Review / Derleme

DOI: 10.4274/tjod.galenos.2022.42272

Turk J Obstet Gynecol 2022;19:152-61

Fertility preservation strategies for cancerous women: An updated review

Kanseri olan kadınlarda doğurganlığı koruma stratejileri: Güncellenmiş bir derleme

Fatemeh Anbari[1,2], Mohammad Ali Khalili[1,2], Maryam Mahaldashtian[1,2], Alireza Ahmadi[3], Maria Grazia Palmerini[4]

[1]*Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*
[2]*Department of Reproductive Biology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*
[3]*Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
[4]*Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italy*

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 potansiyelini geri kazandırmıştır. Kanserli kadınların tedavisinde yeni seçeneklerle ilgili makaleler göz önüne alındığında, 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 prosedürlere odaklanmaktadır. OTC, yumurtalık fonksiyonunu ve doğal hamileliği geri kazanmaya izin veren faydalı bir prosedür olduğunu gördük. Ayrıca, vitrifikasyon ve yavaş dondurma dahil kriyoprezervasyon yöntemlerini karşılaştırarak, yukarıda bahsedilen prosedürleri kullanmanın yeterliliğini bildiren literatürü özetledik. OTC'nin kanserli hastalardaki etkileyici klinik gelişimi 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

Address for Correspondence/Yazışma Adresi: Mohammad Ali Khalili MD,
Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute and Department of Reproductive Biology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
Phone: +983538247085 **E-mail:** Khalili59@hotmail.com **ORCID ID:** orcid.org/0000-0001-9940-749X
Received/Geliş Tarihi: 19.02.2022 **Accepted/Kabul Tarihi:** 07.04.2022

©Copyright 2022 by Turkish Society of Obstetrics and Gynecology
Turkish Journal of Obstetrics and Gynecology published by Galenos Publishing House.

of these patients using methods including, cryopreservation of oocytes, embryos[4], and ovarian tissue (OT)[5] transposition of the ovaries before radiation[6], or in vitro culture (IVC) of ovarian follicles[7]. 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 preservation[8]. 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 reasons[4]. 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 girls[9]. 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 I (MI) stage oocytes (Table 1) were retrieved with a minor or no gonadotropin stimulation[9]. IVM was first applied in patients with polycystic ovarian syndrome with gonadotropin stimulation to avoid ovarian hyperstimulation syndrome[10]. 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 failures[11]. 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. Retrieving immature oocytes and cryopreservation of them is a way for these women to preserve their reproductive ability in the future[12].

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 fertilization[13]. 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 typology and distribution for the proper fertilization and early embryo development[14].

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 oolemma[15], cumulus cells (CCs) through the gap junctions, which is necessary for connecting cytoplasm and nuclear maturation[10], 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 3A[16]. 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 protein[17]. Also, hyaluronan is synthesized by hyaluronan synthase (HAS2) in the plasma membrane and directly extends into the mucous-elastic 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)[18]. 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[19,20].

IVM Culture Medium

Special culture media as the essential IVM media have been applied for both research and clinical purposes[9,19]. The human IVM medium is typically supplemented with serum albumin and gonadotropins[9]. Some studies reported that the use of a patient's serum is more effective and led to significantly

Table 1. IVM studies in which human immature oocytes were cultured in different media or cryopreserved with different methods

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.	-	IVM 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.	[21]
GV	IVM medium supplemented with 75 mIU/mL FSH, 75 mIU/mL LH and 10% SSS for 24-48 hr.	Vitrification	IVF cycle	Vitrification of MII oocytes after IVM was superior.	[66]
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.	[67]
GV	Blastocyst medium (G2) supplemented with 75 IU/L of HMG.	-	IVF cycle	Rescue IVM had negative effects on embryo morphokinetics.	[68]
GV/MI	IVM medium supplemented with 75 mIU/mL FSH, 100 mIU/mL hCG, and 20% HSA with GDF9.	Vitrification	IVM cycle	In cancer patients, vitrification impaired oocyte maturation, viability, subcellular quality after IVM.	[69]
GV	IVM medium supplemented with 75 mIU/mL FSH and 75 mIU/mL LH.	-	IVM cycle	IVM medium supplemented with GDF9 and CCs increased fertilization, embryo development and blastocyst viability rates.	[70]

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)[21], and tissue culture medium 199 (TCM199, Invitrogen, Carlsbad, CA)[21] 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[22], the supplementation with C-Type Natriuretic Peptide to retain gap junctions for a specific time before starting oocyte maturation in vitro[23], with EGF-like growth factors or oocyte secreting factors (GDF-9 and BMP-15), are used[24].

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[25], but further successful items were from vitrification of MII-stage oocytes after the IVM procedure[26].

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[25]. One study reported the first live birth after vitrification of immature oocytes[27]. 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[26]. 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)[28]. 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[29]. Also, it could be used by women in postponing their first pregnancy and menopause[30]. This approach described some diseases, including genetic abnormalities such as Turner's syndrome[31], gynecological diseases[32], systemic and endocrine disorders, autoimmune disease[33], and endometriosis leading to premature ovarian insufficiency[34]. It is the only option available for pre-pubertal girls and women with estrogen-sensitive malignancies[35,36]. However, OTC is a useful procedure that allows for restoring ovarian function and natural pregnancy[37]. 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[38]. 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[39].

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 volum[40]. 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[36]. 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[41]. 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[42]. 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[43].

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[44]. 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[44].

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 orthotropic, although, spontaneous pregnancy is impossible; therefore, subsequent ovarian stimulation and IVF must be performed[44].

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[45].

Ovarian Tissue Freezing Techniques

There are two standard methodologies that have been introduced for cryopreservation procedure, including conventional slow freezing and vitrification[45].

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)[46]. 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[47]. More than 130 live birth was reported from this method; however, its disadvantages are time-consuming and require costly equipment[48].

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[47]. Vitrification is a considerable method due to its quickness, ease, and cost-effectiveness without using special and expensive equipment[30]. It has been reported in a low risk of ice crystal formation in the vitrification method[29]. 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[49]. However, others found no differences in the percentage of apoptotic cells and follicle viability and density between these procedures[21,50]. 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[50]. 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[51]. A disadvantage of vitrification is the direct contact of the sample with nitrogen, therefore, it can lead to viral cross-contamination. Sugishita et al.[20] 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[21]. 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.

Table 2. Basic characteristics and outcomes of studies in terms of comparison between vitrification and slow freezing procedures

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 freeing.	[71]
SF	Leibovitz L-15 (BM1)	FS: 2 M DMSO +10% sss TS: BM1	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.		
Vit	TCM199 with 20% SSS	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)	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 was superior to vitrification in terms of primordial follicle preservation, vascularization, follicular cell proliferation, DNA damage, and AMH expression.	[72]
SF	TCM199 with 5% SSS	FS: Ascending percentage of DMSO (7.5,10 and 12.5) Washing solution: 5% DMSO	Cooling: from 4 °C to -7.0 °C at a rate of -2.0 °C/min, manual 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.		
Vit	Basic cryoprotectants solution with 20% HAS	ES: 7.5% EG and 7.5% DMSO VS: 15% EG,15 % DMSO and 0.25M sucrose WS1: 1 M sucrose, WS2: 0.5 M sucrose, WS3: 0.25 M sucrose, and WS4: 0.125 M sucrose.	ES: 15 min on ice VS: 10 min on ice, then plunged into LN2. Warming: each solution for 5 min.	Slow freezing showed better preservation regardless of the type of follicle. Expression of apoptotic genes was significantly decreased in slow-frozen samples.	[73]
SF	Basic cryoprotectants solution with 2% HAS	FS: 10% DMSO	Cooling: from 0 °C to -8 °C at -2 °C/min, manually seeding, cooling to -40 at 0.3 °C, and -70 °C at 5 °C/min. Thawing: shaking in the water bath at 37 °C for 2 min, then placed in basic cryoprotectants solution for 5 min at RT (three times).		

Table 2. Continued

Method	Basic medium (BS)	Cryoprotectants agent	Equilibrium/Cooling and warming rate	Results	Ref
Vit	"Medium A" supplemented with 0.5% HSA, HEPES (21.8 mM) and glycine (50.0 mM)	solution A: 0.37 M PrOH, 0.37 M EG solution B: 0.75 M PrOH, 0.75 M EG solution C: 1.5 M PrOH, 1.5 M EG supplemented with 0.5 M raffinose WS1: 0.75 M PrOH, 0.75 M EG WS2: 0.37 M PrOH, 0.37 M EG WS3: Medium A	Solution A: 5min at RT, Solution B: 5 min at RT, Solution C: 10 min at +4 °C, then dropped into LN2. Warming: 37 °C water bath for 2 min, WS1: 5 min at 37 °C, WS2: 5 min at 37 °C, WS3: 5 min at 37 °C.	Vitrification preserves follicle and stroma morphology as well as the slow-freezing method and did not increase the rates of follicles and stroma cells with DNA fragmentation	[48]
SF	"Medium A" supplemented with 0.5% HAS with 21.8 mM HEPES and glycine (50.0 mM)	FS1: 3.0 M PrOH and 0.05 M raffinose FS2: 1.5 M PrOH and 0.025 M raffinose TS: Medium A +0.5% HSA	Cooling: Equilibration at 4 °C for 15 min, from 4 °C to -11 °C at -2 °C/min, then lowered to -40 °C at -2 °C/min and from -40 °C to -150 °C at -10 °C/min Thawing: water bath at 37 °C for 2 min, TS: for 5 min at 37 °C (twice).		
Vit	Ham's F10 with 20% HAS	ES: 7.5% EG and 7.5% DMSO VS: 15% EG ,15% DMSO and 0.5 M sucrose WS1: 1 M sucrose, WS2: 0.5 M glucose, Washing solution: BS	ES: 15 min at 38 °C VS: 2 min at RT, then plunged into LN2. Warming: each solution at 38 °C for 5 min.	Slow-freezing and vitrification results showed similar morphological integrity and rates of follicular proliferation and apoptosis.	[74]
SF	Ham's F10 with 20% HAS	FS: 1.5 M DMSO and 0.1 M sucrose	Cooling: from 4 °C to -8 °C at a rate of -2 °C/min, manual seeding, cooling to -40 °C at a rate of -0.3 °C/min, and -30 °C/min to -150 °C Thawing: water bath at 38 °C for 2 min, then washed in Ham's F10 at 38 °C (three times)		
Vit	Leibovitz medium (L-15) with 20% FBS	ES: 7.5% EG and 7.5% DMSO VS: 13.5% EG,13.5% DMSO and 0. 5M sucrose	ES: 10 min at RT VS: 2 min at RT, then plunged into LN2.	Morphologically abnormal primordial follicles and the rates of TUNEL-positive in these cells were lower in vitrification than in slow freezing group.	[75]
SF	Leibovitz medium (L-15) with 10% FBS	FS: 1.5 M DMSO with 0.1 M sucrose	From 4 °C to -7.0 °C at a rate of -2.0 °C/min, held for 5 min manual seeding, held for 10 min, cooling to -40.0 °C at a rate of -0.3 °C/min and -140 °C at a rate of -10 °C/min		

Vit: Vitrification, SF: Slow freezing, ES: Equilibration solution, VS: Vitrification solution, WS: Warming solution, TS: Thawing solution, DS: Diluent solution, FS: Freezing solution, EG: Ethylene glycol, DMSO: Dimethyl sulphoxide, PrOH: 1,2-propanediol, LN2: Liquid nitrogen

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%[56]. 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 it 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[29]. 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.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018;68:7-30.
2. Nagai H, Kim YH. Cancer prevention from the perspective of global cancer burden patterns. J Thorac Dis 2017;9:448-51.
3. Luke B, Brown MB, Missmer SA, Spector LG, Leach RE, Williams M, et al. Assisted reproductive technology use and outcomes among women with a history of cancer. Hum Reprod 2016;31:183-9.
4. Noyes N, Labella PA, Grifo J, Knopman JM. Oocyte cryopreservation: a feasible fertility preservation option for reproductive age cancer survivors. J Assist Reprod Genet 2010;27:495-9.
5. Kometas M, Christman GM, Kramer J, Rhoton-Vlasak A. Methods of Ovarian Tissue Cryopreservation: Is Vitrification Superior to Slow Freezing?-Ovarian Tissue Freezing Methods. Reprod Sci 2021;28:3291-302.
6. Moawad NS, Santamaria E, Rhoton-Vlasak A, Lightsey JL. Laparoscopic Ovarian Transposition Before Pelvic Cancer Treatment: Ovarian Function and Fertility Preservation. J Minim Invasive Gynecol 2017;24:28-35.
7. Yang Q, Zhu L, Jin L. Human Follicle in vitro Culture Including Activation, Growth, and Maturation: A Review of Research Progress. Front Endocrinol (Lausanne) 2020;11:548.
8. Lambertini M, Del Mastro L, Pescio MC, Andersen CY, Azim Jr AH, Peccatori FA, et al. Cancer and fertility preservation: international recommendations from an expert meeting. BMC Med 2016;14:1.
9. Son WY, Henderson S, Cohen Y, Dahan M, Buckett W. Immature Oocyte for Fertility Preservation. Front Endocrinol (Lausanne) 2019;10:464.
10. Son WY, Dahan MH, Monnier P, Holzer H, Nayot D. Early hCG administration as an alternative prevention strategy of ovarian hyperstimulation syndrome during an IVF cycle. Minerva Ginecol 2017;69:207-9.
11. Hourvitz A, Maman E, Brengauz M, Machtinger R, Dor J. In vitro maturation for patients with repeated in vitro fertilization failure due to "oocyte maturation abnormalities". Fertil Steril 2010;94:496-501.

12. Shirasawa H, Kumazawa Y, Sato W, Ono N, Terada Y. In vitro maturation and cryopreservation of oocytes retrieved from intra-operative aspiration during second enucleation for ovarian tumor: A case report. Gynecol Oncol Rep 2016;27;19:1-4.
13. Bos-Mikich A, Ferreira M, Hoher M, Frantz G, Oliveira N, Dutra CG, et al. Fertilization outcome, embryo development and birth after unstimulated IVM. J Assist Reprod Genet 2011;28:107-10.
14. Conti M, Franciosi F. Acquisition of oocyte competence to develop as an embryo: integrated nuclear and cytoplasmic events. Hum Reprod Update 2018;24:245-66.
15. DiLuigi A, Weitzman VN, Pace MC, Siano LJ, Maier D, Mehlmann LM. Meiotic arrest in human oocytes is maintained by a Gs signaling pathway. Biol Reprod 2008;78:667-72.
16. Richani D, Gilchrist RB. The epidermal growth factor network: role in oocyte growth, maturation and developmental competence. Hum Reprod Update 2018;24:1-14.
17. Shuhaibar LC, Egbert JR, Norris RP, Lampe PD, Nikolaev VO, Thunemann M, et al. Intercellular signaling via cyclic GMP diffusion through gap junctions restarts meiosis in mouse ovarian follicles. Proc Natl Acad Sci U S A 2015;112:5527-32.
18. Yokoo M, Kimura N, Sato E. Induction of oocyte maturation by hyaluronan-CD44 interaction in pigs. J Reprod Dev 2010;56:15-9.
19. Son WY, Tan SL. Laboratory and embryological aspects of hCG-primed in vitro maturation cycles for patients with polycystic ovaries. Hum Reprod Update 2010;16:675-89.
20. Sugishita Y, Taylan E, Kawahara T, Shahmurzada B, Suzuki N, Oktay K. Comparison of open and a novel closed vitrification system with slow freezing for human ovarian tissue cryopreservation. J Assist Reprod Genet 2021;38:2723-33.
21. Fesahat F, Dehghani Firouzabadi R, Faramarzi A, Khalili MA. The effects of different types of media on in vitro maturation outcomes of human germinal vesicle oocytes retrieved in intracytoplasmic sperm injection cycles. Clin Exp Reprod Med 2017;44:79-84.
22. Vanhoutte L, Nogueira D, De Sutter P. Prematuration of human denuded oocytes in a three-dimensional co-culture system: effects on meiosis progression and developmental competence. Hum Reprod 2009;24:658-69.
23. Sanchez F, Lolicato F, Romero S, De Vos M, Van Ranst H, Verheyen G, et al. An improved IVM method for cumulus-oocyte complexes from small follicles in polycystic ovary syndrome patients enhances oocyte competence and embryo yield. Hum Reprod 2017;32:2056-68.
24. Ben-Ami I, Komsky A, Bern O, Kasterstein E, Komarovsky D, Ron-El R. In vitro maturation of human germinal vesicle-stage oocytes: role of epidermal growth factor-like growth factors in the culture medium. Hum Reprod 2011;26:76-81.
25. Tucker MJ, Wright G, Morton PC, Massey JB. Birth after cryopreservation of immature oocytes with subsequent in vitro maturation. Fertil Steril 1998;70:578-9.
26. Cohen Y, St-Onge-St-Hilaire A, Tannus S, Younes G, Dahan MH, Buckett W, et al. Decreased pregnancy and live birth rates after vitrification of in vitro matured oocytes. J Assist Reprod Genet 2018;35:1683-9.
27. Chian RC, Huang JY, Gilbert L, Son WY, Holzer H, Cui SJ, et al. Obstetric outcomes following vitrification of in vitro and in vivo matured oocytes. Fertil Steril 2009;91:2391-8.
28. Segers I, Bardhi E, Mateizel I, Van Moer E, Schots R, Verheyen G, et al. Live births following fertility preservation using in-vitro maturation of ovarian tissue oocytes. Hum Reprod 2020;35:2026-36.
29. Lee S, Ozkavukcu S, Ku SY. Current and Future Perspectives for Improving Ovarian Tissue Cryopreservation and Transplantation Outcomes for Cancer Patients. Reprod Sci 2021;28:1746-58.
30. Rivas Leonel EC, Lucci CM, Amorim CA. Cryopreservation of Human Ovarian Tissue: A Review. Transfus Med Hemother 2019;46:173-81.
31. Donnez J, Dolmans MM, Squifflet J, Kerbrat G, Jadoul P. Live birth after allografting of ovarian cortex between monozygotic twins with Turner syndrome (45,XO/46,XX mosaicism) and discordant ovarian function. Fertil Steril 2011;96:1407-11.
32. Donnez J, Jadoul P, Pirard C, Hutchings G, Demylle D, Squifflet J, et al. Live birth after transplantation of frozen-thawed ovarian tissue after bilateral oophorectomy for benign disease. Fertil Steril 2012;98:720-5.
33. Masciangelo R, Bosisio C, Donnez J, Amorim CA, Dolmans MM. Safety of ovarian tissue transplantation in patients with borderline ovarian tumors. Human reproduction (Oxford, England) 2018;33:212-9.
34. Donnez J, García-Solares J, Dolmans MM. Ovarian endometriosis and fertility preservation: a challenge in 2018. Minerva Ginecol 2018;70:408-14.
35. Mirzaeian L, Rafipour H, Hashemi S, Zabihzadeh S, Amanpour SJB, Research CC. Cryopreservation Options to Preserve Fertility in Female Cancer Patients: Available Clinical Practice and Investigational Strategies from the Oncology Guidelines Point of View. Basic Clin Cancer Res 2020;12:42-53.
36. Salama M, Woodruff TK. New advances in ovarian autotransplantation to restore fertility in cancer patients. Cancer Metastasis Rev 2015;34:807-22.
37. Diaz-Garcia C, Domingo J, Garcia-Velasco JA, Herraiz S, Mirabet V, Iniesta I, et al. Oocyte vitrification versus ovarian cortex transplantation in fertility preservation for adult women undergoing gonadotoxic treatments: a prospective cohort study. Fertil Steril 2018;109:478-85.e2.
38. Karimi-Zarchi M, Khalili MA, Binesh F, Mahboubeh VJSAJoC. Ovarian Tissue Reservation and Risk of Reimplantation in a Young Girl with Acute Lymphocytic Leukemia after 6-Month Chemotherapy: A Case Report. South Asian J Cancer 2021;10:112-4.
39. Oktay K, Harvey BE, Partridge AH, Quinn GP, Reinecke J, Taylor HS, et al. Fertility Preservation in Patients With Cancer: ASCO Clinical Practice Guideline Update. J Clin Oncol 2018;36:1994-2001.
40. Peng L. Ovarian tissue freezing and activation after thawing: an update. Middle East Fertil Soc J 2021;26:1-5.
41. Yding Andersen C, Mamsen LS, Kristensen SG. FERTILITY PRESERVATION: Freezing of ovarian tissue and clinical opportunities. Reproduction 2019;158:F27-F34.
42. Pacheco F, Oktay K. Current Success and Efficiency of Autologous Ovarian Transplantation: A Meta-Analysis. Reprod Sci 2017;24:1111-20.
43. Andersen ST, Pors SE, Poulsen LC, Colmorn LB, Macklon KT, Ernst E, et al. Ovarian stimulation and assisted reproductive technology outcomes in women transplanted with cryopreserved ovarian tissue: a systematic review. Fertil Steril 2019;112:908-21.
44. Practice Committee of American Society for Reproductive Medicine. Ovarian tissue cryopreservation: a committee opinion. Fertil Steril 2014;101:1237-43.
45. Arav A, Patrizio P. Techniques of Cryopreservation for Ovarian Tissue and Whole Ovary. Clin Med Insights Reprod Health 2019;13:1179558119884945.

46. Vatanparast M, Khalili MA, Yari N, Omidi M, Mohsenzadeh M. Evaluation of sheep ovarian tissue cryopreservation with slow freezing or vitrification after chick embryo chorioallantoic membrane transplantation. Cryobiology 2018;81:178-84.
47. Amorim CA, Curaba M, Van Langendonckt A, Dolmans MM, Donnez J. Vitrification as an alternative means of cryopreserving ovarian tissue. Reprod Biomed Online 2011;23:160-86.
48. Sanfilippo S, Canis M, Smitz J, Sion B, Darcha C, Janny L, et al. Vitrification of human ovarian tissue: a practical and relevant alternative to slow freezing. Reprod Biol Endocrinol 2015;13:67.
49. Oktem O, Alper E, Balaban B, Palaoglu E, Peker K, Karakaya C, et al. Vitrified human ovaries have fewer primordial follicles and produce less antimüllerian hormone than slow-frozen ovaries. Fertil Steril 2011;95:2661-4.e1.
50. Zhou XH, Zhang D, Shi J, Wu YJ. Comparison of vitrification and conventional slow freezing for cryopreservation of ovarian tissue with respect to the number of intact primordial follicles: A meta-analysis. Medicine 2016;95:e4095.
51. Shi Q, Xie Y, Wang Y, Li S. Vitrification versus slow freezing for human ovarian tissue cryopreservation: a systematic review and meta-anlaysis. Sci Rep 2017;7:8538.
52. Meirow D, Levron J, Eldar-Geva T, Hardan I, Fridman E, Zalel Y, et al. Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. N Engl J Med 2005;353:318-21.
53. Donnez J, Dolmans MM, Demylle D, Jadoul P, Pirard C, Squifflet J, et al. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet 2004;364:1405-10.
54. Lotz L, Dittrich R, Hoffmann I, Beckmann MW. Ovarian Tissue Transplantation: Experience From Germany and Worldwide Efficacy. Clin Med Insights Reprod Health 2019;13:1-8.
55. Leonel ECR, Lucci CM, Amorim CA. Cryopreservation of human ovarian tissue: a review. Transfus Med Hemother 2019;46:173-81.
56. Dolmans MM, von Wolff M, Poirot C, Diaz-Garcia C, Cacciottola L, Boissel N, et al. Transplantation of cryopreserved ovarian tissue in a series of 285 women: a review of five leading European centers. Fertil Steril 2021;115:1102-15.
57. Hanfling SN, Parikh T, Mayhew A, Robinson E, Graham J, Gomez-Lobo V, et al. Case report: two cases of mature oocytes found in prepubertal girls during ovarian tissue cryopreservation. F S Rep 2021;2:296-9.
58. Paulini F, Vilela JM, Chiti MC, Donnez J, Jadoul P, Dolmans MM, et al. Survival and growth of human preantral follicles after cryopreservation of ovarian tissue, follicle isolation and short-term xenografting. Reprod Biomed Online 2016;33:425-32.
59. Dolmans MM, Manavella DD. Recent advances in fertility preservation. J Obstet Gynaecol Res 2019;45:266-79.
60. Telfer EE, Zelinski MB. Ovarian follicle culture: advances and challenges for human and nonhuman primates. Fertil Steril 2013;99:1523-33.
61. White YA, Woods DC, Takai Y, Ishihara O, Seki H, Tilly JL. Oocyte formation by mitotically active germ cells purified from ovaries of reproductive-age women. Nat Med 2012;18:413-21.
62. Fasano G, Demeestere I, Englert Y. In-vitro maturation of human oocytes: before or after vitrification? J Assist Reprod Genet 2012;29:507-12.
63. Mohsenzadeh M, Khalili MA, Nazari S, Jahromi VH, Agharahimi A, Halvaei I. Effect of vitrification on morphology and in-vitro maturation outcome of human immature oocytes. Ital J Anat Embryol 2012;117:190-8.
64. Yazdanpanah F, Khalili MA, Eftekhar M, Karimi H. The effect of vitrification on maturation and viability capacities of immature human oocytes. Arch Gynecol Obstet 2013;288:439-44.
65. Safian F, Khalili MA, Karimi-Zarchi M, Mohsenzadeh M, Ashourzadeh S, Omidi M. Developmental competence of immature oocytes aspirated from antral follicles in patients with gynecological diseases. Iran J Reprod Med 2015;13:507-12.
66. Kasapi E, Asimakopoulos B, Chatzimeletiou K, Petousis S, Panagiotidis Y, Prapas N, et al. Vitrification of Human Germinal Vesicle Oocytes: before or after In Vitro Maturation? Int J Fertil Steril 2017;11:85-92.
67. Madkour A, Bouamoud N, Kaarouch I, Louanjli N, Saadani B, Assou S, et al. Follicular fluid and supernatant from cultured cumulus-granulosa cells improve in vitro maturation in patients with polycystic ovarian syndrome. Fertil Steril 2018;110:710-9.
68. Faramarzi A, Khalili MA, Ashourzadeh S, Palmerini MG. Does rescue in vitro maturation of germinal vesicle stage oocytes impair embryo morphokinetics development? Zygote 2018;26:430-4.
69. Mohsenzadeh M, Tabibnejad N, Vatanparast M, Anbari F, Ali Khalili M, Karimi-Zarchi M. Vitrification has detrimental effects on maturation, viability, and subcellular quality of oocytes post IVM in cancerous women: An experimental study. Int J Reprod Biomed 2019;17:175-84.
70. Chatroudi MH, Khalili MA, Ashourzadeh S, Anbari F, Shahedi A, Safari S. Growth differentiation factor 9 and cumulus cell supplementation in in vitro maturation culture media enhances the viability of human blastocysts. Clin Exp Reprod Med 2019;46:166-72.
71. Labrune E, Jaeger P, Santamaria C, Fournier C, Benchaib M, Rabilloud M, et al. Cellular and Molecular Impact of Vitrification Versus Slow Freezing on Ovarian Tissue. Tissue Eng Part C Methods 2020;26:276-85.
72. 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 MolSci 2019;20:3346.
73. Dalman A, Farahani NSDG, Totonchi M, Pirjani R, Ebrahimi B, Valojerdi MR. Slow freezing versus vitrification technique for human ovarian tissue cryopreservation: An evaluation of histological changes, WNT signaling pathway and apoptotic genes expression. Cryobiology 2017;79:29-36.
74. Klocke S, Bündgen N, Köster F, Eichenlaub-Ritter U, Griesinger G. Slow-freezing versus vitrification for human ovarian tissue cryopreservation. Arch Gynecol Obstet 2015;291:419-26.
75. Xiao Z, Wang Y, Li LL, Li SW. In vitro culture thawed human ovarian tissue: NIV versus slow freezing method. Cryo Letters 2013;34:520-6.