Review Article

Current Opinion in Gynecology and Obstetrics Ovarian Tissue Cryopreservation from Contemporary Indications to Cutting Edge Research- a Review

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Abstract

Over the last three decades, survival rate from malignancies has steadily increased, turning major focus on quality of life in remission. Unfortunately, a major side effect of medical interventions including chemotherapy, radiotherapy, and/or surgery is premature ovarian failure and infertility for many patients. Ovarian tissue cryopreservation is an experimental technique used to preserve reproductive potential in patients who are pre-pubertal, must urgently undergo gonadotoxic treatment or who have other medical conditions requiring treatment that may undermine ovarian reserve and will lead to sub or infertility. We will review the indications, available techniques of tissue harvesting, and methods for freezing and auto transplanting cortical ovarian tissue. We will discuss outcomes, the potential risk of reintroducing malignancy and lastly, cutting-edge research models being developed in the field.

Keywords: Fertility preservation, Ovarian tissue cryopreservation, Indications, Outcomes, Reintroducing malignancy, Prepubertal girls, Experimental models

Introduction

Of all the patients at risk for premature ovarian failure, only a fraction will be referred to fertility preservation consultation, and even a smaller portion undergo a fertility preservation procedure (1,2). Among available techniques, embryo cryopreservation is most commonly used, followed by oocyte cryopreservation however, oocytes freezing has become increasingly prevalent across many demographic groups in recent years. Another method of fertility that is less commonly used is ovarian tissue cryopreservation. For pre-pubertal girls or women who require immediate chemotherapy, cryopreservation of oocytes and/or embryos is not an option. As an alternative, some patients cryopreserve ovarian tissue and undergo autotransplantation once in remission. Although this approach has proved successful, it is comprised of numerous elements, including surgical resection, processing, cryopreservation, thawing, auto-

transplantation, controlled ovarian hyperstimulation (COH) and in vitro fertilization (IVF), all occurring over the span of decades in some cases. Below, we will review these procedural elements and discuss factors that contribute to success.

Indications for Ovarian Tissue Cryopreservation

Ovarian tissue cryopreservation and auto-transplantation is still considered an experimental technique for fertility preservation, however there are calls for it to be re-categorized and applied in routine clinical practice (3). Banking ovarian cortical tissue enables patients who require immediate gonadotoxic treatment, have hormone sensitive malignancies, or otherwise cannot delay their regimen for the purpose of COH. For prepubertal girls, ovarian tissue cryopreservation is the only available option (4,5), and can be implemented with

a relatively simple surgery that results in banking of tissue rich with primordial follicles that can be re-incorporated into patients in adulthood.

Procurement of Ovarian Cortical Tissue

The quiescent ovarian reserve is comprised of primordial follicles that reside within the ovarian cortex. Upon freezing of ovarian cortex, most primordial follicles have a favorable survival rate thanks to their small size and low metabolic rate (6). Therefore, depending on the age and quality of tissue when procured, cortical tissues can contain hundreds of thousands of quiescent follicles. When possible, ovarian tissue should be obtained before a woman initiates treatment, however, for leukemic patients who may harbor cancer cells within the ovarian blood vessels, obtaining ovarian tissue after the first remission and before bone marrow transplant may decrease the risk of recurrence of malignancy upon autotransplantation (7,8). Ovarian tissue is extracted surgically via laparoscopy, mini-laparotomy, possibly at the time of ovarian transposition, or by laparotomy. It may even be performed at the time of excising primary pelvic and abdominal malignancies (3,9). Oophorectomy should be performed in patients undergoing pelvic irradiation or total body irradiation and in those receiving high doses of chemotherapy prone to undermine the ovarian reserve like alkylating agents. This procedure should also be performed in prepubertal girls because of the small size of their ovaries (10). On the contrary, in adults, 4–5 ovarian cortical biopsy samples of ~1 cm in length, 4–5 mm in width and 1.0–1.5 mm in depth are usually taken. It should be taken into consideration that one ovary should be left in situ, as a significant population of quiescent follicles may survive in spite of gonadotoxic treatment, preserving hormonal function and sub-fertility as well as providing a substrate for orthotopic autotransplantation later on (11). Following resection of a single ovary, it is transferred on ice as quickly as possible to the laboratory where it is processed to isolate cortical tissue from the medulla, cut into small slivers and cryopreserved (4,12). As the follicular reserve of the ovary is inversely proportional to age, many groups that routinely freeze ovarian tissue for fertility preservation have set the upper limit for undergoing this treatment at 35 years of age (13).

Methods of Freezing Ovarian Cortical Tissue

Effective preservation and recovery of reproductive potential of human ovaries requires tissue procurement, processing, freezing, thawing, and, following remission,

autologous transplantation. Two methods of ovarian tissue cryopreservation are currently in practice: slow freezing, the primary method (6), and vitrification (14) which is used by more and more groups worldwide. Slow freezing involves the exposure of tissue to cryoprotectant with subsequent slow cooling to approximately -140°C using a programmable freezer, and storage in liquid nitrogen at -196°C. In contrast to slow freezing, which requires 2 to 3 hours, vitrification is almost instantaneous - it involves exposure of the tissue to higher concentrations of cryoprotectants followed by ultra-rapid cooling (15). Owing to superior recovery efficiencies, vitrification has displaced conventional slow freezing as the primary approach to cryopreservation of gametes and embryos (16). Nevertheless, cortical tissue is comprised of diverse cell types, and although tissue is sliced into thin fragments, these dimensions remain large when compared to those of gametes and pre-implantation stage embryos. Post-thaw comparison of cortical tissue viability following slow freezing versus vitrification has revealed comparable preservation of the morphologic integrity, and although oocytes survival was similar between the two methods, granulosa cell survival and the integrity of the stroma were improved with vitrification (16). A recent meta-analysis published by Shi et al. found vitrification may be more effective than slow freezing, with less primordial follicular DNA strand breaks and better preservation of stromal cells (17). Nevertheless, very few live births have been reported following transplantation of tissue that was frozen by vitrification (18,19), as most of the live births in humans to date have been achieved after utilizing slow-freezing (20).

Transplantation of Cortical Ovarian Tissue

The most challenging aspect of restoring the reserve of oocytes within cryopreserved ovarian tissue is the avascular grafting of frozen-thawed cortical fragments. This can be achieved via transplantation into a pelvic (orthotopic) or extrapelvic (heterotopic) site. Orthotopic transplantation is done into the remaining ovary, with thawed ovarian cortex surgically fixed to the medulla after decortication of the ovary. In cases where no ovary remains, cortical fragments are placed in a peritoneal window (21). An advantage of orthotopic grafting is that it enables the possibility of natural conception, however the volume of cortical tissue that can be transplanted is relatively limited, and an invasive procedure is required. Given the nature of the approach, the success of the orthotopic transplantation is confounded by the possibility that pregnancy may have resulted from ovulation of oocytes native to their remaining ovary and

not the transplanted tissue. Although the majority of reported live-births have employed an orthotopic site, volume of transplanted tissue is not often quantified, and it is unclear how many patients have undergone the procedure without conceiving. Hence, efficiency of the procedure remains undetermined.

Heterotopic transplantation, into sites outside the remaining ovary, has been reported in the forearm, abdominal wall, and chest wall and has been associated with reports of the restoration of ovarian function, follicular development, and live birth of twins (22). A limitation of this approach is that pregnancy can only be achieved in conjunction with oocyte retrieval and IVF. A major advantage of heterotopic transplantation is the ability to choose a location that requires minimally invasive procedures during auto-graft and which is easily accessible for monitoring of follicular development and retrieval of oocytes (23-25). Proponents of orthotopic sites have argued that the pelvic cavity provides the optimal environment for follicular development compared with heterotopic sites, as temperature, pressure, paracrine factors and blood supply mimics the physiological setup (13). However, using intraperitoneal space as heterotopic site, Stern et al. have reported pregnancy (26) and a live birth of twins (22), thus providing definitive affirmation of heterotopic transplantation as a viable option.

Outcomes of Cortical Ovarian Tissue Autotransplantation

The first report of a live-born resulting from autotransplantation of human cortical ovarian tissue was at 2004 by the group of Donnez et al. (27), and as of today, there are more than a hundred babies born by employing this approach. Many early studies have defined the timing for restoration of ovarian activity occurring between 3.5 months and 6.5 months after auto graft (20), a finding that is consistent with follicle growth from primordial to antral stages. The mean duration of ovarian function after transplantation is ~4-5 years if follicular density is well-preserved, (20) but up to 10 years of persistence has been reported (28). Unlike the native ovary, the utility of hormonal markers for evaluating ovarian reserve and function is limited. Janse et al. reported that AMH and inhibin B levels may not be associated with the duration of ovarian graft function or probability to achieve a pregnancy (29). Among the different factors influencing ovarian graft longevity, follicular density, which is age-dependent, and cryopreservation prior to gonadotoxic treatment are of paramount importance (30). IVF in women with orthotopically grafted frozen-thawed ovarian

tissue involves a higher risk of empty follicles, abnormal or immature oocytes, and low embryo transfer rates (31). As of now no malformations or any health concerns have been reported in children born after applying this method.

A potential risk of reintroducing malignancy

Although for some patients cryopreserving ovarian tissue might be the only practical option, autotransplantation can carry a substantial risk of reintroducing malignancy. Sonmezer and Oktay (32) have classified malignant disease into three categories, representing low, intermediate, and high risk of ovarian involvement. Later, this classification was modified by Dolmans et al. who re-categorized Ewing sarcoma and Non-Hodgkin lymphoma (NHL) from low to moderate risk (33). The patients that might utilize ovarian tissue cryopreservation vary in age, and throughout life the incidence of cancer varies as well. During childhood, ages 1-14, leukemia accounts for 29% of all cancers, with cancers of brain and nervous system accounting for another 26% and the third most common category being lymphomas and reticuloendothelial neoplasms, at 11%. In adolescence, ages 15-19, the distribution of malignancies differs, with the most common cancer type being lymphoma (21%), brain and nervous system accounting for 17%, and leukemia 14% (34). In adults, there is a shift in incidence again, with breast cancer being the most common malignancy in women, at about 30% of all cancer cases. Indeed, one of every 228 women will suffer from breast cancer before the age of 40 (35). After cancers of the lung, colon, uterus, thyroid and skin, is NHL (4%), followed by leukemia (3%) (34). Interestingly, Dolmans et al. (36) reported that hematologic malignancies are the most common indication for ovarian tissue cryopreservation, at 39.9%, breast cancer being second, at 21.7%.

The risk of reintroducing cancer upon autotransplantation is a matter of concern, and in order to increase safety, exclusion of even a small number of cancer cells within the ovarian tissue is indicated (37). To minimize the risk of transplantation of ovarian tissue contaminated with malignant cells, methods to detect minimal residual disease (MRD) have been developed. Meirow et al. recommend preoperative workup including imaging of the pelvis (sonography, CT scan and/or CT/PET) to exclude ovarian pathology and possible pelvic metastasis. If ovarian tissue is harvested, a meticulous inspection of malignancy in pelvic organs and the abdominal cavity should be performed. Fresh

ovarian tissue cortex and the medulla discarded during preparation for cryopreservation should be evaluated for the presence of malignant cells using multiple histological sections selected randomly. Upon cryopreservation of the tissue, representative sections should be allocated for future investigation. When ovarian tissue is thawed, investigative studies to detect MRD should take place. These included histological evaluation (H&E staining), immuno histochemistry (IHC) staining for anti-CD30 and anti-Ki67. To detect molecular markers in the ovarian cortex PCR, classical RT-PCR and/or quantitative real-time PCR analysis should also be performed. Patients' bone marrow samples or involved lymph nodes stored prior to treatment should simultaneously be evaluated as positive controls (37). Below we discuss some of the more common indications for ovarian tissue cryopreservation among cancer patients and relative risk assessment.

Leukemia: Ovarian metastases have been found in up to 30% of leukemia patients at autopsy (38,39), and also have been detected in frozen-thawed ovarian tissue by RT-qPCR (37,40,41). Although viable malignant cells have been evidenced in ovarian tissue retrieved from women/girls in the active phase of acute leukemia, it has not been demonstrated in patients who received chemotherapy before retrieval of ovarian tissue (7). Recently Shapira et al. reported that harvesting ovarian tissue during complete remission, combined with intense tissue evaluation before transplantation, allowed a safe, successful transplantation in an acute myeloid leukemia survivor (8). It seems that each subtype of leukemia might act differently and relative risk is dependent on how the first course of chemotherapy was performed, the length of time the patient was in remission, and the number of viable malignant cells present that could cause a relapse (33).

Non-Hodgkin Lymphoma: An incidence of 9.8% of ovarian involvement in NHL was found when autopsies were performed (39) and malignant cells were detected by histologic evaluation in 6% of patients (33). While the risk is low, it nevertheless exists and thus warrants caution and further investigation.

Hodgkin Lymphoma (HL): Examination of ovarian cortex from Hodgkin lymphoma (HL) patients using light microscopy (42), IHC (43), and applying markers for detection of MRD (37) did not reveal malignant cells. Moreover, ovarian tissue transplanted into SCID mice did not result in recurrence of lymphoma (42). Among 16 patients with Hodgkin lymphoma who underwent

autotransplantation, none experienced disease recurrence after their ovarian transplantation (30, 44-46). Nevertheless, there is one report on ovarian involvement of a stage III HL within the ovarian cortex (47).

Breast Cancer: No histological evidence of malignant cells has been found in ovarian cortical biopsies from women with breast cancer stage I-IIIa when assessing the tissue using histology and IHC (35,48).

Colorectal Cancer: Metastases from colorectal cancer to the ovaries have been observed in autopsy studies, with the frequency varying from 16.7% to 31.1%, depending on the patient's age (39).

Ewing Sarcoma: No sign of ovarian metastases have been observed in histologic studies of ovarian tissue from patients with Ewing sarcoma (49,50). Nevertheless, when biopsies from multiple patients were analyzed by RT-PCR, one case was suspected of ovarian involvement (51).

In a systematic review of autotransplantation of ovarian tissue from 289 patients with leukemia, lymphoma, Ewing sarcoma, colorectal, gastric, breast, endometrial, and cervical cancer, metastases were common in patients who had leukemia, whereas metastatic disease was less common in most other cancers and was not seen in patients with lymphoma or breast cancer (52). Given the uncertainties regarding transmission of disease, ovarian tissue transplantation is not recommended for patients with blood-borne malignancies, with malignancies that metastasize to the ovary, or with an inherent predisposition to ovarian cancer. However, ovarian tissue transplantation in women with cancers that have a negligible risk of ovarian involvement may be considered for future auto-transplantation (53), although of course, the full scope of potential risk should be discussed with patients before re-implantation. Regardless of the risk posed to patients by auto-transplantation, the brisk pace of research in this field may provide alternative options for isolation of gametes directly from frozen ovarian tissue, or the development of in vitro systems that will minimize the risk of introducing malignant cells to the patient. Towards this end, alternative methods such as in vitro maturation or isolated follicle transplantation (artificial ovary) should be further investigated as future options for patients with leukemia (54).

Ovarian Tissue Cryopreservation Hurdles and Experimental Approaches

The frequency of positive outcomes utilizing ovarian

tissue cryopreservation and re-implantation is increasing (28,55-57), yet graft longevity and follicular output following auto-transplantation remain relatively low (58). Graft ischemia in a 5 to 7-day window post-transplant remains a significant obstacle to maintaining tissue viability, as there is a significant loss of oocytes before graft revascularization. Numerous attempts to improve the viability of ovarian cortical grafts using anti-oxidants (59,60), pro-angiogenic cytokines (61-64), or mechanical manipulations (65), with only moderate improvement (66). As graft resident endothelium is essential for recovery of tissue following xenotransplantation (67), another approach that may abbreviate the ischemic interval is supplementation of grafts with an exogenous source of endothelial cells (ECs) during transplantation. Previous work from our group describes a vascular cell-based strategy that improves graft viability and augments survival of follicular reserve (68). We have also exploited this system to mitigate another detrimental influence of follicular reserve in grafted ovarian tissue -global premature activation. This phenomenon has been described in large animal models (69,70) as well in human isolated follicles, cortical pieces (70), and in the context of transplantation of fresh and thawed human cortical tissue (71). Importantly, administration of AMH mitigates this burnout phenomenon in the context of chemotherapy (72), and utilizing ECs over expressing AMH in the vicinity of the xenograft, we have attenuated activation and growth rate in human xenografts (68).

Modern therapeutic approaches to fertility preservation have cast off established dogmas. For instance, the notion that there is no option for fertility preservation in prepubertal girls who are incapable of responding to hyperstimulation has been definitively refuted, and women who have frozen ovarian tissue during childhood are beginning to return for auto-transplantation (73). Another notion that has been proven false is the idea that patients who have undergone a gonadotoxic treatment will not benefit from ovarian tissue cryopreservation. Patients who have had a relapse of disease can benefit from cryopreservation of tissue prior to initiating second-line treatment, and live birth after cryopreserving ovarian tissue post-chemotherapy regimen was reported in 2005 (74). Even the conservative approach of not transplanting cryopreserved ovarian tissue of a leukemia survivor was recently challenged by Shapira et al (8). Indeed, although cryopreservation of ovarian tissue is still considered experimental by the American Society for Reproductive Medicine (ASRM), the field is progressing at a rapid pace as new ways of salvaging fertility are investigated, and

some prominent advocates have called for integration of ovarian tissue cryopreservation into routine clinical practice.

In summary, ovarian tissue cryopreservation is a promising option for retaining female reproductive options. The major challenges facing this approach relate to avoiding the reintroduction of malignant cells, protection of graft-resident follicles from ischemia and burnout resulting in prolonging the lifespan of ovarian transplant, and improvement of in vitro approaches for activation, survival, growth and maturation of follicles. Importantly, given the brisk pace of advance and lack of clinical standards for application of established approaches, an international registry is absolutely required for better evaluation and improvement of this procedure. According to strong evidence supporting the efficacy of ovarian tissue cryopreservation, patients who are about to commence a gonadotoxic treatment during or before reproductive years should be referred for consultation with an REI immediately upon diagnosis.

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