2024

Comparison of Cryopreservation of Ovarian Tissue Versus Cryopreservation of Oocytes in Fertility Preservation

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by

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Bachelor of Science in Radiologic Sciences, North Dakota State University, 2019

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A Scholarly Project

Submitted to the Graduate Facility of the University of North Dakota

in partial fulfillment of the requirements for the degree of

Master of Physician Assistant Studies

Grand Forks, North Dakota

May 2024

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Acknowledgments

I would like to extend my gratitude to my advisor, Vicki Andvik, and course instructor, Russell Kauffman, for their guidance and expertise on this project. Their support and feedback have been immensely helpful. I would especially like to thank my husband for his unwavering encouragement, patience, and support throughout my entire education. He has been my rock and my biggest fan during this journey, and I cannot thank him enough. I would also like to thank my family and friends for their support during this busy and exciting chapter of my life.

Abstract

This literature review aims to compare ovarian tissue cryopreservation (OTC) and oocyte cryopreservation as methods for fertility preservation. Electronic health science databases including PubMed, Clinical Key, ScienceDirect, and UpToDate were utilized. Seven articles met the inclusion criteria and were analyzed for this comprehensive review. This review indicates that both oocyte and ovarian tissue cryopreservation can effectively preserve fertility, however, ovarian tissue cryopreservation is deemed most effective for prepubertal girls at high risk of iatrogenic primary ovarian insufficiency (POI), or women who are unable to postpone gonadotoxic treatment. Ovarian tissue cryopreservation offers advantages that oocyte cryopreservation does not, such as multiple spontaneous pregnancies from a single transplant and resumption of ovarian function. Ovarian tissue cryopreservation also does not carry the risks associated with ovarian stimulation and delaying gonadotoxic treatment, unlike oocyte cryopreservation. Despite successful birth rates and low surgical risks associated with ovarian tissue cryopreservation, studies reveal a low utilization rate, with women often considering stored ovarian tissue as a "backup plan". There is a need for further additional research on ovarian tissue cryopreservation, especially in the younger age groups, as there was limited participation in follow-up studies. Overall, the studies analyzed in this literature review support ovarian tissue cryopreservation as an effective method of fertility preservation but emphasize the importance of further investigation and follow-up studies.

Keywords: ovarian tissue cryopreservation, oocyte cryopreservation, cryopreservation techniques, ovarian tissue transplantation, fertility methods, cryopreservation safety

Introduction

Premature ovarian insufficiency (POI) is defined as the early depletion of the ovarian reserve and is a leading cause of female infertility. Iatrogenic POI can occur in females following gonadotoxic treatments in women diagnosed with cancer or benign diseases requiring treatment. These treatments include chemotherapy, radiotherapy, and surgery (Cacciottola et al., 2022).

Before recent innovations in fertility preservation, cancer survivors with POI could only achieve parenthood through adoption or donor gametes. Fortunately, now this can be achieved via oocyte cryopreservation, embryo cryopreservation, and ovarian tissue cryopreservation. This literature review aims to compare ovarian tissue cryopreservation (OTC) and oocyte cryopreservation.

Cryopreservation of ovarian tissue can be performed in both pre- and post-pubertal females, and unlike oocyte cryopreservation, requires no ovarian stimulation. This results in avoidance of treatment delays and the risk of ovarian hyperstimulation syndrome. Ovarian tissue cryopreservation also does not require a male partner or sperm donor, and allows for a chance at natural conception, avoiding the financial burden of in vitro fertilization (IVF). Ovarian tissue cryopreservation is the only method suitable for adolescents who have not reached reproductive maturity (Dhonnabhain et al., 2022).

Statement of the Problem

Ovarian tissue cryopreservation (OTC) was previously labeled as an experimental fertility preservation procedure. This label was removed by the American Society for Reproductive Medicine in December 2019; however, the treatment is still considered experimental in many areas of the world. OTC is a unique option for women and adolescents with POI who cannot postpone gonadotoxic treatment. It can be performed during management

of the disease and has low risk of complication. It is also the only option offered for pre-pubertal girls to preserve fertility before highly gonadotoxic treatments, as it does not require ovarian stimulation. OTC has growing success rates, and this literature review aims to compare OTC with oocyte cryopreservation.

Research Question

In women who require fertility preservation, does cryopreservation of ovarian tissue compared to oocyte cryopreservation result in higher rates of successful pregnancy and long-term preservation of fertility?

Methods

A comprehensive literature review was conducted utilizing various online health science databases. These include PubMed, Clinical Key, ScienceDirect, and UpToDate. The keywords used included “ovarian tissue cryopreservation”, “oocyte cryopreservation”, “cryopreservation techniques”, “ovarian tissue transplantation”, “fertility methods”, and “cryopreservation safety.” The articles were limited from 2015 to the present day. Articles that did not focus on ovarian tissue cryopreservation or oocyte cryopreservation were excluded. The resultant articles included meta-analysis, retrospective analysis, prospective analysis, peer-reviewed journal articles, and systematic reviews.

Literature Review

Efficacy of Oocyte Cryopreservation for Infertility

Druckenmiller et al. (2016) performed a retrospective analysis of reproductive-aged cancer patients’ treatment cycles to demonstrate the viability of oocyte cryopreservation as a reproductive choice for patients undergoing gonadotoxic treatments. The various treatment cycles included ovarian stimulation, transvaginal oocyte retrieval, and oocyte cryopreservation. In some cases, the

cycles also involved further oocyte thawing, in vitro fertilization, and embryo transfer. The evaluation criteria involved the number of oocytes retrieved, cryopreserved, and thawed, the response to ovarian stimulation, and pregnancy-related data.

There were 182 oocyte cryopreservation cycles completed by 176 women of child-bearing age between 2005 and 2014 (six women underwent two cycles of ovarian stimulation and oocyte retrieval). These women all presented to the New York University Fertility Center for oocyte cryopreservation. There was a median age of 31 years (interquartile range 24-36). Malignancies of patients include breast (75), gynecologic (51), hematologic (32), and other cancers (18). The average time between the time of consult to oocyte retrieval was 12 days (interquartile range 10-14). The average estradiol level at peak stimulation was 1,446 pg/mL (interquartile range 10-14). Each cycle retrieved 15 oocytes (interquartile range 9-23) and 10 metaphase II oocytes (interquartile range 5-18). Ten patients (comprising 11 cycles) subsequently returned to utilize their cryopreserved oocytes for pregnancy attempts (6% of patients). The survival rate of the thawed oocytes was 86% (confidence interval [CI] 78-94%). Out of 11 thaw cycles, nine yielded embryos suitable for transfer. Per embryo transfer, there was an embryo implantation rate of 27% (CI 8-46%) and a live birth rate of 44% (CI 12-77%). There was a similar likelihood of a live birth utilizing embryos from cryopreserve oocytes between cancer patients (44% [CI 12-77%] per embryo transfer) in this study and non-cancer patients who underwent the same treatment at this center (33% [CI 22-44%] per embryo transfer) (Druckenmiller et al., 2016).

The results of this study demonstrated that oocyte cryopreservation is a feasible technique for fertility preservation in reproductive-age cancer patients needing anti-cancer therapies. It was also demonstrated that adequate ovarian stimulation and oocyte retrieval can be achieved promptly before treatment. Cryopreserving oocytes over embryos allows reproductive autonomy for

patients, regardless of relationship status. Limitations of this study include a small sample size, retrospective data, and only represents data from one fertility center. Strengths of this study include no maternal or neonatal complications reported. Another strength is that the need for a surrogate in women who underwent hysterectomies for cancer treatment in this study did not alter the use of cryopreserved oocytes, indicating that women who must undergo hysterectomies for treatment should still be offered fertility preservation (Druckenmiller et al., 2016).

Cacciottola et al. (2022) reviewed therapeutic methods for both fertility preservation and hormone replacement therapy in young patients with iatrogenic premature ovarian insufficiency (POI). POI may be caused by various treatments, including chemotherapy, radiotherapy, and ovarian surgery. Treatments that cause the greatest risk of developing POI are total body irradiation and chemotherapy before bone marrow transplantation, pelvic irradiation, and some treatments using alkylating agents. Factors that increase the risk of POI include patient age, ovarian reserve, and treatment type. The risk of POI is approximately 10% in patients under the age of 18, and 30-40% in patients under the age of 40 (Cacciottola et al., 2022).

Fertility preservation methods, such as oocyte cryopreservation or ovarian tissue cryopreservation (OTC), are chosen based on the patient's age and pubertal status. Oocyte cryopreservation is the method of choice for post-pubertal patients. Oocyte cryopreservation results in high survival rates and optimal cell competence after thawing. For patients undergoing oocyte cryopreservation due to age-related fertility decline, there is a strong correlation between clinical outcomes and the age of the patient at the time of oocyte cryopreservation. There is a higher likelihood of live birth in women under 35 years of age. For patients undergoing oocyte cryopreservation for oncological reasons, pregnancy rates and live birth rates were comparable to patients undergoing age-related fertility decline. However, cumulative live birth rates were

significantly lower, especially in patients under the age of 35, due to the inability to delay gonadotoxic treatment to perform additional oocyte retrievals. In women with endometriosis undergoing oocyte vitrification, there are fewer oocytes able to be retrieved per patient on average, due to endometriosis progression of POI. Previous ovarian surgery has a negative impact on live birth rates as it limits the number of acceptable oocytes. The main factor of success in oocyte cryopreservation for women at high risk of iatrogenic POI appears to be the age at cryopreservation. This success rate is decreased in patients less than 35 years of age with medical conditions that diminish their fertility. Another determining factor of success is the number of oocytes retrieved, with the ideal rate being 10-15, and this may be difficult in oncology patients who are not able to postpone gonadotoxic treatment to retrieve more oocytes (Cacciottola et al., 2022).

Ovarian tissue cryopreservation (OTC) is a viable method of fertility preservation for prepubertal girls at high risk of iatrogenic POI or women who are unable to postpone or have already begun gonadotoxic treatment. Multiple ovarian biopsies are taken by laparoscopy and slow-frozen. If there is no risk of malignant cell transmission, ovarian fragments are transplanted by laparoscopy to the pelvis via an orthotopic graft to the ovarian medulla or via a peritoneal window. If there is risk of malignant cell transmission, ovarian follicles may either be grown in vitro to obtain mature eggs or used to create an artificial ovary that can then be transplanted to the patient. Pregnancy and live birth rates are around 30% after transplantation. These are associated with age at the time of OTC. Pregnancy and live birth rates are lower if conception is achieved by in vitro fertilization (IVF), while miscarriage rates are higher. The longevity of ovarian function is related to ovarian reserve and patient age at the time of OTC. OTC yields an average ovarian lifespan of 4-5 years, as it is possible to transplant fragments of ovarian tissue in

separate transplantations. Around 55% of patients experience ovarian function for greater than five years. OTC allows for multiple spontaneous pregnancies, an advantage to oocyte cryopreservation. OTC also has the advantage of resuming ovarian function, thus replacing hormone replacement therapy, and alleviating postmenopausal symptoms (Cacciottola et al., 2022).

Efficacy of Cryopreservation of Ovarian Tissue for Infertility

Khattak et al. (2022) performed a systemic review and individual patient data meta-analysis of women who have received ovarian transplants to preserve reproductive and endocrine function. The study included 87 studies and 735 women. All studies that documented fertility or endocrine function outcomes from fresh or frozen-thawed ovarian transplants for at least one participant were included in this review. If more than one study was published from the same center, these were assessed and cross-referenced to avoid and remove duplicates. Studies that reported 5 or more cases of ovarian transplants were included in the statistical analysis, which ended up being 568 women. For this study, data was collected on women's ovarian reproductive function to include pregnancy, live births, and miscarriages. Endocrine function was also assessed through estrogen, progesterone, FSH, LH, and anti-Mullerian hormone (AMH) levels. A return of menstruation with an increase in estrogen (>200 picomoles per liter [pmol/l]) and a decrease in FSH (<25 international units perlite [IU/I] post-transplant) and LH (<15 IU/I post-transplant) defined the return of hormonal ovarian function. For data analysis, for this study to explore heterogeneity, X^2 was used, and significance was $p < 0.05$, where I^2 was used to quantify heterogeneity (Khattak et al., 2022).

For reproductive outcomes following ovarian tissue transplantation, pregnancy, live birth rates, and miscarriage rates were analyzed. For pregnancy analysis, there were 18 studies (547

women) that were included in the meta-analysis. In 184 women, there was at least one pregnancy reported. However, some achieved more than one pregnancy, thus the overall number of pregnancies reported was 290. For frozen transplants, the pregnancy rate was 37% (95% CI: 32-43%). For fresh transplants, it was 52% (95% CI: 28-96%). For live birth rates, there were 17 studies (539 women) included in the meta-analysis. There was at least one live birth reported in 134 women, however, some women achieved more than one birth, thus a total of 166 live births were originally reported. There were case reports documenting 34 later live births, totaling 189 live births. The live birth rate was 28% (95% CI: 24-34%) for frozen transplants and 45% (95% CI: 23-86%) for fresh transplants. For miscarriages, fifteen studies were analyzed that documented miscarriage rates. The miscarriage rate was 37% (95% CI: 30-46%) for frozen transplants and 33% (95% CI: 13-89%) for fresh transplants. The average age for women who had miscarriages at the time of their cryopreservation was 27.8 years (SD: 5.8) (Khattak et al., 2022).

To evaluate endocrine function following ovarian tissue transplantation, estrogen levels, FSH levels, LH levels, AMH levels, and return of menstruation were analyzed. For estrogen levels, eight studies documented pre-transplant levels (104 women) and post-transplant levels (105 women). The average pre-transplant estrogen level was 101.6 pol/l (95% CO: 47.9-155.3), and the average post-transplant estrogen level was 522.4 pmol/l (95% CI: 315.4-729; MD: 228.24; 95% CI: 180.5-276). In 117 women there was >200 pmol/l estrogen levels post-transplantation, with an average time of 19.5 weeks (IQR: 14-24 weeks; range: 5-208 weeks) to reach >200 pmol/l. For FSH, 11 studies documented FSH levels pre-transplantation (136 women) and post-transplantation (132 women). The average pre-transplant FSH level was 68.4 IU/I (95% CI: 52.8-84), and the average post-transplant level was 14.1 IU/I (95% CI: 10.9-17.3;

MD: 61.8; 95% CI: 57-66.6) with substantial heterogeneity, $I^2 = 79\%$ ($p = 0.0001$). The average time frame for FSH levels to decrease to below 25 IU/I was 19 weeks (IQR: 15-26 weeks; range 0.4-208 weeks), which occurred in 135 women (72%). For LH, six studies reported pre-transplantation LH levels (52 women) and post-transplantation LH levels (54 women). The average pre-transplant LH level was 41.5 IU/I (95% CI 32.5-50.5), and post-transplant was 19 IU/I (95% CI: 5.8-32.2; MD: 23.4; 95% CI: 15.6-31.1), heterogeneity $I^2 = 0\%$ ($p = 0.64$). A decrease of LH levels to below 15 IU/I was reported in 46 out of 69 women (67%), with an average time frame of 19.5 weeks (IQR:14-27 weeks; range: 8-156 weeks) to achieve that decrease. For AMH, only one study reported pre-transplantation and post-transplantation AMH levels. In women who had AMH levels of less than 1 ng/ml pre-transplant, 19 pregnancies were reported out of 71 patients. This gives an average pregnancy rate of 27%. Return of menstruation was documented in 273 out of 735 patients, and 196 out of these 273 (72%) reported that they resumed menses. The average time to resume menses was 18 weeks (IQR: 14-22 weeks; range: 3-48 weeks). Graft function duration was reported in 181 women (19 studies) and the average function duration was 2.5 years (IQR: 1.4-3.4 years) (Khattak et al., 2022).

Out of the 735 women in this study review, the patient's age at the time of ovarian tissue retrieval for cryopreservation was only reported in 319 of them. Of the 319 women, 283 were 35 years of age or younger at the time of tissue retrieval, and it was found that pregnancy rates were higher in women whose tissue was retrieved at 35 years or younger. The results of this were statistically significant (Odds Ratio: 0.35; 95% CI: 0.13-0.92; $z = 2.13$; $p = 0.03$, $I^2 = 0\%$). The use of gonadotoxic cancer treatment therapy before tissue cryopreservation was only reported in 122 out of 735 women, and of these, only 56 women (46%) received anti-cancer treatment prior to tissue retrieval. In these 56 women, there were 35 pregnancies and 24 live births. The average

age of women who successfully achieved a live birth and received gonadotoxic treatment prior to tissue retrieval was 29 years (SD: 6) at the time of cryopreservation. Pregnancy rates were noted to increase in women who had cryopreservation at less than 35 years of age. This study's IPD meta-analysis shows a live birth rate from frozen-thawed ovarian transplants to be 28% and 45% from fresh ovarian transplants (95% CI: 23-86%). However, the sample size of the fresh transplant group was small, so it is difficult to determine a significant difference (Khattak et al., 2022).

This study suggests that it is possible to restore ovarian reproductive and endocrine function using fresh or frozen-thawed ovarian transplantation and should be offered as a routine fertility preservation method. Strengths of this study include that it is the first systematic review that included results from both fresh and frozen ovarian tissue transplantation, as well as donor tissue, using IPD meta-analysis. Another strength is that various characteristics that may affect reproductive outcomes, such as age at cryopreservation and the time of the return of hormone levels to premenopausal levels, were considered and analyzed. Chemotherapy before cryopreservation was also considered and analyzed. Limitations of this study include the small number of participants in each study, and the varying time of when hormonal function was assessed, either on the day of transplant or prior to tissue removal. This causes difficulty in adequately assessing the premenopausal status before the transplant. Another limitation is the difficulty of assessing residual hormonal function in women who still have one ovary. Another limitation is many studies do not demonstrate graft longevity to demonstrate the hormonal lifespan of the ovarian tissue. It was also not possible to predict the length of time it would take to conceive naturally in this study, as not all pregnancies were achieved naturally. Another limitation is the clinical heterogeneity of the studies included in the meta-analysis. This study

demonstrated that ovarian tissue cryopreservation and transplantation have promising results in reproductive and endocrine preservation. Therefore, this procedure should be offered to all women who wish to preserve fertility (Khattak et al., 2022).

Poirot et al. (2019) performed a retrospective study including patients 15 years and younger who had gone through ovarian tissue cryopreservation (OTC) before gonadotoxic treatment at a single medical center. In this study, 418 patients aged 15 years and younger went through OTC at this medical center from April 1998 to December 2018. From November 2009 to July 2013, a combination of OTC with freezing of isolated oocytes was also offered to patients. The indications for OTC included any treatment plans that involved high-dose chemotherapy, pelvic or abdominal radiation, ovariectomy, or autologous or allogeneic hematologic stem cell transplant. For tissue retrieval, laparoscopy or mini-laparotomy was performed. In most cases, the entire ovary was removed. The process of retrieval and cryopreservation includes removal of the ovary, isolation of the cortex from the medulla, dividing the cortex into 3 x 5 x 2 mm fragments, placement of fragments into a cryotube, a thirty-minute balancing phase, then placement into the freezer. Immature and mature oocytes were retrieved by aspiration of antral follicles and then placed into the culture medium until freezing (Poirot et al., 2019).

In this study, 313 patients (74.8%) had malignant disease and 105 (25.2%) had benign diseases that required highly gonadotoxic treatments. Of these patients, the average age was 6.9 years (range 0.3-15). The youngest patient in this study to go through OTC was 3.5 months old. The most diagnosed illness in the younger population, with an average age of 3.5 years, was neuroblastoma. The most diagnosed in the older age group, with an average age of 13.5 years, was lymphoma. In children with malignancies, 97 (23.2%) had hematological malignancies. The

most common benign disease requiring gonadotoxic treatment in this study was hemoglobinopathies (68.9%) (Poirot et al., 2019).

Between November 2009 and July 2013, a combination of OTC with freezing of isolated oocytes was offered to patients. This option of having isolated oocytes may increase future fertility restoration for patients. During this time, oocyte isolation for cryopreservation was attempted in 124 patients, and in 50 cases (40.3%) oocytes were able to be obtained from the tissue that was cryopreserved. Three patients returned to request the use of their cryopreserved ovarian tissue, and all three underwent ovarian tissue transplantation. These three patients were all pre-pubertal at the time of OTC. One of the patients underwent transplantation for restoration of endocrine function and spontaneous puberty induction. This patient was 10 years old when undergoing OTC. After three subcutaneous ovarian tissue grafts, this patient was able to achieve endocrine function and spontaneous puberty induction. The two other patients requested the use of their cryopreserved tissue to restore fertility. One patient was 12 years old at the time of OTC. In April 2018, she received an ovarian cortex transplant, however, there has been no return of ovarian function. Thus, a second ovarian transplant is scheduled for this patient. The third patient was 11.2 years old at the time of OTC and had sickle cell disease. She had an ovarian transplant performed in February 2019, with no achievement of pregnancy thus far (Poirot et al., 2019).

Of the 418 patients in this study, 84 had died at the time of follow-up (20.1%). When considering only living patients from this study who are currently 18 years or older, the utilization rate of cryopreserved tissue is 2.2% (3/149). This is lower than expected. No successful pregnancies have been achieved in this study population. OTC is the only option offered for pre-pubertal girls to preserve fertility before highly gonadotoxic treatments. It is

necessary to wait a few more years for more results from this study, especially in the younger patients (<9 years old) at the time of OTC (Poirot et al., 2019).

Safety of Cryopreservation of Ovarian Tissue for Infertility

Leflon et al. (2022) performed a retrospective observational study to determine the gynecological and reproductive health outcomes in women who have undergone ovarian tissue cryopreservation (OTC) before gonadotoxic chemotherapy or radiotherapy. The study was performed from May 2019 to February 2021. It included 87 women, all over the age of 18 who had undergone ovarian tissue cryopreservation from September 2004 to May 2018, prior to gonadotoxic treatments. The mean age was 29.5 (range 18-37 years). The main indications for gonadotoxic treatments for these women were hematological pathologies (43%), including lymphoma 32% (n = 36), leukemia 5% (n = 6), and non-malignant hematological diseases 6% (n = 7). The next most common indications were breast cancer 30% (n = 34), gastrointestinal cancers 7% (n = 8), sarcoma 4% (n = 5), gynecological cancers 4% (n = 4), and one larynx malignancy (Leflon et al., 2022).

A total of 14 women (12%) had died at the time of data collection. Approximately 74% of women completed the follow-up questionnaires which were analyzed more than eighteen months following OTC. The data from the questionnaires revealed that more than 70% of women who planned to become pregnant after cancer treatment succeeded. The data revealed a natural pregnancy rate of about 53%. Eight percent of women underwent ovarian tissue transplantation and six became pregnant and delivered at least once. Eleven women (9.7%) had asked for OTT; however, this was refused for two of them due to the presence of malignant cells on post-thawed fragments. The average age of the nine patients (8.0%) that did undergo OTT after OTC was 26.2 at the time of OTC and 32.5 at the time of OTT (Leflon et al., 2022).

For the OTT, a laparoscopic two-step ovarian tissue auto-transplantation was performed at orthotopic sites in the pelvic cavity. After OTT, 3 women were unsuccessful in restoring ovarian function and required oocyte donation. It took an average of 5.0 ± 1.4 months (range 3.0–7.0) after OTT to restore menstrual function. The average time to achieve pregnancy was 10.3 ± 7.7 months (range 6.0–26.0). Most pregnancies in this study were achieved without OTT despite OTC, but there was a good success rate of pregnancies following OTT. The return rate for OTT in this study was low (8.0%), however, almost 50% of women continued their ovarian tissue storage regardless of if they had pregnancy plans at the time of the follow-up, or if they were able to achieve a natural pregnancy. Many patients viewed their stored ovarian tissue as a "backup" plan (Leflon et al., 2022).

None of the women who underwent OTT had a cancer recurrence. There are several notable strengths of this study. It examines fertility preservation experiences and the gynecologic and reproductive well-being of 87 women who underwent OTC. Another strength is the almost 75% participation rate in follow-up and questionaries, including patients responding to every question on the survey. Another strength is the focus on not only women who underwent OTT, but also on all the women who underwent OTC. This study does have limitations, including the sample size being limited to women who benefited from this follow-up after OTC. Therefore, there are no surveys from women who did not respond to the follow-up questionnaire and some women were also lost to follow-up, though these numbers are low. Another limitation of this study is that the survey relies on the women's memory of their OTC experiences. OTC is an adequate option for fertility preservation, especially prior to gonadotoxic treatments. It can be performed rapidly during the management of the disease and has a low risk of complication. The study revealed that there was a high satisfaction rate in women who underwent OTC. However,

the usage rate of cryopreserved tissue remains quite low, even though OTT has a low surgical risk and low risk of cancer reoccurrence with a successful birth rate. This suggests that additional follow-up studies should be performed on women who underwent OTC (Leflon et al., 2022).

Dolmans et al. (2021) performed a case study review from five European centers' collective experience of transplanting ovarian tissue in 285 women. This review aimed to analyze the indications for and the results of ovarian tissue cryopreservation (OTC) and ovarian tissue transplantation (OTT) in these five centers. It also studied the risk of possible reintroduction of malignant cells through the reimplantation of ovarian tissue from cancer patients. Oocyte cryopreservation provides the highest yield of subsequent pregnancies for women seeking fertility for personal reasons, women with benign diseases, or women undergoing gonadotoxic treatment for cancer that can postpone treatment. Ovarian tissue cryopreservation (OTC) followed by ovarian tissue transplantation (OTT), on the other hand, is specifically indicated for women and adolescents who cannot postpone their cancer treatment. The focus of this study is on OTT outcomes, reproductive outcomes, surgical techniques, the impact of chemotherapy before OTC, endocrine resumption, the risk of cancer relapse, and the lasting function of transplanted tissue (Dolmans et al., 2021).

The selection criteria for this review required a case series with more than 20 subjects undergoing OTT within five European centers. These were further broken down into various cohorts based on location, including the Danish cohort (Andersen's team, 62 patients), Spanish cohort (Diaz's team, 53 patients), French cohort (Poirot's team, 53 patients), Belgian cohort (Donnez and Dolmans' team, 29 patients), and the FertiPROTEKT network which included Germany, Switzerland, and Austria (88 patients). The total number of patients in this case series

review was 285. The mean age of patients in this case review was 29.3 +- 6.2 years (range = 9-44) at the time of OTC and 34.6 +- 5.5 years at the time of the first OTT. 81.2% of women in this series were in premature ovarian insufficiency (POI), and the remaining 18.8% had irregular menses, demonstrating evidence of infertility, had failed in vitro fertilization (IVF), and needed to boost ovarian reserve by OTT. In this series, fifty-nine patients underwent a second OTT, and 7 patients underwent a third. Out of the patients who had their tissue reimplanted, 88.7% had a type of cancer, and 11.3% had a non-cancerous condition. 37.2% of patients with malignant diseases had hematologic cancers, including Hodgkin's lymphoma (24.6%), non-Hodgkin's lymphoma (11.2%), and leukemia (1.4%). The next most common type of malignancy was breast cancer, accounting for 33.3% of cases. Other malignant diseases patients had in this series were digestive tract cancers, cervical cancer, ovarian cancer, Ewing sarcoma, and melanoma. The types of malignancies were different at the five medical centers. In three of them (the Danish cohort, the Spanish cohort, and the FertiPROTEKT network), breast cancer was the most common. Hodgkin's lymphoma was the most common in the other two centers (Belgian cohort and French cohort). Among non-cancerous conditions, the most common were hemoglobinopathies (3.1%), autoimmune diseases (3.1%), and aplastic anemia (1.7%). Fertility preservation is particularly challenging and often the most indicated in breast cancers and hematologic cancers (leukemia, Hodgkin's lymphoma, and non-Hodgkin's lymphoma) due to gonadotoxic treatments causing premature ovarian insufficiency (POI) (Dolmans et al., 2021).

During OTT, ovarian tissue can be transplanted to two types of places in the body, inside the pelvic cavity (orthotopic) or outside of it (heterotopic). Inside the pelvic cavity, the tissue can be transplanted back to the ovarian medulla or a specifically made peritoneal pocket. Outside of the pelvic cavity, ovarian tissue can be transplanted to the abdominal wall muscle or forearm.

Orthotopic reimplantation, performed by either laparoscopy or mini-laparotomy, is the most effective option for restoring fertility and resuming endocrine function. When performing OTT, only a small part of the cryopreserved tissue is encouraged to be re-implanted. In this review, 277 (97.5%) underwent orthotopic transplantation, with 62.7% of these grafted to a specifically made peritoneal window, 16.7% grafted to the medulla of decorticated ovaries, and 20.4% grafted to both the peritoneal window and decorticated ovarian medulla. Five patients underwent heterotopic transplantation via a subcutaneous route to either the forearm or abdominal wall, and 3 patients underwent both methods of transplantation: orthotopic and heterotopic. When analyzing the number of infants born per surgical technique, 30.5% of infants were born via the orthotopic transplantation to the ovarian medulla method, 34.8% of infants were born via orthotopic transplantation to a peritoneal window method, and 34% of infants were born via the combination of both a peritoneal window and decorticated ovarian medulla method. This analysis indicates that the different transplant sites of the orthotopic method all have similar reproductive efficacy. There were no pregnancies achieved via the heterotopic grafting methods, indicating that this method has less reproductive efficacy (Dolmans et al., 2021).

Of the 285 women who underwent transplantation of frozen-thawed ovarian tissue, 26% successfully became pregnant and delivered one or two infants, for a total of 95 infants. There were no significant differences in live birth rates between women who experienced persistent irregular menstruation during OTT (30.6% - 15 out of 49) and women who had amenorrhea prior to OTT (25.4% - 54 out of 212). Comparing natural conception after OTC (40%) with IVF treatment after OTC (36%) showed comparable conception rates, although slightly higher in women who had natural, spontaneous conception. Also, the miscarriage rates were higher in women who underwent IVF (18%) than in women who had natural conception (10%). The

women who did successfully achieve pregnancy were significantly younger at the time of OTC than the women who did not achieve pregnancy, with an average age of 26.9 versus 29.8 years. In women who underwent IVF after OTC, only 50% were able to undergo an embryo transfer, despite numerous attempts at ovarian stimulation. Many of those who were able to undergo a transfer did achieve conception (72%) and live birth (42%), although there was a high miscarriage rate (37%). This may be because after excising and freezing one ovary, only 50% of the ovarian reserve is effectively preserved, and after transplantation in a now postmenopausal woman, only one-third of the tissue (16-17% of the ovarian reserve) is transplanted. Furthermore, the combination of freezing and transplantation may result in only a 30-50% survival rate of follicles, which represents only about 5-8% of the overall ovarian reserve at the time of OTC. Thus, in cases of IVF after OTC, reproductive outcomes may be improved by transplanting more tissue to increase the ovarian reserve, especially in older patients. This study found that women who underwent IVF after OTC and achieved childbirth were younger at the time of OTC than those who did not successfully achieve pregnancy. It is important to recognize that patient age at the time of OTC will affect reproductive outcomes. The results of IVF after OTC are promising and reveal clear fertility potential, but it should be emphasized that the highest conception and live birth rates were achieved in patients who conceived spontaneously, suggesting that IVF should not be initiated immediately when there is a chance of spontaneous conception after OTC and OTT (Dolmans et al., 2021).

Specific considerations of OTT and OTC need to be had if pelvic radiation is part of a patient's treatment plan. In this study of 285 women, 36 women (12.6%) received pelvic radiation before OTT, for a variety of malignancies, including anal cancer (9), colorectal cancer (8), cervical cancer (6), Hodgkin's lymphoma (6), non-Hodgkin's lymphoma (2), leukemia (1),

sickle cell disease (1), vulvar cancer (1), Ewing sarcoma (1), and uterine cancer (1). Out of these 36 women, 9 pregnancies and 7 live births (19%) were achieved. The live birth rates were dependent on the amount of pelvic radiation received. In cases with high doses of pelvic radiation, such as anal and cervical cancer, 0 pregnancies were achieved. In cases with lower doses of pelvic radiation, such as TBI, 50% of women achieved pregnancy and live births. Radiation of the uterus and poor vascularization of the transplant due to pelvic tissue fibrosis are the main reasons for poor outcomes of live birth rates following high doses of pelvic radiation. It is possible to transplant ovarian tissue following pelvic radiation if the radiation dose is relatively low (Dolmans et al., 2021).

It is typically recommended that OTC be performed prior to the start of chemotherapy in patients over the age of 15. Chemotherapy can lead to diminished ovarian reserve, vascular damage leading to ovarian fibrosis, and affect follicles and oocytes. However, this is not always feasible, thus this study examined the outcomes of OTT in patients who underwent chemotherapy prior to OTC. In this study of 285 women, chemotherapy data was only available for 271 patients. Of these 271 women, fifty (18.5%) had chemotherapy before OTC. The rate of ovarian function recovery was not significantly different in patients who had undergone chemotherapy before OTC than in those who had not (90% vs. 85.3%; $p = 0.49$). However, when examining pregnancy rates, a significant difference was noted. Patients who underwent chemotherapy prior to OTC and received ovarian cortex grafts had a pregnancy rate of 50%, while those who did not undergo chemotherapy prior to OTC had a pregnancy rate of 28.1% ($p = 0.004$). However, it is important to consider the median age in women who underwent chemotherapy prior to OTC had a median age of 26 years (range 12-35), while those who did not have a median age of 31 years (range 9-44), thus were significantly different ($P<0.0001$).

Univariate and multivariate analyses of pregnancy outcomes suggest that the results of OTT are not affected by chemotherapy prior to OTC and are no longer a contraindication. Thus, a further study was performed matching patient age and disease and comparing those who underwent chemotherapy prior to OTC to those who did not. When matching up age and disease, it was found that there was no difference in the rate of ovarian function recovery between the two groups. The rate of pregnancy and live birth rates were higher in the group that had received chemo prior to OTC. However, in the group that received chemotherapy prior to OTC, women who had received chemotherapy involving alkylating agents had lower pregnancy rates than women treated without alkylating agents (28.6% vs. 68.4%; $p = .016$), which confirms the harm of alkylating agents on OTT results. This study suggested that fertility preservation guidelines should be updated to state that OTC and OTT results are not affected by prior chemotherapy and that OTC should be the approach chosen when chemotherapy has already begun (Dolmans et al., 2021).

Of the women who underwent OTT, 204 had been diagnosed with premature ovarian insufficiency (POI). Of these women, 181 (88.7%) had endocrine function resumption based on the return of menses. In this study, 59 of 285 (20.7%) underwent a second OTT, and 7 (2.4%) underwent a third procedure due to poor ovarian function recovery. The average time between OTT and return of menses was 4.5 months. A subset of 45 individuals who had undergone OTT more than 5 years ago were assessed to determine the longevity of ovarian function. These findings revealed a five-year ovarian graft survival rate of 55%. The findings also revealed an association between the duration of ovarian function and the age at the time of OTC, regardless of prior chemotherapy exposure prior to OTC. The major determinant of OTT success and graft

longevity appears to be the number of primordial follicles present in the ovarian fragments at the time of OTT, and how many survive the procedure (Dolmans et al., 2021).

Reimplantation of ovarian tissue from cancer patients causing the reintroduction of malignant cells has been a safety concern for many years. Studies from Dolman's group and Anderson's group have shown that hematologic malignancies, especially leukemia, showed the highest risk of spread of malignant cells from OTT. Ovarian tissue from a patient in complete remission is less risk than from a patient in active disease. Thus, it is more beneficial to undergo OTC while patients are in complete remission, especially because reproductive performance nor graft follicle density has been shown to be significantly affected by chemotherapy before OTC. In this study, 2 patients with acute myeloid leukemia underwent OTC following chemotherapy and underwent OTT with no relapse observed. Twelve patients (4.2%) of 285 patients had a relapse following OTT, with 7 having breast cancer, and the rest having cervical cancer, Ewing sarcoma, non-Hodgkin's lymphoma, anal carcinoma, and a CNS tumor. However, all relapses were due to the primary malignancy and not due to the graft, as the relapses were all near the primary location of the cancer (Dolmans et al., 2021).

This is the largest published series analyzing patient data from five different European centers. In this series, the efficacy and safety of OTC followed by OTT have been demonstrated. Almost all women had recovery of endocrine function following OTT. Around one in four women gave birth to a healthy child following OTC and OTT. However, IVF rates were not very high compared to natural conception rates. This study demonstrated that high doses of radiation to the pelvis significantly reduce the successful pregnancy rate. However, this study also revealed that chemotherapy without the use of alkylating agents before OTC does not alter the

pregnancy success rate, indicating that women undergoing chemotherapy may still benefit from OTC and OTT. This study revealed that reproductive outcomes are improved if ovarian tissue is harvested while patients are in complete remission, especially because chemotherapy showed no effect on graft follicle density or reproductive outcomes. In this study, there have been no cases of malignancy relapse that is directly related to the ovarian graft itself, indicating the risk of the reintroduction of malignant cells via OTT to be low. This study should help with the promotion of OTC and OTT for patients who would benefit from it (Dolmans et al., 2021).

Comparison of Oocyte Cryopreservation and Cryopreservation of Ovarian Tissue for Infertility

Dhonnabháin et al. (2022) performed a systematic review to compare obstetric outcomes in patients undergoing cryopreservation of oocytes, embryos, or ovarian cortical tissue before gonadotoxic therapy. Inclusion criteria included women at risk for infertility because of gonadotoxic medical treatment; completion of oocyte, embryo, or ovarian tissue cryopreservation (OTC) procedures; documented follow-up; and articles with original data. Clinical pregnancy rates (CPR), live birth rates (LBR), and miscarriage rates per transfer or transplant were the main outcomes studied. For oocyte and embryo cryopreservation, CPR, LBR, and miscarriage rates were determined by the total number of clinical pregnancies, live births, and miscarriages against the total number of transfer cycles. For ovarian tissue cryopreservation, these were determined by the same but compared to the total number of transplant surgeries. A p-value of $<.05$ was considered to indicate statistical significance (Dhonnabhain et al., 2022).

Thirty-nine studies which were either retrospective or prospective observational studies were analyzed. Within the 39 studies, there were 550 ovarian tissue transplants, 178 embryo transfers, and 102 oocyte transfers. The study found that CPRs were similar between all three options: oocytes (34.9%), embryos (49.0%), and ovarian tissue (43.8%) ($p = .09$). The LBRs also

had no significant differences among the three options: oocytes (25.8%), embryos (35.3%), and ovarian tissue (32.3%) ($p = .11$). The study showed no significant difference in miscarriage rates between oocyte and embryo groups or between oocyte and ovarian tissue cryopreservation groups, but a significant difference and improvement in miscarriage rates in ovarian tissue cryopreservation (7.5%) compared to embryo cryopreservation (16.9%) ($p = .01$). One limitation of this study is the lack of large randomized controlled trials. Another limitation is the low rates of women returning and going through the thawing and application processes. This may be due to women postponing pregnancy until 1-2 years after gonadotoxic treatment due to increased risk of preterm birth and lower conception rates when attempting sooner. Other reasons are not surviving their cancer treatment or having no desire for parenthood after treatment. It is difficult to compare ovarian tissue cryopreservation with oocyte and embryo strategies. When comparing oocyte and embryo strategies, the difference is whether fertilization occurs before (embryo) or after (oocyte) cryopreservation. However, with ovarian tissue cryopreservation, it is a different procedure and process. OTC does carry risks from repeated surgeries but does not carry the risks associated with ovarian stimulation and delaying gonadotoxic treatment like oocyte and embryo strategies. OTC also plays many roles in fertility preservation, such as resuming menses, acting as a hormone replacement tool, and can lead to multiple pregnancies from a singular ovarian tissue transplant. This is also difficult to compare to oocyte and embryo cryopreservation as they are limited to a single conception and live birth per embryo transfer. The results of this study are encouraging for women with a cancer diagnosis who are interested in fertility preservation prior to gonadotoxic therapy. All three strategies have similar chances of achieving pregnancy and live birth, and OTC may even have an advantage as it may allow for multiple pregnancies from a single tissue transplant and a lower miscarriage rate (Dhonnabhain et al., 2022).

Chung et al. (2021) performed a study comparing oocyte cryopreservation (OC) and ovarian tissue cryopreservation (OTC) using a cost-effectiveness model. This study was performed after the American Society for Reproductive Medicine declared in December 2019 that OTC is no longer experimental but rather an alternative fertility preservation option for women receiving gonadotoxic chemotherapy. The percentage of patients who achieved live birth was the primary outcome of success in this study. Incremental cost-effectiveness ratios (ICER) were used for this study. The target population included reproductive-aged women under the age of 40 who had no male partner and were recommended high-risk gonadotoxic therapy. The prospective observational cohort study consisted of 1824 women undergoing gonadotoxic treatment. These women all received the same fertility preservation counseling and had similar BMI, Anti-Mullerian Hormone (AMH), and parity. The average age for OC was 31.7 years and 28.2 years for OTC. This study's most common cancers were lymphoma and breast cancer. Out of this prospective cohort study, 4.8% of patients undergoing OC and 5.5% of patients undergoing OTC returned after an average follow-up of 5 years to utilize their chosen method of fertility preservation, either OC or OTC. After undergoing OC, 1.56% of patients achieved a live birth, and 1.0% of patients achieved a live birth after OTC, with a p-value of <0.05 considered statistically significant differences. Results showed that the estimated cost for oocyte cryopreservation was $16,588 and ovarian tissue cryopreservation was $10,032. Results showed that OC had better results than OTC but with a greater price tag. The ICER of OC was $1,163,954 per live birth. Limitations to this study include that the data was obtained from previous literature, and primarily from a single study. Although it was the largest controlled prospective cohort study, there is still a need for more diverse population data to truly study the cost-effectiveness of each method. Another limitation is that OC cost may be over-estimated and

OTC cost may be underestimated due to the use of financial charges for all oocyte cryopreservation and IVF. This was used this way because many insurances do not cover these services, so charge data is often used in infertility literature to analyze cost-effectiveness. Another limitation is that it did not consider the possibility of women spontaneously conceiving before thawing cryopreserved oocytes and ovarian cortical tissue. The results of this study showed that OC is more clinically successful but is much less cost-effective than OTC. Thus, OTC may be a reasonable cost-effective consideration for women pursuing fertility preservation prior to gonadotoxic chemotherapy (Chung et al., 2021).

Discussion

This literature review has examined and demonstrated that there is evidence that the utilization of ovarian tissue cryopreservation is a sufficient method of fertility preservation and should be offered as an option for women who wish to preserve fertility. Dolman et al. (2021) conducted a comprehensive review and suggests that both oocyte and ovarian tissue cryopreservation can be effective methods of fertility preservation. They highlight that the live birth rates are dependent on factors such as the amount of radiation received and chemotherapy exposure (Dolman et al., 2021). Druckenmiller et al. (2016) and Cacciottola et al. (2022) both support the effectiveness of oocyte cryopreservation as a method for post-pubertal fertility preservation in cancer patients. Furthermore, Cacciottola et al. (2022) highlights the importance of considering the patient's age and pubertal status when choosing between oocyte and OTC. Cacciottola et al. (2022) also supports ovarian tissue cryopreservation as an effective method of fertility preservation for prepubertal girls at high risk of iatrogenic POI or women who are unable to postpone or have already begun gonadotoxic treatment. OTC allows for multiple spontaneous pregnancies, an advantage to oocyte cryopreservation. OTC also has the advantage

of resuming ovarian function, thus replacing hormone replacement therapy, and alleviating postmenopausal symptoms (Cacciottola et al., 2022).

Dhonnabhain et al. (2022) presents a systematic review, indicating that oocyte cryopreservation, embryo cryopreservation, and ovarian tissue cryopreservation have similar clinical pregnancy and live birth rates. OTC also shows a lower miscarriage rate compared to embryo cryopreservation. They highlight that OTC does not carry the risks associated with ovarian stimulation and delaying gonadotoxic treatment, which can be a concern in oocyte cryopreservation (Dhonnabhain et al., 2022). Chung et al. (2021) compares oocyte cryopreservation and OTC, highlighting that oocyte cryopreservation may have better clinical success but is less cost-effective than OTC (Chung et al., 2021).

Poirot et al. (2019) and Leflon et al. (2022) present data indicating a low utilization rate of cryopreserved ovarian tissue (2.2% and 8.0%). Many women in Leflon et al. (2022) study (50%) continued their ovarian tissue storage, however, viewing their stored ovarian tissue as a "backup plan". Leflon et al. (2022) reported a higher natural pregnancy rate and high satisfaction among patients who underwent OTC. Thus, with the low usage rate, even with the low surgical risk, low risk of cancer reoccurrence, and successful birth rate, additional follow-up studies should be performed on women who underwent OTC, especially at a young age (Leflon et al., 2022; Poirot et al., 2019). A similar limitation in many of these studies is the limited participation of women returning for follow-up or utilizing their cryopreserved ovarian tissue, and additional follow-up studies may be beneficial. The studies analyzed in this literature review provide support for ovarian tissue cryopreservation as a safe and efficacious method of fertility preservation.

Conclusion

In summary, the studies analyzed in this literature review demonstrate evidence that ovarian tissue cryopreservation is an adequate method of fertility preservation, with success rates comparable to or slightly less than oocyte cryopreservation, depending on the study. OTC is also a more cost-effective fertility preservation method than oocyte cryopreservation. OTC is especially useful when utilized among cancer patients as it can be performed rapidly during the management of the disease as it is not affected by chemotherapy, thus does not delay treatment for ovarian stimulation like oocyte cryopreservation. OTC is also the only fertility preservation option for prepubertal girls at high risk of iatrogenic premature ovarian insufficiency. OTC also plays many roles in fertility preservation that oocyte cryopreservation does not offer, such as resuming menses, acting as a hormone replacement tool, and can lead to multiple pregnancies from a singular ovarian tissue transplant.

Applicability to Clinical Practice

The information provided in this literature review will be useful for medical providers who are needing to counsel women desiring fertility preservation on the various methods to choose from. Knowing the clinical outcomes, cost-effectiveness, and overall efficacy can significantly aid a patient in choosing the most appropriate fertility method for their individualized care. The information is also useful for women who find themselves in the position of needing or desiring fertility preservation and want to research and compare the available methods for themselves.

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