Ovarian Cryopreservation: An Overview of Current Evidence around the Globe

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Abstract

Advancements in cancer as well as, autoimmunological or hematological conditions treatment have significantly increased the survival rate as well as, the Quality of Life (QoL) for these patients. In addition, Assisted Reproductive Techniques (ART) offers a variety of therapeutic approaches that aim towards fertility preservation and hormonal restoration after treatment. Ovarian Tissue Cryopreservation (OTC) and Ovarian Tissue Transplantation (OTT) have been proposed as an efficient alternative to reserve fertility and hormonal function in cases of prepubertal young girls and women needed to be treated immediately with radiation and/or chemotherapy. In this narrative review, current scientific evidence is summarized in order to explore OTC/OTT. In addition to the qualitative presentation of our findings, we provide cumulative evidence from other published studies to demonstrate the clinical potential and limitations of these procedures.

Keywords: Ovarian tissue cryopreservation, ovarian tissue transplantation, in vitro maturation of ovarian follicles, cancer survivors, fertility preservation, hormonal restoration

Introduction

Over the last decades, the survival rate of patients after cancer treatment has been significantly increased [1]. Consequently, cancer survivors' Quality of Life (QoL) has been improved significantly due to advancements in cancer treatment, as well as to the knowledge gained towards fertility preservation [2]. Except of malignant diseases, both hematological and autoimmune diseases often require radiation, chemotherapy or even bone marrow transplantation [3]. In order to proceed with this treatment combination one must keep in mind that these women are on the verge of Premature Ovarian Failure (POF) induction, which varies extremely from one individual to another [4]. Radiation greater than 2Gy, alkylating agents such as, cyclophosphamide, and total body irradiation prior to bone marrow transplantation can induce POF at approximately all patients [5].

There are different kind of treatments for cancer during childhood such as surgery, radiotherapy and chemotherapy. These treatments have facilitated cure, although gonadal function is usually jeopardised, a fact that has a grave impact on reproductive health [6]. After radiotherapy and/or chemotherapy, especially young women tend to concern more and more about their future fertility [7]. Fertility-related information on the impact of a planned treatment and information on fertility preservation should be provided in oncological and hematological care programs, and in cases of gonadotoxic treatments for treatment of benign diseases that may also impair fertility. Both postponing childbearing and increased risk for malignancies with
age, necessitate immediate and effective management approaches for these women [1].

The first human pregnancy from a frozen-thawed embryo was reported in 1983 and the first live birth in 1984 [8,9]. Ovarian tissue cryopreservation (OTC) emerged as an experimental option to freeze ovarian tissue and currently remains the only pre-treatment fertility preservation option for pre-pubertal children and for patients who cannot delay life-saving therapy [10]. Based on cumulative evidence, the most recent guideline published by the American Society for Reproductive Medicine (ASRM) states that OTC is an “acceptable fertility preservation technique and is no longer considered experimental” [11].

In this narrative review of the literature, we focus on the ovarian cryopreservation and transplantation by providing international data of previous studies on the subject. The aim is to include all scientific evidence and experience gained all these years from different research teams around the globe.

**Material and Methods**

For this narrative review, four major search engines were explored (MEDLINE, Google Scholar, PubMed and EMBASE) up to April 2021, using the following keywords and possible combinations of them: Ovarian tissue cryopreservation; ovarian tissue transplantation; in vitro maturation of ovarian follicles; cancer survivors; fertility preservation. All articles included were only in English language. A number of papers was retrieved, and special attention was given to twelve original studies [1,3,12-21] addressing ovarian cryopreservation and its effectiveness and safety to provide an overview of experience from different fertility units around the world.

All published papers on OTC and OTT in humans were included in this project. Cases involving fresh ovarian tissue transplantation and data from pregnancies and live births from oocyte donations were excluded [14,22]. It was also decided to exclude OTT in patients in whom premature ovarian failure (POF) was diagnosed at the time of OTC [23]. Is worth mentioning that the rationale behind the exclusion of these studies is that a large proportion of these women included, did not present with ovarian follicles at the time of OTC explaining the reason of ovarian function failure after transplantation. Thus, these women represent a different group from those with known ovarian function at the time of OTC.

**Patient’s profile, indications and limitations**

Evidence shows that OTC and OTT are not only reserved for young patients at prepubertal age but also for young adult women who require immediate chemotherapeutic or radiotherapeutic management and to whom possible treatment interventions might jeopardise their ovarian reserve. For these groups of patients OTC remains the only modality available under these age-related and timely manner limitations. More specifically, transplantation of frozen-thawed ovarian tissue has been reported in a total of 430 transplantation procedures involving 394 female patients (Table 1, Figure 1). Forty-seven patients underwent an additional OTT, while seven patients received three subsequent OTT procedures. Data regarding the average time from OTC to the first, second, and third transplantation procedure was available for 156, 38, and 3 patients, respectively (Table 2). The mean age at OTC was 28.9 years (range 9–47 years), while the first transplantation is being performed at a mean age of 33.5 years (range 13–47 years). At the time of the second OTT, the mean age was 33.9 years (range 25–42 years), and at the third 33.7 years (range 26–39 years), (Table 3). Currently, the age threshold to perform OTC is set at 35 years of age, whereas guidelines regarding children or young teenagers younger of less than 17 years of age have not been set yet.

With regards to indications of the reviewed procedures we were able to identify 394 women who had OTC based on published studies. The diagnosis was available in 349, malignant disease in 296 (84,8%) cases and non-malignant disease in 43 (14,2%), figure 1. In 296 of the malignant cases with OTT, the diagnosis at OTC was specified. Among them, breast cancer and haematological malignancies were the main indications for ovarian tissue cryopreservation. 132 out of 296 (44.6%) cases involved haematological cancers, 33 cases out of 296 were diagnosed with gynaecological cancers (11.1%), with breast cancer in 66 cases (22.3%), sarcoma was diagnosed in 21 cases (7.1%), colorectal malignancies occurred in 13 patients (4.4%) and other malignancies had been diagnosed in 31 cases (10.5%). Malignancies remain a major indication for OTC in these patients regardless of their desire for future conception, as it seems that restoring their hormonal status after disease remission is equally desirable for a great number of women [3].

**Technique**

Lessons learnt from animal studies on ovarian tissue cryopreservation (OTC) include experiments in sheep, where autografts of cryopreserved ovarian cortex were sutured on to the ovarian pedicle, with subsequent restoration of
**Table 1:** Data regarding ovarian transplantation from around the globe.

| Country               | No of transplantation | No of women |
|-----------------------|-----------------------|-------------|
| Argentina             | 2                     | 1           |
| Australia             | 23                    | 21          |
| Belgium               | 55                    | 52          |
| China                 | 10                    | 10          |
| Denmark               | 113                   | 89          |
| Finland               | 4                     | 4           |
| France                | 4                     | 4           |
| Germany, Austria      | 76                    | 75          |
| Israel                | 55                    | 52          |
| Italy                 | 4                     | 4           |
| Norway                | 2                     | 2           |
| Netherlands           | 5                     | 5           |
| Poland                | 1                     | 1           |
| Portugal              | 1                     | 1           |
| Russia                | 1                     | 1           |
| South Korea           | 1                     | 1           |
| Spain                 | 24                    | 24          |
| Sweden                | 12                    | 10          |
| Turkey                | 1                     | 1           |
| UK                    | 2                     | 2           |
| USA                   | 34                    | 33          |
| **Total**             | **430**               | **394**     |

**Figure 1:** Distribution of transplantations around the globe.
ovarian function and natural conception [24]. In humans, OTC and ovarian tissue transplantation (OTT) present a significant alternative for young or older women mainly for fertility preservation [16]. Over time, there have been different protocols between fertility institutions, performing both OTC and OTT.

In general, each technique developed aims to remove one ovary or a section of ovarian tissue. The ovarian tissue obtained includes 4 to 5 cortical strips of 1 cm in length, 4–5 mm in width and approximately 0.3-2mm thickness which is adequately prepared before vitrification and finally stored in liquid nitrogen [5,16]. Thawing and transplantation may occur years, or even decades later, when the patient has recovered from the disease [3,4]. Most studies on women undergoing OTC and OTT fail to describe the exact technique used with regards to ovarian graft volume and dimensions.

The dimension of graft plays an important role during cryopreservation. During the freezing process, thinner cortical strips allow better penetration of cryoprotective agents, with smaller possibility of ice crystal formation and injury [25]. In the same time, on transplantation, when dimensions are smaller, the penetration depth for new blood vessels is less and as result ischemia period is smaller [26,27]. On the other hand, grafting smaller fragments can result in excessive follicle activation, resulting in loss of dominant follicles [28]. The data regarding viability of grafted tissue remains inadequate to detect in detail the factors that improve its maintenance. Potentially it is depended on a number of factors including patient’s age, follicular density, amount of tissue grafted, and speed of revascularization that occurs within the transplanted tissue, among others [1]. The duration of grafts potentially plays an important role, although further studies need to conduct to elucidate such hypothesis. At the moment there is no clear guidance about the number of follicles available at the time of transplantation or about the best technique that could allow woman to have the best fertility results [17]. A standardisation of the OTC and OTT techniques needs to be addressed.

A second or even third OTT can be proposed to patients in case of failure of the first attempt, when enough ovarian tissue has been stored. Patient selection will decrease the complications of repeated surgical procedures. Shapira et al. have shown that when patients managed to initially conceive after OTT, may benefit from an additional OTT, if the same patients experienced decreased especially when pregnancy is desired [18].

Ovarian tissue may be grafted either orthotopically and/or heterotopically. When graft is placed in the remaining ovary and/or in peritoneal pockets in the pelvis, the transplantation is considered orthotopic, whereas ovarian grafts in the sub peritoneal space of the abdomen and in the subcutaneous space of the forearm or abdomen are considered heterotopic. The location of the graft site was available in 394, 47 and 7 of the cases during the first, second, and third OTT performed, respectively (Table 2). Based on live birth (LBs), the vast majority of patients with an orthotopic or combined heterotopic/orthotopic graft were successful, while data indicates a single case of successful conception after heterotopic transplantation [29]. Future pregnancy was not desirable in 34 women of the total sample under research. With regards to documentation, it appears that in this subgroup of women, twenty-two women had tissue transplanted orthotopically, eight heterotopically, and in four remaining cases, the information was not available. Although during OTC vitrification was used solely by all institutions, information regarding vascularization or medical treatment used during and after OTT were not available. Future research needs to address the optimal technique that should be performed to increase the success rates of OTT.

**Table 2: Transplantation site of ovarian tissue based on published evidence.**

| Transplantation site                  | First OTT | Second OTT | Third OTT |
|--------------------------------------|-----------|------------|-----------|
| Heterotopic                          | 17 (4.5%) | 6 (12.8%)  | 0 (0%)    |
| Orthotopic                           | 286 (72.5%) | 25 (53.2%) | 7 (100%)  |
| Both heterotopic and orthotopic      | 12 (3%)   | 5 (10.6%)  | 0 (0%)    |
| Unavailable                          | 79 (20%)  | 11(23.4%)  | 0 (0%)    |
| Total                                | 394       | 47         | 7         |

Current evidence suggests no specific time frame between OTC and OTT to achieve optimal results. OTT of 10-year-old or more ovarian tissue grafts have been documented but the literature lacks consistent evidence. If successful OTT could restore ovarian functions within days or months and the viability of the graft is estimated...
approximately 5 years, although studies from single centres suggest ovarian graft function viability of almost a decade. Unfortunately, most studies address conception or live births rather than hormonal status restoration, thus our knowledge so far remains inadequate.

Outcomes of OTT might vary as not all women desire future pregnancies but there have been several women wishing to proceed with this intervention to restore their hormone levels. To avoid any misconceptions, scientific evidence regarding both hormonal function and reproductive outcomes are discussed in detail below.

**Hormonal status after ovarian tissue transplantation**

After revising original published studies, 394 cases of at least one attempt of OTT were identified. The status of endocrine function was available in 300 of the cases, reporting 20 (15%) cases of non-restored function and 280 (85%) hormonally functional cases. Follicular growth or recurrence of menstruation was used to identify the hormonal restoration. The interval from an initial OTT to follicular function was on average 4.0 months.

Regarding cases reporting absence of endocrine restoration, only four cases has been reported to have additional transplantations so far [18,19,30]. In the remaining 47 women who underwent an additional OTT, as well as 7 more women who underwent a third transplantation, endocrine function was reported in 37 cases, while information from the remaining 10 cases was absent. The mean time interval from second and third OTT to follicular function was 3.9 months (SD 1.4 months, range 2–6, n = 13) and 4.5 months (SD 2.1 months, range 3–6, n = 2), respectively.

**Pregnancies and live births (LBs)**

Pregnancies and LBs are sufficient tools to evaluate reproductive outcomes in cases of OTC/OTT, as suggested by literature. More specifically, in a total of 394 women, 236 wished to restore fertility, 34 proceeded with OTT for restoration of the ovarian endocrine function only, 8 patients underwent hysterectomy and for 124 patients, records were absent. This section focuses on pregnancies and LBs as a measure of success in cases of OTC/OTT, while data regarding the method of conception in these cases is discussed later.

Scientific evidence estimates a total of 143 pregnancies which were noted in 103 patients, counting in both biochemical and clinical pregnancies. This resulted in 98 live births by 76 women, and a total of 94 children born [3,5,14,17,20,29-36]. The mean age of patients achieving live birth (LB) or ongoing pregnancy (OGP) (26.4 years (SD 6.3), range 9–38 years) was significantly lower at OTC (P value = 0.0019) compared to patients who failed to conceive (29.6 years (SD 5.4), range 14–39 years) [19].

In addition, data with regards to four legal abortions following OTT has also been published. One occurred due to a genetic diagnosis showing that the embryo carried the same BRCA1 mutation as the mother [37], two further terminations took place because of social reasons [33,38] and a third because of breast cancer relapse [39]. Shapira et al. [18] reports that there was no pregnancy at women with gynaecological cancer. Silber et al. published a case of OTC/OTT which resulted in a pregnancy through fresh ovarian tissue (rather than frozen thawed) obtained by twin siblings [40].

Historically, the very first live birth (LB) achieved spontaneously after OTC and OTT was reported in Belgium in 2004 [41]. In 2005, a team from Israel announced the first case of OTC-OTT achieving LB through ovarian stimulation, although the patient had a history of premature ovarian failure [42]. Same year, one more birth achieved after OTT in the USA [40]. Moreover, in June 2017, Donnez reported 130 LBs after OTC and OTT [43]. In a meta-analysis, 64% of cases had restored their ovarian function, with an ongoing pregnancy or live birth rate of 28.4% [21]. Similar data can be extracted from other teams, for instance, 32.6% by the Spanish group in 2018 [44], 31% by the Danish group in 2015 [17] and 25% by the German FertiPROTEKT network in 2016 [12].

**Method of conception**

One-hundred-three pregnancies and subsequently ninety-eight LBs, which account for 46% and 51% respectively of the total sample, were achieved spontaneously. The couples who achieved spontaneous conceptions that eventually led to live births, 84% of the women were menopausal before OTT. The conceptions achieved by IVF, 75% of the women were menopausal before OTT. Three cases out of the total 98 live births were related to patients who had previously undergone bilateral oophorectomy. In these three cases, two women managed to conceive by IVF after completing OTT in an orthotopic peritoneal pocket [45,46] and the other one had OTT in a heterotopic abdominal pocket [47].

Non-human animal model studies have demonstrated minimal response to ovarian simulation [48] while few only
cases resulted in abnormal genetically oocytes [49,50]. That is why the ovarian stimulation and oocyte retrieval can be considered just after chemotherapy, and even for 6 more months [51]. When chemotherapy delay is not an option, OTC remains the main fertility preservation technique. Some centres though provide OTC only to patient without previous chemotherapy [15]. Shapira reports that women who had chemotherapy were more likely to conceive and deliver at least once (58% vs. 42% and 46% vs. 39%, respectively) despite being of similar age, although the difference was not statistically significant [18], which was also confirmed in a 31-case-study by Poirot et al. [52].

**Recurrence of malignancy**

Although disease remission is a major criterion for undergoing OTT, several studies have reported cases of recurrence of malignancy. Although evidence is not consistent, clinicians should advise patients about this potentially detrimental outcome of OTT and discuss in detail recurrence patterns of specific tumour types.

Kim and colleagues [13] reported recurrence of cervical cancer in a case of OTC/OTT. A single case of recurrence soon after ovarian transplantation has been also noted. Additionally, an incidence of local recurrence of disease in a breast cancer survivor was also reported by Dittrich and colleagues in 2015 [53]. None of these cases reflect recurrence caused by transplantation alone, since the time interval between recurrence and transplantation is minimal.

Stern et al. [29] published a case report describing a case of OCT in a patient treated for a granulosa cell tumour. This patient underwent OTT and finally achieved a twin pregnancy. At the time of caesarean section, an additional suspicious tumour was also noted, involving the diaphragm and a peritoneal deposit located at the left pelvic brim. The tissue was removed, and a granulosa cell tumour was confirmed by histology. Interestingly, analysis of previously grafted tissue revealed no evidence of malignancy. Subsequently, the patient was treated and recovered from the tumour.

In addition [39] reported recurrence of the disease in six patients. Two women formerly diagnosed with breast cancer experienced local relapses. More specifically, one of them was pregnant and had an abortion in the eighth gestational week just prior to receiving chemotherapy [39]. A former Ewing's sarcoma patient had her tissue transplanted at the age of 13 to induce puberty, 4 years after she underwent OTC. Furthermore, one patient experienced a relapse occurring in the hemithorax 4.5 years after transplantation and died subsequently [17]. The remaining frozen tissue was analysed for the possible presence of malignant cells and was found negative.

Moreover, in a case of a patient suffering from Ewing's sarcoma, was diagnosed with Breast rest cancer after transplantation [34], which was considered unrelated to transplantation. Fajau-Prevot et al. reported in 2017 [54] another case of Ewing sarcoma. After OTT, patient conceived spontaneously and delivered a healthy child at term by caesarean section. During the procedure, a 5 × 3 cm benign mucinous cystadenoma was identified and removed from the grafted ovarian tissue. Tissue biopsy was negative for Ewing sarcoma cells, both histologically and by using molecular markers [54].

**Future research**

The aim of this review is to present the worldwide experience on OTC and OTT, as published in peer-reviewed papers. This study identified a total of 430 transplantations performed in a total of 394 patients. The collective data presented in this review offers reassurance on the safety of the procedure. Transplantation to women with a known former malignant or benign diagnosis has been performed 296 times, while recurrence of cancer attributed to OTT remains minimal. Table 4 summarizes current scientific evidence regarding OTC/OTT.

Future research should address hormonal function restoration rather than fertility preservation alone [55,56]. Standardizing the technique of both OTC and OTT is needed thus future studies should focus on describing

| Procedures' time intervals. |  |
|-----------------------------|---|
| **No of patients*** | **Mean age (years)** | **Range (years)** | **Years from cryopreservation to transplantation** |
| Cryopreservation | 353 | 28.9 | 9-47 |
| First OTT | 353 | 33.5 | 13-47 | 4.4 (0.3-13.9) |
| Second OTT | 57 | 33.9 | 25-42 | 6.1 (2.0-14.5) |
| Third OTT | 7 | 33.7 | 26-39 | 10.2 (6.6-12.5) |

*available data for 353 out of 394 patients
optimal evidence-based interventions. Novel agents for revascularization of the transplanted ovarian tissue should also be assessed to establish their role and impact on reproductive outcome.

More studies have compared the results between vitrification and slow freezing after the transplantation of cryopreserved and thawed ovarian tissues [57-60]. Herraiz et al. [57], showed that vitrification preserved a larger population of primordial follicles than slow freezing after transplantation. On the contrary, slow freezing grafts have demonstrated more vascularized area and cell proliferation as compared to vitrification grafts, although no statistical significance was observed [57]. On the other hand, Amorim et al. [58] showed that two vitrification protocols exhibited better preservation of preantral follicles than the conventional slow freezing method after xenotransplantation into ovariectomized mice. At the same time, Lee at all [60] showed that slow freezing for ovarian tissue cryopreservation is superior to vitrification in terms of follicular resilience and by extent survival. Currently, there is no consensus in literature with regards to technique superiority. Further prospective studies need to be conducted in order to elucidate which methods offers optimal results.

Finally, although recurrence of malignancy is reported as minimal, protective strategies and protocols should estimate the risks of OTC/OTT. Safe alternatives such as, in vitro oocyte maturation from the obtained ovarian tissue should be developed and offered to eliminate the risk of transmission of the disease.

**Conclusions**

This study presents the role of OTC/OTT as an effective alternative for fertility preservation, resulting in conception and live birth rates of 60, 5% and 41.5%, respectively. With regards to techniques used when handling harvested tissue, neither tissue dimensions nor surgical approach to transplantation appears to affect reproductive outcomes, however these should be further evaluated in larger studies in the future. It is expected that in the coming years, implementation of OTC/OTT will expand globally, especially now that this technique, traditionally believed as experimental, is implemented in the everyday practice and remains the only fertility preservation approach for specific groups of patients. Undoubtedly, accumulating experience over the years has led OTC-OTT to be recognized as a highly effective alternative for fertility preservation.
Conflict of interest

All authors declare no conflict of interest

References

1. 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. Doi: https://doi.org/10.1016/j.bpobgyn.2020.06.011

2. Anderson RA, Wallace WHB, Telfer EE. Ovarian tissue cryopreservation for fertility preservation: Clinical and research perspectives. Hum Reprod Open. 2017; 2017(1). Doi: https://doi.org/10.1093/hropen/hox001

3. Donnez J, Dolmans MM, Pellicer A, Diaz-Garcia C, Sanchez Serrano M, Schmidt KT. Restoration of ovarian activity and pregnancy after transplantation of cryopreserved ovarian tissue: A review of 60 cases of reimplantation. Fertil Steril. 2013; 99(6):1503-1513. Doi: https://doi.org/10.1016/j.fertnstert.2013.03.030

4. Donnez J, Dolmans MM. Fertility preservation in women. Nat Rev Endocrinol. 2013; 9(12):735-749. Doi: https://doi.org/10.1038/nrendo.2013.205

5. Donnez J, Dolmans MM, Diaz C, Pellicer A. Ovarian cortex transplantation: Time to move on from experimental studies to open clinical application. Fertil Steril. 2015; 104(5):1097-1098. Doi: https://doi.org/10.1016/j.fertnstert.2015.08.005

6. Schover LR, Rybicki LA, Martin BA, Bringelsen KA. Having children after cancer. A pilot survey of survivors' attitudes and experiences. Cancer. 1999; 86(4):697-709. Doi: https://doi.org/10.1002/(sici)1097-0142(19990815)86:4<697::aid-cncr20>3.0.co;2-j

7. Peate M, Meiser B, Friedlander M, Zorbas H, Rovelli S, Sansom-Daly U. 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):1670-1677. Doi: https://doi.org/10.1200/JCO.2010.31.2462

8. Trounson A, Mohr L. Human pregnancy following cryopreservation, thawing and transfer of an eight-cell embryo. Nature. 1983;305(5936):707-709. Doi: https://doi.org/10.1038/305707a0

9. Zeilmaker GH, Alberda AT, van Gent I, Rijkmans CMPM, Drogendijk AC. Two pregnancies following transfer of intact frozen-thawed embryos. Fertil Steril. 1984; 42(2):293-296. Doi: https://doi.org/10.1016/S0015-0282(16)48029-5

10. Wallace WHB, Anderson RA, Irvine DS. Fertility preservation for young patients with cancer: Who is at risk and what can be offered? Lancet Oncol. 2005;6(4):209-218. Doi: https://doi.org/10.1016/S1470-2045(05)70092-9

11. Ethics Committee of the American Society for Reproductive Medicine. Fertility preservation and reproduction in patients facing gonadotoxic therapies: An Ethics Committee opinion. Fertil Steril. 2018; 110(3):380-386. Doi: https://doi.org/10.1016/j.fertnstert.2018.05.034

12. Van der Ven H, Liebenthron J, Beckmann M, Toth B, Korell M, Krüssel J. 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):2031-2041. Doi: https://doi.org/10.1093/humrep/dew165

13. 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):489-493. Doi: https://doi.org/10.1007/s10815-012-9757-3

14. 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. Fertil Steril. 2015; 103(2):462-468. Doi: https://doi.org/10.1016/j.fertnstert.2014.10.045

15. Anderson RA, Mitchell RT, Kelsey TW, Spears N, Telfer EE, Wallace WHB. Cancer treatment and gonadal function: Experimental and established strategies for fertility preservation in children and young adults. Lancet Diabetes Endocrinol. 2015; 3(7):556-567. Doi: https://doi.org/10.1016/S2213-8587(15)00039-X

16. Pages A. Cryostorage of reproductive tissues in the in vitro fertilization laboratory: A committee opinion. Fertil Steril. 2020; 114(3):486-491. Doi: https://doi.org/10.1016/j.fertnstert.2020.06.019

17. Jensen AK, Kristensen SG, Macklon KT, Jeppesen JV, Fedder J, Ernst E, et al. Outcomes of transplantations of cryopreserved ovarian tissue to 41 women in Denmark. Hum Reprod. 2015;30(12):2838-2845. Doi: https://doi.org/10.1093/humrep/dev230

18. Shapira M, Dolmans MM, Silber S, Meirow D. Evaluation of ovarian tissue transplantation: Results from three clinical centers. Fertil Steril. 2020; 114(2):388-397. Doi: https://doi.org/10.1016/j.fertnstert.2020.03.037

19. 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. *Cur Opin Gyn Obs*. 2018; 4(1): 435-445 (2021)

20. Imbert R, Moffa F, Tsepelidis S, Simon P, Delbaere A, Devreker F, et al. Safety and usefulness of cryopreservation of ovarian tissue to preserve fertility: A 12-year retrospective analysis. *Hum Reprod*. 2014; 29(9):1931-1940. Doi: https://doi.org/10.1093/humrep/deu158

21. Pacheco F, Oktay K. Current success and efficiency of autologous ovarian transplantation: A meta-analysis. *Reprod Sci*. 2017; 24(8):1111-1120. Doi: https://doi.org/10.1177/1933719117702251

22. Schmidt KT, Rosendahl M, Ernst E, Loft A, Andersen AN, Dueholm M, et al. Autotransplantation of cryopreserved ovarian tissue in 12 women with chemotherapy-induced premature ovarian failure: The Danish experience. *Fertil Steril*. 2011; 95(2):695-701. Doi: https://doi.org/10.1016/j.fertnstert.2010.07.1080

23. Takae S, Suzuki N. Current state and future possibilities of ovarian tissue transplantation. *Reprod Med Biol*. 2019; 18(3):217-224. Doi: https://doi.org/10.1002/rmb2.12268

24. Baird DT, Webb R, Campbell BK, Harkness LM, Gosden RG. Long-term ovarian function in sheep after ovariectomy and transplantation of autografts stored at −196 C. *Endocrinology*. 1999; 140(1):462-471. Doi: https://doi.org/10.1210/endo.140.1.6453

25. Ferreira M, Bos-Mikich A, Frantz N, Rodrigues J, Brunetto A, Schwartzmann G. The effects of sample size on the outcome of ovarian tissue cryopreservation. *Reprod Domest Anim*. 2010; 45(1):99-102. Doi: https://doi.org/10.1111/j.1439-0531.2008.01261.x

26. Gavish Z, Peer G, Hadassa R, Yoram C, Meirow D. Follicle activation and “burn-out” contribute to post-transplantation follicle loss in ovarian tissue grafts: The effect of graft thickness. *Hum Reprod*. 2014; 29(5):989-996. Doi: https://doi.org/10.1093/humrep/deu015

27. Kagawa N, Silber S, Kuwayama M. Successful vitrification of bovine and human ovarian tissue. *Reprod Biomed Online*. 2009; 18(4):568-577. Doi: https://doi.org/10.1016/S1472-6483(10)60136-8

28. Gavish Z, Peer G, Hadassa R, Yoram C, Meirow D. Follicle activation and “burn-out” contribute to post-transplantation follicle loss in ovarian tissue grafts: The effect of graft thickness. *Hum Reprod*. 2014; 29(8):1828-1828. Doi: https://doi.org/10.1093/humrep/deu119

29. Shapira M, Ra’anani H, Barshack I, Amariglio N, Derech-Haim S, Marciano MN, et al. First delivery in a leukemia survivor after transplantation of cryopreserved ovarian tissue, evaluated for leukemia cells contamination. *Fertil Steril*. 2018; 109(1):48-53. Doi: https://doi.org/10.1016/j.fertnstert.2017.09.001

30. Rodríguez-Villalobos KA, Karlström P, Rezapour M, Castellanos E, Hreinson J, Rasmussen C, et al. Full-term newborn after repeated ovarian tissue transplants in a patient treated for Ewing sarcoma by sterilizing pelvic irradiation and chemotherapy. *Acta Obstet Gynecol Scand*. 2015; 94(3):324-328. Doi: https://doi.org/10.1111/aogs.12568

31. Hoekman EJ, Louwe LA, Rooijers M, Westerlaken LAJ, Klijn NF, Pilgram GSK, et al. Ovarian tissue cryopreservation: Low usage rates and high live‐birth rate after transplantation. *Acta Obstet Gynecol Scand*. 2020; 99(2):213-221. Doi: https://doi.org/10.1016/j.aogs.13735

32. Meirow D, Ra’anani H, Shapira M, Brenghausen M, Derech Chaim S, Aviel-Ronen S, et al. Transplantations of frozen-thawed ovarian tissue demonstrate high reproductive performance and the need to revise restrictive criteria. *Fertil Steril*. 2016; 106(2):467-474. Doi: https://doi.org/10.1016/j.fertnstert.2016.04.031

33. Sánchez-Serrano M, Crespo J, Mirabet V, Cobo AC, Escribá M-J, Pellicer A, et al. Twins born after transplantation of ovarian cortical tissue and oocyte vitrification. *Fertil Steril*. 2010; 93(1):268.e11-268.e13. Doi: https://doi.org/10.1016/j.fertnstert.2009.09.046

34. Milenkovic M, Brännström M, Diaz-Garcia C, Lundin K, Selleskog U, Söderlund B, et al. Spontaneous twin pregnancy with live births after cryopreservation and re-implantation of ovarian tissue. *Gynecol Surg*. 2017; 14(1):9. Doi: https://doi.org/10.1186/s10397-017-1012-6

35. Lambertini M, Ceppi M, Poggio F, Peccatori FA, Azim HA, Ugolini D, et al. Ovarian suppression using luteinizing hormone-releasing hormone agonists during chemotherapy to preserve ovarian function and fertility of breast cancer patients: A meta-analysis of randomized studies. *Ann Oncol*. 2015; 26(12):2408-2419. Doi: https://doi.org/10.1093/annonc/mdv374
444. Oocyte vitrification versus ovarian cortex transplantation in fertility preservation for female cancer patients. *Fertil Steril*. 2015; 96(6):1534-1542. Doi: https://doi.org/10.1016/j.fertnstert.2012.11.057

45. 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. *Fertil Steril*. 2015; 103(2):462-468. Doi: https://doi.org/10.1016/j.fertnstert.2014.10.045

46. Fajau-Prevot C, Le Gac YT, Chevreau C, Cohade C, Gatimel N, Leandri R, et al. Ovarian mucinous cystadenoma after ovarian graft. *Obstet Gynecol*. 2017; 129(6):1035-1036. Doi: https://doi.org/10.1097/AOG.00000000000001990

47. Greve T, Schmidt KT, Kristensen SG, Ernst E, Andersen CY. Evaluation of the ovarian reserve in women transplanted with frozen and thawed ovarian cortical tissue. *Fertil Steril*. 2012; 97(6):1394-1398.e1. Doi: https://doi.org/10.1016/j.fertnstert.2012.02.036

48. Dolmans MM, Marotta ML, Pirard C, Donnez J, Donnez O. Ovarian tissue cryopreservation followed by controlled ovarian stimulation and pick-up of mature oocytes does not impair the number or quality of retrieved oocytes. *J Ovarian Res*. 2014; 7(1):80. Doi: https://doi.org/10.1186/s13048-014-0080-8

49. Herraiz S, Novella-Maestre E, Rodríguez B, Díaz C, Sánchez-Serrano M, Pellicer A, et al. Improving ovarian tissue cryopreservation for oncologic
58. Amorim CA, Dolmans MM, David A, Jaeger J, Vanacker J, Camboni A, et al. Vitrification and xenografting of human ovarian tissue. *Fertil Steril.* 2012; 98(5):1291-1298.e2. Doi: https://doi.org/10.1016/j.fertnstert.2012.07.1109

59. Rahimi G, Isachenko V, Kreienberg R, Sauer H, Todorov P, Tawadros S. Re-vascularisation in human ovarian tissue after conventional freezing or vitrification and xenotransplantation. *Eur J Obstet Gynecol Reprod Biol.* 2010; 149(1):63-67. Doi: https://doi.org/10.1016/j.ejogrb.2009.11.015

60. Lee S, Ryu KJ, Kim B, Kang D, Kim YY, Kim T. Comparison between slow freezing and vitrification for human ovarian tissue cryopreservation and xenotransplantation. *Int J Mol Sci.* 2019; 20(13):3346. Doi: https://doi.org/10.3390/ijms20133346

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