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Cancer and ovarian tissue cryopreservation

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Abstract: Although ovarian tissue cryopreservation is still accepted as an experimental procedure, it has recently become an increasingly resorted technique in highly specialized in vitro fertilization (IVF) clinics. Its indications include many childhood cancers, breast cancer, some autoimmune diseases, hematopoietic stem cell transplantation, and pelvic radiation therapy due to solid tumors. The chemotherapeutics and radiation therapy may diminish ovarian reserve and cause premature ovarian failure. It currently seems as the only option for fertility preservation in children where ovulation induction is ethically not acceptable, and for those when cytotoxic therapy is urgent. There are 2 main methods for transplantation of frozen-thawed ovarian tissues: orthotopic and heterotopic transplantation. Together with its advantages, this method also has some risks, such as reimplantation of the primary tumor, malignant transformation, and loss of follicles. Therefore, a thorough counseling should be provided before going forward with the procedure. Although it is still an experimental method, there are 7 reports of live births after ovarian tissue cryopreservation. In this review, technical details of ovarian tissue cryopreservation, related indications, and methods of ovarian tissue transplantation are discussed and an algorithmic approach to fertility preservation is presented.

Key words: Ovarian cryopreservation, transplantation, fertility preservation

Kanser ve overyan kriyoprezervasyon

Özet: Fertilite koruma yöntemlerinden biri olan overyan doku kriyoprezervasyonu, halen deneysel bir yöntem olarak kabul edilmekle birlikte, son yıllarda gelişmiş tüp bebek merkezlerinde uygulanım alanı bulmaktadır. Endikasyonları arasında pek çok çocukluk çağı kanseri, meme kanseri, bazı otoimmün hastalıklar, hematopoetik kök hücre nakli ve solid tümörler nedeniyle verilen pelvik radyoterapi yer almaktadır. Kemoterapötikler ve radyoterapi overyan rezervi azaltırlar ve prematür over yetmezliğine sebep olabilirler. Özellikle ovulasyon indüksiyonunun etik olarak uygulanamayacağı çocuk hastalarda veya ovülasyon indüksiyon protokolleri için yeterli zamanı olmayan hastalarda overyan doku kriyoprezervasyonu geçerli tek seçenek gibi gözükmektedir. Dondurulmuş-çözülmüş overyan dokuların transplantasyonu için iki yöntem vardır: ortotopik ve heterotopik transplantasyon. Bu metodun avantajları yanında primer kanserin reimplantasyonu, maligniteye dönüşüm ve folikül kaybı gibi riskleri de vardır. Bu nedenle işleme başlanmadan önce eksiksiz bir danışmanlık sunulmalıdır. Hala deneysel bir yöntem olmakla birlikte, overyan doku kriyoprezervasyonu sonrası 7 canlı doğum bildirilmiştir. Bu derlemede overyan doku kriyoprezervasyonu ile ilgili teknik ayrıntılar, endikasyonlor ve transplantasyon yöntemleri tartışılarak, fertilite korunması ile ilgili en son algoritmalar değerlendirilecektir.

Anahtar sözcükler: Overyan kriyopreservazyon, transplantasyon, fertilite korunması

Introduction

Although there was an increasing trend in cancer incidences between 2001 and 2005, the estimated number of women to be diagnosed with a new cancer in 2008 was 692.000 in the United States (1). Based on the rates from 2003 to 2005, 40.35% of men and women born today will be diagnosed with cancer of all sites at some time during their lifetime. So, the lifetime risk of having a cancer is

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approximately 50% (1). According to the data from the Ministry of Health of Turkiye, there were 13.437 new cancer cases in women in 2000 and the incidence was 40.16 per 100.000 (2). However, the cure rates are increasing up to 90% especially for certain types of cancers as a result of the current treatment modalities, such as radiation therapy, chemotherapy, and bone marrow transplantation (3). Thus, more women survive cancer every year but also encounter with the long term devastating side effects of the cancer treatment modalities. These effects may significantly impair the quality of life of the cancer survivors.

With the latest advances in cryobiology, ovarian tissue cryopreservation is rapidly becoming a more widely offered technique by many medical centers around the world. The first trials were initiated in rodent models during the 1950s. However, due to the lack of successful cryoprotectants and modern automated cryopreservation machines, the results were limited. However, after the 1990s, with the availability of modern cryoprotectants and new cryopreservation machines the results became more encouraging. The indications now extend beyond cancer. The objective of this manuscript is to review the indications, technique, risks and benefits, and success rates of ovarian tissue cryopreservation.

Indications

Many chemotherapeutics administered for cancer are gonadotoxic due to utilization of alkylating agents damaging both resting and actively dividing cells. Radiation therapy also causes premature ovarian failure and infertility (4,5). The risk of ovarian failure may be increased by 9 folds with the cyclophosphamide-based combination chemotherapies (6,7). Radiation to the ovaries with the doses more than 6 Gy usually causes permanent infertility (8).

By the access of in vitro fertilization techniques, some cryopreservation methods are developed for future preservation of fertility. These methods aim to preserve oocytes, embryos, and ovarian tissue (9-11). Due to the post-thaw survival and implantation and delivery rates embryo cryopreservation is the first method of choice (12). However, many women who are faced with a decision regarding their future fertility may not have an established partner or desire to use donor sperm for fertilization. In recent years many successful pregnancies have been reported from frozen-thawed oocytes, but the technique still needs to be improved and standardized (9,13,14). The pregnancy rate per frozen-thawed oocyte is 3%-4% (15). Prepubertal children on the grounds of ethical concerns and the women who do not have 2-4 weeks for ovulation induction due to the immediate demand of cancer therapy are not candidates for embryo or oocyte cryopreservation (12).

There are also some non-neoplastic diseases that are treated with chemotherapy and radiation therapy. Chemotherapy and radiation therapy are used to damage the bone marrow in hematopoietic stem cell transplantation (HSCT) for some of these diseases. The indications of HSCT are some cancers, hemoglobinopathies or enzyme deficiencies, and some autoimmune diseases, such as systemic lupus erythematosus and aplastic anemia (Table 1) (12,15). Ovarian tissue cryopreservation may be beneficial for such patients who have future fertility desire.

Childhood cancers

Cancer is the second most common cause of death in children but there are remarkable improvements in the cure rates of many childhood cancers (1). Most common cancers of the childhood period are leukemias, lymphomas, tumors of central nervous system, soft tissue sarcomas, and renal tumors (3,16,17). Acute lymphoblastic leukemia is the most common childhood cancer and more than 95% survive the disease (3). The 5-year survival rate of childhood cancers is approximately 80%, with a higher percentage for lymphomas and Wilm's tumor (3,18). These children who are treated because of cancer need to be protected from gonadotoxic effects of chemotherapeutics.

Ovarian tissue cryopreservation may be the most suitable method for prepubertal female patients receiving chemotherapy, pelvic radiation therapy, HSCT, or oophorectomy for benign diseases (5). Since the number of primordial follicles is the highest in the childhood period, the greatest benefit is expected (19). Furthermore, the problems with oocyte cryopreservation, such as time necessity and ethical issues, are suspended with ovarian tissue cryopreservation (5).

Table 1. Indications for ovarian tissue cryopreservation.

Cancer in children	Hodgkin's and non-Hodgkin's lymphoma Leukemia Ewing's sarcoma Willms' tumor Neuroblastoma Pelvic osteosarcoma Genital rhabdomyosarcoma
Breast cancer	Infiltrative ductal histological subtype Infiltrative lobular? Stage I–III Stage IV?
Cancer of the cervix	Squamous cell carcinoma Adeno/adenosquamous carcinoma?
Autoimmune and hematological diseases	Systemic lupus erythematosus Behçet's disease Steroid resistant glomerulonephritis Inflammatory bowel disease Pemphigus vulgaris Rheumatoid arthritis Progressive systemic sclerosis Juvenile idiopathic arthritis Multiple sclerosis Autoimmune thrombocytopenia Aplastic anemia Sickle cell disease
Benign ovarian disease	Endometriosis Benign ovarian lesions requiring repeated surgeries
Patients receiving pelvic radiation	Solid organ tumors presenting in the pelvis Ewing's sarcoma Osteosarcoma Tumors of the spinal cord Retroperitoneal sarcoma Rectal cancer Benign bone tumors
Prophylactic oophorectomy	BRCA-I-positive patients BRCA-II-positive patients?
Hematopoietic stem cell transplantation	Malignant diseases Genetic, hematological, and autoimmune disorders

Adult cancers

Approximately 8% of female cancers appear in the reproductive period under the age of 40 and the most common cancer of women during reproductive period is breast cancer (20) There were 4307 new breast cancer cases in 2001 with the incidence rate of 12.87 per 100.000 in Turkiye and the estimated new cases for U.S. in 2007 was 178.480 (21,22). Despite the high incidence of the disease, the 5-year survival rate in breast cancer is approximately 90%. Most of the breast cancer patients are subjected to gonadotoxic chemotherapies including cyclophosphamide (23). In breast cancer, unlike other malignancies, there are 6 weeks interval between the surgery and chemotherapy. Therefore, this interval may be a good opportunity for assisted reproductive technologies to supply future fertility. However, classical ovarian stimulation protocols are thought to result in regrowth of breast cancer cells due to the supraphysiological estrogen concentrations. However, recent protocols using tamoxifen and aromatase inhibitors are suggested to be safer (24). Since there is a risk of occult ovarian metastasis, especially in stage IV disease and lobular carcinoma, such patients can be candidates for ovarian tissue cryopreservation (5,11).

Another postulant population for ovarian tissue cryopreservation is cervix cancer patients. This serious health problem affects 500.000 women worldwide every year. Cervix cancer patients who are treated with radiation therapy are candidates for ovarian cryopreservation. Ovulation induction might be risky since there is a risk of bleeding from cervix during the procedure of oocyte retrieval (12). Moreover, 5%-10% of endometrial cancer patients are younger than 40 years of age, and many of these patients undergo hysterectomy and usually salpingo-oopherectomy (25). So, fertility desire makes these patients candidates for ovarian cryopreservation when other established fertility preservation techniques are not applicable.

Autoimmune diseases

Recently, the cytotoxic treatment regimens, especially including cyclophosphamide, are commonly used for autoimmune diseases, such as systemic lupus erythematosus (SLE), steroid resistant glomerulonephritis, and Behçet's disease (26,27). Ovarian tissue cryopreservation may be a good fertility preserving alternative for these patients when treatment is urgent.

HSCT

HSCT is an effective treatment modality for many benign or malignant diseases, such as autoimmune thrombocytopenia, SLE, rheumatoid arthritis, vasculitis, multiple sclerosis, thalassemia, leukemia, lymphoma, and breast cancer. Because of the high dose gonadotoxic chemo-radiotherapy implemented prior to HSCT, these patients are also candidates for ovarian cryopreservation if they do not have enough time for ovarian stimulation protocols (28-30).

Impact of chemotherapeutic agents on ovarian reserve

The damage due to chemotherapeutics diminishes ovarian reserve leading to infertility and premature ovarian failure (POF). The chemotherapy-induced ovarian damage is associated with marked follicle loss and ovarian fibrosis. Besides, chemotherapeutics also affect the ovarian stromal cells that have important endocrine functions, such as post chemotherapy damage repair (31)

The highest risk of primordial follicle death is associated with alkylating agents. These agents are non-cell-cycle-specific and affect the cell regardless of the replication period. The most commonly used alkylating agent is cyclophosphamide and it causes DNA crosslinking in granulosa cells, decreased circulating levels of progesterone and estrogen, and ovarian fibrosis. Follicular destruction with alkylating agents may occur even at very low doses. Relative risk of POF is increased 4-9.3 fold in patients treated with cyclophosphamide (5). The risk levels of ovarian failure due to different chemotherapeutic agents are summarized in Table 2 (32).

Other important factors affecting the degree of gonadal failure are the age of the patient and cumulative dose of the chemotherapeutic agent (32). Similar doses of chemotherapeutics cause a higher incidence of amenorrhea in older women because of the lower ovarian reserve. It is not clear whether the risk of ovarian failure is dependent upon the duration and dose intensity of chemotherapy.

Table 2. The degree of gonadotoxicity of chemotherapeutic agents.

High risk	Moderate risk	Low or no risk
Cyclophosphamide	Cisplatin	Methotrexate
Busulfan	Adriamycin	5-Fluorouracil
Melphalan	Paclitaxel (further studies are required)	Actinomycin D
Procarbazine		Bleomycin
Nitrogen mustard		Vincristine
Chlorambucil		

Techniques for ovarian tissue cryopreservation

Cryopreservation of the ovarian tissue and transplantation was first practiced in animal models after the 1950s with the discovery of cryoprotective agents (33). At that time glycerol was the only available cryoprotectant. Glycerol is a poor cryoprotectant and the success rate with glycerol was low. Only 10% of the primordial follicles survived after the freeze-thaw procedure (34,35). Since the 1990s there are some new and successful cryoprotectants in use, such as propan-l-ol, dimethyl sulfoxide (DMSO) and ethylene glycol (34,36,37). There were no significant differences between the results with ethylene glycol, DMSO, and propylene glycol despite a large number of observations, but survival in glycerol was poor. Results may improve with a longer period of immersion to equilibrate tissue with the cryoprotectant; 30 min is not necessarily optimal but was chosen as a compromise between toxicity and sufficient penetration (34).

Ovarian tissue harvest can easily be performed using laparoscopy (Figure 1). Ovarian cortex is sliced into small fragments after being cleared from the medulla so that cryoprotectants can easily diffuse into cortical tissue (Figure 2). If the thickness of the slices is similar, cryopreservation and revascularization should be equally effective in large and miniature grafts, and good rates of follicular survival can be expected (34). There are 2 main methods to transplant frozen-thawed ovarian tissue. In the orthotopic ovarian transplantation, the frozen-thawed ovarian tissue is grafted into the pelvis. In heterotopic ovarian transplantation the ovarian tissue is grafted subcutaneously into the forearm or abdominal skin (38,39). This method is much less invasive and tissue monitoring is simplified for patients who have risk for cancer recurrence in the ovarian graft (40). The most important advantage of this method is the prevention of cytotoxic effects of chemotherapy and/or radiation therapy in patients who need further treatment.

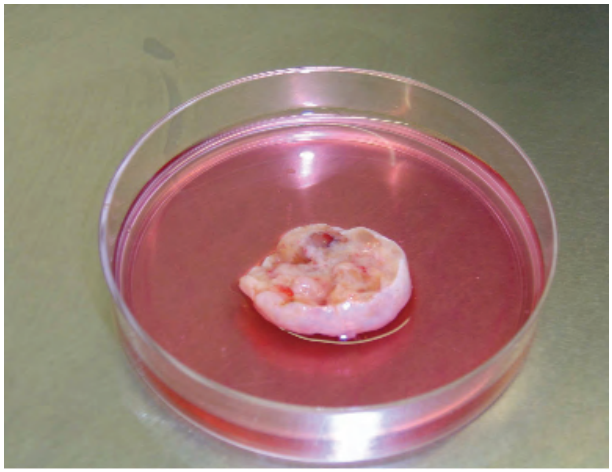

Figure 1. Ovarian tissue removed by laparoscopy.

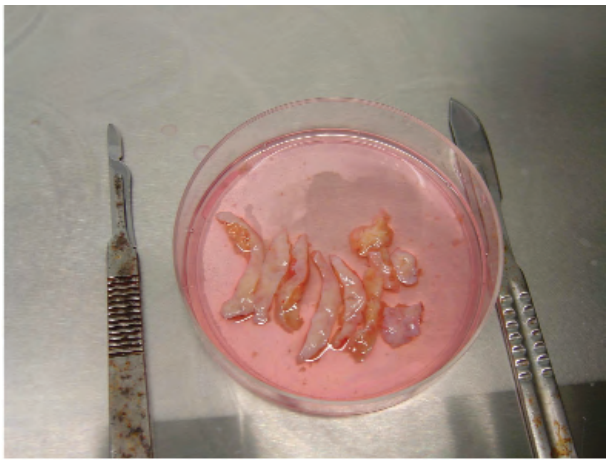

Figure 2. Ovarian cortical fragments cleared from medulla and prepared for cryopreservation.

Although heterotopic transplantation should require IVF to retain fertility, natural conception can occur in the case of orthotopic ovarian transplantation.

The histological observation of mitotic processes in the frozen-thawed samples obtained after transplantation clearly showed that the ovary had been overcoming the freezing injuries by day 3. This could be an indication of the reason the interval between surgery and first litter was not significantly different from that in transfers performed with nonfrozen half ovaries. In comparison with the nonfrozen ovaries, frozen-thawed ovaries may have poor endocrine function and that may cause delivery problems and early fetal death. If the fertilized oocyte was from the residual ovary tissue and the piece of ovary implanted did not ovulate, the corpus luteum that was created could have been too small to support the gestation. (36)

Risks of ovarian tissue cryopreservation

Reimplantation of the primary tumor

One of the most important concerns with ovarian tissue cryopreservation is the plausible reseeding of a tumor. Ideally, ovarian tissue cryopreservation should be performed on patients with a low risk for cancer metastasis to ovaries. Most of the malignancies encountered in the reproductive years do not metastasize to the ovaries. As summarized before by Sönmezer and Oktay, the cancers with high risk of ovarian involvement are leukemias, Burkitt lymphoma, neuroblastoma, and genital rhabdomyosarcoma (Table 3) (5,41-43). Patients with high risk cancers should either not be given the option of ovarian autotransplantation or ovarian tissue harvest should be performed after the first round of chemotherapy in order to ablate any neoplastic cells residing within the ovary (12). However, by this way there is a risk of diminishing the ovarian reserve with each cycle of chemotherapy.

To eliminate the reimplantation of the primary tumor, techniques, such as in vitro maturation of primordial follicles or ovarian tissue xenografting, may become options for patients with high risk (44,45). Regardless of the risk of the cancer involved, a histological assessment for micrometastases must be made on portions of the removed ovarian tissues before cryopreservation to avoid transplanting a tissue that contains cancer (20).

Table 3. The risk of ovarian metastasis.

Low risk	Squamous cell carcinoma of the cervix
	Ewing's sarcoma
	Breast cancer
	Stage I–III
	Infiltrative ductal
	Wilms' tumor
	Non-Hodgkin's lymphoma
	Hodgkin's lymphoma
	Osteogenic sarcoma
	Non-genital rhabdomyosarcoma
Moderate risk	Breast cancer
	Stage IV
	Infiltrative lobular
	Colon cancer (including tumors of rectum and appendix)
	Adeno/adenosquamous carcinoma of the cervix
	Upper gastrointestinal system malignancies
High risk	Leukemia
	Burkitt's lymphoma
	Neuroblastoma
	Genital rhabdomyosarcoma

Malignant transformation

The majority of indications for ovarian tissue removing and cryopreservation are for cancer, and these cancer patients may be at risk for cancer in other organs, such as ovaries. Patients with BRCA-1 and BRCA-2 mutations have 60% and 10%-20% lifetime risk of developing ovarian cancer, respectively (46,47). Since there is no convenient screening method for ovarian cancer, prophylactic salpingo-oopherectomy may be discussed for carriers of BRCA mutations after childbearing or by age 35-40. The incidence of occult ovarian metastasis has been reported between 2% and 18.5% in patients carrying BRCA mutations and undergoing prophylactic oophorectomy (48,49). A combination of intense surveillance and risk-reducing surgery in these patients allows the diagnosis of breast and ovarian cancers at early stages (50).

Heterotopic transplantation of the frozen-thawed ovarian tissue should be offered for such patients as the monitoring and removal of the tissue would be easy and this tissue should be removed back as soon as fertility treatment is complete. However, in vitro maturation and xenotransplantation that may be combined with ovarian cryopreservation in the future are the other options for these patients (5). Nevertheless, there are still some unanswered questions about these procedures. The success rate of growth of primordial follicles from cryopreserved ovarian tissue is a matter of debate. In vitro maturation also has the risk of epigenetic abnormalities secondary to incomplete methylation imprints in immature oocytes (51). Xenotransplantation may have the risk of transmission of prions and/or animal viruses accompanying the retrieved oocytes. More randomized clinical trials need to be carried out for these technologies to clarify the controversies.

Loss of follicles

One of the main limitations with ovarian tissue cryopreservation is the considerable loss of follicles during the initial ischemia and the revascularization periods (32,52). Because of these losses, the life span of the grafts is limited. According to the experimental studies vascular endothelial growth factor and aspirin are not effective in improving revascularization of the grafted tissues (32). Therefore, studies have focused on cryopreservation of the intact human ovary with its vascular pedicle but much more research is needed to clarify the success of this procedure.

Success of ovarian tissue cryopreservation

Human ovarian tissue cryopreservation has been reported since 1996 (34,53,54) and the orthotopic transplantation has been described since 2000 (55). It has been previously reported and our experience confirmed that subsequent to thawing of cryopreserved ovarian tissue, healthy primordial follicles could be observed (Figure 3). There are only 7 reports of live births after ovarian transplantation (Table 4) (39,56-62). In 4 of these cases the pregnancy was achieved by in vitro fertilization (IVF). In another one, there was a spontaneous pregnancy after heterotopic transplantation of the ovarian tissue under abdominal skin of the patient who had been menopausal for 2.5 years after HSCT (59). Ovarian functioning is longer in younger patients with heterotopic transplantation even up to 5 years (59,63)

Because of the potential limited life span of ovarian transplants, the procedure should only be carried out for fertility restoration, and when the patient is ready to conceive and cured of the primary disease. In theory, 1 year may be sufficient after the transplantation to obtain oocytes for IVF and embryo transfer (11) but much more clinical trials are needed to determine the optimal timing.

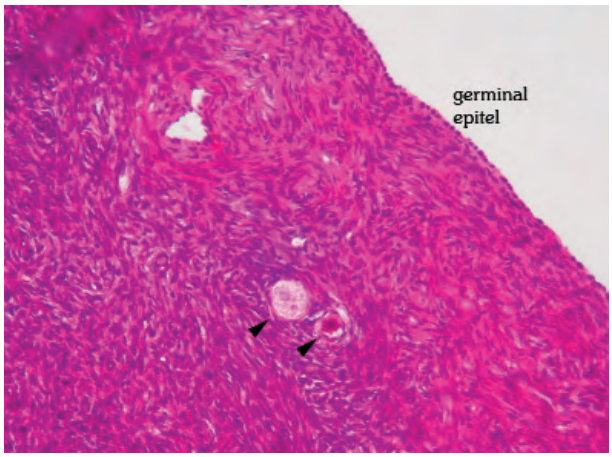

Figure 3. Healthy primordial follicles in the frozen-thawed ovarian tissue.

Table 4. Embryos and Pregnancies Reported From Frozen-Thawed Ovarian Tissue.

Author	Year	Transplantation site	Indication	IVF / spontaneous	Age at cryopr.	Age at transpl.	Outcome
Oktay	2004	Heterotopic	Breast cancer	IVF	30	36	Embryo development
Donnez	2004	Orthotopic	Hodgkin's disease	Spontaneous	25	31	Healthy live birth
Meirow	2004	Orthotopic	Hodgkin's disease	IVF	26	28	Healthy live birth
Demeestere	2006	Orthotopic/ heterotopic	Hodgkin's disease	Spontaneous	24	29	One miscarriage at 7 weeks, one healthy live birth
Oktay	2006	Heterotopic	Hodgkin's disease	Spontaneous	28	32	Healthy live birth
Rosendahl	2006	Orthotopic/ heterotopic	Hodgkin's disease	IVF from heterotopic site	28	30	Biochemical pregnancy
Silber	2008	Orthotopic	Idiopathic premature ovarian failure	Spontaneous	14	28	Ongoing pregnancy
Andersen	2008	Orthotophic	Non Hodgkin's lympohoma	IVF	32	34	Embryo development
Andersen	2008	Orthotopic/ heterotopic	Hodgkin's disease	IVF	25	27	Clinical pregnancy
Andersen	2008	Orthotopic	Hodgkin's diseas	IVF	26	28	Healthy live birth
Andersen	2008	Orthotopic	Ewing's sarkomu	IVF	27	30	Healthy live birth

Conclusion

Ovarian tissue cryopreservation is one of the fertility preserving methods and seems to be more practicable with improving techniques. This method is important particularly for young girls and women who are at risk of losing ovarian reserve due to urgent treatments. However, prior to performing ovarian tissue cryopreservation, clinicians must consider that there are limited data on long-term outcomes of the procedure and the approach to fertility preservation should be individualized. Physicians who are dealing with fertility preservation should work together with medical oncologists, pathologists, and reproductive endocrinologists. Due to the potential risk of reimplantation of the primary cancer and malignant transformation, a thorough counseling should be provided before going forward with the procedure. With the increasing number of cases who get pregnant from frozen-thawed ovarian tissue, we will define our indications more clearly and have the chance to optimize our technique.

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