ASSESSING THE CLINICAL EFFECTIVENESS OF TRANSPLANTING OVARIAN TISSUE

by

DR HAJRA MUMTAZ KHATTAK

A thesis submitted to the University of Birmingham for the degree of

DOCTOR OF PHILOSOPHY

Institute of Metabolism and Systems Research

College of Medical and Dental Sciences

University of Birmingham

May 2022

UNIVERSITY OF
BIRMINGHAM

University of Birmingham Research Archive

e-theses repository

This unpublished thesis/dissertation is copyright of the author and/or third parties. The intellectual property rights of the author or third parties in respect of this work are as defined by The Copyright Designs and Patents Act 1988 or as modified by any successor legislation.

Any use made of information contained in this thesis/dissertation must be in accordance with that legislation and must be properly acknowledged. Further distribution or reproduction in any format is prohibited without the permission of the copyright holder.

Abstract

Aim: To assess the clinical effectiveness of ovarian transplantation in returning reproductive and hormonal function in females.

Methods: Three research methods were applied; 1) evidence synthesis using a systematic review and individual patient data (IPD) meta-analysis, and a systematic review and thematic analysis of qualitative studies, 2) an observational case series and 3) a qualitative thematic analysis study.

Results: The results of systematic review and IPD meta-analysis demonstrated that ovarian tissue cryopreservation (OTC) and transplantation could restore reproductive and hormonal functions in women. The results were significant in females preserving ovarian tissue under the age of 35 years. Through thematic syntheses of studies exploring the experiences of females who had undergone ovarian tissue cryopreservation and transplantation, the experiences were described as emotional, involving complex decision-making, helping them prepare for the long-term consequences of potentially losing their fertility and hormonal function, as well as the OTC procedure being informative. Themes generated as a result of the qualitative interview study showed that women are very keen to have OTC as additional option of fertility preservation. The procedure has its drawbacks in that it is expensive and requires two surgeries nevertheless participants felt it should be offered to young girls and women in whom OTC may be the only option of fertility preservation. Participants with previous cancer who had become menopausal after gonadotoxic cancer treatment, were grateful for the hormonal function from the ovarian transplants. Furthermore, fresh ovarian transplantation in women who underwent bilateral oophorectomy, showed an increase in oestrogen post-transplant. Fresh

ovarian transplants could therefore potentially be beneficial in a select group of women in whom HRT is not an option or they prefer not to take it, to help with symptoms of iatrogenic menopause.

Conclusion: Although being developed for over two decades, ovarian tissue cryopreservation and transplantation is still deemed experimental in most parts of the world. Through this thesis it is demonstrated that many young girls and women can benefit from fertility and hormonal preservation using both fresh and frozen thawed ovarian tissue transplantation.

Dedication

I dedicate this thesis to all the young girls and women with cancer who did not have the opportunity to preserve their fertility.

I would not be the person I am today without the love and support of my friends and family. And so, I would also like to dedicate this work to them.

My aunty Ms Noor Jehan Khattak, whom I fondly call Baji Gul and who brought me up like her own. She taught me to never let anything stop me from achieving my goals.

My parents Mr Javed Khattak and Dr Robina Khattak, for sacrificing their personal accomplishments to give my sisters and I, a better life here in the UK. Papa, thank you for being my biggest supporter, my coach, my best friend and for having so much faith in me. Mama, thank you for being such a strong female role model in my life, your courage has gotten me to where I am today.

Sophia and Sorbi, thank you for being the best cheer leaders throughout this journey and for always lending an ear and offering advice.

T2 and Anee, thank you for endless cups of saffron karak chai and late night chats.

My friends Zunni, Rand, Vai, Sarah, Seuv, Fida and Dorcus for always being there and cheering me on. Our friendship means the world to me.

My new family, Dr Husain, Mum and Aleem and new brother Rameez for being so supportive throughout this journey.

Lastly, I would like to thank the love of my life, my husband, Assad. Thank you for being so kind, so patient and so understanding. Putting up with me during all this could not have been easy!

Acknowledgements

First and foremost, I am extremely grateful to my supervisors for their invaluable advice, continuous support, and patience during my PhD study. Their immense knowledge and plentiful experience have encouraged me throughout my PhD, both on a professional and a personal level. Professor Coomarasamy, thank you for giving me this life changing opportunity. I will forever be indebted to you for believing in me, teaching me and encouraging me. Professor Ioannis Gallos, I am grateful for your support throughout my PhD whether it was teaching me statistics, academic writing or helping with grant applications. Professor Annie Topping, thank you for your wise counsel and encouragement as I developed my skills in qualitative research.

I would also like to extend my sincere thanks to colleagues and friends who have helped make this journey fun and enjoyable:

Catherine Dunlop and Anna Blennerhassett- thank you for providing that 'safe space' for me to pour my heart out when I needed to. I couldn't have gotten through this without your support. The fellows and lecturers at the academic department at Birmingham Women's Hospital for their advice and support throughout my PhD; Justin Chu, Paul Smith, Rima Smith, Hoda Harb, James Cheshire, Pedro Melo, Yealin Chung, Jay Ghosh, Nikki Farmer, Bala Karunakaran, Emily Coley, Nicole Pilarski, Fiona Mackie and Will Parry-Smith. The research nurses and associates who helped me find my feet and helped with participant recruitment; Oonagh Pickering, Rachel Boothe, Shanteela McCooty, Ginnie Iqbal, Faye Andrews and Claire McPake. I thank Sarah Magson, Helen Williams, Hannah Jeffreys, Chloe Trainor and Rajinder Kaur for their help with admin support and grant applications. My senior colleagues Ms Tracey Johnston, Dr Ellen Knox, Ms Ruchira Singh, Ms Pallavi Latthe, Mr Aamir Khan, Ms Deepti Cheema and Ms Sam Pretlove who have provided support throughout my training and enabled me to embark upon this opportunity. My mentor, friend, senior registrar and then educational supervisor Dr Hsu Phern Chong for her endless support and motivation both clinically and academically since I started training in Obstetrics and Gynaecology.

I would like to especially thank the team that helped with the systematic review and individual patient data meta-analysis; Rosie Malhas for being my second reviewer, Laurentiu Craciunas for co-conceiving the concept, encouraging me to aim higher and providing consistent support, kindness and advice, Professor Simon Fishel and Mr Yousri Afifi for their inspiration, and all the international collaborators for providing individual participant data, that made the review possible (Dr Laura Lotz, Professor Ralf Dittrich, Dr Sherman Silber, Dr Debra Gook, Professor Isabelle Demeestere, Dr Inna Marie Dueholm Hjorth and Dr Olga Bystrova). I would like to thank Hannah Woodman for being the second reviewer for the qualitative systematic review, as well as second coder for the qualitative study.

I would also like to express my gratitude to collaborators whom I was fortunate enough to visit during my PhD. Dr Stine Kristensen and Dr Kirsten Macklon who kindly hosted me in their esteemed institution, University Hospital Copenhagen, Righospitalet, where I learnt the ovarian tissue cryopreservation technique using slow freezing on human ovary and observed ovarian tissue retrieval and

transplantation techniques. Professor Christiani Amorim who invited me to visit her laboratory at University Catholique de Louvain, Brussels Belgium. Dr Janice Vilela who showed me the ropes of laboratory research in relation to ovarian tissue and made my stay in Brussels meaningful. None of these travels would have been possible without the generosity of kind funders; Wellbeing of Women, the European Board and College of Obstetrics and gynaecology (EBCOG) travelling fellowship committee and the RCOG EGA Hospital Charity travelling award. Thank you for giving me the opportunity of visiting some of the prestigious centres in the world that offer ovarian tissue cryopreservation and transplantation procedures, which greatly aided my understanding of the subject.

Abbreviations

ACOG American College of Obstetricians and Gynecologists

ART Assisted Reproductive technology

AMH Anti-Mullerian hormone

AML Acute myeloid leukaemia

BFS British Fertility Society

BSO Bilateral salpingo-oophorectomy

CASP Critical Appraisal Skills Programme

COC Combined oral contraceptive

ESHRE European Society of Human Reproduction and Embryology

FSH Follicle stimulating hormone

GnRHa Gonadotropin-releasing hormone agonist

GP General Practitioner

HPO Hypothalamic pituitary ovarian axis

HRT Hormone replacement therapy

IPD Individual patient data

IQR Interquartile range

LH Luteinizing hormone

MD Mean difference

MPA Microscopic polyangiitis

OTC Ovarian tissue cryopreservation

OTCT Ovarian tissue cryopreservation and transplantation

OTT Ovarian tissue transplantation

pHRT pharmacological hormone replacement therapy

POI Premature ovarian insufficiency

PRISMA Preferred Reporting Items for Systematic Reviews and Meta Analyses

SCID Severe combined immunodeficiency

SD Standard Deviation

UK United Kingdom

USA United States of America

USS Ultrasound Scan

Table of Contents

CHAPTER 1: THESIS INTRODUCTION **14**

CHAPTER 2: FRESH AND CRYOPRESERVED OVARIAN TISSUE TRANSPLANTATION FOR MAINTAINING REPRODUCTIVE AND ENDOCRINE FUNCTION: A SYSTEMATIC REVIEW AND INDIVIDUAL PATIENT DATA META-ANALYSIS **32**

Abstract **34**
Background 34
Objective and rationale 34
Search Methods 34
Outcomes 35
Wider implications 36

Introduction **37**

Methods **39**
PROSPERO registration and systematic search 39
Inclusion and exclusion criteria 39
Primary and secondary outcomes 40
Data extraction and quality assessment 41
IPD requests 41
Data analysis and presentation 41

Results **42**
Reproductive outcomes after ovarian tissue transplantation 50
Endocrine function after ovarian tissue transplantation 53
How fresh and frozen-thawed transplants differ in restoring hormonal and fertility outcomes 58
Duration of graft function 59
Outcomes based on factors that affect fertility and likelihood of return of endocrine function 60
Surgical approaches for ovarian tissue retrieval and transplantation 67
Risks of surgery 71
Risk of subsequent cancers in the ovarian graft 71
Worldwide activity 71

Discussion **72**
Strengths 78
Limitations 79
Implications for clinical practice 81
Implications for further research 81

References **84**

CHAPTER 3: FRESH OVARIAN TISSUE AUTO-GRAFTING IN MAINTAINING ENDOCRINE FUNCTION: A CLINICAL SERIES. **99**

Abstract **100**

Introduction **102**

Methods **108**
Study Design 108
Consent process 109
Follow up 110

Results **112**
Participants 112
Surgical technique 114
Outcomes 116
Long term follow up 119

Discussion **121**
Strengths of the study 122
Limitations of the study 122
Implications of findings 123
Unanswered questions and future research 123

Conclusion **124**

References **125**

CHAPTER 4: EXPERIENCES OF YOUNG GIRLS AND WOMEN UNDERGOING OVARIAN TISSUE CRYOPRESERVATION: A SYSTEMATIC REVIEW AND THEMATIC SYNTHESIS **132**

Abstract **134**

Methods **137**
Systematic search of the literature 138
Quality assessment and appraisal 140
Data extraction 141
Data synthesis 141

Results **142**
Included studies 142
Results of meta-synthesis 144

Discussion **154**

Conclusion **160**

CHAPTER 5: WHY WOMEN CONSIDER OVARIAN TISSUE CRYOPRESERVATION FOR PRESERVING REPRODUCTIVE AND HORMONAL FUNCTION: A QUALITATIVE ENQUIRY **167**
Introduction 169
Methods 170
Analysis 170
Results 170

Conclusion 171

Introduction 172
Methods 174
Results 184
Discussion 197
Conclusion 210
References 211

CHAPTER 6: THESIS DISCUSSION AND WIDER IMPLICATIONS 221

List of figures

Figure 1. Anatomical structure of the ovary. Adapted from Silvestris et al. 2020. (3) 17
Figure 2. Histological structure of the developing follicle, also depicting signalling pathways and mediators of ovulation. Adapted from Williams and Erickson, 2000.(4) 18
Figure 3. Overview of ovarian tissue cryopreservation and transplantation technique. Adapted from Andersen et al. 2019. 22
Figure 4. Sites of ovarian transplantation. Adapted from (Taylan and Oktay 2019). 23
Figure 5. PRISMA flow diagram of search and selection strategy. 44
Figure 6. Meta-analysis of reproductive outcomes from fresh and frozen ovarian transplants: pregnancy rates. 53
Figure 7. Meta-analysis of reproductive outcomes from fresh and frozen ovarian transplants: live birth rates. 54
Figure 8. Meta-analysis of reproductive outcomes from fresh and frozen ovarian transplants: miscarriage rates. 54
Figure 9. Evidence of return of hormonal function after human ovarian transplantation shown by an increase in oestrogen (pmol/l) post- transplant. 55
Figure 10. Evidence of return of hormonal function after human ovarian transplantation shown by a decrease in FSH (IU/l) post-transplant 56
Figure 11. Evidence of return of hormonal function after human ovarian transplantation shown by a decrease in LH (IU/l) post-transplant. 57
Figure 12. Oestrogen (pmol/l) in participants having received fresh ovarian transplants. E2 = oestrogen, IV = inverse variance). 60
Figure 13. FSH (IU/L) in participants having received fresh ovarian transplants. 61
Figure 14. Comparisons of pregnancy rates in participants who had ovarian tissue cryopreserved before the age of 35 years, to those who had ovarian tissue cryopreservation after 35 years of age. Comparison of return of hormonal function (FSH). 62
Figure 15. Comparisons of return of hormonal function (FSH) in participants who had ovarian tissue cryopreserved before the age of 35 years, to those who had ovarian tissue cryopreservation after 35 years of age. 62
Figure 16. Comparison of post transplant oestrogen in participants who received chemotherapy before cryopreservation to those who did not. 65
Figure 17. Comparison of post-transplant FSH in participants who received chemotherapy before cryopreservation to those who did not. 65
Figure 18. Comparison of pregnancy rates in participants who received chemotherapy before cryopreservation to those who did not. 65
Figure 19. Evidence of return of endocrine function in participants with cervical cancer. 67
Figure 20. Meta-analysis comparing pregnancy rates using slow freezing or vitrification as a method of cryopreservation. 68

Figure 21. Meta-analysis comparing live birth rates using slow freezing or vitrification as a method of cryopreservation. 68
Figure 22. Worldwide ovarian transplantation activity based on cases reported in the literature. 74
Figure 23. Recruitment strategy. 115
Figure 24. Study flow chart. 117
Figure 25. Fresh ovarian autografting technique. 119
Figure 26. Follicle Stimulating Hormone (FSH) at baseline (before transplantation) and at 6 months post-transplant. 120
Figure 27. Oestrogen at baseline (before transplantation) and at 6 months post-transplant. 122
Figure 28. Anti-Mullerian Hormone (AMH) at baseline (before transplantation) and at 6 months post-transplant. 122
Figure 29. Follicle Stimulating Hormone (FSH) and Oestrogen in participants at 18 months post ovarian transplantation. 124
Figure 30. Evidence of follicles in the transplanted ovarian autograft (patient 2). 124
Figure 31. PRISMA Flow diagram of search and selection strategy. 148
Figure 32. Schematic representation of generated themes. 149
Figure 33. Study flow diagram. 185
Figure 34. Schematic representation of generated themes. OTC = ovarian tissue cryopreservation. 191

List of tables

Table 1. Summary of research studies within the PhD thesis. 26
Table 2. Study characteristics for patients who received ovarian transplantation. 45
Table 3. Pregnancy and live births in relation to FSH (IU/L). 57
Table 4. Pregnancy and live births in relation to oestrogen (pmol/l). 59
Table 5. Details of mode of conception for pregnancies post ovarian transplantation. 63
Table 6. Number of pregnancies and live births in women with 5 most common cancers (reported at participant level data). 67
Table 7. Overview of ovarian tissue transplantation technique. 70
Table 8. Reproductive and endocrine outcomes based on site of transplant. 72
Table 9 Reported cases of fresh ovarian transplantation. 109
Table 10. PICoS framework to develop eligibility criteria. 142
Table 11. Study characteristics table for included studies. 147
Table 12. Participant demographics. 190

Publications and presentations

Papers accepted for publication directly from this doctoral research

Khattak H, Malhas R, Craciunas L, Afifi A, Amorim CA, Fishel S, Silber S, Gook D, Demeestere I, Bystrova O, Lisyanskaya A, Manikhas G, Lotz L, Dittrich R, Colmorn LB, Macklon KT, Hjorth IMD, Kristensen SG, Gallis I, Coomarasamy A. Fresh and cryopreserved ovarian tissue transplantation for preserving reproductive and endocrine function: a systematic review and individual patient data meta-analysis. *Hum Reprod Update* 2022. (Chapter 2)

Khattak H, Gallos I, Coomarasamy A, Topping A. Why are women considering ovarian tissue cryopreservation to preserve reproductive and hormonal ovarian function? a qualitative study protocol. *BMJ open* 2022. (Chapter 5).

Khattak H, Amorim CAA. What are my options? Fertility preservation methods for young girls and women. *Fertil Steril*. 2022;S0015-0282(22)00251-5.

Khattak H, Woodman H, Afifi Y, Amorim CAA, Fishel S, Gallos I, Coomarasamy A, Topping A**.** Experiences of young girls and women undergoing ovarian tissue cryopreservation: A systematic review and thematic synthesis. *J Psychom Obstet Gynaecol.* 2022 Dec;43(4):502-516. (Chapter 4).

Published conference abstracts

Khattak H. Fresh ovarian autotransplantation to preserve ovarian and endocrine function and delay premature menopause: a demonstration of the technique. Abstracts from the 2019 ISFP meeting in NYC. ***Journal of Assisted Reproduction and Genetics* 37, 987– 1015(2020).** (Poster presentation).

Khattak H, Malhas R, Craciunas L, Afifi Y, Fishel S, Amorim C, Gallos I, Coomarasamy A. Reproductive and endocrine outcomes after fresh and frozen-thawed ovarian tissue transplantation based on age and anti-cancer therapy: A systematic review and individual patient data meta-analysis. ***Human Reproduction*, Volume 36, Issue Supplement_1, July 2021.** (Oral presentation).

Conference presentations

Khattak H. Reproductive and endocrine outcomes of ovarian transplantation and worldwide activity. Dirmas Birmingham National conference, University of Cairo, Egypt.April 2021.

Khattak H. Ovarian tissue cryopreservation and transplantation- Oncofertility and beyond. Trainees Research Talent Hunt, World Association of Trainees in Obstetrics and Gynaecology (WATOG). 17th & 18th July 2021.

Awards, Fellowships and Grants during this doctoral research

Entry level Research Scholarship. Wellbeing of Women.

European Board and College of Obstetrics & Gynaecology (EBCOG) travelling fellowship.

Royal College of Obstetricians and Gynaecologists (RCOG), EGA Hospital Charity travelling Fellowship.

Training

Basic statistics and epidemiology, London School of Hygiene and Tropical Medicine (2021)

The Nottingham systematic review course, University of Nottingham **(**2021)

Qualitative Research Methods, University of Oxford (2021)

Cryopreservation of ovarian tissue, European Board and College of Human Reproduction and Embryology (ESHRE, 2021)

CHAPTER 1: THESIS INTRODUCTION

Ovarian reproductive and endocrine function

The ovary is a multilayer organ that is constantly evolving and has two important roles; producing oocytes (reproductive function) and steroidogenesis, production of oestrogen and progesterone (hormonal function). The latter plays an important role in regulating the hypothalamic-pituitary-ovarian (HPO) axis in controlling the menstrual cycle, ovulation and ultimately fertilisation. A highly coordinated and complex set of processes must occur in order for the ovaries' steroidogenic and ovulatory roles to be realised, which is follicular development.

Structure of the ovary

Human ovaries are paired, oval structures that are about 3cm each, one on each side of the uterus situated intraperitoneal in the pelvic cavity. (1) Macroscopically, the ovary can be divided into an outer shell, the cortex and the inner medulla (Figure 1). (2) The cortex consists of ovarian follicles whereas the medulla largely provides blood and nerve supply. Follicles in the ovarian cortex can be regarded as the functional units of the ovary and consist of oocytes (microscopic). (2) The oocyte is enveloped by granulosa cells, that are responsible for the generation of sex hormones (oestrogen and progesterone) as well as a variety of growth factors that interact with the oocyte throughout development (Figure 2). (1-4)

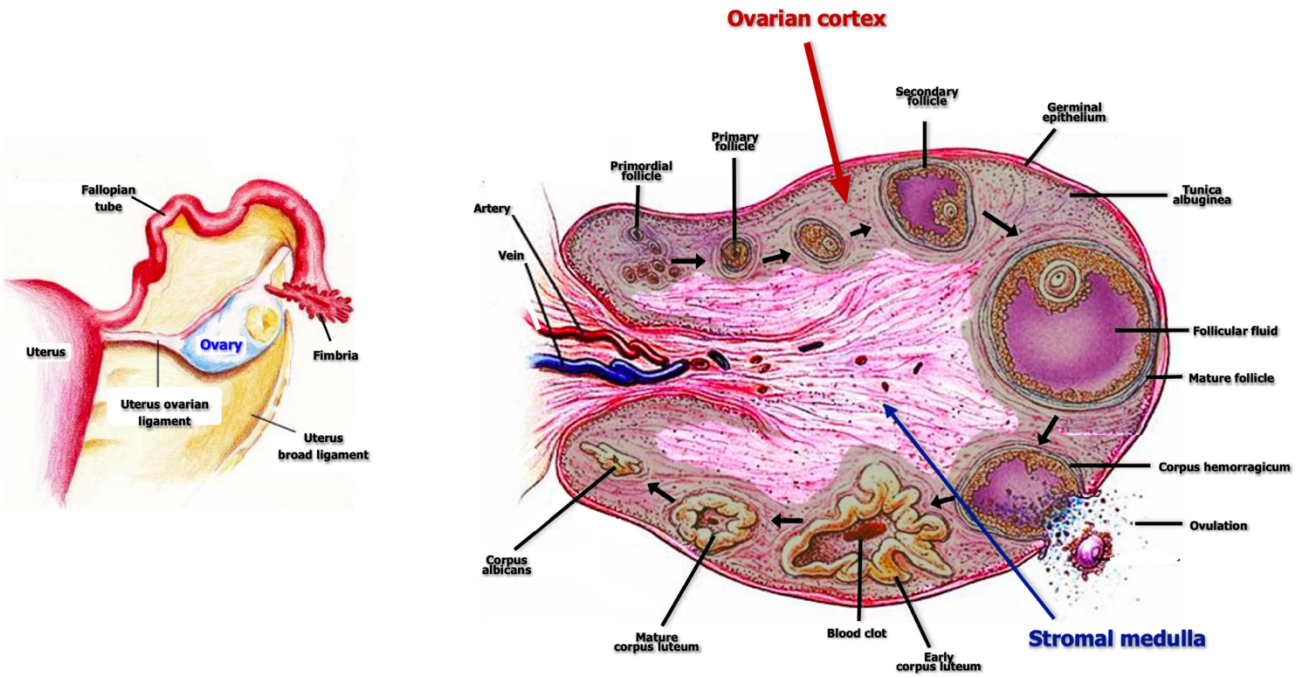


Figure 1. Anatomical structure of the ovary. Adapted from Silvestris et al. 2020. (3)

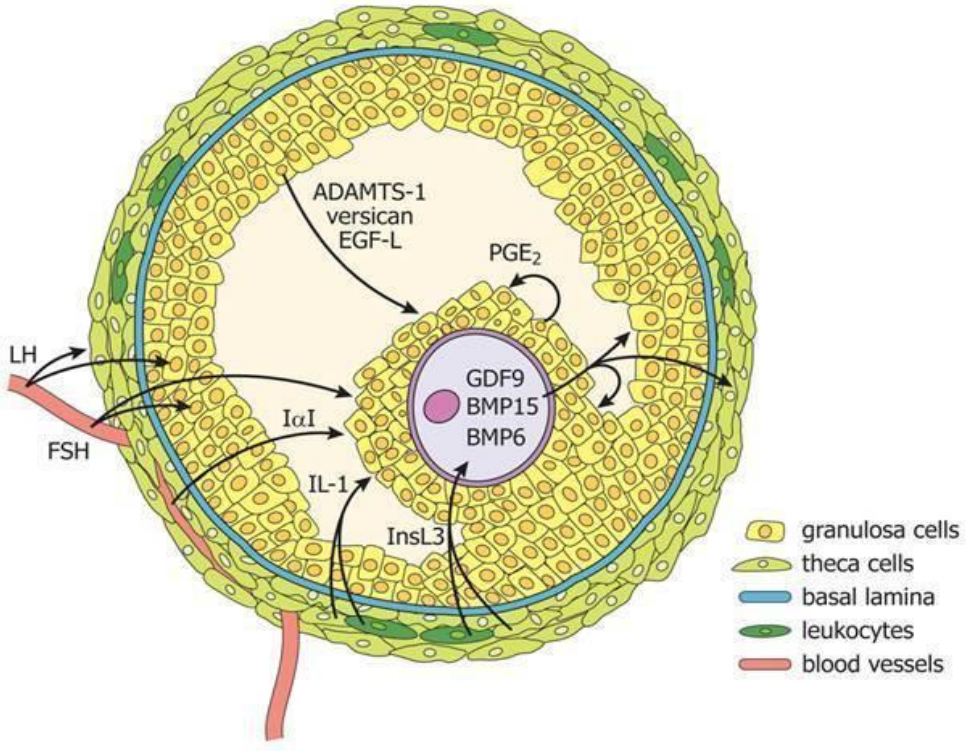


Figure 2. Histological structure of the developing follicle, also depicting signalling pathways and mediators of ovulation. Adapted from Williams and Erickson, 2000.(4)

Ovarian function during the life course of a female

The primitive ovary begins to develop at approximately the 7th week in the developing embryo. The development involves migration of germ cells to the specified sex gland. In females, these are oogonial cells that are mitotically active. The oogonial cells rapidly proliferate and take over the ovary, reaching their peak at 20 weeks of gestation, and are approximately 6–7 million in number. (2) The follicles then undergo atresia at an exponential rate such that about only 1 million remain at birth. The atresia continues throughout and approximately only 300,000–500,000 primordial follicles remain at puberty. (2) Each month, a further thousands of follicles undergo atresia at ovulation and approximately 25,000 follicles are left when a woman is 37 years old. (2,5) This 'ovarian reserve' decreases further until the age of menopause when menstruation ceases and no further functioning oocytes are left. (6) The average age of menopause in the UK is 51 years. (7) About 1% of women will undergo menopause before the age of 40; this is termed as premature menopause and this could be due to gonadotoxic anti-cancer treatments, genetics or surgery, but many causes of premature menopause remain unknown. (7)

Female fertility preservation

Female fertility preservation involves techniques that offer women the option to have biological children at a time point of their choosing. Originally these techniques were developed for females who were at risk of losing their fertility (usually because of gonadotoxic cancer treatment). The main techniques that have been used for this process are pharmacological protection (such as gonadotropin releasing hormone, GnRHa), ovarian transposition, embryo and oocyte cryopreservation and ovarian tissue cryopreservation. (8–10)

The need for female fertility preservation

Around 0.9 million females of reproductive age were diagnosed with cancer globally in 2020 and a significant increase has been noted over the last decade. (11) Ground breaking research has led to improved diagnostic and anti-cancer treatment options, which have significantly reduced mortality in young female cancer patients. (12) This has allowed female cancer survivors to focus on their quality of life more, especially on the choice of having biological children. (10,13,14) Gonadotoxic cancer treatments, however, can render young females infertile due to cancer treatments not specifically targeting the cancer cell line but also the reproductive tissue. Infertility can also lead to a disruption in hormonal milieu resulting in early menopause. (15) The reproductive system is somewhat neglected when patients with cancer are offered treatment because the focus is mainly on survival rather than on life after cancer. A collaboration between oncologists and reproductive medical specialists is therefore imperative to ensure both needs are attended to. Indeed, one such collaboration has led to the term ‘oncofertility’, which was coined in 2007 and aims to ‘bridge the gap between oncology and reproductive endocrinology’. (16) In addition, there may be reasons other than cancer for why females may opt for fertility preservation and these include benign haematological diseases, autoimmune disorders, benign gynaecological conditions such as ovarian cysts and endometriosis, or the wish to preserve fertility for personal reasons. (17–19)

History of fertility preservation

In the past, embryo cryopreservation was the only option of fertility preservation for couples. The first pregnancy from cryopreserved embryos was reported in 1983 and this was followed by a live birth two years later (from a frozen blastocyst). (9,20,21)

Although embryo cryopreservation has become a routine procedure in the world of assisted reproductive technology (ART), it comes with a prerequisite of having a male partner in order for females to consider undertaking the procedure. With the development of oocyte cryopreservation in the 1980's followed by the routine use in infertility treatments thereafter, it has negated the need for a male partner as a fertility preservation option for many women. However, for women with cancer, the ovulation induction process to retrieve oocytes is not always feasible due to time sensitive commencement of anti-cancer therapies. Furthermore, although the use of GnRH agonist is an option, current recommendation does not regard it to be an equivalent or alternative to cryopreservation procedures, and hence should only be used in conjunction or in cases when cryopreservation of reproductive material is not viable. (8) This leaves young women with limited options to preserve their fertility for the future.

State of the art: ovarian tissue cryopreservation and transplantation (OTCT)

Parrott et al. reported on the first case of successful fertility restoration after transplantation of ovarian tissue in mice in 1960 but it was not until the 1990's that the procedure progressed to larger animals. (22) Gosden et al. reported a live birth from transplanted cryopreserved ovarian tissue in sheep in 1994. (23) The encouraging results led to xenotransplantation of human ovarian tissue to severe combined immunodeficient (SCID) mice and the follicular survival in ovarian grafts made it possible to offer this novel method to female cancer patients. (24) The first orthotopic ovarian transplant was reported in the year 2000 and the development of the first embryo, reported by the same group in 2004. (24,25) The first live birth from ovarian transplantation, however, was reported in 2004 by Donnez et al. (26) A year

later, another live birth was reported in a patient who had received anti-cancer treatment before cryopreservation of ovarian tissue. (27) Ovarian tissue cryopreservation is an alternative method to oocyte and embryo cryopreservation that has been shown to return not only reproductive, but also hormonal function in females. (19,28,29) Although it was developed two decades ago, ovarian tissue cryopreservation and transplantation is still considered experimental in most parts of the world except in the USA, where the American Society of Reproductive Medicine (ASRM) has removed the experimental label recently. (10,13,30)

OTCT technique

Ovarian tissue cryopreservation involves removing either the whole ovary or part of the ovary, dissecting the cortex from the medulla and cryopreserving the cortex in liquid or vapour nitrogen using slow freezing or vitrification techniques (Figure 3). (31) When the patient wishes to conceive, the tissue can be thawed and transplanted back either at a heterotopic site (such as the pelvic side wall) or orthotopic site (the remaining ovary) as shown in Figure 4. (5,32) Many women have successfully conceived following ovarian tissue transplantation, with over 200 live births reported. (33)

Figure 3. Overview of ovarian tissue cryopreservation and transplantation technique. Adapted from Andersen et al. 2019. (34)

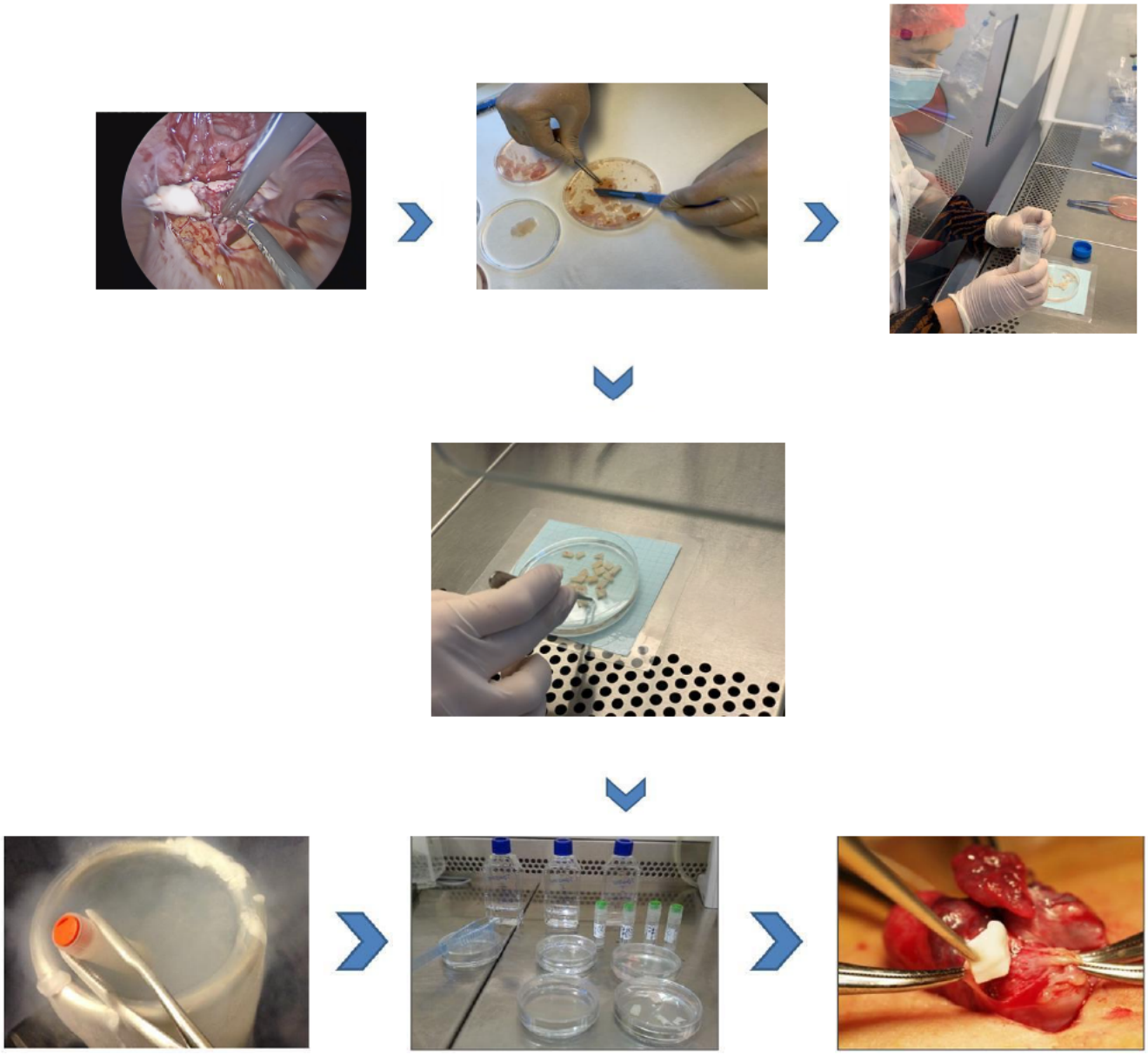
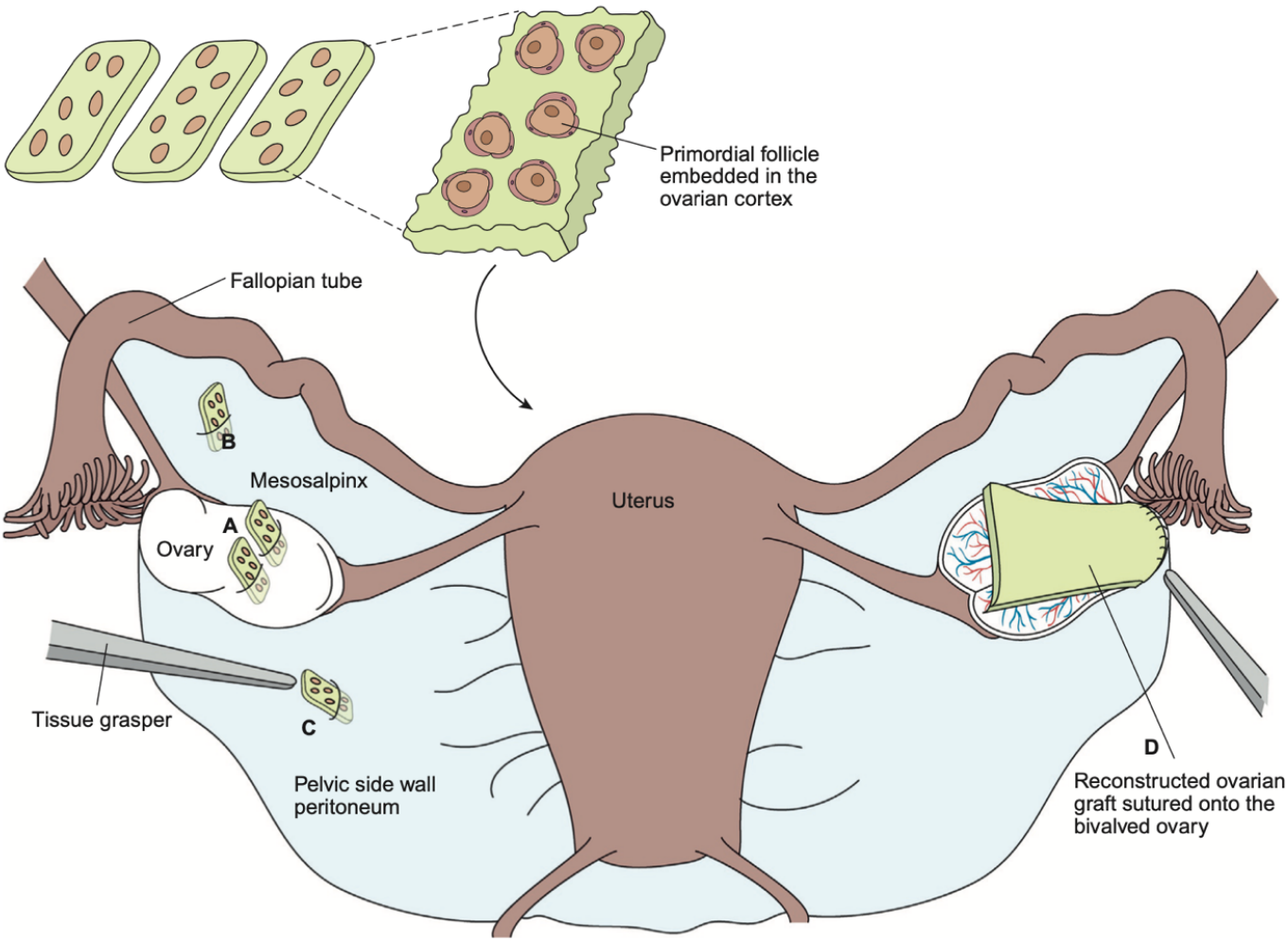


Figure 4. Sites of ovarian transplantation. Adapted from Taylan and Oktay 2019. (5)



Preservation of ovarian hormonal function

Although importance is given to preserving reproductive function in females, those who undergo cancer treatment also end up suffering from early menopause, which significantly adds to the burden of post cancer quality of life. Being in menopause predisposes these young women to osteoporosis, cardiovascular risk and mental health problems. (7) Furthermore, women are now living almost half of their lives in menopause due to improvements in quality of life that have led to increased life expectancy, not to mention cure of diseases that were not available decades ago. Pharmacological hormone replacement therapy (pHRT) is currently the only

treatment to help women with menopausal symptoms. (7) Pharmacological HRT however is not an option for all women such as those known to have or are suspected of having oestrogen dependent cancer, endometrial hyperplasia, venous thromboembolism or reasons such as simply not wanting to be on HRT. (7) Recent controversies around the use of HRT have resulted in cautiousness from clinicians and patients regarding its use. (34) The notion of using ovarian transplants to help with menopausal symptoms or to even delay the onset of menopause is not without its controversy but it does provide an option for women in whom pHRT is not viable. (35–37)

Perspectives on fertility preservation

There is great discrepancy in how care for cancer survivors is provided, especially in terms of fertility preservation. Hammarberg et al. explored the experiences of healthcare professionals in established clinics that provided advanced fertility preservation techniques for young female cancer survivors. (38) The results of the study highlighted lack of communication between oncologists and reproductive medicine specialists as well as challenges in managing expectations of individuals undergoing fertility preservation. (39) However, there is a gap in the literature on studies that aim to understand the experiences and expectations of females who consider OTC as a method of fertility preservation in particular. (38) It is imperative that the views and differing perspectives of women from a diverse range of backgrounds are explored on this important topic such that individualised, patient-centred care is provided accordingly in the future.

Purpose of thesis

Although ovarian tissue cryopreservation is offered in many fertility centres worldwide (on an experimental basis), there is great discrepancy in its delivery. Not all patients have access to these procedures and those that do, are faced with the dilemma of whether it will achieve the desired outcomes (live birth and return of hormonal function). The aim of this thesis was to collate reliable data on the effectiveness of ovarian tissue transplantation to guide healthcare professionals in providing the best evidence-based care to women who seek to undergo this method of fertility and hormonal preservation. The lived experiences of women who have undergone this procedure, in addition to the views, motivations and expectations of women who have considered this procedure, will provide new insights into the decision-making process with regards to opting for this procedure.

Thesis overview

The aim of this work is to explore in detail the effectiveness of transplanting ovarian tissue in returning reproductive and endocrine function in young girls and women.

The following chapters (with brief descriptions) are compiled in the thesis:

- Chapter 2- a systematic review and individual patient data (IPD) meta-analysis of reproductive and endocrine outcomes from fresh and frozen-thawed ovarian transplants.

- Chapter 3- an observational case series to assess the feasibility of fresh ovarian transplantation in preventing early menopause.

- Chapter 4- a thematic synthesis of qualitative studies that address the experiences of young girls and women who either considered or underwent ovarian tissue cryopreservation or transplantation (or both).
- Chapter 5- a pragmatic narrative qualitative study exploring the views, motivations and expectations of women who have considered or are considering ovarian tissue cryopreservation as a method of fertility and hormonal preservation.
- Chapter 6- an in-depth discussion of the results including reproductive and endocrine outcomes from ovarian tissue transplantation, the experiences and views of females who are considering or have undergone ovarian tissue cryopreservation and transplantation, and finally the novel use of fresh ovarian transplants in females with benign conditions will be considered.

The summary of research studies and the methodology applied in this thesis, as per PICO format, is displayed in Table 1.

Table 1. Summary of research studies within the PhD thesis

Population	**Intervention or factor of interest**	**Comparison**	**Outcomes**	**Study design**	**Chapter**
Prepubertal girls with cancer, females who received gonadotoxic anti-cancer therapies, infertile females, females with genetic abnormalities such as turner's syndrome,	Fertility preservation using ovarian tissue cryopreservation and having received ovarian transplants (fresh, frozen-thawed or donor)	n/a	Pregnancy rates, live birth rates, miscarriage rates	Systematic review and individual patient data meta-analysis	2

females at risk of, or with premature ovarian insufficiency					
Young girls and women	Considering or undergone ovarian tissue cryopreservation	n/a	Lived experience	Systematic review of qualitative studies and thematic syntheses	3
Females aged 18-45	Considering or undergone ovarian tissue cryopreservation	n/a	Views, motivations and expectations	Pragmatic qualitative interview study	4
Females aged 18-45	Fresh ovarian tissue transplantation (autografting)	n/a	FSH, LH, Oestrogen, progesterone, AMH, quality of life assessment	Observational study	5

References

1. Gibson E, Mahdy H. Anatomy, Abdomen and Pelvis, Ovary. 2022 Jul 25. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2022 Jan. PMID: 31424771.

2. Oktem O, Oktay K. The ovary: Anatomy and function throughout human life. Ann N Y Acad Sci. 2008;1127:1–9.

3. Silvestris E, De Palma G, Canosa S, Palini S, Dellino M, Revelli A, et al. Human ovarian cortex biobanking: A fascinating resource for fertility preservation in cancer. Int J Mol Sci. 2020;21(9).

4. Williams CJ, Erickson GF. Morphology and Physiology of the Ovary. MDText.com, Inc., South Dartmouth (MA); 2000. Available from: http://europepmc.org/abstract/MED/25905186

5. Taylan E, Oktay K. Chapter 30 - Autologous Transplantation of Human Ovarian Tissue. In: Leung PCK, Adashi EYBT-TO (Third E, editors. The ovary. Third. Academic Press; 2019. p. 493–500.

6. Committee on Obstetric Practice. ACOG CO 589 - Female Age-Related Fertility Decline. Obstet Gynecol. 2002;99(4):679–80.

7. Guideline C. NCC-WCH Menopause. 2015;(November):1–283. Available from: https://www.ncbi.nlm.nih.gov/books/NBK327156/pdf/Bookshelf NBK327156.pdf%0Ahttps://www.nice.org.uk/guidance/ng23/evidence/full-guideline-559549261

8. Preservation TEGG on FF, Anderson RA, Amant F, Braat D, D'Angelo A, Chuva de Sousa Lopes SM, et al. ESHRE guideline: female fertility preservation†. Hum Reprod Open. 2020 Oct 3;2020(4):hoaa052.

9. Fisch B, Abir R. Female fertility preservation: Past, present and future. Reproduction. 2018;156(1):F11–27.

10. Yasmin E, Balachandren N, Davies MC, Jones GL, Lane S, Mathur R, et al. Fertility preservation for medical reasons in girls and women: British fertility society policy and practice guideline. Hum Fertil. 2018;

11. International Agency for Research on Cancer (IARC), World Health Organization (WHO). Estimated number of new cases in 2020, all cancers, females, ages 0-39. 2021.

12. Nisker J, Baylis F, McLeod C. Choice in fertility preservation in girls and adolescent women with cancer. Cancer. 2006;107(7 SUPPL.):1686–9.

13. The American Society for Reproductive Medicine (ASRM). Fertility preservation in patients undergoing gonadotoxic therapy or gonadectomy: a committee opinion. Fertil Steril. 2019;112(6):1022–33.

14. Yee S, Abrol K, McDonald M, Tonelli M, Liu KE. Addressing oncofertility needs: views of female cancer patients in fertility preservation. J Psychosoc Oncol. 2012;30(3):331–46.

15. Robins J. Oncofertility: Fertility Preservation for Cancer Survivors. Vol. 91, Fertility and Sterility. 2009. 1966–1966 p.

16. Woodruff TK. Oncofertility: A grand collaboration between reproductive medicine and oncology. Reproduction. 2015;150(3):S1–10.

17. Condorelli M, Demeestere I. Challenges of fertility preservation in non-oncological diseases. Acta Obstet Gynecol Scand. 2019;98(5):638–46.

18. Donnez J, Dolmans M-M. Fertility Preservation in Women. N Engl J Med. 2017;377(17):1657–65.

19. Donnez J DM. Fertility preservation in women. Nat Rev Endocrinol 2013;9(12)735-749.

20. Cohen J, Simons RF, Fehilly CB, Fishel SB, Edwards RG, Hewitt J, et al. Birth after replacement of hatching blastocyst cryopreserved at expanded blastocyst stage. Vol. 1, Lancet (London, England). England; 1985. p. 647.

21. Trounson A, Mohr L. Human pregnancy following cryopreservation, thawing and transfer of an eight-cell embryo. Nature. 1983 Oct;305(5936):707–9.

22. Parrot DMV. the Fertility of Mice With Orthotopic Ovarian Grafts Derived From Frozen Tissue. Reproduction. 1960;1(3):230–41.

23. Gosden RG, Baird DT, Wade JC, Webb R. Restoration of fertility to oophorectomized sheep by ovarian autografts stored at -196 degrees C. Hum Reprod. 1994 Apr;9(4):597–603.

24. Oktay K, Karlikaya G. Ovarian Function after Transplantation of Frozen, Banked Autologous Ovarian Tissue. N Engl J Med. 2000;342:25.

25. Oktay K, Buyuk E, Veeck L, Zaninovic N, Xu K, Takeuchi T, et al. Embryo

development after heterotopic transplantation of cryopreserved ovarian tissue. Lancet. 2004;363(9412):837–40.

26. Donnez PJ, Dolmans MM, Demylle D, Jadoul P, Pirard C, Squifflet J, et al. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet. 2004;364(9443):1405–10.

27. Meirow D, Levron J, Eldar-Geva T, Hardan I, Fridman E, Zalel Y, et al. Pregnancy after Transplantation of Cryopreserved Ovarian. N Engl J Med. 2005;353(3):318–21.

28. Dolmans MM, Donnez J. Fertility preservation in women for medical and social reasons: Oocytes vs ovarian tissue. Best Pract Res Clin Obstet Gynaecol. 2021;70:63–80.

29. Gellert SE, Pors SE, Kristensen SG, Bay-Bjørn AM, Ernst E, Yding Andersen C. Transplantation of frozen-thawed ovarian tissue: an update on worldwide activity published in peer-reviewed papers and on the Danish cohort. J Assist Reprod Genet. 2018;35(4):561–70.

30. Marin L, Bedoschi G, Kawahara T, Oktay KH. History, Evolution and Current State of Ovarian Tissue Auto-Transplantation with Cryopreserved Tissue: a Successful Translational Research Journey from 1999 to 2020. Reprod Sci. 2020;27(4):955–62.

31. Rivas Leonel EC, Lucci CM, Amorim CA. Cryopreservation of Human Ovarian Tissue: A Review. Transfus Med Hemotherapy. 2019;46(3):173–81.

32. Donnez J, Dolmans MM. Transplantation of ovarian tissue. Best Pract Res Clin

Obstet Gynaecol. 2014;28(8):1188–97.

33. Dolmans MM, von Wolff M, Poirot C, Diaz-Garcia C, Cacciottola L, Boissel N, et al. Transplantation of cryopreserved ovarian tissue in a series of 285 women: a review of five leading European centers. Fertil Steril. 2021;115(5):1102–15.

34. Yding Andersen C, Mamsen LS, Kristensen SG. Freezing of ovarian tissue and clinical opportunities. Reproduction. 2019;158(5):F27–34.

35. Langer RD, Hodis HN, Lobo RA, Allison MA. Hormone replacement therapy–where are we now? Climacteric. 2021;24(1):3–10.

36. Andersen CY, Kristensen SG. Novel use of the ovarian follicular pool to postpone menopause and delay osteoporosis. Reprod Biomed Online. 2015;31(2):128–31.

37. Donnez J, Dolmans MM. Natural hormone replacement therapy with a functioning ovary after the menopause: dream or reality? Reprod Biomed Online. 2018;37(3):359–66.

38. Hammarberg K, Kirkman M, Stern C, McLachlan RI, Gook D, Rombauts L, et al. Cryopreservation of reproductive material before cancer treatment: a qualitative study of health care professionals' views about ways to enhance clinical care. BMC Health Serv Res. 2017;17(1):1–10.

39. Gracia CR, Chang J, Kondapalli L, Prewitt M, Carlson CA, Mattei P, et al. Ovarian tissue cryopreservation for fertility preservation in cancer patients: Successful establishment and feasibility of a multidisciplinary collaboration. J

Assist Reprod Genet. 2012;29(6):495–502.

CHAPTER 2: FRESH AND CRYOPRESERVED OVARIAN TISSUE TRANSPLANTATION FOR MAINTAINING REPRODUCTIVE AND ENDOCRINE FUNCTION: A SYSTEMATIC REVIEW AND INDIVIDUAL PATIENT DATA META-ANALYSIS

Preamble

A version of this chapter has been published in *Human Reproduction Update* journal.

Khattak H, Malhas R, Craciunas L, Afifi Y, Amorim CA, Fishel S, Silber S, Gook D, Demeestere I, Bystrova O, Lisyanskaya A, Manikhas G, Lotz L, Dittrich R, Colmorn LB, Macklon KT, Hjorth IMD, Kristensen SG, Gallos I, Coomarasamy A. Fresh and cryopreserved ovarian tissue transplantation for preserving reproductive and endocrine function: a systematic review and individual patient data meta-analysis. Hum Reprod Update. 2022 Feb 24:dmac003. doi: 10.1093/humupd/dmac003. Epub ahead of print. Erratum in: Hum Reprod Update. 2022 Mar 14; PMID: 35199164.

Abstract

Background

Ovarian tissue cryopreservation involves freezing and storing of surgically retrieved ovarian tissue in liquid or vapour nitrogen below -190ºC. The tissue can be thawed and transplanted back with the aim of restoring fertility or ovarian endocrine function. The techniques for human ovarian tissue freezing and transplantation have evolved over the last 20 years, particularly in the context of fertility preservation in pre-pubertal cancer patients. Fresh ovarian tissue transplantation, using an autograft or donor tissue, is a more recent development; it has the potential to preserve fertility and hormonal function in women who have their ovaries removed for benign gynaecological conditions. The techniques of ovarian tissue cryopreservation and transplantation have progressed rapidly since inception; however, the evidence on the success of this intervention is largely based on case reports and case series.

Objective and rationale

The aim of this study was to systematically review the current evidence by incorporating study-level and individual patient-level meta-analyses of women who received ovarian transplants, including frozen-thawed transplant, fresh or donor graft.

Search Methods

The review protocol was registered with PROSPERO (CRD42018115233). A comprehensive literature search was performed using MEDLINE, EMBASE, CINAHL and Cochrane Central Register of Controlled Trials from database inception to

October 2020. Authors were also contacted for individual patient data if relevant outcomes were not reported in the published manuscripts. Meta-analysis was performed using inverse-variance weighting to calculate summary estimates using a fixed-effects model.

Outcomes

The review included 87 studies (735 women). Twenty studies reported on ≥ 5 cases of ovarian transplants and were included in the meta-analysis (568 women). Fertility outcomes included pregnancy, live birth and miscarriage rates, and endocrine outcomes included oestrogen, FSH and LH levels. The pooled rates were 37% (95% CI 32-43%) for pregnancy, 28% (95% CI 24 – 34%) for live birth, and 37% (95% CI 30– 46%) for miscarriage following frozen ovarian tissue transplantation. Pooled mean for pre-transplant oestrogen was 101.6 pmol/l (95% CI: 47.9 to 155.3), which increased post-transplant to 522.4 pmol/l (95% CI: 315.4 to 729; mean difference 228.24; 95% CI: 180.5 to 276). Pooled mean of pre-transplant FSH was 66.4 IU/L (95% CI: 52.8 to 84), which decreased post-transplant to 14.1 IU/L (95% CI: 10.9 to 17.3; mean difference 61.8; 95% CI: 57 to 66.6). The median time to return of FSH to a value <25 IU/L was 19 weeks (interquartile range 15 – 26 weeks; range 0.4 – 208 weeks). The median duration of graft function was 2.5 years (interquartile range 1.4 to 3.4 years; range 0.7-5 years). The analysis demonstrated that ovarian tissue cryopreservation and transplantation could restore reproductive and hormonal functions in women. Further studies with larger samples of well characterised populations are required to define the optimal retrieval, cryopreservation and transplantation processes.

Wider implications

Ovarian tissue cryopreservation and transplantation may not only be effective in restoring fertility but also the return of reproductive endocrine function. Although this technology was developed as a fertility preservation option, it may have the scope to be considered for endocrine function preservation.

Key words

Ovarian tissue; cryopreservation; transplantation; premature ovarian insufficiency; fertility preservation; menopause; oncofertility

Introduction

There is an increase in the number of young girls and women diagnosed with cancer globally. In 2020 alone, approximately 0.9 million cases of new cancers were diagnosed worldwide in women aged 0-39 years (crude and age-standardised incidence rate per 100,000) (1). Ground-breaking research into anti-cancer therapies has resulted in a significant increase in survival rates of young female cancer participants, which has brought into focus the need for maintaining quality of life in these women (2–4). Unfortunately, gonadotoxic anti-cancer therapies pose substantial risk to the fertility prospects of girls and young women. Oocyte cryopreservation can be offered to post-pubertal women; however, this is not an option for pre-pubertal girls. Over the last two decades, a new method of fertility preservation has been developed to cryopreserve ovarian cortical tissue, and this option has the advantage of being suitable for pre-pubertal girls. The surgically retrieved ovarian tissue is prepared by separating the cortex from the medulla. The ovarian cortex, which contains thousands of primordial follicles in girls and young women, is then cut into strips, dehydrated in cryoprotectant solution and cryopreserved (frozen) using controlled rate freezing (slow) or vitrification (ultrarapid). The cryopreserved tissue is stored in vials and once the cancer treatment is concluded and the patient is deemed disease-free by their oncologist, the tissue can be thawed and transplanted back to restore fertility and endocrine function. Transplantation process may involve surgically transplanting ovarian tissue onto the remaining ovary (orthotopic), pelvic side wall, subcutaneously or intramuscularly (heterotopic). To date, thousands of girls and young women have had their ovarian tissue cryopreserved and for those not given the option there is a significant level of regret (5–7). Ovarian tissue cryopreservation and transplantation

have shown promise in preserving fertility and restoring endocrine function (8–13). The frozen-thawed ovarian tissue grafts are capable of endocrine function, producing viable oocytes for up to 7 years or even longer (12,14–16). This procedure has therefore enabled women to have their biological children, while restoring physiological ovarian hormonal function.

Fresh ovarian transplants have also been used to establish endocrine function and fertility in recipients with premature ovarian insufficiency (POI). The first successful fresh human ovary transplantation was reported in 2005 in monozygotic twins (10). The same centre performed further fresh transplants in eight participants resulting in 11 healthy babies. In recent years, multiple centres have reported a series of fresh ovarian transplants with evidence of endogenous hormone production (12,17–24).

Despite ovarian tissue cryopreservation and transplantation being available for two decades, there is a marked variation in the delivery of this procedure worldwide. Most of the data are based on case reports from specialised centres with expertise in providing this procedure, but there are many unpublished cases. The objective of this review was to synthesize the existing evidence on the use of fresh and cryopreserved ovarian tissue transplantation, using study-level data and individual patient data (IPD).

Methods

PROSPERO registration and systematic search

The review protocol was registered with PROPERO (CRD42018115233) on 15th November 2018 (25). A comprehensive literature search was performed using

MEDLINE, EMBASE, CINAHL and Cochrane Central Register of Controlled Trials from database inception to October 2020.

The databases were searched using the following key words and medical subject heading (MeSH) terms: ovarian, cryopreservation, transplantation, fresh transplantation, pregnancy, live birth and ovarian function. The search strategy for MEDLINE is attached to the appendix 1. The search was completed by screening the reference lists of all relevant publications.

Inclusion and exclusion criteria

Inclusion and exclusion criteria for studies were established before the literature search was conducted. Study selection was carried out by two independent reviewers (H.K. and R.M.). Any disagreements about inclusion were resolved by consensus or arbitration by a third reviewer (L.C.)

All studies that reported fertility or endocrine outcomes from either fresh or frozen-thawed ovarian transplants for at least one participant were included. These comprised cohort studies, observational studies, case reports, case series, conference abstracts and grey literature (irrespective of country of origin, affiliations of authors, language or year of publication). Commentaries, editorials, correspondence and letters were excluded. When more than one publication originated from the same centre, population or cohort, reports were individually assessed to identify and remove duplicates. This was also double checked by cross referencing and contacting the authors directly for clarification, and with the most recent or complete publication being selected.

Primary and secondary outcomes

For each patient identified and included in the study, the aim was to collect data on ovarian reproductive function, such as pregnancy, live births and miscarriages, and endocrine function, such as oestrogen, progesterone, FSH, LH and anti-Müllerian hormone (AMH) levels. The return of hormonal function was defined by an increase in oestrogen, and a decrease in FSH and LH along with return of menstruation. Although the accuracy of values of FSH has not been assessed robustly in the literature, for the purpose of this review the ESHRE guidelines on management of women with POI is used as a reference (26). A return of hormonal activity was described as women having achieved an FSH of < 25 IU/l post-transplant, LH of < 15 IU/L and oestrogen of >200 pmol/l. Furthermore, characteristics of participants that could have potential modifier effects on return of reproductive and ovarian endocrine function were pre-specified. These included variables such as age at cryopreservation, age at transplantation, cryopreservation before gonadotoxic chemotherapy, amount of ovary transplanted and site of transplant. Studies that only reported an aggregate for the cohorts were grouped separately.

Data extraction and quality assessment

Data extraction was performed in duplicate using a pre-defined piloted proforma. Both H.K. and R.M. extracted the data. Modified Newcastle-Ottawa scale was used for assessing the quality of the studies (27).

IPD requests

The authors of case reports and cohort studies were contacted and requested to provide IPD if the relevant outcomes were not available in the publication. If we were

unsuccessful in acquiring IPD, the studies were included in the aggregate data meta-analysis. Patient-level data were requested from 16 centres. The data were provided for 220 women from six centres (USA, Belgium, Australia, Russia, Germany and Denmark).

Data analysis and presentation

Mean and SD, or median, interquartile range (IQR) and range were used to summarise the data from studies that reported ≥ 5 cases. All the outcomes were converted to a standard unit for analysis; for FSH and LH, international units per litre (IU/L) and for oestrogen, picomoles per litre (pmol/l). The pooled outcomes were calculated as mean difference (MD) for FSH, LH and oestrogen using the inverse variance method with 95% CIs and a fixed effects model (28). Risk Ratios (RRs) with 95% CIs were calculated for pregnancy rates in relation to age (≤ 35 years and > 35 years) at retrieval, reported as dichotomous variables with the inverse variance method under the fixed-effects model. Review Manager 5.3 was used for calculating MDs and RRs (29).

To explore heterogeneity, $\chi 2$ test was used and significance was set at $P < 0.05$, where I^2 was used for quantifying heterogeneity (30). Meta-analysis for pooled estimates for overall pregnancy rates, live birth rates and miscarriages were calculated using inverse-variance weighting to calculate the fixed-effects summary estimates. Statistical analyses were performed using Stata statistical package (Version 17, StataCorp, College Station, TX, USA) and Review Manager (Revman) software (29).

Results

A total of 20,566 records were identified through the literature search. After removing 679 duplicates and addition of 10 studies from sources outside the search, 19,897 titles and abstracts were screened. After excluding studies that were not relevant, 198 full text articles were assessed for eligibility. Out of these 87 studies (735 women) were included in the review. We were able to extract IPD for 355 women and study-level data for 380 women. Studies that reported ≥ 5 cases of ovarian transplants were included in the statistical analysis (568 women). The characteristics of studies included in the meta-analysis are reported in Table 2. The PRISMA flow chart for the study is presented in Figure 5.

Figure 5. PRISMA flow diagram of search and selection strategy.

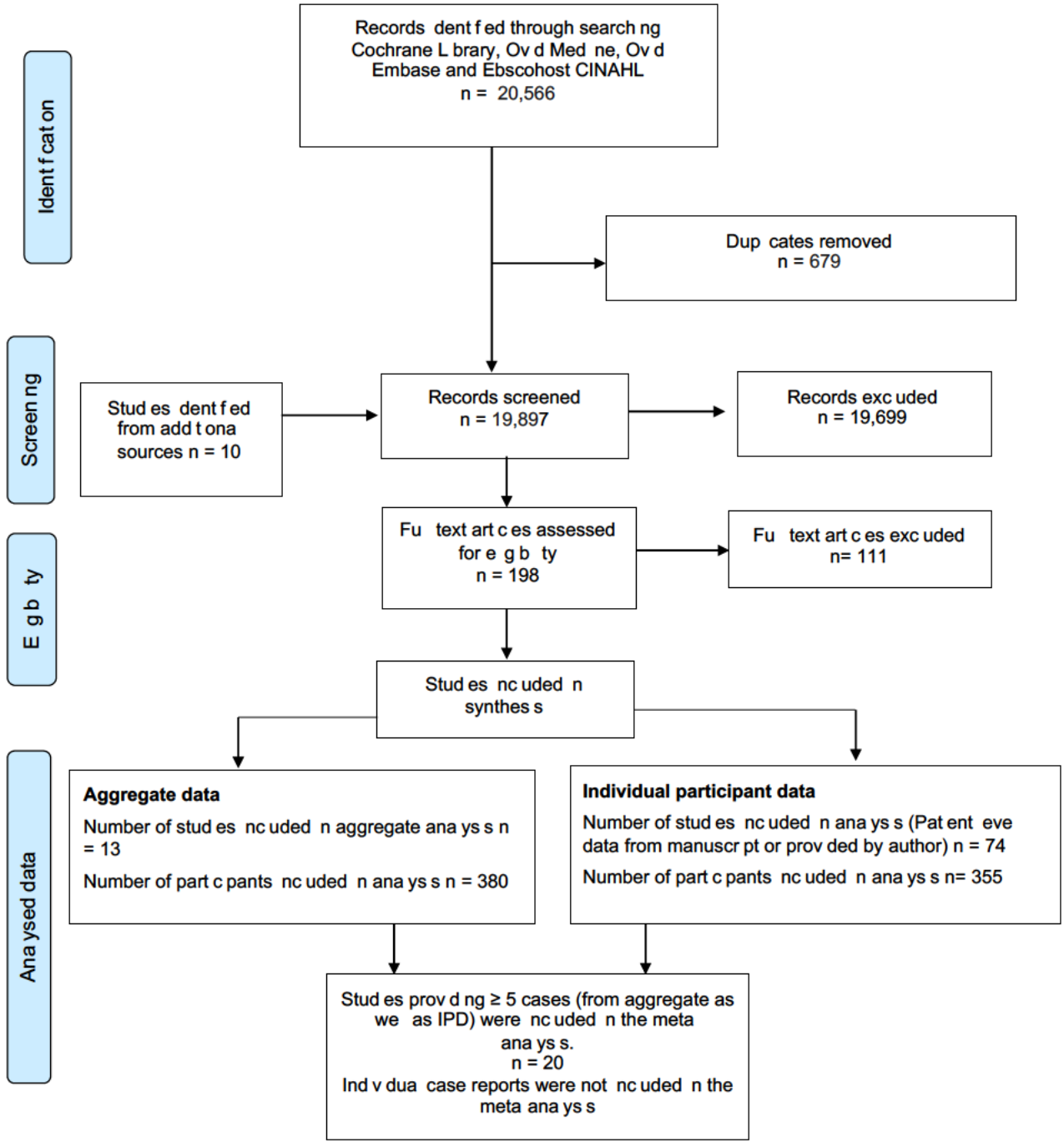


Table 2. Study characteristics for patients who received ovarian transplantation.

References (Country of origin)	Women undergoing fertility preservation (n)	Age (Mean, SD) Cryopreservation Transplantation	Type of ovarian transplants (Fresh or frozen, n, size of transplant)	Surgical technique		Reproductive and endocrine outcomes + follow up reported
				Surgical approach for transplantation (n)	Site of transplant (n)	
Case series/Cohort studies (Studies with ≥ 5 patients)						
Beckman et al. 2017* + IPD unpublished data[6] (Germany)	AML (1), Anaplastic carcinoma (5), Antisynthetase Syndrome (1), Breast cancer (28), cervical cancer (3), CML (3), Dysgerminoma (1), endometrial cancer (1), HL (29), Ovarian cancer (7), POI (3), Rectal cancer (3), SLE (1), social freezing (1)	**Cryopreservation** 30.5 (5.4) **Transplantation** 35.3 (4.9)	Frozen (86) Amount of tissue transplanted not described (Size 3x2x1mm)	Laparoscopy (86)	Remaining ovary + Pelvic side wall (4) Pelvic side wall (82)	FSH, LH and oestrogen at baseline and post transplant Return of menstrual activity Pregnancies Live births
Bystrova et al. 2019 (Russia)	Cervical cancer (9) Endometrial Cancer (1)	**Cryopreservation** 30.8 (4.4) **Transplantation** 31.8 (4.4)	Frozen (10) 10 fragments (5x5mm)	SC + Laparoscopy (3) SC (7)	SC abdominal wall (6), SC forearm + abdominal wall (1), SC forearm (1), SC abdominal wall + peritoneum (2)	FSH and LH at baseline and post transplant Oestrogen post transplant 3 year follow up
Cacciatore et al. 2001 and Cacciatore et al. 2013 (Spain)	TAH + BSO (4) Dermoid cyst (1)	**Cryopreservation** 20[3] **Transplantation** 44 (7.8)	Fresh (3) Frozen (2) Size of fragment transplanted (1x1x1 cm)	Laparoscopy (1), SC (2), IM (2)	SC arm (2), IM Rectus abdominis muscle (2), Pelvic side wall (1)	FSH and Oestrogen at baseline and post transplant Return of menstruation Pregnancies Live births 1 year follow up
Diaz Garcia et al. 2018 (Spain)	Breast cancer (31) HL (9), Rectal cancer (2), Medulloblastoma (1) Persistent trophoblastic disease (1)	**Cryopreservation** 34.3 (7.2) **Transplantation** 38.9 (4.1)	Frozen (44)	Laparoscopy (1) Laparotomy (41)	Subcortical pouches (24), Cortical microsurgical sutures (26), Subperitoneal pouches (27)	Pregnancies Live births
Donnez et al. 2011* (Donnez et al. 2004; Donnez et al. 2005; Donnez et al. 2006; Donnez et al. 2007; Cambon et al. 2008; Donnez et al. 2008; Donnez and Squifflet 2010; Donnez et al. 2011; Donnez and Squifflet 2011)[4] (Belgium)	AML (1) Beta Thalassemia (1) Breast cancer (1) BSO (1) HL (4) MPA (1) Neuroectodermal tumour of right orbit (1) NHL (2) Sickle cell anaemia (4) Turner's syndrome (1)	**Cryopreservation** 24 (5.1) **Transplantation** 29.7 (4.7)	Fresh (2) Fresh donor (4) Frozen (13) Size of tissue not given	Mini laparotomy (4), 2 step laparoscopy (2) Laparoscopy (3) Laparotomy (2) Not given (8)	Remaining ovary (10), Previous ovary +Pelvic side wall (4), Remaining ovary +SC (1), Pelvic side wall (4)	FSH at baseline and post transplantation Oestrogen at baseline and post transplant Return of menstruation Pregnancies Live births

Gook et a . 2019 (Stern et a . 2011, Stern et a . 2013; Stern et a . 2014) + IPD (unpub shed data) (Austra a)	Auto mmune po yarter t s (1) B ce ymphoma (1) Breast cancer (1) Cerv ca cancer (9) Cyst (1) Desmo d Tumour (1) Endometr a cancer (1) Endometr os s (1) Ew ng's sarcoma (2) Gynaeco og ca Cancer (2) HL (4) NHL (3) NMDA encepha t s (1) Pe v c Sarcoma (1) Sarcoma (1) SLE (1) Soc a freez ng (2) Wegner's (2)	**Cryopreserva tion** 29.1 (6.1) **Transplantati on** 35.5 (5.7)	Frozen (35) Average amount of t ssue transp ant = 330.1 mm^3	Laparoscopy (35)	Rema n ng ovary + Pe v c s de wa (21), Pe v c s de wa (14)	FSH and oestrogen at base ne and post transp ant Pregnanc es L ve b rths
Hoekman et a . 2019, K jn et a . 2019 (The Nether ands)	Breast Cancer (2) Hodgk n's ymphoma (2) Non Hodgk n's ymphoma (1) Ew ng's sarcoma (1)	**Cryopreserva tion** 27 (4.7) **Transplantati on** 33.4 (5.8)	Frozen (7) Average of 8 p eces transp ants (10×5×1 mm)	Not reported	Rema n ng ovary (6) Rema n ng ovary + per tonea wa (1)	Return of menstruat on Pregnanc es L ve b rths
Imbert et al. 2014* (Demeestere et a . 2015 + IPD prov ded by author) (Be g um)	Breast cancer (3) Recta cancer (1) HL (3) NHL (2) Sarcoma (1) S ck e ce anaem a (1) Wegner's (1)	**Cryopreserva tion** 27.5 (5.8) **Transplantati on** 33.1 (5.1)	Frozen (12) Amount and s ze of transp anted t ssue not g ven	Not prov ded	Pe v c s dewa +SC (2), Rema n ng ovary + Pe v c s de wa (2), Rema n ng ovary + pe v c s de wa + SC (1)	FSH and oestrogen at base ne and post transp ant Return of menstruat on Pregnanc es L ve b rths 5 months fo ow up
Janse et a . 2011 (Be g um and The Nether ands)	S ck e ce anaem a (2) AML (1) HL (1) Wegner's (1) NHL (1) Endometr os s (1)	**Cryopreserva tion** 23 (5.4) **Transplantati on** 29.6 (3.6)	Frozen (6) Fresh (1) S ze of transp ant not g ven	Not reported	Rema n ng ovary (6), Per tonea w ndow (1)	FSH at base ne and post transp ant Oestrogen at base ne and post transp ant Return of menstruat on Pregnanc es 2.5 year fo ow up
Jensen et al. 2015* (Schm dt et a . 2004; Schm dt et a . 2005; Rosendah et a . 2006; Andersen et a 2008; Schm dt et a . 2011; Andersen, C. Y., et a . 2012; Greve et a .2012; Ernst et a 2013; Mack on et a . 2014, Hjorth et a 2020)[1] (Denmark)	Breast cancer (12) HL (5) NHL (5) Cerv ca cancer (3) Ap ast c anaem a (2) Ew ng sarcoma (2) Paroxysma nocturna haemog ob nur a (2) Sarcoma (2) Haemo yt c uraem c syndrome (1) Ovar an cancer (1) Co on cancer (1) Ana cancer (1) Auto mmune Sma Vesse Vascu t s (1) Morbus Behcet (1) Chor ocarc noma (1) Wegener's Granu omatos s (1)	**Cryopreserva tion** 30 (5.8) **Transplantati on** 33.7 (5.6)	Frozen (58) (5x5x1mm)	M n aparotomy (19) Laparoscopy + m n aparotomy (24) Laparoscopy (15)	Rema n ng ovary (19), Rema n ng ovary + Per tonea pocket (18), Per tonea pocket (15), Rema n ng ovary + Abdom na wa (3) Rema n ng ovary +Per tonea pocket +Abdom na wa (3),	FSH at base ne and post transp ant Oestrogen at base ne and post transp ant Return of menstruat on Pregnanc es L ve b rths 10 year fo ow up

Me row et a . 2016 (Israe)	AML (1) Breast Cancer (3) CML (1) Ew ng's sarcoma (2) HL (9) NHL (4)	**Cryopreserva tion** 28.7 (7.5) **Transplantati on** 34.4 (6.9)	Frozen (20) Amount of transp ant not g ven. (5x10x1.5mm)	M n aparotomy (20)	Rema n ng ovary (20)	FSH and oestrogen at at base ne and post transp antat on Return of menstruat on Pregnanc es L ve b rths Fo ow up 7 to 141 months (mean 3.18 years)
Oktay et al. 2016* (Oktay et e . 2003; Oktay et e 2004; Oktay et e 2011) (USA)	Cerv ca cancer (1) Ben gn ovar an cysts (1) Breast cancer (1) HL (1) hemophagocyt c ymphoh st ocytos s (1) NHL (1)	**Cryopreserva tion** 27 (4.7) **Transplantati on** 34.5 (2.4)	Frozen (4) Fresh (2) Amount of t ssue transp anted not g ven. (5x5x2 mm)	Laparoscopy (1) Robot c ass sted aparoscopy (1) SC (2) IM (2)	Rema n ng ovary (2), SC (2), IM (2)	FSH at base ne and post transp ant Oestrogen post transp ant Return of menstrua act v ty Pregnanc es L ve b rths 2 year fo ow up
Po rot et a . 2019 (France)	Beta tha assem a (1) Cerv ca cancer (1) Ew ng's sarcoma (1) HL (14) NHL (8) Ovar an cancer (3) Pseudomyxoma per tone (1) Shwachman D amond syndrome (1) S ck e ce anaem a (1)	**Cryopreserva tion** 26.2 (5.8) **Transplantati on** 33.5 (4.8)	Frozen (31) Amount of ovary transp anted, med an 16 (range 4 32) (6 × 4 × 1.5 mm)	Laparoscopy (31)	Pe v c s de wa (27), SC (3), SC+ Pe v c s de wa (1)	Pregnanc es L ve b rths Fo ow up 2.2 years (range: 0.6 12.0)
Roux et a . 2019 (France)	HL (51), NHL (21), Ew ng's Sarcoma (5), AML (5), ALL (3), Mye opro ferat ve syndrome (1), Synov a sarcoma (2), Recta Cancer (2), Myxof brosarcoma (1), Abdom na myoma (1), Sp na ependymoma (1), Neurob astoma (1), Mesothe um (1), Border ne ovar an, Cancer (4), Breast, Cancer (5), Cerv ca Ca (1), S ck e ce d sease (2), Tha assem a (2), Fancon Anaem a (1), Per arter t s nodosa (1), System c mastocytos s (1), Schwachman D amond syndrome (1), Neuro upus (1)	**Cryopreserva tion** 26.3 (5.8) **Transplantati on** 32.8 (4.6)	Frozen (114) Amount and s ze of t ssue not g ven.	2 step aparoscopy (69) Not descr bed (45)	Rema n ng ovary (105)	Return of ovar an act v ty Pregnanc es L ve b rths 6 months fo ow up
Sanchez et a . 2007 (Spa n)	Hysterectomy + BSO (12)	**Transplantati on** 40.5 (2.9)	Fresh (12)	Laparoscopy (12)	Contra atera ovary (12)	AMH and FSH at base ne and post transp antat on Fo ow up 2 years

Silber 2010*; S ber 2008 (USA)	POI (9)	**Transplantation** 32.5 (8)	Fresh (9) (Donor t ssue) Amount of t ssue transp anted and str p s ze not g ven	M n aparotomy (9)	Rec p ent ovary (8), Who e ovary transp ant (1)	FSH, LH and oestrogen at base ne and post transp antat on Return of menstrua act v ty Pregnanc es L ve b rths
S ber et a . 2018 (USA)	Bra n tumour (1) HL (4) Leukaem a (3) MS (1) POI (3) Synov a cancer (1)	**Cryopreservation** 23.8 (4) **Transplantation** 31.2 (3.9)	Frozen (12) Frozen donor (1) 25% of thawed t ssue transp anted (10x10x1.5mm)	M n aparotomy (13)	Rema n ng ovary (13)	FSH and AMH at base ne and post transp ant Oestrogen at base ne and post transp ant Return of menstruat on Pregnanc es L ve b rths Fo ow up >5 years
Suzuk et a . 2015 (Japan)	POI (37)	**Cryopreservation** 37 (4.7) **Transplantation** 37 (4.7)[5]	Frozen (37) An average of 60 ovar an cubes transp anted ($1.5mm^3$)	Laparoscopy (37)	Serosa of fa op an tubes n (37)	Pregnanc es L ve b rths
Suzuk et a . 2019 (Japan)		**Cryopreservation** 35.5 (3.8) **Transplantation** 40.6 (3.7)	Frozen (8) 11 fragments transp anted (mean) S ze of fragments not g ven		Rema n ng ovary + pe v c s de wa (6), Rema n ng ovary (2)	AMH at base ne and post transp ant Pregnanc es Ongo ng pregnanc es
Van der ven et a . 2016[2] (Germany, Austr a and Sw tzer and)	Breast cancer (14) HL (10) Other (14) Ben gn d sease (2)	**Cryopreservation** 31 (5.9) **Transplantation** 35 (5.2)	Frozen (40) 15 20% of ovar es transp anted S ze not g ven	Laparotomy (2) Laparoscopy (38)	Rema n ng ovary (4), Per tonea pocket (30), Rema n ng ovary + per tonea pocket (6)	Return of menstrua act v ty Pregnanc es L ve b rths Fo ow up > 1 year
Zha et a . 2016 (Japan, Ch na and US)	POI (14)	**Transplantation** 29.2 (4.2)	Fresh (14)	Laparoscopy (14)	Serosa of fa op an tube (14)	Pregnanc es L ve b rths Fo ow up 1 year
Case reports/case series (Studies with < 5 patients)						
Akar et a . 2011 (USA)	HL	**Cryopreservation** 32 **Transplantation** 38	Frozen (1) 11 str ps	Robot c ass sted aparoscopy	Rema n ng ovary + pe v c s de wa	FSH, LH and oestrogen at base ne and post transp ant Return of menstruat on 6 month fo ow up
A mod n et a . 2015 (Braz)	POI	Transp antat o n 31	Fresh 4 fragments (2x5x1.5mm)	Laparotomy	Rec p ent ovary	FSH, LH and oestrogen at base ne and post transp ant Return of menstruat on 18 months fo ow up
Azem et a . 2012 (Israe)	HL	**Cryopreservation** 28 **Transplantation** 38	Frozen	M n aparotomy	Pe v c s de wa	FSH and LH at base ne and post transp ant Oestrogen post transp ant Pregnancy L ve b rth

Burme ster et a . 2013 (Austra a)	Breast Cancer (1) Not spec f ed (1)	**Cryopreserva tion** 38(1.4) **Transplantati on** 45 (3.8)	Frozen (2) 10 fragments (5x5x1mm)	M n aparotomy	Rema n ng ovar es	Oestrogen at base ne and post transp ant FSH post transp ant Return of menstruat on Pregnancy L ve b rth
Bystrova 2019 (Russ a)	HL (1)	**Cryopreserva tion** 26 **Transplantati on** 28	Frozen 2 fragments (5x5 mm)	M n aparotomy	Rema n ng ovary	FSH, LH and AMH at base ne and post transp ant Return of menstruat on Pregnancy L ve b rth
Dun op et a . 2016 (UK)	W m's tumour	**Cryopreserva tion** 22 **Transplantati on** 32	Frozen 8 fragments (10x10x5mm)	Laparoscopy	Rema n ng ovary	FSH, LH, AMH and oestrogen post transp ant Pregnancy L ve b rth
Fabbr et a 2018 (Ita y)	Cyst c teratoma	**Cryopreserva tion** 24 **Transplantati on** 34	Frozen 15 str ps (10x2mm)	Laparoscopy + SC	Rema n ng ovary + pe v c s de wa + SC abdom na	FSH, LH and oestrogen post transp ant
Fabbr et a . 2014 (Ita y)	Co orecta Cancer (1) HL (1) Breast Cancer (1)	**Cryopreserva tion** 28.6 (5.5) **Transplantati on** 36.5 (5.7)	Frozen Average of 22 str ps (s ze not g ven).	Laparoscopy (2) SD (1)	Rema n ng ovary + pe v c s de wa (2), SD abdom na wa (1)	FSH and LH at base ne and post transp ant Oestrogen post transp ant Return of menstruat on
Fàbregues et a . 2017 (Spa n)	Breast cancer (3) Nasopharyngea cancer (1)	**Cryopreserva tion** 35.3 (2.8) **Transplantati on** 40.3 (3.6)	Frozen Average of 10 p eces transp anted. S ze not g ven.	Laparoscopy (4)	Rema n ng ovary	FSH, LH and oestrogen at base ne on y. Return of menstruat on L ve b rth
Fajau Prevot et a . 2017 (France)	Ew ng's Sarcoma	**Cryopreserva tion** 32 **Transplantati on** 37	Frozen 21 str ps (1.5mm)	2 step Laparoscopy	Rema n ng ovary + Pe v c s de wa	AMH, FSH and LH at base ne and post transp ant. Return of menstruat on Pregnancy L ve b rth
Ha x a et a . 2019 (Ch na)	POI	**Cryopreserva tion** 28 **Transplantati on** 29	Frozen Amount not g ven (1x1x1mm)	Laparoscopy	Pe v c s de wa	Pregnancy L ve b rth
H ders et a . 2004 (The Nether ands)	Cerv ca cancer	**Transplantati on** 29	Fresh	SC	SC (arm)	Not g ven
Isachenko et a . 2012 (Germany)	HL	**Cryopreserva tion** 25 **Transplantati on** 32	Frozen Max 8 str ps 2.5x1x1mm	Laparoscopy	Pe v c s de wa	Return of menstruat on Pregnancy L ve b rth
Isachenko et a . 2013 (Germany)	Ew ng's Sarcoma	**Cryopreserva tion** 25 **Transplantati on** 30	Frozen 9 p eces 5x3x1mm	Laparoscopy	Pe v c s de wa	FSH and oestrogen at base ne and post transp ant Return of menstruat on Pregnanc es L ve b rths

Kim et al. 2012 (K m et a 2004; K m et a . 2009) (South Korea)	Cerv ca Cancer (3) Breast Cancer (1) HL (1)	**Cryopreserva tion** 31 (4.1 **Transplantati on** 35.8 (4)	Frozen 8 20 p eces 5x5x1mm	IM	Between rectus musc e and rectus sheath	FSH at base ne and post transp ant 10 year fo ow up
K ran et a . 2004 (Turkey)	TAH + BSO	**Transplantati on** 44	Fresh 10 str ps 4x20x2mm	IM	Rectus musc e	FSH post transp ant 6 months fo ow up
K se eva et a . 2015 (Russ a)	Thyro d cancer	**Cryopreserva tion** 24 **Transplantati on** 26	Frozen Amount and s ze of t ssue not g ven	Laparoscopy	Rema n ng ovary	FSH, LH and AMH at base ne and post transp ant Pregnancy L ve b rth
Kodama et a . 2010 (Japan)	Caesarean hysterectomy	**Transplantati on** 34	Fresh	Laparotomy	Pe v c s dewa	FSH, LH and oestrogen post transp ant 4.5 year fo ow up
Kr stensen et a . 2017 (Denmark)	Ovar an Cancer	**Cryopreserva tion** 23 **Transplantati on** 32	Frozen 18 p eces		Laparoscop c ass sted m n aparotomy	FSH, LH and oestrogen post transp ant Menstruat on Pregnancy L ve b rth
Laufer et a . 2010 (USA)	W ms' tumour (3)	**Transplantati on)** 3 (1.8)	Fresh Amount of t ssue transp anted, and str p s ze not g ven	IM	De to d musc e (ax a)	Menstruat on (menarche) 10 year fo ow up
Lee at a . 2018 (Korea)	Recta Cancer	**Cryopreserva tion** 30 **Transplantati on** 34	Frozen Amount not descr bed S ze 5x5x1mm	Laparoscopy	Pe v c s de wa	FSH, LH, AMH and oestrogen at base ne and post transp ant Pregnancy
Lorenzo et a . 2016 (Argent na)	Ask n Tumour	**Cryopreserva tion** 28 **Transplantati on** 32	Frozen 12 str ps 2mm	Laparoscopy	Rema n ng ovary	Menstruat on Pregnancy L ve b rth
Mardes c et a . 2017 (Prague)	Breast Cancer	**Cryopreserva tion** 29 **Transplantati on** 36	Frozen Amount of t ssue transp anted, and s ze not g ven	Laparoscopy	Rema n ng ovary	FSH, LH, AMH and oestrogen at base ne and post transp ant Menstruat on Pregnancy
Mathews et a . 2018 (UK and Denmark)	Beta Tha assem a	**Cryopreserva tion** 9 **Transplantati on** 21	Frozen 7 fragments, 1 2mm	Laparoscopy	Rema n ng ovary + pe v c s de wa	FSH, LH, AMH and oestrogen at base ne and post transp ant Menstruat on Pregnancy L ve b rth
Mhatre et a . 2006	Turner's syndrome (2) POI (1)	**Donor age** 26,38 and 21 **Recipient age** 17,15,22	Fresh (donor) Var ous amount of t ssue S ze nor g ven	Laparotomy	Host ovary	9 months to 3 years fo ow up
M enkov c et a . 2017 (Sweden)	HL	**Cryopreserva tion** 27 **Transplantati on** 31	Frozen 7 str ps S ze not g ven	M n aparotomy	Rema n ng ovary + pe v c s de wa	FSH, LH, AMH and oestrogen at base ne and post transp ant Menstruat on Pregnancy L ve b rth
Mu er et a . 2012 (Germany)	HL	**Cryopreserva tion** 25	Frozen Amount of t ssue transp anted, and s ze not g ven	Laparoscopy	Pe v c s de wa	FSH, LH, AMH and post transp ant Menstruat on

		Transplantation 30				Pregnancy L ve b rth
Ozkavakcu et a . 2019 (Turkey)	ALL	**Cryopreservation** 20 **Transplantation** 27	Frozen 9 str ps 0.5x1x1cm	Laparoscopy	Pe v c s de wa	FSH at base ne and post transp ant Pregnancy L ve b rth
Povoa et a . 2016 (Portuga)	Adnexectomy (secondary to a mass) + congen ta absence of r ght ovary	**Cryopreservation** 18 **Transplantation** 28	Frozen 8 fragments 2x2mm	Laparoscopy	Pe v c s de wa	FSH and oestrogen at base ne and post transp ant Menstruat on
Radford et a . 2001 (UK)	HL	**Cryopreservation** 36 **Transplantation** 37	Frozen 2 str ps 1x5cm	Laparoscopy	Rema n ng ovary + pe v c s de wa	FSH, LH and oestrogen at base ne and post transp ant Menstruat on
Radwan et a . 2016 (Po and)	Cerv ca cancer	**Cryopreservation** 28 **Transplantation** 29	Frozen 2 fragments 5x5x1mm	Laparoscopy	Pe v c s de wa	FSH, LH, AMH and oestrogen at base ne and post transp ant Menstruat on
Reve et a . 2011 (Israe)	Beta Tha assem a	**Cryopreservation** 19 **Transplantation** 23	Frozen 36 fragments 1.5x3x1.5mm	Laparotomy	Rema n ng ovary + pe v c s de wa	Pregnancy L ve b rth
Revelli et al. 2013* (B as n et a . 2015) (Ita y)	Beta Tha assem a	**Cryopreservation** 21 **Transplantation** 29	Frozen 17 fragments 5x5x1mm	2 step aparoscopy	Rema n ng ovary	FSH, LH and oestrogen at base ne and post transp ant Menstruat on Pregnancy L ve b rth
Rodr guez Wa berg et a . 2014 (Sweden)	Ew ng's Sarcoma	**Cryopreservation** 23 **Transplantation** 31	Frozen 97 p eces 1x3x5mm	Laparoscopy	Rema n ng ovary	FSH, LH and oestrogen at base ne and post transp ant Pregnancy L ve b rth
Shap ra et a . 2018 (Israe)	AML	**Cryopreservation** 19 **Transplantation** 32	Frozen 6 str ps 1 2mm^2	M n aparotomy	Rema n ng ovary	FSH, LH and oestrogen at base ne and post transp ant Pregnancy L ve b rth
Tamm ste et a . 2019 (Eston a)	Breast cancer	**Cryopreservation** 28 **Transplantation** 30	Frozen 5 p eces 2x5x2mm	Submuscu ar	Submuscu ar Pe v c wa	AMH, and at base ne and post transp ant Menstruat on Pregnancy L ve b rth
Tanbo et a . 2015 (Norway)	HL (1) T ce ymphoma (1)	**Cryopreservation** 28 (5.7) **Transplantation** 32 (2.8)	Frozen Amount of ovary transp anted not descr bed S ze 5x5x1mm	M n aparotomy	Rema n ng ovary	Pregnancy L ve b rth
Wø ner Hanssen et a . 2005 (Sweden)	Sjogren's syndrome	**Cryopreservation** 32 **Transplantation** Not g ven	Frozen Amount of t ssue transp anted not g ven 2x2x1mm	IM	**I**M	Not g ven

Y n et a . 2009 (Ta wan)	Acc denta TAH + BSO	**Transplantati on** 32	Fresh	Laparotomy	Pfannenst e nc s on s te	FSH, LH and oestrogen post transp ant 51 months fo ow up

Note:
Studies originating from the same group of authors were cross checked for references and duplication of patients identified Some studies may therefore have less patients than originally reported (due to removal of duplications and reporting in other studies)
* = Pr mary study. [1] Pr mary study used to report outcomes wh st others used to gather data n nd v dua pat ents where poss b e. [2] Data prov ded by D ttr ch et a . not nc uded n th s study (as IPD prov ded). [3] Age at cryopreservat on n= 1. [4] A stud es pub shed by the author n pr mary study comb ned. [5] Transp antat on a few days after IVA, freez ng and thaw ng. [6] IPD data prov ded by Professor R D ttr ch. *SC*, Subcutaneous; *IM*, ntramuscu ar; *SD*, Subderma ; *CML*, Chron c Mye o d Leukaem a; *HL*, Hodgk n ymphoma; *NHL*, Non Hodgk n ymphoma; *AML*, Acute Mye o d Leukaem a; *PNH*, Paroxysma Nocturna Haemog ob nur a; *TAH + BSO*, Tota abdom na hysterectomy + b atera sa p ngo oophorectomy; *MPA*, M croscop c po yang t s; *IVA*, In v tro act vat on; *ALL* Acute ymphocyt c eukaem a

Reproductive outcomes after ovarian tissue transplantation

Pregnancies

Eighteen studies (547 women) were included in meta-analysis for reproductive outcomes and at least one pregnancy was reported in 184 women (Figure 6). The pregnancy rate for frozen transplants was 37% (95% CI 32-43%) and for fresh transplants was 52% (95% CI 28-96%). Some women achieved more than one pregnancy giving an overall of 290 pregnancies reported in the literature.

Live births

Seventeen studies (539 women) were included in the meta-analysis and at least one live birth was reported in 134 women (Figure 7). The live birth rate for frozen transplants was 28% (95% CI 24-34%) and for fresh transplants was 45% (95% CI 23-86%). Some women achieved more than one live birth giving a total of 166 live births from women included in meta-analysis. The median number of live births per patient from frozen-thawed transplant was 1 (range 1-4) and the median number of live births per patient from fresh transplant was also 1 (range 1-3). Apart from the 17 studies, we also found case reports that described a further 34 live births. Overall, 189 live births have been reported in the literature.

Miscarriages

Fifteen studies reported miscarriage rates. The mean age at cryopreservation in women who had miscarriages was 27.8 years (SD 5.8). Miscarriage rate for frozen transplants was 37% (95% CI 30– 46%) and for fresh transplants was 33% (95% CI 13-89%) as presented in Figure 8.

Figure 6. Meta-analysis of reproductive outcomes from fresh and frozen ovarian transplants: pregnancy rates.

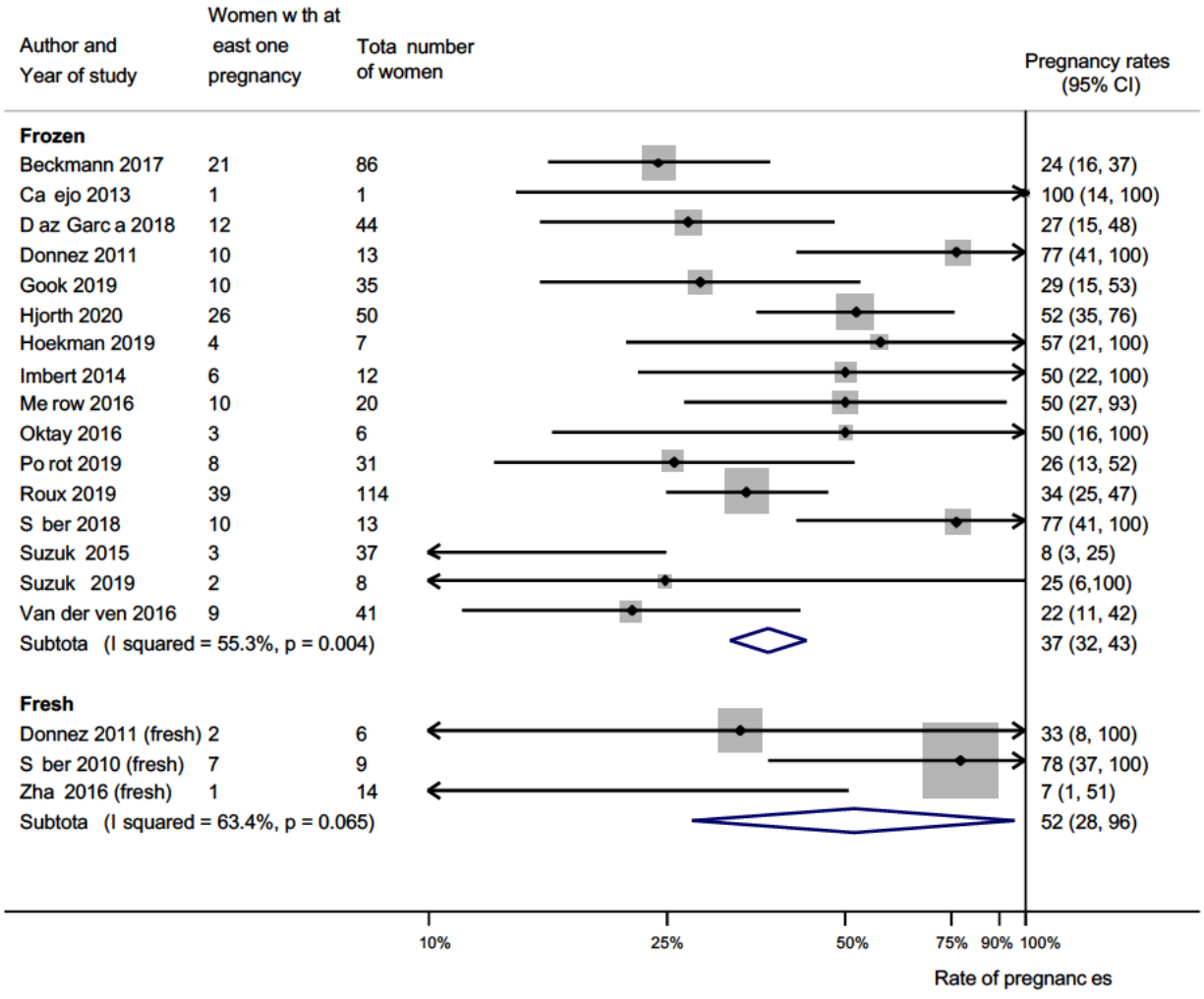


Figure 7. Meta-analysis of reproductive outcomes from fresh and frozen ovarian transplants: live birth rates.

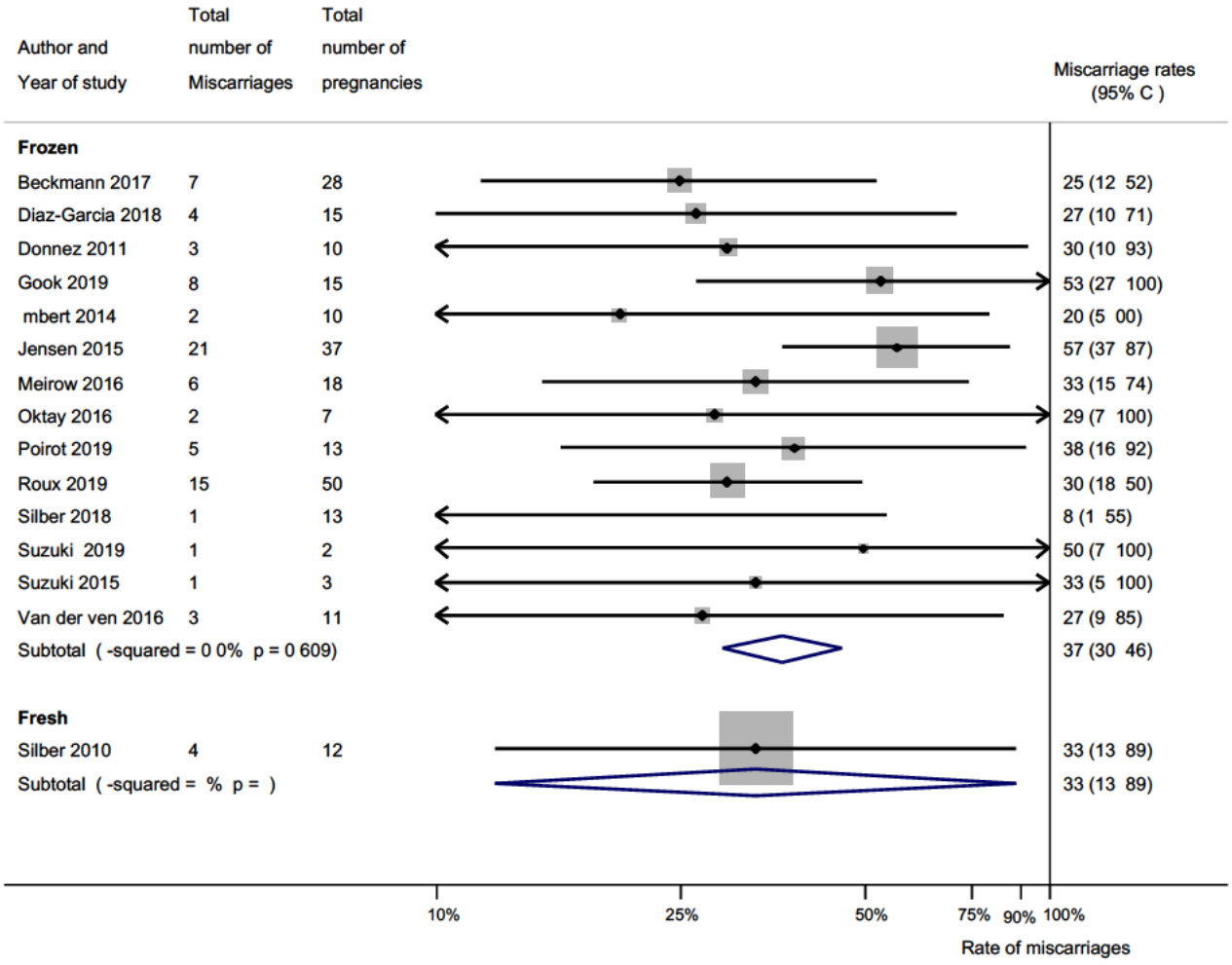
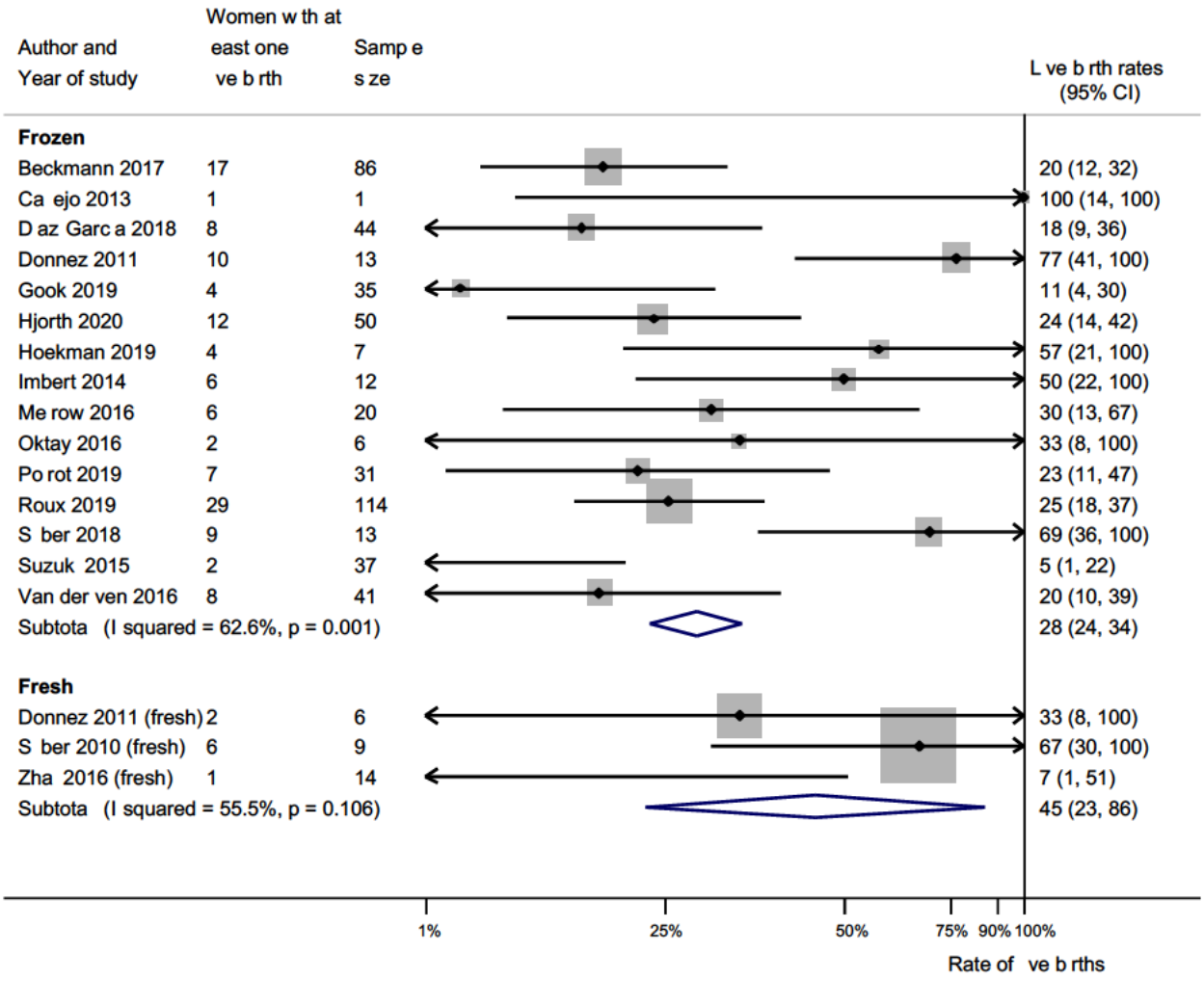


Figure 8. Meta-analysis of reproductive outcomes from fresh and frozen ovarian transplants: miscarriage rates.



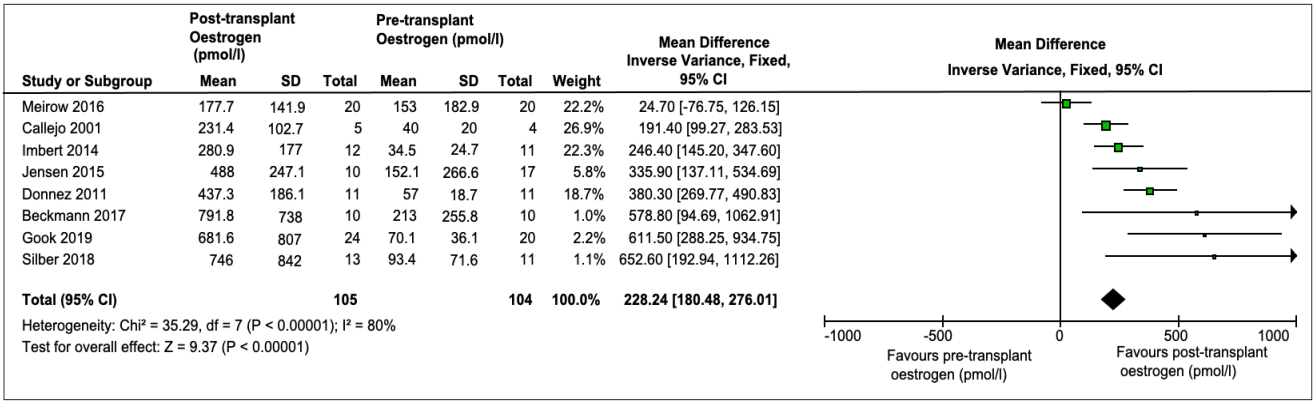
Endocrine function after ovarian tissue transplantation

Oestrogen

Eight studies (Figure 9) reported the levels of oestrogen pre transplantation (104 women) and post-transplantation (105 women). Pooled mean for pre-transplant oestrogen was 101.6 pmol/l (95% CI: 47.9 to 155.3), which increased post-transplant to 522.4 pmol/l (95% CI: 315.4 to 729; MD 228.24; 95% CI: 180.5 to 276). An increase in oestrogen of >200 pmol/l was noted in 117 women (75%) post graft. The

median time to return of oestrogen to a value >200pmol/l was 19.5 weeks (IQR 14 – 24 weeks; range 5 – 208 weeks).

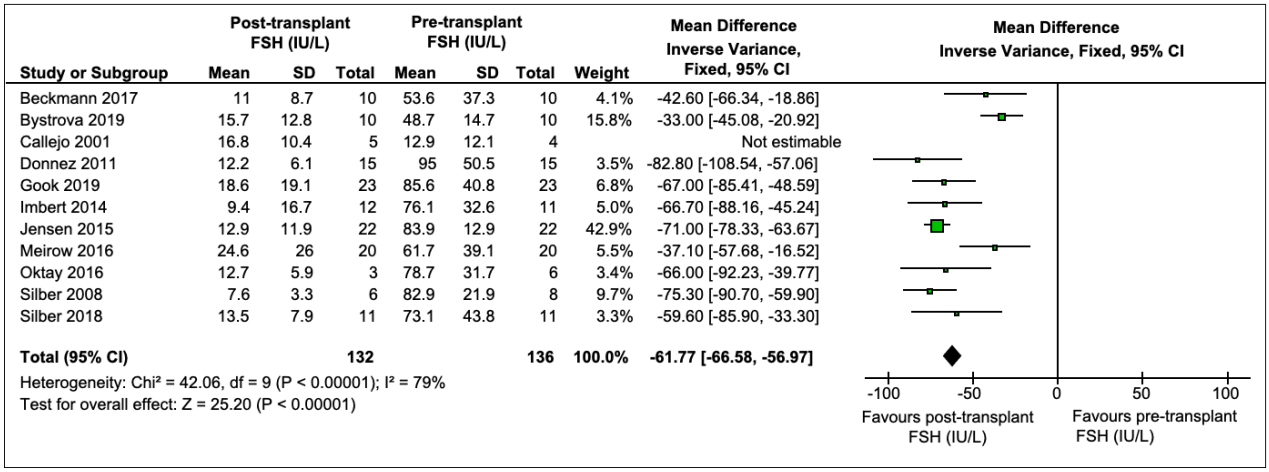
Figure 9. Evidence of return of hormonal function after human ovarian transplantation shown by an increase in oestrogen (pmol/l) post- transplant.



FSH

Eleven studies (Figure 10) reported FSH pre transplantation (136 women) and post transplantation (132 women). Pooled means of pre-transplant FSH was 68.4IU/L (95% CI: 52.8 to 84), which decreased post-transplant to 14.1 IU/L (95% CI: 10.9 to 17.3; MD 61.8; 95% CI: 57 to 66.6) with substantial heterogeneity, I^2 = 79%, (P=0.0001). Overall FSH levels post-transplant were reported for 187 out of 735 women. A decrease in FSH below 25 IU/L was achieved in 72% (135/187 women). The median time to return of FSH to a value <25 IU/L was 19 weeks (IQR 15 – 26 weeks; range 0.4 – 208 weeks).

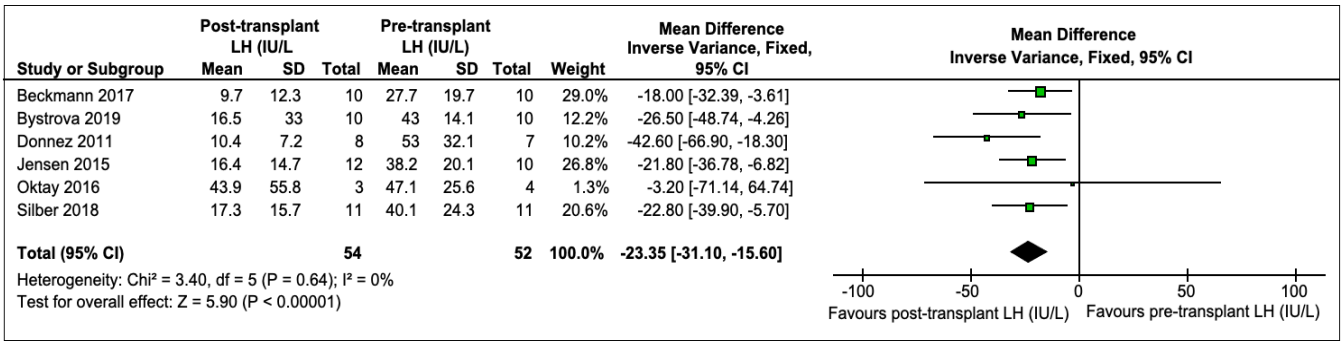
Figure 10. Evidence of return of hormonal function after human ovarian transplantation shown by a decrease in FSH (IU/l) post-transplant



LH

Six studies (Figure 11) reported LH pre transplantation (52 women) and post transplantation (54 women). Pooled mean for pre-transplant LH was 41.5 IU/L (95% CI: 32.5 to 50.5), which decreased post-transplant to 19 IU/L (95% CI: 5.8 to 32.2; MD 23.4; 95% CI: 15.6 to 31.1), heterogeneity I^2 = 0% (P=0.64). Overall, LH values post transplantation were described in 69 out of 735 women. A decrease in LH below 15 IU/L was achieved in 46 out of 69 women (67%). The median time to return of LH to a value <15 IU/L was 19.5 weeks (IQR 14 – 27 weeks; range 8 –156 weeks).

Figure 11. Evidence of return of hormonal function after human ovarian transplantation shown by a decrease in LH (IU/l) post-transplant.



Additionally, we were able to collate reproductive outcomes based on whether participants achieved an FSH of less than or equal to 25 IU/L and compared to those who had FSH of greater than 25 IU/L post transplantation. It was found that an FSH of ≤ 25 IU/L was reported in 128 women, 64 (50%) of whom were able to achieve at least one pregnancy and 44 (34%) at least one live birth (Table 3).

Table 3. Pregnancy and live births in relation to FSH (IU/L).

FSH level post transplantation (IU/L)	Total number of participants, n	FSH in whom pregnancy was reported, IU/L, Mean (SD)	Women with at least one pregnancy	Women with at least one live birth	Studies
≤ 25	128	11.1 (5.4)	64	44	Andersen, et al. 2012; Anderson et al. 2008; Azem et al. 2012; Burmeister, et al. 2013 Bystrova et al. 2019; Callejo et al. 2001; Callejo et al. 2013; Demeestere et al. 2006; Dittrich 2008; Dittrich 2012; Donnez and Squifflet 2011; Donnez and Squiflet 2010; Donnez et al 2004; Donnez et al 2006; Donnez et al. 2007; Donnez et al. 2012; Dunlop et al. 2016; Ernst 2013; Fàbregues et al. 2017; Fajau-Prevot et al. 2017; Greve et al. 2012; Gook 2019; Imbert et al. 2014; Demesteere et al. 2015; Silber et al. 2018; Isachenko et al. 2013; Janse et al. 2011; Kiran et al. 2004; Kristensen et al. 2017; Mardesic et al. 2017; Mathews et al. 2018; Meirow et al. 2016; Meirow et al. 2005; Milenkovic et al. 2017; Muller et al. 2012; Oktay et al. 2003; Oktay et al. 2016; Ozkavakcu et al. 2019; Povoa et al. 2016; Radwan et al. 2016; Rodrigeuz-Walberg et al. 2014; Rosendahl et al. 2006; Schmidt et al. 2011; Silber et al. 2005; Stern et al. 2013; Tammiste et al. 2019; Yin et al. 2009; Beckmann et al. 2017
> 25	43	48.5 (29)	11	8	Almodin et al. 2015; Andersen et al. 2012; Anderson et al. 2008; Bystrova et al. 2019; Callejo et al. 2001; Donnez et al. 2011; Donnez et al. 2008; Fabbri et al. 2018; Fabbri et al. 2014; Greve et al. 2012; Gook 2019; Imbert et al. 2014; Silber et al. 2018; Janse 2011; Kim et al 2004; Kodama et al 2010; Lee at al 2018; Meirow et al. 2016; Radford et al. 2001; Revelli et al. 2013; Schmidt et al. 2011; Shapira et al. 2018; Schmidt et al. 2004; Beckmann et al. 2017

For reproductive outcomes in relation to levels of oestrogen, we only assessed the data of participants who were truly menopausal before transplantation (levels of oestrogen <100pmol/l) (31). Data post-transplant was then divided into two

categories; participants who achieved oestrogen level of ≥200pmol/l post transplantation and those who did not. It was found that 56 participants achieved an oestrogen of ≥200pmol/l post transplantation, 19 (34%) of whom achieved at least one pregnancy and 15 (27%) at least one live birth (Table 4)

Table 4. Pregnancy and live births in relation to oestrogen (pmol/l).

Oestrogen level post transplantation (pmol/l)	**Total number of participants, n**	**Oestrogen in whom pregnancies were reported, pmol/l, Mean (SD)**	**Women with at least one pregnancy**	**Women with at least one live birth**	**Studies**
<200	21	121 (34.8)	7	4	Almodin et al. 2015; Bystrova et al. 2019; Ernst et al. 2012; Imbert et al. 2014; Silber et al. 2018; Meirow et al. 2016; Oktay 2016; Rosendahl 2006
≥200	56	652.3 (724.4)	19	15	Anderson et al. 2008; Burmeister et al. 2013; Bystrova et al. 2019; Callejo et al 2001; Callejo et al. 2013; Donnez et al. 2010; Donnez et al. 2004; Donnez et al. 2006; Donnez et al. 2007; Donnez et al. 2011; Donnez et al. 2008; Fabbri et al.2014; Gook 2019; Imbert et al. 2014 Silber et al. 2018; Isachenko et al. 2013; Janse et al. 2011; Mardesic et al. 2017; Meirow et al. 2016; Milenkovic et al. 2017; Povoa et al. 2016; Radford et al. 2001; Radwan et al. 2016; Revelli et al. 2013; Rodriguez-Walberg et al. 2014; Silber et al. 2005; Stern et al. 2013; Schmidt et al. 2004; Beckmann et al. 2017

AMH

Only one study provided enough data for AMH pre and post transplantation. (32) We found that even in those women who had an AMH of < 1ng/ml pre transplant, 19 pregnancies were observed in 71 patients (pregnancy rate = 27%).

Return of menstruation

Menstrual activity was reported as an outcome in 273 out of 735 women. It was noted that 196 out of 273 (72%) women were reported to have resumed menstruation. The median time to return of menstrual activity was 18 weeks (IQR 14-22 weeks; range 3 – 48 weeks). The return of menstrual activity coincides with that of hormonal function (median time to return of FSH to a value of <25 IU/L is 19 weeks and LH < 15 IU/L was 19.5 weeks).

How fresh and frozen-thawed transplants differ in restoring hormonal and fertility outcomes

Through the literature search we identified 45 fresh transplants, 11 of which used a graft from a donor (twin sister). Fifteen pregnancies and eight live births were reported for participants receiving fresh ovarian transplantation. Two studies that included five or more participants receiving fresh transplants were included in meta-analysis (Figures 12 and 13). Pooled mean for oestrogen before transplantation was 54.8 pmol/l (SD 7.6) and after fresh ovarian transplant, 403.3 pmol/l (SD 128.3), (MD, 307.31; 95% CI: 159.78 to 454.85; z = 4.08; I^2 = 0%), as shown in Figure 12. Three studies reported FSH in women having received fresh transplants but only two

studies described outcomes in women who were menopausal (FSH > 25 IU/L) at the time of transplantation (Figure 12). Pooled mean for FSH in these women pre-transplant was 83.9 IU/L (SD 2.9) and post-transplant 9.1 IU/L (SD 2.1), (MD, 74.65; 95% CI: -49.91 to 99.39; z = 5.91; I^2 0%).

Figure 12. Oestrogen (pmol/l) in participants having received fresh ovarian transplants. E2 = oestrogen, IV = inverse variance)

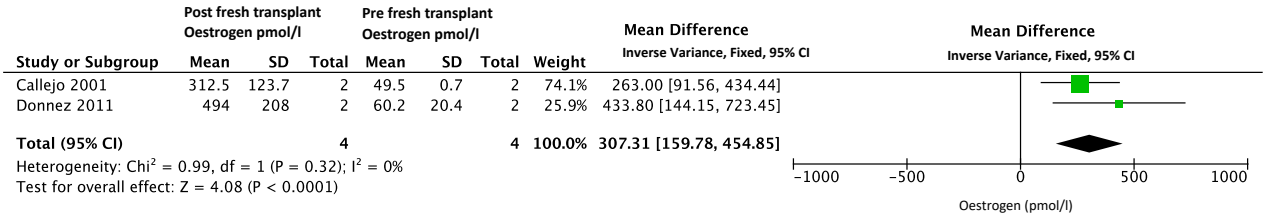
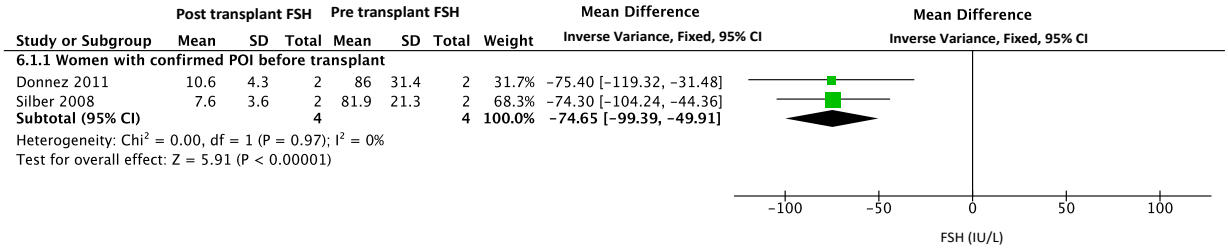


Figure 13. FSH (IU/L) in participants having received fresh ovarian transplants.



Duration of graft function

Duration of the ovarian graft function was reported in 19 studies (181 women). In 15 studies, the authors reported the exact duration (or median duration if more than one patient was reported). The median duration of function was 2.5 years (IQR 1.4 to 3.4 years), range 0.7-5 years. The mean age at cryopreservation for this group of women was 27.1 years (SD 6.8). A further three studies including 26 women with a mean age at cryopreservation 30.3 years (SD 2.5), reported a range with pooled duration of function being 1.2-7.7 years. A case series of three participants who

received fresh ovarian transplants (patient's own ovary) between the ages of 1-5 years experienced menarche as well as 13-15 years of duration of function. (33)

Outcomes based on factors that affect fertility and likelihood of return of endocrine function

Age at ovarian tissue retrieval for cryopreservation

It was found that out of 735 women included in the review, age was provided for 319 women at participant level data. Of these, 283 had their ovarian tissue retrieved for cryopreservation at ≤35 years of age. A subgroup of four studies that reported data on participants age at cryopreservation and transplantation (Figures 14 and 15) were included in meta-analysis. We found that pregnancy rates were higher in participants in whom ovarian tissue was cryopreserved at ≤35 years of age, with results being statistically significant (Odds Ratio, 0.35; 95% CI: 0.13 to 0.92; z = 2.13; P 0.03, I^2 = 0%). Return of hormonal function is shown as a decrease in FSH and was lower in the group that had ovarian tissue frozen at ≤35 years of age (MD, 4.38; 95% CI: -4.29 to 13.05; z = 0.99; P 0.32, I^2 = 0% (Figure 14).

Figure 14. Comparisons of pregnancy rates in participants who had ovarian tissue cryopreserved before the age of 35 years, to those who had ovarian tissue cryopreservation after 35 years of age. Comparison of return of hormonal function (FSH).

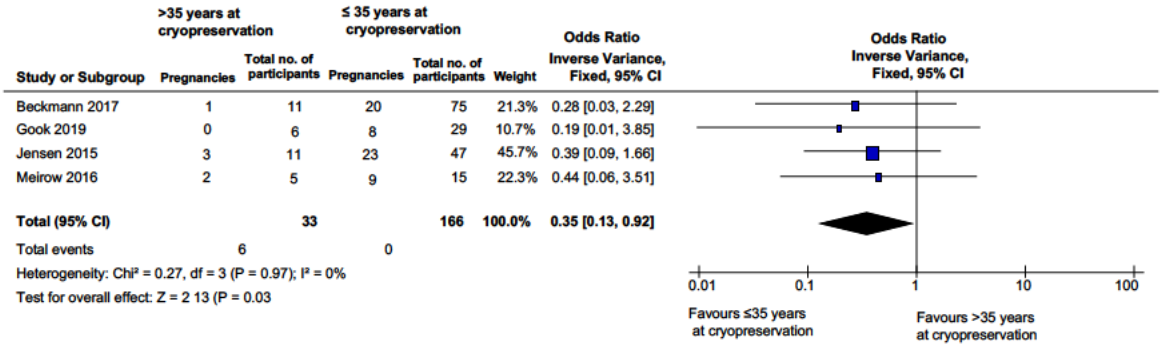
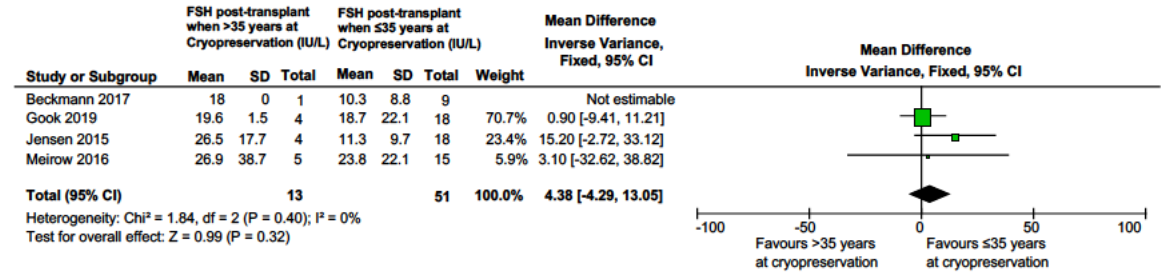


Figure 15. Comparisons of return of hormonal function (FSH) in participants who had ovarian tissue cryopreserved before the age of 35 years, to those who had ovarian tissue cryopreservation after 35 years of age.



Mode of conception

Mode of conception was provided clearly in 276 pregnancies. It was noted that 190 (69%) pregnancies were conceived naturally whereas ART was used for 90 pregnancies (Table 5).

Table 5. Details of mode of conception for pregnancies post ovarian transplantation

Source	Conception	
	Spontaneous	Assisted
Azem 2012		1
Burmeister 2013		1
Callejo 2013		1
Diaz Garcia 2018	7	8
Dolmans 2013	4	1
Donnez 2011	7	8
Donnez and Squifflet 2011	1	
Donnez et al 2004	1	
Donnez et al 2005		1
Donnez et al 2011	1	
Donnez et al 2011 (2)	7	5
Dunlop 2016	1	
Ernst 2013	1	
Fàbregues 2017	1	
Fajau-Prevot 2017	1	
Gook 2019	14	
Haixia 2019	1	
Hoekman 2019	4	2
Imbert 2014 (+IPD)	10	
IPD R Dittrich (Beckmann M.W. 2017)	19	10
Isachenko 2012		1
Janse 2011	2	
Jensen 2015	13	23
Kiseleva 2015		1
Klijn 2019	1	1
Lorenzo 2016	1	1
Meirow 2016	6	10
Milenkovic 2017	1	
Muller et al 2012		1
Oktay 2011	4	
Oktay 2016		2
Ozkavakcu 2019	1	
Poirot 2019	12	1
Revel 2011		1
Revelli 2013	1	
Rodrigeuz- Walberg 2014	1	
Rosendahl 2006		1
Roux 2019	27	
Schmidt 2011	1	2
Shapira 2018	1	1
Silber 2007	4	
Silber 2008	8	
Silber 2018	13	
Suzuki 2015		3
Suzuki 2019	2	
Tammiste 2019	1	
Tanbo 2015	1	

Van der ven 2016	9	2
Zhai 2016		1
Total	**190**	**90**

Anti-cancer therapy before retrieval

Whether a participant received anti-cancer therapy before retrieval of ovarian tissue was reported in 122 out of 735 participants. It was found that 56 out of 122 patients (46%) had received anti-cancer treatment before ovarian tissue cryopreservation. Thirty-five pregnancies and 24 live births were reported in these women. In women with live births, 11 had Hodgkin's lymphoma, five had non-Hodgkin's lymphoma, one had microscopic polyangiitis (MPA), one had acute myeloid leukaemia (AML) and one had Wilms' tumour. Mean age at cryopreservation for women who received chemotherapy before tissue freezing and achieved live birth was 29 years (SD 6). We were able to perform meta-analysis on data from five studies that reported reproductive and endocrine outcomes based on whether the women received anti-cancer treatment before cryopreservation or not. Although the results were not statistically significant, a decrease in FSH, an increase in oestrogen and increased pregnancy rates were noted in participants who did not receive anti-cancer therapy before cryopreservation (Figures 16, 17 and 18)

Figure 16. Comparison of post-transplant oestrogen in participants who received chemotherapy before cryopreservation to those who did not.

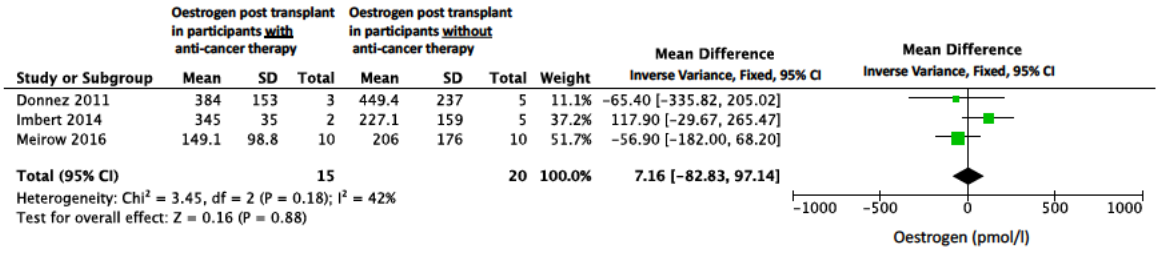


Figure 17. Comparison of post-transplant FSH in participants who received chemotherapy before cryopreservation to those who did not.

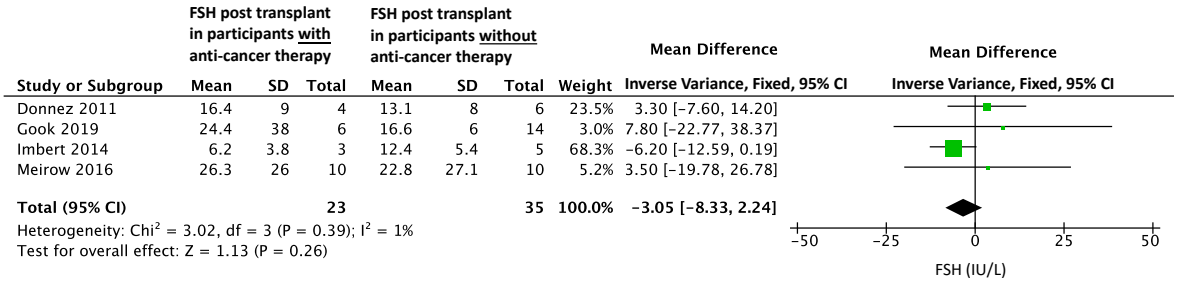
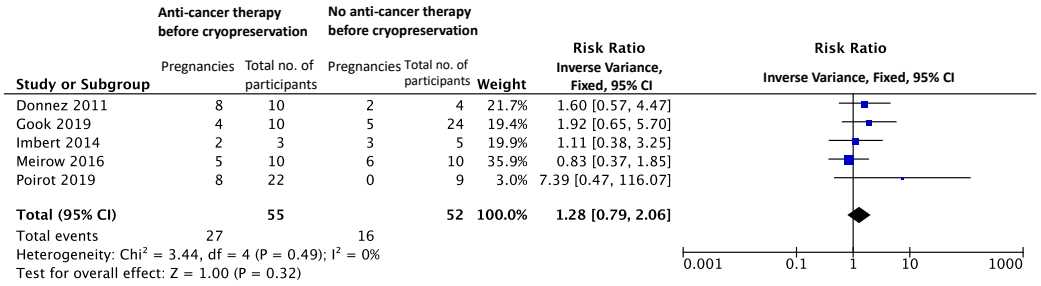


Figure 18. Comparison of pregnancy rates in participants who received chemotherapy before cryopreservation to those who did not.



Type of cancer

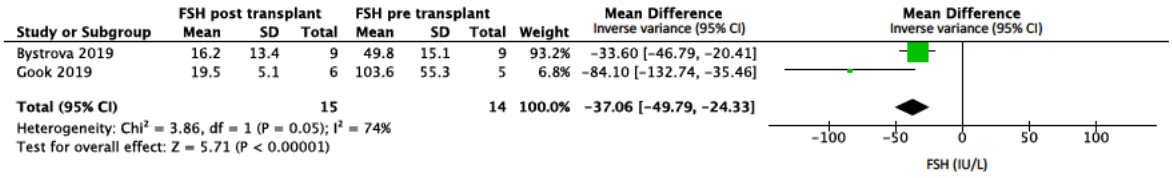
The five most common cancers at participant level included breast cancer, cervical cancer, non-Hodgkin's lymphoma, ovarian cancer and sickle cell anaemia. There was insufficient IPD across the cohorts to be able to perform a meta-analysis and provide pregnancies and live birth rates based on type of cancer. The sums of pregnancies and live birth rates in women with the five most common cancers based on IPD are described in Table 6. Of all the cancer cases reported, we found no live births in women who had suffered with cervical cancer. Given that cervical cancer affects women of reproductive age, transplanting cryopreserved ovarian tissue may help with symptoms of early menopause. To assess endocrine function, we

conducted meta-analysis on two studies that included more than five participants that had cervical cancer (Figure 19). There was a significant reduction in FSH levels; pooled mean for pre transplant FSH was 69.1 IU/L (95% CI: 44.5-93.5) which decreased to 17.6 IU/L (95% CI: 11.7-32.6; MD 37.1; 95% CI: 49.8 to 24.3). The return of hormonal function in this cohort is therefore promising and ovarian tissue cryopreservation and transplantation may be beneficial to these women to help with their menopausal symptoms.

Table 6. Number of pregnancies and live births in women with 5 most common cancers (reported at participant level data).

Type of malignancy	Number of women	Pregnancies	Live births
Breast Cancer	54	14	13 (1 set of twins)
Cervical Cancer	28	0	0
Non-Hodgkin's lymphoma	27	11	7
Ovarian cancer	12	4	4
Sickle cell anaemia	9	3	3

Figure 19. Evidence of return of endocrine function in participants with cervical cancer.



Amount of ovary transplanted to achieve reproductive or endocrine function

There was inconsistency in how the amount of ovarian tissue transplanted was measured. The authors described the amount of the ovarian tissue in volume, strips,

fragments, pieces, biopsies or sections. Not all authors reported a 3-dimensional measurement of the ovarian graft. Owing to the variation in size of the ovary and follicular count at baseline, it is not possible to estimate the optimal amount of tissue for achieving desirable reproductive and hormonal outcomes.

Slow freezing versus vitrification

In our review we found 13 cases in whom vitrification was used to cryopreserve ovarian tissue (34–36). Five pregnancies and two live births were reported. Based on the data available, meta- analysis showed that the cumulative pregnancy rate for ovarian tissue cryopreserved using slow freezing was 37% as compared to vitrification, which was 44% (Figures 20 and 21).

Figure 20. Meta-analysis comparing pregnancy rates using slow freezing or vitrification as a method of cryopreservation

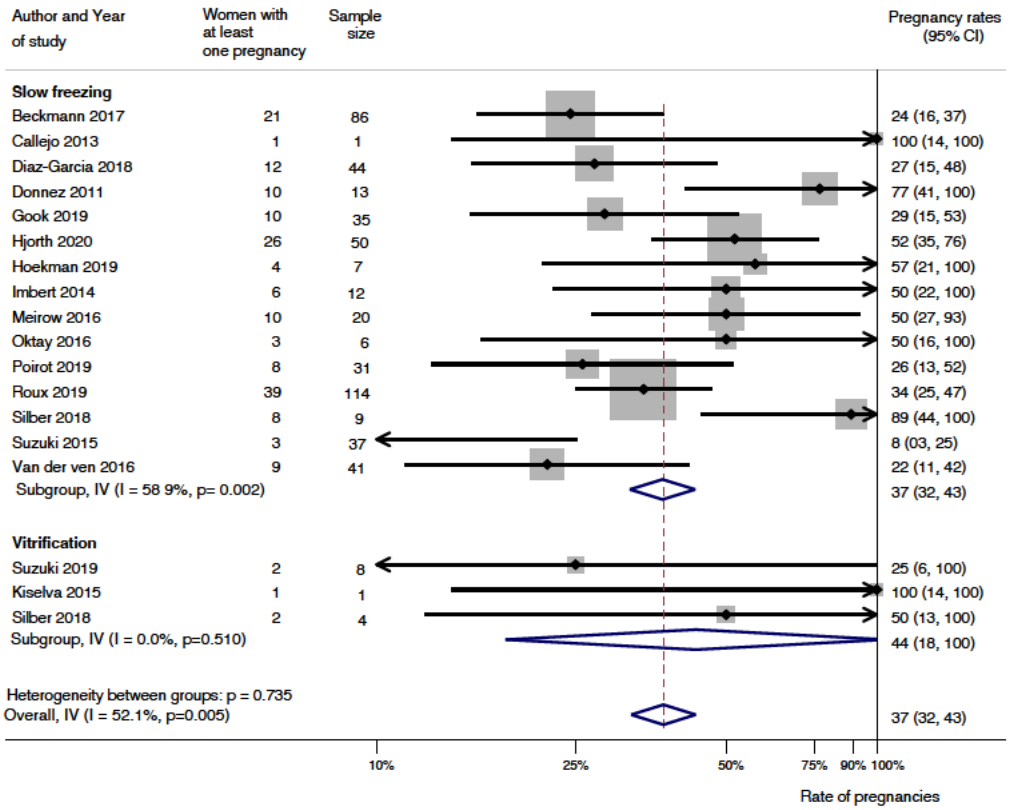
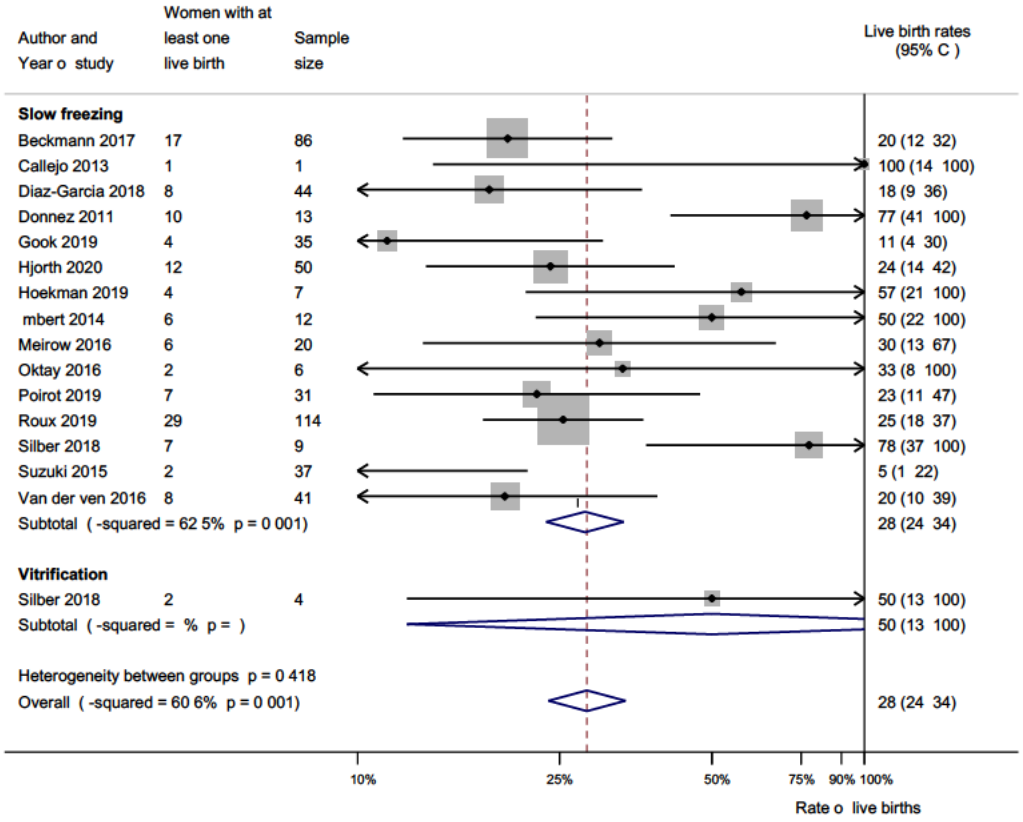


Figure 21. Meta-analysis comparing live birth rates using slow freezing or vitrification as a method of cryopreservation.



Surgical approaches for ovarian tissue retrieval and transplantation

Surgical technique

The surgical approach for retrieval of ovarian tissue was reported for 237 out of 735 women, with 225 women (95% having had laparoscopy and 12 (5%) having had laparotomy. The surgical approach for transplant was reported for 323 women where 205 (64%) had their ovarian tissue transplanted using laparoscopy and 95 (29%) had laparotomy. Other sites and techniques are listed in Table 7. The site of transplant was explicitly reported in 440 participants and described as transplant onto a remaining ovary (orthotopic), pelvic side wall or peritoneal pocket (heterotopic),

transplant on two sites (orthotopic + heterotopic), transplant at three sites (remaining ovary, pelvic side wall and abdominal wall), subcutaneous, subdermal and intramuscular (rectus abdominis and deltoid muscles).

Table 7. Overview of ovarian tissue transplantation technique.

Surgical Approach	Number of patients	Source
Laparoscopy	205	Bystrova 2019; Janse 2011; Poirot 2019; Zhai 2016; Callejo, J., et al. (2013); Donnez et al 2011 (2); Donnez et al 2012; Gook 2019; Beckmann M.W. 2017 + IPD; Oktay 2004; Oktay 2016; Diaz; Garcia et al. 2018; Dunlop et al. 2016; Fabbri et al 2018; Fabbri et al. (2014); Fàbregues et al. (2017); Fajau-Prevot et al 2017; Haixia 2019; Isachenko et al. 2012; Isachenko et al. 2013; Kiseleva et al 2015 ;Lee at al 2018; Lorenzo 2016; Mardesic 2017; Mathews et al 2018; Muller et al 2012; Ozkavakcu 2019; Povoa et al 2016; Radford 2001; Radwan 2016 Revelli 2013
Intramuscular (IM)	6	Oktay 2003; Kim et al 2012; Hilders et al 2004; Laufer 2010; Tammiste 2019
Laparotomy/ Mini laparotomy	95	Almodin et al (2015); Andersen, C. Y., et al. (2012); Donnez et. Al 2008; Meirow 2005; Revel 2011; Tryde Schmidt 2004; Sanchez et al. 2007; Diaz Garcia et al. 2018; Mhatre et al. 2003; Kim et al. 2012; Azem et al 2012; Burmeister, L., et al. (2013); Donnez et al 2011; Donnez et al 2011 (2); Donnez and Squiflet 2010;Ernst 2012; Meirow (2016); Milenkovic 2017; Shapira 2018; Silber 2008; Tanbo 2015 Anderson 2008; Kristensen 2017; Rosendahl 2006; Greve et al.(2012)
Subcutaneous	12	Oktay 2011; Bystrova 2019; Kiran 2004
Submuscular	1	Tammiste et al. 2019

Transplant on to remaining ovary

Of the 440 women, 175 had ovarian tissue transplanted onto their remaining postmenopausal ovary. FSH levels pre and post transplantation were reported in 55 participants, with mean FSH of 71.5 IU/L (SD 44.6) pre transplant and 25.3 (SD

28.5) post transplantation. Oestrogen at pre transplantation and post-transplant was reported in 33 of the 175 participants. Mean oestrogen before transplant was 104.9 pmol/l (SD 143.7) and post-transplant 387.5 pmol/l (SD 419.6). Fifty-two pregnancies and 34 live births have been recorded from transplant onto the remaining ovary.

Pelvic side wall or peritoneal pocket

Ovarian tissue was transplanted onto the pelvic side wall or peritoneal pockets in 184 women. Oestrogen levels at baseline and post-transplant were described in 22 participants. The mean oestrogen level before transplant was 207.5 pmol/l (SD 245.1) and post-transplant 1204.4 pmol/l (SD 1164.3). FSH was reported in 25 participants, with mean pre-transplant FSH of 58.8 IU/L (SD 38.5) and post-transplant 14.4 IU/L (SD 13.2). Forty-one pregnancies and 37 live births were reported.

Transplant onto two sites: pelvic side wall and remaining ovary

Fifty participants had their ovarian tissue transplanted in the pelvic peritoneum as well as the remaining ovary. The mean oestrogen level pre transplantation in these participants was 172 pmol/l (SD 144) and post-transplant 1922 pmol/l (SD 3257.9), reported in 14 participants. FSH was reported in 24 participants with mean pre-transplant 84.7 IU/L (SD 47.6) and post-transplant 20.5 IU/L (SD 21.1). Twenty-eight pregnancies and 10 live births were reported.

Transplant onto three sites: pelvic side wall, remaining ovary and abdominal wall

Eight participants received ovarian transplantation in three sites during one operation. FSH was reported in seven participants with a mean of 62.9 IU/L (SD

28.1) pre transplant and 10.8 IU/L (SD 8.5) post-transplant. There was insufficient data to report on oestrogen post transplantation. Four pregnancies and one live birth were reported. The rest of the transplant sites (abdominal, subcutaneous and intramuscular) are described in Table 8.

Table 8. Reproductive and endocrine outcomes based on site of transplant.

Site of transplant	No. of participants in whom transplant site reported	Endocrine function		Reproductive function		Studies
		Oestrogen n, pre transplant, post-transplant Mean (SD) (pmol/l)	FSH n, pre transplant, post-transplant Mean (SD) (IU/L)	Pregnancies	Live births	
Remaining ovary	175	33, 104.9(143.7), 387.5(419.6)	55, 71.5(44.6), 25.3(28.5)	52	34	Greve et al. 2012; Janse et al. 2011; Meirow et al. 2016; Schmidt et al. 2005; Schmidt et al. 2011; Sanchez et al 2007; Andersen et al. 2012; Andersen et al. 2008; Donnez and Squifflet 2011; Donnez et al. 2005; Donnez et al. 2007; Donnez et al. 2011; Suzuki 2019; Oktay 2016; Almodin et al. 2015; Burmeister et al. 2013; Ernst et al. 2012; Fàbregues et al. 2017; Lorenzo 2016; Mardesic 2017; Revelli 2013; Diaz-Garcia 2018; Rodrigeuz-Walberg et al. 2014; Shapira et al. 2018; Tanbo et al. 2015; Schmidt et al. 2004; Roux et al. 2019
Pelvic side wall or peritoneum	184	22, 207.5(245.1), 1204.4(1164.3)	25, 58.8(38.5) 14.4 (13.2)	41	37	Greve et al. 2012; Poirot et al. 2019; Schmidt et al. 2011; Zhai et al. 2016; Callejo et al. 2013; Donnez et al. 2004, Donnez et al. 2005; Donnez et al. 2011; Donnez et al. 2012; Gook et al. 2019; Bekcman et al. 2017; Azem et al. 2012; Isachenko et al 2012; Kristensen 2017; Lee et al. 2018; Muller et al. 2012; Ozkavakcu et

						al. 2019; Povoa et al. 2016; Radwan et al. 2016; Kodama et al. 2010; Diaz-Garcia 2018, Roux 2019
Pelvic side wall/peritoneum + remaining ovary	50	14, 172(144), 1922(3257)	24, 84.7 (47.6) 20.5 (21.1)	28	10	Greve et al. 2012; Imbert et. Al 2014 Schmidt et al. 2011; Donnez et al. 2006; Donnez et al 2011; Gook 2019; Suzuki et al. 2019; Beckmann et al. 2017; Akar et al. 2011; Ernst et al. 2013; Fabbri et al. 2014; Fajau-Prevot et al. 2017; Mathews et al. 2018; Milenkovic et al. 2017; Radford et al. 2001; Revel et al. 2011
Pelvic side wall + abdominal wall + remaining ovary	8	-	7, 62.9 (28.1) 10.8 (8.5)	4	1	Greve et al. 2012; Schmidt 2005; Schmidt et al. 2011; Anderson et al. 2008
Intramuscular (IM)	6	-	-	-	-	Oktay 2003; Hilders et al 2004; Laufer 2010; Tammiste 2019
Subcutaneous	14	-	-	4	3	Oktay 2011; Bystrova 2019; Callejo 2001; Kiran 2004
Submuscular	3	-	-	-	1	Callejo 2001; Tammiste et al. 2019

Risks of surgery

The included studies did not report any specific complications related to ovarian transplantation other than those of gynaecological laparotomy and laparoscopic surgery. The only complications reported thus far were those of skin infection and injury to surrounding organs (37–39). The largest dataset from 'FertiProtekt' network

(a collaborative network of German speaking countries) that included 71 transplantations in 58 participants did not highlight any ovarian transplantation related complications (32).

Risk of subsequent cancers in the ovarian graft

Through our literature search, we found two cases of cancers reported in the transplanted ovarian graft. A case report diagnosed the recurrence of granulosa cell tumour in a patient at caesarean section delivery. The patient had not received any adjuvant chemotherapy before oophorectomy for ovarian tissue cryopreservation (40). In another case, a patient who was treated for Ewing's sarcoma and had ovarian tissue cryopreserved before receiving chemotherapy, presented with an ovarian mucinous cystadenoma in the transplant (41).

Worldwide activity

The ovarian tissue cryopreservation and transplantation procedure was noted to be the most prominent in Europe, especially in Belgium, Denmark, France, Germany and Spain. A collaborative network of German speaking countries called 'Fertiprotekt' which includes Germany, Switzerland and Austria, forms one of the largest databases of ovarian cryopreservation and transplantation procedures. Figure 19 displays the worldwide activity.

Figure 22. Worldwide ovarian transplantation activity based on cases reported in the literature. Note: The blue pins represent the countries that have specialised centres offering ovarian tissue transplantation. The number of ovarian transplant cases in various countries that are published are as follows: Argentina (1), Australia (37), Belgium (26), China (1), Denmark (80), Estonia (1), France (162), Germany (92), Holland (1), India (3), Israel (24), Italy (5), Japan (8), Korea (12), Poland (1), Portugal (1), Prague (2), Russia (13) Spain (71), Sweden (3), Taiwan (1), Turkey (3), UK (3), USA (38), Multi centre collaborations (65).



Discussion

This meta-analysis includes data from 568 women at individual level, as well as study level, and suggests that ovarian reproductive and endocrine function could be restored using fresh or frozen-thawed ovarian transplantation. The pooled results show a significant decrease in FSH and LH, and an increase in oestrogen post ovarian transplant. The median time to return of FSH to a value of <25 IU/L was 19 weeks, that of LH to a value of <15 IU/L was 19.5 weeks and menstruation was 18 weeks. A total 189 live births were reported in this systematic review. Two recent cohort studies that included 67 participants reported greater than 50% pregnancy rates and more than 40% live birth rates (39,42). Our IPD meta-analysis, however, showed that the live birth rate from frozen-thawed ovarian transplants is 28%, which is in keeping with a recent study published by Dolmans et al. 2021 and previous systematic reviews (6,43–45). The meta-analysis also showed that the live birth rates from fresh transplants was 45% (95% CI 23-86%). However, it is not possible to comment on the difference between fresh and frozen transplants owing to the very small sample size of fresh transplants. Although more miscarriages are reported in the fresh transplant group, sample size is extremely small for a comparison with frozen-thawed transplants. There is limited evidence on whether cryodamage or loss of follicles post transplantation result in less favourable oocytes being fertilised and hence the pregnancy ending in miscarriage. The evidence however suggests that the main concern when transplanting tissue is the loss of follicles caused by ischemia when retransplanting and hence the delay in revascularisation (46). Further research is required to explore whether there is an increased risk of miscarriage from fresh ovarian transplants. Furthermore, pregnancy rates were noted to be higher in participants in whom ovarian tissue was cryopreserved at ≤ 35 years. The results

also suggest that higher concentrations of oestrogen are achieved when pelvic sites and more than one site is used for transplantation. Whether the ovarian tissue is transplanted onto the remaining ovary or pelvic peritoneal pockets, a similar number of live births was achieved (34 live births for transplant on to remaining ovary and 37 if transplanted at a close proximity to the ovary in a peritoneal pocket). It was estimated that ovarian grafts had a median duration of function of 2.5 years (IQR 1.4 to 3.4, range 0.7-5 years). This should be interpreted with caution, however, as the duration of graft function may correlate with the number of transplantations. Moreover, the procedure of ovarian tissue retrieval and transplantation is safe and the only risks associated are known intraoperative and postoperative complications for operative gynaecological laparoscopy and laparotomy (32). Likely variables such as the amount of the tissue transplanted and follicle density which may influence oestrogen level, longevity of function and pregnancy could not be assessed owing to a lack of, or variable, information reported. We found that most centres used slow freezing as a method of ovarian tissue cryopreservation with only 13 cases reported using vitrification. Evidence of pregnancies being achieved shows that vitrification could be considered as a method of cryopreserving ovarian tissue. A systematic review and meta-analysis conducted to assess the proportion of morphologically intact tissue after cryopreservation showed vitrification to be superior to slow freezing (47). However, the protocols used for vitrification vary significantly and are not validated to support a change in practice. The data therefore needs to be interpreted with caution as further studies are required to draw definitive conclusions.

Although most centres will perform a unilateral oophorectomy for fertility preservation in women who require gonadotoxic anti-cancer therapies, through this review we were not able to ascertain whether this alone puts young girls and women at risk of

premature menopause (entering menopause before the age of 40 years). There is evidence to suggest that unilateral oophorectomy appears to reduce the age of menopause by 1-1.8 years (48–51). As for the reproductive outcomes in women who have undergone unilateral oophorectomy, recent evidence suggests that for unassisted reproduction, the outcomes were similar to those with two ovaries but when using ART, reduced live birth rates were reported. This is relevant and reassuring for healthy women who wish to preserve their fertility for social reasons, and who may be worried about their fertility if wishing to try naturally (51). Chemotherapy before tissue retrieval, although reported in a small number of women having tissue transplanted, did not appear to compromise ability to conceive and achieve a live birth, which is very encouraging. This evidence is also supported by a recent study where the authors conclude that anti-cancer therapy before cryopreservation should not be considered a contraindication to opting for this method of fertility preservation (42). Many experts have commented on the possibility of recurrence of primary cancer or even emergence of new cancer in the ovarian graft (52,53). Although patients with cancers that have a high chance of recurrence should be treated with caution, in cases of recurrence it is not always possible to prove that the cancer definitely originated in the transplanted graft.

When examining the type of cancer and related pregnancies and live births achieved, it was interesting to note that no pregnancies were achieved in the cervical cancer cohort: this in keeping with a recently published study in which no pregnancies were reported in this group either (43). A study conducted by Anderson *et al.* (2018) showed that the pregnancy rates were noted to be the lowest in women suffering from cervical cancer as compared to other cancers (standardized incidence ratio = 0.34, 95 % CI: 0.31-0.37) (54). Cervical cancer affects relatively young

women and there has been a steep increase in the incidence of cervical cancer in recent years (55). Fertility preservation in females with cervical cancer is usually achieved by surgery (radical trachelectomy) but is only offered to those with early-stage disease, a good prognosis and ideally those who do not require adjuvant anti-cancer therapy in addition to surgery (56). This limits the candidature for fertility preservation in this cohort. Furthermore, there is evidence to suggest that treatment for cervical cancer (radiotherapy or chemotherapy) compromises uterine function as most women would need to undergo radiotherapy involving the uterus, resulting in fibrosis and scarring (55,57). There is also evidence to suggest that the dose and site of radiation (pelvic or total body) significantly impacts pregnancy outcomes (57). Women who have had a fertility-sparing surgery may therefore still not be able to conceive (58). Offering fertility preservation in this cohort needs to be carefully considered as they may not be able to conceive despite standard methods of fertility preservation before treatment. The return of hormonal function in this cohort, however, is promising and perhaps ovarian tissue cryopreservation could be considered to preserve hormonal function in these women. Furthermore, there is much debate as to whether AMH is valuable in predicting pregnancy and live birth rates in women undergoing OTC. Our data suggests that having a low AMH pre-transplantation does not predict a poor reproductive outcome in young girls and women who want to consider ovarian tissue cryopreservation as a method of fertility preservation. Ideally one needs to measure AMH pre-retrieval, pre-transplant and at various time points post-transplant to be able to accurately predict pregnancy and live birth rates. Another factor to consider is the assay used for analysing the blood test, that adds further confounding to the results. For the reasons mentioned, the

predictability of reproductive outcomes based on AMH alone should be interpreted with caution.

Additionally, through this review we found that more pregnancies were achieved naturally as compared to using ART. Furthermore, recent studies have shown promising results from IVM of immature oocytes in ovarian cortical grafts. A study conducted on ovarian cortical tissue from 25 women demonstrated an unexpectedly high number of metaphase II (MII) oocytes being generated without stimulation (59). This gives further hope to many young girls and women who cannot undergo ovarian stimulation before chemotherapy, for achieving motherhood from ovarian tissue cryopreservation and transplantation.

Ovarian tissue cryopreservation and transplantation may still be deemed as experimental by some centres, however many experts are now considering the potential use of this procedure in clinical practice and offering it as a routine fertility preservation method (60–62). Evidence suggests that ovarian tissue cryopreservation may be particularly applicable to pre-pubertal girls and those at high risk of POI who require immediate gonadotoxic anticancer therapy and cannot wait for oocyte retrieval (63,64). As per recommendation by the American Society for Reproductive Medicine, ovarian tissue cryopreservation is an acceptable technique for preserving fertility and is no longer considered experimental (61). A fertility preservation guideline recently published by the ESHRE also recommends offering ovarian tissue cryopreservation in patients undergoing moderate to high risk gonadotoxic treatment, if patient prefers this method over oocyte and embryo freezing (65). A guideline by the British Fertility Society (BFS) on fertility preservation for medical reasons in females describes the limitations of this technique when

applied to adolescents and children. With increasing evidence of live births and return of endocrine function however, the BFS concludes that ovarian tissue freezing should be considered in pre-pubertal girls (63,66). The BFS committee also agreed on the advantages of this technique with regards to the possibility of natural conception (especially if the ovarian tissue is transplanted in the pelvis, close to the fallopian tube) and proposed its use in post-pubertal patients, especially if oocyte cryopreservation is not possible. Furthermore, the possibility of continued hormone production from these transplants in order to prevent menopause has been emphasised by various experts in the literature (7,66,67). Therefore ovarian transplants could also potentially be used as cell-based hormonal replacement therapy.

Strengths

We performed a comprehensive search of the literature and synthesised the evidence from all studies available in this systematic review. While previous reviews have shed light on the outcomes of this procedure in the form of reproductive or hormonal function, the results have mainly been restricted to frozen-thawed transplants. This is the first systematic review that included outcomes from both fresh, frozen and donor ovarian tissue transplantation using unique IPD meta-analysis. Through a broad literature search, we identified all the reported ovarian transplant cases in addition to adding participant-level data through collaboration with centres that are established in providing this procedure. We also considered grey literature that includes an account of unsuccessful cases in various centres, conference proceedings and unpublished cases. This allowed inclusion of 87 studies

and 735 women. To our knowledge, this is the largest collation of ovarian tissue transplantation outcomes to date. Our meta-analysis model can be updated, as such having the potential to create a worldwide network of ovarian cryopreservation and transplantation activity.

Furthermore, various relevant outcomes that determine reproductive and endocrine function, such as age at cryopreservation and transplantation technique, were analysed in addition to the time of return of hormonal function to premenopausal levels. We were also able to collate the outcomes based on disease, chemotherapy prior to tissue retrieval, and site of transplant.

Limitations

Most of the studies included small numbers of participants, which reduces our confidence in true success rates of this procedure. In some studies, hormonal function was described as being assessed immediately before tissue retrieval whereas others assessed on the day of transplant, just prior to the procedure. For studies that did not explicitly describe the timing of hormonal tests, it is not possible to assess accurately the premenopausal status before the transplant. Also, in women having a remaining ovary, albeit menopausal, there is uncertainty regarding residual hormonal function from that ovary. Many studies also failed to mention the longevity of the graft to give an accurate estimate of the hormonal lifespan of the ovarian tissue. We were also unable to calculate the time to pregnancy. Not all pregnancies were conceived naturally and so it was not possible to predict how long it would have taken to conceive naturally. Through this review, 45 cases of fresh ovarian transplants were identified but meta-analysis was only possible for two studies with a very small sample size (four women). Although the results were

reassuring in terms of decreasing FSH to a level of <25 IU/L and levels of oestrogen increasing to >200pmol/l, it was not possible to make a comparison with frozen-thawed transplants owing to such a small sample size.

Furthermore, clinical heterogeneity of the studies included in the meta-analysis resulted in weakness in our analysis. To overcome this, we endeavoured to gather IPD from authors and six centres agreed to provide this and further clarification. Collecting IPD was one of the significant challenges of this project. In order to help authors who may have a lack of time, funding or organisational support, we offered assistance in data collation and also sent them an outcome spreadsheet to assist with data collection (68). From the 10 authors and centres that did not share data, six did not respond to emails despite reminders and four centres informed us that they were awaiting further publications or were simply not keen on collaboration despite a positive initial response. A scoping review that involved assessing the outcomes of IPD requests found that for academic studies eligible for IPD requests, only 33% provided the data (69). We have managed to acquire data from 38% of the centres. Furthermore, we had requested data for 493 participants, from centres that had previously reported cohorts of five or more patients: we received data for 220 patients from six centres, which gives us a response rate of 45%. But despite the novelty of IPD meta-analysis on this topic, it has its limitations. When more than one publication originated from the same centre, population or cohort, there was a possibility for double counting the patients. For this reason, reports were individually assessed by two reviewers independently to identify and remove duplicates. This was also double checked by cross referencing and contacting the authors directly for clarification, and with the most recent or complete publication being selected. To ensure that the IPD and aggregate samples were a random sample from the

population of interest, we compared the studies and conducted a meta-analysis of reproductive outcomes. We found that the live birth rate for studies that provided aggregate data was 28% (95% CI 23-35%) compared to that for IPD, which was 30% (95% CI 23-38%). This supports the assumption that the data are missing at random and the ones that did not provide IPD are not systematically different from the ones that provided it (Appendix 2, 3 and 4).

Despite the efforts of gathering IPD, due to the heterogeneity of the included studies, the available outcome measures in this study need to be interpreted with caution. In this review, we measured the success of ovarian transplantation using various outcome measures such as pregnancy rate, live birth rate and return of hormonal function however there are limitations to these outcome measures that can impact the clinical interpretation of study results. Reproductive outcome measures such as pregnancy and live birth rate in women (whether undergoing cancer treatment or not) are affected by many factors such as age, the quality of oocytes, extent of damage to the ovaries from gonadotoxic treatment etc. Although we have conducted a meta-analysis to provide pregnancy and live birth rates, the data used is largely heterogenous. Similarly, endocrine outcome measures using hormone levels such as FSH, LH and AMH were measured at different time points and may not have accurately detected changes in the ovarian reserve and fertility potential. Furthermore, although we endeavoured to provide reproductive outcomes based on markers of ovarian reserve such as FSH, it should be noted that FSH is not a predictor of live birth in patients who undergo OTC. While FSH is used as a marker of ovarian reserve and can provide information about a woman's fertility potential, it is not a reliable indicator of the likelihood of achieving a successful pregnancy. Other factors such as age, AMH, overall health, and the type of gonadotoxic treatment

used, need to be considered when assessing a woman's fertility and likelihood of a successful pregnancy.

Lastly, the ovarian transplant procedure is carried out worldwide in different centres with variable protocols and some degree of heterogeneity is to be expected. This review also highlights how this procedure is currently provided, showing lack of consensus in the delivery of this technique as well as an urgent unmet need for a worldwide registry.

Implications for clinical practice

Through previous publications and this review, we can conclude that ovarian tissue transplantation has shown promising results in preserving ovarian reproductive and endocrine function. This procedure should no longer be considered experimental, and it should be offered to women who wish to preserve their fertility out of the research context. The potential use of transplants in preserving hormonal function is an area that needs to be explored further. There is currently no robust guidance for clinicians or patients about this procedure and its potential uses. With its potential use in alleviating menopausal symptoms and improving quality of life, there is an urgent unmet need for a robust policy and standard. The results of this review will hopefully guide clinicians in advising women about the benefits and shortcomings of this procedure until a formal guideline is produced.

Implications for further research

Further data with larger studies that include participants with results that were not successful need to be included to give an accurate success rate of this procedure. Furthermore, a robust guideline for the optimal size of the tissue graft and surgical

procedure for retrieval and transplantation of ovarian tissue needs to be produced to guide centres with this technique. There is also an urgent unmet need for a worldwide registry to provide a database that records all cases of ovarian transplantation. Lastly, predefined outcome sets need to be determined to ensure all randomized control trials, cohort studies or case series report similar outcomes for ovarian transplantation.

Data availability statement

The data underlying this article are available in the article and its online supplementary material.

Acknowledgements

We thank Mr Derick Yates (Library & Knowledge Service, Birmingham Women's Hospital) for his assistance in designing the search strategy and literature search.

Authors' roles

H.K. and A.C. designed the study. H.K. ran the literature search, extracted the data, liaised with authors for participant level data, performed association analysis and drafted the manuscript. R.M. extracted the data as a second reviewer and critical revised the manuscript. I.G. contributed to developing the search strategy, literature search, drafting the manuscript and statistical analysis related to reproductive outcomes. L.C. contributed to study design, data interpretation and critical analysis of the manuscript. S.S., D.G., I.D., O.B., A.L., G.M., L.L, R.D, L.B.C., K.T.M., I.M.D.H. and S.G.K. helped in collating data from their respective centres and contributed to participant level data. Y.A., C.A., and S.F. interpreted the data and

critically reviewed the manuscript. All authors approved the final version of the manuscript.

Funding

This study and H.K. were supported through a Wellbeing of Women entry-level scholarship, award reference ELS904 (Registered Charity No.: 239281).

Conflict of interest

None.

References

1. International Agency for Research on Cancer (IARC), World Health Organization (WHO). *Estimated number of new cases in 2020, all cancers, females, ages 0-39.* Available from: https://gco.iarc.fr/today/online-analysis-table?v=2020&mode=population&mode_population=continents&population=900&populations=900&key=asr&sex=2&cancer=39&type=0&statistic=5&prevalence=0&population_group=0&ages_group%5B%5D=0&ages_grou

2. Stam H, Grootenhuis M, Last B. Social and emotional adjustment in young survivors of childhood cancer. *Supportive Care in Cancer* 2001; **9**: 489–513.

3. Langeveld N, Stam H, Grootenhuis M, Last B. Quality of life in young adult survivors of childhood cancer. *Supportive Care in Cancer* 2002; **10**: 579–600.

4.

5. Nieman CL et al. Fertility Preservation and Adolescent Cancer Patients: Lessons from Adult Survivors of Childhood Cancer and Their Parents. In: Woodruff TK, Snyder KA (eds) Oncofertility Fertility Preservation for Cancer. Cancer Treatment and Research. 2007. Springer, Boston, MA, pp. 201-217.

6. Jayasuriya S, Peate M, Allingham C, et al. Satisfaction, disappointment and regret surrounding fertility preservation decisions in the paediatric and adolescent cancer population. *J Assist Reprod Genet* 2019; **36**: 1805-1822.

7. Gellert SE, Pors SE, Kristensen SG, Bay-Bjørn AM, Ernst E, Andersen CY. Transplantation of frozen-thawed ovarian tissue: an update on worldwide activity published in peer-reviewed papers and on the Danish cohort. *Journal of Assisted Reproduction and Genetics* 2018; **35**: 561–570.

8. Andersen CA, Mamsen LS, Kristensen SG. Freezing of ovarian tissue and clinical opportunities. Reproduction 2019; **158**: 27–34.

9. Donnez PJ, Dolmans MM, Demylle D, Jadoul P, Pirard C, Squifflet J, Langendonckt Van A. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. *Lancet* 2004; **364**: 1405–1410.

10. Meirow D, Levron J, Eldar-Geva T, Hardan I, Fridman E, Zalel Y, Dor J et al. Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. *New England Journal of Medicine* 2005; **353**: 318–321.

11. Silber SJ, Lenahan, KM, Levine DJ, Pineda JA, Gorman KS, Friez MJ, Gosden RG. Ovarian transplantation between monozygotic twins discordant for premature ovarian failure. *N Engl J Med* 2005; **353**: 58–63.

12. Oktay K, Türküolu I, Rodriguez-Wallberg KA. Four spontaneous pregnancies and three live births following subcutaneous transplantation of frozen banked ovarian tissue: What is the explanation? *Fertility and Sterility* 2011; **95**: 804.e7-804.e10.

13. Andersen CY, Silber SJ, Berghold SH, Jorgensen JS, Ernst E. Long-term duration of function of ovarian tissue transplants: Case reports. *Reproductive BioMedicine Online* 2012; **25**: 128–132.

14. Silber S, Pineda J, Lenahan K, Derosa M, Melnick J. Fresh and cryopreserved ovary transplantation and resting follicle recruitment. *Reproductive BioMedicine Online* 2015; **30**: 643–650.

15. Grynberg M, Poulain M, Sebag-Peyrelevade S, Le Parco S, Fanchin R, Frydman N. Ovarian tissue and follicle transplantation as an option for fertility preservation. *Fertility and Sterility* 2012; **97**: 1260–1268.

16. Donnez J, Dolmans MM. Ovarian Cortex Transplantation: 60 Reported Live Births Brings the Success and Worldwide Expansion of the Technique towards Routine Clinical Practice. *J Assist Reprod Genet* 2015; **32**: 1167–70.

17. Jensen AK, Kristensen SG, Macklon KT, Jeppesen JV, Fedder J, Ernst E, Andersen CY. Outcomes of transplantations of cryopreserved ovarian tissue to 41 women in Denmark. *Human Reproduction* 2015; **30**: 2838–2845.

18. Callejo J, Salvador C, Miralles A, Vilaseca S, Lailla JM, Balasch J. Long-term ovarian function evaluation after autografting by implantation with fresh and frozen-thawed human ovarian tissue. *Journal of Clinical Endocrinology and Metabolism* 2001; **86**: 4489–4494.

19. Donnez J, Squifflet J, Dolmans MM, Martinez-Madrid B, Jadoul P, Van Langendonckt A. Orthotopic transplantation of fresh ovarian cortex: A report of two cases. *Fertility and Sterility* 2005; **84**: 1018.e1-1018.e3.

20. Mhatre P, Mhatre J. Orthotopic ovarian transplant - Review and three surgical techniques. *Pediatric Transplantation* 2006; **10**: 782–787.

21. Sánchez M, Alamá P, Gadea B, Soares SR, Simón C, Pellicer A. Fresh human orthotopic ovarian cortex transplantation: Long-term results. *Human Reproduction* 2007; **22**: 786–791.

22. Silber SJ, Derosa M, Pineda J, Lenahan K, Grenia D, Gorman K, Gosden RG. A series of monozygotic twins discordant for ovarian failure: Ovary transplantation (cortical versus microvascular) and cryopreservation. *Human Reproduction* 2008; **23**: 1531–1537.

23. Donnez J, Dolmans MM, Squifflet J, Kerbrat G, Jadoul P. Live birth after allografting of ovarian cortex between monozygotic twins with Turner syndrome (45,XO/46,XX mosaicism) and discordant ovarian function. *Fertility and Sterility* 2011; **96**: 1407–1411.

24. Almodin CG, Almodin PM, Radaelli MRM, Minguetti-Câmara VC, Ceschin, AP, Ribeiro RC. The first ovarian tissue transplant between monozygotic twin sisters discordant for ovarian function in Latin America. *Jornal Brasileiro de Reproducao Assistida* 2015; **19**: 29–32.

25. Silber S, Kagawa N, Kuwayama M, Gosden R. Duration of fertility after fresh and frozen ovary transplantation. *Fertility and Sterility* 2010; **94**: 2191–2196.

26. Khattak H, Malhas R, Craciunas L, Fishel S, Amorim C, Afifi Y, Gallos I and Coomarasamy A. To review the effects of fresh and cryopreserved ovarian tissue transplantation for preserving fertility and endocrine function. *PROSPERO 2018* CRD42018115233 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42018115233

27. European society of human reproduction and embryology (ESHRE) Guideline Group on POI, Webber L, Davies M, Anderson R, Bartlett J, Braat D, Cartwright B, Cifkova R, S. Keizer-Schrama de M, Hogervorst E, Janse F, Liao L, Vlaisavljevic V, Zillikens C, Vermeulen N. ESHRE Guideline: management of women with premature ovarian insufficiency. *Human Reproduction* 2016; **31**: 926–937.

28. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Available to download from Ottawa Hospital Research Institute's website: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp

29. Demets DL. Methods for combining randomized clinical trials: strengths and limitations. *Stat Med* 1987; **6**: 341–350.

30. Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre. The Cochrane Collaboration, 2014.

31. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002; **21**: 1539-1558.

32. Middle JG, Kane JW. Oestradiol Assays: Fitness for Purpose? *Annals of Clinical Biochemistry* 2009; **46**: 441–56.

33. Beckmann MW, Dittrich R, Lotz L, van der Ven K, van der Ven HH, Liebenthron J, Toth B. Fertility protection: complications of surgery and results of removal and transplantation of ovarian tissue. *Reproductive BioMedicine Online* 2017b; **36**: 188–196.

34. Laufer MR, Upton J, Schuster SR, Grier H, Emans SJ, Diller L. Ovarian Tissue Autologous Transplantation to the Upper Extremity for Girls Receiving Abdominal/Pelvic Radiation: 20-year Follow-up of Reproductive Endocrine Function. *Journal of Pediatric and Adolescent Gynecology* 2010; **23**: 107–110.

35. Kiseleva M, Malinova I, Komarova E, Shvedova T, Chudakov K, Kaprin A. The First Russian Case of Pregnancy after Orthotopic Transplantation of Vitrified Ovarian Tissue. *Gynecological Endocrinology* 2015; **31**: 91–92.

36. Silber SJ, DeRosa M, Goldsmith S. et al. Cryopreservation and transplantation of ovarian tissue: results from one center in the USA. *J Assist Reprod Genet* 2018; **35**: 2205–2213.

37. Iwahata Hi, Takae S, Iwahata Y, et al. Ovarian tissue transplantation: 8 case experiences of single fertility preservation center in Japan. Abstracts from the 2019 ISFP meeting in NYC. *J Assist Reprod Genet* 2020; **37**: 987–1015.

38. Rosendahl M, Schmidt KT, Ernst E, Rasmussen PE, Loft A, Byskov AG, Andersen CY et al. Cryopreservation of ovarian tissue for a decade in Denmark: A view of the technique. *Reproductive BioMedicine Online* 2011; **22**: 162–171.

39. Dolmans MM, Jadoul P, Gilliaux S, Amorim CA, Luyckx V, Squifflet J, Van Langendonckt A. A review of 15 years of ovarian tissue bank activities. *Journal of Assisted Reproduction and Genetics* 2013; **30**: 305–314.

40. Hoekman EJ, Louwe LA, Rooijers M, van der Westerlaken LAJ, Klijn NF, Pilgram GSK, de Kroon CD, Hilders CG. Ovarian Tissue Cryopreservation: Low Usage Rates and High Live-Birth Rate after Transplantation. *Acta Obstetricia et Gynecologica Scandinavica* 2020; **99**: 213–21.

41. Stern CJ., Gook D, Hale LG, Agresta F, Oldham J, Rozen G, Jobling T. Delivery of twins following heterotopic grafting of frozen-thawed ovarian tissue. *Human Reproduction* 2014; **29**: 1828.

42. Fajau-Prevot C, Le Gac YT, Chevreau C, et al. Ovarian Mucinous Cystadenoma After Ovarian Graft. *Obstet Gynecol* 2017; **129**: 1035-1036.

43. Shapira M, Dolmans MM, Silber S, and Meirow D. Evaluation of Ovarian Tissue Transplantation: Results from Three Clinical Centers. *Fertility and Sterility* 2020; **114**: 388–97.

44. Dolmans, MM et al. Transplantation of Cryopreserved Ovarian Tissue in a Series of 285 Women: A Review of Five Leading European Centers. *Fertility and Sterility* 2021; **115**: 1102–15.

45. Lotz L, Dittrich R, Hoffmann I, Beckmann MW. Ovarian tissue transplantation: experience from Germany and worldwide efficacy. *Clinical Medicine Insights: Reproductive Health* 2019; **13:** 1-8.

46. Sheshpari S, Shahnazi M, Mobarak H, Ahmadian S, Bedate AM, Nariman-Saleh-Fam Z, Mahdipour M et al. Ovarian function and reproductive outcome after ovarian tissue transplantation: A systematic review. *J Transl Med* 2019; **17**: 396.

47. Lee J, Kong HS, Kim EJ, Youm HW, Lee JR, Suh CS, Kim SH. Ovarian injury during cryopreservation and transplantation in mice: a comparative study between cryoinjury and ischemic injury. *Human reproduction* 2016; **31**(8): 1827–1837.

48. Shi Q, Xie Y, Wang Y et. Al. Vitrification versus slow freezing for human ovarian tissue cryopreservation: a systematic review and meta-anlaysis. *Scientific Reports* 2017; **7**: 8538.

49. Cramer D and Huijuan X. Predicting Age at Menopause. *Maturitas* 1996; **23**: 319–26.

50. Yasui T, Hayashi K, Mizunuma H, Kubota T, Aso T, Matsumura Y, Lee JS, Suzuki S. Factors associated with premature ovarian failure, early menopause and earlier onset of menopause in Japanese women. *Maturitas* 2012; **72**: 249–255.

51. Bjelland, E K. Wilkosz P, Tanbo TG, Eskild A. Is Unilateral Oophorectomy Associated with Age at Menopause? A Population Study (the HUNT2 Survey). *Human Reproduction* 2014; **29**: 835–41.

52. Gasparri, ML et al. Biological Impact of Unilateral Oophorectomy: Does the Number of Ovaries Really Matter? *Geburtshilfe und Frauenheilkunde* 2021; **81**: 331–338.

53. Dittrich R, Hackl J, Lotz L, Hoffmann I, Beckmann MW. Pregnancies and live births after 20 transplantations of cryopreserved ovarian tissue in a single center. *Fertility and Sterility* 2015; **103**: 462-468.
54. Kristensen SG, Giorgione V, Humaidan P, Alsbjerg B, Bjørn A-M B, Ernst E, Andersen CY. Fertility preservation and refreezing of transplanted ovarian tissue—a potential new way of managing patients with low risk of malignant cell recurrence. *Fertility and Sterility* 2017; **107**: 1206-1213.
55. Anderson RA, Brewster DH, Wood R, Nowell S, Fischbacher C, Kelsey TW, Wallace HB. The impact of cancer on subsequent chance of pregnancy: a population-based analysis. *Human Reproduction* 2018; **33**: 1281–1290.
56. Arbyn M, Weiderpass E, Bruni L, de Sanjosé S, Saraiya M, Ferlay J, Bray F. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. *The Lancet. Global health* 2020; **8**(2): e191–e203.
57. Bentivegna E, Sebastien G, Amandine M, Cyrus C, Alexandra L, Philippe M. Oncological Outcomes after Fertility-Sparing Surgery for Cervical Cancer: A Systematic Review. *The Lancet Oncology* 2016; **17**(6): e240–53.
58. Teh WT, Stern C, Chander S, and Hickey M. The Impact of Uterine Radiation on Subsequent Fertility and Pregnancy Outcomes. *BioMed Research International* 2014; 482968.
59. Somigliana E, Mangili G, Martinelli F, Noli S, Filippi F, Bergamini A, Bocciolone L, Buonomo B, Peccatori, F. Fertility preservation in women with cervical cancer. *Critical reviews in oncology/hematology* 2020; **154**: 103092.
60. Nikiforov D, Junping C, Cadenas J, et al. Improving the maturation rate of human oocytes collected ex vivo during the cryopreservation of ovarian tissue. *J Assist Reprod Genet* 2020; **37**: 891-904.
61. Donnez J, Dolmans MM, Diaz-Garcia C, Pellicer A. Ovarian cortex transplantation: Time to move on from experimental studies to open clinical application. *Fertility and Sterility* 2015; **104**: 1097–1098.

62. Practice Committee of the American Society for Reproductive Medicine. Fertility preservation in patients undergoing gonadotoxic therapy or gonadectomy: a committee opinion. *Fertility and Sterility* 2019; **112** :1022-1033.

63. Donnez J, Dolmans M. Fertility preservation in women. *Nature Reviews Endocrinology* 2013; **9**: 735-749.

64. Lambertini M, Del Mastro L, Pescio MC, Andersen CY, Azim HA, Peccatori FA, Costa M, Revelli A, Salvagno F et al. Cancer and fertility preservation: International recommendations from an expert meeting. *BMC Medicine* 2016; **14**: 1–16.

65. Matthews SJ, Picton H, Ernst E, Andersen CY. Successful pregnancy in a woman previously suffering from β-thalassemia following transplantation of ovarian tissue cryopreserved before puberty. *Minerva Ginecol* 2018; **70**: 432-435.

66. European society of human reproduction and embryology (ESHRE) Fertility preservation guideline group. Female fertility preservation. 2020. Available from: https://www.eshre.eu/Guidelines-and-Legal/Guidelines/Female-fertility-preservation

67. Yasmin E, Balachandren N, Davies MC, et al. Fertility preservation for medical reasons in girls and women: British fertility society policy and practice guideline. *Hum Fertil (Camb)* 2018; **21**: 3-26.

68. Donnez J, Dolmans MM. Natural hormone replacement therapy with a functioning ovary after the menopause: dream or reality? *Reproductive BioMedicine Online* 2018; **37**: 359–366.

69. Nevitt SJ, Marson AG, Davie B, Reynolds S, Williams L, Smith CT. Exploring Changes over Time and Characteristics Associated with Data Retrieval across Individual Participant Data Meta-Analyses: Systematic Review. *BMJ* 2017; **357**: j1390.

70. Ventresca M, Schünemann HJ, Macbeth F et al. Obtaining and managing data sets for individual participant data meta-analysis: scoping review and practical guide. *BMC Med Res Methodol* 2020; **20:** 113.

71. Beckmann MW, Dittrich R, Lotz L, Oppelt PG, Findeklee S, Hildebrandt T, Müller A. Operative techniques and complications of extraction and transplantation of ovarian tissue: the Erlangen experience. *Archives of Gynecology and Obstetrics* 2017a; **295**: 1033–1039.

72. Bystrova O, Lapina E, Kalugina A, Lisyanskaya A, Tapilskaya N, Manikhas G. Heterotopic transplantation of cryopreserved ovarian tissue in cancer patients: a case series. *Gynecological Endocrinology* 2019; **35**: 1043–1049.

73. Bystrova O, Lapina E, Kalugina A, Lisyanskaya A, Manikhas GM. Spontaneous pregnancy after transplantation of cryopreserved ovarian tissue: a case report. *Problemy reproduktsii* 2019; **25**: 51-54.

74. Callejo J, Salvador C, González-Nuñez S, Almeida L, Rodriguez L, Marqués L, Lailla JM. Live birth in a woman without ovaries after autograft of frozen-thawed ovarian tissue combined with growth factors. *Journal of Ovarian Research* 2013; **6**: 1–6.

75. Diaz-Garcia C, Domingo J, Garcia-Velasco JA, Herraiz S, Mirabet V, Iniesta I, Pellicer A. Oocyte vitrification versus ovarian cortex transplantation in fertility preservation for adult women undergoing gonadotoxic treatments: a prospective cohort study. *Fertility and Sterility* 2018; **109**: 478-485.

76. Dolmans MM, Donnez J, Camboni A, Demylle D, Amorim C, Van Langendonckt A, Pirard, C. IVF outcome in patients with orthotopically transplanted ovarian tissue. *Human Reproduction* 2009; **24**: 2778–2787.

77. Donnez J, Dolmans MM, Demylle D, Jadoul P, Pirard C, Squifflet J, Van Langendonckt A. Restoration of ovarian function after orthotopic (intraovarian and periovarian) transplantation of cryopreserved ovarian tissue in a woman treated by bone marrow transplantation for sickle cell anaemia: Case report. *Human Reproduction* 2006; **21**: 183–188.

78. Donnez J, Dolmans MM, Pirard C, Van Langendonckt A, Demylle D, Jadoul P, Squifflet, J. Allograft of ovarian cortex between two genetically non-identical sisters: Case Report. *Human Reproduction* 2007; **22**: 2653–2659.

79. Donnez J, Dolmans MM. Fertility preservation in women. *New England Journal of Medicine* 2017; **377**: 1657–1665.

80. Donnez J, Silber S, Andersen CY, Demeestere I, Piver P, Meirow D, Dolmans MM. Children born after autotransplantation of cryopreserved ovarian tissue. A review of 13 live births. *Annals of Medicine 2011*; **43**: 437–450.

81. Donnez J, Squifflet J, Jadoul P, Demylle D, Cheron AC, Van Langendonck A, Dolmans MM. Pregnancy and live birth after autotransplantation of frozen-thawed ovarian tissue in a patient with metastatic disease undergoing chemotherapy and hematopoietic stem cell transplantation. *Fertility and Sterility* 2011; **95**: 1787.e1-1787.e4.

82. Donnez J, Squifflet J, Pirard C, Jadoul P, Dolmans MM. Restoration of ovarian function after allografting of ovarian cortex between genetically non-identical sisters. Human Reproduction 2010; **25**: 2489–2495.

83. Gook D et al. Experience with Transplantation of Human Cryopreserved Ovarian Tissue to a Sub-Peritoneal Abdominal Site. *Human reproduction (Oxford, England)* 2021; **36**(9): 2473–83.

84. Haixia D, Ningxia S, Wen L. The first livebirth of orthotopic transplantation of cryopreserved ovarian tissues.

85. Hilders CG, Baranski AG, Peters L, Ramkhelawan A, Trimbos JB. Successful human ovarian autotransplantation to the upper arm. *Cancer* 2004; **101**: 2771–2778.

86. Hjorth IMD, Kristensen SG, Dueholm M, Humaidan P. Reproductive outcomes after in vitro fertilization treatment in a cohort of Danish women transplanted with cryopreserved ovarian tissue. *Fertility and Sterility* 2020; **114**: 379-387.

87. Imbert R, Moffa F, Tsepelidis S, Simon P, Delbaere A, Devreker F, Demeestere I. Safety and usefulness of cryopreservation of ovarian tissue to preserve fertility: A 12-year retrospective analysis. *Human Reproduction* 2014; **29**: 1931–1940.

88. Janse F, Donnez J, Anckaert E, H. de Jong F, Fauser BCJM, Dolmans MM. Limited Value of Ovarian Function Markers following Orthotopic Transplantation of Ovarian Tissue after Gonadotoxic Treatment. *The Journal of Clinical Endocrinology & Metabolism* 2011: **96**; 1136–1144.

89. Kiran G, Kiran H, Coban YK, Guven AM, Yuksel M. Fresh autologous transplantation of ovarian cortical strips to the anterior abdominal wall at the Pfannenstiel incision site. *Fertility and Sterility* 2004; **82**: 954–956.

90. Kiran G, Kiran H, Coban YK, Guven AM. Ovarian cortical transplantation may be an alternative to hormone therapy in patients with early climacterium. *Fertility and Sterility* 2005; **84**: 1509.e1-1509.e3.

91. Kodama Y, Sameshima H, Ikenoue T, Ikeda T, Kawagoe Y. Successful fresh whole ovarian autotransplantation without vascular anastomosis. *Fertility and Sterility* 2010; **94**: 2330.e11-2330.e12.

92. Lee JR, Lee D, Park S, Paik EC, Kim SK, Jee BC, Kim SH et al. Successful in vitro fertilization and embryo transfer after transplantation of cryopreserved ovarian tissue:Report of the first Korean case. *Journal of Korean Medical Science* 2018; **33**: 1–7.

93. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, Moher D et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ* 2009; **339**: b2700.

94. Meirow D, Ra'anani H, Shapira M, Brenghausen M, Derech Chaim S, Aviel-Ronen S, Dor J et al. Transplantations of frozen-thawed ovarian tissue demonstrate high reproductive performance and the need to revise restrictive criteria. *Fertility and Sterility* 2016; **106**: 467–474.

95. Milenkovic M et al. Spontaneous Twin Pregnancy with Live Births after Cryopreservation and Re-Implantation of Ovarian Tissue. *Gynecological Surgery* 2017; **14**(1): 4–6.

96. Oktay K, Bedoschi G, Pacheco F, Turan V, Emirdar V. First pregnancies, live birth, and in vitro fertilization outcomes after transplantation of frozen-banked ovarian tissue with a human extracellular matrix scaffold using robot-assisted minimally invasive surgery. *American Journal of Obstetrics and Gynecology* 2016; **214**: 94.e1-94.e9.

97. Oktay K, Buyuk E, Rosenwaks Z, Rucinski J. A technique for transplantation of ovarian cortical strips to the forearm. *Fertility and Sterility* 2003; **80**: 193–198.

98. Oktay K, Oktem O. Ovarian cryopreservation and transplantation for fertility preservation for medical indications: report of an ongoing experience. *Fertility and Sterility* 2010; **93**: 762–768.

99. Ozkavavukcu S, Sonmezer M. First ongoing pregnancy following frozen-thawed ovarian tissue re-transplantation in Turkey in a patient with cured acute lymphoblastic leukemia. Abstracts from the 2019 ISFP meeting in NYC. *J Assist Reprod Genet* 2020; **37**: 987–1015.

100. Poirot C, Brugieres L, Yakouben K, Prades-Borio M, Marzouk F, de Lambert G, Dalle JH. Ovarian tissue cryopreservation for fertility preservation in 418 girls and adolescents up to 15 years of age facing highly gonadotoxic treatment. Twenty years of experience at a single center. *Acta Obstetricia et Gynecologica Scandinavica* 2019; **98**: 630–637.

101. Poirot C, Fortin A, Lacorte JM, Akakpo JP, Genestie C, Vernant JP, Boissel N et al. Impact of cancer chemotherapy before ovarian cortex cryopreservation on ovarian tissue transplantation. *Human Reproduction* 2019; **34**: 1083–1094.

102. Póvoa A, Xavier P, Calejo L, Soares S, Sousa M, Silva J, Beires J et al. First transplantation of cryopreserved ovarian tissue in Portugal, stored for 10 years: An unexpected indication. *Reproductive BioMedicine Online* 2016; **32**: 334–336.

103. Radford JA, Lieberman BA, Brison DR, Smith ARB, Critchlow JD, Russell SA, Shalet SM et al. Orthotopic reimplantation of cryopreserved ovarian cortical strips after high-dose chemotherapy for Hodgkin's lymphoma. *Lancet* 2001; **357**: 1172–1175.

104. Radwan P, Abramik A, Wilczyński J, Radwan M. Successful autotransplantation of cryopreserved ovarian tissue with recovery of the ovarian function. *Ginekologia Polska* 2016; **87**: 235–240.

105. Revel A, Laufer N, Ben Meir A, Lebovich M, Mitrani E. Micro-organ ovarian transplantation enables pregnancy: A case report. *Human Reproduction* 2011; **26**: 1097–1103.

106. 108. Revelli A, Marchino G, Dolfin E, Molinari E, Delle Piane L, Salvagno F, Benedetto C. Live birth after orthotopic grafting of autologous cryopreserved ovarian tissue and spontaneous conception in Italy. *Fertility and Sterility* 2013; **99**: 227–230.

107. Rodriguez-Wallberg KA, Karlström PO, Rezapour M, Castellanos E, Hreinsson J, Rasmussen C, Hovatta O. Full-term newborn after repeated ovarian tissue transplants in a patient treated for Ewing sarcoma by sterilizing pelvic irradiation and chemotherapy. *Acta Obstetricia et Gynecologica Scandinavica* 2015; **94**: 324–328.

108. Rosendahl M, Simonsen MK, Kjer JJ. The influence of unilateral oophorectomy on the age of menopause. *Climacteric* 2017; **20**: 540-544.

109. Roux C, Pech JC, Amiot C, et al. Ovarian tissue transplantation in France. Data from the GRECOT (Research and study group for ovarian and testicular cryopreservation) register. Abstracts from the 2019 ISFP meeting in NYC. *J Assist Reprod Genet* 2020; **37,** 987–1015.

110. Schmidt KLT, Andersen CY, Starup J, Loft A, Byskov AG, Andersen AN. Orthotopic autotransplantation of cryopreserved ovarian tissue to a woman cured of cancer - Follicular growth, steroid production and oocyte retrieval. *Reproductive BioMedicine Online* 2004; **8**: 448–453

111. Shapira M, Raanani H, Barshack I, Amariglio N, Derech-Haim S, Marciano MN, Meirow, D et al. First delivery in a leukemia survivor after transplantation of cryopreserved ovarian tissue, evaluated for leukemia cells contamination. *Fertility and Sterility* 2018; **109**: 48–53.

112. Silber SJ. Ovary cryopreservation and transplantation for fertility preservation. *Molecular Human Reproduction* 2012; **18**: 59–67.

113. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC. StataCorp. 2015.

114. Suzuki N, Yoshioka N, Takae S, Sugishita Y, Tamura M, Hashimoto S, Kawamura K et al. Successful fertility preservation following ovarian tissue vitrification in patients with primary ovarian insufficiency. *Human Reproduction* 2015; **30**: 608–615.

115. Tammist, T, Kask K, Padrik P, Idla K, Rosenstein K, Jatsenko T, Salumets A. A case report and follow-up of the first live birth after heterotopic transplantation of cryopreserved ovarian tissue in Eastern Europe. *BMC Women's Health* 2019; **19**: 1–8.

116. Tanbo T, Greggains G, Storeng R, Busund B, Langebrekke A, Fedorcsak P. Autotransplantation of cryopreserved ovarian tissue after treatment for malignant disease - The first Norwegian results. *Acta Obstetricia et Gynecologica Scandinavica* 2015; **94**: 937–941.

117. Van Der Ven H, Liebenthron J, Beckmann M, Toth B, Korell M, Krüssel J, Dittrich R et al. Ninety-five orthotopic transplantations in 74 women of ovarian tissue after cytotoxic treatment in a fertility preservation network: Tissue activity, pregnancy and delivery rates. *Human Reproduction* 2016; **31**: 2031–2041.

118. von Wolff M, Germeyer A, Liebenthron J, Korell M, Nawroth F. Practical recommendations for fertility preservation in women by the FertiPROTEKT network. Part II: fertility preservation techniques. *Archives of Gynecology and Obstetrics* 2018; **297**: 257–267.

119. Wølner-Hanssen P, Hägglund L, Ploman F, Ramirez A, Manthorpe R, Thuring A. Autotransplantation of cryopreserved ovarian tissue to the right forearm 4 1/2 years after autologous stem cell transplantation. *Acta Obstetricia et Gynecologica Scandinavica* 2005; **84**: 695–698.

120.

121. Yin CS, Huang KC, Chiu HM. A 51-month follow-up of fresh autologous hemi-ovary transplantation at the Pfannenstiel incision site after hysterectomy and accidental bilateral salpingo-oophorectomy in a premenopausal woman. *Taiwanese Journal of Obstetrics and Gynecology* 2009; **48**: 69–71.

122. Zhai J, Yao G, Dong F, Bu Z, Cheng Y, Sato Y, Sun Y. In vitro activation of follicles and fresh tissue auto-transplantation in primary ovarian insufficiency patients. *Journal of Clinical Endocrinology and Metabolism* 2016; **101**: 4405–4412.

CHAPTER 3: FRESH OVARIAN TISSUE AUTO-GRAFTING IN MAINTAINING ENDOCRINE FUNCTION: A CLINICAL SERIES.

Preamble

The original study was given favourable opinion by research and ethics committee on 30th November 2018.

A substantial amendment was sought and approved on 5th June 2019.

A further non substantial amendment was sought and approved on 6th March 2020.

Due to the COVID-19 pandemic, it was not possible to follow up patients. A substantial amendment was therefore sought so that patients could be followed up at any time point when presented for their appointment. The amendment was granted on 19th February 2021.

Abstract

Background: Many women undergo surgery for removal of either their ovaries or a significant part of the ovaries for non-malignant indications for example endometriosis and ovarian cysts. Consequently, total or significant loss of ovarian function can occur and cause premature menopause and permanent fertility loss with significant compromise on quality of life. Although hormonal replacement therapy (HRT) is the only available treatment for premature menopause, frozen thawed ovarian transplantation has been shown to return ovarian endocrine function in women with cancer as well as those with benign conditions. Furthermore, fresh ovarian transplantation has been used previously and can potentially prevent menopausal symptoms by providing endogenous hormones. The aim of this study was to assess the return of ovarian endocrine function in patients having undergone fresh ovarian tissue transplantation.

Methods: Participants undergoing unilateral or bilateral oophorectomy, who had consented to ovarian tissue transplantation were approached to take part in this study. Hormonal function was assessed at follow up appointments over a 2-year period using blood tests: follicle stimulating hormone (FSH), luteinizing hormone (LH), progesterone and anti-mullerian hormone (AMH). Graft viability was assessed using ultrasound scans. Symptoms of menopause were assessed using the quality of life questionnaire (MenQol).

Results: This observational study included 14 women aged 29 to 45 (Mean 36.1 years, SD 3.9) who underwent fresh ovarian autotransplantation to preserve hormonal function in a tertiary hospital. The size of the graft was approximately

10x10x1mm. Participants had follow up blood tests that included FSH, LH, oestrogen and progesterone as well as ultrasound scans to assess follicular activity in the ovarian graft. At 6 months, oestrogen post transplantation was recorded in five patients, four of whom had oestrogen levels of <100pmol/l before surgery. The mean oestrogen level post-transplant was 334.2pmol/l (SD 187.2pmol/l). FSH at 6 months was reported in five participants. Mean baseline FSH was noted to be 3.4 IU/L (SD 1.7). Mean FSH post-transplant was 18.9 (SD 15.3). The mean age of participants in this cohort was 35.6 years (SD 1.9). LH was also reported in 5 participants. Mean baseline LH was 2.02 IU/L (SD 2) and post-transplant 14.1 (SD 12.5). Longer follow up was only available for 2 participants (loss to follow up and lack of appointment availability due to the pandemic).

Conclusion: Elevated levels of oestrogen post-surgery indicate preservation of ovarian endocrine function using fresh ovarian autografts. Return of ovarian endocrine function using ovarian transplants needs to be explored further as it could help with symptoms of menopause in young women undergoing bilateral oophorectomy. Further research should include larger sample sizes and observation over longer periods of time to ascertain how long the grafts will work for. Future research is also required to assess the best transplantation techniques, optimal graft sizes and transplantation site to provide adequate hormone levels to prevent early onset of menopause.

Introduction

Premature ovarian insufficiency (POI)

Premature ovarian insufficiency is defined as amenorrhea which is caused by loss of ovarian function before the age of 40. Premature ovarian insufficiency affects 1% of women under 40 years and 0.1% of women under 30 years in the UK. (1–3) Premature ovarian insufficiency is different to natural menopause in that management options and patient needs vary considerably.

Causes of POI

Various causes of POI have been described and divided into those that appear as primary amenorrhea or those that result in secondary amenorrhea (depending on if occurred before or after menarche). Pre-menarche POI could be caused by chromosomal abnormalities or genetic defects such as Turner's syndrome, whereas POI post menarche could be due to iatrogenic factors such gonadotoxic anti-cancer therapy or surgery, but many causes remain unknown. (1,4) In premenopausal women, surgical primary ovarian insufficiency (POI) is the most common cause of ovarian hormone deficit. (5)

Iatrogenic POI

Ovarian failure as a result of medical treatment is termed iatrogenic POI. Interventions such as chemotherapy, radiotherapy or surgical removal of ovaries can

result in POI. Young women may undergo bilateral oophorectomy for issues like prevention of breast and ovarian cancer, pelvic pain and severe endometriosis, which results in early onset of menopause. For non-malignant diseases such as the ones mentioned above, there is often variation in practice when conducting bilateral salpingo-oophorectomies (BSO). (6) To avoid ovarian cancer from occurring later in life, bilateral salpingo-oophorectomy is typically offered at the time of hysterectomy for non-malignant conditions. (6) However, because of the possible risks associated with the loss of ovarian hormone production, this treatment is increasingly being advised against. (6) It is estimated that about 23% of women between ages of 40-44 undergo elective BSO with hysterectomy. (7,8) There is a fine balance to be achieved between offering BSO considering the age and risks of malignancy. (5)

There is evidence to suggest that surgical menopause is linked to worsened symptoms from oestrogen depletion such as hot flushes, night sweats, vaginal dryness and loss of libido, as compared to natural menopause. (9) One reason for this is the abrupt loss of ovarian function as compared to the gradual onset with natural menopause. (9,10) Women who experience ovarian insufficiency as a result of oophorectomy therefore present a unique situation which can often be debilitating for young women.

The need for bilateral oophorectomy in benign gynaecological conditions

Although it is recommended that healthy premenopausal ovaries be retained unless there is a risk of ovarian cancer, it is not always possible to do so. Non-oncological reasons for oophorectomy include pelvic pain, endometriosis and benign ovarian

masses. Young women in particular who suffer with severe endometriosis and pelvic pain usually opt for bilateral oophorectomy as a last resort. (11)

Consequences of surgical POI

Depletion of oestrogen as a result of POI increases the risk of osteoporosis, cardiovascular disease, cognitive decline, loss of libido, dementia, and a concomitant increase in morbidity and mortality. (2,3) Women with ovarian conservation before the natural age of menopause, have a reduced long-term risk of cognitive decline and dementia compared to women who undergo bilateral oophorectomy. (7,12–14) Symptoms experienced during surgical menopause are similar to those experienced by women who develop POI due to other reasons, however, iatrogenic POI involves a more rapid development of symptoms due to abrupt deficiency of oestrogen, progesterone and testosterone, and a disruption of the hypothalamic-pituitary-ovarian axis. (10) One study that compared the health-related quality of life in women having undergone surgical menopause, with those who had natural menopause, found that symptoms of menopause were worse in women after surgical menopause. (15)

Treatment options for POI

Therapy choices and patient needs for premature ovarian insufficiency differ from those for typical menopause. Currently the only treatment options for young women with POI are either the combined oral contraceptive (COC) or hormone replacement therapy (HRT), with HRT being the only option for women undergoing surgical menopause. A Cochrane review conducted on the topic of post-surgical HRT for women with endometriosis concluded that exogenous hormones could potentially

result in further pain and disease recurrence. Although the notion should not deprive young women from using HRT, further studies are required to make definitive assumptions. (16)

HRT for women with surgical menopause

Although HRT is currently the best recognised pharmaceutical option for aiding with symptoms of menopause, there is evidence to suggest that oestrogen alone cannot counteract the symptoms of menopause. Various preparations of HRT are available; however it is not suitable for all women, especially who are well below the age of natural menopause as they may require higher than usual doses. (17)

Ovarian tissue transplantation (OTT) as a means of endogenous hormone production

A recent fertility preservation technique of ovarian tissue cryopreservation (OTC) and transplantation (OTT) that has shown promise in returning reproductive function in women, has also been shown to return ovarian endocrine function. (18–23) The systematic review conducted on the topic (Chapter 2) also alludes to the potential of return of endocrine function using ovarian transplants. Use of ovarian transplants for endocrine function alone however is not reported widely.

Fresh ovarian autografting

Leporrier et al. 1987 described whole ovary transplantation to the forearm of a patient with Hodgkin's disease, to avoid pelvic irradiation to the ovary. This new technique provided successful outcomes for patient as resumption of menstruation as well as follicular growth were noted. Since then, there have been many cases of

fresh ovarian tissue transplantation, successful in restoring fertility and endocrine function. (24–30) One study in particular described a case series of 25 women who underwent fresh and frozen ovary transplantation where FSH levels returned to normal and menstrual cycles resumed in all women. Live births were also reported. (27,31) Although fresh autografts are expected to be just as effective in restoring ovarian function as cryopreserved tissue, the role of fresh autografts in preserving ovarian endocrine function is explored in only a handful of studies. Donnez et al. reported fresh ovarian autografting in women with severe endometriosis, where histological examination revealed survival of primordial follicles. (32) This however is the only study that reports using fresh autografts for a benign disease. The list of fresh ovarian transplants as reported in the literature are displayed in Table 9.

Table 9 Reported cases of fresh ovarian transplantation.

Study	**Number of reported cases**	**Type of fresh transplant**	**Disease/Reasons for transplant**	**Site of transplant**	**Outcome**
Leporrier et al. 1987(24)	1	Autograft (whole ovary)	Hodgkin's disease	Forearm	Menstruation and follicular development
Marconi et al. 1997 (25)	1	Autograft (accidental at surgery)	Endometriotic cyst	Abdominal wall trocar site	Follicular growth
Almodin et al. (2015) (33)	1	Donor	POI	Recipient ovary	Return of hormonal function and follicular development. No pregnancy reported
Oktay et al. 2003 (28)	2	Autograft	Stage IIIB squamous cell cervical carcinoma, n =1 Recurrent benign ovarian cysts, n =1	Subcutaneous	Return of hormonal function and follicular development. No pregnancy reported
Laufer et al. 2010 (34)	3	Autograft	Wilms tumour	Subcutaneous (axilla), n = 1, deltoid muscle belly, n = 1	Menarche, follicular development and

				Triceps muscle belly, n = 1	return of hormonal function
Kodama et al. 2010 (35)	1	Autograft	Caesarean hysterectomy (accidental bilateral oophorectomy)	Retroperitoneum	Return of hormonal function
Kiran et al. 2004 (29)	1	Autograft	Fibroids	Anterior abdominal wall	Return of hormonal function and follicular development
Donnez et al. 2010 (36)	3	Donor	POI due to: Beta-thalassemia major, n = 1 Homozygous sickle cell anemia, n = 1 Acute myelogenous leukemia, n = 1 (all required bone marrow transplant)	Not explicitly described	Return of hormonal function
Hilders et al. 2004 (37)	1	Autograft	Cervical cancer	Upper arm	Not given
Donnez et al. 2011 (38)	1	Donor	Turner syndrome	Ovarian hilus	Restoration of ovarian activity and pregnancy
Donnez et al. 2005 (32)	2	Autograft	Severe endometriosis	Peritoneal window (pelvic side wall)	Follicular growth
Callejo et al. 2001 (39)	3	Autograft	Fibroids	Subcutaneous (arm) = 2 Rectus abdominis muscle = 1	Return of ovarian endocrine function
Silber et al. 2005 (26)	1	Donor	POI	Medulla of the recipient	Return of ovarian endocrine function, follicular development and live birth
Silber et al. 2008 (31)	8	Donor	POI = 7 Acute lymphoblastic leukaemia =1	Medulla of the recipient	Return of ovarian endocrine function, follicular development and live births
Zhai et al. 2016 (40)	1	Autograft (in vitro activated)	POI	Beneath the serosa of both fallopian tubes	Follicular growth and hormonal function (although modulated with GnRH agonists for ART)

Yin et al. 2009 (41)	1	Autograft	Uterine fibroids and dense pelvic adhesions	Subcutaneous tissue of the Pfannenstiel incision	Preservation of ovarian endocrine function and follicular development
Sanchez et al. 2007 (42)	12	Autograft	Fibroids = 10, endometrial polyps = 1, menorrhagia = 1	Medulla of the contralateral ovary	Preservation of ovarian endocrine function and follicular development

The fresh autografting procedure was approved to be applied clinically at Birmingham Women's Hospital as a new procedure, and an alternative for ovarian conservation in women with endometriosis. Through this study we aimed to preserve ovarian endocrine function by transplantation of fresh ovarian cortical strips during routine bilateral oophorectomies. For young women undergoing oophorectomies, this was thought to be their only hope of endogenous hormone production from the ovary, preventing POI and its symptoms. The aim of this study is therefore to assess the effectiveness of fresh ovarian transplants in women undergoing BSO for benign disease.

Methods

Study Design

This was an observational study of women having surgery that was likely to lead to significant compromise of ovarian reserve (for e.g. oophorectomy or ovarian cystectomy for endometriosis). These women had healthy strips of the ovarian cortex removed and implanted into a peritoneal fold, subdermal or vaginal with the aim to preserve ovarian function. Women with documented menopause or POI were excluded. All women were offered baseline tests for their hormone profile (follicle stimulating hormone (FSH), Oestrogen, progesterone and luteinising hormone (LH) as part of their routine care and anti-müllerian hormone (AMH) where available.

Women were also requested to complete a validated quality of life, menopause specific questionnaire at follow up appointments (in person, virtually or over the phone, electronically or by post).

Inclusion criteria

- Women
- Age: 18-45 years
- Undergoing surgery (or having undergone surgery previously) for the removal of one or both ovaries and received fresh ovarian transplantation
- Patients will be offered tests and investigations prior to the surgery: FSH, LH, oestrogen, progesterone and AMH (if possible) and ultrasound scan (USS) for visibility of follicles and if possible follicular count

Exclusion criteria:

- Women suffering from menopause or premature ovarian failure (this did not include patients on GnRH agonists)
- Women younger than 18 or older than 45 years.
- Women who require fertility treatments.
- Women who are pregnant at the time of the study investigations.
- Women who are participants in other interventional studies or trials.
- Women suffering from cancer

Consent process

The notes of all the women who are attending the clinic were screened. Women with the potential of removal of one or both their ovaries were given the patient information sheet whilst they waited. The doctor or research nurse in Gynaecology outpatient clinic verified eligibility for participation in the study, provided verbal and written information and instructions about the study and obtained written consent. If during consultation it was confirmed that these patients were to have a surgery for removal of their ovaries, they were invited to take part in the study. If the patient had any questions, they were answered during consultation. If the patient required time to consider participation, the consent was sought either on the day of the surgery or at a follow up appointment. All participants were consented before the COVID-19 pandemic. For patients that had ovarian transplant surgery before this study commenced but not recruited previously, were also approached during their follow up appointment (telephone, virtual or in person). These patients had the option of completing the consent form in person, over the phone, electronically or by post. Recruitment strategy is shown in Figure 23.

Surgical technique

The steps of ovarian tissue retrieval and transplantation are as follows (Figure 24):

1. Patient was prepared for routine laparoscopic surgery
2. Vasopressin is injected into the ovarian cortex followed by a stay stitch to manipulate the ovary
3. Curved micro scissors were then used to remove the cortex
4. The medulla is carefully dissected, and the cortex is trimmed to 1x1cm (1mm thick)

5. The tissue was transplanted back to the pelvic side wall using two to four simple inverted stitches.

Follow up

Hormonal function was assessed at follow up appointments, over a 2-year period using blood tests; FSH, LH, progesterone and AMH. The follow up was to be done at two time points with blood tests, ultrasound assessment of the ovarian autograft and assessment of menopausal symptoms using a validated quality of life questionnaire (Menqol). Any patients suffering with menopausal symptoms were started on HRT and not included in the analysis. Graft viability was assessed using an ultrasound scan. Due to the COVID-19 pandemic, an amendment was sought due to appointments being held virtually or over the phone.

Figure 23. Recruitment strategy.



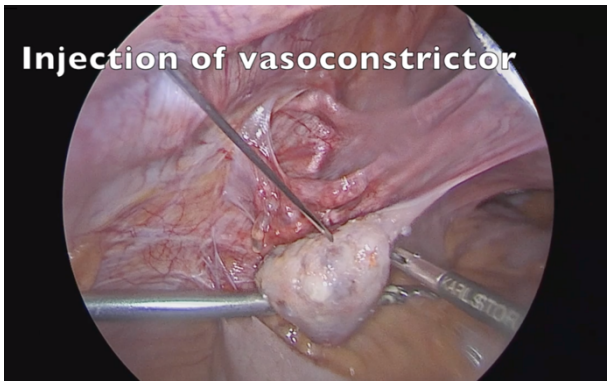
Results

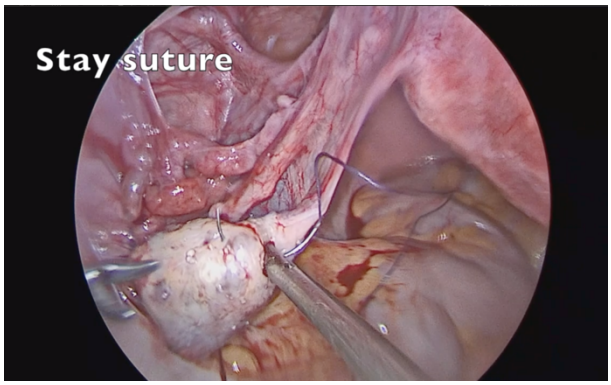
Participants

Twenty-one patients were assessed for eligibility and 14 were recruited to the study. Of the 14 patients, 13 had a laparoscopic approach to the surgical procedure and one had an open abdominal approach. Four patients had unilateral oophorectomies but two of them had previously had an oophorectomy. Originally the patients were to be followed up at 6 and 12 months but due to availability of clinic appointments, an amendment was sought, after which patients were followed up when they came for a routine outpatient appointment with their clinical care provider.

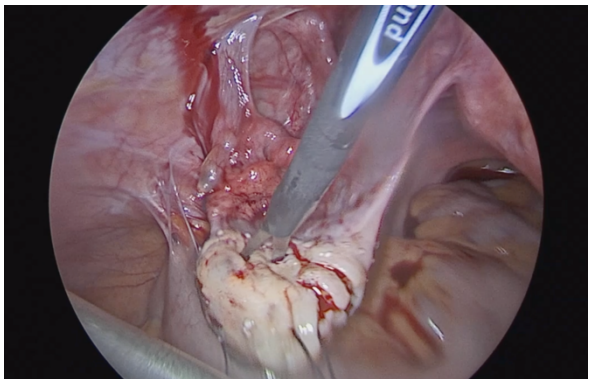
The mean age at transplantation surgery was 36.1 years (SD 3.9). Underlying reasons for surgery were endometriosis and pelvic pain. The study flow chart is shown below (Figure 25).

Figure 24. Fresh ovarian autografting technique.

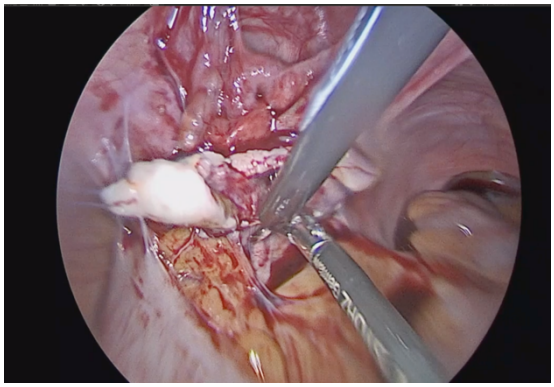




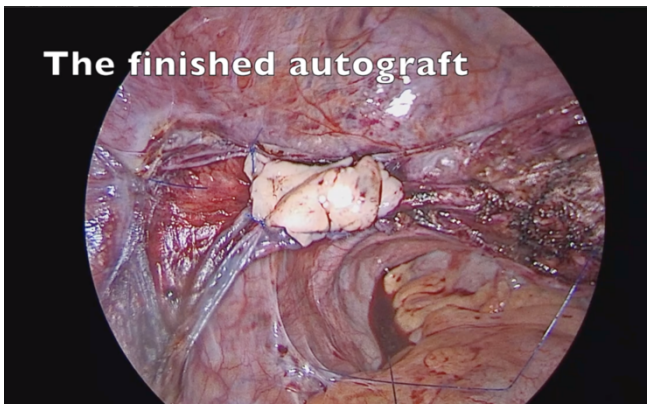
After routine entry into the abdominal cavity, vasopressin is injected into the ovarian cortex followed by a stay stitch to manipulate the ovary.



Curved micro scissors are then used to remove the cortex.

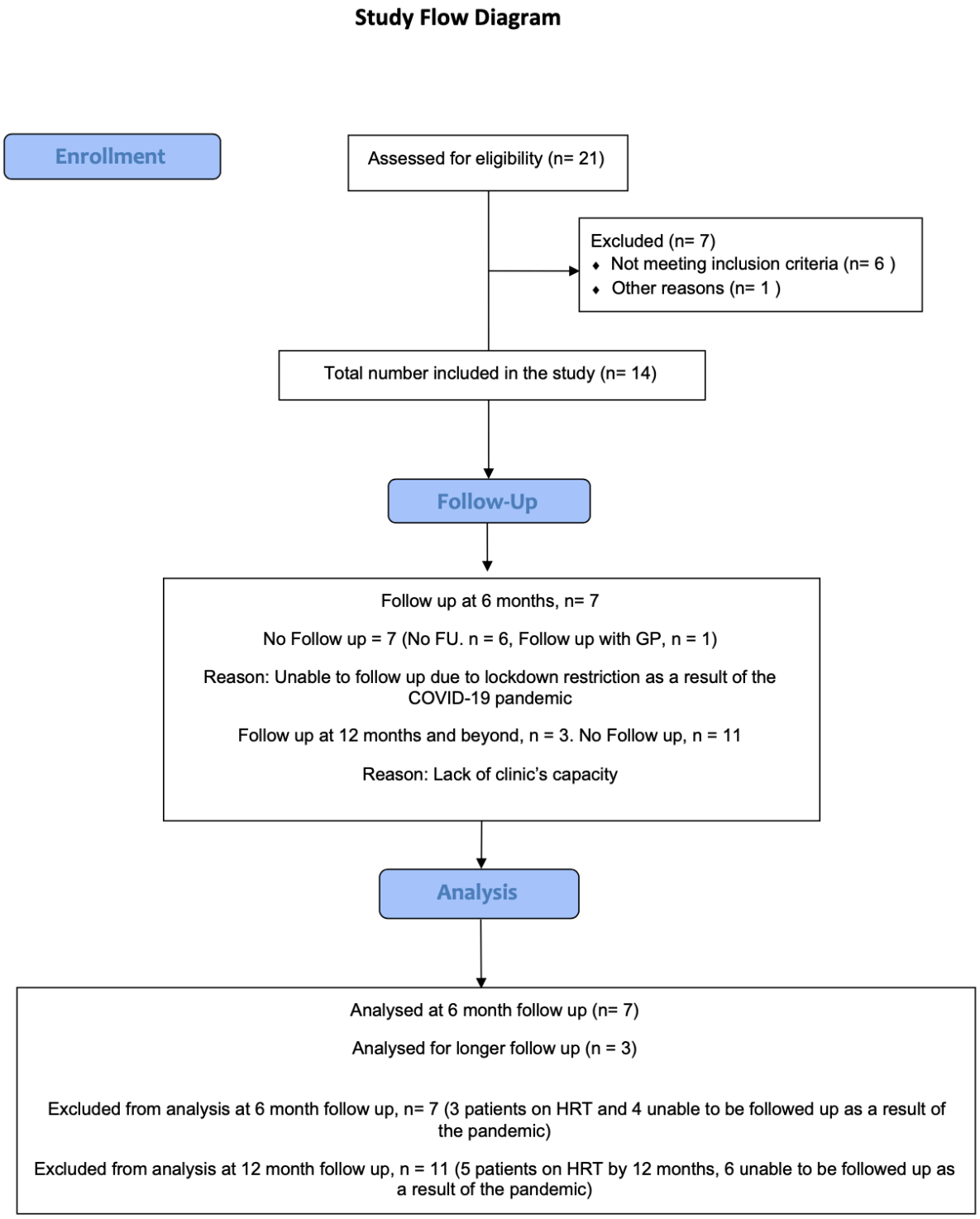


The medulla is carefully dissected, and cortex trimmed to 1x1cm (1mm thick).



The tissue is transplanted back to pelvic side wall using two to four simple inverted stitches.

Figure 25. Study flow chart.



Sites of grafts

All grafts were cut to approximately 10 x 10 x 1mm in size by the surgeon before transplantation. Seven patients (50%) had transplantation to left pelvic side wall, five (36%) to right pelvic side wall and two participants (14%) had transplantation to more than one site (one participant with transplantation to left pelvic side wall, supraumbilical, port site and one to left pelvic side wall and subcutaneous tissue in abdomen).

Outcomes

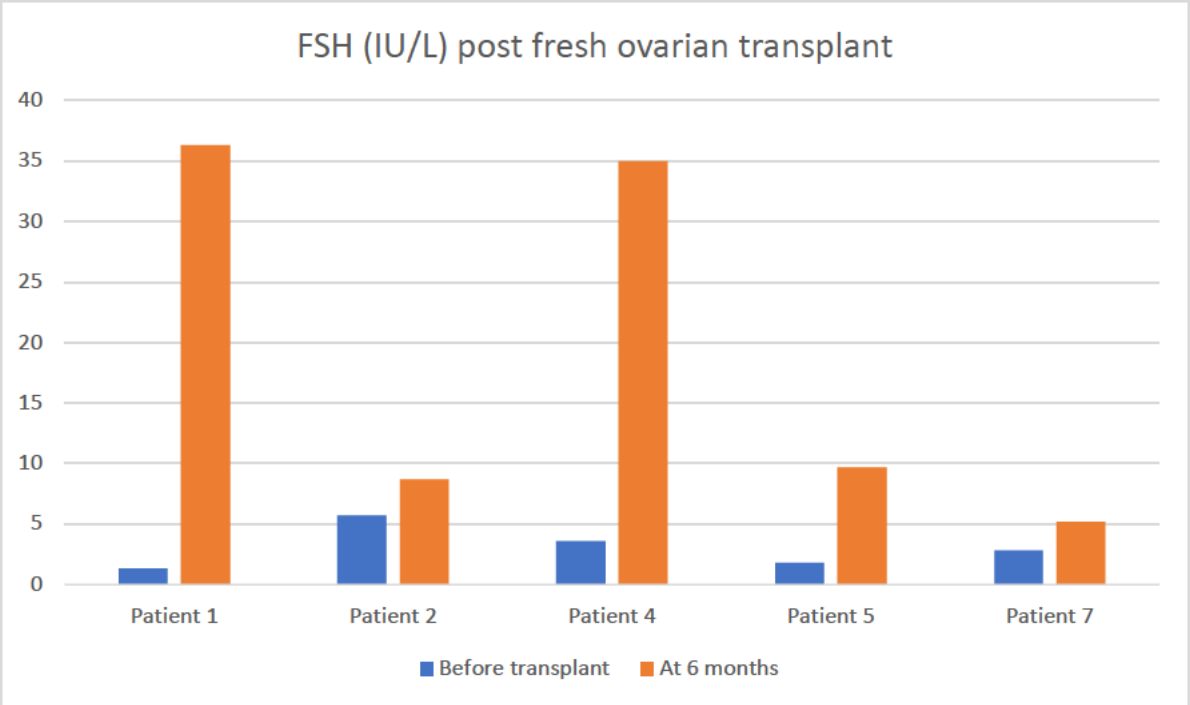
6 month follow up

Seven patients were followed up at 6 months before the COVID-19 pandemic. The graft did not work for one patient, and she was already on HRT at this stage (patient 3).

Follicle stimulating hormone (FSH) and Luteinizing Hormone (LH)

FSH at 6 months was reported in five participants. Mean baseline FSH was noted to be 3.4 IU/L (SD 1.7). Mean FSH post-transplant was 18.9 (SD 15.3). The mean age of participants in this cohort was 35.6 years (SD 1.9). LH was also reported in the same five participants. Mean baseline LH was 2.02 IU/L (SD 2) and post-transplant 14.1 (SD 12.5). Below, Figure 26 shows a graphical representation of FSH at baseline (before the transplantation procedure took place in these patients) and at 6 months follow up.

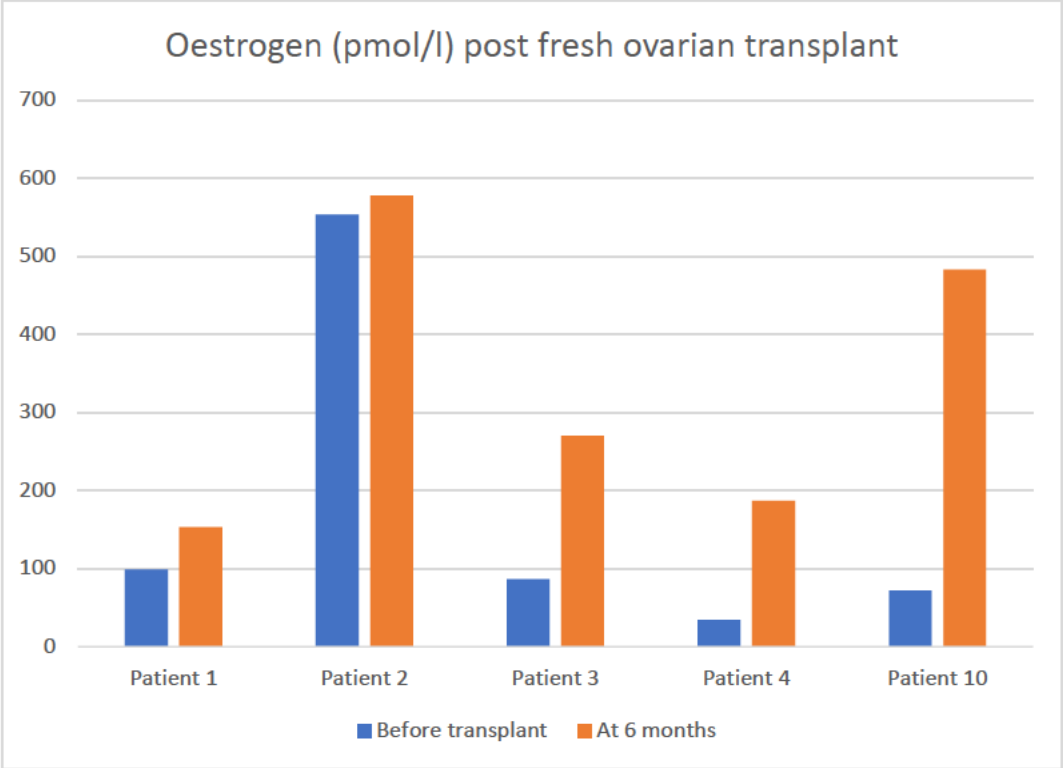
Figure 26. Follicle Stimulating Hormone (FSH) at baseline (before transplantation) and at 6 months post-transplant.



Oestrogen

Of the 13 patients that had bilateral salpingo-oophorectomies, 5 had baseline oestrogen levels and the other 8 were on gonadotropin-releasing hormone agonist (GnRHa) for symptoms of endometriosis and, therefore, had low oestrogen levels. Oestrogen post transplantation was recorded in 5 patients, 4 of whom had oestrogen levels of <100pmol/l before surgery. The mean oestrogen level post-transplant was 334.2pmol/l (SD 187.2pmol/l). The mean age of participants was 34.4 years (SD 3.6 years). Below, Figure 27 represents the level of oestrogen in patients both pre and 6 months post transplantation.

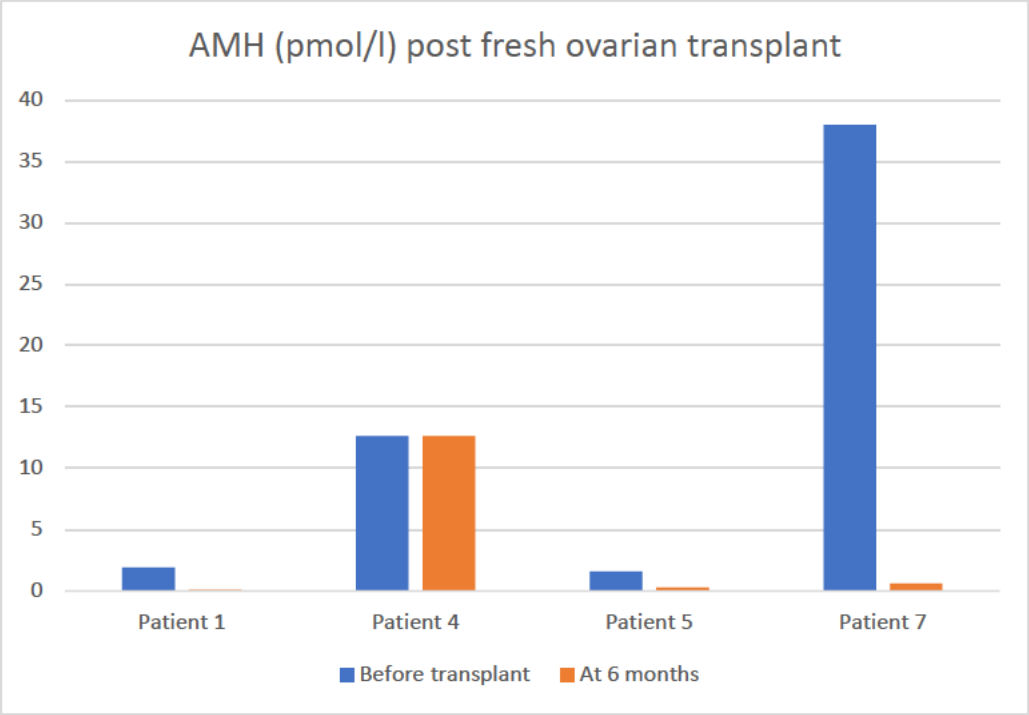
Figure 27. Oestrogen at baseline (before transplantation) and at 6 months post-transplant.



Anti-Mullerian Hormone (AMH)

AMH was done in eight patients before transplantation. Post transplantation AMH was, however, reported in four participants. Mean AMH in this group was 13.5pmol/l (SD 17.1) which was noted to be 3.45 (SD 6.1) post transplantation. The average age of participants was 35.8 years (SD 2.2). Figure 28 shows the levels of AMH pre and 6 months post ovarian transplantation.

Figure 28. Anti-Mullerian Hormone (AMH) at baseline (before transplantation) and at 6 months post-transplant.



Ultrasound scan assessment

Seven participants received ultrasound scans, 3 of whom were also scanned a year later.

1. Visibility of graft

   In all of the participants scanned, the graft was visible.

2. Presence of antral follicles

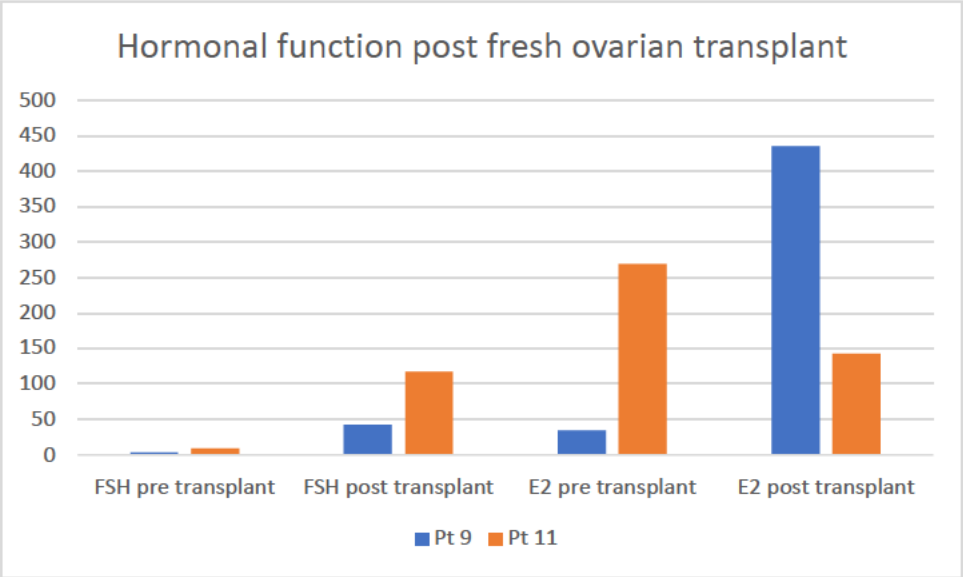
   Ovarian follicles were noted in grafts of all the patients at 6 months post pelvic ultrasound scan.

Long term follow up

Patients were given instructions to seek medical advice if they suffered from symptoms of menopause. Due to long wait times for appointments at secondary and tertiary level care, these patients were advised to see their GP and start hormonal

replacement therapy if symptoms of menopause were unbearable. Due to the COVID-19 pandemic, there was a large backlog of patients with other gynaecological concerns to be seen and unfortunately the clinic that followed up with these patients did not have the capacity to see them within the time frame of this project. Ultimately, only three patients had follow ups after a year and two of them had blood tests. All three participants had ultrasound scans. Figure 29 represents oestrogen and FSH levels in the two patients at baseline and at 18 months post ovarian transplants.

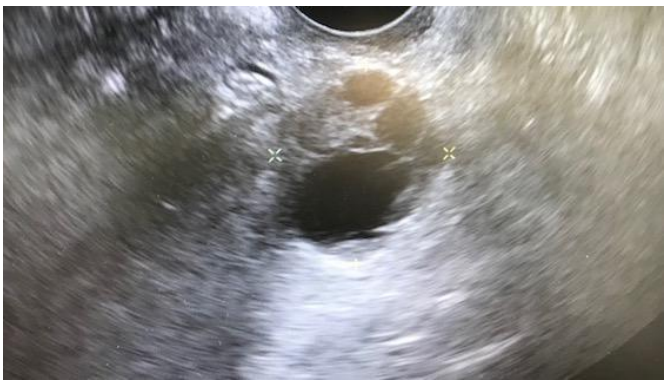
Figure 29. Follicle Stimulating Hormone (FSH) and Oestrogen in participants at 18 months post ovarian transplantation.



Ultrasound scan assessment

Three participants had a scan beyond 12 months. In all of the participants, the graft was visible and follicular activity was noted (Figure 30).

Figure 30. Evidence of follicles in the transplanted ovarian autograft (patient 2).



Lastly, due to the COVID-19 pandemic, patients were lost to follow up which resulted in lack of quality-of-life questionnaires being completed. Only four participants completed the questionnaire at 6 months post-surgery. Of the four participants that completed the questionnaire, two (50%) reported hot flashes and night sweats, 1 (25%) reported vaginal dryness and 1 (25%) reported experiencing poor memory. No participants reported lack of sexual desire and only 1 (25%) reported avoiding intimacy.

Discussion

Through this study it is demonstrated that women with chronic pelvic pain and endometriosis, in whom ovarian conservation is not an option, fresh ovarian autografting could potentially preserve ovarian endocrine function. The use of ovarian tissue autografts to preserve endocrine function has been applied previously however not in women of reproductive age who suffer with endometriosis. (27,29,33,35,41) It is recommended that premenopausal women with low genetic risk of ovarian cancer should have their ovaries preserved. (43) Ovarian conservation however is not always possible due to reasons including benign

ovarian tumours, tubal ovarian abscess that is not resolved with antibiotics, adnexal torsion resulting in necrosis and endometriosis. (44) In a small cohort of women where this applies, fresh ovarian autografting could therefore offer a potential solution. In this study it is demonstrated that although the FSH and LH remained high after the transplant, return of oestrogen and graft visibility was seen in participants at follow up, proving the proof of concept. Evidence suggests that bilateral oophorectomy results in a sudden depletion of ovarian hormones resulting in severe menopausal symptoms. (9,10,44,45) Transplantation of ovarian autografts either at orthotopic sites (within the pelvic cavity) or heterotopic (outside the pelvic cavity) may help counteract the abrupt loss of ovarian endocrine function. The results from quality-of-life questionnaire demonstrate that 50% of the participants reported hot flashes and night sweats, but symptoms such as vaginal dryness and poor memory were only reported in 25% of participants. Additionally, no participants reported lack of sexual desire. The results from the questionnaire suggest that in the short term, ovarian grafting has the potential to help with symptoms of menopause. For women in whom ovarian conservation and HRT is not an option, or they prefer not to conserve their ovaries or take HRT, fresh ovarian grafting could help provide endogenous oestrogen, bridging the gap until the natural age of menopause.

Strengths of the study

Previously ovarian autografting had only been applied in young girls and women with no previous other gynaecological conditions. Additionally, ovarian autografting works as an alternative to HRT, does not require additional surgery, and allows for endogenous hormone production. While the initial sample size may have been

considered small, this holds its own advantages including addressing the research question in a shorter space of time as needed with this study, obtaining ethical approvals, and testing a novel hypothesis with limited resources, money, patients, etc. As a proof of concept, it has been demonstrated that ovarian autografting in women with endometriosis, can potentially preserve ovarian endocrine function.

Limitations of the study

Selection bias is a potential limitation in this study due to the lack of follow-up for many patients. The Covid-19 pandemic made it harder to follow up with patients in a timely manner, as most patients had their follow-up during lockdowns when only emergency admissions were seen at the hospital providing ovarian transplantation. Even after clinics resumed, there was a backlog of new patients to be seen, which further delayed follow-up appointments. Because many patients were not followed up, the study sample may not be representative of the broader population of patients who undergo ovarian transplantation, potentially introducing selection bias. As a result, the findings of this study may not be generalisable to the broader population and should be interpreted with caution.

Furthermore, the study was initially set to recruit participants prospectively however due to the pandemic this was not possible, and an amendment was sought. Outcomes were therefore collected retrospectively from patients that met the inclusion criteria, and which may have caused inappropriate exclusion of participants, further adding to selection bias.

Implications of findings

Young women who suffer with endometriosis and pelvic pain do opt bilateral oophorectomies (17,45,46). This depletion in oestrogen results in iatrogenic menopause. Pharmacologic treatment, such as HRT, is an option, although it may not be appropriate for many young girls and women, and it is not without side effects. (5,17,45) Additionally, in women under the age of 50, bilateral salpingo-oophorectomy with non-malignant hysterectomy has previously appeared to be linked to an increase in all-cause mortality. (6) This study fills an identified gap in the literature by reporting a case series on ovarian autografting in women with benign gynaecological conditions such as pelvic pain and endometriosis. This study demonstrates the success in return of ovarian endocrine function as depicted by an increase in oestrogen post fresh ovarian autografts in women of reproductive age. The novel technique used in this study could potentially provide an alternative to HRT for select patients who want to maintain endogenous ovarian endocrine function.

Unanswered questions and future research

While this study design can provide preliminary results, further research is necessary to demonstrate and explore this issue more directly through replicated studies with larger sample sizes, meticulous follow up of participants receiving ovarian transplants and follow up for a longer period. The effect of HRT versus fresh ovarian autografts is another area of research that needs to be explored further. Especially the quality of life in young women after BSO and ovarian transplants needs to be assessed, and whether ovarian transplants could provide a smoother transition into natural menopause in comparison to those taking HRT.

Conclusion

Data on ovarian autografting are increasingly encouraging as evident by the return of oestrogen and presence of follicles in the ovarian grafts months after post transplantation. Fresh ovarian transplants have the potential to maintain endocrine function in a select group of women in whom HRT is not a feasible option. The study has started laying the foundations of understanding, and the current research will provide important and novel information, especially regarding use of ovarian autografts as an alternative to HRT in young women. Future research needs to be focused on longer term follow up to assess the longevity of the graft and optimal transplantation sites.

References

1. Barriga PP, Montiel CG. Premature ovarian insufficiency in adolescents : an update. 2021;56(2):2–11.
2. Guideline C. NCC-WCH Menopause. 2015;(November):1–283. Available from: https://www.ncbi.nlm.nih.gov/books/NBK327156/pdf/Bookshelf NBK327156.pdf%0Ahttps://www.nice.org.uk/guidance/ng23/evidence/full-guideline-559549261
3. POI TEGG on, Webber L, Davies M, Anderson R, Bartlett J, Braat D, et al. ESHRE Guideline: management of women with premature ovarian insufficiency†. Hum Reprod. 2016 May 1;31(5):926–37. Available from: https://doi.org/10.1093/humrep/dew027
4. Fenton A. Premature ovarian insufficiency: Pathogenesis and management. J Midlife Health. 2015;6(4):147.
5. Sarrel PM, Sullivan SD, Nelson LM. Hormone replacement therapy in young women with surgical primary ovarian insufficiency. Fertil Steril. 2016 Dec;106(7):1580–7.
6. Cusimano MC, Chiu M, Ferguson SE, Moineddin R, Aktar S, Liu N, et al. Association of bilateral salpingo-oophorectomy with all cause and cause specific mortality: population based cohort study. BMJ. 2021;375. Available from: https://www.bmj.com/content/375/bmj-2021-067528
7. Faubion SS, Kuhle CL, Shuster LT, Rocca WA. Long-term health consequences of premature or early menopause and considerations for management. Climacteric. 2015;18(4):483–91.
8. Wright JD, Herzog TJ, Tsui J, Ananth C V, Lewin SN, Lu Y-S, et al. Nationwide trends in the performance of inpatient hysterectomy in the United States.

Obstet Gynecol. 2013 Aug;122(2 Pt 1):233–41.

9. Benshushan A, Rojansky N, Chaviv M, Arbel-Alon S, Benmeir A, Imbar T, et al. Climacteric symptoms in women undergoing risk-reducing bilateral salpingo-oophorectomy. Climacteric. 2009;12(5):404–9.

10. Morrison JH, Brinton RD, Schmidt PJ, Gore AC. Estrogen, Menopause, and the Aging Brain: How Basic Neuroscience Can Inform Hormone Therapy in Women. J Neurosci. 2006 Oct 11;26(41):10332 LP – 10348. Available from: http://www.jneurosci.org/content/26/41/10332.abstract

11. Group T members of the EGC, Becker CM, Bokor A, Heikinheimo O, Horne A, Jansen F, et al. ESHRE guideline: endometriosis†. Hum Reprod Open. 2022 Jan 1;2022(2):hoac009. Available from: https://doi.org/10.1093/hropen/hoac009

12. Rocca WA, Bower JH, Maraganore DM, Ahlskog JE, Grossardt BR, de Andrade M, et al. Increased risk of cognitive impairment or dementia in women who underwent oophorectomy before menopause. Neurology. 2007 Sep;69(11):1074–83.

13. Ryan J, Scali J, Carrière I, Amieva H, Rouaud O, Berr C, et al. Impact of a premature menopause on cognitive function in later life. BJOG An Int J Obstet Gynaecol. 2014;121(13):1729–39.

14. Shuster LT, Gostout BS, Grossardt BR, Rocca WA. Prophylactic oophorectomy in premenopausal women and long-term health. Menopause Int. 2008 Sep 1;14(3):111–6. Available from: https://doi.org/10.1258/mi.2008.008016

15. Bhattacharya SM, Jha A. A comparison of health-related quality of life (HRQOL) after natural and surgical menopause. Maturitas. 2010;66(4):431–4.

Available from: http://dx.doi.org/10.1016/j.maturitas.2010.03.030

16. Al Kadri H, Hassan S, Al-Fozan HM, Hajeer A. Hormone therapy for endometriosis and surgical menopause. Cochrane Database Syst Rev. 2009;(1).
17. Zanello M, Borghese G, Manzara F, Esposti ED, Moro E, Raimondo D, et al. Hormonal replacement therapy in menopausal women with history of endometriosis: A review of literature. Med. 2019;55(8):1–9.
18. Yding Andersen C, Mamsen LS, Kristensen SG. Freezing of ovarian tissue and clinical opportunities. Reproduction. 2019;158(5):F27–34.
19. Donnez J, Dolmans MM, Demylle D, Jadoul P, Pirard C, Squifflet J, et al. Restoration of ovarian function after orthotopic (intraovarian and periovarian) transplantation of cryopreserved ovarian tissue in a woman treated by bone marrow transplantation for sickle cell anaemia: Case report. Hum Reprod. 2006;21(1):183–8.
20. Kim SS. Assessment of long term endocrine function after transplantation of frozen-thawed human ovarian tissue to the heterotopic site: 10 year longitudinal follow-up study. J Assist Reprod Genet. 2012;29(6):489–93.
21. ASRM. Fertility preservation in patients undergoing gonadotoxic therapy or gonadectomy: a committee opinion. Fertil Steril. 2019;112(6):1022–33. Available from: https://doi.org/10.1016/j.fertnstert.2019.09.013
22. Donnez J, Dolmans MM. Fertility preservation in women. N Engl J Med. 2017;377(17):1657–65.
23. Yasmin E, Balachandren N, Davies MC, Jones GL, Lane S, Mathur R, et al. Fertility preservation for medical reasons in girls and women: British fertility society policy and practice guideline. Hum Fertil. 2018;

24. Leporrier M, von Theobald P, Roffe JL, Muller G. A new technique to protect ovarian function before pelvic irradiation. Heterotopic ovarian autotransplantation. Cancer. 1987 Nov;60(9):2201–4.

25. Marconi G, Quintana R, Rueda-Leverone NG, Vighi S. Accidental ovarian autograft after a laparoscopic surgery: case report. Fertil Steril. 1997 Aug;68(2):364–6.

26. Silber SJ, Lenahan KM, Levine DJ, Pineda JA, Gorman KS, Friez MJ, et al. Ovarian transplantation between monozygotic twins discordant for premature ovarian failure. N Engl J Med. 2005;353(1):58–63.

27. Silber S, Kagawa N, Kuwayama M, Gosden R. Duration of fertility after fresh and frozen ovary transplantation. Fertil Steril. 2010;94(6):2191–6. Available from: http://dx.doi.org/10.1016/j.fertnstert.2009.12.073

28. Oktay K, Buyuk E, Rosenwaks Z, Rucinski J. A technique for transplantation of ovarian cortical strips to the forearm. Fertil Steril. 2003;80(1):193–8.

29. Kiran G, Kiran H, Coban YK, Guven AM, Yuksel M. Fresh autologous transplantation of ovarian cortical strips to the anterior abdominal wall at the Pfannenstiel incision site. Fertil Steril. 2004;82(4):954–6.

30. Kiran G, Kiran H, Coban YK, Guven AM. Ovarian cortical transplantation may be an alternative to hormone therapy in patients with early climacterium. Fertil Steril. 2005;84(5):1509.e1-1509.e3.

31. Silber SJ, Derosa M, Pineda J, Lenahan K, Grenia D, Gorman K, et al. A series of monozygotic twins discordant for ovarian failure: Ovary transplantation (cortical versus microvascular) and cryopreservation. Hum Reprod. 2008;23(7):1531–7.

32. Donnez J, Squifflet J, Dolmans MM, Martinez-Madrid B, Jadoul P, Van

Langendonckt A. Orthotopic transplantation of fresh ovarian cortex: A report of two cases. Fertil Steril. 2005;84(4):1018.e1-1018.e3.

33. Almodin CG, Almodin PM, Radaelli MRM, Minguetti-Câmara VC, Ceschin AP, Ribeiro RC. The frst ovarian tissue transplant between monozygotic twin sisters discordant for ovarian function in Latin America. J Bras Reprod Assist. 2015;19(1):29–32.

34. Laufer MR, Upton J, Schuster SR, Grier H, Emans SJ, Diller L. Ovarian Tissue Autologous Transplantation to the Upper Extremity for Girls Receiving Abdominal/Pelvic Radiation: 20-year Follow-up of Reproductive Endocrine Function. J Pediatr Adolesc Gynecol. 2010;23(2):107–10. Available from: http://dx.doi.org/10.1016/j.jpag.2009.09.005

35. Kodama Y, Sameshima H, Ikenoue T, Ikeda T, Kawagoe Y. Successful fresh whole ovarian autotransplantation without vascular anastomosis. Fertil Steril. 2010;94(6):2330.e11-2330.e12. Available from: http://dx.doi.org/10.1016/j.fertnstert.2010.03.059

36. Donnez J, Squifflet J, Pirard C, Jadoul P, Dolmans MM. Restoration of ovarian function after allografting of ovarian cortex between genetically non-identical sisters. Hum Reprod. 2010;25(10):2489–95.

37. Hilders CG, Baranski AG, Peters L, Ramkhelawan A, Trimbos JB. Successful human ovarian autotransplantation to the upper arm. Cancer. 2004;101(12):2771–8.

38. Donnez J, Dolmans MM, Squifflet J, Kerbrat G, Jadoul P. Live birth after allografting of ovarian cortex between monozygotic twins with Turner syndrome (45,XO/46,XX mosaicism) and discordant ovarian function. Fertil Steril. 2011;96(6):1407–11. Available from:

http://dx.doi.org/10.1016/j.fertnstert.2011.09.012

39. Callejo J, Salvador C, Miralles A, Vilaseca S, Lailla JM, Balasch J. Long-term ovarian function evaluation after autografting by implantation with fresh and frozen-thawed human ovarian tissue. J Clin Endocrinol Metab. 2001;86(9):4489–94.

40. Zhai J, Yao G, Dong F, Bu Z, Cheng Y, Sato Y, et al. In vitro activation of follicles and fresh tissue auto-transplantation in primary ovarian insufficiency patients. J Clin Endocrinol Metab. 2016;101(11):4405–12.

41. Yin CS, Huang KC, Chiu HM. A 51-month follow-up of fresh autologous hemi-ovary transplantation at the Pfannenstiel incislon site after hysterectomy and accidental bilateral salpingo-oophorectomy in a premenopausal woman. Taiwan J Obstet Gynecol. 2009;48(1):69–71. Available from: http://dx.doi.org/10.1016/S1028-4559(09)60039-5

42. Sánchez M, Alamá P, Gadea B, Soares SR, Simón C, Pellicer A. Fresh human orthotopic ovarian cortex transplantation: Long-term results. Hum Reprod. 2007;22(3):786–91.

43. ACOG Practice Bulletin No. 89: Elective and Risk-Reducing Salpingooophorectomy. Obstet Gynecol. 2008;111(1). Available from: https://journals.lww.com/greenjournal/Fulltext/2008/01000/ACOG Practice Bul letin No 89 Elective and.39.aspx

44. Rodriguez M, Shoupe D. Surgical Menopause. Endocrinol Metab Clin North Am. 2015 Sep;44(3):531–42.

45. Garg A, Robinson L. Surgical menopause: A toolkit for healthcare professionals. Post Reprod Heal. 2021;27(4):222–5.

46. Secoșan C, Balint O, Pirtea L, Grigoraș D, Bălulescu L, Ilina R. Surgically

induced menopause—A practical review of literature. Med. 2019;55(8):1–10.

CHAPTER 4: EXPERIENCES OF YOUNG GIRLS AND WOMEN UNDERGOING OVARIAN TISSUE CRYOPRESERVATION: A SYSTEMATIC REVIEW AND THEMATIC SYNTHESIS

Preamble

A version of this chapter is published in Psychosomatic Journal of Obstetrics and Gynaecology.

Khattak H, Woodman H, Afifi Y, Amorim CA, Fishel S, Gallos I, Coomarasamy A, Topping A. Experiences of young girls and women undergoing ovarian tissue cryopreservation: a systematic review and thematic synthesis. *J Psychosom Obstet Gynaecol.* 2022 Dec;43(4):502-516.

Abstract

The aim of this study was to explore the experiences of young girls and women who underwent or considered ovarian tissue cryopreservation (OTC) using a systematic review of qualitative studies with thematic synthesis framework. Major electronic databases: MEDLINE, EMBASE, the Cochrane Library, CINAHL and PsycINFO were searched from 1946 to May 2020 and reference lists of relevant articles were hand searched. Any studies that described a qualitative inquiry and highlighted the experiences of women with regards to OTC were included. Two independent reviewers (H.K. and H.W.) screened the title and abstracts and made a selection against inclusion criteria. Main outcomes measures were experiences of women who have considered and/or undergone ovarian tissue cryopreservation (OTC), decision making in women who underwent or considered OTC and patient education. Nineteen studies were assessed for full text eligibility and four were included in analysis. 144 verbatim quotations from 85 participants in high income countries (UK, USA and Denmark) were included. Two studies adopted grounded theory approach, one phenomenology and one inductive content analysis. Four themes were generated; participants described their experiences as emotional, involving complex decision-making, helping them prepare for the long-term consequences of potentially losing their fertility and hormonal function, as well as their experience being educational. Additionally, the more practical aspects of the procedure such as OTC being invasive as well as costs implications were highlighted. Women and young girls are often involved in making time-sensitive decisions whether or not to undergo OTC. Healthcare professionals involved in the care of young girls and women undergoing this method need to also take into consideration the emotional wellbeing

of the patients as well as the time and expertise it requires to help them make an informed decision.

Key words: Female fertility preservation; ovarian tissue cryopreservation; experiences; qualitative research; thematic analysis

Introduction

Ovarian tissue cryopreservation (OTC) is an emerging method for fertility preservation that has been utilised by thousands of young girls and women worldwide, whether they are treated for cancer or benign gynaecological conditions (1-3). While some experts still consider OTC experimental, many stakeholders and policy makers are exploring the possibility of routinely using this procedure for women and prepubertal girls (4,5). However, there remains limited information on the patient experience of this procedure, despite technical advancements over the past two decades.

Initially developed as a fertility preservation option for young girls and women with cancer, OTC has since been used in women with non-malignant diseases who are at risk of premature menopause (3,6-8). OTC involves retrieving a portion of the ovary and freezing it in liquid or vapour nitrogen. The tissue is typically collected before the administration of gonadotoxic anti-cancer therapy and transplanted once treatment is completed. The first live birth resulting from OTC and transplantation was reported in 2004 (9). Since then, over 200 live births have been reported globally (1,7,8). OTC has provided comfort to many young girls and women, particularly because it remains the only fertility preservation option for prepubertal girls.

Recent improvements in treatment, prognosis, and quality of life post-cancer treatment have sparked greater interest in post-treatment fertility. Many individuals seek information from their healthcare providers as their primary source of information, while others rely on online resources (12). However, the adequacy of

information provided to young girls and women to make informed decisions has been criticised, highlighting the need for improvement when discussing fertility preservation (10,11). Compared to embryo and oocyte cryopreservation, OTC is a newer alternative method of fertility preservation that warrants further investigation to better understand how women and young girls make decisions about undergoing the procedure. Although several studies have been conducted to provide success rates, technique-related information, patient satisfaction surveys, and healthcare providers' opinions on the matter (1,2,11,15-19), quantitative methods may not provide sufficient depth on their own. Qualitative methods, on the other hand, aim to comprehend lifestyle, systems, policies, and human environments through human interaction, as opposed to the analysis of numbers and statistical figures (20). Synthesising qualitative research enables data to be combined across various contexts, and ultimately allows researchers to create new theoretical or conceptual models, identify research gaps, inform primary studies, and provide evidence for the development, implementation, and evaluation of health interventions (20). Despite OTC's well-developed scientific potential, the qualitative evidence remains limited. This review was conducted to shed light on women and young girls' experiences of undergoing OTC for fertility preservation and their interactions with healthcare professionals.

Methods

The protocol was registered on the International Prospective Register of Systematic Reviews (PROSPERO) Database (registration number: CRD42020192882). The review was guided by recommendations to enhance transparency in reporting the synthesis of qualitative research (ENTREQ) as described in Appendix 10 (21,22). The ENTREQ statement aids researchers to report aspects of the steps which

structure the synthesis of qualitative health research. These include; searching and selecting qualitative research, quality appraisal, and methods for synthesising qualitative findings (21,22).

Systematic search of the literature

A search strategy was developed in consultation with an information specialist (Appendix 11). The eligibility criteria for inclusion of studies were devised using the population, indicator or phenomenon of interest, context and study design organising framework, as shown in Table 10 (23).

Table 10. PICoS framework to develop eligibility criteria.

PICoS Indicator	Eligibility criteria
Population / Participants	Young girls or women who considered or underwent ovarian tissue cryopreservation
Indicators / phenomenon of Interest	Experiences relating to ovarian tissue cryopreservation
Context	Any women or young girls who considered or underwent ovarian tissue cryopreservation as a method of fertility or hormonal preservation
Study design	Qualitative research designs including (but not limited to) thematic analysis, grounded theory, phenomenological analysis and mixed method studies with qualitative components.
Exclusion criteria	Women who underwent other fertility preservation methods such as oocyte and embryo cryopreservation

Major electronic databases: MEDLINE, EMBASE, the Cochrane Library, CINAHL and PsycINFO were sought and reference lists of relevant articles were hand

searched. Any studies that described a qualitative inquiry and highlighted the experiences of women with regards to OTC were included. Abstracts retrieved through the search were first screened to remove duplicates. Subsequently, two independent reviewers (H.K. and H.W.) screened the title and abstracts and made a selection against inclusion criteria. This was followed by the screening of full text articles by two reviewers. Any further articles identified through hand searching and that were deemed relevant by either or both of the reviewers were also included in the full-text review. Disagreements were resolved with the help of a third reviewer (AT) until consensus was reached.

Quality assessment and appraisal

All included studies were critically appraised using 'The Critical Appraisal Skills Programme' CASP qualitative checklist (24) and are displayed in Appendix 12. All the studies were appraised for their validity, evaluation of the methodological quality and an assessment of external validity. Critical appraisal was conducted independently by two reviewers (H.K. and H.W.). The findings from the two reviewers were compared and any disagreements were discussed and re-reviewed. The studies included were assessed as high quality. All included studies had clearly described aims and the qualitative methods employed in all the studies were appropriate. Although the data were collected to address the stated research questions, the relationship between researcher and participants was not described and only two studies discussed recruitment details such as data saturation (25,26). One study reported that ethical approval was not required (as per their national legislation) however the study was attested by their Regional Committee on Health Research (27).

Data extraction

A data extraction form was developed *a priori* in Microsoft Excel to facilitate data extraction. One review author (H.K.) extracted and charted all the data, the second author (H.W.) then verified the extracted data. Any differences were resolved through discussion until consensus was reached. If differences were not resolved by discussion, a third reviewer (A.T.) was approached. Extracted materials were based on the inclusion criteria and a framework of predefined outcomes of interest. Information gathered from the selected studies included: author; year of publication; country of study; study type; setting; relevant background and impetus for the study; methodological approach and specified methods; patient characteristics and demographics; main findings including themes and narratives relating to the experience of OTC and key relevant discussion points. Data were mainly analysed (coded) by one reviewer, but regular meetings were held with the review team to ensure that the extracted data appropriately reflected the original data.

Data synthesis

The process of thematic analysis and meta-synthesis process as described by Thomas and Harden (2008) was used and involved the following steps (28).

Step 1: Coding

Student ID:
PhD Met+Sys Re T Lab A300

Data from primary studies were coded line by line and examined for meaning. This helped identify the translation of codes between studies. An Excel spread sheet was used to organize the extracted descriptive and qualitative data.

Step 2: Theme development

The codes generated in step 1 were assessed for meanings. These meanings were further organized into categories. The properties of these categories were then analysed.

Step 3: Theme generation

Categories generated were compared between studies and any similarities or difference was examined. Similar categories were merged such that substantive themes were generated merging higher-level constructs. This enabled the development of higher order abstractions of the phenomena.

Results

Included studies

Through our literature search, we found 17 potential studies, but only two studies were eligible for inclusion in the analysis. Two further studies, including an unpublished doctoral thesis, were found through hand searching. Four studies were included in the thematic analysis, representing experiences of 85 females in the form of 144 verbatim quotes. The included studies were conducted in Denmark, UK and two in USA. The age range of the

Student ID:
PhD Met+Sys Re T Lab A300

participants at the time of interview was 13-42 years, with all participants undergoing the procedure as a fertility preservation option due to cancer. The study design included two studies that adopted the grounded theory, one phenomenology and one inductive content analysis. The characteristics of included studies are given in Table 11. A Preferred Reporting Items for Systematic Reviews and Meta- Analyses (PRISMA) diagram of the search and study selection is presented in Figure 31.

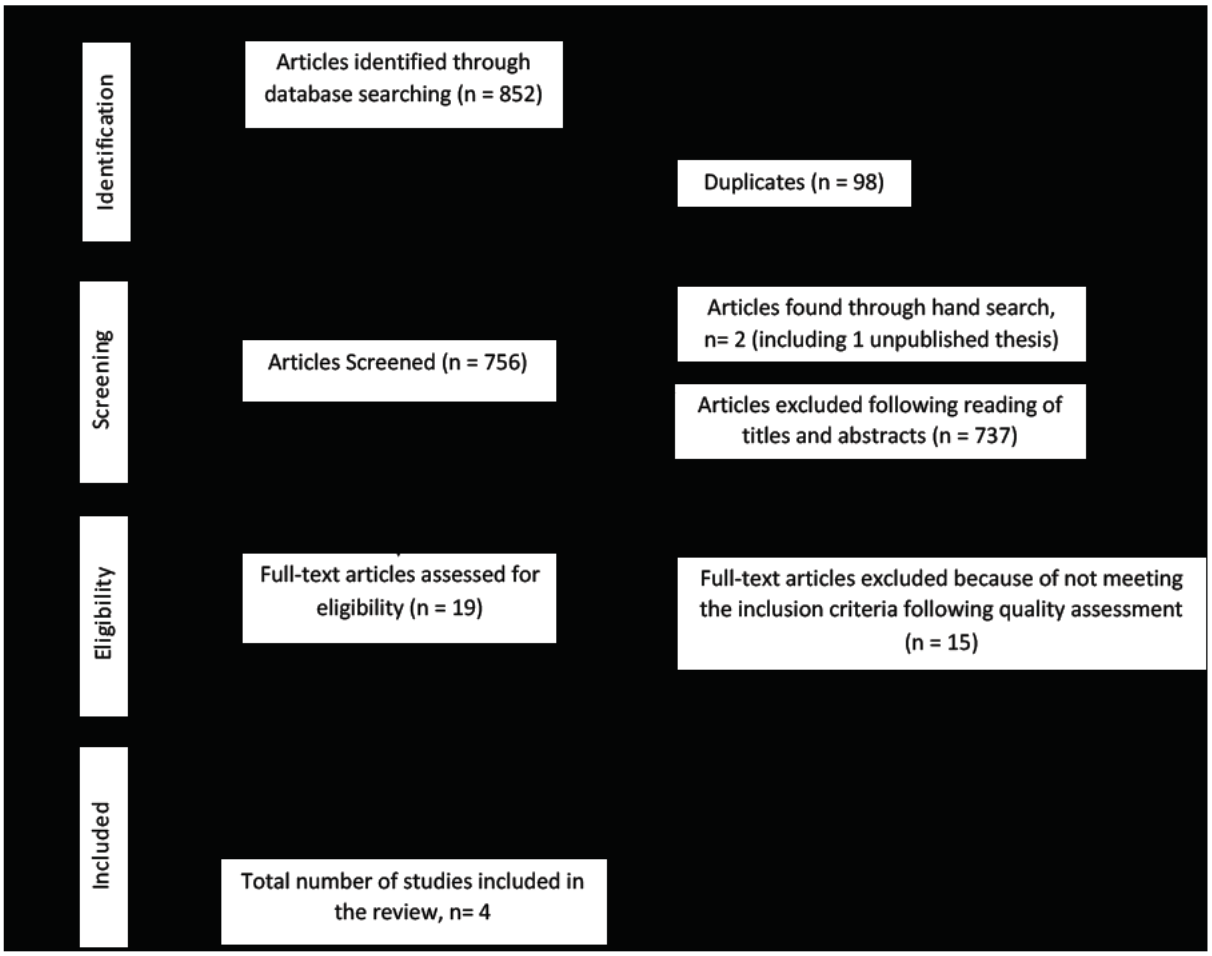
Table 11. Study characteristics table for included studies.

Author Year Country	**Aim(s)**	**Sample/Population**	**Study design/methods**	**Findings/Themes**	**Specific outcomes**	**Quality appraisal score**
M tche et a . 2020 USA	To determ ne pat ents' know edge and fee ngs about the r cryopreserved ovar an t ssue	8 fema es Mean age at the t me of nterv ew = 25.8 ± 6.1 (Range 19 37 years) Mean age at OTC = 20.4 ± 7.5 (range 13 35 years)	Qua tat ve nterv ew study, nduct ve content ana ys s (generat on of themes)	Lack of understand ng Fert ty ntent ons Hormona funct on Fee ngs about OTC (pos t ve and negat ve)	Ident f cat on of s gn f cant know edge gap among pat ents w th cryopreserved ovar an t ssue, about ts uses, benef ts as we as a strong des re for mproved pat ent educat on.	H gh
Hershberg er et a . 2013 USA	To prov de an n depth descr pt on of the dec s on mak ng process that women who are d agnosed w th cancer undergo as they dec de whether to accept or dec ne fert ty cryopreservat on.	27 fema es Mean age at the t me of nterv ew = 28.7 (Range 19 40)	Qua tat ve grounded theory approach	Four phases of dec s on mak ng process Ident ty Contemp ate Reso ve Engage	C ar f cat on of under y ng process that women w th cancer undergo about fert ty cryopreservat on To mprove qua ty of care for women w th cancer by mp ement ng appropr ate y t mes nformat on and ta ored deve opmenta and contextua counse ng to support dec s on mak ng.	H gh

Tennyson et a . 2017 (unpub sh ed thes s) UK	To exp ore the ved exper ences of teenagers and young adu ts w th cancer who have been offered ovar an t ssue cryopreservat on.	8 fema es Mean age at the t me f nterv ew = 17.5 (Range 13 22)	Interpretat ve phenomeno og ca qua tat ve study	Sma procedure, b g mean ng Improv ng adjustment to cancer Reduc ng the mpact of cancer	To exp ore the ved exper ences of teenagers and young adu ts w th cancer who have been offered ovar an t ssue cryopreservat on	H gh
Bach et a . 2020 Denmark	Futures and fears n the freezer: Dan sh women's exper ences w th ovar an t ssue cryopreservat on and transp antat o n	42 fema es Age range at the t me of nterv ew = 15 42 years	Qua tat ve sem structured n depth nterv ews, grounded theory ana yt ca approach	Dec s on mak ng: OTC as a hope n the dark Storage: opportun t es n the freezer Transp antat on: nav gat ng r sk, chances and outcomes Surp us t ssue: hav ng 'someth ng n the bank' for the future	Exper ences w th OTC and transp antat on, nc ud ng the r ref ect ons on ong term storage of t ssue and the use of surp us t ssue	H gh

Figure 31. PRISMA Flow diagram of search and selection strategy.

Student ID:
PhD Met+Sys Re T Lab A300



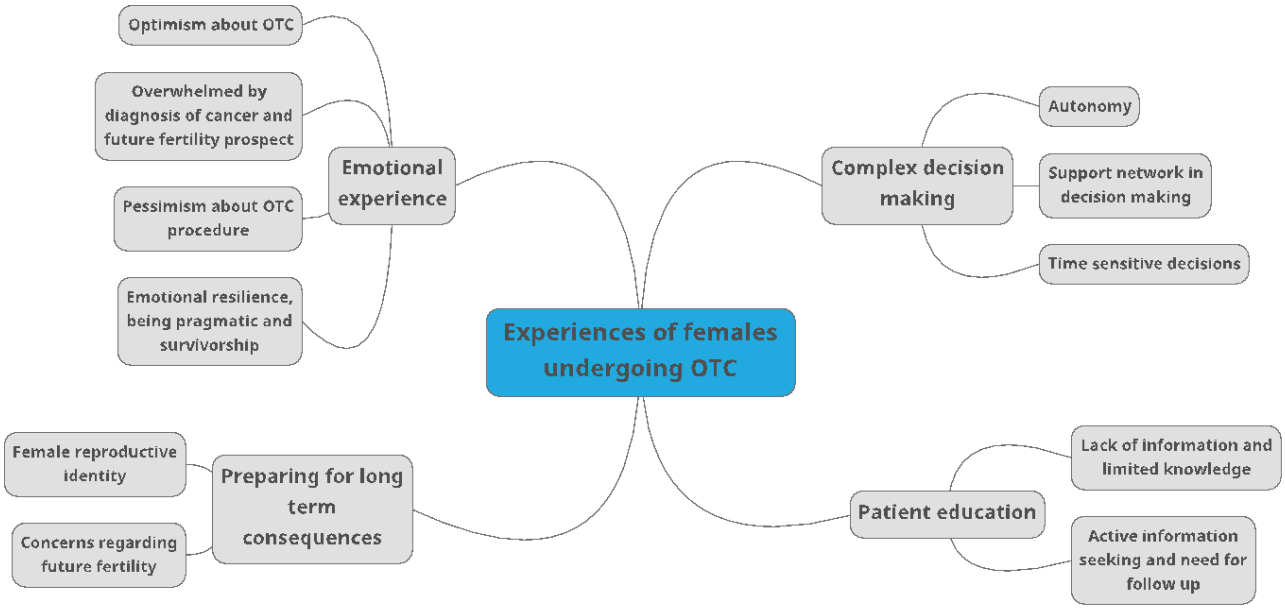
Results of meta-synthesis

We identified four themes associated with experiences of young girls and women who underwent or considered having ovarian tissue cryopreservation. Participants described their experiences as emotional, involving complex decision-making, educational and helping them prepare for the long-term consequences of potentially losing their fertility and hormonal function. Additionally, the more practical aspects of the procedure such as cost

Student ID:
PhD Met+Sys Re T Lab A300

implications and the invasive nature were also highlighted. A schematic representation of the generated themes is shown in Figure 32.

Figure 32. Schematic representation of generated themes.



An emotional experience

Participants described the process of undergoing fertility preservation as very emotional. There was a great deal of overwhelm, but the idea of 'banking' something for the future formed part of a sense of hope for the future, about survival and was reassuring.

Overwhelmed by diagnosis and future fertility prospects

For women of childbearing age, the diagnosis of cancer can come with bad news about losing their potential for motherhood. Women described this as very challenging and an added burden to their disease.

> ***'Everything was happening in a whirlwind.'***
>
> (Mitchell, Whiting-Collings, & Christianson, 2020, p. 2)

> ***'…it was devastating, absolutely devastating.'***
>
> (Hershberger et al., 2013, p. 63)

Emotional resilience, being pragmatic and survivorship

Some participants dealt with the situation pragmatically, thinking of the next steps and sometimes making difficult choices.

> ***'Then comes cancer. Alright, you know what, we can handle this. We're educated enough, that, you know what, this is not all dead set. This is a bump in the road and we will face it head on and we'll fight, and years from now we'll look back and it'll be a memory. And that's okay. But then you tell us on top of it, that we can't have children. We can't have a family ...you know, as tears rolled down my cheeks–which was not me what-so-ever–I can deal with the cancer, but throw this [loss of fertility] on top of it and it was devastating, absolutely devastating.'***
>
> (Hershberger et al., 2013, p. 63)

Optimism and being grateful for having the option

Participants described the feelings of having undergone OTC as something that had provided hope, reassurance and an opportunity to 'save' their future.

'Gave me one less thing to worry about with everything else going on'

(Tennyson, 2017, p. 86)

Pessimistic about OTC procedure

Ovarian tissue cryopreservation is an invasive procedure requiring surgery and some participants felt this might impact their already deteriorating health. Participants found it to be an aggressive cure for preserving fertility. Also, the fact that they heard 'stories' of women who had conceived despite having chemotherapy somewhat challenged their view about the necessity for fertility preserving intervention.

"If I were to go back, I'm not sure if I would have said yes…because I know of many stories of women who received chemotherapy and didn't have any problems reproducing after that.

(Mitchell, Whiting-Collings, & Christianson, 2020, p. 3)

Attitudes of young adults towards fertility preservation

The analysis included young participants, most of whom described being coerced into having the ovarian tissue cryopreservation procedure. They felt they were too young to make these decisions and something they had not thought about before.

'I was a little mad about having to do it when I didn't have any interest in having kids.'

(Mitchell, Whiting-Collings, & Christianson, 2020, p. 3)

'but my parents were very adamant like 'maybe you'll change your mind in the future', but I didn't really care.'

(Mitchell, Whiting-Collings, & Christianson, 2020, p. 3)

Complex decision-making

Participants appeared to find decision making about fertility and dealing with the news of a cancer diagnosis complex. Although their diagnosis was something they did not have control over, a decision about fertility preservation, provided a sense of being in charge of their destiny. Ovarian tissue cryopreservation was therefore seen as autonomous although it meant making time-sensitive decisions often with the support of family, friends and input from healthcare professionals.

Autonomy

Participants felt that the option of preserving fertility gave them a sense of control over their body. This was described in various ways as some participants felt empowered whilst others felt some coercion that the decision was made for them and they had to conform.

> ***'[it] let me have some control'***
>
> (Mitchell, Whiting-Collings, & Christianson, 2020, p. 2)

> ***'It was about having the option. That I regained a bit of free will..'***
>
> (Bach, Macklon, & Kristensen, 2020, p. 558)

In contrast another participant described how she perceived that the decision was determined by whether she already had children and she just complied but ultimately was appreciative:

> ***'It was like "Do you have a child?" "No, I do not." "Okay, you have to go to the fertility clinic." I actually didn't have a lot of influence on that. And today, I mean, I'm really happy about that, because I think if I had to think about it... the pros and cons, then I don't know what I would have done.'***
>
> (Bach, Macklon, & Kristensen, 2020, p. 558)

Many of the young girls in the included studies that were diagnosed with cancer had not considered the consequences of their illness or made a connection between cancer treatment and threat to future fertility. Often the decision to preserve fertility was taken by their parents or guardians and made some feel that their autonomy was taken away.

> ***'you're just told like you don't really have an option'***
>
> (Tennyson, 2017, p. 87)

Support network in decision making

The complex decision-making involved support from family, friends and advice of the health care professional. Some participants felt if it was not for this support network, they might have not made the decision to go for OTC.

> ***'She (my mother) was the one – her and my dad – were the ones who helped me make a lot of the big decisions.'***
>
> (Hershberger et al., 2013, p. 64)

> ***'[Being offered OTC showed] how kind of how important you are to the medical team and how much they care for you.'***
>
> (Tennyson, 2017, p. 84)

Student ID:
PhD Met+Sys Re T Lab A300

Time sensitive decisions

Participants also felt that they were making decisions sometimes under immense pressure. This was primarily due to the urgency of starting anti-cancer treatments.

> ***'It's tough to make this decision under the gun.'***
>
> (Hershberger et al., 2013, p. 64)

Preparing for the long-term consequences

Women saw ovarian tissue freezing as something that gave them a future, which involved surviving cancer and leading a 'normal' life. It also meant exploring what fertility meant and how they defined themselves as having, or not, a reproductive identity. For most participants, this was something they had not previously considered. It was evident that to some participants, fertility seemed to form a significant part of womanhood.

Female reproductive identity

Some participants regarded fertility as an important characteristic of being a female. It was not something they considered might be endangered as a result of a cancer diagnosis and anti-cancer therapies. Ovarian tissue cryopreservation gave these participants the option of preserving this aspect of 'femaleness' after they became aware that it was at risk. Undergoing OTC meant making a choice that would have consequences beyond the here and now.

> ***'It was like having the possibility of reclaiming my body or reclaiming my life including all the choices I had. So, it was not tied to a specific wish to have another child in that way.'***

(Bach, Macklon, & Kristensen, 2020, p. 558)

Participants recognized that banking ovarian tissue might help long term with symptoms caused by hormonal imbalance and not just fertility. Many expressed concerns about developing treatment related early onset of menopause and the ovarian tissue provided security, hope for the future, a future that might involve living a 'normal life' with children.

> ***'I was pretty insistent about wanting the last part of my tissue implanted. And if they couldn't find room, apparently my ovary is pretty small, which they always grumble about... then they could put it wherever, because I had, you know, a feeling like I had to have my body assembled again. I mean, that it had to be whole again in some way.'***
>
> (Bach, Macklon, & Kristensen, 2020, p. 562)

In the account above, women conceptualized wholeness or intactness closely with fertility and normality. Whereas in contrast some focused on the assault of the removal and transplant of the ovarian tissue as an invasive procedure, removal of what constituted a woman and how in retrospect she should not have opted for it.

> ***'I mean it is kind of a drastic procedure, I feel. You know you're removing like a piece of woman's you know reproductive or hormonal system down there.'***
>
> (Mitchell, Whiting-Collings, & Christianson, 2020, p. 3)

Fertility was seen as precious and now threatened ***'One of those things that you take for granted.'*** (Tennyson, 2017, p. 82), and the option of ovarian tissue cryopreservation offered a consolation that softened the cancer diagnosis. Although fertility preservation

was a small part of dealing with cancer, it nevertheless contributed to the wellbeing of women after treatment.

Concerns regarding future fertility prospects

The potential for loss of fertility was concerning and causing further anxiety in addition to dealing with a cancer diagnosis.

> ***'That… has been a paralyzing fear of mine - not getting pregnant.'***
>
> (Mitchell, Whiting-Collings, & Christianson, 2020, p. 3)

Other anxieties were related to costs associated with restoring the tissue.

> ***'Anxiety… because you don't know if it's going to work, you don't know if you're going to be able to use that tissue, you don't know if financially you will have resources'***
>
> (Mitchell, Whiting-Collings, & Christianson, 2020, p. 2)

It also reinforced the damaged identity brought by cancer, causing further disruption with life-long consequences and unexpected losses.

> ***'One of the things that startled me the most when I was diagnosed was how it might affect my fertility. Not because I may never be able to bear a child, which I want to do, but because it made me realize how this diagnosis was going to be truly life changing and could affect me the rest of my life.'***
>
> (Hershberger et al., 2013, p. 62)

Whereas others could reconcile future loss by getting through the cancer journey and surviving. Fertility preservation was secondary, put away temporarily and thought about in the future (especially for younger participants).

'is this even what I should be worrying about. [?]… should I just be focusing on getting better?'

(Tennyson, 2017, p. 86)

Patient education

Ovarian tissue cryopreservation is a relatively new fertility preservation method and only offered by a limited number of centres. Our analysis highlighted the lack of information provided about this new technique to the participants. A number of study participants felt they had received limited information about follow up, specifically what they needed to do when ready to consider re-implantation of the preserved ovarian tissue.

Lack of information and limited knowledge

Many participants reported an overall lack of understanding about ovarian tissue cryopreservation as a fertility preservation technique. This was highlighted as lack of information provided to the participants during fertility preservation counselling. It is unclear whether this was due to the fact that these young girls and women had discussions about OTC immediately prior to commencing gonadotoxic anti-cancer treatment, and that there was often an imperative to make decisions quickly.

'Before I tried artificial conception... wow, was I naïve…'

(Bach, Macklon, & Kristensen, 2020, p. 561)

Participants also did not seem to recall the details of the procedure. Some participants mistook ovarian tissue freezing for freezing of oocytes due to the complex jargon used by healthcare professionals.

> ***I think they (froze) eggs… Oh, they didn't freeze eggs?***
>
> (Mitchell, Whiting-Collings, & Christianson, 2020, p.3)

> ***'I was talking to doctors and they were using a lot of jargon'***
>
> (Hershberger et al., 2013, p. 62)

Active information seeking and need for follow up

Participants were keen for health care professionals to follow them up to discuss the future of their tissue. At the time of OTC, women were facing a cancer diagnosis, a barrage of information and potential decisions about treatment for cancer that included risks to fertility. However, their primary concern was the current treatment and not their future fertility. After completion of cancer treatment, women started to think more about a future that included having biological children and therefore wanted information about OTC and its implications at that point.

> ***'I have probably been given a lot of information which I have forgotten…'***
>
> (Bach, Macklon, & Kristensen, 2020, p. 559)

> ***'It would be helpful if there was patient follow-up-whether that be something as simple as a newsletter, or a quarterly email, or just something biannual that patients could know about what is happening'.***

(Mitchell, Whiting-Collings, & Christianson, 2020, p.3)

The included studies represented the perspectives of a wide range of female participants of different ages. Lack of knowledge appeared more pronounced in the younger group. This seemed to be related to fertility and risks of infertility not imagined.

> ***'I think I just assumed growing up that everyone can have kids'.***
>
> (Tennyson, 2017, p. 80)

And a second issue was the complexity of the information presented.

> ***'Maybe they need someone to break it down in a way that I can understand for people who are younger'.***
>
> (Mitchell, Whiting-Collings, & Christianson, 2020, p. 3)

Participants therefore expected healthcare professionals to follow them up meticulously as most of the information provided before the OTC procedure was forgotten.

Discussion

In this review, we included four studies that highlighted the experiences of young girls and women undergoing ovarian tissue cryopreservation. The results were analysed using 144 verbatim quotes from 85 participants. We identified four themes associated with experiences of young girls and women who underwent or considered ovarian tissue cryopreservation. The experiences were described as emotional, involving complex decision-making, educational and helping them prepare for the long-term consequences of potentially losing their fertility and hormonal function.

Evidence suggests that there is a great variability across studies when it comes to patient satisfaction in relation to receiving infertility related information associated with cancer (29). Our results echo these findings as most participants would have liked more information before making a decision to undergo ovarian tissue cryopreservation. We found that overall, participants did not understand the technical aspects of the ovarian tissue cryopreservation procedure. They also did not seem to remember much of the information provided, mostly because they were under pressure and had to make other decisions in relation to cancer treatment. The European Society of Human Reproduction and Embryology (ESHRE) in their recent guideline on fertility preservation makes a strong recommendation for clinicians to provide patients with clear information on the impact of their disease (cancer or benign) and treatments on fertility (30). This must also include decisional aids where required. Previous systematic reviews highlight the need for the provision of decision aids as early on as a week after diagnosis in the fertility preservation process (31). There is evidence to suggest that participants preferred written information in addition to oral information such as leaflets that they can take home. Relevant websites dedicated to fertility preservation have also shown to help patients understand the procedure better (29,31,32). Furthermore Anazodo et al. stipulated that having more than one form of decision aid helps patients compare fertility preservation options (33). Through this study, we found that the information provided to participants at the time of fertility preservation needs to be accessible and perhaps in more than one format so it can be easily comprehended by all age groups. Most participants were making decisions under

pressure and so a healthcare professional trained to conduct these consultations are required to have these discussions. We also found that there was a discrepancy in how these young girls and women were followed up. Participants felt the need to have a longer follow up because they may forget they have a tissue ‘banked’ for future use.

Furthermore, younger participants expressed frustration towards how the information was provided and the use of medical jargon. Through this review we found that lack of knowledge and information was more pronounced in the younger patients. The ESHRE guideline on fertility preservation also stressed the need for providing age-specific information, although this has only been considered as a ‘good practice point’ (30). Another study that examined the provision of oncofertility care globally, recommended age-specific information as being useful for young adolescents. We however suggest providing clear, age-specific information to young adolescents as absolutely necessary. Furthermore, since younger participants were unable to fully understand the impact of their disease on fertility, their parents or guardians were having to make these decisions on their behalf. Most young participants therefore felt frustrated with being infantilized, as if their autonomy was taken away. Interestingly, none of the included studies mention the use of Gillick competence or equivalent fitness and whether these were considered in participants before making fertility preservation decisions (34,35).

Overall, participants felt grateful for having the option of ovarian tissue cryopreservation especially since oocyte cryopreservation was not a feasible option for them. Participants

also highlighted how making decisions under pressure was not always possible without the support of family and friends. Healthcare professionals in particular played an important role in the decision-making process. All the studies included, stated how input from the clinician and nurses as well as how the information about the procedure was provided, were key factors in accepting ovarian tissue cryopreservation as a fertility preservation option. Evidence suggests that patients find the decision-making process more manageable if they have adequate knowledge about fertility preservation options (36).

Furthermore, women saw ovarian tissue cryopreservation as an opportunity for the future. Young girls and women and who had not considered becoming mothers, were suddenly faced with the dilemma of being deprived off this right. Being offered ovarian tissue cryopreservation was therefore seen as a salvation. Some participants however did comment on the invasive nature of the procedure and something they would have reconsidered had they had more time and information. We also found that ovarian tissue preservation was provided free of charge in two settings (27,37), whereas participants who had to pay through their insurance, were worried whether spending all this money will help them in the future since there no guarantee they will be able to conceive after transplantation (25,26).

Strengths

To the best of our knowledge, this is the first systematic review that gathers qualitative evidence on the experiences of women undergoing ovarian tissue cryopreservation.

Student ID:
PhD Met+Sys Re T Lab A300

Through a robust systematic search and hand searching of grey literature, we included four studies in the thematic analysis. Although ovarian tissue cryopreservation is being offered widely in specialized centers, patient experience has been limited to survey questionnaires only. This review captures various aspects of patient experience as well as provides recommendations for future research and guidance that can be applied by clinicians in routine practice.

Limitations

There was a lack of true qualitative research on the topic of ovarian tissue cryopreservation and this limited our analysis to only four studies. Through our literature search we found that researchers deemed patient surveys as qualitative research and if there were any true qualitative studies, they mainly focused on the views of health care professionals rather than women that opt for ovarian tissue cryopreservation. This limited us in providing a complete picture of patient experience. In the studies included, all participants interviewed were suffering with cancer. These women were primarily focusing on survivorship, with fertility preservation playing a small role in cancer survival. Furthermore, the possibility of hormonal preservation from this method was only mentioned in one study. It is therefore not possible to comment on whether participants were, or not informed about this. If they were provided with information about hormonal function, we cannot ascertain whether participants simply could not recall this information or return of hormonal function was not important to them.

Student ID:
PhD Met+Sys Re T Lab A300

Implications for practice

Ovarian tissue cryopreservation should be provided by specialised centres only, with healthcare professionals particularly trained in carrying out consultations related to fertility preservation. The decision to undergo this method of fertility preservation for both cancer and benign reasons should be taken by a multidisciplinary team of reproductive endocrinologists, oncologists, surgeons, psychologists, and nurses. The centres that provide this novel technique, should provide participants with written material as well as online visual aids (websites and e-learning). There needs to be a robust policy in place for follow up of these patients. This may be complex for young girls as they are likely to have had their ovarian tissue cryopreserved when much younger. Policy makers need to reach a consensus regarding a time frame for follow up for girls who have had their tissue retrieved before puberty.

Furthermore, there is an urgent unmet need for a universal guideline for health care professionals involved in the care of women requiring ovarian tissue cryopreservation. Various fertility preservation societies have provided guidance on who to offer this procedure to, however there is a significant discrepancy in how this procedure is provided globally.

Young adolescents who wish to undergo this procedure pose a challenge for health care professionals. This cohort requires particular attention when it comes to the provision of information. Additionally, clinicians need to consider capacity and competence in these

young girls as well as guidance on when to involve parents in the decision-making process. There is currently no robust guidance on assessing capacity and competence in young adolescent girls when it comes to considering ovarian tissue cryopreservation as a fertility preservation method.

Lastly, there is a need to establish a global registry and research database to generate evidence-based information regarding the return of hormonal and reproductive function from transplantation of the cryopreserved ovarian tissue.

Conclusion

This study addresses the experiences of young girls and women regarding a new fertility preservation method. Ovarian tissue cryopreservation is gaining popularity due to not requiring ovarian stimulation and therefore preventing a delay in starting anti-cancer treatment. Women who have considered or undergone this procedure have described their experiences as emotional, requiring complex decision making and having to make time sensitive decisions. Healthcare professionals involved in the care of young girls and women undergoing this method therefore need to take into consideration the emotional wellbeing of the patients as well as the time and expertise it requires to help them make an informed decision. Furthermore, given the novel nature of this procedure, young girls or women undergoing this method of fertility preservation should be given clear information about what the procedure entails and followed up meticulously.

References

1. Gellert SE, Pors SE, Kristensen SG, Bay-Bjørn AM, Ernst E, Yding Andersen C. Transplantation of frozen-thawed ovarian tissue: an update on worldwide activity published in peer-reviewed papers and on the Danish cohort. J Assist Reprod Genet. 2018;35(4):561–70.
2. Sheshpari S, Shahnazi M, Mobarak H, Ahmadian S, Bedate AM, Nariman-Saleh-Fam Z, et al. Ovarian function and reproductive outcome after ovarian tissue transplantation: A systematic review. J Transl Med. 2019;17(1). Available from: https://doi.org/10.1186/s12967-019-02149-2
3. Dolmans MM, Donnez J. Fertility preservation in women for medical and social reasons: Oocytes vs ovarian tissue. Best Pract Res Clin Obstet Gynaecol. 2021;70:63–80. Available from: https://doi.org/10.1016/j.bpobgyn.2020.06.011
4. ASRM. Fertility preservation in patients undergoing gonadotoxic therapy or gonadectomy: a committee opinion. Fertil Steril. 2019;112(6):1022–33. Available from: https://doi.org/10.1016/j.fertnstert.2019.09.013
5. Yasmin E, Balachandren N, Davies MC, Jones GL, Lane S, Mathur R, et al. Fertility preservation for medical reasons in girls and women: British fertility society policy and practice guideline. Hum Fertil. 2018;
6. Yding Andersen C, Mamsen LS, Kristensen SG. Freezing of ovarian tissue and clinical opportunities. Reproduction. 2019;158(5):F27–34.

Student ID:
PhD Met+Sys Re T Lab A300

7. Kristensen SG, Andersen CY. Cryopreservation of Ovarian Tissue: Opportunities Beyond Fertility Preservation and a Positive View Into the Future. Front Endocrinol (Lausanne). 2018;9:347. Available from: https://www.frontiersin.org/article/10.3389/fendo.2018.00347
8. Almodin CG, Almodin PM, Radaelli MRM, Minguetti-Câmara VC, Ceschin AP, Ribeiro RC. The frst ovarian tissue transplant between monozygotic twin sisters discordant for ovarian function in Latin America. J Bras Reprod Assist. 2015;19(1):29–32.
9. Donnez PJ, Dolmans MM, Demylle D, Jadoul P, Pirard C, Squifflet J, et al. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet. 2004;364(9443):1405–10.
10. Shapira M, Dolmans MM, Silber S, Meirow D. Evaluation of ovarian tissue transplantation: results from three clinical centers. Fertil Steril. 2020;114(2):388–97. Available from: https://doi.org/10.1016/j.fertnstert.2020.03.037
11. Dolmans MM, von Wolff M, Poirot C, Diaz-Garcia C, Cacciottola L, Boissel N, et al. Transplantation of cryopreserved ovarian tissue in a series of 285 women: a review of five leading European centers. Fertil Steril. 2021;115(5):1102–15. Available from: https://doi.org/10.1016/j.fertnstert.2021.03.008
12. Schover L. Psychosocial aspects of infertility and decisions about reproduction in young cancer survivors: a review. Med Pediatr Oncol. 1999;33:53–59.
13. Partridge A, Gelber S PJ. Web-based survey of fertility issues in young women with

breast cancer. J Clin Oncol. 2004;22:4174–4183.

14. Shin S, Kim J, Kim D, Kim H, Lee S, Kim SH, et al. The Importance of Education regarding the Effects of Anticancer Treatment on Fertility and Ovarian Function in the Female Patients with Breast Cancer. J menopausal Med. 2019 Dec;25(3):142—148. Available from: https://europepmc.org/articles/PMC6952705

15. Lotz L, Barbosa PR, Knorr C, Hofbeck L, Hoffmann I, Beckmann MW, et al. The safety and satisfaction of ovarian tissue cryopreservation in prepubertal and adolescent girls. Reprod Biomed Online. 2020;40(4):547–54. Available from: https://doi.org/10.1016/j.rbmo.2020.01.009

16. CS - Sullivan-Pyke, CA C, M P, CR G, JP. G. - Ovarian tissue cryopreservation (OTC) in prepubertal girls and young women: an analysis of parents' and patients' decision-making. - J Assist Reprod Genet. 2018;

17. Forman EJ, Anders CK, Behera MA. A nationwide survey of oncologists regarding treatment-related infertility and fertility preservation in female cancer patients. Fertil Steril. 2010;94(5):1652–6. Available from: http://dx.doi.org/10.1016/j.fertnstert.2009.10.008

18. Vesali S, Navid B, Mohammadi M, Karimi E, Omani-Samani R. Little information about fertility preservation is provided for cancer patients: A survey of oncologists' knowledge, attitude and current practice. Eur J Cancer Care (Engl). 2019;28(1):1–8.

19. Quinn GP, Vadaparampil ST, King L, Miree CA, Wilson C, Raj O, et al. Impact of physicians' personal discomfort and patient prognosis on discussion of fertility

preservation with young cancer patients. Patient Educ Couns. 2009;77(3):338–43.

20. Watkins DC. Qualitative Research: The Importance of Conducting Research That Doesn't "Count." Health Promot Pract. 2012 Mar 1;13(2):153–8. Available from: https://doi.org/10.1177/1524839912437370

21. Tong A, Sainsbury P, Craig J. Consolidated criteria for reporting qualitative research (COREQ): a 32-item checklist for interviews and focus groups. Int J Qual Heal Care. 2007 Dec 1;19(6):349–57. Available from: https://doi.org/10.1093/intqhc/mzm042

22. Tong A, Flemming K, McInnes E, Oliver S, Craig J. Enhancing transparency in reporting the synthesis of qualitative research: ENTREQ. BMC Med Res Methodol. 2012;12(1):181. Available from: https://doi.org/10.1186/1471-2288-12-181

23. Stern C, Jordan Z, Mcarthur A. Developing the review question and inclusion criteria: The first steps in conducting a systematic review. Am J Nurs. 2014;114(4):53–6.

24. CASP. Critical Appraisal Skills Programme (2018). CASP (Qualitative) Checklist. [online] Available at: https://casp-uk.net/wp-content/uploads/2018/03/CASP-Qualitative-Checklist-2018 fillable form.pdf. Date Accessed: 30.11.2020. 2018.

25. Hershberger PE, Finnegan L, Pierce PF, Scoccia B. The Decision-Making Process of Young Adult Women with Cancer Who Considered Fertility Cryopreservation. JOGNN - J Obstet Gynecol Neonatal Nurs. 2013;42(1):59–69. Available from: http://dx.doi.org/10.1111/j.1552-6909.2012.01426.x

26. Mitchel C, Whiting-Collings L, Christianson M. Understanding patients' knowledge and feelings regarding their cryopreserved ovarian tissue: A qualitative interview

study. J Adolesc Young Adult Oncol. 2020;9(4):502–7.

27. Bach AS, Schmidt KT, Kristensen SG. Futures and fears in the freezer: Danish women's experiences with ovarian tissue cryopreservation and transplantation. Reprod Biomed Online. 2020;41(3):555–65. Available from: https://doi.org/10.1016/j.rbmo.2020.06.009

28. Thomas J, Harden A. Methods for the thematic synthesis of qualitative research in systematic reviews. BMC Med Res Methodol. 2008;8:1–10.

29. Goossens J, Delbaere I, Lancker A Van, Beeckman D, Verhaeghe S, Hecke VA. Cancer patients' and professional caregivers' needs, preferences and factors associated with receiving and providing fertility-related information: a mixed-methods systematic review. Int J Nurs Stud. 2014;51(2):300–319.

30. ESHRE. Female Fertility Preservation Guideline. 2020.

31. Shen S, Zelkowitz P, Rosberger Z. Cancer and fertility: optimizing communication between patients and healthcare providers. Curr Opin Support Palliat Care. 2019 Mar;13(1):53—58. Available from: https://doi.org/10.1097/SPC.0000000000000413

32. Ehrbar V, Urech C, Alder J, Harringer K, Rochlitz RZDC, Tschudin S. Decision-making about fertility preservation-qualitative data on young cancer patients' attitudes and needs. Arch Womens Ment Heal. 2016;19(4):695–9.

33. Anazodo A, Laws P, Logan S, Saunders C, Travaglia J, Gerstl B, et al. How can we improve oncofertility care for patients? A systematic scoping review of current international practice and models of care. Hum Reprod Update. 2019;25(2):159–79.

34. Taylor JF, Ott MA. Fertility Preservation after a Cancer Diagnosis: A Systematic Review of Adolescents', Parents', and Providers' Perspectives, Experiences, and Preferences. J Pediatr Adolesc Gynecol. 2016;29(6):585–98.

35. Quinn GP, Murphy D, Knapp C, Stearsman DK, Bradley-Klug KL, Sawczyn K, et al. Who decides? Decision making and fertility preservation in teens with cancer: a review of the literature. J Adolesc Health. 2011/03/15. 2011 Oct;49(4):337–46. Available from: https://pubmed.ncbi.nlm.nih.gov/21939862

36. Peate M, Meiser B, Friedlander M, Zorbas H, Rovelli S, Sansom-Daly U, et al. It's now or never: fertility-related knowledge, decision-making preferences, and treatment intentions in young women with breast cancer--an Australian fertility decision aid collaborative group study. J Clin Oncol. 2011;29(13):1670–7.

37. Tennyson R. Paper B: An interpretative phenomenological analysis study into the experience of teenagers and young adults offered ovarian tissue cryopreservation following a cancer diagnosis. 2017.

CHAPTER 5: WHY WOMEN CONSIDER OVARIAN TISSUE CRYOPRESERVATION FOR PRESERVING REPRODUCTIVE AND HORMONAL FUNCTION: A QUALITATIVE ENQUIRY

Student ID:
PhD Met+Sys Re T Lab A300

Preamble

When originally designing the study that would contribute to this doctoral thesis, the plan was to undertake a qualitative study to explore with women attending a non-publicly funded clinic to discuss OTC. Due to the COVID-19 pandemic, the service temporarily ceased, and it was not possible for women to attend the clinic. In order not to miss the opportunity to explore why women might consider or understand what motivated those who had undergone fertility preservation to choose OTC, amendments were made to original protocol. The study described in this chapter presents the views, motivations and expectations from women recruited through social media and through online message boards who indicated a willingness to discuss OTC or had undergone the procedure. An amendment to the original ethical approval, granted on 14/09/2020 (ERN 19-1578A) was therefore sought and granted on 19/01/2021, (ERN 19-1578A).

The protocol for this study has been published in BMJ Open, which is added to the appendix (Appendix 19).

Khattak H, Gallos I, Coomarasamy A, Topping AE. Why are women considering ovarian tissue cryopreservation to preserve reproductive and hormonal ovarian function? A qualitative study protocol. BMJ Open 2022;0:e051288. doi:10.1136/bmjopen-2021-051288

Student ID:
PhD Met+Sys Re T Lab A300

Abstract

Introduction

Many women who are in a position to control their fertility, are delaying childbirth for various reasons including career aspirations, lack of long-term relationships, or not feeling ready for motherhood. Currently two established fertility preservation options are accessible to women and young girls - oocyte cryopreservation (egg freezing) and embryo cryopreservation. Ovarian tissue cryopreservation (OTC) provides another option and involves removing sections of ovarian tissue and cryopreserving it for future use. Subsequently, often several years later, the ovarian tissue is transplanted back onto the remaining ovary, pelvic side wall or subcutaneous tissue sites in forearm. OTC was originally developed for women and young girls undergoing cancer treatments that were potentially damaging to the ovaries. This technique offers many young girls and women, who would otherwise be infertile, the chance at some point in the future, to have biological children. In addition to fertility preservation, this surgical technique can also be used to restore ovarian endocrine function thus could be used as another strategy for those women experiencing premature menopause and associated debilitating symptoms. In healthy women, OTC has the potential to prolong time to natural age of menopause remaining women are fertile for longer. In theory, endocrine function of ovaries could also help transition through symptoms of natural menopause. However, OTC is still considered experimental in most countries, is an invasive technique, involving two operations with

Student ID:
PhD Met+Sys Re T Lab A300

concomitant risks, and not commonly available in the UK through NHS services could be costly. It is, therefore, important to understand perceptions of women who are interested in, or have undergone this procedure, and how they view its potential benefits and risks, so that evidence-based and women-centred care can be provided for women in the future.

Methods

This study used a qualitative enquiry approach to explore the knowledge, motivation, and decision-making who expressed an interest in and or had sought ovarian tissue cryopreservation for fertility or hormonal preservation. A pragmatic approach underpinned the theoretical orientation to frame the qualitative study design. Data was collected from women who responded after information about the study was disseminated via social media platforms, online patient information groups and relevant organisations. Women who responded to the call were invited to participate in an in-depth semi-structured interview.

Analysis

Participant interviews were conducted using Zoom software, audiotaped and, if consented, video recorded. The recordings were transcribed verbatim and analysed using thematic analysis supported by NVivo© software (version12).

Results

Student ID:
PhD Met+Sys Re T Lab A300

Twenty one participants were interviewed (age range 22-43 years) including nine women who identified as single, 11 married (or in a long term, stable relationship) and one was separated. All 21 participants had undergone formal education to age 18 years. Nineteen were university graduates and two were currently attending higher education. All but two were employed. Participants were from a range of different ethnic backgrounds. Six participants had previously been diagnosed with cancer, five of whom were residents outside the UK and had undergone OTC procedure in their resident country. Sixteen participants indicated they were interested in OTC for non-medical (social reasons) or had awareness of the procedure as a fertility preservation option. Five themes emerged as a result of the thematic synthesis: OTC provides another option for female fertility preservation, it provides insurance in the future, fertility is not a priority now, awareness and availability of OTC procedure and financial considerations if wishing to undergo OTC.

Conclusion

This study highlights the perceptions of women who have considered or undergone OTC. Whether women considered OTC for social reasons or due to fertility ablating cancer treatments, OTC was seen as another option of fertility preservation. Key findings included participants' concerns about the costs associated with the OTC procedure; although it was also argued that it should be made available to those in whom it is the only option for fertility preservation or have the means to do so. Lastly, participants who underwent OTC procedure (all US based) perceived that not all members of the multidisciplinary team were up to date with information about provision of OTC.

Student ID:
PhD Met+Sys Re T Lab A300

Key words: Female fertility preservation; hormonal preservation; ovarian tissue cryopreservation; decision making; menopause; qualitative; thematic analysis

Introduction

Advances in anti-cancer therapies have resulted in an increase in the number of young women surviving cancer. A consequence of ovary-damaging treatments as a result of anti-cancer therapies is that patients may be left subfertile or infertile. (1) The field of fertility preservation is evolving, with more practise innovation, service usage, and improved results driving the therapeutic landscape forward. Fertility preservation offers women that have survived cancer with the chance to have children. (2–4). Advances in fertility preservation techniques also means that the advantages presented by fertility restoration may not be limited to just women suffering from cancer. Fertility preservation for non-medical reasons is termed as 'elective' or 'social' freezing. In recent decades, the average age of women giving birth to their first child has gradually increased. (5) Various reasons have been offered to explain this trend including increased levels of participation in higher education, career aspirations, lack of suitable partner or sustained relationship with someone who wishes to co-parent, and financial considerations. (5–7). Hence some women may wish to consider fertility preservation to extend their fertility for social reasons.

Although oocyte and embryo freezing are well established fertility preservation methods, several centres worldwide are now offering a new procedure that involves ovarian tissue

Student ID:
PhD Met+Sys Re T Lab A300

cryopreservation (OTC). Unfortunately for women who are starting cancer therapy, oocyte freezing can be a lengthy process involving controlled ovarian stimulation that can cause delays to initiation of cancer treatment. Ovarian tissue cryopreservation offers an alternative option, first used clinically over two decades ago and has been utilised by thousands of women worldwide. (8–11) Whilst the availability of OTC has increased since its introduction as an innovative technology for female fertility preservation, its availability in the UK remains patchy, with only a handful of centres offering this option to national health service (NHS) patients. (4,12,13) In the UK, when available, OTC procedures are normally only offered to women with cancer prior to fertility ablating treatment. The paucity of the service, paired with the small patient group to whom it is available, disadvantages women for whom OTC is the only fertility preservation option, for example those needing to start anti-cancer treatment imminently. Globally, some centres are offering OTC routinely for the preservation of fertility (mainly for women with cancer but also for social reasons as mentioned above) but due to OTC requiring trained staff and a dedicated biobank availability is variable. (12,14) Evidence suggests that re-implantation of thawed previously frozen ovarian tissue can produce a return of hormonal function in women; hence it could be used to preserve hormonal function as well. (15,16) In chapter two, we conducted a systematic review and individual patient data meta-analyses to address the reproductive and endocrine outcomes from ovarian transplantation. Through this study, we found that the live birth rate from OTC and subsequent transplantation procedure was 28%. (17) Furthermore, the study also highlighted the return of endocrine function from transplanting ovarian tissue. Additionally,

ovarian tissue cryopreservation and transplantation procedures have also been used in women experiencing premature ovarian insufficiency (POI) to restore reproductive function. (18) Therefore, OTC followed by transplantation offers a wide range of women the possibility of pregnancy, to have biological children and experience motherhood, in addition to preserving and or restoring their hormonal function. Ovarian tissue cryopreservation for social reasons or preservation in women experiencing POI, has its critics. (19) However, alongside the clinical evidence of benefit, it is also crucial to gain the perspectives of women (which hasn't been previously explored) to enable informed discussion regarding who is eligible for or should have access to OTC.

Recently, there has been increased awareness regarding fertility preservation methods and they are now also being offered to healthy women who wish to delay childbirth. (20–24) For example, age related oocyte preservation is now considered acceptable as a strategy for delaying pregnancy for personal reasons, but this technique does not preserve ovarian hormonal function. (3,25,26) In comparison to age-related oocyte preservation, OTC has the potential to offer women with natural ovarian hormonal function and postponing menopausal symptoms. (16,26) Hence there is a need to explore the underlying values, beliefs and reasons that motivate women to seek ovarian tissue cryopreservation for hormone-related purposes.

Methods

Theoretical framework

Women have a longer life expectancy as compared with few decades ago and can spend nearly half of their lives in menopausal state, which for some mean experiencing debilitating post-menopausal symptoms for an extended period of time. (27) Reduced levels of oestrogen due to menopause increases cardiovascular risk and osteoporosis, and for some, can have a negative impact on mental wellbeing. (28,29) Understanding the acceptability of a procedure such as OTC in the imaginations of women who are not yet menopausal but may have fertility 'biological clock' anxieties warrants attention. One possible explanation for using OTC is the notion of future proofing, as OTC offers the potential of hormonal 'insurance'. Menopause was once something experienced but not spoken of and is starting to increasingly being portrayed as a negative life event in both the medical literature and mainstream media. (30–35) This may provide some traction for requests to preserve ovarian hormonal function through an interventional approach. Some women may wish to pre-empt the anticipated menopausal changes both physically and psychologically, by choosing to intervene and prolong their fertility prior to menopause. How these influences manifest and or if they engender a menopausal anticipatory anxiety is currently unknown.

Theory underpinning decision making

In behavioural psychology two systems of thinking are described as important in influencing the decisions we make; 'System 1' thinking is fast, influenced by emotions and is intuitive.

This system enables humans to differentiate between danger and safety. (36) In comparison, ‘System 2’ is slower and functions through reasoning. It is used as a backup and is often difficult to actuate. While ‘System 1’ operates reflexively, normally activation of ‘System 2’ requires conscious processing. Often these two systems interact with each other rather than operate independently, with System 2 overriding System 1 (if it is intermittently overruled). The crucial point is when System 1 does not allow for System 2 to take over. (37) This concept of fast and slow thinking could be applied to how women may make decisions when considering OTC to preserve their fertility or hormonal function. The proposition is that women may use system 1 when initially seeking information or attending a first consultation about the procedure, whereas system 2 may come into play when a more considered decision is required such as when finally deciding whether or not to undergo the procedure.

A concept that may modulate ‘System 1’ or ‘System 2’ thinking in the case of these women is that of biographical disruption. Biographical disruption, a term that was first coined in 1982 by Michael Bury to describe how events or occurrences such as an illness can cause a profound change that affects how individuals understand themselves. This has been used to understand response to chronic illness where an individual may feel that their condition has created a major disruption to their self-identity, causing them to redefine their ‘biography’. (38) This concept could be extended to women experiencing menopause, or to women who will ultimately become menopausal, by framing menopause as a state where women may be challenged to redefine their identity. A qualitative study conducted by de

Salis et al. 2018 examined the women's experiences of menopause in women and offered three main explanatory narratives. Menopause was defined either as 1) a normal process where no transition occurs in the women or in her societal interactions; 2) as a struggle, associated with loss of identity and distress; and 3) menopause as release from the messy business of menstruation and therefore a liberating experience. (29) In the wider medical literature, menopause has been associated with ageing and other risks such as osteoporosis and cardiovascular disease and often constructed as an illness that is treatable with hormone replacement therapy (HRT). (31,39–41) Although the notion of medicalisation is contentious in itself; not least because is it often presented as medical dominance over 'a lay docile' female population to that of consumerism where users determine or resist medicalisation. (42) HRT is not an unproblematic solution for managing menopausal symptoms and the side effects and consequences of HRT are well known with women enabling them to be 'active participants in decision-making' about this risks. (42) In some cases, women have resisted the rationality of evidence-based medicine and risk narratives when presented by healthcare providers and negotiated the competing discourses to obtain solutions that they perceive are best for them. This has exposed women to making decisions that involve balancing acceptability with risks of hormone replacement treatments.

Anticipation of the biographical disruption which menopause might induce, may worry some women in their younger years and function as the background interference to 'System 1' thinking. This interference may be influenced by experiences, emotions and memories associated with learning about menopause. This menopausal system 1 thinking could be

overruled by ‘System 2’ thinking (influenced by facts, logic and evidence) when a woman is making a decision about whether or not to undergo OTC. However, it could very well be the reversed for example, ‘System 1’ sends suggestions to ‘System 2’ regarding menopause as a biological disruption which then turns these thoughts into beliefs. Irrespective, awareness of the symptoms of menopause has gained greater profile in recent years likely fuelled by media interest and widely accessible information on the topic. Whether these influences encourage women to seek out information deliberately through ‘System 2’ thinking and, in some cases, opt for a procedure that can potentially delay, or even reverse menopause remains unknown. Women who seek to preserve their fertility or hormonal function may have various personal reasons as to why they choose to do so and this study sought to explore these perspectives. Throughout this study we explored whether or not menopause was seen as an anticipation of biographical disruption in women’s lives that pre-empted them to bank their ovarian tissue for future use.

Study design

Pragmatic Qualitative Descriptive Design was used to test and develop the theories underpinning this body of research. (43)

Pragmatist theory

Pragmatism can be defined as a link between ‘human knowing’ and ‘human action’ and explores how beliefs are held and shared through behaviours. (44) Pragmatism incorporates the idea that two individuals may share the same experience, but their

worldviews of the situation may not be the same. The extent to which an experience is similar, depends on the degree of shared view. This phenomenon therefore allows an individual to hold a unique, as well as a shared, view of an experience. (43,44) This study was designed to purposively recruit women from varying backgrounds, ethnicities and ages, to collate their views and experiences of women in relation to fertility and hormonal preservation in thinking about OTC. Pragmatist philosophy proposes that the meaning of actions and beliefs are found in consequences. (43) Actions in pragmatism are 'fluidly' linked to consequences, and so, they are open to change. If the situation pertaining to an action change, so does the consequence of the action, even though the action remains unchanged. (43,44)

Participant selection

Participants originally were to be recruited from one UK based clinic that offers this procedure on a private basis. It was however not possible to recruit from the clinic due to closures as a result of the COVID-19 pandemic. Hence participants were recruited through social media platforms (Facebook, Twitter and Instagram). Snowball sampling was used.

Participants were all women who were aware of and interested in, or had considered, or undergone ovarian tissue freezing for fertility and/or hormonal preservation. Details of the study were posted on Twitter, Facebook and Instagram of the researcher as well as through patient information groups such as fertility network UK. These posts were

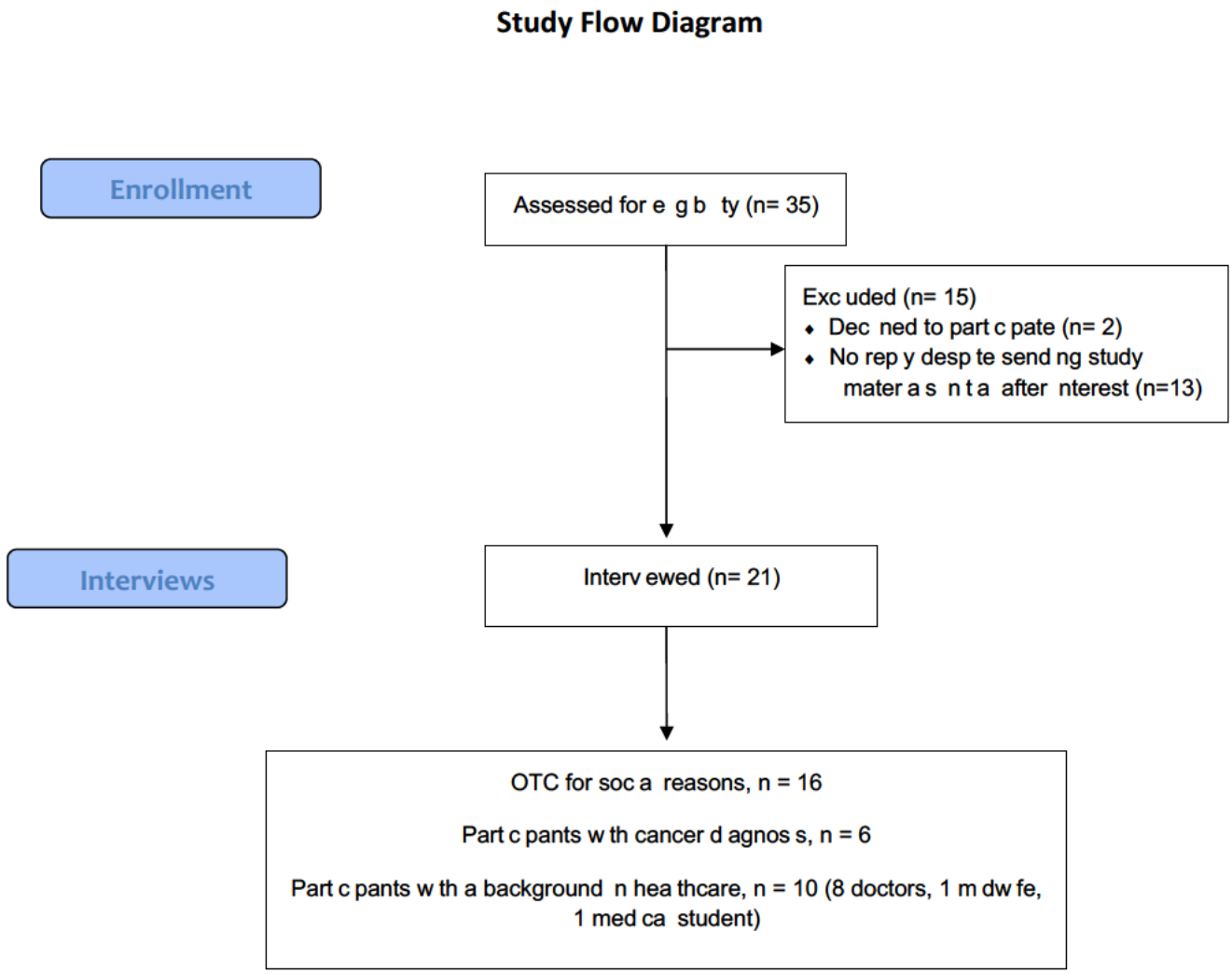
encouraged to be shared. Potential participants responded to the call by contacting the researcher directly via email, which was provided in the post. The sampling was purposive in nature, in that anyone who was born female at birth, aged of 18-45 years and was thinking about fertility preservation and considering OTC, was eligible. The age range was chosen because we wanted to reach an adult population that included both women of reproductive age and those who had not yet reached menopause. Women who had heard of ovarian tissue cryopreservation and were interested in it or undergone it as a fertility or hormonal preservation method, were deemed eligible.

Non-participation

Thirty-one individuals showed interest after contacting the researcher on social media platforms and emails. Twenty-one participants, however, proceeded to be interviewed. The study flow diagram is shown in Figure 33.

Student ID:
PhD Met+Sys Re T Lab A300

Figure 33. Study flow diagram



Recruitment

Recruitment and sampling continued until data saturation point was reached, at 21 participants.

Data collection

Interview guide

A topic guide to frame the interviews was developed for the study (Appendix 17). This was not piloted prior to commencing interviewing and minor adjustments were made following the first three interviews. The interview adopted a semi-structured approach, starting with open ended questions to understand individual perspectives of the women about fertility, fertility preservation and menopause followed by specific questions about awareness of the different fertility preservation techniques. No follow up interviews were conducted.

Audio/visual recording

All interviews were audio recorded. If the participants permitted and consented, video recordings were also included. The video recordings were not used in the data analysis but saved on a secure university drive should they need to be referred to in the future. The recordings were transcribed automatically on Zoom, and the transcripts were checked

again manually, and anonymised. Each participant was assigned a pseudonym to protect their anonymity which was used in the reporting of the results. (45) Transcripts were not returned to participants for comment or corrections. Data was analysed using thematic analysis, supported using NVivo software version 12. (46) On average, the duration of the interview was 36 minutes (range 23 to 55 minutes). Reflective field notes were made following each interview and these were used to assist in analysis, stored as part of the study data set. Reflexivity aided critical thinking, as well as questioning assumptions and identifying the degree that the researcher's ideas, actions, and judgments affected coding and analysis. (47)

Interviews were conducted until saturation was reached after the 21st interview. The definition of saturation used in this study was the 'the point in coding when you find that no new codes occur in the data'. (48–50) In practice this was the point that mounting instances of the same codes, but no new ones were applied to the data.

Data analysis

The transcripts were coded line by line. Data was coded by one person and confirmed by a second coder. The method described for thematic analysis by Braun and Clarke 2006 was used. (51) This involved what has been described as the "complete coding" method first working systematically through the data and coding data chunks that were relevant to

the research questions. Subsequently, refining codes as the analysis evolved. All transcribed data were checked prior to commencing coding which helped with familiarisation of data. This was followed by initial coding. Coding was theory driven in that codes particular to women's perspective on OTC for fertility and hormonal preservation were identified. When all the data had been coded, the codes were grouped into categories. These categories were then collapsed to form the basis of potential overarching themes. Appendix 18 shows an example of the codes identified during the analysis, as well as how the original theoretical orientation were used to frame coding and theme development.

The researcher (H.K) conducted the interviews, and completed the analysis and coding, then a second coder reviewed the coding; any cases of disagreement were discussed in conjunction with the first researcher, and mutually agreed. Derivation of themes from codes was an iterative process.

Results

Description and size of sample

21 participants (age range 22-43 years) were interviewed, of which nine identified as single, 11 married or in a civil partnership or long term, stable relationship and one woman described her status as separated. Nineteen participants were university graduates with two currently undertaking higher education. All but two were employed. Participants self-

identified their ethnic backgrounds including Black Caribbean (n=2), Asian (n=11) and White (n=8). Six participants had previously been diagnosed with cancer, five of whom had undergone OTC outside the UK. Sixteen participants were considering OTC for social reasons. Detailed demographic information is presented in Table 12. Eleven participants had a background in healthcare (7 doctors, 1 midwife, 1 nurse, 1 radiographer and 1 medical student). Sixteen participants were interested in use of OTC for social reasons.

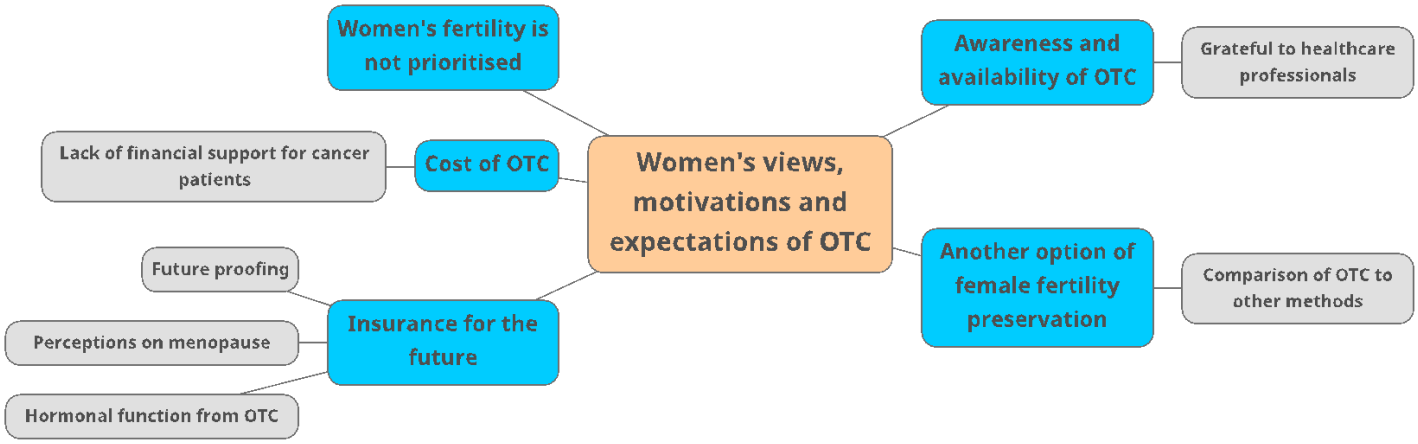
Table 12. Participant demographics.

No.	Pseudonym	Age	OTC experience	Marital status	Education	Profession	Country of residence	Ethnicity
1	Faria	32	Interested in OTC	Single	University	Banker	UK	Asian-South Asian
2	Alex	22	Interested in OTC	Single	College/sixth form	Student	India	Asian-South Asian
3	Donna	37	Interested in OTC	Single	University	Not given	UK	Black African
4	Andrea	35	Interested in OTC	Single	University	Charity sector	UK	Asian-Chinese
5	Vinita	31	Interested in OTC	Long term relationship	University	Doctor	UK	Asian-South Asian
6	Casey	33	Interested in OTC	Married	University	Doctor	UK	Caucasian
7	Denise	38	Interested in OTC	Married	University	Midwife	UK	Caucasian
8	Rita	32	Interested in OTC	Single	University	Not given	UK	Not given
9	Debora	30	Interested in OTC	Married	University	Doctor	UK	Black African
10	Faye	31	Interested in OTC	Single	University	Doctor	UK	Asian-South Asian
11	Gina	31	Interested in OTC	Single	University	Doctor	Turkey	Not given

12	Nina	35	Interested in OTC	Single	University	Not given	UK	Asian-South Asian
13	Veronica	33	Interested in OTC	Single	University	Teacher	India	Asian-South Asian
14	Ruth	33	Undergone OTC	Married	University	Self employed	USA	Not given
15	Emma	23	Interested in OTC	Single	College/Sixth form	Student	UK	Caucasian
16	Alice	40	Undergone OTC	Married	University	Not given	USA	Caucasian
17	Danika	34	Undergone OTC	Married	University	Scientist	USA	Caucasian
18	Amelia	43	Undergone OTC	Married	University	Nurse	USA	Caucasian
19	Lucy	34	Undergone OTC	Married	University	Radiographer	USA	Caucasian
20	Lina	31	Interested in OTC	Separated	University	Doctor	UK	Asian-South Asian
21	Anya	32	Interested in OTC	Married	University	Doctor	UK	Asian-South Asian

Five themes were developed from the thematic analysis. These were: OTC provides another option of female fertility preservation, OTC provides insurance for the future, female fertility is not a priority, awareness and availability of OTC procedure, and cost of OTC. The themes are represented in Figure 34.

Figure 34. Schematic representation of generated themes. OTC = ovarian tissue cryopreservation.



Themes

Theme: Another option of fertility preservation

Women with cancer who were not eligible for oocyte cryopreservation because there was urgency to start cancer treatment expressed gratitude that OTC was provided as an option as it also meant that their cancer treatment was not delayed due to fertility preservation.

> ***'So that [OTC] was kind of like my only option, given the short timeline...so I was grateful to have that hope out there.'***
>
> Amelia, 43 (undergone OTC)

In healthy women who had the desire to preserve their fertility using OTC, it gave them a choice between oocyte cryopreservation and OTC.

'I think the fact that we have more options is really encouraging and it's helpful for a lot of women out there.'

Lina, 31 (interested in OTC)

Subtheme: Comparison of OTC to other FP methods

Participants compared OTC with what they understood about other available methods of fertility preservation, such as oocyte cryopreservation and embryo cryopreservation, and described OTC to be more 'natural. For participants interested in OTC, the notion of potentially avoiding exogenous hormones for oocyte collection was attractive as forcing ovulation was perceived as 'unnatural'. The added benefit of potential hormonal preservation that could help with menopausal symptoms in the future, was also appealing and considered closer to the natural bodily function as compared to taking pharmacological treatment for menopausal symptoms. Some participants had undergone other assisted reproductive techniques as well as OTC. In these women the OTC procedure both for conception as well as return of hormonal function was again deemed as being more 'natural'.

'I think the fact that you actually take a part of your ovary, freeze it and transplant it back, you can get some of that function back rather than trying [assisted reproductive technology] to do things, I mean not that it's a more natural thing to do, but it's very close to how your natural fertility works, or how your natural hormones work…as opposed to egg freezing.'

Anya, 32 (interested in OTC)

'This felt so natural…so wonderful to feel like my body is working you know. Being able to just naturally conceive and not have to like have all those procedures and the egg retrieval, all that stuff like at such a young age is just…such a [great thing] …something to [really] embrace'.

Ruth, 33 (undergone OTC)

Theme: Awareness and availability of OTC

One of the issues voiced by the participants who had required cancer care was that they felt that some of the healthcare professionals involved in their cancer care were unaware of the OTC procedure, particularly their oncologists. This may be that preserving fertility is not always a top priority for oncologists at the time of cancer diagnosis when the clinical imperative might be initiating cancer treatment.

'I think that there's like hardly any awareness about it when I talk to people about this, they've never heard of it, they have no idea even, my oncologist.'

Alice, 40 (undergone OTC)

'I would have loved to be guided by my oncologist, even by my doctor that removed my ovarian tissue and even by a patient organization to say hey, these are the doctors that know what they are doing when it comes to this and actually have proven success in doing that stuff.'

Danika, 34 (undergone OTC)

A participant who had undergone OTC and was also a patient advocate for OTC expressed her concerns about healthcare professionals failing to emphasise the importance of fertility preservation, suggesting that some young women who are at risk of losing their fertility due to treatment may miss out an opportunity to preserve their reproductive function.

> ***'But I met other cancer survivors, where the oncologist never even told them that they were going to lose their fertility.'***
>
> Danika, 34 (undergone OTC)

In the UK it is expected that clinicians will have a fertility preservation discussion prior to initiation of cancer treatment. (4) None of the participants who had OTC were based in the UK, it is therefore not possible to ascertain whether the discussions take place routinely in the UK. Participants who were thinking about OTC for social reasons were surprised by the lack of awareness of the procedure and wondered why it was not widely publicised.

> ***'Makes you think why...why hasn't it been thought about like years ago.'***
>
> Debora, 30 (interested in OTC)

> ***'I mean if I hadn't heard about it from a colleague, I'm not sure if I would have known about it.'***
>
> Nina, 35 (interested in OTC)

One participant suggested it was linked to gender disparity in both information and availability of fertility preservation procedures between males and females and offered it as a potential reason for the lack of awareness about OTC.

> ***'Because we always know that the female health falls behind males all the time.'***
>
> Amelia, 43 (undergone OTC)

Even though OTC has been described in the evidence for more than two decades, it is still not widely available. (9,13,17) One participant who sought to explore options to preserve her fertility was told that OTC could not be offered to her because the clinic did not provide it.

> ***'He [doctor] made it clear at the time that it's not something that the clinic he was working at offered, and that I would need to go elsewhere, which was a little bit disappointing especially as it was something I was quite interested in.'***
>
> Nina, 35 (interested in OTC)

> ***'I'm quite curious as to why this is not more of a well-known thing, you know why it is not a routine and we talk about IVF and IVF is a thing, we've always known that you can freeze something but I'm just quite curious this is not routine practice.'***
>
> Rita, 32 (interested in OTC)

Women in this study seemed to be inquisitive about OTC as a fertility and hormonal preservation option however were surprised by the limited availability of this procedure.

Subtheme: Grateful to healthcare professionals

Participants who had undergone OTC as a result of cancer treatment to prevent impairment to their fertility were grateful that they were undergoing treatment in centres that offered OTC or had their care managed by healthcare professionals who were able to refer them. They felt indebted to what they perceived as 'forward-thinking' healthcare professionals, who had enabled their access to fertility preservation using OTC.

> ***'When I was meeting with the [bone marrow] transplant physician the nurse practitioner, who worked with him had just gone to a conference where this doctor talked about this experimental ovarian tissue freezing that worked in sheep and rats. I was grateful for that nurse who happened to just go to that conference at the right time.'***
>
> Amelia, 43 (undergone OTC)

> ***'I was lucky, fortunate that I had a primary doctor that saw the big picture and knew me. If I hadn't had her, I wouldn't have this miracle inside of me right now.'***
>
> Alice, 40 (undergone OTC)

Theme: Fertility is not a priority

Participants considering OTC for fertility preservation gave a variety of reasons for wanting to delay motherhood including not being ready, financial reasons and focusing on career progression. Not having a partner or being in a legal relationship was not necessarily a

reason for delaying motherhood as there were also participants who identified as married, wished to delay childbearing.

> ***'I'm actually at a stage in my life, that I feel children are not something that I'm thinking about right now.'***
>
> Veronica, 33 (interested in OTC)

> ***'I've always kind of felt like 35 was the time that I would really need to start thinking about this stuff a bit more seriously.'***
>
> Casey, 33 (considering OTC)

The women who had undergone cancer treatment had not really had thoughts about their fertility at the forefront prior to diagnosis and treatment initiation. Also, these women perceived that fertility was not always a priority for the healthcare professionals looking after them.

> ***'I don't think I really thought about it, until she [the nurse] had mentioned that. I feel most times in cancer treatments like you're just so worried about the disease that sometimes things like fertility get brushed over.'***
>
> Amelia, 43 (undergone OTC)

> ***'I feel like they are one track minded, like when I try to talk to my oncologist, I love her dearly she's amazing, but when I tried to talk to her about my fertility and hormones post cancer and how I'm feeling, does she***

care? I think the bottom line is I don't have cancer and that is what is important to her.'

Alice, 40 (undergone OTC)

Theme: Insurance of fertility and hormonal function in the future

For participants who had previously had cancer, OTC was a form of insurance that protected their fertility and hormonal function for the future. It provided hope, a sense of normalcy, a future that included the possibility of having biological children and restarting hormonal function.

'It gave me that hope in the midst of everything else that was going on. It just was kind of that light that I needed.'

Amelia, 43 (undergone OTC)

'Just having that [hope] that whole time, I felt like really kept me going, and I was like it'll all be okay, everything will get back to normal. Like in a couple of years it will feel like this was just a really bad dream.'

Lucy, 34 (undergone OTC)

<u>Subtheme: Future proofing</u>

OTC was also seen as a form of security for the future. Some described OTC as empowering as it offered the prospect of not being constrained by a time-limited reproductive lifespan.

'Fertility preservation is not just about postponing [or] having children, which is important, having children at the right time, with the right partner...when you're ready, for me that is a big deal…not having to panic about it when that time comes.'

Donna, 37 (interested in OTC)

'I had always considered that I would have completed my family by my age and it had never occurred to me, as I was growing up, that I would be in the situation where I'm not married and I have not started [family] you know, never mind completed my family. So in that way this is something that I need to seriously consider it if that's something I still want in life, which it is.'

Nina, 35 (interested in OTC)

Subtheme: Perceptions on menopause

What menopause meant for participants emerged through discussion and was a recurring narrative in the interviews with women interested in OTC. Attitudes towards menopause seemed to be particularly influenced by the experiences of close family members.

'I sort of dread menopause and I associate it with aging.'

Rita, 32 (interested in OTC)

'My mom has really struggled with menopause and menopausal symptoms.'

Donna, 37 (interested in OTC)

'Like I saw first-hand what was happening to a very sane, beautiful woman, what she [my mother] was going through.'

Gina, 31 (interested in OTC)

Menopause was perceived as negative by some of the participants. However, for some interfering in an inevitable process was perceived as 'unnatural' and women should not 'play with nature' unless at risk of early menopause.

'I would be willing to embrace the natural progression of things unless someone was able to tell me you're going to go through menopause the age of 42 and I'll be like actually I'll take whatever is on the market (laughs) to delay that a little bit.'

Debora, 30 (interested in OTC)

These participants however did see benefit in the use of OTC in young girls and women who are at risk of early menopause due to fertility ablating cancer treatments or those at risk of POI.

'I also know that women who are aware of it, who maybe don't want to hit menopause, and early menopause like in early 40s or you know if they have a family history or hereditary [disease] that can happen with menopause, so if they want to prevent that then that [OTC] could be an option for them.'

Alice, 40 (undergone OTC)

Subtheme: Hormonal function from OTC

Participants who had OTC due to cancer diagnosis and re-transplantation, found the return of hormonal function to bring benefits, one being reduction in menopausal symptoms that occurred as a consequence of treatment.

> ***'So that's why we tried to keep each ovarian tissue section in as long as we could because it gave me those hormone levels that I wasn't going to be able to get otherwise. You know HRT is not an option for me.'***
>
> Amelia, 43 (undergone OTC)

> ***'The main reason I wanted it was to get me out of menopause.'***
>
> Lucy, 34 (undergone OTC)

Some participants who had undergone OTC and subsequent transplantation, also had remaining tissue stored in the bank. Because the transplantation had helped with menopausal symptoms, one participant was very keen on having further transplantation of stored tissue, not for having children but to help manage the symptoms of menopause such as hot flushes and vaginal dryness.

> ***'I do consider having another transplant because not necessarily to have babies, but for the hormone replacement.'***
>
> Ruth, 33 (undergone OTC)

Theme: Cost of OTC

One of the concerns raised by participants considering OTC for social reasons was the potential costs associated with the double surgical procedure of harvesting and then re-implantation and storage. This had an influence on deciding whether or not to undergo OTC. Participants seemed to weigh up the perceived benefits of what the procedure offered in terms of value for money against providing the option of fertility preservation.

> ***'I think the only deterring factor would be the finance it's very, very pricey from what I understand. I would if money wasn't a huge determining factor.'***
>
> Andrea, 35 (interested in OTC)

> ***'I would want to know the full scope of [OTC procedure] …because you have invested emotionally and financially and physically.'***
>
> Faria, 32 (interested in OTC)

Some participants acknowledged that the cost might be prohibitive but that should not mean that the procedure should be unavailable for fertility preservation.

> ***'Yes, so there'll be an element of expense to it, but if that's what a woman is willing to do, then it at least should be an option...'***
>
> Nina, 35 (interested in OTC)

<u>Subtheme: Lack of financial support for young girls and women with cancer</u>

There was a view across the interviews that female fertility received less attention which was perceived to be similar to other conditions relating to women's health.

> ***'So if you're male and you want to freeze your sperm, yes you go ahead and do that it's covered [by health insurance], but for females it is not an option.'***
>
> Amelia, 43 (undergone OTC)

> ***'It's about what we believe and listen to you know, everybody having that same opportunity.'***
>
> Lina, 31 (interested in OTC)

As participants who had undergone OTC were not based in the UK, they did not have access to a national healthcare coverage as part of the treatment package for cancer. Participants were therefore grateful for the generosity of some healthcare professionals who did not charge for the OTC procedure. Whereas others had to pay for the procedure themselves and were frustrated that procedures such as IVF had funding whereas OTC did not.

> ***'Of course, like insurance doesn't cover it so you know, trying to get funds for, but [ovarian transplant surgeon] was great and did their services for free, essentially, so I was able to have that.'***
>
> Lucy, 34 (undergone OTC)

'Like there's organisations that will sponsor IVF you know, like this, you can apply for grants for that and there isn't that right now for the ovarian transplant. It's just too new and unheard of.'

Ruth, 33 (undergone OTC)

Discussion

This study was undertaken to understand the views, motivations and expectations of girls and women who considered and or had undergone OTC as a method of fertility and or hormonal preservation. Although not universally available to all, women are intrigued by OTC. Participants unanimously described that they were considering OTC because of how it offered 'another' option for female fertility preservation. OTC was considered to be more natural compared to other fertility preservation methods, albeit for different reasons. For example, those who were interested in OTC for hormonal preservation, viewed the lack of exogenous hormones required, as increasing the 'naturalness' of OTC. Whereas participants who had undergone OTC and other assisted reproductive techniques such as oocyte cryopreservation and attempts of in vitro fertilisation (IVF), considered OTC to be 'more natural' although it involved invasive surgical procedures, a minimum of two and sometimes more surgical interventions. Participants who were interested in OTC and were familiar with the processes involved, also regarded the idea of being able to conceive without taking exogenous hormones for oocyte retrieval for IVF followed by embryo transfer, as 'natural'. For those that had undergone transplantation, the return of hormonal

function was reported to be as close to natural. This was even more pronounced in those that underwent early menopause due to cancer treatment and were prescribed hormone replacement therapy (HRT) until they received the transplantation of their ovarian tissue.

The reasoning for considering or undergoing OTC varied based on whether OTC was considered for social reasons, or whether the participant was at risk of infertility due to anti-cancer treatment. For participants with cancer the *'fear of losing fertility'* was the underlying motivation for considering OTC. For those participants who were interested in OTC for social reasons, they described their motivations in terms of *'insurance of fertility for the future'* although *'fertility was not a priority'* at the time of interview, it often came up and was described as *'not ready for motherhood'*. There seemed to be a cognitive dissonance as on the one hand these women did not prioritise motherhood but on the other, perhaps had an underlying fear of not being ready. Ovarian tissue cryopreservation was approached as *'insurance for return of fertility should it be necessary'*.

Those participants who had undergone OTC expected 'more information' than what they received from the healthcare professionals who supported them through the process. Some participants felt discussion of OTC when facing the likelihood of treatment induced infertility as an option was *'by chance'* rather than as part of an open discussion of available options with their healthcare provider. Although they now expressed feeling

'grateful for OTC', they were surprised that oncologists failed to prioritise fertility and seemed unaware of OTC during discussions about fertility preservation at the time.

Participants that were interested in OTC for social reasons expected their healthcare provider to explain the likely *'success rates'* of the procedure during discussions. This was linked to a perception that the procedure is remained experimental in some countries. Only those participants who were healthcare professionals and/or those that had undergone the procedure already, appeared to understand the risks involved with the procedure. There was consensus that there was a *'lack of awareness'* about OTC coupled with limited availability. The likelihood that hormonal function would return after ovarian tissue transplantation was an area that the participants felt needed to be investigated further. They perceived that this might benefit young girls and women with POI by providing them with their 'own' hormones and by aiding with symptoms of early menopause, or in cases of healthy women, help with symptoms of menopause. Women with cancer, who had already undergone OTC had a better understanding of the technique. All participants described that at the time of considering OTC, decision-making was felt to be overwhelming. Participants interested in OTC for social reasons, appeared to have only a vague understanding of the procedure regarding tissue extraction, storage and re-implantation.

<u>Female fertility and the need for fertility preservation</u>

Student ID:
PhD Met+Sys Re T Lab A300

Females are born with a finite supply of oocytes that deplete at an exponential rate, with only 25, 000 follicles remaining from the 1-2 million by the time a woman is 37 years old. (52). This supply depletes more quickly in young girls and women who undergo gonadotoxic anti-cancer treatments. (1) Fortunately, advances in ART have been made, resulting in oocyte cryopreservation and OTC. Oocyte cryopreservation is not applicable in pre-pubertal girls due to requiring ovulation induction, which is not possible or ethical in this population. Despite the OTC technique being developed for over two decades, many young girls and women do not have access to it. Possibly because information about the technique has been poorly disseminated and or no requests have made the consequence is women are either not referred routinely or do not follow through even if they are referred. This impacts on demand and ultimately on the availability of fertility preservation and newer techniques such as OTC. (53)

Immediately following a cancer diagnosis, the priority for clinicians and possibly the patient will be to start anti-cancer treatment as rapidly as possible, and this will take precedence over matters of fertility preservation. (54) This haste to treatment initiation could ultimately lead to the fertility potential of a woman to be jeopardised as discussed in the review of the literature presented in Chapter 4. This study illuminates the challenges encountered by women who reported some consternation that healthcare professionals had limited knowledge about the OTC procedure. Indeed, some of the participants accessed OTC by chance. Of the six participants who had previously been treated for cancer, five underwent

OTC. The one cancer survivor who did not undergo fertility preservation did discuss it with their oncologist but did not have a consultation with a reproductive medicine specialist. Upon enquiring further, she could not recollect the reasoning behind not seeing a specialist for fertility preservation. Evidence suggests that many oncologists either fail to talk about fertility preservation with their female patients or do so ineffectively. (53,55–58) It can only be conjecture that this may have been the case with this participant.

Various factors have been identified that confound access to female fertility preservation. Age, financial status and education appear to influence who are referred for and who have access to fertility preservation treatments. (54,57) The decision on which method(s) for fertility preservation is used with young girls or women is undertaken by a multidisciplinary team of health care professionals and it is imperative that they are up to date with information about provision of OTC so that patients can make an informed choice. As none of the participants who had OTC prior to initiation of cancer treatment were UK based it is beyond this study to determine whether discussions related to fertility preservation take place routinely. Furthermore, healthcare professionals may have a lack of expertise with infertility risks, unfamiliarity with fertility preservation techniques, few clinical perspectives of fertility preservation and lack of referral protocols, which all function to negatively influence fertility discussion practices. (54)

Financial implications

Lack of provision of OTC is further compounded by cost. Ovarian tissue cryopreservation is still considered experimental in most parts of the world and is not readily available through the NHS for the most part or insurance schemes elsewhere, making it a particularly prohibitive procedure. A study conducted on economic evaluation on OTC and oocyte cryopreservation found that the estimated cost of OTC procedure in order to achieve at least one live birth was $10,032 (approximately £8130). (50) The private company that offers this procedure in the UK, estimates for the costs to be £2890 for 'opportunistic' surgery (where the woman is having surgery for another procedure and the tissue is retrieved opportunistically) or from £6000 for primary standard care (exclusive surgery for the OTC procedure). (60) There is lack of homogenous funding for OTC procedure in the UK, whether at the individual level or organisational level. (13) Regulatory and funding bodies should consider the overall benefits derived from OTC to ensure adequate funding and service provision is in place, especially for young women with cancer.

Views, motivations and expectations

Overall participants expressed dissatisfaction with the absence of OTC as an option for young girls and women. This was the case for both cancer survivors and those interested in OTC for social reasons. Furthermore, motivations for undergoing OTC were clear for

women who were suffering with cancer i.e., preserving fertility that may otherwise be lost following cancer treatment. Women who were interested in undergoing OTC for social reasons had a vague understanding of the procedure, for instance they may not be as motivated to understand the procedure as compared to female cancer patients in whom there was an immediate threat to fertility. Whilst participants interested in OTC were intrigued by it, only participants who were healthcare professionals truly understood the procedure and it's risks in detail. As a result, there is an even greater dearth of understanding about the numerous alternatives open to women who wish to preserve their fertility.

Hormonal preservation with OTC

Participants who had gone through gonadotoxic cancer treatments discussed the debilitating symptoms associated with premature menopause. Depletion of hormonal function after anti-cancer therapies is therefore an area that needs to be investigated further, both on a clinical and research front. Different types of cancers and their subsequent treatments are linked with either a greater or lesser risk of premature menopause. (1) We were not able to ascertain if those participants who had cancer treatment had a discussion about the possibility and risk of early menopause with their oncologist, as they had no recollection of it being mentioned. All the participants in this study who had undergone OTC became menopausal after anti-cancer treatments. At the

time of interview, these participants had already received ovarian tissue transplantation and all reported improved menopausal symptoms post transplantation. Participants with previous cancer who underwent OTC, did not recollect giving much thought at the time of OTC surgery to future fertility and/or hormonal preservation as they were dealing with the existential threat posed by a cancer diagnosis. Participants were mostly grateful that they had an option. Most of these women are now advocates for the availability of the OTC procedure and have suggested that conversations with women undergoing cancer treatment should involve discussion of hormonal as well as fertility preservation.

Women who were considering OTC to ensure their fertility for reasons not linked to cancer had mixed views about delaying menopause with OTC. Although participants considering OTC for hormonal preservation expressed anxieties about menopause, these anxieties seemed to be influenced by the experience of other women, particularly close family relatives, who had gone through menopause. One participant, whose mother and sister had both experienced early menopause, was keen to undergo OTC and expressed frustration at the limited availability of the procedure for women like herself.

Mitigating against an anticipated biographical disruption

Fear of infertility after cancer, or knowledge of reduced fertility, can have detrimental impact on female identity and psychological well-being, impact on survivorship, and can

cause biographical disruption. (61) Those participants who had previously been diagnosed with cancer underwent cancer treatment in their early twenties and had already experienced feeling different to and being ostracised amongst their peers. Their main motivation underpinning the decision for OTC was fear of the future beyond cancer treatment, possibly not being able to live what they perceived as a ‘normal’ life, which included having biological children. OTC offered hopefulness against what was felt as an additional consequential loss from cancer.

Participants interested in OTC for social reasons appeared to view pregnancy as something for the future and not necessarily at a point when they were likely to be more fertile. This resonates with the current literature indicating there has been a rapid increase in the average age of first-time mothers in the last few decades. In the US, the percentage of women aged 35-44 who become first-time mothers has grown more than five-fold in the previous four decades. (62) Within Europe, Italy has one of the highest mean age of first-time mothers (30.6 years). (63) These developments are most likely due to an increase with women entering higher education in large numbers, entering the full-time workforce, and using contraceptive techniques to protect against unwanted pregnancy, and our cohort reflects this. (64)

Eight of the 11 healthcare professional women who participated were in their 30’s, did not have children and indicated they were not thinking of planning pregnancy in very near

future. One had a child previously but conceived using assisted reproductive techniques. Despite expressing an awareness of their declining reproductive capacity, these women were still opting to postpone childbearing. Yet ultimately experiencing motherhood was viewed as an important aspect of an imagined future identity. The idea of childlessness was deemed as unacceptable, with OTC offering a form of indemnification, providing an opportunity to extend their fertility and therefore slowing the biological clock and providing space to achieve career and life goals. The anticipated biographical disruption to these informants was part of a potential future that was ultimately childless. OTC mitigated against this and offered future proofing.

Women are choosing to 'delay childbearing' but insuring against infertility are increasing their options. Women in our cohort seemed to be well aware they have a finite reproductive lifespan but not necessarily aware of their reproductive health. The phenomenon of optionality is more complex in that women are now choosing to delay childbearing due to reasons beyond their control (lack of partner, lack of supportive work-place etc). (7,21)

Young women in this study who were interested in OTC as a way of preserving their fertility also verbalised concerns about a future and anticipated menopause negatively. They associated it with ageing, decreased libido, increased risk of osteoporosis and cardiovascular conditions. Participants saw menopause as a bundle of debilitating

symptoms and how OTC could potentially help them live a healthy lifestyle in their later years.

Decision-making and OTC

Recruitment from social media with its global reach helped us understand the views, motivations and expectations of women regarding OTC procedure. This was supplemented by an analysis of results through a behavioural psychology lens lending to 'System 1' and 'System 2' thinking of the brain's two operating systems. Combining a pragmatist approach to qualitative inquiry with how fast and slow thinking shapes the perception and choice of women regarding OTC procedure has allowed for a critical analysis around this important subject matter. Systems thinking theory (thinking fast and slow) was apparent in the accounts of the participants in this study. System 1 (reflexive) thinking was used when initially considering OTC and often involved a 'gut reaction', a solution for solving a reproductive and hormonal function concern. However, all participants discussed questioning their initial response which involved them in re-considering whether the decision needed to be taken at pace, an example of System 2 (conscious) thinking. Participants saw OTC as potentially empowering for women, it offered an alternative female fertility preservation method and something that should be available to those who wanted to preserve or extend their fertility. Those participants who were healthcare professionals (and particularly those who worked in women's health) were

positive about OTC as an option for women who were at risk of loss of fertility or hormonal function.

What was unclear from this study was whether women were conscious of the time limited nature of female fertility. The average age of participant in this study considering OTC for social reasons was 32.8 years (SSD 4.7), an age by which female fecundity decreases at an exponential rate. When discussing OTC and other fertility preservation methods, participants expressed hopefulness about their fertility regardless of their age. It was perceived that OTC was suitable for others and should be an option but was not foreground to personal declining fertility. System 1 and 2 thinking was evident; however there is much more overlap between the two, especially with aspects of motherhood which appeal to emotion whereas cost or availability of OTC are things which clearly influence System 2. Ultimately, the myriad of factors affecting woman's decision mean they must integrate 1 and 2 type thinking to arrive at a measured conclusion in which they understand they may not be able to achieve all their aspirations and require sacrifice of one for another. This is ultimately dependent on the woman's circumstance, as outlined by pragmatist philosophy.

Strengths

In several respects, the methodology and conclusions we used in this study build on past studies that address the experience of women undergoing OTC (as described in chapter

4). Previous literature focused mainly on the acceptability and perception of healthcare professionals towards it with only a handful of studies addressing the experiences of women who have actually undergone OTC. To best of our knowledge this is the first study exploring women's perspectives of OTC as a fertility and hormone preservation method, using qualitative research methods. Through this study we have uniquely examined the views of women on a newer method of fertility preservation. We recruited women who were interested in OTC as well as those who have experienced it, giving us with distinct insights, using a pragmatic method and purposive sampling.

Limitations

The main limitation of this study was being unable to recruit from the clinic as a result of the COVID-19 pandemic and therefore social media channels were used to identify potential participants. Because of its potential to reach vast audiences, social media is increasingly being utilised for recruiting to research studies, however in our study it may have resulted in exclusion of the views of women who do not use such platforms. (65) Recruiting participants through social media platforms can have several limitations that may affect the generalisability of the study results. One such limitation is the potential for selection bias. For example, participants who use social media may differ in important ways from those who do not, such as age, gender, socio-economic status, and health status. This can lead to an unrepresentative sample that may not accurately reflect the wider UK population, which can limit the external validity of the study. Furthermore, there

are concerns about privacy and confidentiality when recruiting participants through social media platforms. It is important to ensure that the recruitment process does not compromise the confidentiality of the participants' personal information.

Additionally, all the participants who volunteered to participate in this study who had undergone OTC had positive outcomes. Only one failed to conceive but was nevertheless satisfied with the level of return of hormonal function. A further limitation was also the fact that women who had undergone OTC, had done so many years previously. We were therefore relying on the recall of participants, and this may have been biased given most had positive outcomes and therefore may not remember how they truly felt at the time of OTC.

Furthermore, most of the participants were residents in high income countries who may have had greater access to cutting edge cancer treatments and fertility preservation options. The findings therefore may not be applicable in other settings. Participants with cancer highlighted the costs associated with this procedure but were living in a country with no universal healthcare coverage. Although the provision of OTC in the UK appears to be based on post code lottery, when available, the procedure is funded for medical reasons (cancer). The findings in relation to cost for cancer patients may therefore not be directly applicable to the health system in the UK.

The sample size, characteristics of participants included and study findings may not be generalisable to the wider population of women however, the findings are still relevant to a particular group of women - those who are part of the workforce. Women in the workforce may face unique challenges when it comes to balancing career progression and motherhood. Fertility preservation using OTC is not the ultimate insurance but nevertheless does provide women with an additional option of fertility preservation.

Lastly, none of the participants who were interested in OTC for social reasons had undergone OTC. Given they were merely considering it and the limited availability, this may simply be wishful thinking. Only one participant had sought advice about OTC availability but had been disappointed.

Conclusion

OTC could potentially provide women with an alternative approach for fertility and/or hormonal preservation. Healthy women who wish to consider OTC for fertility or hormonal preservation (or both) should be made fully aware of the potential risks and benefits associated with the procedure and ultimately likely success rates. Participants in this study felt that those who want to pursue OTC after understanding the risks, and consent, should be given the option to do so. This procedure is currently not widely available, with a few centres offering an OTC service. There is also limited awareness regarding OTC among oncologists and other clinicians involved in counselling women facing loss of fertility. This

could mean many cancer patients missing the opportunity of preserving their fertility due to lack of awareness of both the patients and healthcare professionals. Women undergoing cancer ablating treatment should be counselled for OTC as this may be their only option of preserving their fertility and having biological children in the future. Furthermore, hormonal preservation using OTC in females with cancer is an area that requires further exploration. Oncologists and reproductive medicine specialists should liaise closely to be able to offer young girls and women with cancer, a chance at bearing children. To overcome the perceived hurdles to reproductive dialogues with young cancer patients and to improve care, active initiatives such as regular and persistent interprofessional collaboration and communications that can allow fertility preservation education are needed. Further research should include mapping oncofertility services in the UK to understand the barriers to provision of OTC. The viability of OTC as a hormonal preservation option needs to be investigated further especially women at risk of early menopause as a result of gonadotoxic anti-cancer therapies.

References

1. Spears N, Lopes F, Stefansdottir A, Rossi V, De Felici M, Anderson RA, et al. Ovarian damage from chemotherapy and current approaches to its protection. Hum Reprod Update. 2019 Nov;25(6):673–93.
2. ASRM. Fertility preservation in patients undergoing gonadotoxic therapy or gonadectomy: a committee opinion. Fertil Steril. 2019;112(6):1022–33. Available from: https://doi.org/10.1016/j.fertnstert.2019.09.013
3. Preservation TEGG on FF, Anderson RA, Amant F, Braat D, D'Angelo A, Chuva de

Sousa Lopes SM, et al. ESHRE guideline: female fertility preservation†. Hum Reprod Open. 2020 Oct 3;2020(4):hoaa052. Available from: https://doi.org/10.1093/hropen/hoaa052
4. Yasmin E, Balachandren N, Davies MC, Jones GL, Lane S, Mathur R, et al. Fertility preservation for medical reasons in girls and women: British fertility society policy and practice guideline. Hum Fertil. 2018;
5. Mills M, Rindfuss RR, McDonald P, te Velde E, Force on behalf of the ER and ST. Why do people postpone parenthood? Reasons and social policy incentives. Hum Reprod Update. 2011 Nov 1;17(6):848–60. Available from: https://doi.org/10.1093/humupd/dmr026
6. . Dolmans, M.-M. and Manavella DD. Recent advances in fertility preservation. J Obs Gynaecol Res, 45 266-279. 2019; Available from: doi:10.1111/jog.13818
7. Donnez J, Dolmans MM. Fertility preservation in women. N Engl J Med. 2017;377(17):1657–65.
8. Van DerVen H, Liebenthron J, Beckmann M, Toth B, Korell M, Krüssel J, et al. Ninety-five orthotopic transplantations in 74 women of ovarian tissue after cytotoxic treatment in a fertility preservation network: Tissue activity, pregnancy and delivery rates. Hum Reprod. 2016;31(9):2031–41.
9. Gellert SE, Pors SE, Kristensen SG, Bay-Bjørn AM, Ernst E, Yding Andersen C. Transplantation of frozen-thawed ovarian tissue: an update on worldwide activity published in peer-reviewed papers and on the Danish cohort. J Assist Reprod Genet. 2018;35(4):561–70.
10. Oktay K, Oktem O. Ovarian cryopreservation and transplantation for fertility preservation for medical indications: report of an ongoing experience. Fertil Steril. 2010;93(3):762–8. Available from: http://dx.doi.org/10.1016/j.fertnstert.2008.10.006
11. Sheshpari S, Shahnazi M, Mobarak H, Ahmadian S, Bedate AM, Nariman-Saleh-Fam Z, et al. Ovarian function and reproductive outcome after ovarian tissue transplantation: A systematic review. J Transl Med. 2019;17(1). Available from: https://doi.org/10.1186/s12967-019-02149-2
12. Amorim CA, Leonel ECR, Afifi Y, Coomarasamy A, Fishel S. Cryostorage and retransplantation of ovarian tissue as an infertility treatment. Best Pract Res Clin Endocrinol Metab. 2018;33(1):89–102.
13. Latif S, Martins Da Silva S, Davies M, Mavrelos D, Foo X, Sangster P, et al. Fertility preservation provision in the NHS: a national assessment of care policies. Hum Fertil. 2022;0(0):1–6. Available from: https://doi.org/10.1080/14647273.2022.2045519

14. Shenfield F, de Mouzon J, Scaravelli G, Kupka M, Ferraretti A, Prados F, et al. Oocyte and ovarian tissue cryopreservation in European countries: statutory background, practice, storage and use†. Hum Reprod Open. 2017;2017(1):1–9.
15. Andersen CY, Kristensen SG. Novel use of the ovarian follicular pool to postpone menopause and delay osteoporosis. Reprod Biomed Online. 2015;31(2):128–31. Available from: http://dx.doi.org/10.1016/j.rbmo.2015.05.002
16. Donnez J, Dolmans MM. Natural hormone replacement therapy with a functioning ovary after the menopause: dream or reality? Reprod Biomed Online. 2018;37(3):359–66. Available from: https://doi.org/10.1016/j.rbmo.2018.05.018
17. Khattak H, Malhas R, Craciunas L, Afifi Y, Amorim CA, Fishel S, Silber S, Gook D, Demeestere I, Bystrova O, Lisyanskaya A, Manikhas G, Lotz L, Dittrich R, Colmorn LB, Macklon KT, Hjorth IMD, Kristensen SG, Gallos I, Coomarasamy A. Fresh and cryopreserved ovarian tissue transplantation for preserving reproductive and endocrine function: a systematic review and individual patient data meta-analysis. Hum Reprod Update. 2022 May 2;28(3):400-416. doi: 10.1093/humupd/dmac003. Erratum in: Hum Reprod Update. 2022 Mar 14.
18. Silber SJ, Derosa M, Pineda J, Lenahan K, Grenia D, Gorman K, et al. A series of monozygotic twins discordant for ovarian failure: Ovary transplantation (cortical versus microvascular) and cryopreservation. Hum Reprod. 2008;23(7):1531–7.
19. Kolibianaki EE, Goulis DG, Kolibianakis EM. Ovarian tissue cryopreservation and transplantation to delay menopause: facts and fiction. Maturitas. 2020 Dec;142:64–7.
20. Varlas VN, Bors RG, Albu D, Penes ON, Nasui BA, Mehedintu C, et al. Social Freezing: Pressing Pause on Fertility. Int J Environ Res Public Health. 2021 Jul;18(15).
21. Dolmans MM, Donnez J. Fertility preservation in women for medical and social reasons: Oocytes vs ovarian tissue. Best Pract Res Clin Obstet Gynaecol. 2021;70:63–80. Available from: https://doi.org/10.1016/j.bpobgyn.2020.06.011
22. Stoop D, Cobo A, Silber S. Fertility preservation for age-related fertility decline. Lancet. 2014;384(9950):1311–9. Available from: http://dx.doi.org/10.1016/S0140-6736(14)61261-7
23. Alteri A, Pisaturo V, Nogueira D, D'Angelo A. Elective egg freezing without medical indications. Acta Obstet Gynecol Scand. 2019;98(5):647–52.
24. Hammarberg K, Kirkman M, Pritchard N, Hickey M, Peate M, McBain J, et al. Reproductive experiences of women who cryopreserved oocytes for non-medical reasons. Hum Reprod. 2017;32(3):575–81.

25. BP J, S S, A M, JR S, P S, J BN. The dawn of a new ice age: social egg freezing. Acta Obs Gynecol Scand. 2018;97(6):641–7.
26. Kristensen SG, Andersen CY. Cryopreservation of Ovarian Tissue: Opportunities Beyond Fertility Preservation and a Positive View Into the Future. Front Endocrinol (Lausanne). 2018; 9:347. Available from: https://www.frontiersin.org/article/10.3389/fendo.2018.00347
27. Roser M, Ortiz-Ospina E, Ritchie H. Life Expectancy. OurWorldInData.org. 2013. Available from: https://ourworldindata.org/life-expectancy
28. Mishra GD, Brown WJ, Dobson AJ. Physical and mental health: Changes during menopause transition. Qual Life Res. 2003;12:405–12.
29. de Salis I, Owen-Smith A, Donovan JL, Lawlor DA. Experiencing menopause in the UK: The interrelated narratives of normality, distress, and transformation. J Women Aging. 2018;30(6):520–40. Available from: https://doi.org/10.1080/08952841.2018.1396783
30. Hardy C, Thorne E, Griffiths A, Hunter MS. Work outcomes in midlife women: the impact of menopause, work stress and working environment. Women's Midlife Heal. 2018;4(1):1–8.
31. Cherney K. Effects of Menopause on the Body. 2020 [cited 2022 Apr 12]. Available from: https://www.healthline.com/health/menopause/hrt-effects-on-body
32. Shoebridge A, Steed L. Discourse about menopause in selected print media. Aust N Z J Public Health. 1999 Oct;23(5):475–81.
33. Carlson ES, Li S, Holm K. An analysis of menopause in the popular press. Health Care Women Int. 1997;18(6):557–64.
34. Hust SJT, Andsager JL. Medicalization vs. adaptive models? Sense-making in magazine framing of menopause. Women Health. 2003;38(1):101–22.
35. Diller V. Menopause in the Media: It's About Time! 2013.
36. Kahneman D. Thinking, fast and slow. New York: Farrar, Straus and Giroux; 2011.
37. Richie, M. and Josephson S. Quantifying Heuristic Bias: Anchoring, Availability, and Representativeness. Teach Learn Med. 2017;30(1):67–75.
38. Bury M. Chronic illness as biographical disruption. Sociol Health Illn. 1982;4(2):167–82. Available from: https://doi.org/10.1111/1467-9566.ep11339939
39. Rocca WA, Bower JH, Maraganore DM, Ahlskog JE, Grossardt BR, de Andrade M, et al. Increased risk of cognitive impairment or dementia in women who underwent oophorectomy before menopause. Neurology. 2007 Sep;69(11):1074–83.
40. Hoga L, Rodolpho J, Gonçalves B, Quirino B. Women's experience of menopause: a systematic review of qualitative evidence. JBI Evid Synth. 2015;13(8). Available

from:
https://journals.lww.com/jbisrir/Fulltext/2015/13080/Women s experience of meno pause a systematic.18.aspx
41. Langer RD, Hodis HN, Lobo RA, Allison MA. Hormone replacement therapy–where are we now? Climacteric. 2021;24(1):3–10. Available from: https://doi.org/10.1080/13697137.2020.1851183
42. Ballard K, Elston M. Medicalisation: A Multi-dimensional Concept. Soc Theory Heal. 2005;(3):228–241. Available from: https://doi.org/10.1057/palgrave.sth.8700053
43. Morgan DL. Pragmatism as a Paradigm for Social Research. Qual Inq. 2014 Feb 3;20(8):1045–53. Available from: https://doi.org/10.1177/1077800413513733
44. Goldkuhl G. Pragmatism vs interpretivism in qualitative information systems research. Eur J Inf Syst. 2012 Mar 1;21(2):135–46. Available from: https://doi.org/10.1057/ejis.2011.54
45. Inc ZVC. Zoom Video Communications Inc. 2016. Available from: https://d24cgw3uvb9a9h.cloudfront.net/static/81625/doc/Zoom-Security-White-Paper.pdf
46. QSR International. NVivo Qualitative Data Analysis Software. 12 ed. QSR Int Pty Ltd. 2018.
47. Mauthner NS, Doucet A. Reflexive Accounts and Accounts of Reflexivity in Qualitative Data Analysis. Sociology. 2003 Aug 1;37(3):413–31. Available from: https://doi.org/10.1177/00380385030373002
48. Urquhart C. Grounded Theory for Qualitative Research: A Practical Guide. Thousand Oaks: Sage; 2013. p194.
49. Saunders B, Sim J, Kingstone T, Baker S, Waterfield J, Bartlam B, et al. Saturation in qualitative research: exploring its conceptualization and operationalization. Qual Quant. 2017/09/14. 2018;52(4):1893–907. Available from: https://pubmed.ncbi.nlm.nih.gov/29937585
50. Braun V, Clarke V. To saturate or not to saturate? Questioning data saturation as a useful concept for thematic analysis and sample-size rationales. Qual Res Sport Exerc Heal. 2021;13(2):201–16. Available from: https://doi.org/10.1080/2159676X.2019.1704846
51. Braun, V. and Clarke V. Using thematic analysis in psychology. Qual Res Psychol. 2006;3(2):77-101.
52. ACOG. Female age-related fertility decline. Fertil Steril. 2014 Mar 1;101(3):633–4. Available from: https://doi.org/10.1016/j.fertnstert.2013.12.032
53. Bann CM, Treiman K, Squiers L, Tzeng J, Nutt S, Arvey S, et al. Cancer Survivors'

Use of Fertility Preservation. J Womens Health (Larchmt). 2015 Dec;24(12):1030–7.
54. Covelli A, Facey M, Kennedy E, Brezden-Masley C, Gupta AA, Greenblatt E, et al. Clinicians' Perspectives on Barriers to Discussing Infertility and Fertility Preservation with Young Women with Cancer. JAMA Netw Open. 2019;2(11):1–13.
55. Bastings L, Baysal O, Beerendonk CCM, Braat DDM, Nelen WLDM. Referral for fertility preservation counselling in female cancer patients. Hum Reprod. 2014 Oct;29(10):2228–37.
56. Klock SC, Zhang JX, Kazer RR. Fertility preservation for female cancer patients: early clinical experience. Fertil Steril. 2010 Jun;94(1):149–55. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0015028209005937
57. Quinn GP, Vadaparampil ST, Bell-Ellison BA, Gwede CK, Albrecht TL. Patient-physician communication barriers regarding fertility preservation among newly diagnosed cancer patients. Soc Sci Med. 2008 Feb;66(3):784–9.
58. Yee S, Abrol K, McDonald M, Tonelli M, Liu KE. Addressing oncofertility needs: views of female cancer patients in fertility preservation. J Psychosoc Oncol. 2012;30(3):331–46.
59. Chung EH, Lim SL, Myers E, Moss HA, Acharya KS. Oocyte cryopreservation versus ovarian tissue cryopreservation for adult female oncofertility patients: a cost-effectiveness study. J Assist Reprod Genet. 2021 Sep;38(9):2435-2443. doi: 10.1007/s10815-021-02222-0. Epub 2021 May 11.
60. Profam Ltd. Available from: http://www.profam.co.uk
61. Ussher JM, Perz J, Miller A, Patterson P, Wain G, Hobbs K, et al. Threat of biographical disruption: The gendered construction and experience of infertility following cancer for women and men. BMC Cancer. 2018;18(1):1–17.
62. Fritz R, Jindal S. Reproductive aging and elective fertility preservation. J Ovarian Res. 2018;11(1):1–8.
63. Eurostat. Births and Fertility: Over 5 million babies born in the EU in 2015. Available from: https://ec.europa.eu/eurostat/documents/2995521/7898237/3-08032017-AP-EN.pdf/b17c1516-faad-4e65-b291-187826a7ac88#:~:text=On%20average%20in%20the%20EU,2001%20to%201.58%20in%202015.
64. Collins J, Evers H, Golombok S, Hannaford P, Jacobs HS, La Vecchia C, et al. Social determinants of human reproduction. Hum Reprod. 2001;16(7):1518–26.
65. Benedict C, Hahn AL, Diefenbach MA, Ford JS. Recruitment via social media: advantages and potential biases. Digit Heal. 2019;5:1–11.

CHAPTER 6: THESIS DISCUSSION AND WIDER IMPLICATIONS

Student ID:
PhD Met+Sys Re T Lab A300

The overall goal of this thesis was to explore the effectiveness of ovarian transplantation as well as how ovarian tissue transplants may be used to improve female reproductive and endocrine function. In order to assess the effectiveness of ovarian transplants, it is important to first define what effectiveness in healthcare means. Effectiveness is the ability of an intervention to have a meaningful effect on patients in normal clinical conditions. Hence, the thesis also explored the role of ovarian tissue cryopreservation in this process, which is a prerequisite to ovarian tissue transplantation, in addition to an exploration of the perceptions of women who were interested in this procedure as a fertility or hormonal preservation. The following phenomena were examined:

1. Current evidence on the use of fresh and frozen ovarian transplantation

2. Could fresh ovarian autografts be used to preserve hormonal function?
3. What are the experiences of young girls and women who have undergone ovarian tissue harvesting for fertility or hormonal preservation?
4. Why are women interested in ovarian tissue cryopreservation as a method of preserving fertility and hormones?

The findings from each of the above phenomena are reported in this summary. Furthermore, wider implications of the thesis and recommendations for future practice will also be discussed.

Student ID:
PhD Met+Sys Re T Lab A300

Fresh and frozen ovarian transplantation: summary of findings and future work

This study generated interesting findings in relation to the use of ovarian transplants (fresh, frozen-thawed and donor) for preserving reproductive and endocrine outcomes in women. The multicentre, international collaboration between our centre, Australia, Belgium, Denmark, Germany, Russia and USA, resulted in a collection of 735 cases of ovarian transplants reported to date. Through previous publications and this review, we can conclude that ovarian tissue transplantation has shown promising results in preserving ovarian reproductive and endocrine function. This procedure should no longer be considered experimental, and it should be offered to women who wish to preserve their fertility out of the research context. However, the potential use of transplants in preserving hormonal function is an area that needs to be explored further. There is currently no robust guidance for clinicians or patients about this procedure and its potential uses. With its potential use in alleviating menopausal symptoms and improving quality of life, there is an urgent unmet need for robust policy and standard around this procedure. The results of this review will hopefully guide clinicians in advising women about the benefits and shortcomings of this procedure until a formal guideline is produced.

Large scale studies with higher sample sizes of participants need to be orchestrated in the future to give an accurate success rate of this procedure. Additionally, a robust guideline for the optimal size of the tissue graft and surgical procedure for retrieval and transplantation of ovarian tissue needs to be produced to guide centres with best practices

around this technique. Furthermore, there was great heterogeneity in reporting of outcomes from ovarian transplants which made it difficult to conduct meta-analysis. It is therefore recommended that a predefined outcome set be determined to ensure all randomised control trials, cohort studies or case series report similar outcomes for ovarian transplantation.

Lastly, around 300 pregnancies and 200 live births have now been reported worldwide from transplantation of ovarian tissue. There is however no data on the obstetric outcomes for these pregnancies. Future research should include meticulously following up with these women and creating a global database to record the long-term outcomes for women who received the ovarian transplantation and children born as a result.

Fresh ovarian autografting: summary of findings and future work

In this study, a novel technique of fresh ovarian autografting to prevent symptoms of iatrogenic menopause was explored. This study falls under the umbrella of current evidence on the use of fresh and frozen ovarian transplantation to answer the question of whether or not fresh ovarian autografts can be used to preserve hormonal function. Furthermore, the study fills an identified gap in the literature by reporting a case series on ovarian autografting in women with benign gynaecological conditions such as pelvic pain and endometriosis. For young women with severe endometriosis, hysterectomy with bilateral saplingo-oophorectomy is a last resort. Depletion in oestrogen because of

oophorectomy causes iatrogenic menopause. Pharmacologic treatment, such as HRT, is an option but is not without side effects. This study demonstrates the success in return of ovarian endocrine function as depicted by an increase in oestrogen post fresh ovarian autografts in women of reproductive age. While the effect of HRT versus fresh ovarian autografts is another area of research that needs to be explored further, the novel technique used in this study could potentially provide an alternative to HRT for select patients who want to maintain endogenous ovarian endocrine function. This study design can provide preliminary results, however, further research is necessary to demonstrate and explore this issue more directly through replicated studies with larger sample sizes, meticulous follow up of participants receiving ovarian transplants and follow up over a longer period of time.

Experiences of females undergoing OTC: summary of findings and future work

This systematic review of qualitative studies highlighted various themes in relation to the experiences of young girls and women who had considered or undergone OTC (with the view of transplantation in the future). Ovarian tissue cryopreservation as a fertility preservation technique is still considered a fairly new technique and considered experimental in most parts of the world. Because OTC is only used in cancer patients or those who are at danger of losing their fertility due to benign diseases, the circumstances in which OTC and transplantation are considered are not always pleasant for the patient.

The experiences of women revealed that the circumstances in which they underwent or considered OTC were emotive, and decision making became that much more complex. Through the process of consideration for OTC and future transplantation, these women become better prepared for the consequences of potential infertility in the future. This however would not be possible without equipping these women with all the relevant evidence and information needed to support their decision-making process. It was therefore concluded that OTC should only be provided by specialised centres, with healthcare professionals particularly trained in carrying out consultations related to fertility preservation. The decision to undergo this method of fertility preservation for both cancer and benign reasons should be taken by a multidisciplinary team of reproductive endocrinologists, oncologists, surgeons, psychologists, and nurses. The centres that provide this novel technique, should provide participants with written material as well as online visual aids (websites and e-learning) to help them understand the risks and benefits. There needs to be a robust policy in place for follow up of these patients. This may be complex for young girls as they are likely to have had their ovarian tissue cryopreserved when much younger. Policy makers need to reach a consensus regarding a time frame for follow up for girls who have had their tissue retrieved before puberty.

Furthermore, there is an urgent unmet need for a universal guideline for health care professionals involved in the care of women requiring OTC. Various fertility preservation societies have provided guidance on who to offer this procedure to, however there is a

significant discrepancy in how this procedure is provided globally. Establishing a gold standard for these set of guidelines in the future will enable good practice to be available to all.

While challenges around this procedure are plentiful in nature, young adolescents who wish to undergo this procedure pose a unique challenge for healthcare professionals in particular. This cohort requires close attention when it comes to the provision of information. Additionally, clinicians need to consider capacity and competence in these young girls as well as guidance on when to involve parents in the decision-making process. There is currently no robust guidance on assessing capacity and competence in young adolescent girls when it comes to considering OTC as a fertility preservation method.

Why are women interested in ovarian tissue cryopreservation as a method of preserving fertility and hormones?

This study explored the views, motivations and expectations of women who were interested in or underwent OTC. The results of the thematic analysis were interesting in that women unanimously felt that OTC and subsequent transplantation, provided women with another option of fertility preservation (whether women want to consider it for social reasons or due to fertility ablating cancer treatments). Although the procedure is not without its risks such as requiring potentially two surgeries and the uncertainty regarding

longevity of the transplanted grafts, women felt it was important for this technique to become readily available as an option for women. Healthy women who wish to consider OTC for fertility or hormonal preservation (or both) should specifically be made aware of the procedure's potential risks and success rates. Furthermore, the procedure is not widely available and centres that provide it are few and far between. This leads to many cancer patients missing the opportunity of preserving their fertility due to lack of awareness on both the patients' and healthcare professionals' sides. Women facing cancer ablating treatment need to be considered for OTC as this may be their only option of preserving their fertility and having biological children in the future. Furthermore, hormonal preservation using OTC in females with cancer is an area of study that needs to be explored in more detail to truly understand the risks and benefits associated with this technique. There is lack of funding for OTC procedures on a personal level and organisational level and this needs to be mitigated for young girls and women with cancer. The decision on methods for fertility preservation in young girls or women is undertaken by a multidisciplinary team of health care professionals and it is imperative that they are up to date with information about the provision of OTC so that young girls and women can make an informed choice regarding their fertility. Oncologists and reproductive medicine specialists need to work closely together to be able to offer young girls and women with cancer a chance at motherhood. The lack of attention to this subject matter around fertility preservation represents both a unique opportunity and an important challenge for us to engage more emphatically and scientifically with this area of study. To overcome the

perceived hurdles to reproductive dialogues with young cancer patients and to improve care, active initiatives such as regular and persistent interprofessional collaboration and communications that can advocate for fertility preservation education are also needed.

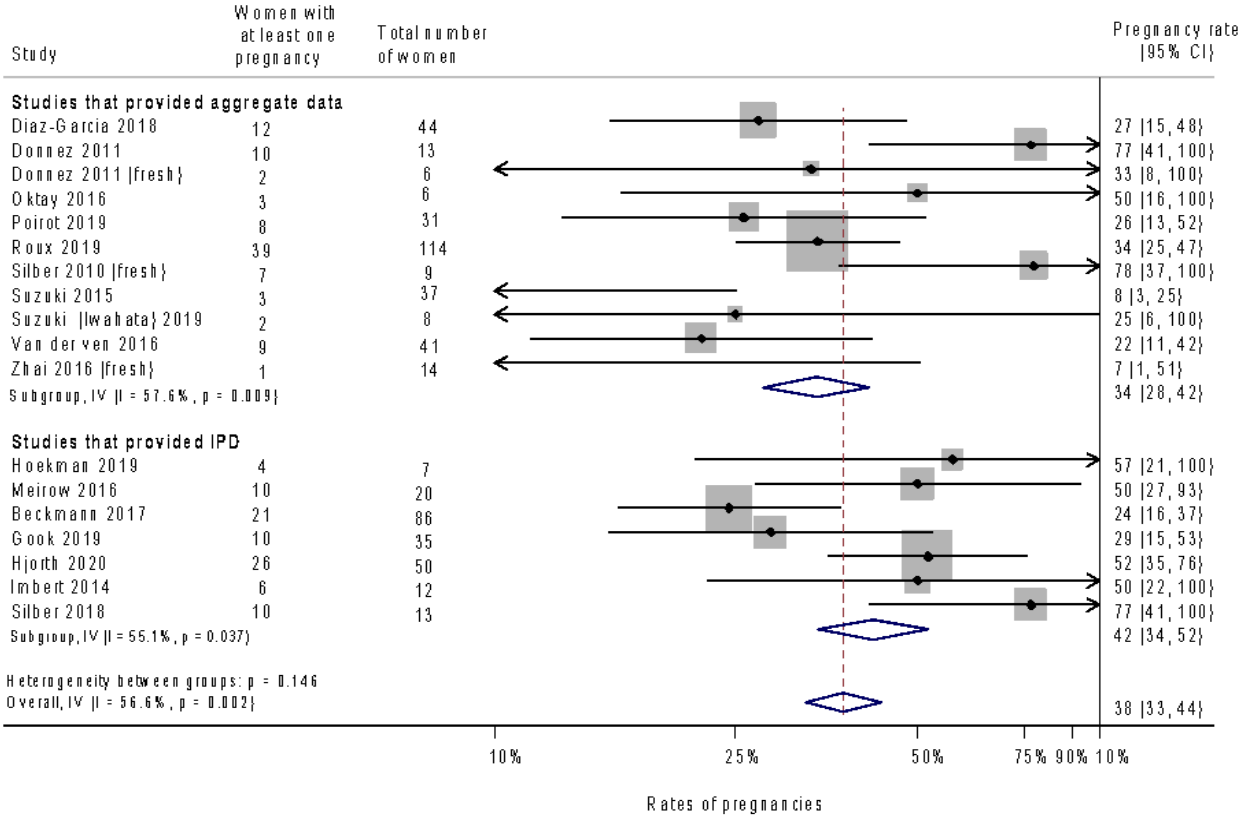
APPENDIX

Appendix 1. Search Strategy

1 Humans/
2 Female/
3 exp ADULT/
4 exp Middle Aged/
5 3 or 4
6 (human or female* or woman or women or adult* or middle aged).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
7 1 or 2 or 5 or 6
8 1 and 2 and 5
9 (human* and (female* or woman or women) and (adult* or middle aged)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
10 8 or 9 (
11 exp Tissue Donors/ or tissue donor*.mp.
12 tissue transplants.mp. or exp Transplants/
13 tissue transplantation.mp. or exp Tissue Transplantation/
14 exp Transplantation, Autologous/
15 exp AUTOGRAFTS/ or autograft*.mp.
16 exp Transplantation, Heterotopic/ or heterotopic transplant.mp.
17 exp Allografts/
18 exp Heterografts/
19 (allograft* or autograft* or xenograft* or orthotopic or heterotopic or autotransplant or autotransplantation or transplant*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
20 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19
21 exp OVARY/ or ovary.mp.
22 (ovarian or ovaries).mp.
23 21 or 22
24 10 and 20 and 23

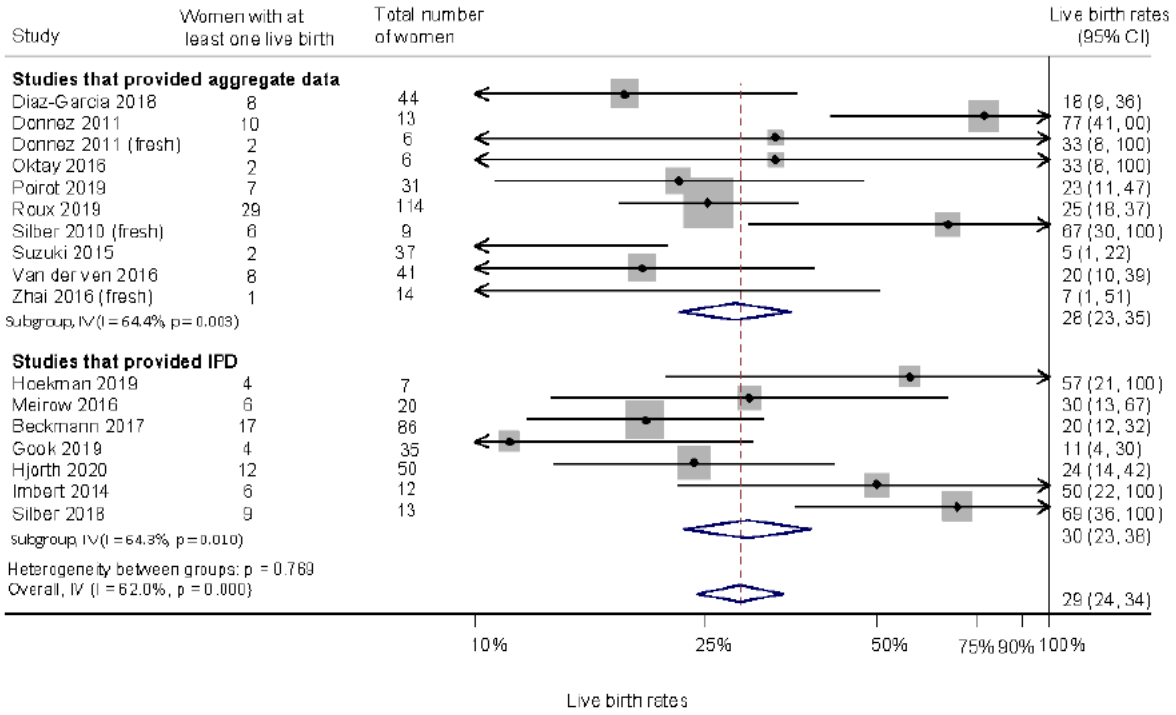
Student ID:
PhD Met+Sys Re T Lab A300

Appendix 2. Meta-analysis comparing pregnancy rates between studies that provided aggregate data vs studies that provided participant level data.



Student ID:
PhD Met+Sys Re T Lab A300

Appendix 3. Meta-analysis comparing live birth rates between studies that provided aggregate data vs studies that provided participant level data.



Appendix 4. Characteristics of studies with and without participant level data.

Characteristics of studies with and without participant level data									
IPD data	Assoc ated pub cat on(s)	Number of part c p ants	Age at cryopreser vat on (Mean, SD)	Age at transp a ntat on (Mean, SD)	Cryoprese rvat on techn que	Transp ant at on techn que	Informat on prov ded on endocr ne funct on (Yes/No)	Pregna ncy rates (%)	L ve b rth rates (%)
Austra a	Gook et a . 2019	35	29.1 (6.1)	35.5 (5.7)	S ow freez ng	Laparosco py	Yes	29	11
Be g um	Imbert et a . 2014; Demeestere et a . 2015)	12	27.5 (5.8)	33.1 (5.1)	S ow freez ng	2 step aparosco py	Yes	50	50
Denmark	Hjorth et a . 2020, Jensen et a . 2015	64	30 (5.8)	33.7 (5.6)	S ow freez ng	Laparosco py + m n - aparotom y	Yes	52	24

Germany	Beckman et a . 2017	86	30.5 (5.4)	35.3 (4.9)	S ow freez ng	Laparosco py	Yes	24	20
Russ a	Bystrova et a . 2019	10	30.8 (4.4)	31.8 (4.4)	S ow freez ng	Laparosco py	Yes	n/a	n/a
USA	S ber et a . 2018; S ber et a . 2010, S ber et e . 200	13	23.8 (4)	31.2 (3.9)	S ow Freez ng + V tr f cat o n	M n - aparotom y + aparosco py	Yes	77	69
Israe	Me row et a . 2016	20	28.7 (7.5)	34.4 (6.9)	S ow freez ng	M n - aparotom y	Yes	50	30
The Nether ands	Hoekman et a . 2019	7	27 (4.7)	33.4 (5.8)	S ow freez ng	Not exp c t y g ven	No	57	57
Non IPD data	Assoc ated pub cat on(s)	Number of part c p ants	Age at cryopreser vat on (Mean, SD)	Age at transp a ntat on (Mean, SD)	Cryoprese rvat on techn que	Transp ant at on techn que	Informat on prov ded on endocr ne funct on (Yes/No)	Pregna ncy rates (%)	L ve b rth rates (%)
Be g um	Donnez et a 2004; Donnez et a . 2005; Donnez et a . 2006; Donnez et a . 2007; Cambon et a . 2008; Donnez et a . 2008; Donnez and Squ ff et 2010; Donnez et a . 2011; Donnez and Squ ff et 2011)	17	24 (5.1)	29.7 (4.7)	S ow freez ng	Laparosco py	Yes	77	30
Ch na	Zha et a . 2016	14	n/a (fresh transp ant at on)	29.2 (4.2)	V tr f cat o n	Laparosco py	No	7	7
France	Po rot et a . 2019	31	26.2 (5.8)	33.5 (4.8)	S ow freez ng	Laparosco py	No	26	23
France	Roux et a . 2019; Am ot et a . 2017	114	26.3 (5.8)	32.8 (4.6)		Laparosco py	No	34	25
Japan	Suzuk et a . 2015	37	37 (4.7)	37 (4.7)	V tr f cat o n	Laparosco py	No	8	5
Japan	Suzuk et a . 2019	8	35.5 (3.8)	40.6 (3.7)	V tr f cat o n	Not exp c t y g ven	Yes	25	Not g ven
Russ a	L syanskya et a . 2009 (conference abstract)	16	Not g ven	Not g ven	Not g ven	Not g ven	Not g ven	Not g ven	Not g ven
Spa n	D az-Garc a et a . 2018	44	34.3 (7.2)	38.9 (4.1)	S ow freez ng	Laparosco py + m n - aparotom y	No	27	18
USA	Oktay et a . 2016; Oktay et e . 2003;	6	27 (4.7)	34.5 (2.4)	S ow freez ng	Laparosco py	Yes	50	33

	Oktay et e 2004; Oktay et e 2011								
USA	K m et a . 2012	6	31 (4.1	35.8 (4)	S ow freez ng	Between the rectus musc e and the rectus sheath	No	4 embryo s but no pregnan c es reporte d	Not g ven

Student ID:
PhD Met+Sys Re T Lab A300

Appendix 5. Protocol for Ovarian transplant study (Chapter 3).

Ovarian transplantation for preserving endocrine and reproductive function (OysTER)

Table of Contents

Student ID:
PhD Met+Sys Re T Lab A300

Ovarian transplantation for preserving endocrine and reproductive function (OysTER) 250**Signature page** 251**Chief investigator details** 252**Sponsor statement** 252**Key Study/Trial Contacts** 252Chief Investigator 252Study/Trial Team 253**Appendix** 253**Study summary** 254Research objective 254Recruitment target and process 254Duration of project 255Full title of the study 255Short study title 256Primary objective 256Secondary objectives 256Background and Rationale 256Why is it important 257Study Design 257Consent process 258Treatment/study process 259Withdrawal process 259Data Management process (data management, storage and access) 259Data analysis 260Patient & Public Involvement 260Protocol Compliance 260Definition of End of Study and Archiving 261**References** 261

Signature page

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to adhere to the signed University of Birmingham's Sponsorship CI declaration.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

Chief Investigator:



Signature: .....

Date: 21/05/19

Name: (please print): Arri Commarasamy

Student ID:
PhD Met+Sys Re T Lab A300

Chief investigator details
Prof. Arri Coomarasamy
Professor of Gynaecology
MBChB, MD, FRCOG
University of Birmingham
3rd Floor Birmingham Women's Hospital, Mindelsohn Way
Birmingham
B15 2TG

[redacted] 21/05/19

… ....

Signature Date

Sponsor statement
Where the University of Birmingham takes on the sponsor role for protocol development oversight, the signing of the IRAS form by the sponsor will serve as confirmation of approval of this protocol.

Funding
No funding has been sought for this study.

Key Study/Trial Contacts

Chief Investigator

Prof. A Coomarasamy
Professor of Gynaecology
MBChB, MD, FRCOG
University of Birmingham
3rd Floor Birmingham Women's Hospital, Mindelsohn Way
Birmingham
B15 2TG

Study/Trial Team

Principal Investigator
Dr Hajra Khattak
Clinical Research Fellow
BMBS, BMedsci, DFSRH
University of Birmingham
3rd Floor Birmingham Women's Hospital, Mindelsohn Way
Birmingham
B15 2TG

Academic Supervisor
Dr I Gallos
Clinical Scientist, Consultant Obstetrician & Gynaecologist
DMS, MD, MRCOG
University of Birmingham
3rd Floor Birmingham Women's Hospital, Mindelsohn Way
Birmingham
B15 2TG

Statistician
Dr Aurelio Tobias
Institute of Environmental Assessment and Water Research
Spanish Council for Scientific Research (CSIC)
C/Jordi Girona 18-26
Barcelona, Spain
E-08034

Appendix
OTC- Ovarian tissue cryopreservation
POI - Premature ovarian insufficiency
IVF- In vitro fertilization
HRT - Hormone replacement therapy
USS- Ultrasound Scan
LH -Luteinising Hormone
FSH -Follicle stimulating Hormone

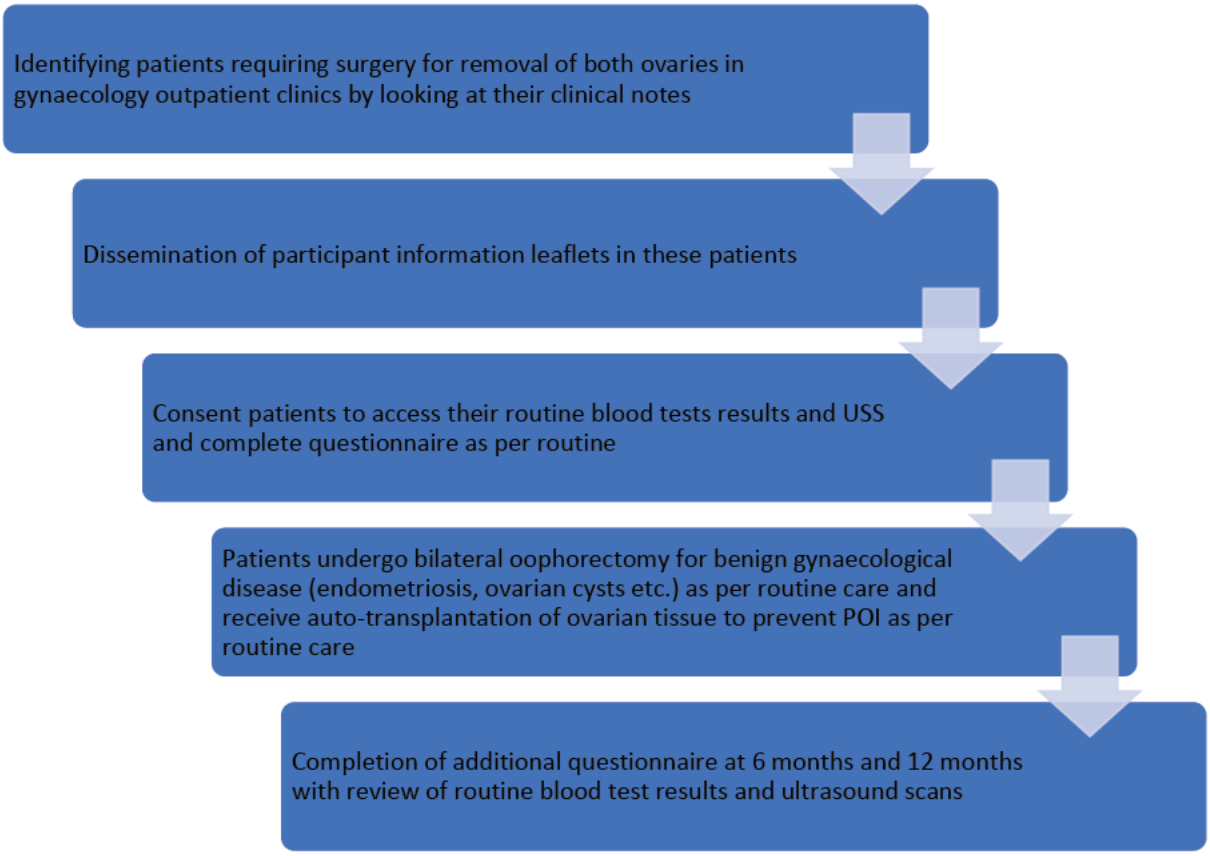
AMH -Anti Müllerian Hormone
BWH- Birmingham Women's Hospital
NICE –National Institute of Clinical Excellence
GnRH- Gonadotrophin releasing hormone

Study summary

Research objective

To assess the effectiveness of ovarian tissue transplantation.

Recruitment target and process



Duration of project

The study will last for 2 years and the participants will be requested to consent to take part and complete a quality of life questionnaire.

The study is being conducted in line with the principles of GCP.

Full title of the study

To describe the outcomes of fresh ovarian tissue auto-grafting in maintaining fertility and endocrine function in a clinical series.

Student ID:
PhD Met+Sys Re T Lab A300

Short study title

Ovarian transplantation for preserving endocrine and reproductive function (OysTER).

Primary objective

1. Assessing the return of ovarian endocrine function in patients having undergone ovarian tissue transplantation (by analysing the data from the hormonal tests and ultrasound scan).
2. Assessing their quality of life using a validated questionnaire.

Secondary objectives

1. Assessing the longevity of the ovarian graft:
The study will give an indication of how long the ovarian graft is functional for during the study period (as it will be indicated by the hormonal tests)

2. Assessment of the surgical technique:
Ovarian transplantation being a new procedure, may pose technical challenges when performing the transplant. This may indirectly have an affect on the functionality of the graft. The tests results therefore may give an indication of how the technique affected ovarian graft function.

Background and Rationale

Many women undergo surgery for removal of their ovaries or a significant part of the ovaries for non-malignant indications e.g. endometriosis and ovarian cysts. This can sometimes also be accompanied with removal of their womb. Consequently, total or significant loss of ovarian function can occur and cause premature menopause and permanent fertility loss with significant compromise on the quality of life.

Student ID:
PhD Met+Sys Re T Lab A300

Why is it important

Premature ovarian insufficiency (POI) is a devastating condition for young women and even more distressing if the cause is iatrogenic and can potentially be prevented. There is evidence to suggest that fresh ovarian tissue transplantation has been successful in restoring fertility and endocrine function. [1,2] Silber et al, in one of the few studies available, described a case series of 25 women after fresh and frozen ovary transplantation where FSH levels returned to normal and menstrual cycles resumed in all women. [3] They described 20 live babies following 11 fresh and 13 cryopreserved ovary transplantations (live birth rate of over 70%). [3] Furthermore, ovarian surgery (ovarian cystectomy and oophorectomy) is a recognisable cause of compromising ovarian reserve and the main reason of iatrogenic POI and fertility loss. [3 5] Fresh autografts are expected to be at least as effective in restoring ovarian function as cryopreserved tissue.[1]

I therefore aim to test a hypothesis in my primary study whereby the ovarian reserve can be preserved if the ovarian tissue is resected prior to performing surgery on the ovary and transplanting it on to the pelvic sidewall, subdermal transplantation as well as in the vagina . The idea is to resect the 'surgically untouched' strip of ovarian cortex, before it is being operated on and transplanted onto the pelvic peritoneum, under the skin (in the abdomen or the arm) or in the vagina. For patients undergoing oophorectomy, this may be the only hope of endogenous hormone production from the ovary, preventing POI and its symptoms in young women. The fresh autografting procedure is already approved to be applied clinically at Birmingham Women's Hospital as a new procedure and this study will help prove the restoration of ovarian function for these girls or women and support a change in the surgical practice aiming to preserve ovarian function and fertility.

Study Design

This will be an observational study of women having surgery that is likely to lead to significant compromise of ovarian reserve (for e.g. oophorectomy or ovarian cystectomy for endometriosis). These women will have healthy strips of the ovarian cortex removed and implanted into a peritoneal fold, subdermally or vaginally with the aim to preserve ovarian function. We will exclude women with documented menopause or POI. All women will be offered baseline tests for their hormone profile (follicle stimulating hormone (FSH),

Oestrogen as part of their routine care and anti müllerian hormone (AMH) costs permitting. Women will also be requested to complete a validated quality of life questionnaire (at 6 and 12 month follow up appointments).

Inclusion criteria

- Women
- Age: 18-45
- Undergoing surgery for the removal of one or both ovaries
- Patients will be offered tests and investigations prior to the surgery:
- FSH, LH, Progesterone and AMH (if possible)
- USS for antral follicle count

Rationale: Children are unlikely to be referred with benign gynaecological conditions requiring removal of both of the ovaries. Young girls aged 16-17 may require the help of their parents/guardians to consent and therefore females under 18 therefore will not be included. It is a new procedure and at this stage it is important that informed consent is sought from individuals who are adults and have full capacity.
Exclusion criteria:

- Women suffering from menopause or premature ovarian failure (this does not include patients on GnRH analogues)
- Women younger than 18 or older than 45 years.
- Women who require fertility treatments.
- Women who are pregnant at the time of the study investigations.
- Women who are participants in other interventional studies or trials.
- Women suffering from cancer

Rationale: The aim of this study is to assess the efficacy of ovarian transplants. Women who already are suffering from menopause are likely to have non-functioning or suboptimal functioning ovaries. Transplantation of their own ovaries is not feasible in this group.

Consent process

For women who agree to participate, the doctor in Gynaecology outpatient clinic will verify eligibility for participation in the study, provide verbal and written information and instructions about the study and obtain written consent.

Consenting and assessment for eligibility will take place within the hospital clinic by a doctor who has received study-specific training in obtaining research consents and assessing patient eligibility and will hold a valid GCP certificate.

Treatment/study process

The notes of all the women who are attending the clinic will be screened. Women with the potential of removal of both their ovaries will be given the patient information sheet whilst they wait. If during consultation it is confirmed that these patients are to have a surgery for removal of their ovaries, they will be invited to take part in the study. Should the patient have any questions, these can be answered during consultation. If the patient requires time to consider participation, the consent can be sought either on the day of the surgery or at a follow up appointment. They will also be informed that their initial decision to participate in the study is not irreversible and they may reconsider it at any point with no consequence on their routine care. Patients will not be obliged to give reasons for their withdrawal from the study.

Withdrawal process

Written consent will be sought from patients. Patients will be reassured that they can withdraw from the study at any time, without giving reasons. Withdrawal will not affect their clinical care. This is also stated in participant information leaflet.

Data Management process (data management, storage and access)

Paper records will be accessible only to authorised researchers, Sponsor representatives and individuals within the direct care team of participants and stored at all times in a securely locked filing cabinet.
At the study office, any paper records (such as consent forms and case report forms) will be stored securely behind three levels of security. The records will be stored in a locked filing cabinet inside a locked room, which is located on a corridor with restricted swipe card access.

The Study Office belongs to the University of Birmingham and is located within Birmingham Women's Hospital. The computers are linked to the University's network and all electronic data will be stored and accessed from the University's servers.
Anonymised research data generated from the study will be stored on the dedicated secure data storage system. Only individuals with the necessary approvals will be able to access any of these data. Laboratory research data and records will be stored according to local regulations and requirements. This data will be anonymised.

Data analysis

Outcome data will be gathered to assess return and continuation of ovarian function by testing for ovarian reserve markers (AMH, FSH and oestrogen) at 6 months and 12 months post-operatively. Reference ranges appropriate for the age will be used to define restoration of ovarian function. Data will be analysed descriptively.
Furthermore, patients will be asked to complete a questionnaire at 6 month and 12 month appointments regarding patient experience of side effects related to surgery, menopausal-like symptoms, return of menses and effect on pre-existing symptoms of benign disease e.g. endometriosis related pelvic pain. The data from the same questionnaire filled out at initial consultation will also be included in the analysis.
The minimum number of patients to be included is 30 over a 12-month period with a follow up of another 12 months.

Patient & Public Involvement

Patients, service users, and/or their carers may be involved in dissemination of findings. We intend to contact charities and share our results and these charities may disseminate our findings.

Protocol Compliance

Monitoring and auditing of conducting of research will be carried out as per Standard Operating Procedures of University of Birmingham

Definition of End of Study and Archiving

The trial will end in October 2020 (two years of recruitment and follow up) by which the follow up for last patient shall be concluded. All the data collected will be stored for 10 years.

References

1. Silber SJ, Lenahan KM, Levine DJ, Pineda JA, Gorman KS, Friez MJ, et al. Ovarian transplantation between monozygotic twins discordant for premature ovarian failure. N Engl J Med 2005;353:58–63.
2. Silber, Sherman et al. Duration of fertility after fresh and frozen ovary transplantation. *Fertility and Sterility* , Volume 94 , Issue 6 , 2191 – 2196.
3. Silber S. Ovarian tissue cryopreservation and transplantation: scientific implications. *Journal of Assisted Reproduction and Genetics* 2016 33(12), 1595–1603.
4. Moorman P et al. Effect of Hysterectomy With Ovarian Preservation on Ovarian Function. *Obstetrics and Gynecology* 2011, 118(6), 1271–1279.
5. Hirokawa W et al. The post-operative decline in serum anti-Müllerian hormone correlates with the bilaterality and severity of endometriosis. *Hum Reprod* 2011; **26:904**–10.
6. Goodman, L.R. et al. Evaluation of ovarian reserve before and after surgery in women with ovarian endometrioma compared to pelvic endometriosis and negative controls: a prospective cohort trial. *Fertility and Sterility*, Volume 104 , Issue 3 , e174.

Appendix 6. The Menopause-specific Quality of Life (MENQOL) questionnaire.





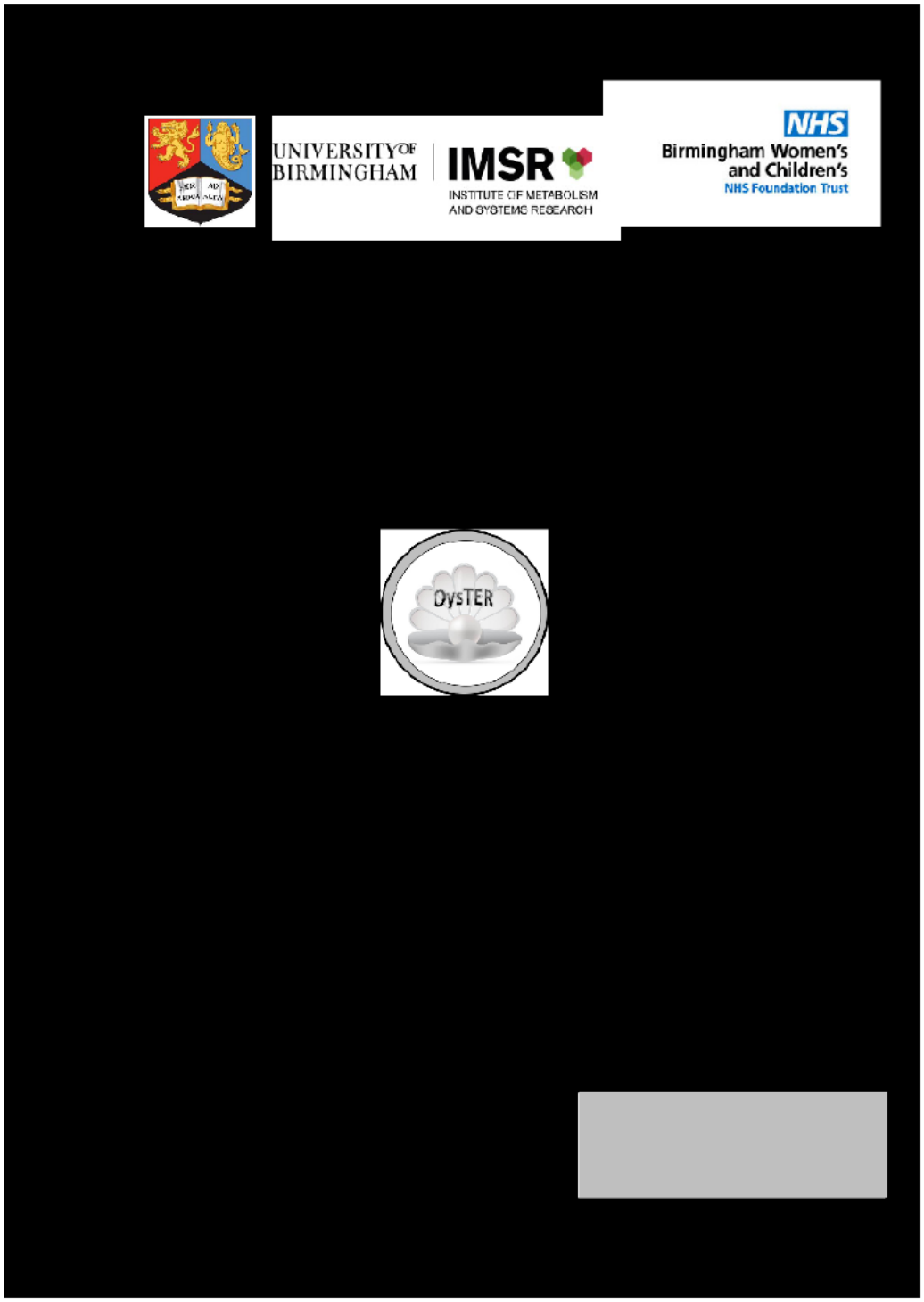
The Menopause-specific Quality of Life (MENQOL) Questionnaire.
Reprinted from Hilditch et al[3] © 1996, with permission from Elsevier.

The Menopause Specific Quality of Life Questionnaire

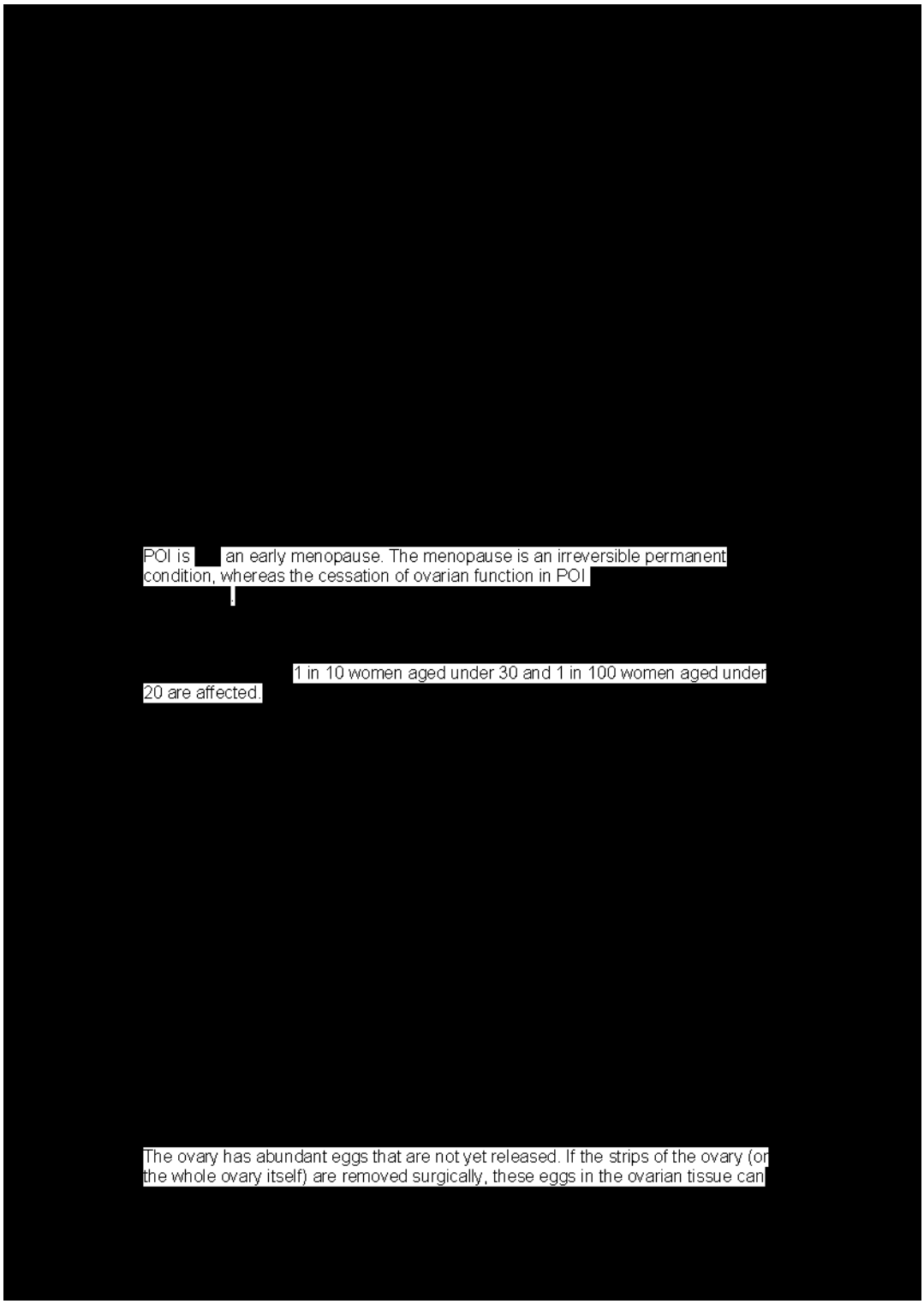
For each of the following items, indicate whether you have experienced the problem in the **PAST MONTH**. If you have, rate how much you have been *bothered* by the problem.

				Not at all bothered							Extremely bothered
				0	1	2	3	4	5	6	
1.	HOT FLUSHES OR FLASHES	☐ No	☐ Yes	→	0	1	2	3	4	5	6
2.	NIGHT SWEATS	☐ No	☐ Yes	→	0	1	2	3	4	5	6
3.	SWEATING	☐ No	☐ Yes	→	0	1	2	3	4	5	6
4.	BEING DISSATISFIED WITH MY PERSONAL LIFE	☐ No	☐ Yes	→	0	1	2	3	4	5	6
5.	FEELING ANXIOUS OR NERVOUS	☐ No	☐ Yes	→	0	1	2	3	4	5	6
6.	EXPERIENCING POOR MEMORY	☐ No	☐ Yes	→	0	1	2	3	4	5	6
7.	ACCOMPLISHING LESS THAN I USED TO	☐ No	☐ Yes	→	0	1	2	3	4	5	6
8.	FEELING DEPRESSED, DOWN OR BLUE	☐ No	☐ Yes	→	0	1	2	3	4	5	6
9.	BEING IMPATIENT WITH OTHER PEOPLE	☐ No	☐ Yes	→	0	1	2	3	4	5	6
10.	FEELINGS OF WANTING TO BE ALONE	☐ No	☐ Yes	→	0	1	2	3	4	5	6
11.	FLATULENCE (WIND) OR GAS PAINS	☐ No	☐ Yes	→	0	1	2	3	4	5	6
12.	ACHING IN MUSCLES AND JOINTS	☐ No	☐ Yes	→	0	1	2	3	4	5	6
13.	FEELING TIRED OR WORN OUT	☐ No	☐ Yes	→	0	1	2	3	4	5	6
14.	DIFFICULTY SLEEPING	☐ No	☐ Yes	→	0	1	2	3	4	5	6
15.	ACHES IN BACK OF NECK OR HEAD	☐ No	☐ Yes	→	0	1	2	3	4	5	6
16.	DECREASE IN PHYSICAL STRENGTH	☐ No	☐ Yes	→	0	1	2	3	4	5	6
17.	DECREASE IN STAMINA	☐ No	☐ Yes	→	0	1	2	3	4	5	6
18.	FEELING A LACK OF ENERGY	☐ No	☐ Yes	→	0	1	2	3	4	5	6
19.	DRYING SKIN	☐ No	☐ Yes	→	0	1	2	3	4	5	6
20.	WEIGHT GAIN	☐ No	☐ Yes	→	0	1	2	3	4	5	6
21.	INCREASED FACIAL HAIR	☐ No	☐ Yes	→	0	1	2	3	4	5	6
22.	CHANGES IN APPEARANCE, TEXTURE, OR TONE OF YOUR SKIN	☐ No	☐ Yes	→	0	1	2	3	4	5	6
23.	FEELING BLOATED	☐ No	☐ Yes	→	0	1	2	3	4	5	6
24.	LOW BACKACHE	☐ No	☐ Yes	→	0	1	2	3	4	5	6
25.	FREQUENT URINATION	☐ No	☐ Yes	→	0	1	2	3	4	5	6
26.	INVOLUNTARY URINATION WHEN LAUGHING OR COUGHING	☐ No	☐ Yes	→	0	1	2	3	4	5	6
27.	CHANGE IN YOUR SEXUAL DESIRE	☐ No	☐ Yes	→	0	1	2	3	4	5	6
28.	VAGINAL DRYNESS DURING INTERCOURSE	☐ No	☐ Yes	→	0	1	2	3	4	5	6
29.	AVOIDING INTIMACY	☐ No	☐ Yes	→	0	1	2	3	4	5	6

Appendix 7. Patient information leaflet and consent form (OysTER study).



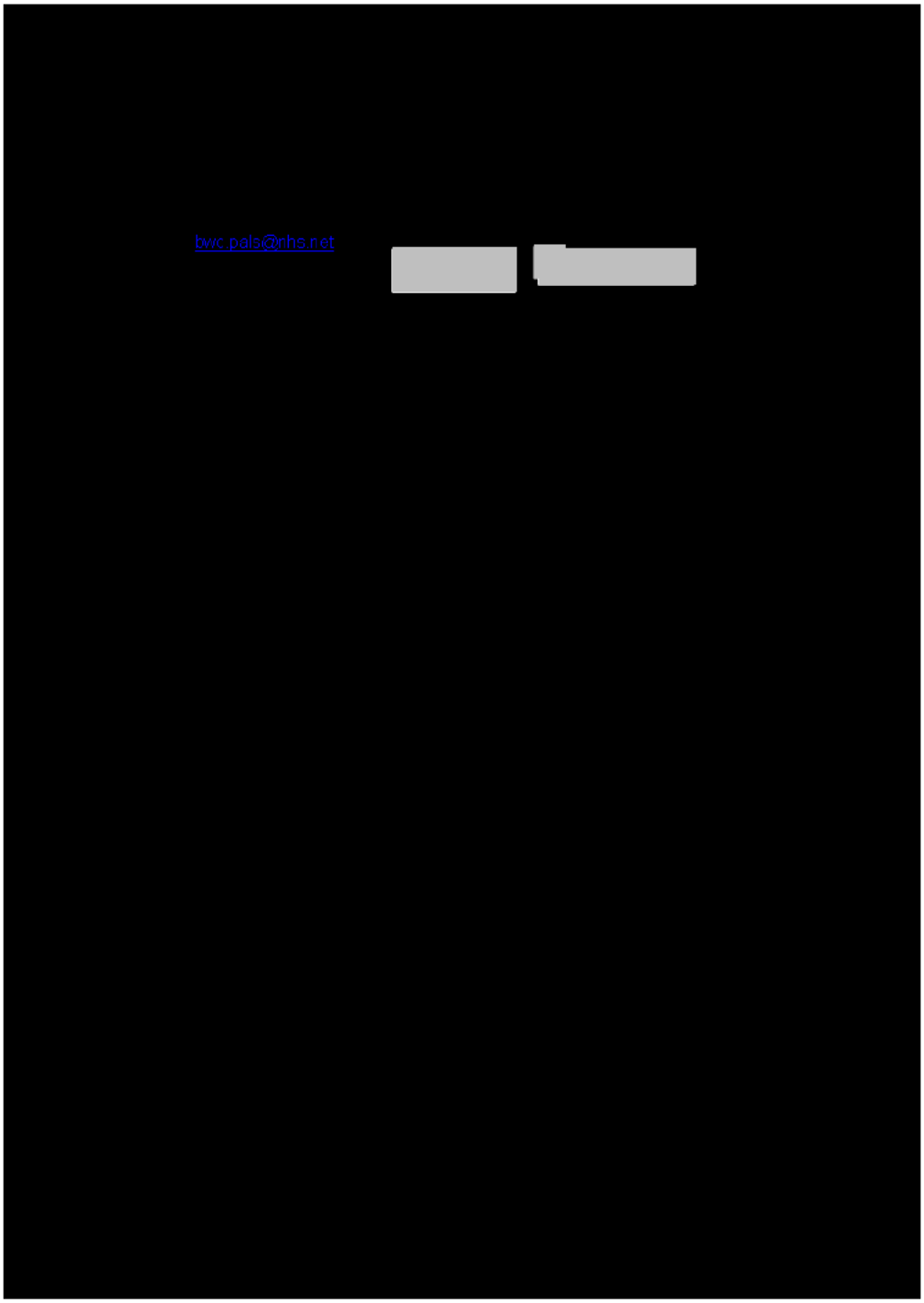
Student ID:
PhD Met+Sys Re T Lab A300



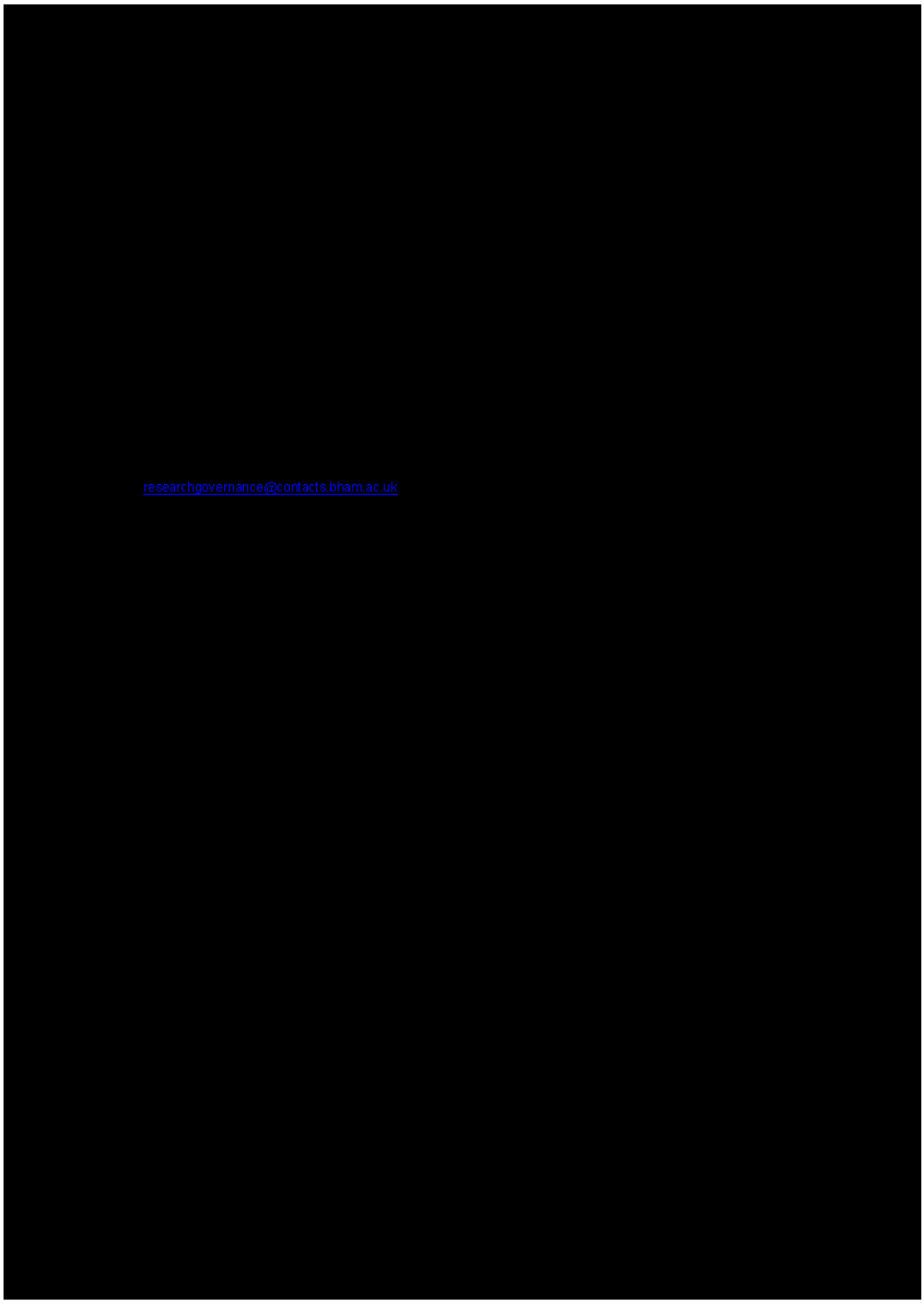
Student ID:
PhD Met+Sys Re T Lab A300

be frozen and transplanted back at a later stage. Once transplanted, the ovarian tissue can release eggs. This procedure has been demonstrated in patients suffering from cancer and has resulted in return of the hormonal function of the ovary as well as resulted in pregnancy and live births. Furthermore, the strips of the ovary can also be transplanted back at the time of the removal (fresh autografting). In your case, you have had/will have the fresh ovarian transplant. The main goal of ovarian tissue transplantation is the restoration of hormonal function of the ovary. This idea can potentially prevent the need for hormonal treatment in patients suffering from POI.

researchgovernance@contacts.bham.ac.uk



Student ID:
PhD Met+Sys Re T Lab A300



Student ID:
PhD Met+Sys Re T Lab A300

CONSENT FORM

Study Number:

Participant Identification Number for this trial:

Please initial box

1. I confirm that I have read the information sheet version …… for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the University of Birmingham, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
4. I understand that the information collected about me will be used to support other research in the future, and may be shared anonymously with other researchers. ☐
5. I give permission for the information collected to be used to support other ethically approved research in the future, and may be shared anonymously with other researchers. ☐
6. I would like to be informed of the results of this study. I thereby give permission to be contacted using the details listed below for this purpose. ☐
7. I am happy to be contacted about participating in future regarding ethically approved research. I give permission to be contacted using the details listed below for this purpose. ☐
8. I agree to take part in the above study. ☐

Please complete the information below to confirm how you would like to be contacted

By Post: …… By Phone: ……

…… By Email: ……

……

Name of Participant	Date	Signature
Name of Person taking consent	Date	Signature

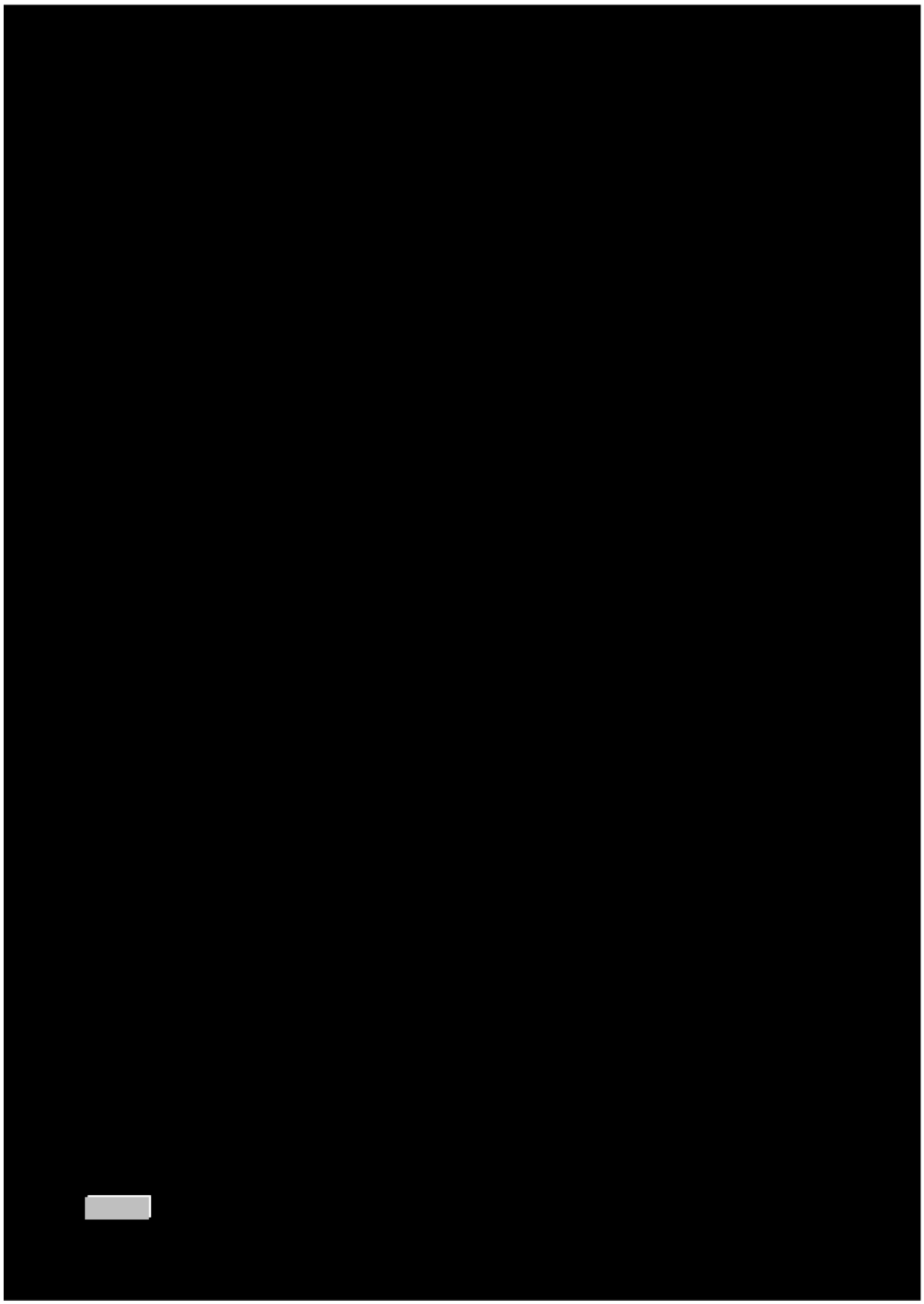
IRAS Help

obtain local agreement

HRA website

researchgovernance@contacts.bham.ac.uk

hra.approval@nhs.net



Appendix 8. Ethical approval for OysTER study.

Ymchwil Iechyd a Gofal Cymru
Health and Care Research Wales

NHS
Health Research Authority

Pro essor Arri Coomarasamy
Pro essor o Gyanecology
University o Birmingham
Academic Unit
3rd Floor Birmingham Women's Hospital Mindelsohn Way
Birmingham
B15 2 G

Email: hra.approval@nhs.net
Research-permissions@w ales.nhs.uk

30 November 2018 RE SSUE

Dear Pro essor Coomarasamy

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title	**Ovarian transplantation for preserving endocrine and reproductive function**
IRAS project ID	**246841**
Protocol number	**n/a**
REC reference	**18/EM/0338**
Sponsor	**University of Birmingham**

am pleased to con irm that HRA and Health and Care Research Wales (HCRW) Approval has been given or the above re erenced study on the basis described in the application orm protocol supporting documentation and any clari ications received You should not expect to receive anything urther relating to this application

How should I continue to work with participating NHS organisations in England and Wales?
You should now provide a copy o this letter to all participating NHS organisations in England and Wales as well as any documentation that has been updated as a result o the assessment

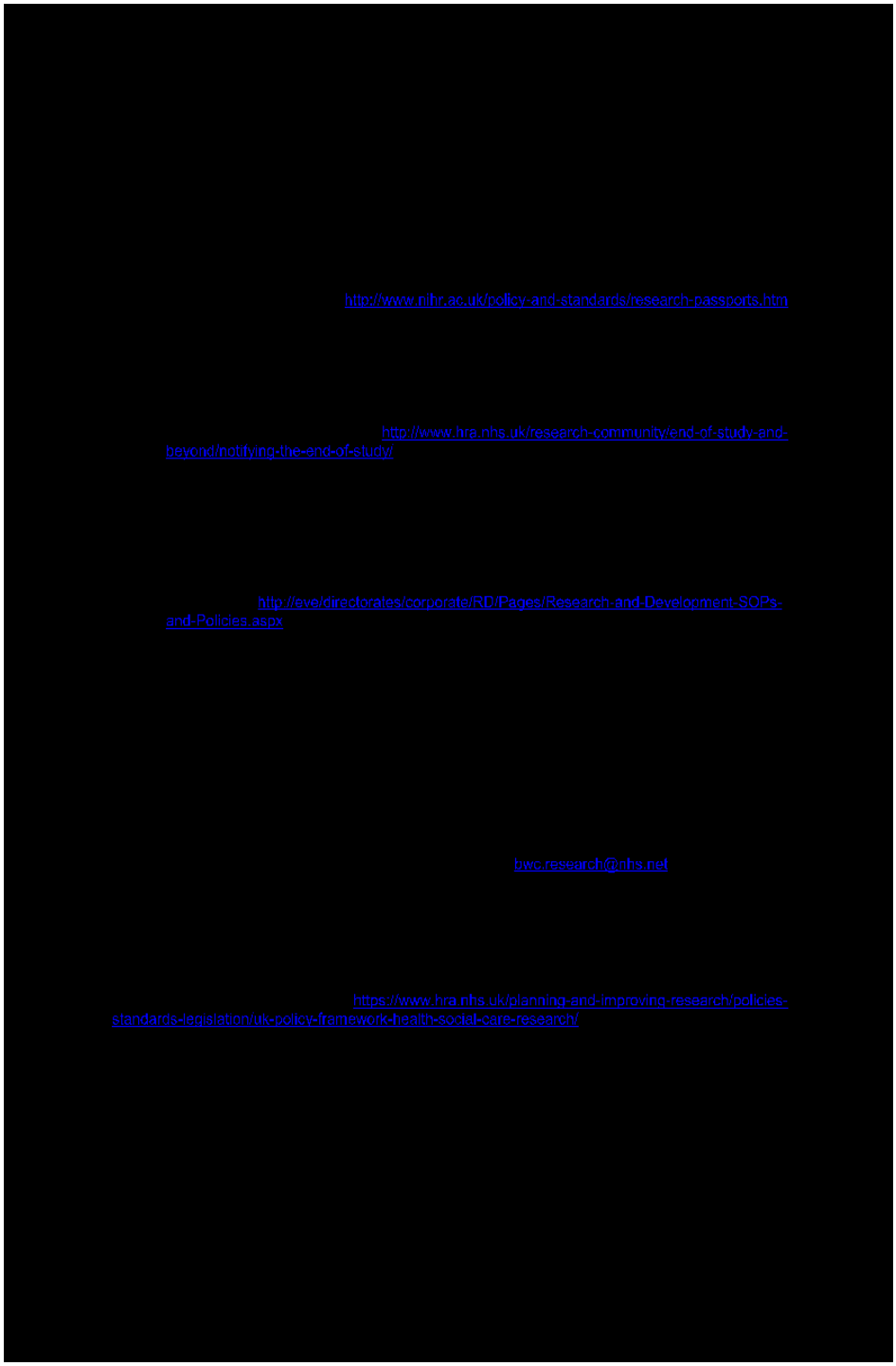
his is a single centre study completed as part o an educational project he R&D o ice will con irm to you when the study can start ollowing issue o HRA and HCRW Approval

t is important that you involve both the research management unction (e g R&D o ice) supporting each organisation and the local research team (where there is one) in setting up your study Contact details o the research management unction or each organisation can be accessed here

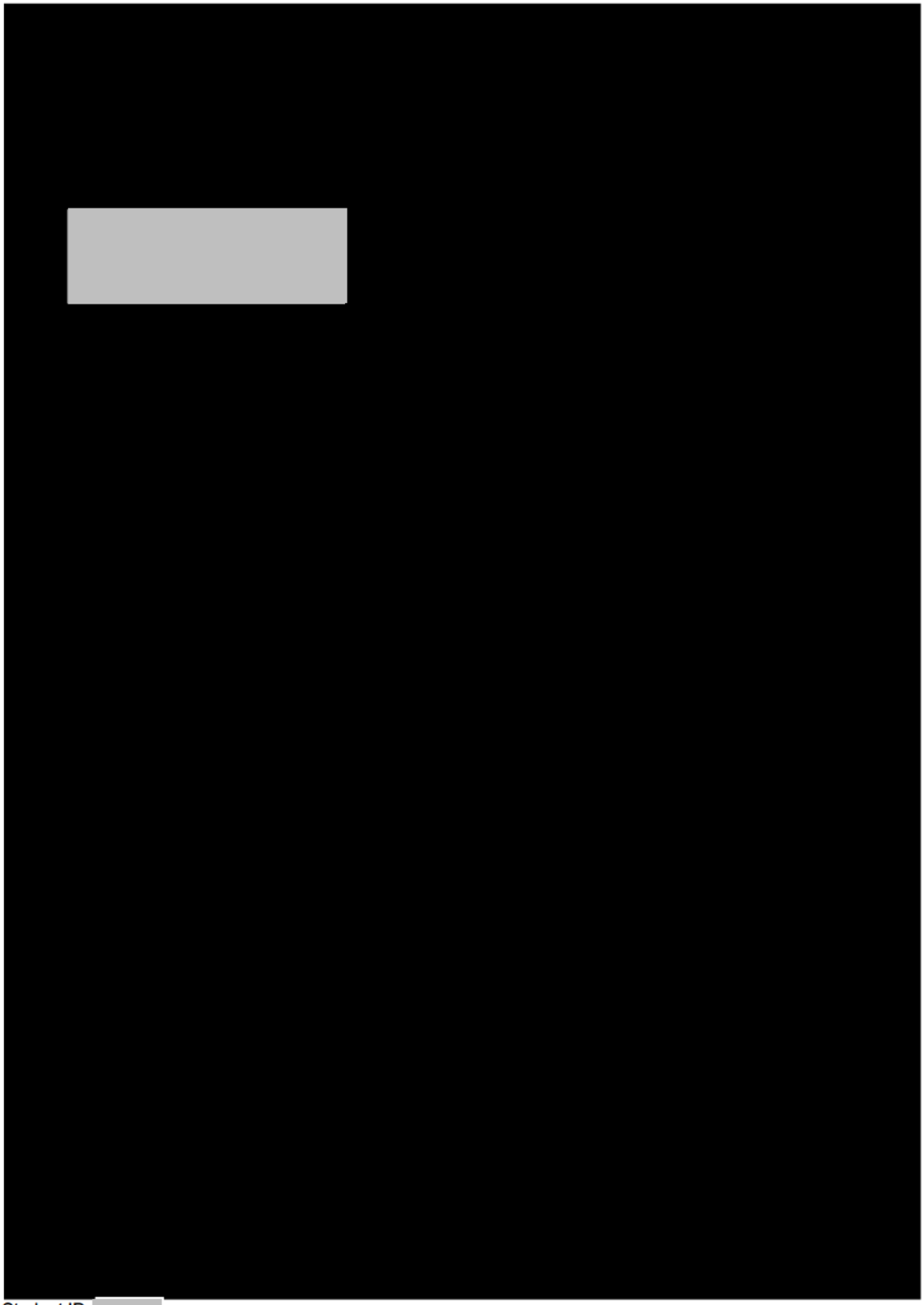
How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?
HRA and HCRW Approval does not apply to NHS/HSC organisations within the devolved administrations o Northern reland and Scotland



Student ID:
PhD Met+Sys Re T Lab A300



Student ID:
PhD Met+Sys Re T Lab A300



Student ID:
PhD Met+Sys Re T Lab A300

Appendix 9. Amendments to OysTER study.

A Research Ethics Committee established by the Health Research Authority

NHS
Health Research Authority

East Midlands - Nottingham 1 Research Ethics Committee
The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS
Tel:

Please note: This is the favourable opinion of the REC only and does not allow the amendment to be implemented at NHS sites in England until the outcome of the HRA assessment has been confirmed.

05 June 2019

Dear Hajra Khattak

Study title:	**Ovarian transplantation for preserving endocrine and reproductive function**
REC reference:	**18/EM/0338**
Protocol number:	**n/a**
Amendment number:	**1**
Amendment date:	**21 March 2019**
IRAS project ID:	**246841**

The above amendment was reviewed at the meeting of the Sub-Committee held on 23 April 2019 by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

The members of the Sub Committee were satisfied with the responses submitted by the researcher and were content to issue a Favourable Opinion for the Amendment.

The Sub-Committee have reviewed this Amendment and have raised the following points:

A Research Ethics Committee established by the Health Research Authority

Document	Version	Date

https://www.hra.nhs.uk/planning-and-improving-research/learning/



A Research Ethics Committee established by the Health Research Authority



IRAS 246841. Amendment categorisation and implementation information

nrescommittee.EastMidlands-Nottingham1@nhs.net <noreply@hra.org.uk>

[redacted]

Amendment Categorisation and Implementation Information

Dear Professor Coomarasamy,

IRAS Project ID:	246841
Short Study Title:	Assessing the effectiveness of transplanting ovarian tissue
Date complete amendment submission received:	06.03.20
Amendment No./ Sponsor Ref:	06.03.20 - updated PIS (vers 1.8) and Protocol (vers 3.2)
Amendment Date:	06 March 2020
Amendment Type:	Non-substantial
Outcome of HRA and HCRW Assessment	**This email also constitutes HRA and HCRW Approval for the amendment,** and you should not expect anything further.
For NHS/HSC R&D Office information	
Amendment Category	**C**

Thank you for submitting an amendment to your project. We have now categorised your amendment and please find this, as well as other relevant information, in the table above.

What should I do next?

If you have participating NHS/HSC organisations in any other UK nations that are affected by this amendment **we will** forward the information to the relevant national coordinating function(s).

You should now inform participating NHS/HSC organisations of the amendment.

- For NHS organisations in England and/or Wales, this notification should include the NHS R&D Office, ICRN (where applicable) as well as the local research team.

When can I implement this amendment?

You may implement this amendment **immediately**. Please note that you may only implement changes described in the amendment notice.

Who should I contact if I have further questions about this amendment?

If you have any questions about this amendment please contact the relevant national coordinating centre for advice:

- England – amendments@hra.nhs.uk
- Northern Ireland – research.gateway@hscni.net
- Scotland – nhsg.NRSPCC@chs.net
- Wales HCRW.amendments@wales.nhs.uk

Additional information on the management of amendments can be found in the IRAS guidance.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/.

Please do not hesitate to contact me if you require further information.

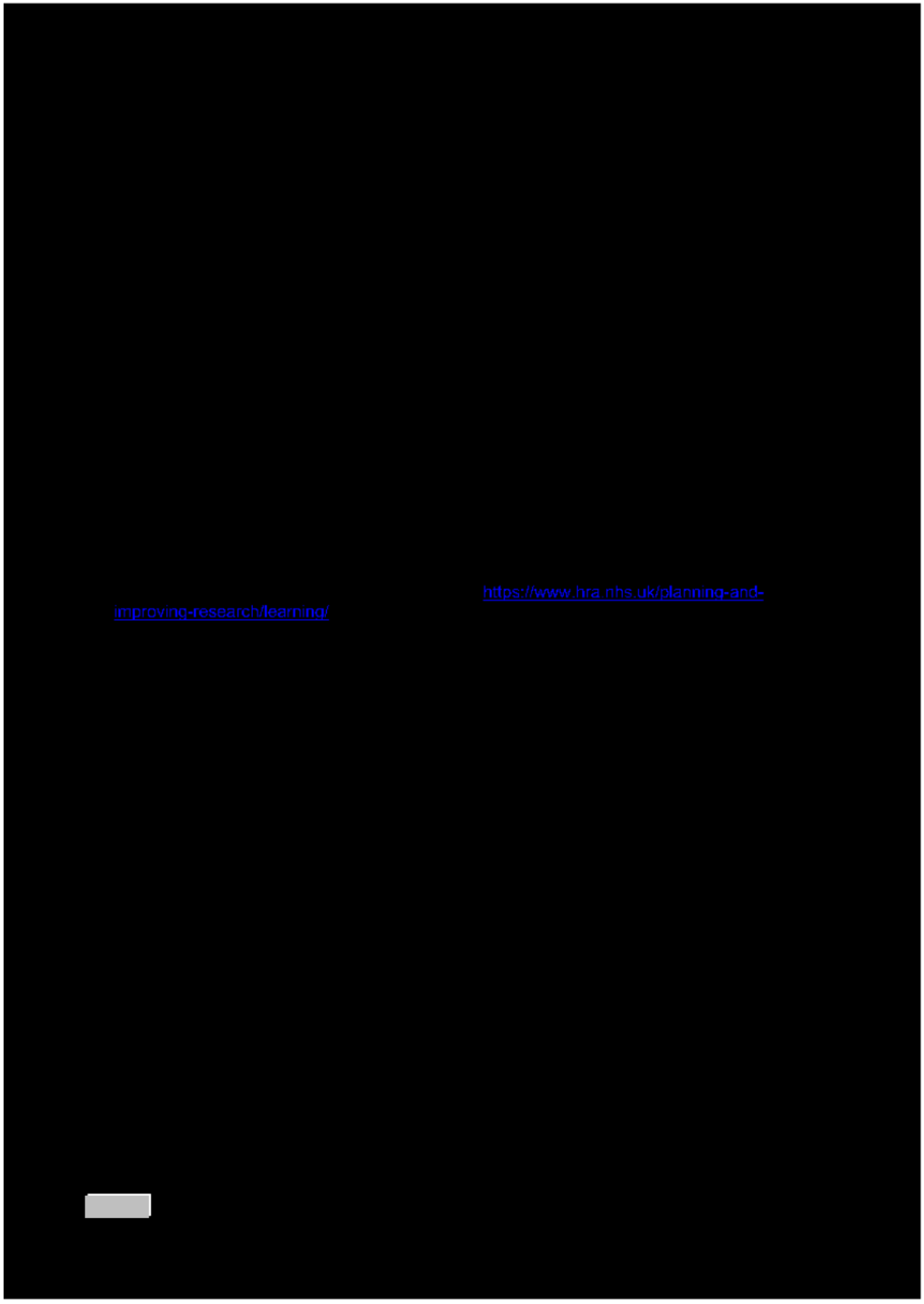
Kind regards

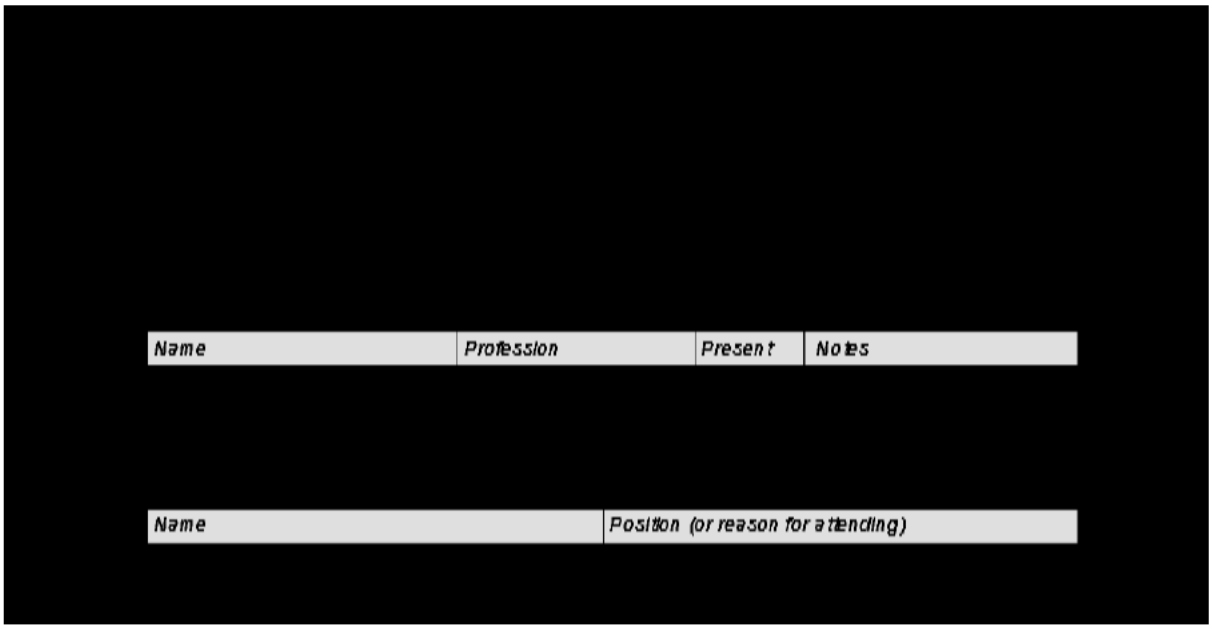
Ms Helen Poole
Approvals Specialist
Health Research Authority
Ground Floor | Skipton House | 80 London Road | London SE1 6LH
E.amendments@hra.nhs.uk
W. www.hra.nhs.uk

Sign up to receive our newsletter | IRA Latest.

NHS
Health Research Authority

Document	Version	Date


https://www.hra.nhs.uk/planning-and-improving-research/learning/



Name	Profession	Present	Notes

Name	Position (or reason for attending)

Student ID:
PhD Met+Sys Re T Lab A300

Appendix 10. Enhancing Transparency in Reporting the Synthesis of Qualitative Research (ENTREQ) Guidelines Checklist.

No	Item	Guide and description	Page No.
1	Aim	State the research question the synthesis addresses.	See Chapter 4
2	Synthesis methodology	Identify the synthesis methodology or theoretical framework which underpins the synthesis, and describe the rationale for choice of methodology (e.g. meta-ethnography, thematic synthesis, critical interpretive synthesis, grounded theory synthesis, realist synthesis, meta-aggregation, meta-study, framework synthesis	See Chapter 4
3	Approach to searching	Indicate whether the search was pre-planned (comprehensive search strategies to seek all available studies) or iterative (to seek all available concepts until they theoretical saturation is achieved).	See Chapter 4
4	Inclusion criteria	Specify the inclusion/exclusion criteria (e.g. in terms of population, language, year limits, type of publication, study type).	See Chapter 4
5	Data sources	Describe the information sources used (e.g. electronic databases (MEDLINE, EMBASE, CINAHL, psycINFO, Econlit), grey literature databases (digital thesis, policy reports), relevant organisational websites, experts, information specialists, generic web searches (Google Scholar) hand searching, reference lists) and when the searches conducted; provide the rationale for using the data sources.	See Chapter 4
6	Electronic search strategy	Describe the literature search (e.g. provide electronic search strategies with population terms, clinical or health topic terms, experiential or social phenomena related terms, filters for qualitative research, and search limits).	See Chapter 4 and Appendix 11
7	Study screening methods	Describe the process of study screening and sifting (e.g. title, abstract and full text review, number of independent reviewers who screened studies).	See Chapter 4
8	Study characteristics	Present the characteristics of the included studies (e.g. year of publication, country, population, number of participants, data collection, methodology, analysis, research questions).	Table 11
9	Study selection results	Identify the number of studies screened and provide reasons for study exclusion (e,g, for comprehensive searching, provide numbers of studies screened and reasons for exclusion indicated in a figure/flowchart; for iterative searching describe reasons for study exclusion and inclusion based on modifications t the research question and/or contribution to theory development).	Figure 31
10	Rationale for appraisal	Describe the rationale and approach used to appraise the included studies or selected findings (e.g. assessment of conduct (validity and robustness), assessment of reporting (transparency), assessment of content and utility of the findings).	Appendix 12
11	Appraisal items	State the tools, frameworks and criteria used to appraise the studies or selected findings (e.g. Existing tools: CASP, QARI, COREQ, Mays and Pope [25]; reviewer developed tools; describe the domains assessed: research team, study design, data analysis and interpretations, reporting).	See Chapter 4 and

			Appendix 12
12	Appraisal process	Indicate whether the appraisal was conducted independently by more than one reviewer and if consensus was required.	See Chapter 4 and Appendix 12
13	Appraisal results	Present results of the quality assessment and indicate which articles, if any, were weighted/excluded based on the assessment and give the rationale.	143, Appendix 12
14	Data extraction	Indicate which sections of the primary studies were analysed and how were the data extracted from the primary studies? (e.g. all text under the headings “results /conclusions” were extracted electronically and entered into a computer software).	See Chapter 4
15	Software	State the computer software used, if any	See Chapter 4
16	Number of reviews	Identify who was involved in coding and analysis.	See Chapter 4
17	Coding	Describe the process for coding of data (e.g. line by line coding to search for concepts)	See Chapter 4
18	Study comparison	Describe how were comparisons made within and across studies (e.g. subsequent studies were coded into pre-existing concepts, and new concepts were created when deemed necessary)	See Chapter 4
19	Deviation of themes	Explain whether the process of deriving the themes or constructs was inductive or deductive	See Chapter 4
20	Quotations	Provide quotations from the primary studies to illustrate themes/constructs, and identify whether the quotations were participant quotations of the author’s interpretation.	See Chapter 4
21	Synthesis output	Present rich, compelling and useful results that go beyond a summary of the primary studies (e.g. new interpretation, models of evidence, conceptual models, analytical framework, development of a new theory or construct).	See Chapter 4

Appendix 11. Search strategy for systematic review and thematic synthesis (Chapter 4).

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) <1946 to May 14, 2020>

Search Strategy:

1 exp Women/ (36213)

2 Female.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] (8774352)

3 (female* or women or woman or girl*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] (9000195)

4 1 or 2 or 3 (9000195)

5 exp Cryopreservation/ (36818)

6 (cryopreservation or freezing).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] (71392)

7 5 or 6 (82918)

8 exp Ovary/ (90740)

9 exp Ovarian Follicle/ (27551)

10 8 or 9 (90740)

11 (ovarian tissue or ovaries or ovarian tissue or ovarian graft or ovarian autograft or ovary graft).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] (37877)

12 10 or 11 (107790)

13 4 and 7 and 12 (2394)

14 exp Patient Satisfaction/ or exp Patient-Centered Care/ (106431)

15 exp Patient Satisfaction/ or patient experience.mp. or exp "Surveys and Questionnaires"/ (1084274)

16 14 or 15 (1099765)

17 patient involvement.mp. or exp Patient Participation/ (27030)

18 decision making.mp. or exp Decision Making/ (311005)

19 (decision* or decide or decision research).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] (459944)

20 exp Health Knowledge, Attitudes, Practice/ or exp Health Services Research/ (267488)

21 qualitative research.mp. or exp Qualitative Research/ (66855)

22 ethnography.mp. or exp Anthropology, Cultural/ (162058)

23 exp Nursing Methodology Research/ or grounded theory.mp. or exp Grounded Theory/ or exp Attitude to Health/ or exp Adaptation, Psychological/ (539962)

24 exp Qualitative Research/ or phenomenology.mp. (62519)

25 case studies.mp. or exp Case Reports/ (2128245)

26 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 (4185148)

27 13 and 26 (237)

Appendix 12. Assessment of quality of included studies using the Critical Appraisal Skills Programme (CASP) tool.

Author(s)	Aims	Method appropriate	Design appropriate	Recruitment appropriate	Data collection appropriate	Prior experience/ relationship	Ethical approval	Data Analysis	Clear statement of findings	Value of research
Bach et al. 2020	Yes	Yes	Yes	Yes	Yes	Unclear	No	Yes	Yes	Yes
Hershberger et al. 2013	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	Yes	Yes
Mitchel et al. 2020	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	Yes	Yes
Tennyson 2017 (unpublished thesis)	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	Yes	Yes

Appendix 13. Ethical approval for qualitative study (Chapter 5)

From: Susan Cottam (Research Support Services) [redacted]
Sent: 14 September 2020 12:55
To: Arri Coomarasamy (Metabolism and Systems Research)
[redacted]
Cc: Anne Topping (Nursing) <[redacted]; Hajra Khattak (PhD Metab + System FT Lab A300) <[redacted]
Subject: Application for Ethical Review ERN_19-1578

Dear Professor Coomarasamy

Re: "To explore the views, motives and expectations of patients considering undergoing ovarian tissue cryopreservation"
Application for Ethical Review ERN_19-1578

Thank you for your application for ethical review for the above project, which was reviewed by the Science, Technology, Engineering and Mathematics Ethical Review Committee.

On behalf of the Committee, I confirm that this study now has full ethical approval.

I would like to remind you that any substantive changes to the nature of the study as described in the Application for Ethical Review, and/or any adverse events occurring during the study should be promptly brought to the Committee's attention by the Principal Investigator and may necessitate further ethical review.

Please also ensure that the relevant requirements within the University's Code of Practice for Research and the information and guidance provided on the University's ethics webpages (available at https://intranet.birmingham.ac.uk/finance/accounting/Research-Support-Group/Research-Ethics/Links-and-Resources.aspx) are adhered to and referred to in any future applications for ethical review. It is now a requirement on the revised application form (https://intranet.birmingham.ac.uk/finance/accounting/Research-Support-Group/Research-Ethics/Ethical-Review-Forms.aspx) to confirm that this guidance has been consulted and is understood, and that it has been taken into account when completing your application for ethical review.

Please be aware that whilst Health and Safety (H&S) issues may be considered during the ethical review process, you are still required to follow the University's guidance on H&S and to ensure that H&S risk assessments have been carried out as appropriate. For further information about this, please contact your School H&S representative or the University's H&S Unit at healthandsafety@contacts.bham.ac.uk.

Kind regards

Susan Cottam
Research Ethics Manager
Research Support Group
C Block Dome
Aston Webb Building
University of Birmingham
Edgbaston B15 2TT
Tel: 0121 414 8825
Email: s.l.cottam@bham.ac.uk
Web: https://intranet.birmingham.ac.uk/finance/RSS/Research-Support-Group/integrity-ethics-governance/Research-Ethics/index.aspx

Please remember to submit a new Self-Assessment Form for each new project.

Click Research Governance for further details regarding the University's Research Governance and Clinical Trials Insurance processes, or email researchgovernance@contacts.bham.ac.uk with any queries relating to research governance.

Appendix to study Appendix 14. Amendment qualitative (Chapter 5). 15. Participant information

Application for amendment ERN_19-1578A

Susan Cottam [redacted]
Tue 19/01/2021 13:33
To:
Cc: [redacted]

Dear Professor Coomarasamy and Professor Topping

Re: "A qualitative study exploring why women consider ovarian tissue cryopreservation"
Application for amendment ERN_19-1578A

Thank you for the above application for amendment, which was reviewed by the Science, Technology, Engineering and Mathematics Ethical Review Committee.

On behalf of the Committee, I can confirm that this amendment now has full ethical approval.

I would like to remind you that any substantive changes to the nature of the study as now amended, and/or any adverse events occurring during the study should be promptly brought to the Committee's attention by the Principal Investigator and may necessitate further ethical review. A revised amendment application form is now available at https://intranet.birmingham.ac.uk/finance/accounting/Research-Support-Group/Research-Ethics/Ethical-Review-Forms.aspx . Please ensure this form is submitted for any further amendments.

Please also ensure that the relevant requirements within the University's Code of Practice for Research and the information and guidance provided on the University's ethics webpages (available at https://intranet.birmingham.ac.uk/finance/accounting/Research-Support-Group/Research-Ethics/Links-and-Resources.aspx) are adhered to and referred to in any future applications for ethical review. It is now a requirement on the revised application form (https://intranet.birmingham.ac.uk/finance/accounting/Research-Support-Group/Research-Ethics/Ethical-Review-Forms.aspx) to confirm that this guidance has been consulted and is understood, and that it has been taken into account when completing your application for ethical review.

Please be aware that whilst Health and Safety (H&S) issues may be considered during the ethical review process, you are still required to follow the University's guidance on H&S and to ensure that H&S risk assessments have been carried out as appropriate. For further information about this, please contact your School H&S representative or the University's H&S Unit at healthandsafety@contacts.bham.ac.uk.

If you require a hard copy of this correspondence, please let me know.

Kind regards

Susan Cottam
Research Ethics Manager
Research Support Group
C Block Dome

leaflet for qualitative study (Chapter 5).



A qualitative study exploring why women consider ovarian tissue cryopreservation

INFORMATION FOR PARTICIPANTS

Lead researcher

Dr Hajra Khattak

Contact: hajra.khattak@nhs.net

(Clinical Research Fellow)

Principal Investigator

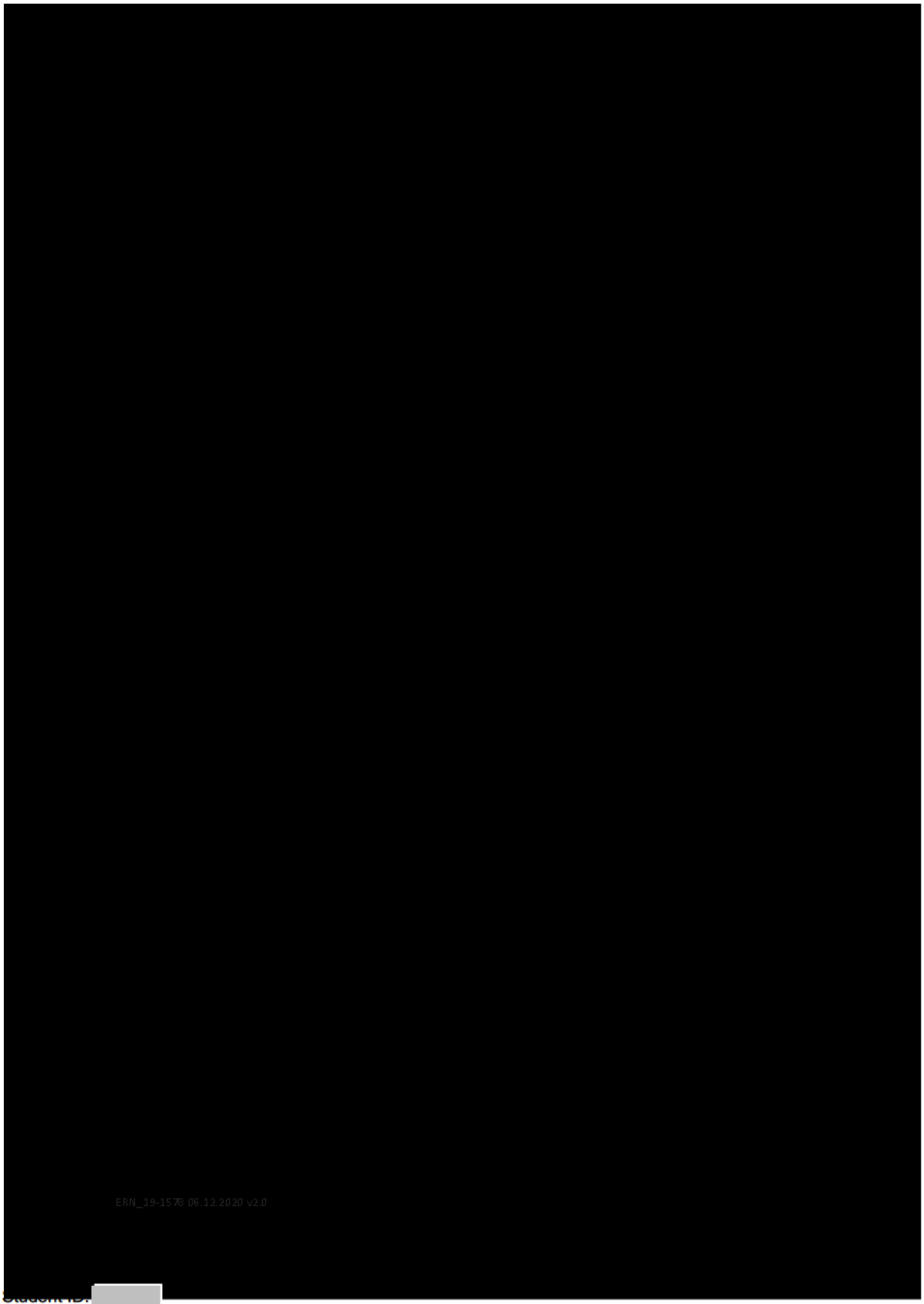
Professor Arri Coomarasamy

Co-investigator

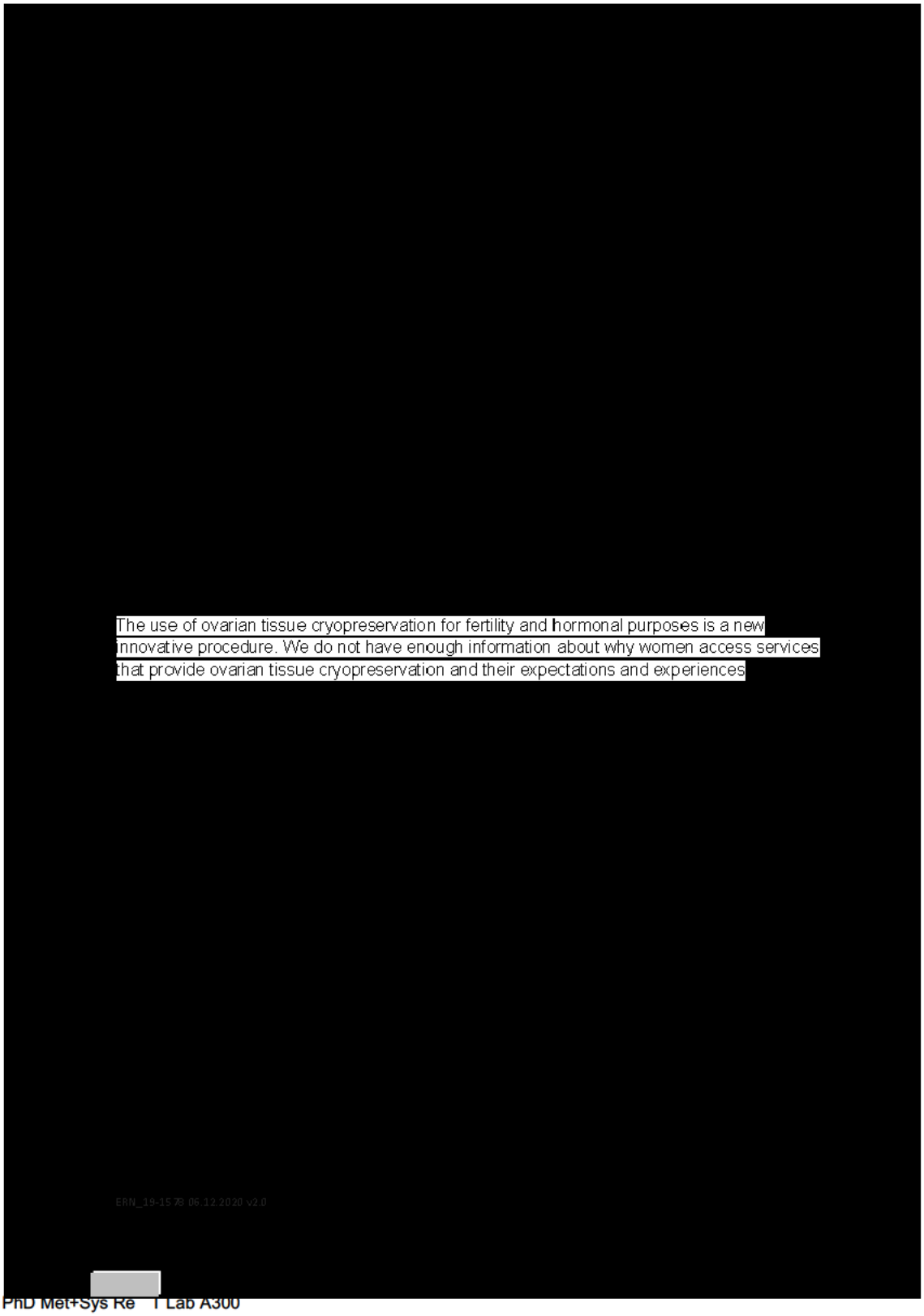
Professor Annie Topping

ERN_19-1578 06.12.2020 v2.0

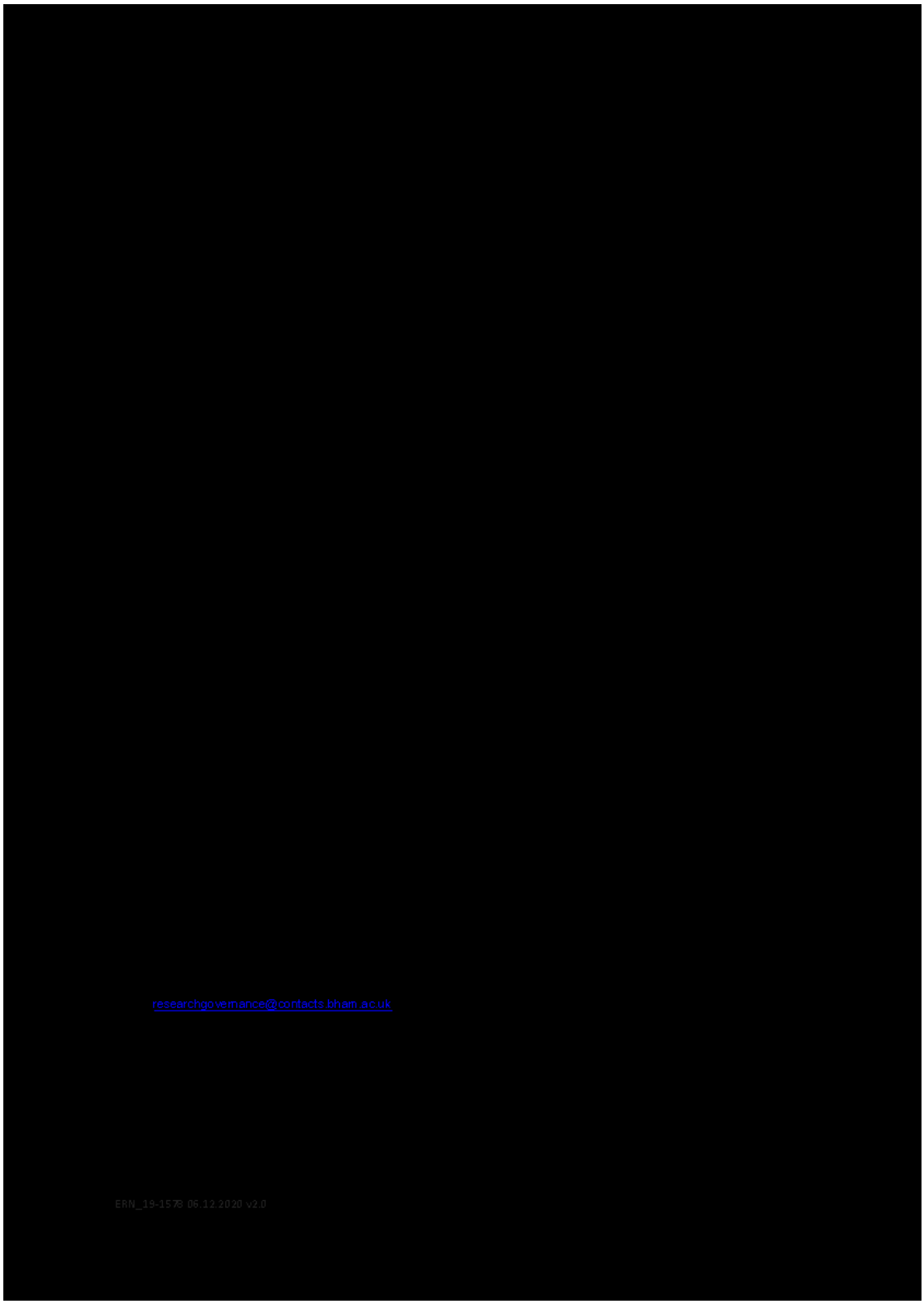
Student ID:
PhD Met+Sys Re T Lab A300



PhD Met+Sys Re T Lab A300



PhD Met+Sys Re T Lab A300



Student ID:
PhD Met+Sys Re T Lab A300

researchgovernance@contacts.bham.ac.uk

Appendix 16. Interview consent form.

Participant no:



A qualitative study exploring why women consider ovarian tissue cryopreservation

INTERVIEW CONSENT FORM

Please initial ALL boxes for 'Participant' I confirm that:	**Participant initials**	**PI initials (for remote consent)**
I have read and understood the information sheet version ___ dated _____ for the research project named above. I have had enough time to consider the information and to ask questions. I am happy with any answers I have been given. I understand that my participation in the interview is voluntary and that I am free to withdraw my participation at any time, without giving any reasons. I know this will not affect my legal rights.		
I agree to the researcher audio-recording and video-recording the session. **OR** I agree to the researcher only audio-recording the session. (Delete as appropriate) I understand that data collected during the study will be looked at by the research team and that all information will remain confidential, and that no personal information will be used which may identify me in the final report or scientific publications.		
I understand that all data collected will be stored in a locked filing cabinet at the University of Birmingham and on password-protected university computers or drives in line with the Data Protection Act.		
I understand that data collected during the study may be looked at by individuals from regulatory authorities and representatives of the sponsor who have a duty to monitor the quality of the research, and I give permission for this. I understand that if any disclosures are made during the sessions that suggest malpractice, misconduct, or that someone is in danger of harm, this information will be shared with the appropriate personnel.		
I give permission for my anonymised data to be used in future studies and secondary analysis.		
I agree to take part in this research study.		

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Name of person taking consent	Date	Signature
Consent taken and confirmed in:	Person	Remotely by PI

One copy of consent form (electronic or print) given to the participants, one kept in the site file, one added to patient notes.

ERN_19-1578 v2.0 11.09.2020

Student ID:
PhD Met+Sys Re T Lab A300

Appendix 17. Interview guide

Research questions

Views, motivations and expectations of women considering ovarian tissue cryopreservation (OTC)

Why women seek ovarian tissue cryopreservation?

Why women choose after consultation to proceed, or not with, ovarian tissue cryopreservation?

What is the impact of a diagnosis of sub-optimal fertility on women who seek ovarian tissue cryopreservation?

What motivates women to seek ovarian tissue cryopreservation

What women understand about ovarian tissue cryopreservation

The decision-making process once they have undergone initial assessment and aware of their fertility and eligibility for OTC

The impact of their decision-making and meaning of fertility and/or hormonal preservation.

Topics covered

- Women's understanding of ovarian tissue freezing.
- How did they find out about it.
- What made them want to explore whether this procedure is for them.
- Depending on the results from blood tests and scan the following would be explored:

-Intention to proceed with the procedure
-What they hope to gain from OTC. If this is fertility related, it would be explored further.
Any issues arising as result will be discussed. If it is related to hormonal purpose, concerns related to menopause will be explored.

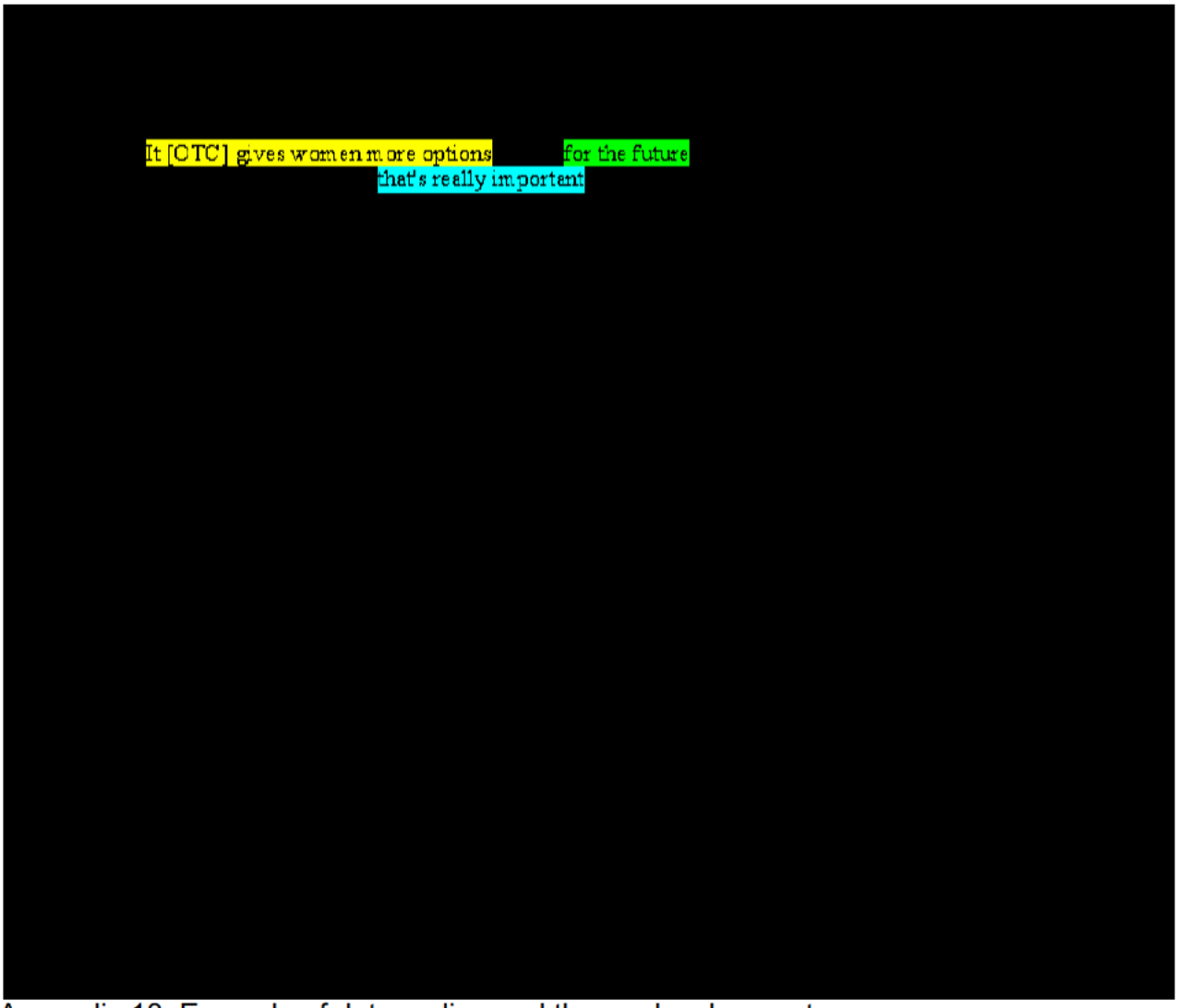
- If the women are not eligible for the procedure, it will be explored how that has made them feel.

Women with marginal results

Furthermore, any women with marginal results to be explored separately. Their intention to proceed with the procedure despite marginal results for ovarian tissue cryopreservation will be explored. The following topics will be covered:

Student ID:
PhD Met+Sys Re T Lab A300

- What do they hope to gain from undergoing OTC (fertility preservation or hormonal preservation or both).
- If opting for fertility preservation, what are their reasons for delaying motherhood.
- If hormonal, what is it about menopause that worries them.
- Do you think you may still wish to go ahead despite knowing that you may not have the desired results given your test results?



Appendix 18. Example of data coding and theme development.

BMJ Open

Why are women considering ovarian tissue cryopreservation to preserve reproductive and hormonal ovarian function? A qualitative study protocol

Hajra Khattak ,[1] Ioannis Gallos,[1] Arri Coomarasamy,[1] AE Topping [2]

To cite: Khattak H, Gallos I, Coomarasamy A, *et al*. Why are women considering ovarian tissue cryopreservation to preserve reproductive and hormonal ovarian function? A qualitative study protocol. *BMJ Open* 2022;12:e051288. doi:10.1136/bmjopen-2021-051288

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2021-051288).

Received 15 March 2021
Accepted 28 March 2022

Check for updates

© Author(s) (or their employer(s)) 2022. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

[1]Tommy's National Centre for Miscarriage Research, Institute of Metabolism and Systems Research, University of Birmingham College of Medical and Dental Sciences, Birmingham, UK
[2]Institute of Clinical Sciences, School of Nursing, University Hospitals Birmingham NHS Foundation Trust, University of Birmingham, Birmingham, UK

Correspondence to
Dr Hajra Khattak;
hajra.khattak@nhs.net

ABSTRACT

Introduction Current fertility preservation options available to women are oocyte cryopreservation (egg freezing) or embryo cryopreservation. A newer procedure, ovarian tissue cryopreservation (OTC), has become available in some centres, which offers another option for women and girls considering fertility preservation. These procedures are commonly offered to women about to undergo treatments for cancer. OTC involves removing sections of ovarian tissue and cryopreserving it for future reimplantation, often several years later. OTC offers girls and women who may become infertile with optionality and the possibility of pregnancy. OTC has potential for other applications, including restoring ovarian endocrine function beyond biological menopause. This is not without controversy but has led to some women considering undergoing the procedure for purposes of ovarian hormonal preservation (conservation of ovarian endocrine function). OTC is invasive, involves two surgical procedures with concomitant risks and can be costly. Understanding why women may consider and ultimately undergo OTC is timely, so that evidence-based and women-centred care can be provided.

Methods A pragmatic narrative qualitative design will be used. A purposive sample of women aged 18–45 who are considering, or have sought, OTC will be recruited over 1-year period. Potential participants will be approached via a clinic that offers OTC on a private basis or via social media.

Analysis Participant interviews will be audio and, if consented, video recorded. These will be conducted face-to-face or virtually. The recordings will be transcribed verbatim and analysed using a thematic analysis approach supported by NVivo software.

Ethics and dissemination Ethical approval has been granted by the Institutional Ethical Review ERN_19–1578A. We expect to disseminate the findings of this study through journal articles, conference presentations and multimedia to public.

Strengths and limitations of this study

- This study seeks to provide an in-depth understanding of the decision-making process in women in relation to fertility or hormonal preservation.
- A rigorous study design has been developed to explore any concerns women express seeking individual control over temporal aspects of loss of fertility and menopause.
- Use of purposive sampling will include recruitment of women from different backgrounds facilitating a diverse and broad sample.
- Sample may not be representative given recruitment from only one clinic.
- Social media recruitment excludes the views of women who do not use such platforms.

INTRODUCTION

A consequence of advances in anticancer therapies is that the number of young women surviving cancer has increased; however, the treatment may leave some subfertile or infertile. Fertility preservation is an approach often offered to young women before commencement of cancer treatment to future proof fertility post-treatment and improve the quality of life for these women. Advances in fertility preservation techniques have increased the potential for women to seek these techniques for reasons other than cancer, what could be termed as social reasons. The average age of women having their first child has increased in the recent decades.[1 2] Various reasons have been offered, including lack of partner, increased access to education, employment and career progression, access to contraception and financial considerations, which have provided women with greater choice and control over their fertility.[1 3 4] Oocyte and embryo freezing are established fertility preservation methods.[5–7] several centres worldwide are offering a newer procedure that involves cryopreserving ovarian tissue.[8–11] The availability of cryopreservation of ovarian tissue has increased since its introduction two decades ago, but access remains limited.[12 13] For women who are planned to undergo cancer therapy, the processes involved in oocyte freezing can be lengthy involving a period of controlled

BMJ Open: first published as 10.1136/bmjopen-2021-051288 on 13 April 2022. Downloaded from http://bmjopen.bmj.com/ on May 18, 2022 by guest. Protected by copyright.

Appendix 19. Published protocol for qualitative study (Chapter 5).

Student ID:
PhD Met+Sys Re T Lab A300

ovarian stimulation that can cause delays to planned treatment. Ovarian tissue cryopreservation (OTC) is an alternative approach that has been used by many women worldwide.[9 14–16] The availability of this procedure is limited in the UK with only a handful of centres offering this service on the national health service (NHS).[17–22] In the UK, the OTC procedure is normally only offered to women with a cancer diagnosis prior to commencing fertility ablating treatment. Globally, some centres are now offering OTC not only for the preservation of fertility largely to women with cancer but also for social reasons.[23 24] This interest for social reasons is linked with the evidence that reimplanted previously frozen ovarian tissue can produce a return of hormonal function, hence could be used for preserving hormonal function for longer.[25 26] OTC and transplantation procedures have also been used with women who are at risk of developing premature ovarian insufficiency (POI), including non-cancer indications such as oophorectomy, benign ovarian tumours, Turner syndrome and benign haematological conditions.[20–22] This technique, therefore, offers patients with a wide variety of illnesses, a chance to have biological children and experience motherhood, in addition to preserving their hormonal function. Prior to wider adoption; however, it is crucial that alongside the clinical evidence of benefit, we understand patient experience associated with this procedure. This will inform the provision of best care to these patients.

OTC has largely been used as a fertility preservation method in women treated for cancer. The consequences of gonadotoxic therapies (eg, chemotherapeutic agents) not only affect reproductive outcomes but also cause iatrogenic menopause. Fertility preservation methods are also offered to healthy women who may wish to delay pregnancy. Age-related oocyte preservation is now gaining greater acceptability as a strategy for controlling timing of pregnancy for social reasons.[14 27] The technique does not preserve ovarian hormonal function in women with normal ovarian hormonal function but offers the potential for extended preservation, hence, the need to explore the reasoning underpinning the decision to seek OTC for hormonal purposes. It is important to note that given the experimental nature of this procedure, OTC for social reasons is not widely available or even allowed in some countries.

Women are now living longer and almost of half their life in a menopausal state.[30] This can impact on the physical health of women, incurring increased cardiovascular risk and osteoporosis, and for some impact on mental well-being.[31 32] Although pharmacological interventions are available, OTC has the potential to provide physiological hormonal production and delay menopause.[25 28 29] One explanation for social interest in OTC is the notion of future proofing, as it offers the potential of hormonal 'insurance'. Menopause is increasingly portrayed as a negative life event in both the medical literature and popular media.[33–42] Some evidence suggests that how a woman experiences menopause is largely based on her sociocultural background and/or their female family members' experience with menopause.[43] Furthermore, postmenopausal women reportedly held more positive perceptions of menopause compared with those in a perimenopausal or premenopausal stage.[44] This may provide some explanation of why some young girls and women may wish to preserve tissue that could extend their ovarian hormonal function. Women may be pre-empting the biographical disruption that results from the changes they perceive they will experience during menopause.[45] What is unknown is whether anticipatory concern is based on personal experience and/or information they have gathered from the media, news or healthcare professionals or indeed a combination.

Biographical disruption in relation to menopause

Biographical disruption, a term that was first coined in 1982 by Michael Bury, to describe how events or occurrences such as illness cause a profound change that affects how the individual understands themselves. Normally applied in relation to long-term illness where the individual may feel that the illness has created a major disruption to their self-identity, causing them to redefine their 'biography'.[46] This concept could be applied to women experiencing negative effects of menopause, or for women who will ultimately become menopausal, by framing menopause as a state, which women may feel challenged to redefine their identity. A qualitative study conducted by de Salis *et al* examined the experiences of menopause in women and identified three main explanatory narratives. Menopause was defined either as a normal process where no transition occurs in the women or in her societal interactions; as a struggle, associated with loss of identity and distress and/or menopause as release from the messy business of menstruation and, therefore, a liberating experience.[33] In the literature, menopause has been associated with ageing and related risks such as osteoporosis and cardiovascular disease unless hormone replacement therapy (HRT) is prescribed.[45] HRT is not an unproblematic solution and issues with HRT are well known.[47] This presents some women with decisions balancing acceptability and risks of life-long hormone replacement.

Anticipation of the biographical disruption engendered by menopause, therefore, may worry some women in their younger years. Awareness of the symptoms of menopause has gained greater profile in recent years possibly fuelled by media interest, and whether this influences women to seek out and, in some cases, opt for a procedure that can potentially delay or even reverse menopause remains unknown. Women who seek to preserve their fertility or hormonal function may have many reasons, and this study aims to explore these phenomena. Through this study, we intend to explore how menopause is seen by these women and examine if anticipation of a biographical disruption pre-empts women to bank their ovarian tissue for future use.

BMJ Open: first published as 10.1136/bmjopen-2021-051288 on 13 April 2022. Downloaded from http://bmjopen.bmj.com/ on May 18, 2022 by guest. Protected by copyright.

Student ID:
PhD Met+Sys Re T Lab A300

Theoretical framework

The philosophy of Pragmatism alongside decision-making theory, as described by Kahneman's System 1 and 2 thinking, will be used to frame this study.[19]

Pragmatist theory

Pragmatism can be defined as a link between 'human knowing' and 'human action'[49] and how beliefs are held and shared. Pragmatism recognises that although two individuals may experience the same situation, their worldviews of the situation may not be the same. The extent to which the experience is similar depends on the degree of shared view. This phenomenon provides a unique individual, as well as a shared, view of the experience.[19 50] Through interviewing a wide range of women (age range 18–45), we believe we will collate a unique account of the views and experiences of women who consider or choose fertility and hormonal preservation using OTC. Furthermore, as per the pragmatist position, meaning of actions and beliefs is found in consequences.[50] In the context of this study, this relates to the decisions participants make after they have had an initial consultation where they are being assessed for eligibility for the OTC procedure. For participants recruited via social media, these theoretical assumptions will help illuminate the thinking employed by women when deciding whether or not to opt for this procedure based on their current knowledge. Their responses will be analysed alongside their demographics such as age, occupation, relationship status, whether they already have children or if they have an interest in becoming a parent later in life, and employment. As actions are 'fluidly' linked to consequences such that they are open to change, if the situation pertaining to action changes, so would the consequence of the action, even though the action remains unchanged.[24 50] Based on this assumption, we aim to understand what women decide when and if their circumstances change (such as being ineligible for OTC after the initial consultation).

A pragmatist approach to qualitative inquiry involves using multiple resources as well as multiple methods of data collection. Data analysis can involve using various qualitative approaches.[51] Recruitment from a clinic that offers this procedure in addition to worldwide recruitment from social media will help us understand the views, motivations and expectations of women regarding OTC procedure in a wide variety of settings.

Thinking fast and slow (in relation to decision-making)

In behavioural psychology, two systems of thinking are described; 'system 1' and 'system 2' that ultimately influence the decisions we make. 'System 1' is fast, influenced by emotions and intuitive. This system enables humans to differentiate between danger and safety.[28] In comparison, 'system 2' is slower and functions through reasoning. It is used as a backup and often difficult to actuate. While 'system 1' operates automatically, normally 'system 2' remains in the background. Often these two systems interact with each other rather than operate independently. System 2 overriding system 1 (if it is intermittently over-ruled). The crucial point is when system 1 does not allow for system 2 to take over.[52] This concept of fast and slow thinking potentially could be applied to how women may make decisions when considering OTC to preserve their fertility or hormonal function. The proposition is that although women may use system 1 when seeking information and attending an initial consultation about the procedure, whereas system 2 may come into play when a more considered decision is required when finally deciding whether to undergo the procedure. It is envisaged that understanding women's decision-making will influence the quality of information and care provided to these women and better manage expectations.

AIMS AND OBJECTIVES

Aim

To explore the participants' perceptions, concerns, expectations and reasoning behind considering OTC.

Objectives

The objectives of this study are to interview women who have considered OTC as a method of fertility and hormonal preservation and gain an insight into:

1. What motivates women to seek OTC?
2. What women understand about OTC?
3. Gain an in-depth understanding of the decision-making process in women who have considered having OTC and may or may not have undergone initial assessment about eligibility for OTC.
4. The impact of their decision-making and meaning of fertility or hormonal preservation.

METHODS AND ANALYSIS

Study design

Pragmatic qualitative descriptive design.

Data collection

This study will use qualitative enquiry to explore decision-making process associated with seeking OTC for preserving fertility and hormonal function. The data will be collected using semistructured interviews. A topic guide will be used to frame the interviews but will not drive the enquiry (online supplemental file), as these will take the form of 'a conversation with a purpose'.[53] The interviews will be conducted by the lead researcher, following confirmation of consent and will be audio and/or video conferencing recorded. Participants will be given the option to be interviewed in person (subject to COVID-19 restrictions) or using telecommunication such as telephone or video teleconferencing. The interviews will be supplemented with clinical and demographic information provided as part of their clinical care and will be extracted from their clinical records. This will include age and investigations such as blood tests and ultrasound scan performed to ascertain suitability for OTC.

BMJ Open: first published as 10.1136/bmjopen-2021-051288 on 13 April 2022. Downloaded from http://bmjopen.bmj.com/ on May 18, 2022 by guest. Protected by copyright.

Student ID:
PhD Met+Sys Re T Lab A300

Student ID:
PhD Met+Sys Re T Lab A300

Data analysis

All interviews irrespective of mode, or participant, will be audio-recorded, downloaded and saved as sound files to a secure server prior to transcription. Transcription will be undertaken by a trusted supplier bound by a secure data transfer agreement through a password-protected server. Following transcription anonymisation will seek to remove any identifiable information and each transcript will be allocated a unique identifier.

All transcribed interview data will be analysed using thematic analysis.[14] Themes generated from the interview data will be divided into groups based on participants' reasoning for considering OTC (medical or social). Transcripts will first be imported into NVivo software to facilitate data management.[15] After familiarisation, reading and rereading each transcript and checking against sound file, coding will be undertaken. Sections of text will be named, and these will ultimately form a series of codes. These codes will be organised into tentative groupings (themes), and through an iterative process of sorting, resorting and reallocation form a tentative thematic map. This thematic schema may undergo numerous stages of refinement ultimately surfacing an analysis of the women's accounts irrespective of whether they ultimately underwent the procedure or not. This study is designed and will be reported in line with the Consolidated Criteria for Reporting Qualitative Studies.[16]

Study setting

The study will take place in a clinic that currently offers this technique on a private healthcare basis (in a non-NHS setting). Study information will also be shared on social media platforms. Due to restrictions as a result of COVID-19 pandemic, participants will be interviewed over the phone and/or video telecommunication if not possible to be interviewed in person.

Sample and recruitment

All women who book a consultation to discuss OTC within the study period will be approached by a member of the clinical team. For recruitment from the internet participants will contact the study coordinator themselves.

Inclusion criteria

- Women.
- Ability to provide informed consent.
- Aged 18–45 years.
- Considering OTC.

Exclusion criteria

Women who have already made a decision to opt for other methods of fertility preservation such as oocyte and embryo freezing will be excluded.

Sampling

The sampling will be purposive in nature based on the following categories (across full age range of 18–45):

1. Women who have heard of OTC and have considered it as a fertility or hormonal preservation method.
2. Women seeking OTC who are deemed eligible by the clinician based on ultrasound scan and blood tests and decide to proceed with the procedure.
3. Women who are deemed eligible for OTC by the clinician but decide to not go for OTC.
4. Women who are deemed ineligible for OTC based on their tests but are still keen to preserve their reproductive and endocrine function.
5. Women suffering from cancer seeking OTC as a mean of fertility preservation or having already had the consultation or procedure.

Recruitment

Based on comparable studies, it is anticipated that a sample size of 12–24women who are considering OTC will be adequate to answer the study questions, covering the age range and categories specified. Recruitment and sampling, however, will continue until data saturation point is reached, estimated at 24 participants.[17]

Prospective recruitment

Women who are interested in ovarian tissue freezing contact a central office. Once an appointment is booked for consultation, they are normally sent written information regarding the procedure. Alongside this information, they will be sent a participant information sheet (PIS) for this study (either via email or in post) by clinic staff. Furthermore, women coming for consultation will also be provided with a PIS by the clinic staff if not received prior. For prospective recruitment, women will be approached by the consulting clinician or other clinical staff they meet for their initial appointment to discuss ovarian tissue freezing, to ascertain if they are interested in participating in the study. Should the woman express a willingness to participate and agrees to be interviewed following their OTC consultation, the lead researcher will speak to the patient. This will provide participants with an opportunity to ask questions. If the woman agrees to take part, the lead researcher will provide informed consent. Furthermore, any healthcare professional involved in the care of the participant, named on the delegation log and trained in line with Good Clinical Practice (GCP) will also be able to take consent and recruit women to the study.[18] Participants agreeing to take part on the day will be given the option of conducting a face-to-face interview. If they prefer, they can also be interviewed remotely using audio and/or telecommunication at a later date. For online recruitment, study details and invitation to take part will be posted on social media platforms and forums. The participants who are interested will be encouraged to approach the researcher via email, who will then share the study documents (participant information leaflet and consent form).

Retrospective recruitment

Women who have previously attended the clinic and have already had their consultation to discuss and/or undergo OTC will be contacted by a member of clinic

BMJ Open: first published as 10.1136/bmjopen-2021-051288 on 13 April 2022. Downloaded from http://bmjopen.bmj.com/ on May 18, 2022 by guest. Protected by copyright.

Student ID:
PhD Met+Sys Re T Lab A300

staff via telephone, email or postproviding them with information about the study. This information will also provide an invitation to participate in the study, a PIS and consent form that will include contact details of the lead researcher. Patients can then contact the lead researcher via email if interested in participating, to ask questions and if they wish to proceed and organise an interview. Participants can opt for a virtual interview using audio and or video telecommunication or be interviewed in person (depending on restriction related to COVID-19 pandemic). Participants will be given the option of completing the electronic consent form using an electronic signature or returning it via post. For those with virtual interviews, and not having returned a completed consent form prior, the consent process will be undertaken at the beginning of the interview, audio-recorded and signed by the researcher conducting the interview.

Sample identification

The participants will be identified by the lead researcher, associated clinicians, healthcare professionals trained in GCP and research nurses. This will be done when patients enquire about the ovarian tissue freezing service from the central office (via email, by telephone or post). Posters explaining the study will be displayed, and information leaflets, will also be available in clinics. For online recruitment, the lead researcher will post on various social media platforms such as Facebook, Twitter and Instagram. Participants interested in the study will approach the researcher independently. Participants will then be selected according to the inclusion criteria.

Consent

Informed consent will be obtained by the lead researcher or healthcare staff involved in the care of the woman who are GCP trained, prior to participants recruited or being interviewed. Participants will be provided with written information such as leaflets and consent form that explains the study usually a few weeks before their consultation with care provider. The study information will be sent along with all other clinical information. Participants will ideally be given 24 hours from reviewing study information before they can participate. For those who have not received this information before their consultation will be given the patient information sheet and consent form on the day of their consultation. Participants can agree to take part in the study directly after their consultation (ie, less than 24 hours) if it means it is less burden on them in terms of time commitment. These women will also be given the chance to go away and think about the interviews if they required time, in which case interviews will be held remotely. For patients who have already had their consultation will be sent details about the study. The clinical care provider will advise the patient to contact the researcher if they are interested. If these women agree to participate, the consent form will be sent to them via email or post. The consent form can be returned via email using an electronic signature or via post. Participants will also be given the option of being able to complete the consent form prior to their interview (it will be audio-recorded and consent form signed by the lead researcher). For participants recruited via the internet, study documents will be sent over email or social media messaging. All patients will be assessed for capacity and only those deemed capable will be recruited to the study. Participants will be encouraged to ask questions. Participants will be informed that they can withdraw at any time up to 14 days following the interview, without giving any reason. However, they should withdraw more than 14 days after the interview, the data collected about them will be retained. This will be explained in the PIS and consent form. Participants recruited via the clinic will also be assured that this will not affect their medical care in any way.

Research governance

Assessment and management of risk

Women accessing this clinic are normally self-referrals and only a minority may have been referred by another clinician. Preserving fertility and hormonal sufficiency can be an emotive subject, and this may be compounded if a woman is already experiencing a condition such as cancer, or may believe they have or will discover through the assessment process to identify eligibility for OTC, signs of early menopause. The ethical implications of interviewing women whose fertility may be in doubt or challenged (due to cancer treatment or other conditions) have been discussed during the development of this study. The well-being of women accessing this procedure, and agreeing to participate, is paramount and takes precedence over any knowledge to be gained. Should a woman become emotionally distressed during an interview,and does not wish to continue, the interview will be terminated. As the interviewer is a clinician, with advanced communication skills and a trained interviewer, any immediate distress will be managed. The participant will be signposted to relevant support services such as the Daisy Network who provide support for women with POI or back to the care of their General Practitioner (GP). If during the process of being investigated for the procedure, participants unexpectedly found that they were experiencing early menopause diagnosed through clinical history and investigations, they will be referred to the relevant healthcare professionals and support groups.[39] If these women wish to continue to take part in the study, they will be given the option of interview at a later date when they feel comfortable and ready.

Regulatory review and Compliance

This study will take place in a clinic of non-NHS healthcare provider. Before any research activities commence, the research team will obtain the approval of the relevant research and ethics committees. Arrangements will be put in place with the study site provider organisation for issuing permission to conduct the study. For any amendments to the study, an application form will be completed

BMJ Open: first published as 10.1136/bmjopen-2021-051288 on 13 April 2022. Downloaded from http://bmjopen.bmj.com/ on May 18, 2022 by guest. Protected by copyright.

Student ID:
PhD Met+Sys Re T Lab A300

and submitted to the University of Birmingham Research Ethics. Amendments will only be implemented once approved.

Protocol compliance

Serious deviations from the study protocol will be reported without delay by the doctoral researcher to the chief investigator and sponsor and onwards as appropriate. The chief investigator will ensure that such deviations are investigated and appropriate actions taken. The responsible research and ethics committee will be notified as soon as possible of any serious breach that could undermine public confidence in the research. Procedures for recording and reporting serious adverse events will be clearly documented in the site file.

Data protection and patient confidentiality

All participants will be allocated an identifier, and this will be linked to all data. All identifiable study information will be held securely on an encrypted and password-protected computers at the University of Birmingham and separately from data. Data files will only be accessible by members of the research team and regulatory bodies. The face-to-face and telephone interviews will be undertaken using a password-protected recorder, and those using n video conferencing will be recorded using the record programme function using a password-protected PC. All sound files will be downloaded to a study-specific secure server at the University of Birmingham and recording deleted from the device and programme. Each sound file (with identifier) will be transferred securely to an agreed transcription service with confidentially agreements in place. Following transcription, every effort will be made to remove any recognisable information to anonymise the transcripts. Participants will be informed that anonymous verbatim quotations may be included in any manuscripts generated from audio-recordings and/or transcripts to report study findings.

All study researchers, clinicians and nurses will uphold the principles and comply with the requirements of the EU General Data Protection Regulation 2016/679 and Data Protection Act 2018 in the collection, storage, processing and disclosure of personal information. All study researchers, clinicians, nurses and midwives will also maintain up to date GCP training. The data protection measures of this study will adhere to relevant policies and procedures of the University of Birmingham. All study data collected on paper will be held securely in a locked room or locked cabinet at the university that is accessible only to the research team and relevant regulatory authorities. All study data collected or otherwise generated in electronic form will be held securely on encrypted and password protected in university-owned computers or University of Birmingham's research data stores and drives, which will also provide data backup. Any audio recordings to be externally transcribed will be released only to a specialist transcription supplier and subject to a Confidentiality Agreement to prevent disclosure of any personally identifiable information to third parties. The audio recordings will be transferred via a secure server with user identifiers and passwords. Subsequently, any personally identifiable data within transcripts will be removed by the research team and substituted by anonymised unique identification references. All study data will be held securely in the custody of the chief investigator for 10 years after first publication of the results, in accordance with the University of Birmingham Research Data Management Policy.

ETHICS AND DISSEMINATION

Study findings will be owned by the University of Birmingham and disseminated in order to contribute to understanding of the experience of, and motives and expectations of women seeking ovarian tissue freezing. Study findings may be used to inform the development of future funding proposals, facilitate the continuous professional development of healthcare practitioners and development of referral pathways and guidelines.

The participants will be informed of the outcomes and provided with a lay summary on request. Study findings will be reported in the form of a doctoral research thesis and academic manuscript/s, to be supplemented by presentation/s and relevant conferences and report to interested charities and appropriate women's support networks. The manuscript/s will be also made openly available via a dedicated online repository hosted by the University of Birmingham for this purpose.

Patient and public involvement

It was not possible to involve patients or the public in the design, or conduct, or reporting or dissemination plans of our research.

Limitations of the study

Because recruitment is to take place from only one clinic, the sample may not be representative. Furthermore, participants may be restricted in access to the clinic due to restrictions because of the pandemic and this may affect recruitment. Finally, social media recruitment excludes the views of women who do not use such platforms.

Contributors HK is a doctoral student, also training in Obstetrics and Gynaecology and is supervised by a team that is experienced in conducting qualitative research. AC is her lead supervisor, with AFT and IG being co-supervisors. The lead researcher (HK) has received training to conduct the interviews. AC is the principal investigator. All the authors were involved in designing this study. HK and AFT have contributed to gaining ethical approvals and drafting the initial version of this manuscript. All authors approved the final version.

Funding This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. HK's PhD includes an entry level scholarship from Wellbeing of Women (Registered Charity Number: 239281).

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

BMJ Open: first published as 10.1136/bmjopen-2021-051288 on 13 April 2022. Downloaded from http://bmjopen.bmj.com/ on May 18, 2022 by guest. Protected by copyright.

Student ID:
PhD Met+Sys Re T Lab A300

Student ID:
PhD Met+Sys Re T Lab A300

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/.

ORCID iDs
Hajra Khattak http://orcid.org/0000-0002-7330-3825
AE Topping http://orcid.org/0000-0002-0111-2341

REFERENCES

1 Matthews TJ, Hamilton BE. Delayed childbearing: more women are having their first child later in life. *NCHS Data Brief* 2009;21:1–8.
2 OECD. Family database: age of mothers at childbirth and age-specific fertility 2018.
3 Mills M, Rindfuss RR, McDonald P, *et al*. Why do people postpone parenthood? reasons and social policy incentives. *Hum Reprod Update* 2011;17:848–60.
4 Collins J, Evers H, Golombok S, *et al*. Social determinants of human reproduction. *Hum Reprod* 2001;16:1518–26.
5 Goldman KN, Grifo JA. Elective oocyte cryopreservation for deferred childbearing. *Curr Opin Endocrinol Diabetes Obes* 2016;23:458–64.
6 Varlas VN, Bors RG, Albu D, *et al*. Social freezing: Pressing pause on fertility. *Int J Environ Res Public Health* 2021;18. doi: 10.3390/ijerph18158088. [Epub ahead of print: 30 07 2021].
7 Fritz R, Jindal S. Reproductive aging and elective fertility preservation. *J Ovarian Res* 2018;11:1–8.
8 Donnez M-M, von Wolff M, Poirot C, *et al*. Transplantation of cryopreserved ovarian tissue in a series of 285 women: a review of five leading European centers. *Fertil Steril* 2021;115:1102–15.
9 Gellert SE, Pors SE, Kristensen SG, *et al*. Transplantation of frozen-thawed ovarian tissue: an update on worldwide activity published in peer-reviewed papers and on the Danish cohort. *J Assist Reprod Genet* 2018;35:561–70.
10 Lotz L, Dittrich R, Hoffmann I, *et al*. Ovarian tissue transplantation: experience from Germany and worldwide efficacy. *Clin Med Insights Reprod Health* 2019;13:1179558119886735.
11 Donnez J, Dolmans M-M. Fertility preservation in women. *Nat Rev Endocrinol* 2013;9:735–49.
12 ESHRE Guideline Group on Female Fertility Preservation, Anderson RA, Amant F, *et al*. ESHRE guideline: female fertility preservation. *Hum Reprod Open* 2020;2020:hoaa052.
13 Anderson RA, Davies MC, Lavery SA, *et al*. Elective egg freezing for non-medical reasons: scientific impact paper No. 63. *BJOG* 2020;127:e113–21.
14 Sheshpari S, Shahnazi M, Mobarak H, *et al*. Ovarian function and reproductive outcome after ovarian tissue transplantation: a systematic review. *J Transl Med* 2019;17:398.
15 Oktay K, Oktem O. Ovarian cryopreservation and transplantation for fertility preservation for medical indications: report of an ongoing experience. *Fertil Steril* 2010;93:762–8.
16 Van der Ven H, Liebenthron J, Beckmann M, *et al*. Ninety-five orthotopic transplantations in 74 women of ovarian tissue after cytotoxic treatment in a fertility preservation network: tissue activity, pregnancy and delivery rates. *Hum Reprod* 2016;31:2031–41.
17 Anderson RA, Baird DT. The development of ovarian tissue cryopreservation in Edinburgh: translation from a rodent model through validation in a large mammal and then into clinical practice. *Acta Obstet Gynecol Scand* 2019;98:545–9.
18 Complete Fertility Southampton. Ovarian tissue cryopreservation, 2016. Available: https://www.completefertility.co.uk/fertility-treatments-services/fertility-preservation/ovarian-tissue-cryopreservation
19 [illegible] University Hospital NHS Foundation Trust. Ovarian tissue cryopreservation - Future Fertility Programme Oxford, 2021. Available: https://www.ouh.nhs.uk/future-fertility/patients/ovarian.aspx
20 UCL new ovarian tissue freezing programme launched. 2019. Available: https://www.ucl.ac.uk/womens-health/news/2019/jan/new-ovarian-tissue-freezing-programme-launched
21 Yasmin E, Balachandren N, Davies MC. Fertility preservation for medical reasons in girls and women: British fertility Society policy and practice guideline. *Hum Fertil* 2018.
22 Amorim CA, Leonel FOR, Afifi Y, *et al*. Cryostorage and retransplantation of ovarian tissue as an infertility treatment. *Best Pract Res Clin Endocrinol Metab* 2019;33:89–107.
23 Profam. Profam. [cited 2021 Dec 4], 2021. Available: https://www.profam.co.uk
24 Oktay K. Innovation fertility preservation and IVF, 2021. Available: https://www.fertilitypreservation.org/contents/fertility-preservation-details/elective-ovarian-tissue-freezing-for-future-transplantation
25 Andersen CY, Kristensen SG. Novel use of the ovarian follicular pool to postpone menopause and delay osteoporosis. *Reprod Biomed Online* 2015;31:128–31.
26 Donnez J, Dolmans M-M. Natural hormone replacement therapy with a functioning ovary after the menopause: dream or reality? *Reprod Biomed Online* 2018;37:359–66.
27 Kim SS, Lee WS, Chung MK, *et al*. Long-term ovarian function and fertility after heterotopic autotransplantation of cryobanked human ovarian tissue: 8-year experience in cancer patients. *Fertil Steril* 2009;91:2349–54.
28 Schmidt KLT, Andersen CY, Loft A, *et al*. Follow-up of ovarian function post-chemotherapy following ovarian cryopreservation and transplantation. *Hum Reprod* 2005;20:3539–46.
29 Callejo J, Salvador C, Miralles A, *et al*. Long-term ovarian function evaluation after autografting by implantation with fresh and frozen-thawed human ovarian tissue. *J Clin Endocrinol Metab* 2001;86:4189–94.
30 Silber SJ, DeRosa M, Pineda J, *et al*. A series of monozygotic twins discordant for ovarian failure: ovary transplantation (cortical versus microvascular) and cryopreservation. *Hum Reprod* 2008;23:1531–7.
31 Donnez J, Martinez-Madrid B, Jadoul P, *et al*. Ovarian tissue cryopreservation and transplantation: a review. *Hum Reprod Update* 2006;12:519–35.
32 Donnez J, Jadoul P, Pirard C, *et al*. Live birth after transplantation of frozen-thawed ovarian tissue after bilateral oophorectomy for benign disease. *Fertil Steril* 2012;98:720–5.
33 Oktay K, Bedoschi G, Berkowitz K, *et al*. Fertility preservation in women with Turner syndrome: a comprehensive review and practical guidelines. *J Pediatr Adolesc Gynecol* 2016;29:409–16.
34 Jones BP, Saso S, Manic A, *et al*. The dawn of a new ice age: social egg freezing. *Acta Obstet Gynecol Scand* 2018;97:641–7.
35 Kristensen SG, Andersen CY. Cryopreservation of ovarian tissue: opportunities beyond fertility preservation and a positive view into the future. *Front Endocrinol* 2018;9:347.
36 Roser M, Ortiz-Ospina E, Ritchie H. Life expectancy. OurWorldInData.org, 2013. Available: https://ourworldindata.org/life-expectancy
37 Mishra GD, Brown WJ, Dobson AJ. Physical and mental health: changes during menopause transition. *Qual Life Res* 2003;12:405–12.
38 de Salis I, Owen-Smith A, Donovan JL, *et al*. Experiencing menopause in the UK: the interrelated narratives of normality, distress, and transformation. *J Women Aging* 2018;30:520–40.
39 Yding Andersen C, Mamsen LS, Kristensen SG. Fertility preservation: freezing of ovarian tissue and clinical opportunities. *Reproduction* 2019;158:F27–34.
40 Guillemin MN. Managing menopause: a critical feminist engagement. *Scand J Public Health* 1999;27:273–8.
41 Gannon L, Ekstrom B. Attitudes toward menopause: the influence of sociocultural paradigms. *Psychol Women Q* 1993;17:275–88.
42 Wadsworth G. Hearing midlife voices: Assessing different methods for researching women's experiences of menopause and midlife. *Womens Stud Int Forum* 2000;23:645–54.
43 Hoga L, Rodolpho J, Gonçalves B. Women's experience of menopause: a systematic review of qualitative evidence. *JBI Evid Synth* 2015;13.
44 Dashti S, Bahri N, Fathi Najafi T, *et al*. Influencing factors on women's attitudes toward menopause: a systematic review. *Menopause* 2021;28:1102–200.
45 Bury M. Chronic illness as biographical disruption. *Sociol Health Illn* 1982;4:167–82.
46 Langer RD, Hodis HN, Lobo RA, *et al*. Hormone replacement therapy - where are we now? *Climacteric* 2021;24:3–10.
47 Cagnacci A, Venier M. Ashman A. The controversial history of hormone replacement therapy. *Medicina* 2019;55:602.

Student ID: [illegible]
PhD Met+Sys Re[illegible]T Lab A300

BMJ Open: first published as 10.1136/bmjopen-2021-051288 on 13 April 2022. Downloaded from http://bmjopen.bmj.com/ on May 18, 2022 by guest. Protected by copyright.

Appendix 20. Published paper.

What are my options? Fertility preservation methods for young girls and women

In the last few decades, female fertility preservation has advanced dramatically. Embryo cryopreservation was once the sole option, and it came with the drawback of requiring a male partner or someone willing to donate sperm. Oocyte cryopreservation has solved this dilemma to a certain extent. However, this strategy is not applicable in prepubertal girls. Ovarian tissue cryopreservation, which involves retrieving strips of ovarian tissue and cryopreserving them in liquid or vapor nitrogen, followed by transplantation, is an alternative technique that has addressed this problem, but it is also not without risks. Of the 3 options available to females, ovarian tissue cryopreservation is still considered experimental in most of the world (1).

Although most fertility centers offer embryo and oocyte cryopreservation, ovarian tissue cryopreservation is not widely available. In young girls and women, where embryo and oocyte cryopreservation are not an option, the lack of availability of ovarian tissue cryopreservation results in a missed opportunity for fertility preservation in this cohort. There is evidence to suggest that live births from ovarian tissue cryopreservation are now promising (28%). However, setting up an ovarian tissue cryopreservation service is time-consuming and requires expertise, local and national approvals, and access to a storage bank. Furthermore, ovarian tissue cryopreservation and transplantation is invasive, requiring 2 surgeries and surgical expertise, and expensive. Therefore, the lack of funding is another issue and may result in depriving women a chance at motherhood.

Women invest emotionally, financially, and physically in fertility preservation to increase their chances of having a biologic child later in life. As a result, the fertility preservation strategy most suited to women must also be financially viable. To date, there has been no economic evaluation of the available techniques; in fact, there has been no comparison. In a recent study, Dhonnabáin et al. (2) compared, in detail, the reproductive outcomes of the 3 available female fertility preservation techniques and provided a thorough summary. The study also investigated the utilization rate of cryopreserved reproductive tissue and quoted it to be between 5% and 10% (2). In addition to such a low return rate, there is much more work to be done in this area because the current available studies are lacking in breadth and quality, which makes it even more cumbersome to make comparisons. An interesting observation was the number of participants who underwent ovarian tissue transplantation. Of the 3 available methods, ovarian tissue cryopreservation and transplantation had the highest number of participants despite being considered experimental. No significant difference was observed in the live birth rates when the 3 female fertility preservation techniques were compared. Furthermore, ovarian tissue cryopreservation was shown to have significantly less miscarriage rates compared with the more established embryo cryopreservation method. The findings of this study are timely and relevant and pave the way for removing the experimental label from ovarian tissue cryopreservation and transplantation for young girls and women who are at risk of losing their fertility owing to gonadotoxic cancer therapies.

Females have a limited reproductive lifespan. This is reduced even further when young girls or women undergo inevitable gonadotoxic fertility ablating anticancer treatments. In 2020, nearly 0.9 million instances of new malignancies were reported globally in females aged 0–39 years (crude and age-standardized incidence rate per 100,000) (3). With the limited availability of fertility preservation options, many young girls and women remain deprived. Indeed, where applicable, females should be offered all 3 methods and given a choice. However, it is not always possible to do so because of the lack of availability of the procedure or financial constraints. Although the study by Dhonnabáin et al. (2) has limitations, it is a starting point that provides a comparison between the various available fertility preservation techniques. Centers that do not provide this technique could perhaps collaborate with biobanks and centers that do offer all 3 techniques. There is reassuring evidence from some of the largest cohorts of ovarian transplantation in Europe that transporting ovarian tissue to a centralized bank is feasible (4).

Furthermore, the survival of individuals with cancer has brought into focus the improvement in the quality of life after cancer. Having biologic children forms part of the quality of life after survival; thus, there is a need to make young women aware of the gonadotoxic anticancer therapies that can result in premature ovarian insufficiency. There is now increasing evidence that transplanting ovarian tissue also results in the return of ovarian endocrine function (1). In addition, when comparing various fertility preservation techniques, young girls and women need to be explicitly informed about the return of a hormonal function (albeit irregular) (1). In addition to fertility preservation, young girls and women undergoing ovarian tissue cryopreservation who wish to preserve their hormonal function using this approach should be allowed to do so as long as they fully understand the risks.

Adolescent girls and women with cancer make fertility preservation decisions on the basis of physician expertise and knowledge (5). As a result, it is vital that the healthcare professionals who work with these women keep abreast of the latest research and fertility preservation strategies. It is also imperative that these professionals are equipped with the most up-to-date evidence when it comes to the success rates of these procedures. With already difficult decision making during cancer diagnosis, selecting the best fertility preservation option becomes even more challenging. There is hope that with the start of studies that have begun to compare the available female fertility preservation techniques, young girls and women in the near future will be able to choose from all 3 techniques and decide on a method best suited to their individual needs.

Student ID: [redacted]
PhD Met+Sys Re T Lab A300

u
h

https://doi.org/10.1016/j.fertnstert.2022.04.010



DIALOG: You can discuss this article with its authors and other readers at https://www.fertstertdialog.com/posts/55068

REFERENCES

https://ec.europa.eu/eurostat/databrowser/view/tps00199/default/table?lang=en&category=t_demo.t_demo_pop.t_demo_pop_pro.t_demo_pop_pro_pop (accessed 28 May 2021).

Jensen AK, Kristensen SG, Macklon KT, Fedder J, Ernst E, et al. Outcomes of transplantation of cryopreserved ovarian tissue to 41 women in Denmark. Hum Reprod 2015;30:2196–204.

Peate M, Meiser B, Hickey M, Friedlander M. The fertility-related concerns, needs and preferences of younger breast cancer patients during their cancer journey. Breast Cancer Res Treat 2009;117:487–501.

Peddie VL, Porter MA, Barrett R, Culligan D, MacDonald DG, King D, et al. Factors affecting decision making about fertility preservation after cancer diagnosis: a qualitative study. BJOG 2012;119:1048–57.

Student ID:
PhD Met+Sys Re T Lab A300