Fertility preservation strategies for cancerous women: An updated review

Kanseri olan kadınlarda doğurganlığı koruma stratejileri: Güncellenmiş bir derleme

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Abstract

Due to the increase in cancer among young women, the risk of premature ovarian insufficiency with subsequent infertility has been raised. Fertility preservation restores reproductive potential along with increasing life expectancy in these patients. Given the articles on new options for treating cancerous women, we searched the keywords, including fertility preservation, in vitro maturation (IVM), and ovarian cryopreservation. This review focuses on the currently available procedures, including in (IVM) of retrieved immature oocytes, oocyte, embryo, and ovarian tissue cryopreservation (OTC). OTC is a helpful procedure that restores ovarian function and natural pregnancy. Also, we summarized the literature that reported the qualification of using the abovementioned procedures, comparing the cryopreservation methods including vitrification and slow freezing. Due to the impressive clinical development of OTC in cancerous patients, it is recommended as a standard treatment in cryopreservation strategies.

Keywords: Fertility preservation, in vitro maturation, ovarian tissue cryopreservation, ovarian tissue transplantation

Öz

Genç kadınlar arasında kanserin artması nedeniyle, erken yumurtalık yetmezliği ve ardından kısırlık riski artmıştır. Doğurganlığın korunması, bu hastalarda yaşam beklentisinin artmasıyla birlikte üreme potansiyeline geri kazandırılmıştır. Kanserli kadınların tedavisinde yeni seçeneklerle ilgili makaleler göz önüne alınmadı, doğurganlığın korunması, in vitro olgunlaştırma (IVM) ve yumurtalık kriyoprezervasyonu gibi anahtar kelimeleri aradık. Bu derleme, elde edilen immatür oositlerin IVM ve oositin, embriyonun ve yumurtalık dokusunun kriyoprezervasyonu (OTC) dahil olmak üzere şu anda mevcut olan proscedürlerde odaklanmaktadır. OTC, yumurtalık fonksiyonunu ve doğal hamileliği geri kazanmaya izin veren faydali bir prosedür olduğunu gördük. Ayrıca, vitrifikasyon ve yavaş donuruma dahl kriyoprezervasyon yöntemlerini karşılaştırarak, yukarıda bahsedilen prosedürleri kullanmanın yeterliliğini bildiren literaturu özellek. OTC'nin kanserli hastalardaki etkiliyeti klinik gelişimini nedeniyle, OTC kriyoprezervasyon stratejisinde standart bir tedavi olarak önerilmektedir.

Anahtar Kelimeler: Doğurganlığın korunması, in vitro olgunlaştırma, yumurtalık dokusu kriyoprezervasyonu, yumurtalık dokusu nakli

Introduction

Cancer incidence in different age groups, especially in adolescents and young women, has shown a slight increase since the 1970s(1). Although the survival rates from cancer have improved, cancer is still one of the leading health concerns, especially in young people(2). Fertility preservation is an approach used to protect cancer patients from the risk of infertility due to medical treatments, as radiotherapy, chemotherapy, and surgery. Cancer therapies are, in fact, harmful to reproductive function. The treatments used in these patients correlated with a high percentage of losing follicular numbers, especially in young women(3). Nowadays, this treatment allows us to maintain the reproductive potential.
of these patients using methods including, cryopreservation of oocytes, embryos⁹, and ovarian tissue (OT)¹⁰ transposition of the ovaries before radiation¹⁰, or in vitro culture (IVC) of ovarian follicles⁷. As recommended by the American Society of Clinical Oncology (ASCO) and the European Society for Medical Oncology, embryo/oocyte cryopreservation a known technique in fertility preservation⁰. Nevertheless, embryo cryopreservation needs a sperm source, it is not a suitable option for single women. Also, there are other numerous limitations to embryo production, cryopreservation, and storage due to ethical, religious, and social reasons⁴. However, vitrification of oocytes recovered from stimulated in vitro fertilization (IVF) cycles causes a delay in cancer treatment, due to the time necessary for controlled ovarian hyperstimulation (COH)⁹. Other fertility preservation techniques, such as ovarian tissue cryopreservation (OTC) and in vitro maturation (IVM) of immature oocytes, can be implemented immediately in cancer therapy, even in underage girls⁹. The purposes of this review are to explain the up-to-date knowledge about current developments of IVM, the clinical employment of OTC, and transplantation in cancerous women.

**Methodology**

MEDLINE-PubMed (http://www.ncbi.nlm.nih.gov/PubMed), Google Scholar (https://scholar.google.com/), Scopus (https://www.scopus.com) and ISI web of science (http://apps.lib.wosg.ir/WOS) databases were applied for extracting available human original and review studies, from 2010-August 2021. The keywords were used: “fertility preservation,” “in vitro maturation,” “ovarian cryopreservation” and “ovarian transplantation.” We mentioned 75 pieces of scientific literature consisting of reviews, original, guidelines, and recommendations, which have addressed the issue of fertility cryopreservation recently.

**IVM in Reproductive Medicine**

**Indications of IVM**

In the IVM process, the immature germinal vesicle (GV) and metaphase 1 (MI) stage oocytes (Table 1) were retrieved with a minor or no gonadotropin stimulation⁹. IVM was first applied in patients with polycystic ovarian syndrome with gonadotropin stimulation to avoid ovarian hyperstimulation syndrome¹⁰. Furthermore, IVM is a useful technique for patients who are concerned about the long periods of hormonal stimulation, and in cycles of recurrent oocyte maturation arrest, poor embryo quality, or IVF failures¹¹. IVM may also be suitable for women who cannot have sufficient time to obtain fully mature oocytes before cancer therapy. IVM avoids the increased estrogen levels in women with hormone-sensitive tumors, which are seen in COH cycles. Retraining immature oocytes and cryopreservation of them is a way for these women to preserve their reproductive ability in the future¹².

**Oocyte Maturation**

Nuclear and cytoplasmic maturation are two essential processes for oocyte maturation. The steps of nuclear maturation can be referred to as the meiotic resumption, indicated by the germinal vesicle breakdown, chromatin aggregation, the organization of the meiotic spindle, chromosome separation with the extrusion of the first polar body, progression to MII, and meiotic re-arrest before fertilization¹³. Cytoplasmic maturation is necessary to obtain a capacity for insemination, and early embryogenesis, subsequently, it provides conditions for implantation, pregnancy, and normal fetal development. This process includes numerous metabolics such as the accumulation of mRNA, proteins and substrates which all are needed to achieve the oocyte developmental competence and structural variations in the organelle typeology and distribution for the proper fertilization and early embryo development¹⁴.

**Molecular Mechanism of in vivo Oocyte Maturation**

Oocyte maturation in vivo is an intricate mechanism regulated through hormonal pathways, interactions with circumambient somatic cells, and gene expression, which is regulated by transcription factors. The elevation of cyclic adenosine 3’, 5’-monophosphate (cAMP) levels can prevent oocyte maturation. The high intra-oocyte cAMP concentration inactivates the meiosis promoting factor (MPF), thus blocking meiotic development. A drop in the cAMP levels stimulates the luteinizing hormone (LH) surge, resulting in the oocytes being released from the inhibitory milieu of the follicle, and maturation occurs. There are three sources of high cAMP levels within the oocyte. It includes the oocyte itself via G-protein coupled receptors on the oolemma¹⁵, cumulus cells (CCs) through the gap junctions, which is necessary for connecting cytoplasm and nuclear maturation¹⁶, and guanosine 3,5-cyclic monophosphate (cGMP), which is produced in the mural and CCs, crosses through gap junctions into the oocyte and inhibits cAMP hydrolysis by the oocyte-specific phosphodiesterase 3A¹⁶. Is mentioned that cAMP and cGMP, are the main molecules that play a key role in controlling mammalian oocyte meiosis. After the LH surge, another factor induced by mural granulosa cells is the epidermal growth factor (EGF). LH activation of mural granulosa cells induces the expression of the EGFs binding to their receptors in CCs; thus, mitogen-activated protein kinase (MAPK) is activated. The increased activation of MAPK may lead to the synthesis of meiosis resumption-inducing factor (s) and the blocking of gap junctions via a gap junction protein¹⁷. Also, hyaluronan is synthesized by hyaluronan synthase (HAS2) in the plasma membrane and directly extends into the mucous-elastica extracellular matrix (ECM). After that, the COCs are interrupted, which cessation the transportation of cAMP and resulting in the activation of MPF. Furthermore, oocytes secret soluble factors, such as growth differentiation factor-9 (GDF-9), bone morphogenetic protein 15 (BMP-15), and BMP-6. These growth factors stimulate the HAS2 gene expression and
cumulus expansion in the presence of the follicle-stimulating hormone (FSH). In standard IVM cycles, the immature COCs are isolated from antral follicles and subsequently saturated in a culture medium without cAMP-modulating agents. Standard IVM mediums typically include FSH or other additives such as EGF, EGF-p, and/or LH/hCG. In this system, FSH significantly improves MII rates, intra-oocyte cAMP levels decrease, and stimulation of the meiotic process begins.

**IVM Laboratory Procedures**

The laboratory procedure for IVM cycling is time-consuming and technically challenging. First, the COCs were collected from a follicular environment by searching them into a Petri dish under a stereomicroscope or using a cell strainer composed of nylon mesh with 70-μm pores to collect more oocytes with a small number of CCs. All handling procedures should be performed in optimal conditions such as warm stages or plates at 37 °C. In the IVM cycles treated with human chorionic gonadotropin (hCG) priming, in vivo matured oocytes may be retrieved at oocyte collection. However, in the IVM cycles without hCG priming, in vivo matured oocytes cannot be recovered on the day of retrieval. The retrieved COCs are usually transferred to an IVM culture medium supplemented with hormones and growth factors. They were cultured for 24-30 h (day 1) to 48 h (day 2) then, the matured oocytes were cryopreserved or inseminated with partner spermatozoa.

**IVM Culture Medium**

Special culture media as the essential IVM media have been applied for both research and clinical purposes. The human IVM medium is typically supplemented with serum albumin and gonadotropins. Some studies reported that the use of a patient’s serum is more effective and led to significantly

| Oocyte stage | IVM medium | Cryopreservation method | Type of COH | Results | Ref |
|--------------|------------|------------------------|-------------|---------|-----|
| GV/MI        | IVM medium supplemented with 0.075 IU/mL FSH and 0.075 IU/mL LH for 24-48 hr. | Vitrification | IVF cycle | IVM is effective strategy, if done before oocyte vitrification. | [62] |
| GV/MI        | Hams F10 supplemented with 0.75 IU LH, 0.75 IU FSH and 40% FF. | Vitrification | IVF cycle | GV-stage vitrification followed by IVM is superior to that performed in MI. | [63] |
| GV/MI        | Ham’s F10 supplemented with 0.75 IU FSH, 0.75 IU LH and 40% HFF. | Vitrification | IVF cycle | IVM of fresh immature oocytes is better than of vitrified ones, with higher maturation and viability. | [64] |
| GV/MI        | Ham’s F10 supplemented with 0.75 IU LH, 0.75 IU FSH and 40% FF. | - | IVF cycle | In patients with gynaecological diseases, oocytes maturation after IVM from unstimulated ovaries showed good developmental competence. | [65] |
| GV           | Ham’s F10 medium supplemented with 0.75 IU of LH and 0.75 IU of FSH with 40% HFF. | - | IVF cycle | IVM with blastocyst medium was superior in ICSI cycles. | [66] |
| GV           | IVM medium supplemented with 75 mlU/mL FSH, 75 mlU/mL LH and 10% SSS for 24-48 hr. | Vitrification | IVF cycle | Vitrification of MII oocytes after IVM was superior. | [67] |
| GV/MI        | Simple IVM system (S-IVM), autologous follicular fluid (AFF-IVM), HFF (HFF-IVM), and HFF with CCs isolated from non PCOS women. | - | IVF cycle | In PCOS patients, HFF/CGC-IVM protocol significantly increased IVF outcomes. | [68] |
| GV           | Blastocyst medium (G2) supplemented with 75 IU/L of HMG. | - | IVF cycle | Rescue IVM had negative effects on embryo morphokinetics. | [69] |
| GV/MI        | IVM medium supplemented with 75 mlU/mL FSH, 100 mlU/mL hCG, and 20% HSA with GDF9. | Vitrification | IVF cycle | In cancer patients, vitrification impaired oocyte maturation, viability, subcellular quality after IVM. | [70] |
| GV           | IVM medium supplemented with 75 mlU/mL FSH and 75 mlU/mL LH. | - | IVF cycle | IVM medium supplemented with GDF9 and CCs increased fertilization, embryo development and blastocyst viability rates. | [71] |

GV: Germinal vesicle, MI: Metaphase I; IVM: In vitro maturation; COH: Controlled ovarian hyperstimulation; IVF: In vitro fertilization; FSH: Follicle-stimulating hormone, LH: Luteinizing hormone; GV: Germinal vesicle, MI: Metaphase I.
higher rates of maturation and pregnancy compared to the use of a donor’s FF and serum substitute supplement. Serum may have some relevant factors for oocyte maturation, such as EGF. Commercial IVM media, such as SAGE (Cooper Surgical) IVM medium, MediCult IVM medium (MediCult, Origio, Måløv, Denmark), and tissue culture medium 199 (TCM199, Invitrogen, Carlsbad, CA) have the advantage of immature oocytes culture. Recently, improved culture systems to mimic the in vivo maturation process, such as the use of 3-D culture systems, the supplementation with C-Type Natriuretic Peptide to retain gap junctions for a specific time before starting oocyte maturation in vitro, with EGF-like growth factors or oocyte secreting factors (GDF-9 and BMP-15), are used.

**IVM Oocyte Cryopreservation**

Oocytes retrieved from IVF/IVM cycles can be cryopreserved using the slow-cooling or vitrification approaches. In theory, there are two methods for immature oocyte cryopreservation before IVM (at GV or MI-stage) or after IVM (MII-stage) (Table 1). The first successful pregnancy and live birth were reported after slow-cooling of immature human oocytes, but further successful items were from vitrification of MII-stage oocytes after the IVM procedure.

**Clinical Outcomes in IVM Cycles**

Limited studies have reported live births after the cryopreservation of IVM oocytes. The first live birth was achieved using the slow-cooling method at the GV stage oocytes recovered from IVM cycles. One study reported the first live birth after vitrification of immature oocytes. Later, the McGill reproductive center reported five gravidities with live births after vitrification at MII-stage after IVM of immature oocytes collected from hCG-primed IVM cycles. In their study, MII oocytes obtained from IVM cycles had significantly lower recovery and fertilization rates after vitrification than in vivo MII oocytes generated from IVF cycles. Additionally, implantation, clinical pregnancy, and live birth rates were lower in IVM-oocytes vitrification groups. Nevertheless, in cancer patients, there are limited studies of successful pregnancies or live births after cryopreservation of IVM oocytes, employing either slow-cooling or vitrification methods. Previous studies reported that the mean oocyte maturation rate of 39% ±23% was achieved after the collection of 1,220 COCs from 77 patients who were done oophorectomy for OTC (maturation rate of 22% in pre-menarche children and 42% in adult patients). Only three live births from OT oocyte in vitro maturation (OTO-IVM) in women have been reported in this literature. The rate of live births from OTO-IVM per embryo transfer was 43% in the aforementioned study.

**OTC**

**Indications for OTC**

OTC and ovarian tissue transplantation (OTT) is the best choice for women undergoing chemoradiotherapy who cannot delay the start of these therapies or are ineligible for ovarian stimulation. Also, it could be used by women in postponing their first pregnancy and menopause. This approach described some diseases, including genetic abnormalities such as Turner’s syndrome, gynecological diseases, systemic and endocrine disorders, autoimmune disease, and endometriosis leading to premature ovarian insufficiency. It is the only option available for pre-pubertal girls and women with estrogen-sensitive malignancies. However, OTC is a useful procedure that allows for restoring ovarian function and natural pregnancy. It is an important evaluation of cumulative factors, such as adequate ovarian reservation, level of AMH hormone, age of patients, and previous treatment regimens for performing this technique. This approach was suggested in some guidelines, such as ASCO 2018, which mentioned OTC as a standard treatment for these cases, due to the rapid improvement of the OT freezing technique.

**Transplantation to the Patient**

**Cortical OTT**

For the cryopreservation of cortical OT, strips of the ovarian cortex removed throughout the menstrual period by laparoscopy or laparotomy. The OT contains many primordial and immature follicles numbers that can be protected by freezing. However, it is more complicated than embryo and oocyte freezing due to different cell types and permeability to water and cell volume. After removal of the medulla, the cortex is divided into several strips of approximately equal size for grafting (10x5 with 1-mm-thick piece) or slices (4x2 with 1-mm-thick piece) which allow penetrating the cryoprotectant agents (CPAs) into the thin layer of the cortex. Using larger pieces of the cortex (5x5x1 mm) may prepare better conditions for OTT. It is worth mentioning that too small pieces of the cortex are difficult to fixed to the underlying surface and subsequently oxygenation and normal re-vascularization were disturbed. Ovarian cortical pieces could be transplanted into patients after treatment of the disease or could be done IVM of obtained follicles from OTs. Almost all the healthy live births were achieved following this method. According to a previous meta-analysis study, the reestablishment of ovarian activity rate was 63.9%, and live birth was reported 57.5% by autotransplantation method in women younger than 30 years at the time of OTT. A survival rate of 84% was reported in follicles after frozen-thawed OTs. However, up to 72% of the follicles are disrupted due to ischemia and reperfusion injury after OTT.
Orthotropic Transplantation of Cortical Tissue

Orthotropic transplantation involves transplanting strips of OT into the remaining portion of the ovary or the peritoneum of the ovarian fossa. The advantage of this procedure becomes possible by natural conception and has provided a suitable environment for follicular development. However, the number of grafted fragments limited by the remaining ovarian size, also, it may increase ischemia and follicle atresia after grafting due to avascular condition. However, the first pregnancy was reported in 2004 using this method, and so far most live births have been from this transplantation.

Heterotopic Transplantation of Cortical Tissue

Heterotopic transplantation refers to the grafting of cortical OT into extra-pelvic sites such as the forearm, abdominal, and chest wall. The transplanted tissue can easily removed or replaced when necessary. Contrary to the orthotropic method, it avoids major abdominal surgery and, has no limit to the number of grafted fragments. Although this technique is less invasive than orthotopic, although, spontaneous pregnancy is impossible; therefore, subsequent ovarian stimulation and IVF must be performed.

Transplantation of the Whole Ovary

In theory, the transplantation of thawed whole ovaries can lead to vascular anastomosis in the ovarian pedicle, however, the ischemia and follicle atresia is reduced due to vascular grafting. As a result, it may provide a more significant follicular reserve and a longer lifetime for an organ transplant. Although, it had some problems, such as a large mass of OT, creating a non-homogeneous cooling rate between different layers of OT. Problems associated with mass and cold transfer eventually increase the probability of ice formation. The multi-thermal gradient technique provides a possible way to overcome the ischemic damage to the whole ovary.

Ovarian Tissue Freezing Techniques

There are two standard methodologies that have been introduced for cryopreservation procedure, including conventional slow freezing and vitrification.

Conventional Slow Freezing

In this technique, a controlled cooling machine is used to OT slowly until -140 °C at low rates (~1 °C/min) before plunging it into liquid nitrogen (LN2). Ovarian sample as a complex tissue has different types of cell and ECM. The slow freezing method can prepare a higher equilibration period to allow the release CPA release into the inner complex tissue areas. Slow freezing helps osmotic adjustments between extra and intracellular fluids with CPAs during freezing/thawing procedures. Most times the combination of permeating CPAs, such as glycerol, dimethyl sulfoxide, ethylene glycol, and 1,2-propanediol, and non-permeating CPAs as sucrose, trehalose, and raffinose, was used to protect against cell damage caused by the production of ice crystals and hypertonicity during cryopreservation. More than 130 live birth was reported from this method; however, its disadvantages are time-consuming and require costly equipment.

Vitrification

The vitrification procedure was introduced with an ultrafast cooling rate (~20,000 °C/min) by direct plunging into LN2 and a high concentration of CPAs. The concentration of CPA was the most critical cause of cell damage; however, it is recommended to use a combination of two or more CPAs. Vitrification is a considerable method due to its quickness, ease, and cost-effectiveness without using special and expensive equipment. It has been reported in a low risk of ice crystal formation in the vitrification method. Nevertheless, there is still no optimal protocol for vitrification. As a result, data about the vitrification technique in human OT is still limited, and some centers may prefer to perform slow freezing for OTC. Thus, the superiority between vitrification and slow freezing for OTC remains unresolved. Some studies showed a lower percentage of apoptotic cells and higher viability of primordial follicles after the slow freezing procedure. Additionally, the frozen-thawed cortical tissue could produce a higher number of hormones AMH in tissue culture after the slow freezing method. However, others found no differences in the percentage of apoptotic cells and follicle viability and density between these procedures.

The viability rate of primordial follicles should be assessed after different cryopreservation methods. This assessment is performed using staining assays such as hematoxylin-eosin and trypan blue solution. By staining, the state of primordial follicle quality, including intact nucleolus, clear cytoplasm, and round shape, density, and viability, can be examined. A meta-analysis of 14 studies in 2017 suggested that less primordial follicular DNA damage and better conditions for the preservation of stromal cells after vitrification. A disadvantage of vitrification is the direct contact of the sample with nitrogen, therefore, it can lead to viral cross-contamination. Sugishita et al. recently introduced a new closed vitrification system. According to this study, none of the cryopreservation methods, including slow freezing, conventional vitrification, and closed vitrification didn’t show any significant difference in terms of DNA damage and apoptosis pathway in both primordial and primary follicles compared with a fresh baseline control group. Nevertheless, only three live births have been reported from the vitrification procedure. A summary of the main properties and outcomes regarding the comparison of vitrification and slow freezing are presented in Table 2.
| Method | Basic medium (BS) | Cryoprotectants agent | Equilibrium/cooling and warming rate | Results | Ref |
|--------|-------------------|-----------------------|-------------------------------------|---------|-----|
| Vit    | Leibovitz L-15 (BM1) | ES: 5.58% (1M) EG, 3.55% (0.5M) DMSO, 0.125 M sucrose and 2.50% SSS, VS1: 11.16% (2M) EG, 7.10% (1M) DMSO, 0.25 M sucrose with 5.00% SSS, VS2: 22.32% (4M) EG, 14.20% (2M) DMSO, 0.5 M sucrose and 10% SSS, WS1: BM1 with 0.8 M sucrose, WS2: BM1 with 0.4 M sucrose, WS3: sucrose-free BM1 | Equilibration solution: 5 min at RT, VS1: 7 min at RT, VS2: 4 °C for 10 min, then plunged into LN2. Warming: each solution for 5 min at RT. | More intact follicles showed after vitrification and the apoptotic primordial follicles were no significant between 2 groups. Vitrification led to gene overexpressed (gene expression of granulosa cells, oocytes and cellular cycle) compared to slow freezing. | [71] |
| SF     | Leibovitz L-15 (BM1) | FS: 2 M DMSO +10% sss, TS: BM1 | ES: 7.5% EG, 7.5% DMSO in VS: 20% EG, 20% DMSO and 0.5 M sucrose, TS: 55 mL HEPES, 20 mL SSS, and 34.24 g sucrose, DS: 65 mL HEPES, 20 mL SSS, and 17.12 g sucrose, Washing solution: (40 mL HEPES and 10 mL SSS) | Cooling: 10 min at RT, from 20 °C to -35 °C at -2 °C/min, semi-automatic seeding at -11 °C, cooling to -150 °C at 25 °C/min. Thawing: shaking in the water bath at 37 °C for one min, then placed in TS for 5 min at RT. | Slow freezing was superior to vitrification in terms of primordial follicle preservation, vascularization, follicular cell proliferation, DNA damage, and AMH expression. | [72] |
| Vit    | TCM199 with 20% SSS | FS: Ascending percentage of DMSO (7.5,10 and 12.5), Washing solution: 5% DMSO | ES: 25 min at RT, VS: 15 min at RT, then plunged into LN2. Warming: TS: 5 min at RT, DS: 5 min at RT, Washing: 5 min at RT (twice). | Slow freezing showed better preservation regardless of the type of follicle. Expression of apoptotic genes was significantly decreased in slow-frozen samples. | [73] |
| SF     | TCM199 with 5% SSS | FS: 10% DMSO | ES: 15 min on ice, VS: 10 min on ice, then plunged into LN2. Warming: each solution for 5 min. Cooling: from 0 °C to -8.0 °C at a rate of -2.0 °C/min, manually seeding, cooling to -40.0 °C at a rate of -0.3 °C/min and -140 °C at a rate of -10 °C/min. Thawing: shaking in the water bath at 37 °C, then placed in washing solution for 10 min at RT. | Slow freezing showed better preservation regardless of the type of follicle. Expression of apoptotic genes was significantly decreased in slow-frozen samples. | [73] |
Clinical Outcomes

The first successful human live birth from orthotropic transplantation was reported in 2004, and Meirow et al. (52) reported a second live birth in 2005 (53). Up until now, due to the impressive development of the OT freezing technique, specially, the ovarian cortex implantation method, more than 130 healthy babies have been born since, 2017 worldwide (54, 55). This statistic has been mentioned in 200 cases until 2021 (56).

Andersen et al. (43) investigated the clinical outcome rate of the 3 largest cohort studies in Belgium, Denmark, and Israel. They reported that pregnancy rates varied from 3.9% to 19.3% and live birth rates from 3.9% to 14% per cycle (43). In other studies, the live birth rate was reported from 25.4% to 30.6% (50). Also, the cumulative clinical pregnancy and the cumulative live birth and clinical ongoing pregnancy rates were 57.5% and 37.7%, respectively (42). The lack of consensus could be due to the timing of initiation of ART from OTT, patient’s age, type of ovarian stimulation protocol, and
Overall, the strategy of centers regarding providing services to these patients. A systematic review showed that clinical outcomes were considerably lower in women undergoing OTT than in IVF cycles\(^{(43)}\). However, there are few reports on the prevalence of pregnancy in pre-pubertal girls. Recent literature reported only two cases of live births who underwent OTC. One case was 14 years old with sickle cell disease that was underwent autologous tissue transplantation for her at the age of 24 years, and pregnancy was achieved spontaneously. Another case was a girl at the age of 9 years old with beta-thalassemia. After the treatment process, she returned for OTT and achieved a live birth undergoing the IVF program\(^{(57)}\).

**Future Perspectives on Eliminating the Risk of Malignant Cell Transmission**

Alternative approaches have been introduced for the deletion of malignant cells in certain types of cancer with high metastasis potential, such as leukemia, Burkitt’s lymphoma, neuroblastoma, and ovarian tumors. The artificial ovary technique is one of the new approaches that are known, as primordial follicles isolated from OTs and transferred onto a scaffold-like ovarian organ. The development of human pre-antral follicles was seen after grafting of primordial follicles inside a fibrin scaffold and, respectively, xenografted in nude mice\(^{(58)}\). Future studies attempt to find a three-dimension-printed artificial ovary to restore both endocrine and reproductive function in animals\(^{(59)}\). Another approach is to isolate immature oocytes and perform IVM in the ART lab\(^{(59)}\). The main challenge is maintaining the interaction between the oocytes and the somatic cells that surround them\(^{(60)}\). The acquisition of meiotic, s developmental conditions, and genome imprinting are important factors that should be considered for this issue. Oocytes differentiated from ovarian stem cells (OSCs) may be another option for the mentioned conditions. Studies have shown that OSCs have been retrieved from mice that are suitable for fertilization and implantation, as well as embryo development and live births in an animal model\(^{(61)}\). However, due to the scarcity of OSCs and their ethical issues use of these cells in the clinical application was insufficient, for this reason, this technique is not currently used in clinical practice, especially in cancer patients\(^{(20)}\). These aforementioned techniques are still in a research setting and can be used for female fertility preservation in the near future.

**Conclusion**

Due to the impressive clinical development of OTC in cancerous patients, it is recommended as a standard treatment in cryopreservation strategies. However, OTC was a useful procedure that allows for restore ovarian function and natural pregnancy. However, IVM treatment does not require high gonadotropin stimulation and it is not necessary to take more than 48 h for the decision to perform. Therefore, when patients are unable to delay the chemotherapy, retrieving immature oocytes from the antral follicles and the IVM method may be a good approach. A combination of IVC of isolated OSCs, small follicles, and an artificial ovary technique could eliminate the risk of malignant cell transmission. These approaches could be a good fertility preservation strategy for cancer patients in future studies.

**Ethics**

**Peer-review:** Internally and externally peer-reviewed.

**Authorship Contributions**

Concept: M.A.K., Design: M.A.K., Data Collection or Processing: F.A., M.A.K., M.M., A.A., M.G.P., Analysis or Interpretation: F.A., M.A.K., M.M., A.A., M.G.P., Literature Search: F.A., M.A.K., M.M., A.A., M.G.P., Writing: F.A., M.A.K., M.M., A.A., M.G.P.

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