B	0.95 ± 0.04	0.05
Anoxic period	A	65.7 ± 8.0	N.S.
Anoxic period	B	69.0 ± 10.8	0.05

n1 = 5 (number of rabbits in Group A)
n2 = 10 (number of rabbits in Group B)

For Legend: See Table 5.1

TABLE 8.2

Incidence and grade of adhesion at two week inspection following allografting the ovary

Adhesion - Grade	Group A	%	Group B	%
0	3	60	5	50
1	1	20	3	30
2	1	20	1	10
3	0	0	1	10

n1 = 5 (number of animals in non-immunosuppressed Group A)

n2 = 10 (number of animals in immunosuppressed Group B)

TABLE 8.3

Results of post-transplantation assessments of ovarian allografts

Group	No. of recipients	Post transplant adhesion	Number of non-rejected grafts				Results of 1st mating attempt 4 weeks post-transplantation			Results of 2nd mating attempt 10 weeks post-transplantation		
			1st Week	2nd Week	6th Week	14th Week	Mated	Ovulated	Pregnant	Mated	Ovulated	Pregnant
A	5	2	3	2	2	2	2	2	0	2	2	1
B	10	5	10	10	4	4	4	4	1	4	3	1

grafts showed adhesion formation, while in five out of 10 in Group A, adhesions were seen.

(b) Two weeks post-transplantation: In Group A, three grafts were rejecting; the rejection was early in one and advanced (swelling and haemorrhages) in two (the two which showed early rejection in the first post-transplantation week). Two grafts appeared normal and so was the condition of the genitalia. In Group B, all the grafts appeared normal, as did the genitalia.

(3) Results of mating (4 weeks following transplantation)

In Group A, two out of five animals accepted the male and mated (these were the animals with non-rejecting grafts). In Group B, four out of 10 animals mated (these, on later assessments, turned out to be the animals with the non-rejecting grafts).

(4) Two week post-mating graft assessment (Table 8.4)

In Group A, three grafts showed very advanced rejection (swelling, haemorrhage and necrosis). The genitalia of the animals with these grafts were atrophic. The two remaining grafts showed ovulation and corpora lutea formation; only one of these two grafts showed Grade 2 adhesions. In Group B, four grafts appeared normal and so was the condition of the genitalia of the animals with these grafts. All these four grafts ovulated and produced corpora lutea, but only one graft achieved pregnancy. However, the animal did not deliver at the expected date. Exploratory laparotomy in this animal showed degenerated products of conception (Fig. 8.10). Of the other three viable non-rejecting grafts which did not achieve pregnancy, one showed Grade 1

TABLE 8.4

Ovulatory function and pregnancy rate of allografted ovaries

Group	No. of allograft	Mated	Ovulation %	Pregnancy %
A	5	2	2 (40%)	1† (20%)
B	10	4	4 (40%)	1* (10%)

Group A = 5 animals (non-immunosuppressed allograft)

Group B = 10 animals (immunosuppressed allograft)

* Animal in Group B got pregnant twice; first pregnancy was unsuccessful while the second pregnancy was successful.

† Animals in Group A got pregnant on the second mating.

Note: Mating was attempted in all the animals of both groups.

adhesions, another showed Grade 2 adhesions and a third was free of adhesions. The remaining six grafts of Group B were swollen, red to grey or white in colour, and had a curious rather hard leathery feel on texture (Figs. 8.8). The genitalia of these six animals showed evidence of atrophy (pale and shrunken).

(5) Results of the second mating of the animals with the viable non-rejecting allografts

In Group A, one animal achieved pregnancy and delivered four normal litters. In this animal there were no adhesions. The other animal ovulated, formed corpora lutea, showed adhesions (Grade 2) and did not achieve pregnancy. In Group B, only one animal achieved pregnancy (the same animal which achieved an unsuccessful pregnancy by the first mating) and delivered two normal litters. This animal did not show adhesions formation. In the other three viable non-rejecting allografts in this group, one showed few fine mottlings and did not ovulate, and the other two ovulated, formed corpora lutea and were surrounded by adhesions (Fig. 8.19).

(6) Comparing the number of the surviving grafts in the two groups, it can be seen that at the sixth post-transplantation week assessment, two out of five grafts in the non-immunosuppressed group were viable, and four out of 10 grafts in the immunosuppressed group were viable.

(7) Comparing the results of mating (at the fourth week post-transplantation) in the two groups, it can be seen that two out of

five animals in the non-immunosuppressed group mated and four out of 10 in the immunosuppressed group mated. It appears that the number of viable grafts and the number of animals which mated were the same in each group.

(8) Hormonal assessment (Table 8.5)

All animals showed decline in the peripheral plasma progesterone level compared with the pre-operation level ($P < 0.01$). The level of progesterone, however, began to rise after the sixth post-operative day.

Both the declining progesterone levels, during the first six post-operative days and the recovering (rising) levels after that tended to be higher in the non-immunosuppressed group than in the immunosuppressed group, but the difference did not reach statistical significance.

(9) Comparing the pregnant animals in both groups, one can see (Fig. 8.3) that the decline in peripheral plasma progesterone levels in the immediate post-operative interval was less and the rise after that and during pregnancy, more in the non-immunosuppressed than in the immunosuppressed animals. Also the number of corpora lutea and the number of litters (five and four respectively) in the non-immunosuppressed animal were higher than those in the immunosuppressed animal (three and two respectively).

(10) Results of body weight assessment

It can be seen (Table 8.6 and Fig. 8.4) that animals of both groups showed loss of weight in the immediate post-operative period. Body

TABLE 8.5

Mean peripheral plasma progesterone level in ng/ml + S.D. following allografting the ovary in both non-immunosuppressed and immunosuppressed groups

Group	Base Value	Post operative days				F	df	P
		6	12	24	30*			
Group A	1.6 ± 0.2	0.3 ± 0.06	0.5 ± 0.2	0.5 ± 0.5	0.86 ± 1	1.40	1,13	N.S. 0.05
Group B	1.59 ± 0.1	0.14 ± 0.04	0.3 ± 0.1	0.3 ± 0.28	0.63 ± 0.6			

* Animals of both groups were mated on the 28th post-operative day.

n1 = 5 (number of animals in Group A)

n2 = 10 (number of animals in Group B)

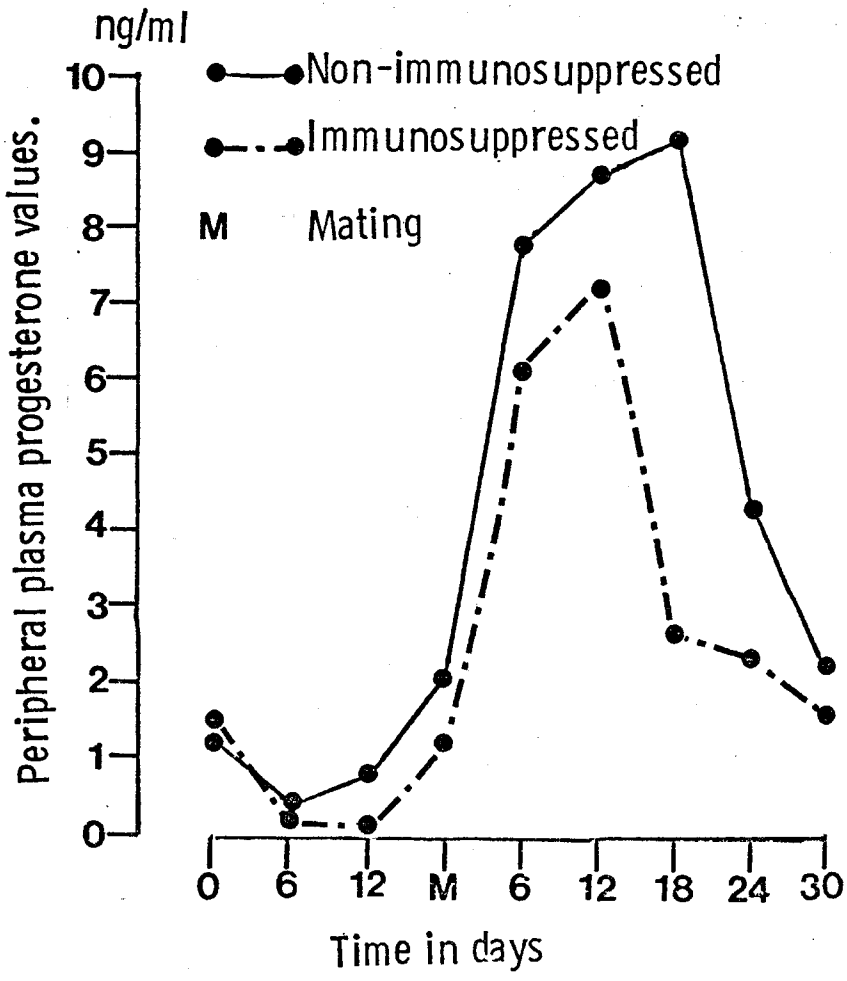


Fig.8.3

Combined graphic representation showing peripheral plasma progesterone values in a non-immunosuppressed and in a CyA-immunosuppressed ovarian allograft rabbits in the postoperative period and during pregnancy.

TABLE 8.6

Mean body weight in Kilograms + S.D. in non-immunosuppressed compared with immunosuppressed allograft

Group	Base Value	Weeks post grafting				F	df	P
		One week	2 weeks	3 weeks	4 weeks			
Non-immunosuppressed	4.5 ± 0.5	4.08 ± 0.6	4.1 ± 0.6	4.3 ± 0.5	4.6 ± 0.5	11.73	1,13	S. 0.01
Immunosuppressed	4.6 ± 0.6	3.5 ± 0.4	2.9 ± 0.4	3.2 ± 0.6	3.6 ± 0.6			

n1 = 5 "Non-immunosuppressed allografts"

n2 = 10 "Immunosuppressed allografts"

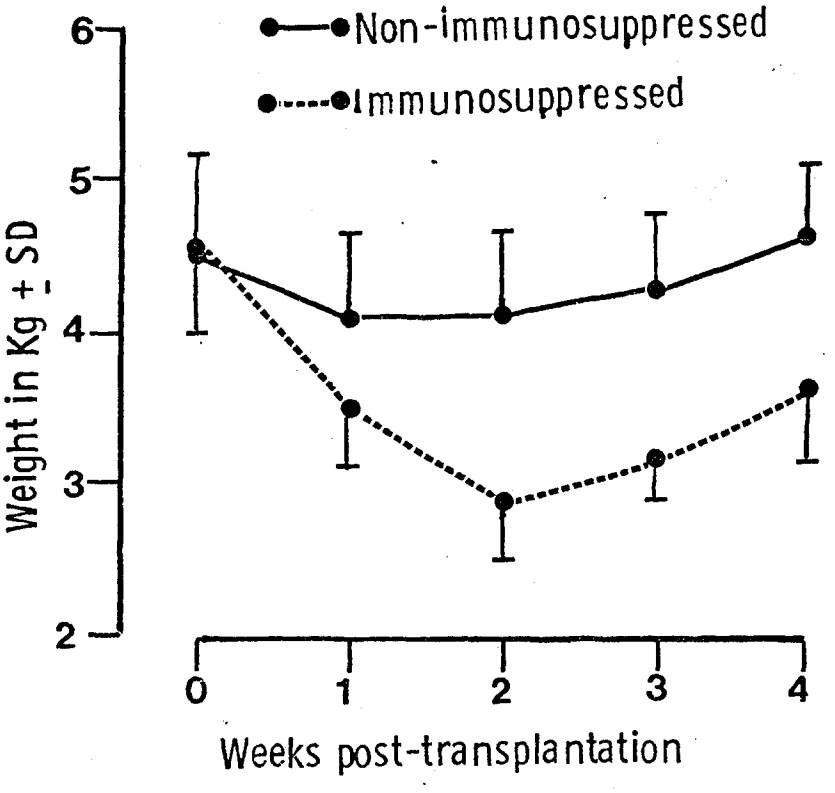


Fig. 8.4

Graphic representation of weight loss in Kg in the immunosuppressed and non-immunosuppressed groups.

weight rose again afterwards to the pre-operative level in non-immunosuppressed group, while it failed to do so in the immunosuppressed group. The immunosuppressed group showed significant decline ($P<0.01$) in weight compared with the non-immunosuppressed group, although some gain in weight was observed in the immunosuppressed group after cessation of CyA treatment.

(11) Results of histological studies

A. Light microscopical examination

(1) The non-immunosuppressed group

The rejected graft showed cellular infiltration typical of vascular rejection; mononuclear cells, lymphocytes and occasionally neutrophil infiltrates were seen invading the follicles (beginning at the periphery) and causing degeneration and necrosis of all the elements of the follicles (Fig.8.6) and of the ovary.

In the surviving graft, lymphocytic infiltration was seen in sections from wedge biopsy specimens taken one week post-transplantation (Fig.8.5). The lymphocytic infiltration resolved later. The blood vessels were dilated and congested but no haemorrhage or extravasation were seen and the different elements of the ovarian structure were remarkably preserved, and remained so till the end of the experiment (Fig. 8.12).Periovarian adhesions were seen in the surviving graft which did not achieve pregnancy.

(2) Immunosuppressed group

(a) Although the macroscopical appearance of the grafts was normal during the two weeks of CyA treatment (Fig.8.7), microscopical examination showed thickening of the tunica albuginea and degeneration of oocytes and follicular cells of some of the primary follicles. In the secondary follicles, the follicular cells were tall and cylindrical with the nuclei lying against the basement membrane. The tertiary follicles looked normal. There was thickening in the arterial wall, involving mainly the media. No sign of cellular infiltration was seen (Fig. 8.9).

(b) The histological appearance of the grafts four weeks after cessation of CyA administration showed degeneration and destruction of the follicular cells and oocytes of the primary follicles, mononuclear cell infiltration (Fig. 8.14) and blood vessel thickening affecting mainly the media (Fig. 8.15). Few Graafian follicles could be seen.

(c) Examination of the viable grafts at the termination of the experiment revealed

(i) One graft showed corpora lutea, normal primary and mature follicles; some of the follicles showed degeneration changes, particularly in the oocytes. There was thickening of the tunica albuginea and of the theca, and blood vessel wall. This was the graft which achieved pregnancy (Figs. 8.13).

(ii) Another graft (Fig. 8.18) showed multiple variable-sized cysts containing blood "chocolate cysts". The wall of these cysts varied in thickness from thin fibrotic covered by one or two rows of flattened

follicular cells to completely fibrotic wall lined by more or less squamous-like epithelial elements. Graafian follicles of normal morphological appearance were occasionally seen. The subgerminal capsule, tunica albuginea, was thick.

(iii) The other two surviving grafts showed extensive adhesions with new blood vessel formation, marked thickening of the theca (Fig.8.19) thickening of blood vessel wall, degenerative changes in follicles and oocytes, and an increase in the fibrous tissue elements of the stroma.(Figs. 8.16, 8.17).

B. Transmission electron microscopy

This identified the type of cellular infiltrate and the histological type of rejection.

(1) In the non-immunosuppressed group, first and second week biopsy specimens from the graft showed that the predominant type of cell in the cellular infiltrate was the lymphoblast (Fig.8.21), characterised by loosely packed euchromatin nucleus and large cytoplasmic volume containing many organelles, One or two plasma cells were occasionally seen. They were characterised by extensive development and dilatation of granular endoplasmic reticulum. There were many circulating lymphocytes (T-cells) (Fig. 8.20), with their characteristic heterochromatic nucleus and a plasma membrane which shows small projections (pseudo podia) which probably serve for cell attachment to the surface and for cell-to-cell contact.

Specimens examined six weeks post-transplantation showed the appearance of more plasma cells and neutrophils (Fig. 8.22) in addition

to lymphocytes. The neutrophil characteristically showed lobulated nucleus and distinctly granular cytoplasm. The general picture is that of a terminal stage of acute rejection with extensive tissue necrosis, which attracted neutrophils.

(2) In the immunosuppressed group: specimens taken during CyA treatment showed no cellular infiltration and normal structure (Fig. 8.23). Specimens taken six weeks after transplantation (i.e. four weeks after stopping CyA) showed extensive destruction of tissue elements, fibrosis and some lymphocytic infiltration (Fig. 8.24). The surviving ovaries showed no cellular infiltration (Fig. 8.25).



Fig. 8.5 Photomicrograph of a biopsy specimen of non-immunosuppressed ovarian allograft one week post-transplantation showing lymphocytic infiltration.

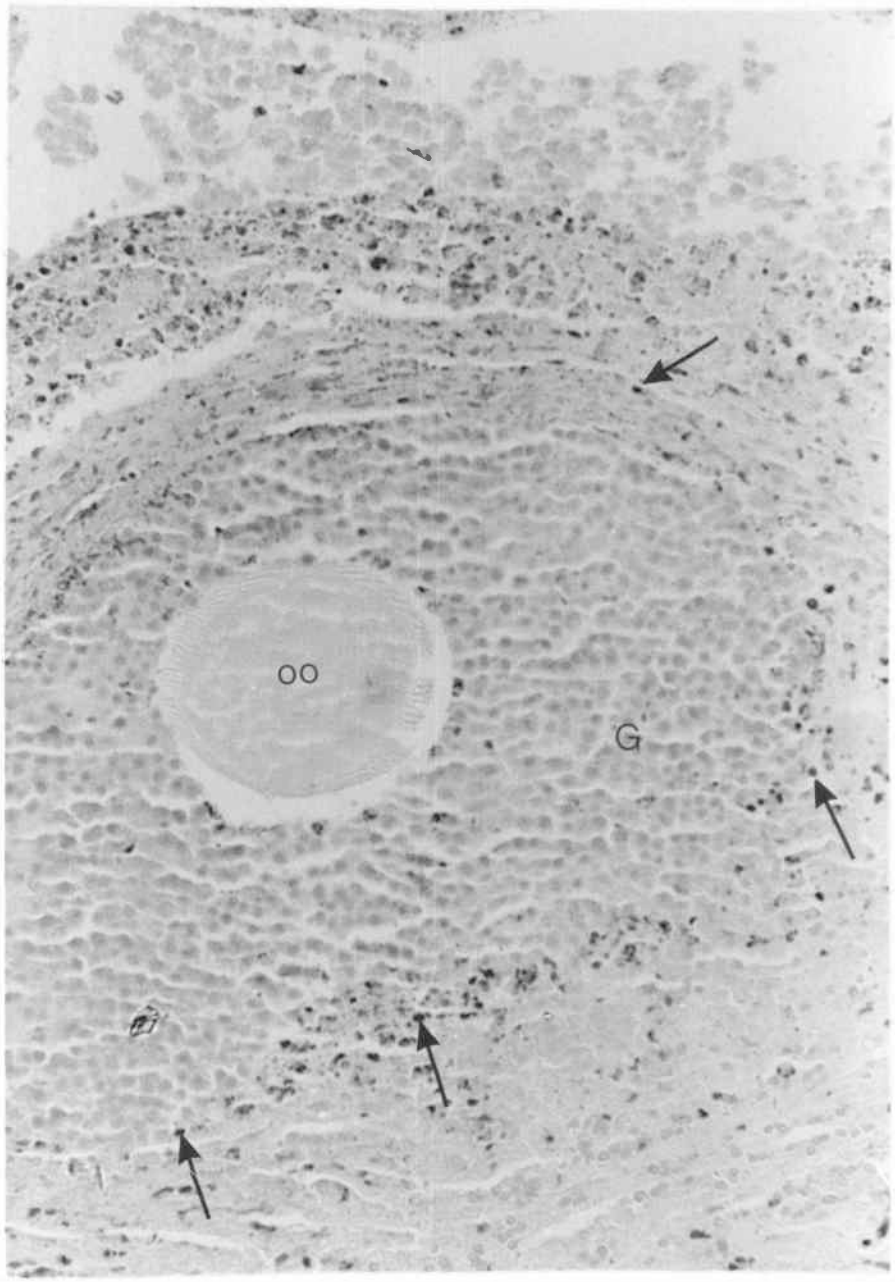


Fig. 8.6 Photomicrograph of a section obtained by biopsy from non-immunosuppressed ovarian allograft two weeks post-transplantation showing advanced rejection, with lymphocytic infiltration (arrows) mostly at the periphery of the follicle, granulosa cells (G) and oocytes (oo) showing necrotic changes.

X 20

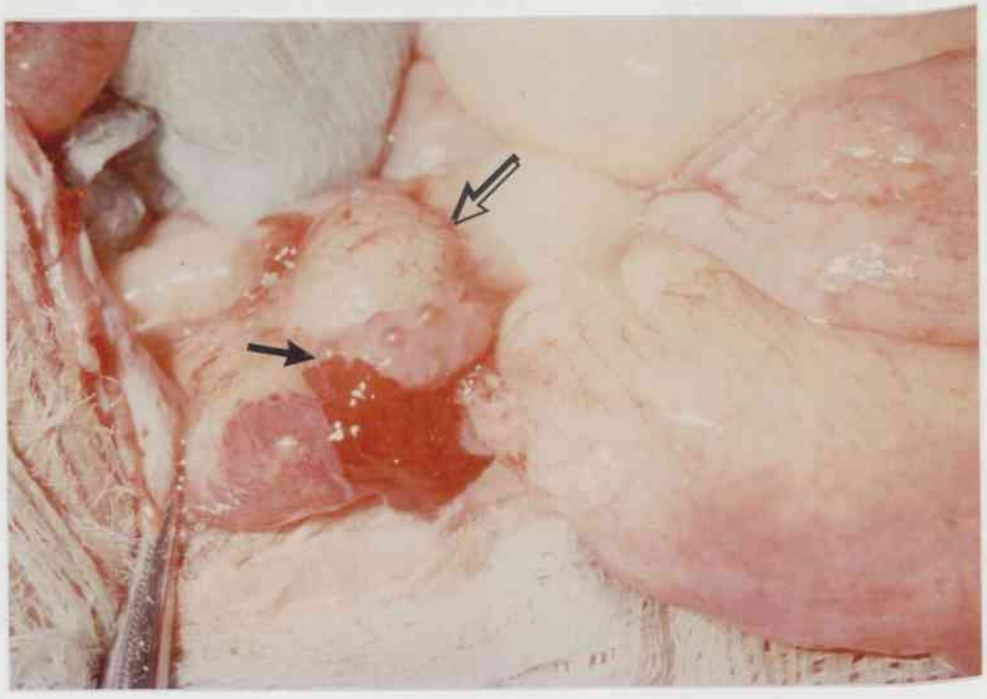


Fig. 8.7 Photograph of ovarian allograft (under the operating microscope) after one week of CyA treatment, showing normal appearance. Ovarian allograft with follicles (black arrow), fimbria, suture line between grafted and recipient peritoneum (hollow arrow)



Fig. 8.8 Photograph of ovarian allograft (under the surgical microscope x 10), showing early rejection, 4 weeks after stopping CyA. Whitish/grey coloured surface with network of blood vessels. It felt hard and leathery.

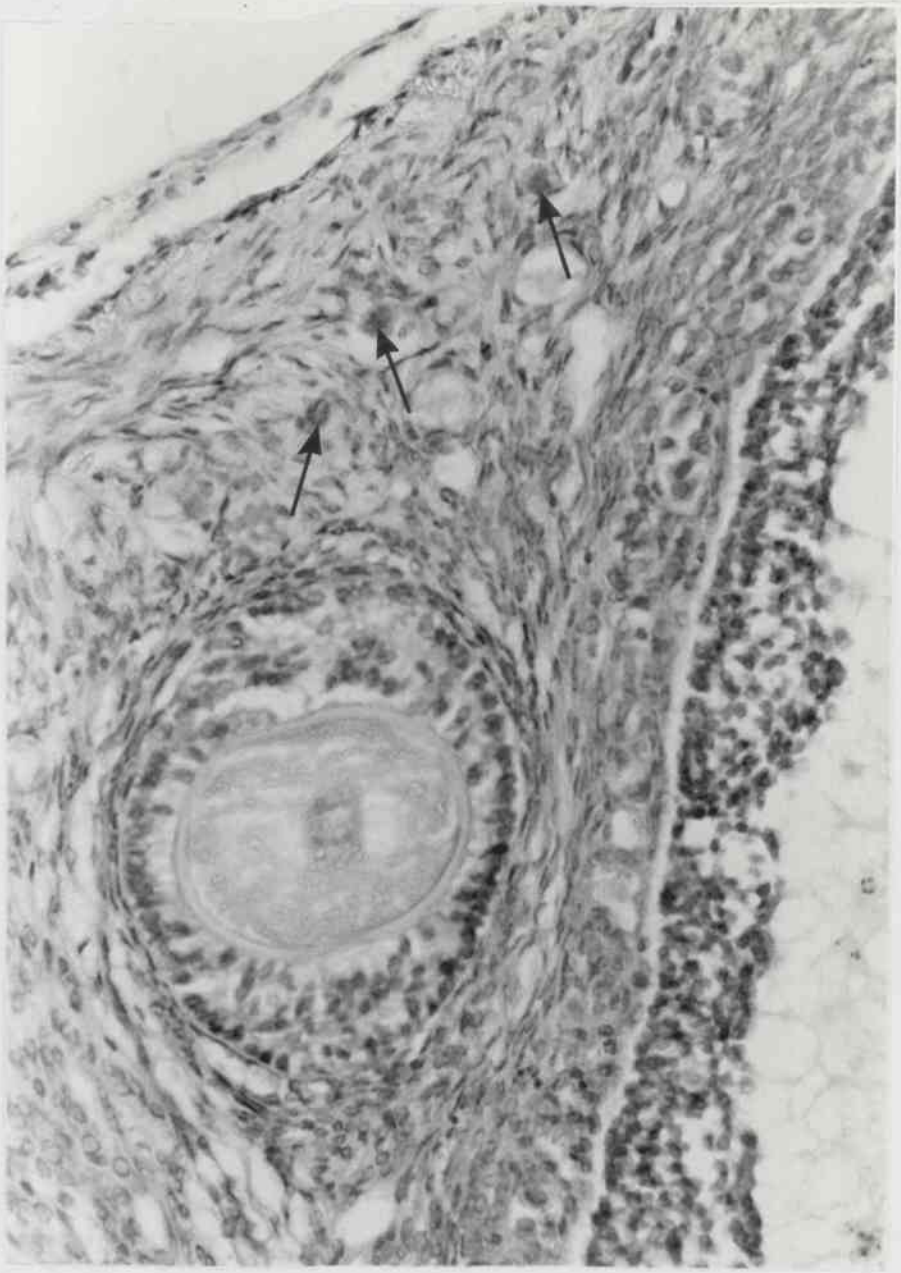


Fig. 8.9 Photomicrograph of a biopsy specimen from immunosuppressed ovarian allograft two weeks post-transplantation showing some degenerated primary follicles (with their oocytes) (arrows). Secondary follicle shows tall cylindrical granulosa cells with basal nuclei. No lymphocytic infiltration. There is increased fibrous element.

X 20

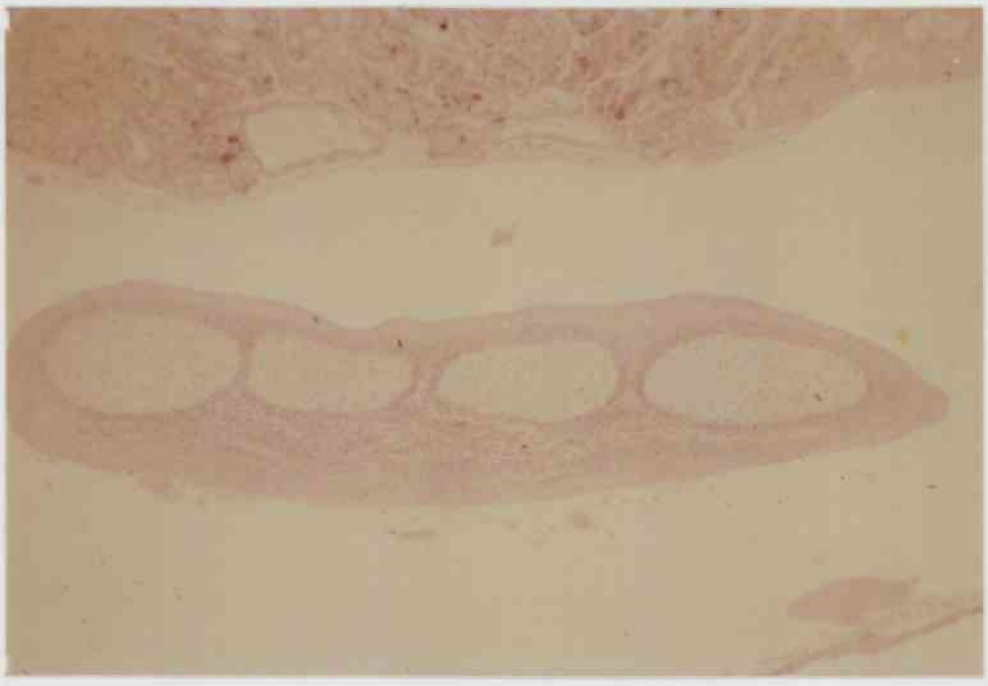


Fig. 8.10 Photomicrograph of undelivered product of conception (removed by hysterotomy) of the first pregnancy of a CyA-immuno-suppressed rabbit with ovarian allograft, showing part of the back bone

H & E X 10

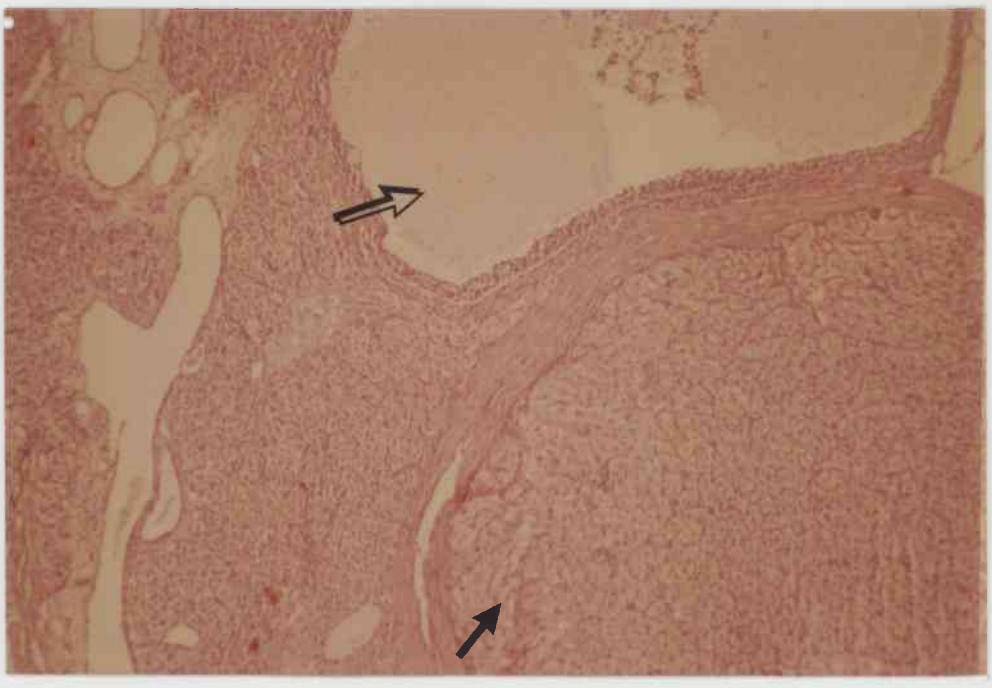


Fig. 8.11 Photomicrograph of a section of viable functioning ovarian non-immunosuppressed allograft showing normal corpus luteum (black arrow) and mature follicles (hollow arrow)

H & E X 10

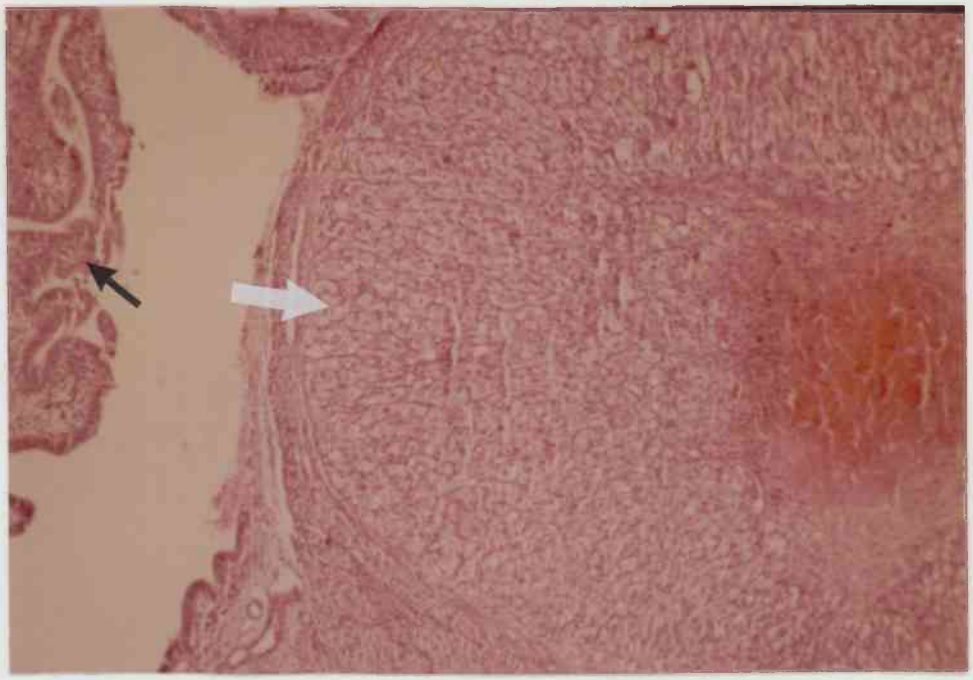


Fig. 8.12 Photomicrograph of a section of non-immunosuppressed ovarian allograft (which achieved pregnancy) showing normal corpus luteum (white arrow) and a part of the fimbria ovarica (black arrow)

X 4

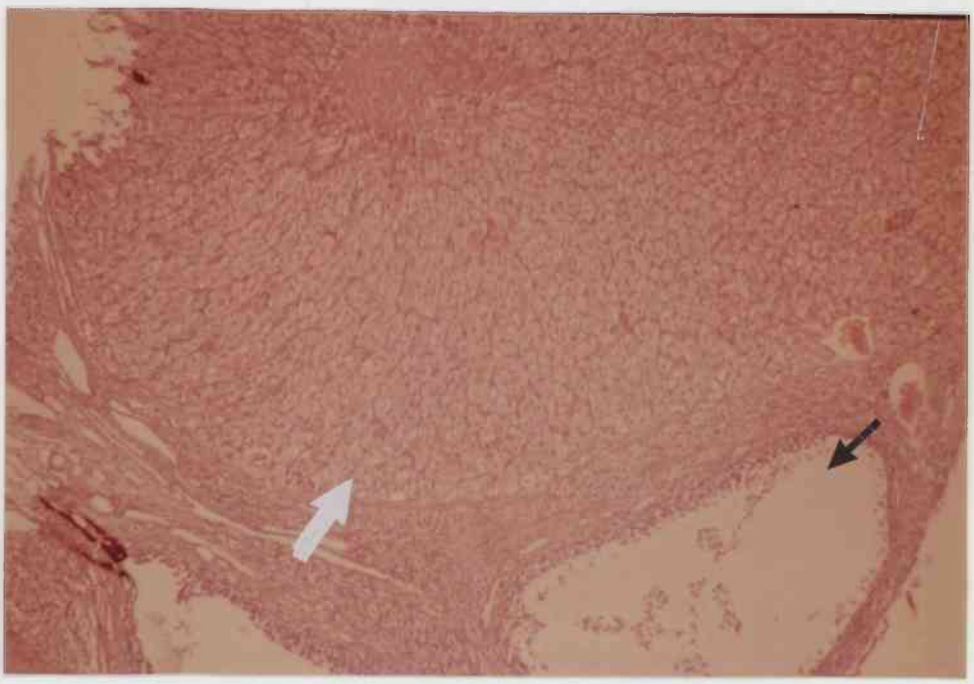


Fig. 8.13 Photomicrograph of a section of immunosuppressed ovarian allograft (which achieved pregnancy), 14 weeks after stopping CyA treatment, showing normal corpus luteum (white arrow) and normal mature follicle (black arrow)

X 4

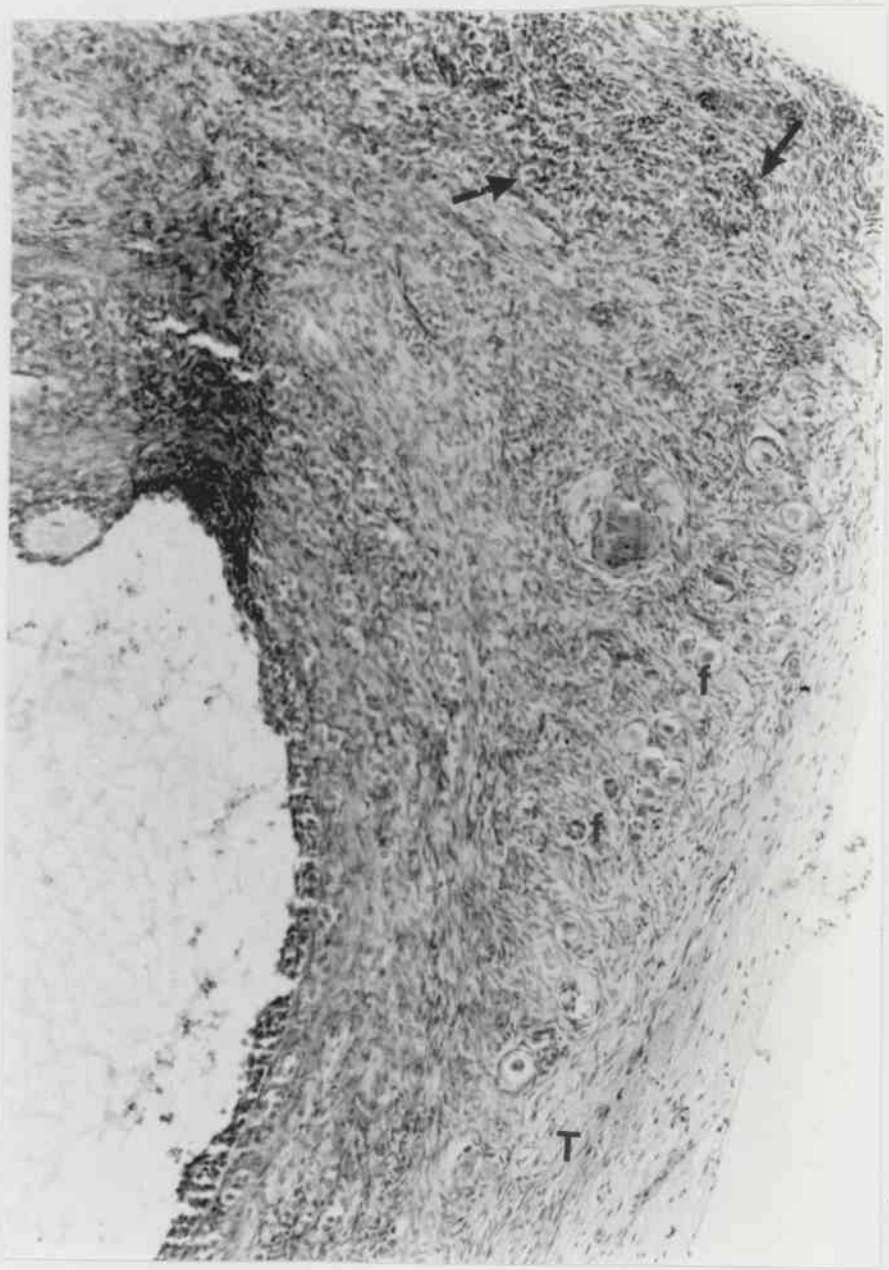


Fig. 8.14 Photomicrograph of a section of immunosuppressed ovarian allograft (4 weeks after cessation of CyA treatment) showing degeneration of primary follicles and their oocytes (f), lymphocytic infiltration (arrows) and excessive fibrous thickening of the tunica albuginea (T)

X 10

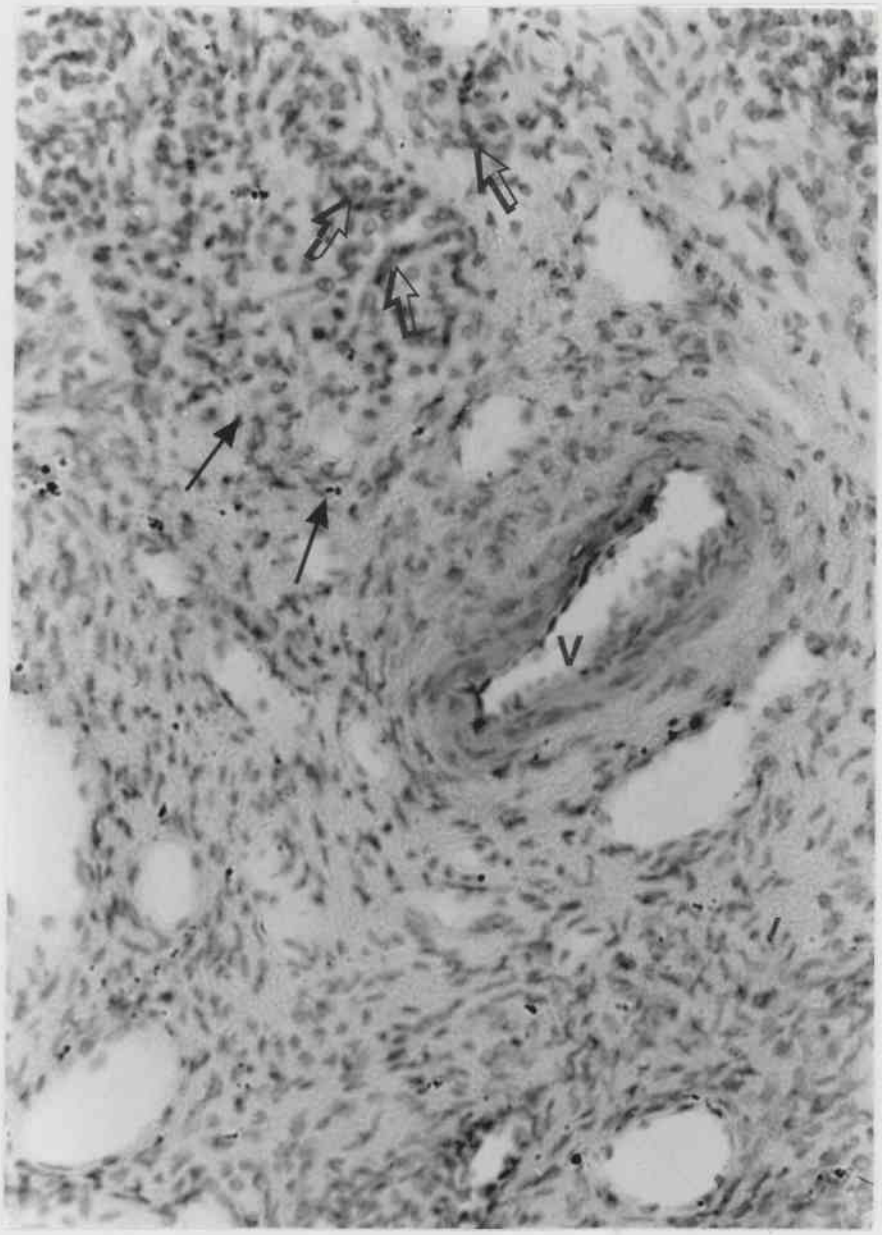


Fig. 8.15 Photomicrograph of a section of immunosuppressed ovarian allograft taken 4 weeks after cessation of CyA treatment, showing lymphocytic infiltration (arrows), vessel wall thickening (v) and biopsy suture surrounded by foreign body tissue reaction (hollow arrow)

X 20

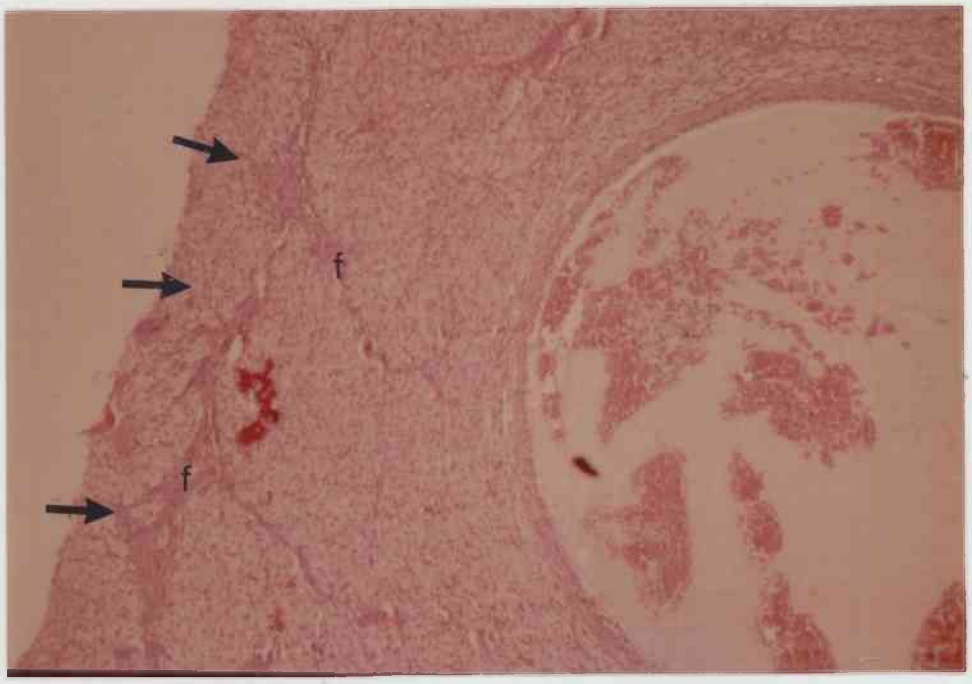


Fig. 8.16 Photomicrograph of immunosuppressed ovarian allograft 10 weeks post-stopping CyA treatment, showing thick tunica albuginea (black arrow), fibrosed parenchyma (f) and thin-walled (follicular or luteal) cyst filled with blood

X 4

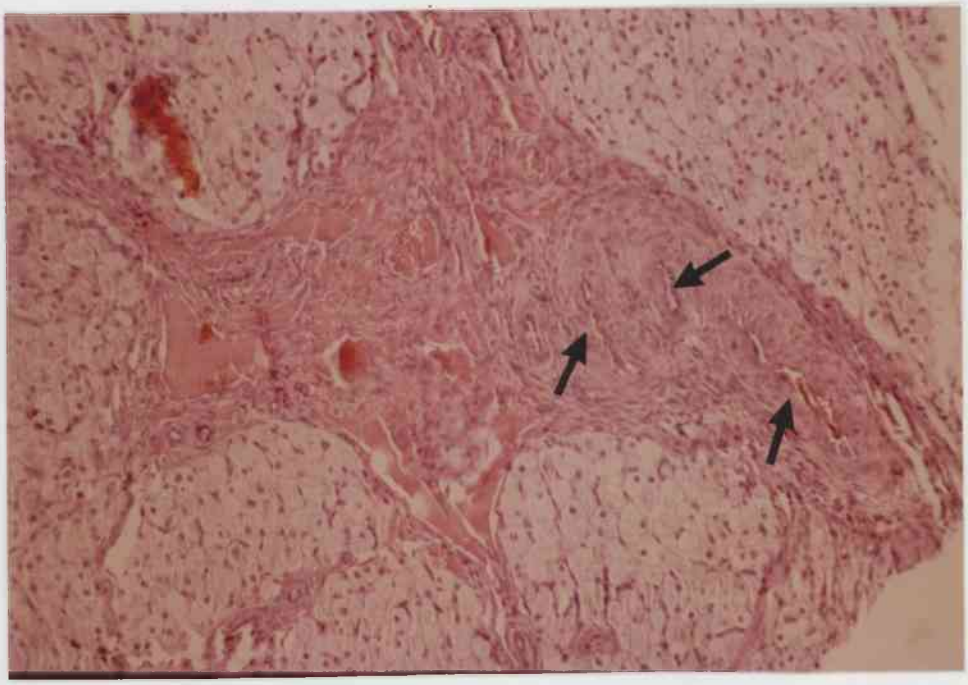


Fig. 8.17 Photomicrograph of immunosuppressed ovarian allograft 10 weeks post-stopping CyA treatment, showing excessive thickening of blood vessel's wall, specially the media (arrows)

X 10

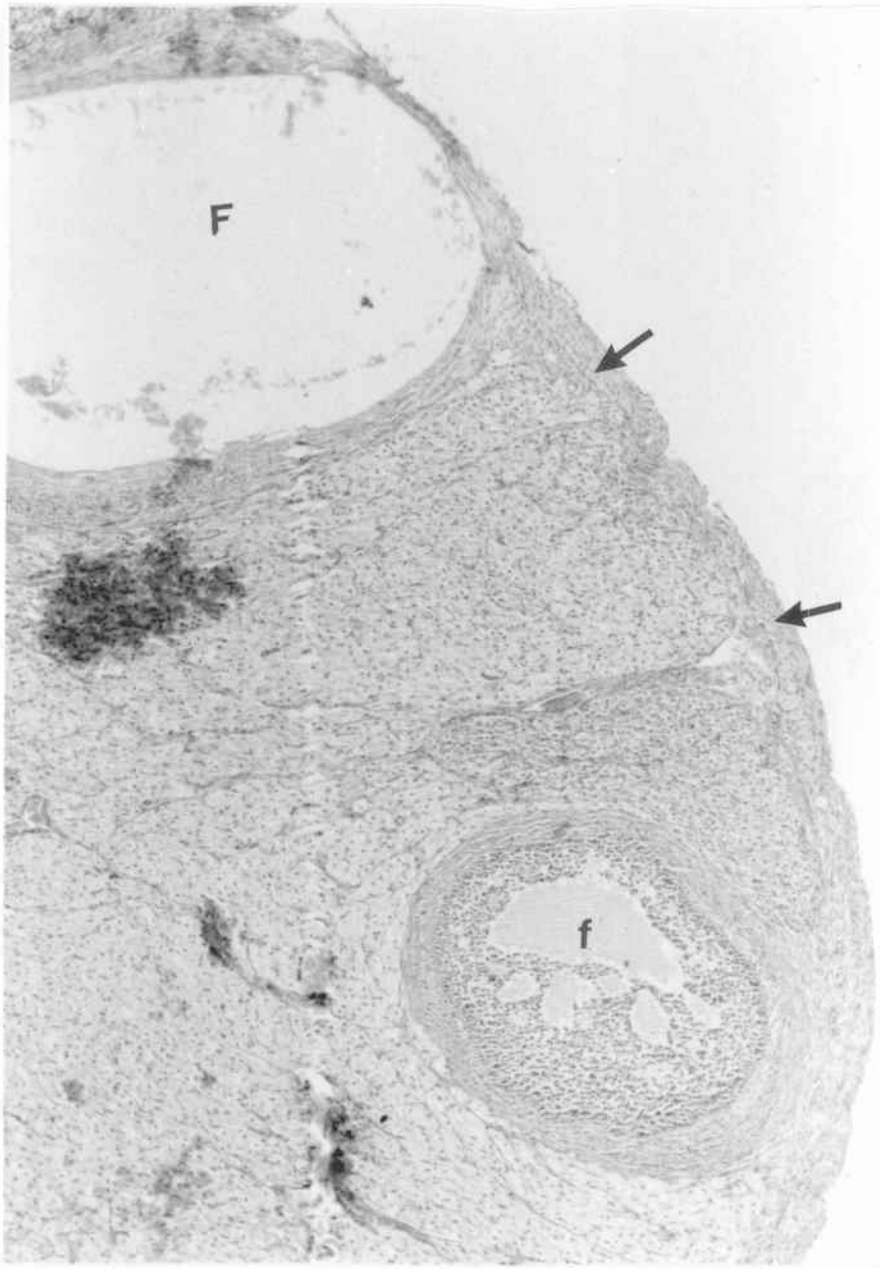


Fig. 8.18 Photomicrograph of a section from ovarian allograft (10 weeks after stopping CyA treatment), showing dilated cystic follicles (F) containing blood (chocolate cysts) with thin fibrotic wall, tertiary follicle of normal morphological appearance (f), thickening and fibrosis of tunica albuginea (arrows)

X 4

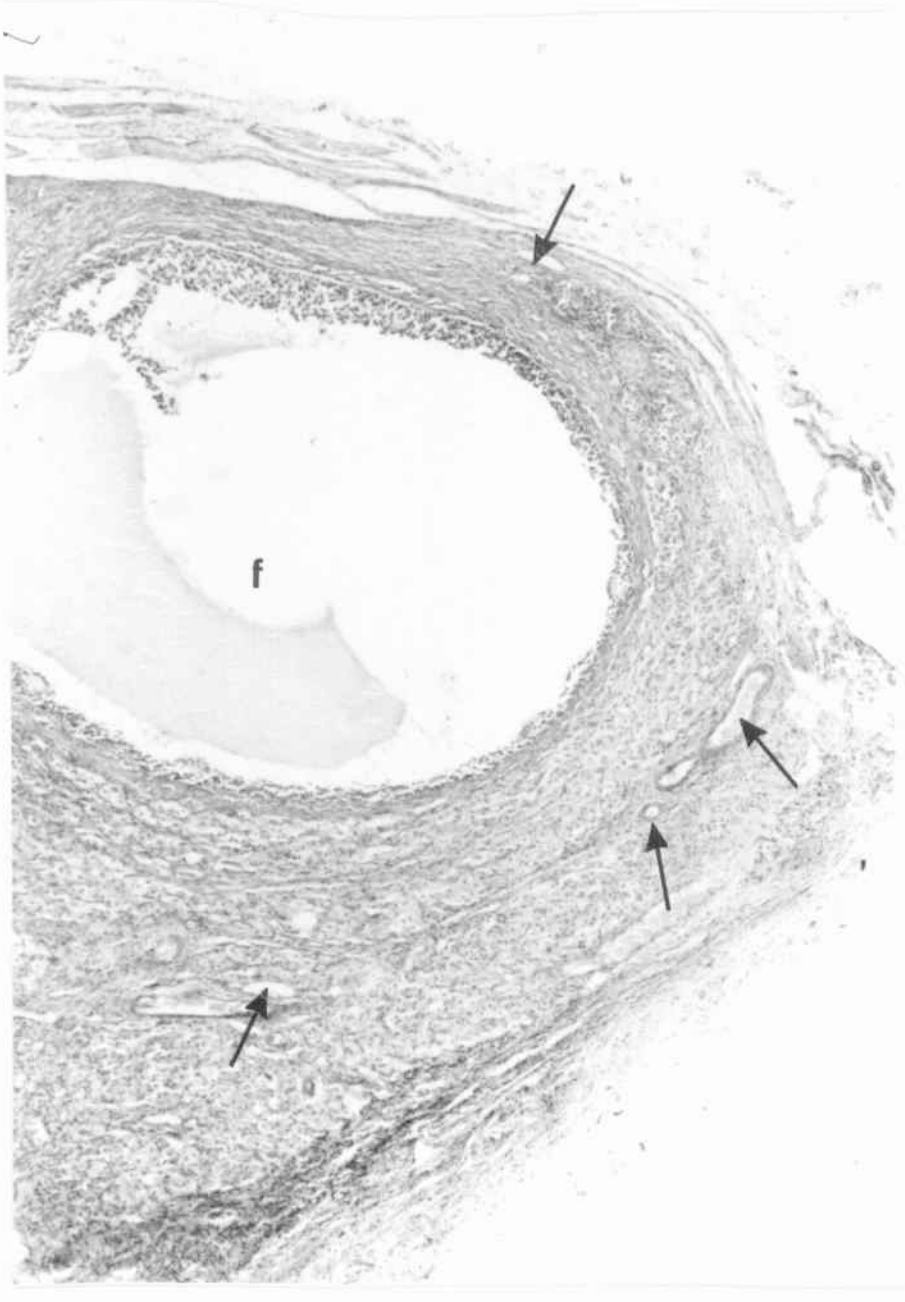


Fig. 8.19 Photomicrograph of immunosuppressed ovarian allograft, 10 weeks after stopping CyA treatment, showing bands of periovarian adhesions with new blood vessel formation (black arrows), normal mature follicle (f).

X 4

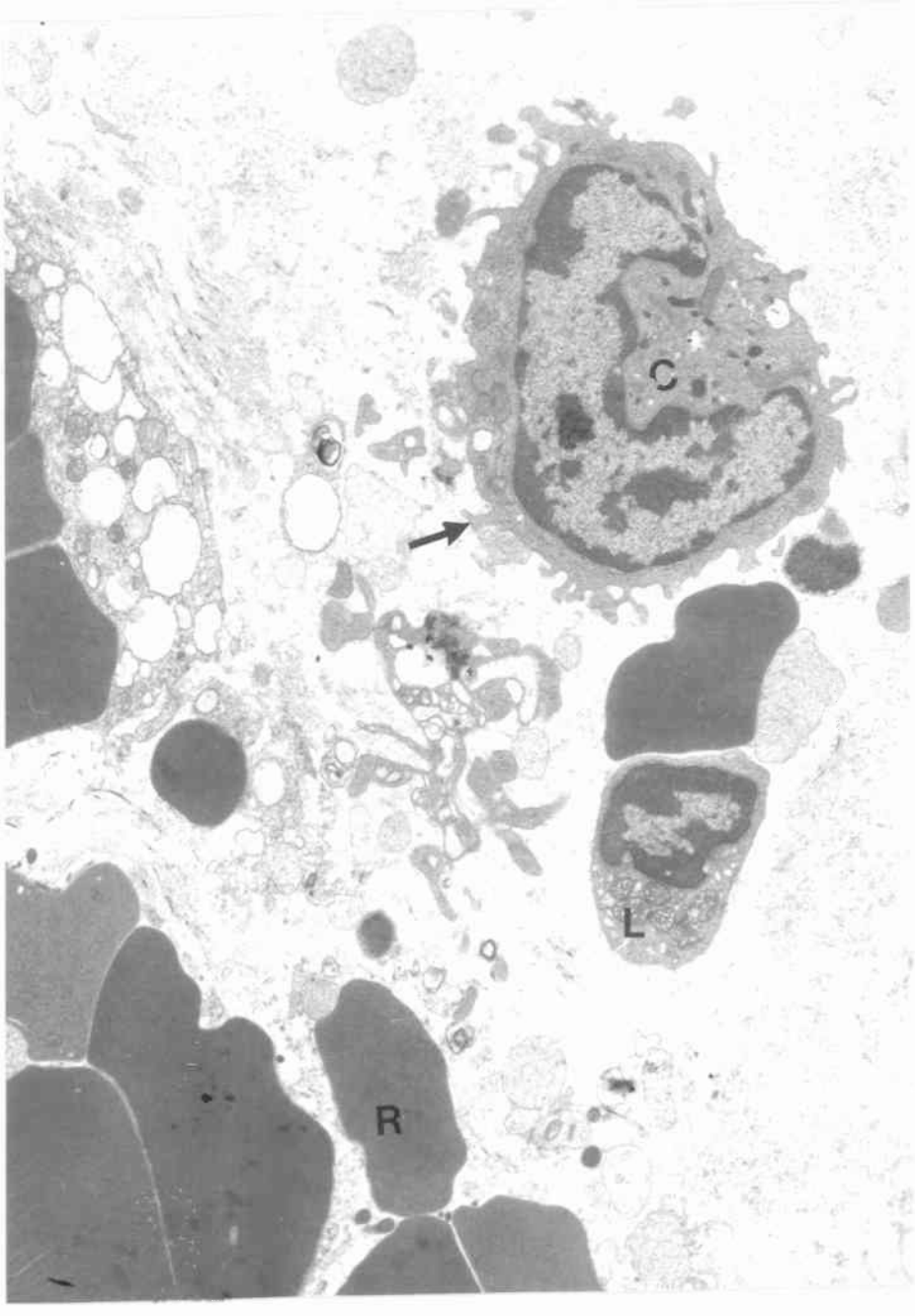


Fig. 8.20 Transmission electron micrograph of non-immunosuppressed ovarian allograft one week post-transplantation showing extravasation of red cells (R), circulating lymphocyte (C), with pseudopodia (arrow) and small lymphocyte (L)

X 3,360

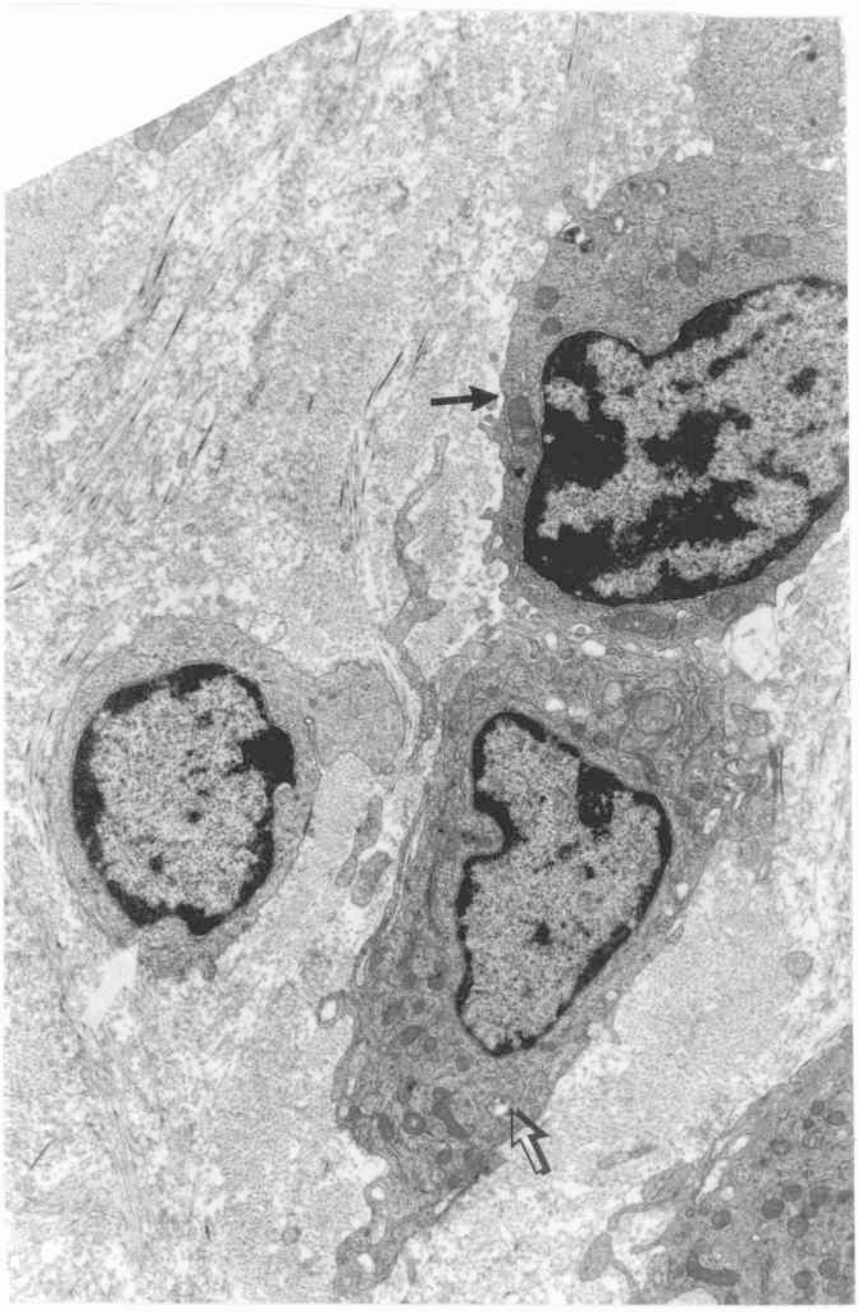


Fig. 8.21 Transmission electron micrograph of non-immunosuppressed ovarian allograft 2 weeks post-transplantation - lymphoblast (black arrow), lymphocyte (white arrow) and plasma cell (hollow arrow)

X 8,400

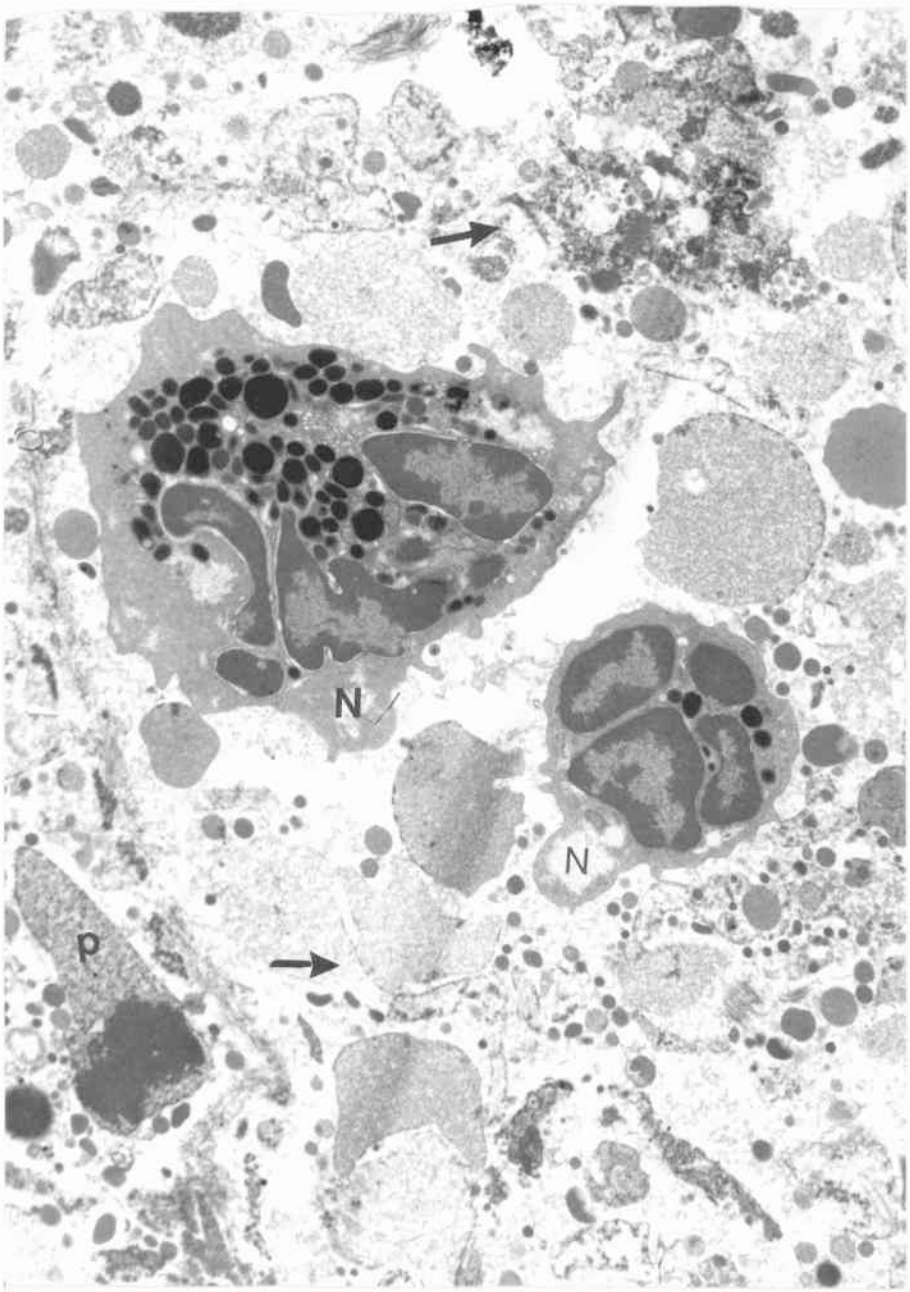


Fig. 8.22 Transmission electron micrograph of non-immunosuppressed rejected ovarian allograft 6 weeks after transplantation showing plasma cell (P) and neutrophils (N), with extensive tissue necrosis (arrow)

X 5,250

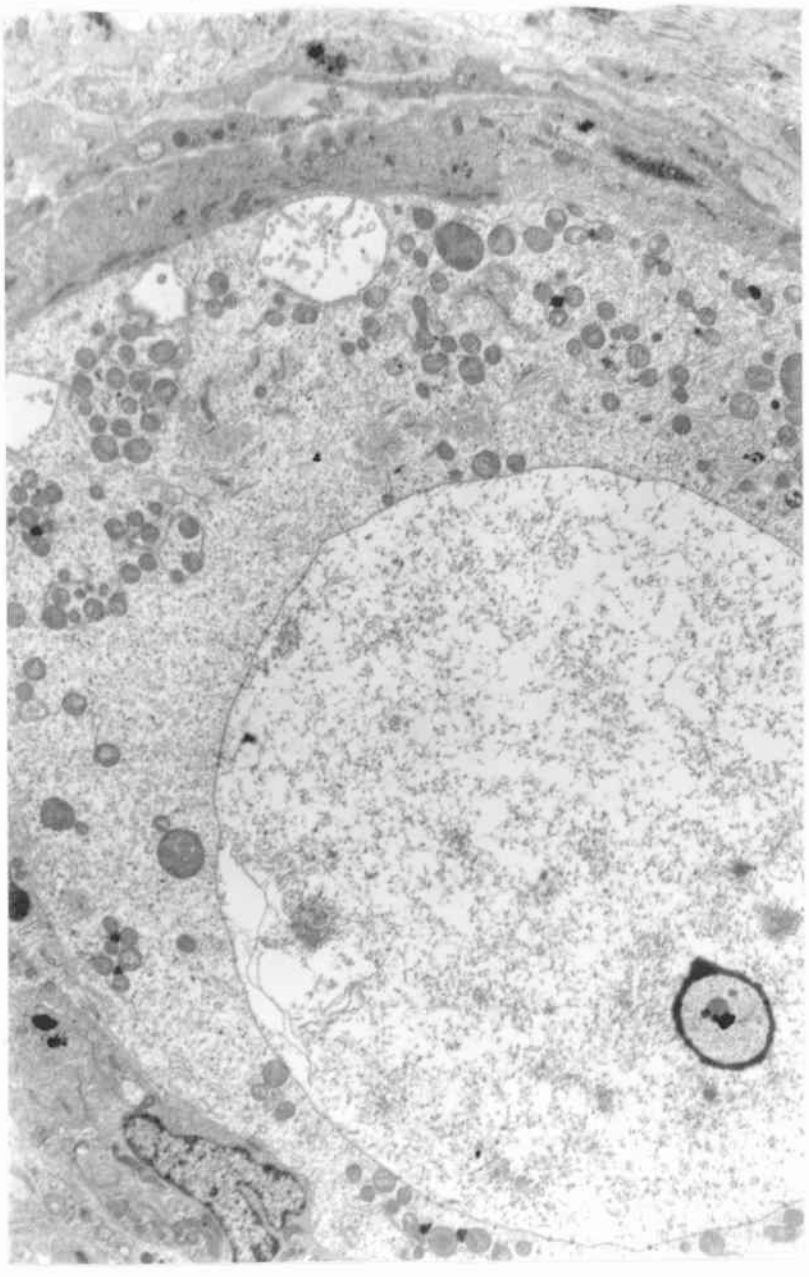


Fig. 8.23 Transmission electron micrograph of immunosuppressed ovarian allograft under CyA treatment showing normal structure of primary follicle.

X 5,250

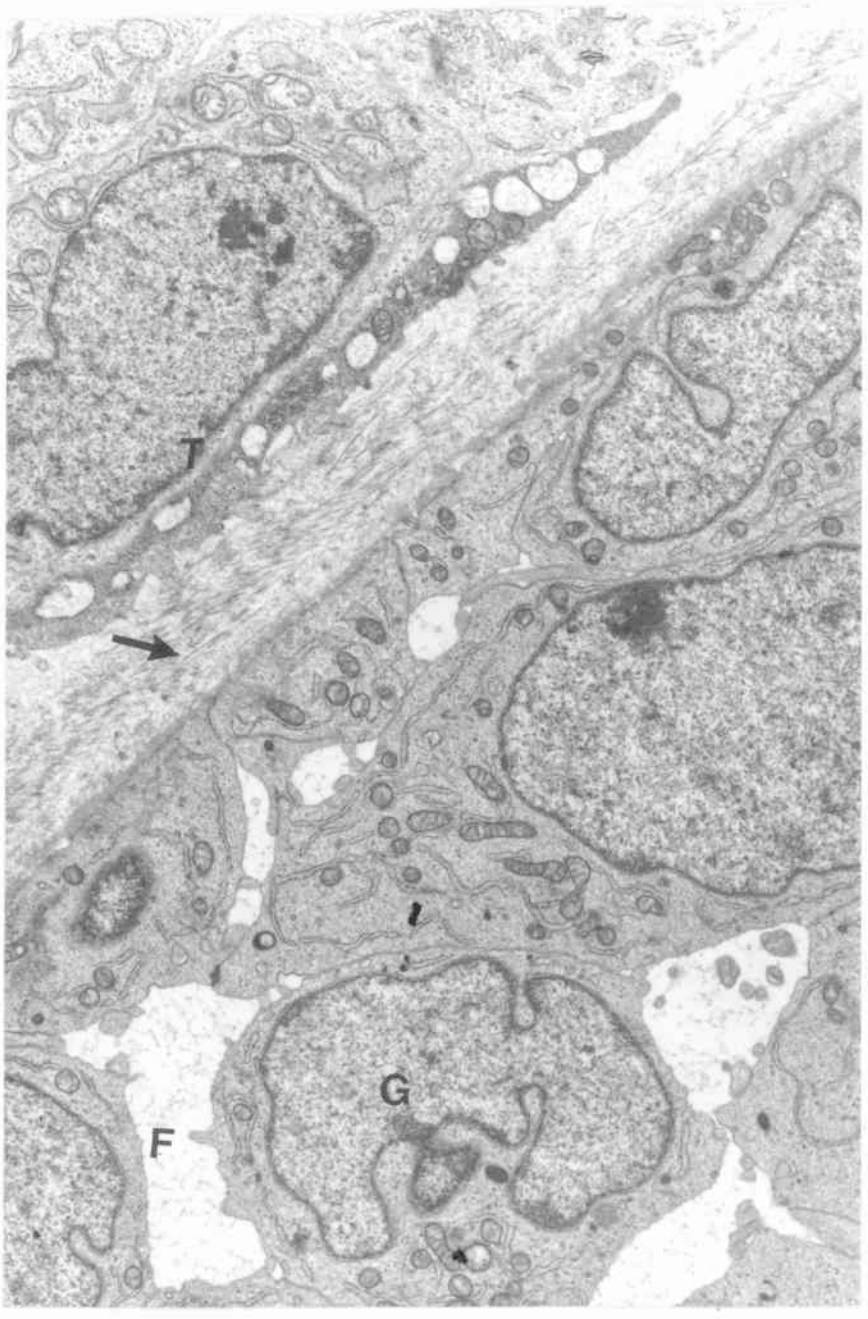


Fig. 8.25 Transmission electron micrograph of the surviving immunosuppressed ovarian allograft showing normal granulosa cells (G), follicular fluid (F), thickened theca (T) and fibrosis (arrow)

X 8,400

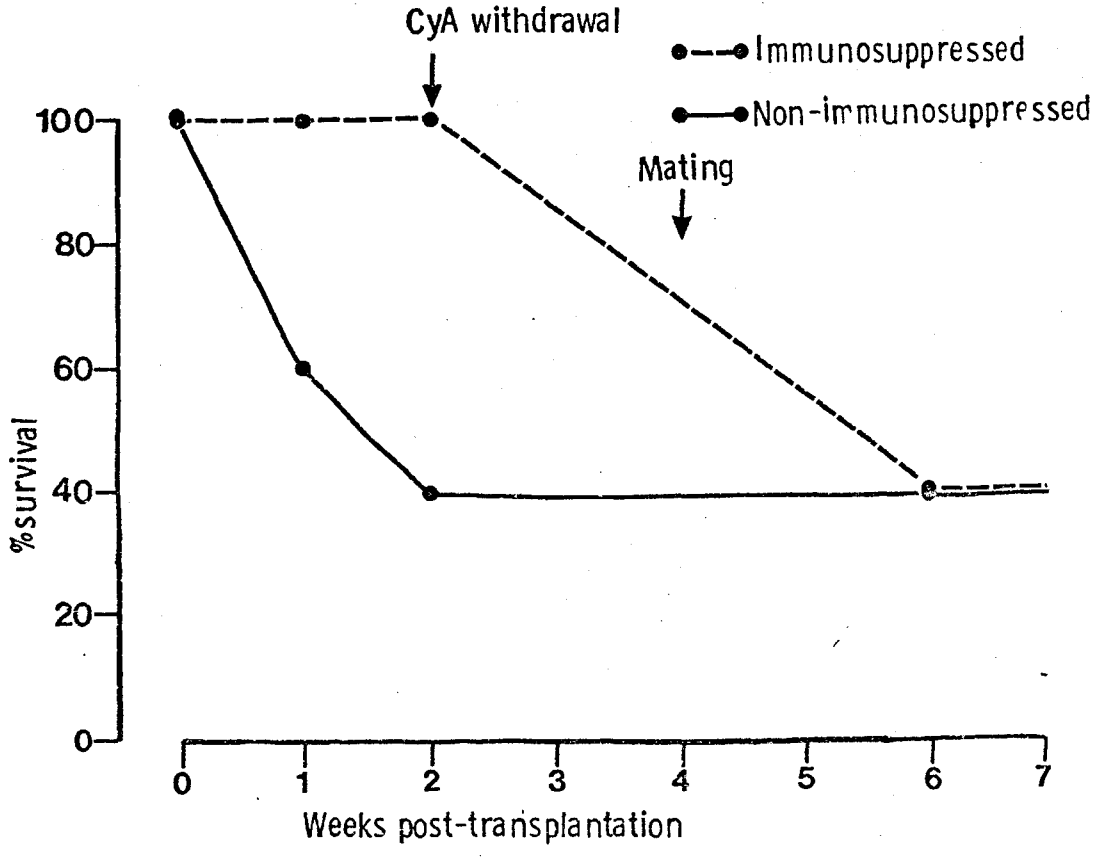


Fig. 8.26

To show percent graft survival in weeks in the immunosuppressed and the non-immunosuppressed groups.

Discussion

In view of the multifaceted objective of this study, it appears more appropriate to consider and discuss the results under a number of separate headings and to end by summing up a conclusion.

1. Transplantation procedure: The procedure used in this study proved to be highly satisfactory in terms of vascular patency rates (100%), uniform achievement of surgically successful graft and paucity of adhesion formation relative to the extensiveness of the operation (Tables 8.4, 8.2).

2. Normothermic anoxia: As in the autograft experiment (p.179), the immediate effect of the anoxia which the graft sustained during transplantation was on the function of the graft causing a significant decline ($P < 0.01$) in peripheral plasma progesterone levels in the immediate post-operative period(Table 8.5). The effect of anoxia was transient and the peripheral plasma progesterone level gradually rose again after the sixth post-operative day, indicating that ovarian tissue can tolerate relatively long periods of ischaemia (69 $\pm$10.8 min.). It also appears from Table (8.5) that the peripheral plasma progesterone levels tended to be lower in the immunosuppressed group. This point will be referred to and explained later in the discussion.

3. Body weight: Both gain in weight (Hamilton et al, 1982) and loss of weight (Gratwohl et al, 1981)were reported in animals on Cyclosporin A. The result of this study showed that in the group of animals on CyA (Group B), there was a significant decline in weight ($P < 0.01$) compared with the group of animals not on CyA treatment (Fig.8.4, Table 8.6).The weight loss under CyA treatment was discussed in Chapter 6.

4. The non-immunosuppressed group: The results showed that two out of five grafts survived and functioned normally and that one achieved successful pregnancy. These gratifying results were unexpected for it has long been accepted that ovarian allografts are rejected as readily as any other tissue (Krohn, 1977), and that within five to six days of grafting host lymphocytes invade the graft and bring about its total destruction in three weeks or so (Harris and Eakins, 1949). Green et al (1982) reported that non-immunosuppressed ovarian allografts in the rabbit were always rejected; rejection appearing six days after grafting and leading to complete necrosis of the graft in three weeks. The three rejected ovaries in the non-immunosuppressed group of our study conformed to this picture; acute rejection with lymphocytic infiltration appearing on the sixth or seventh post-transplantation day and proceeding unabated to almost complete destruction of the graft in three to four weeks.

In the two non-immunosuppressed ovaries which survived, normal ovarian structure was preserved to the end of the experiment (more than 16 weeks in some). However, in one surviving graft lymphocytic infiltration was seen in the first post-transplantation biopsy specimen, one week after grafting (Fig. 8.5), but the infiltration resolved later and the animal achieved pregnancy. This is in full agreement with the conclusions of Jacobs (1974) and of Linder (1961), that lymphocytic infiltration of ovarian allograft, even if severe, may not be a good criterion of imminent rejection, nor does it preclude long survival.

As to the question of why and how two allografts in this Group (and four in the immunosuppressed group) were accepted, a straightforward

explanation is not immediately available. However, two hypothetical possibilities may first be mentioned only to be refuted. One, is that we were dealing with a highly inbred group of rabbits, among which the immunological gap had been narrowed by inbreeding. This possibility cannot be accepted in view of the complexity of the histocompatibility system in the rabbit (Cohen and Tissot, 1974) which ensures rejection of grafts exchanged even between highly inbred rabbits (Harrison and Bartlett, 1970). The second hypothetical possibility is that we were dealing with rabbits with complete antigenic similarity. This possibility must also be refuted, as it would lead to the assumption that out of 25 (10 donors and 15 recipients) randomly selected rabbits in this study, six pairs were genetically identical.

We believe that the explanation most probably lies in an immuno-manipulative step incorporated in the transplantation procedure and aimed at the elimination of the "passenger leucocytes" by gentle repeated flushing of the graft vasculature with Ringer's solution. The passenger leucocytes are leucocytes and macrophages residing in the graft at the time of transplantation and are carried over to the host with the graft. These leucocytes, particularly the lymphocytes which are rich in transplantation antigens, are believed to be an important component of the antigenic stimulus of the graft, constitute a barrier to allo-transplantation and contribute to the vulnerability of the graft to the attack of the recipient immune system, though they are not essential to the development of rejection (Billingham, 1971; Lafferty and Woolnough, 1977). Depletion of the passenger lymphocytes by various means (pre-treatment with Cyclophosphamide: Guttman et al, 1980: culture in Cyclophosphamide containing medium: Lafferty and Woolnough,

1977; radio-isotopes: Talmage et al, 1976) prolonged graft survival of other organs. In fact, the improvement in survival of ovarian allografts after a period of maintenance in tissue culture (Jacobs, 1974; Leuker and Sharpton, 1974) may be explained by the dissolution of the passenger leucocytes (Russell and Cosmi, 1979) or to the loss of their antigenicity (Opelz and Tarasaki, 1974). It may be said, to explain the development of rejection in the other grafts, that the method did not eliminate all the passenger leucocytes from all the grafts and that relatively few leucocytes are required to activate the recipient response (Steinmuller, 1967; Talmage et al, 1976). Indeed, some of the grafts were probably not "adequately" flushed , as I was worried that the vascular endothelium might be injured. However, other yet unknown factors may also be important in the explanation of the survival of the grafts and the whole subject deserves further study.

It appears, at the moment, that the technique we described for the elimination of the passenger leucocytes was effective, physiological, not difficult to perform even with vessels within the microsurgical range, economic and potentially applicable to future clinical ovarian transplantation. If this technique for graft survival prolongation turns out to be as promising as we hope it will be, then there will probably be no need, in ovarian transplantation, for immunosuppressants with their possible toxic and embryopathic effects.

5. The immunosupressed group: The results of this group showed that all immunosuppressed grafts appeared normal and on microscopical examination showed no evidence of cellular infiltration, the hallmark of rejection, while under CyA treatment. Four weeks after the with-

drawal of the drug, however, six grafts were found to be almost completely rejected. This showed that CyA effectively suppressed graft rejection but that it failed to induce graft acceptance or provide extended immunosuppression after discontinuation of the drug. These results are in contrast to a study by Green et al (1982) in which they reported prolongation of ovarian allograft survival, in rabbit, long after withdrawal of CyA. Our results, however, agree with those of Gratwohl et al (1981) working with skin grafts in rabbit, and with those of Deeg et al (1980) working with skin grafts in dog, in which it was shown that survival of skin grafts depended on continued administration of CyA and that rejection appeared soon after CyA withdrawal.

In fact, if we had accepted failure to mate as evidence of ovarian failure, which the observation of Palka and Sawyer (1966) on ovariectomized rabbits seemed to suggest, then the fate of the graft would have been known by the fourth week (the mating date) (Fig. 8.26). The four rabbits which mated were those found later to have viable ovaries. This finding confirms the importance of the ovaries to sexual receptivity in the rabbit.

As it can be seen from the results of this group, CyA does not, really, prolong ovarian graft survival beyond the course of the treatment and the survival of four grafts in this group probably has an explanation similar to that outlined for the two surviving grafts in the non-immunosuppressed group.

The results of the histological examination of both the rejected and non-rejected grafts of the immunosuppressed grafts showed widespread

structural changes involving almost all the elements of the structure of the ovary. They included: thickening of the tunica albuginea, extensive deposition of fibrous tissue in the stroma, thickening of the theca and of the blood vessel walls, formation of multiple blood-containing "chocolate" cysts and the presence of sticky exudative, thick surface with abnormal fine blood vessels. None of these structural changes were encountered in the non-immunosuppressed grafts (rejected and non-rejected). The absence of these afore-mentioned structural alterations from the non-immunosuppressed grafts raised our suspicion of drug toxic effect. The fact that gonadotoxic effects of CyA have not been reported, prompted us to carry out a separate study of the effect of CyA on the ovary in the rabbit (Experiment IV, Chapter 6). The result of that study confirmed our suspicion that CyA was indeed responsible for the devastating effects on the grafted ovary, including the particularly severe oocyte destruction observed in these grafts. The demonstration of these effects of CyA also offered a plausable explanation of some observations made in the study. One observation was related to the rabbit in the immunosuppressed group, which achieved two pregnancies - the first pregnancy did not succeed because the chemically injured ovary could not support it, as intact ovaries are indispensible for the maintenance of pregnancy in the rabbit (Amoroso and Perry, 1977). The products of conception under such circumstances are not evacuated and often undergo resorption (Amoroso and Finn, 1962). The failure of the products of conception to be delivered, necessitated exploratory hysterotomy to demonstrate them (Fig.8.10). As we have seen from Experiment IV, the effects of CyA on the ovary are slowly and partially reversible after withdrawal of the drug, and it was, therefore,

possible that the ovary in this animal made enough recovery to support the second pregnancy. Another observation that can be seen from Table 8.5 relates to the tendency of the peripheral plasma progesterone, even early in the experiment, to be lower in the immunosuppressed than in the non-immunosuppressed, suggesting that the immunotherapeutic benefits conferred by the effect of CyA on the graft (by suppressing rejection), were more than nullified by the gonadotoxic effects.

The similarity of the histological changes demonstrated in the allograft of this study, to those reported in biopsy and autopsy specimens of ovaries of patients who received cytotoxic and immunosuppressive drugs for a non-gonadal disorder and the possibility that CyA may cause, in the human, similar damage to that caused in the rabbit ovary, has already been discussed in relation to Experiment IV (Chapter 6). It may be added here, however, that whatever the virtues of CyA may be, it certainly cannot be used in ovarian transplantation.

In conclusion, this study demonstrated, in an animal model, that the three major barriers to human ovarian allotransplantation, difficult vascular anastomosis, graft rejection and the relatively toxic immunosuppressive agents required for what is essentially a non-life saving measure, are, in fact, not insurmountable. A 100% patency rate was achieved in the anastomosis of vessels of 0.7 mm in diameter; these vessels are much smaller than the human ovarian vessels. Ovaries were successfully transplanted across major histocompatibility antigens and immunosuppressive drugs were not required, and their possible toxic effects on the recipient were thus avoided.

This study also highlighted the importance of eliminating passenger lymphocytes to the survival of the graft and dsscribed a simple effective method for depleting these leucocytes. In the language of immunology this means that measures to improve graft survival should be directed not only against the recipient immune responses but also, and more profitably, to the donor graft before transplantation. This is in full agreement with Lafferty and Woolnough's (1977) conclusion that indefinite allograft survival across a major histocompatibility barrier can be achieved by treatment of the donor tissue prior to its transplantation to the recipient.

The present experiment clearly illustrated that it was important to discern the direct action of the immunosuppressant on the graft from its immunotherapeutic effects. It also demonstrated, the hitherto, unreported serious toxic effects of CyA on the ovary and pointed the clinical implication of that.

CHAPTER 9

CONCLUSION

In essence this is an interdisciplinary study concerned with transplantation of an organ, the ovary, the functional survival of which must secure dual function, endocrine and generative. The challenges involved fall in the realm of several fields of the science of medicine. The disruption of the anatomical relations of the graft, including deprivation of neural connections and lymphatic drainage, the transient period of devascularization (period of normothermic anoxia), the task of anastomosing extremely small vessels and ensuring adequate graft vascular supply and, above all, having to face the actions of several arms of the recipient immune response which, if not subdued by some means, is certain to bring about total destruction of the graft in a short time, are the main challenges involved in grafting.

As this study has clearly shown, disruption of anatomical relations can be kept to a minimum by exploiting the potentials of microsurgery. The tubovarian relationship was restored to optimum and ovum pick up and transport were not disturbed. Vessels with microvascular calibre were anastomosed without difficulty and effective blood flows to match the varying metabolic demands of the ovary were ensured. The graft rejection reaction was shown not to be insurmountable and 40% graft survival and a pregnancy was achieved without resorting to immunosuppressants.

The studies in this Thesis touched on a topic which is still controversial, namely transplantation of the ovary in the human. The

transplantation procedure appears to be highly satisfactory, and the uncertainty of the success of microvascular anastomosis, previously considered a major barrier to human ovarian transplantation (Wood, 1982) can now be dispelled. In fact, it is likely that the operation will be easier in the human for the vessels are larger than those in the rabbit and adequate ovarian function in man is possible with blood supply only from the ovarian vessels (Merril, 1970).

With autotransplantation, the question is simple and centres on the existance of the proper indications, which are outlined in Chapter 7 and on the feasibility of the surgical technique.

When it comes to allotransplantation, the problem is somewhat different. The use of transplantation for other than life saving measures is still debatable and although the restoration of reproduction is of extreme importance to certain individual patients, yet it is widely believed that its equation with life-saving transplantation is not acceptable. It is only after watching these patients taking so many risks in the pursuit of even the slimmest hope of achieving pregnancy, that one believes that the equation may often be acceptable. In fact, it appears that transplantation of the ovary meets the requirement of an "ideal" situation for transplantation which, according to Matracru (1971), requires that (1) the physiological integrity of the donor should not suffer, and (2) the physiological integrity of the recipient should be restored by the transplant. Many patients who are undergoing the operation of hysterectomy in their reproductive years might become donors without jeopardy to their life or physical or emotional wellbeing. Specific modification of the operative technique might allow the ovary to

be removed and be used for transplantation. This approach has the advantage of allowing ample time for tissue-matching and selection of the most compatible donor. A mother who has completed her family planning may donate an ovary to her infertile daughter; it is even possible, where early marriage and large families are the rule, for an elder sister, who has completed her family planning, to donate an ovary to her younger infertile sister. This would greatly improve the chances of survival of the graft because of the tendency of the histocompatibility (transplantation) antigens to be inherited en bloc, so that the daughter shares one haplotype with her mother and has a 25% chance to have identical histocompatibility and a 50% chance to share one haplotype with her sister (Robbins et al, 1981a). The advantages of exchanging transplant between relatives do not only lie in the more favourable outcome of grafts so exchanged (Calne, 1982), but also in that instead of the negative psychological reaction to donation which is sometimes seen in graft donors (Eklund et al, 1982), a positive psychological reaction is the rule in the majority of related donors (Kamstra-Hennen et al, 1981). Besides, the newborn will not be completely "foreign" to the mother.

The present study appears to provide an optimistic view that ovarian allotransplantation, as an unconventional mode of therapy, will be justified in cases where the ovaries are irrepairably damaged or totally lacking. We also envisage that the transplantation operation will be satisfactory and that reliable monitoring of graft viability and function will not be a problem. The highly sensitive radioimmunoassay technique of ovarian steroid estimation in the peripheral plasma and even in ovarian effluent collected by retrograde catheterization of

the ovarian vein (Kirschner and Jacobs, 1971), the use of ultrasonography to visualise the ovary and follow the maturation of the follicle (Buttery et al, 1983; Kidd et al, 1981) and possibly to perform a percutaneous ultrasonically guided needle biopsy of the graft, similar to that used for aspiration of human follicles (Lenz and Lauritsen, 1982) and the laparoscopic examination (Marik , 1976), possibly with ovarian biopsy, afford a very reliable means of monitoring the ovarian graft.

The arguments against human ovarian transplantation centres on the fact that the newborn who is the product of an ovarian transplant is completely foreign to the recipient and is equivalent to a healthy adopted child. That may be so, talking in immunological language, but whether he would be equivalent to an adopted child or not should be left to the "mother" to decide. Besides, we have already entertained the possibility that donor ovaries may be obtained from mothers or sisters of the recipients, in which case the newborn would not be totally "foreign" to the mother.

Suggestions for future research

Ovarian transplantation provides a unique opportunity to study a remarkable combination of immunological, endocrinological, gametogenical and life-producing capability in an organ system. In this study, as in any other study, more points often emerge that need answering than those that were answered by the study, and the following are some of the lines of future research in ovarian transplantation which are suggested by the results of the present study.

(1) Extension of the ovarian allotransplantation experiments, in the rabbit, to include a larger number of graft recipients, longer follow-up and the use of different immunosuppressive agents to confirm and extend the result of the present study.

(2) Evaluation of the preliminary observation made in this study on the probable importance of eliminating "passenger leucocytes" to graft survival.

(3) In view of the known fact that once a transplanted ovary has survived for a prolonged period, the recipient would accept skin grafts, which would otherwise be rejected (Goldman, 1974), an effect which is specific to the ovary (Krohn, 1977), it is suggested, that now that survival of ovarian graft to an indefinite period is possible, to investigate this observation further and also to find out if allografts of other organs can also be accepted following ovarian transplantation.

(4) Study the mechanism of action of CyA on the ovary and the relevance of the changes observed to some of the pathological conditions of the ovary, particularly those in which excessive fibrosis is a feature (e.g. Stein-Leventhal Syndrome) and the relevance of that to the frequent development of hirsutism in patients who received CyA treatment (Hamilton et al, 1982).

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