

Review Article



Toward precision medicine for preserving fertility in cancer patients: existing and emerging fertility preservation options for women

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ABSTRACT

As the number of young cancer survivors increases, quality of life after cancer treatment is becoming an ever more important consideration. According to a report from the American Cancer Society, approximately 810,170 women were diagnosed with cancer in 2015 in the United States. Among female cancer survivors, 1 in 250 are of reproductive age. Anticancer therapies can result in infertility or sterility and can have long-term negative effects on bone health, cardiovascular health as a result of reproductive endocrine function. Fertility preservation has been identified by many young patients diagnosed with cancer as second only to survival in terms of importance. The development of fertility preservation technologies aims to help patients diagnosed with cancer to preserve or protect their fertility prior to exposure to chemo- or radiation therapy, thus improving their chances of having a family and enhancing their quality of life as a cancer survivor. Currently, sperm, egg, and embryo banking are standard of care for preserving fertility for reproductive-age cancer patients; ovarian tissue cryopreservation is still considered experimental. Adoption and surrogate may also need to be considered. All patients should receive information about the fertility risks associated with their cancer treatment and the fertility preservation options available in a timely manner, whether or not they decide to ultimately pursue fertility preservation. Because of the ever expanding number of options for treating cancer and preserving fertility, there is now an opportunity to take a precision medicine approach to informing patients about the fertility risks associated with their cancer treatment and the fertility preservation options that are available to them.

Keywords: Cancer; Chemo- or radiation therapy; Fertility Preservation; Technologies; Precision Medicine; Oocyte

Funding

This work was supported by the Center for Reproductive Health after Disease (P50HD076188) from the National Institutes of Health National Center for Translational Research in Reproduction and Infertility (NCTRI) (to SYK and TKW) and by a grant of the Korea Healthcare Technology R&D Project, Ministry of Health and Welfare, Republic of Korea (HI12C0055) (to SKK and JRL).

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

INTRODUCTION

During embryonic development, germ cells proliferate and reach a maximal number (6–7 million) at 20 weeks of gestation [1,2]. This number decreases due to atresia to 1 million at birth and to 400,000 oocytes around puberty. During the reproductive lifespan, this finite number of oocytes—called the ovarian reserve—further decreases with each menstrual cycle, ultimately reaching 1,000 at the time of menopause (**Fig. 1A**) [3,4]. Endocrine function and fertility depend on the cyclical recruitment and development of oocytes from the ovarian reserve. The ovarian follicle secretes hormones that are necessary for not only oocyte development and fertility, but also endocrine function that impacts other systems, such as the cardiovascular system and bone. Thus, preservation of the ovarian reserve is necessary to maintain overall women's health (**Fig. 1B**). Unfortunately, chemo- and radiation therapies that are effective in eliminating cancer cells also destroy growing oocytes in the ovary (**Fig. 1B**). The toxic environment also negatively affects the oocytes in the ovarian reserve, causing death or abnormal activation leading to atresia (**Fig. 1C**), all of which results in depletion of ovarian follicles and hormonal imbalance (**Fig. 1D**) [5,6].

The degree of depletion of ovarian follicles caused by cancer treatment depends on the dose and type of chemotherapy and radiation, fractionation scheme, and irradiation field used [7–9]. Oocytes are one of the most sensitive cells in the human body to radiation; indeed, 50% of immature human oocytes can be destroyed by exposure to 2 Gy of radiation [8,10]. Exposure to abdominal radiation of 20 to 30 Gy in childhood or total-body irradiation of 15 Gy leads to loss of ovarian function (premature ovarian failure, POF) and increased risk of adverse pregnancy outcomes in up to 90% of patients [11,12]. Chemotherapy that includes alkylating agents such as cyclophosphamide (Cy) or busulfan affects somatic cells in other tissues as well as the oocyte and the ovarian follicle.

In men with cancer, current options for preserving fertility prior to or during chemo- and radiation therapy include gonadal shielding and sperm banking (semen cryopreservation). Testis-sparing surgery can also preserve fertility in male cancer patients. In women with cancer, fertility preservation strategies include controlled ovarian stimulation (COS) followed by *in vitro* maturation (IVM) of the retrieved oocytes, which can then be either cryopreserved or fertilized *in vitro* (IVF) to produce embryos for cryopreservation [13]. Ovarian tissue cryopreservation is an experimental option, with the thawed tissue used for transplantation at later date. Several emerging technologies available in the near future include *in vitro* ovarian follicle culture, ovarian follicle transplantation (artificial ovary), *in vitro* activation of ovarian follicles, and the use of fertoprotective agents (**Fig. 2**). In this review, the various existing and emerging technologies for the preservation of fertility and restoration of reproductive function in women with cancer will be discussed, with the aim of illustrating how cancer treatments and fertility preservation options can be tailored to meet the needs and desires of individual patients.

EXISTING TECHNOLOGIES

In women diagnosed with cancer, there are two standard options for fertility preservation: cryopreservation of mature oocytes or embryos (**Fig. 2A**). Ovarian tissue cryopreservation for later transplantation is still considered to be experimental and can only be performed under

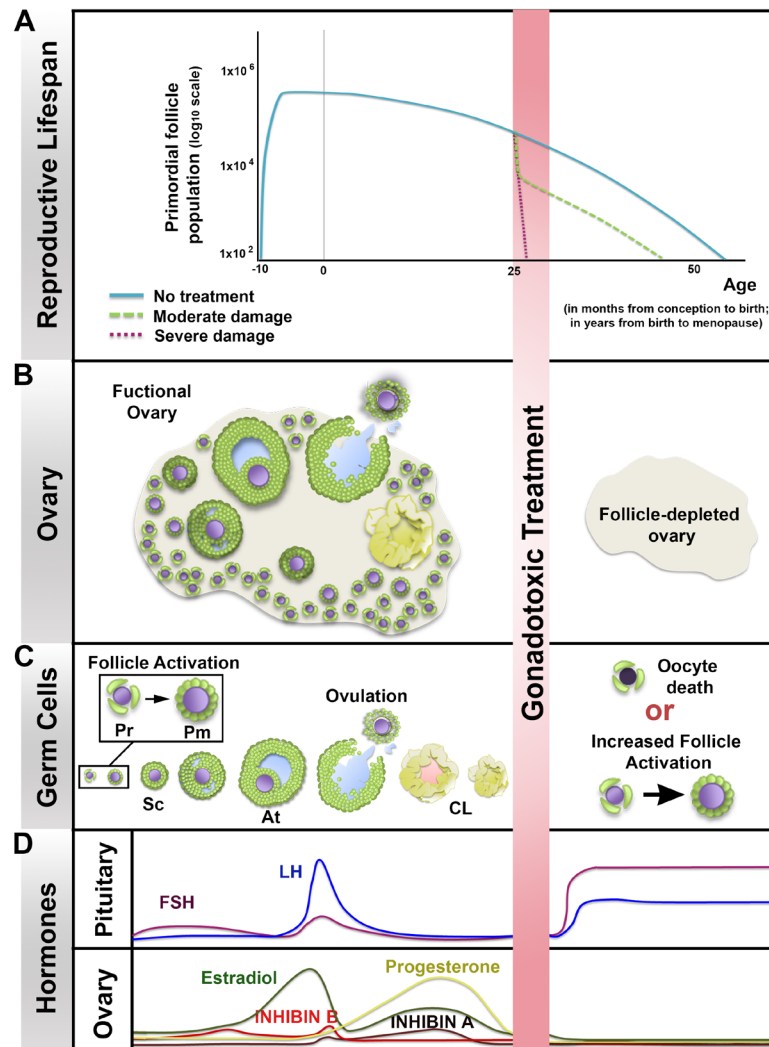


Fig. 1. Consequences of gonadotoxic treatment in reproductive-aged women. (A) The primordial follicle population—the ovarian reserve—can be represented by a parabolic curve across the female lifespan in which activation or death of primordial ovarian follicles occurs progressively with each menstrual cycle, from puberty to the menopause. Reprinted from Wallace and Kelsey [4]. (B–D) The growth of follicles with each cycle maintains hormonal balance necessary for overall women’s health. Gonadotoxic stress or treatment, such as chemotherapy or radiation therapy (red bar across all panels), induces a rapid decrease in the highly sensitive primordial follicles of the ovarian reserve (A, C), resulting in a follicle-depleted ovary (B) and premature ovarian failure (POF). Depletion of the ovarian reserve disrupts normal endocrine function and the production of hormones such as follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, progesterone, inhibin B, and inhibin A (D), leading to hormonal imbalance similar to that seen in postmenopausal women.

IRB approval. Selecting the optimal method for fertility preservation depends on timing of cancer treatment, treatment regimen, cancer type, age of the patient, and presence or absence of a partner.

1. Embryo cryopreservation

Embryo cryopreservation is the most widely used method in infertility clinics, resulting in thousands of births each year from frozen embryos (Fig. 2A). Human embryos are highly resistant to damage by freezing, with reported survival and implantation rates of over 90%

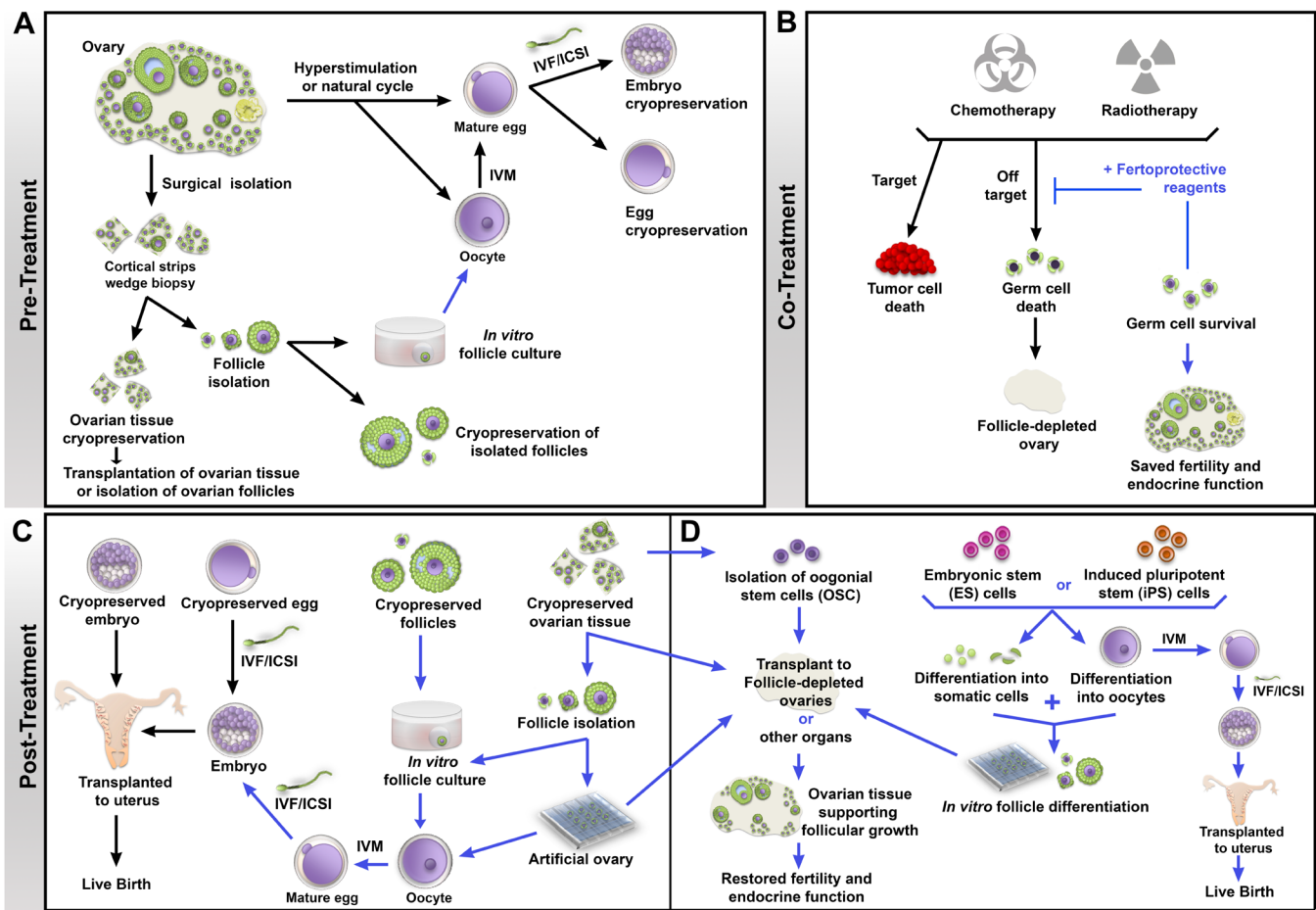


Fig. 2. Schema of fertility preservation approaches in cancer patients. Each procedure is marked with a line, either black (established) or blue (investigational). (A) Patients undergoing a natural or hyperstimulated cycle produce mature eggs that can be matured *in vitro* (IVM) and either cryopreserved or fertilized by *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) to produce embryos that are cryopreserved. In patients who cannot undergo controlled ovarian stimulation (e.g., prepubertal girls or women with hormone-sensitive cancers) or in those who must start cancer treatment immediately, strips of ovarian cortex can be removed and cryopreserved, or individual follicles can be isolated from the tissues and cryopreserved. Investigational methods are focusing on use of thawed ovarian tissues for transplantation or *in vitro* growth of follicles, followed by IVM and IVF/ICSI. (B) During treatment, gonadotoxicity can be mitigated by the use of fertoprotective reagents that induce death in tumor cells while preventing off-target effects in other tissues, resulting in preserved fertility and endocrine function. (C) After cancer treatment, cryopreserved embryos can be thawed and transferred into the uterus, or cryopreserved eggs can be thawed and used for IVF/ICSI and the resulting embryos transferred to the patient. On the investigational side (blue arrows), cryopreserved follicles can be grown in culture, matured *in vitro*, and fertilized to produce embryos for transfer. Ovarian tissue can be transplanted back into the patient, or follicles can be isolated and used to produce embryos or cultured on a three-dimensional bioplotting scaffold as an artificial ovary for transplantation back into patients. Transplantation of ovarian tissue or follicles within an artificial ovary has the potential of restoring fertility as well as endocrine function after cancer treatment. (D) In patients who were unable to cryopreserve eggs, embryos, or ovarian tissue prior to treatment, researchers are now investigating the possibility of using oogonial stem cells (OSCs) to repopulate follicle-depleted ovaries, or differentiating follicle somatic cells and oocytes from embryonic stem (ES) cells or induced pluripotent stem (iPS) cells to assemble follicles *de novo* for transplantation or IVM and IVF/ICSI to create embryos for transfer.

and 30%, respectively [14]. Moreover, the live birth rate per embryo transfer has been reported to be 20%, while the accumulated pregnancy rate from cryopreservation of multiple embryos has been reported to be more than 60% in infertility patients [15]. Since the entire embryo cryopreservation procedure requires approximately 2 weeks, it is not an option for women who have aggressive cancers and must start cancer treatment immediately. Because women must undergo COS to produce mature eggs, this option is also not recommended for women who have hormone-sensitive cancers and is not possible in prepubertal girls. For women without a partner, donated sperm can be used to create embryos.

2. Oocyte cryopreservation

In 2013, the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology announced that modern procedures for oocyte cryopreservation should no longer be considered experimental [16]. This method provides an alternative to embryo cryopreservation for those women who do not have a partner or do not want to use donated sperm. In addition, the cryopreserved oocyte is the legal property of the woman, which avoids the complex custody issues often associated with cryopreserved embryos. Like embryo cryopreservation, however, cryopreservation of oocytes involves hormonal stimulation of the ovary (COS), so this method is not available to women with aggressive cancers that require immediate treatment or hormone-sensitive cancers, or for prepubertal girls. Collected oocytes can be cryopreserved as mature MII eggs or as immature germinal vesicle (GV) oocytes.

1) Mature oocyte cryopreservation

The first live birth from a cryopreserved oocyte was reported by Chen [17] in 1986. Due to improved freezing and thawing techniques, the pregnancy rate with cryopreserved oocytes has improved considerably. Recently, high survival rates ($\geq 90\%$) and pregnancy rates (40% to 55%) have been reported in egg donation programs [18]. A number of studies have reported no differences in the pregnancy rate or implantation rate of embryos obtained from cryopreserved mature oocytes compared with those from fresh oocytes, and further, no noticeable differences in chromosomal abnormalities have been reported [19-21]. At the present time, mature oocyte cryopreservation is a preferred fertility preservation method if the individual is postpubertal and if chemotherapy or radiation treatment can be delayed to allow time for the full procedure.

2) Immature oocyte cryopreservation and *in vitro* maturation

Aspiration of immature oocytes followed by IVM is an option for patients who are unable to undergo ovarian stimulation, including prepubertal girls, women with hormone-sensitive cancers, and patients with polycystic ovarian syndrome (PCOS) who cannot undergo stimulation due to ovarian hyperstimulation syndrome. Aspiration of immature oocytes offers the advantage of allowing immediate cancer treatment, since COS is not required. In addition, it is thought that less damage is caused by cryopreservation of immature oocytes than mature oocytes, as immature oocytes lack the spindle and nuclear membrane that protect the chromatin. However, studies have reported that cryopreserved mature oocytes achieved better outcomes compared to IVM of cryopreserved immature oocytes [22]. For this reason, immature oocytes are often matured by IVM and then cryopreserved as mature oocytes [22,23]. There have been only a few reports of successful birth using cryopreserved oocytes or embryos from immature oocytes that were matured *in vitro* [24]. So far, this technique has mainly been used in PCOS patients, and data on efficacy in cancer patients are not available.

3. Ovarian stimulation protocols used for embryo or mature oocyte cryopreservation

1) Random-start COS

In a conventional COS protocol for embryo or oocyte cryopreservation, cycles require up to 5 weeks, since ovarian stimulation must be started on day 2 to 3 of the menstrual cycle (Fig. 2A). In many cases, this time frame is appropriate, since many patients undergo tumor

resection prior to chemo- or radiation therapy. That said, some patients need to start cancer treatment sooner and therefore random start COS protocols have been developed. In this procedure, ovarian stimulation is initiated irrespective of the phase of the cycle [25-27]. This random start protocol is based on the recent concept that there are multiple waves of follicle recruitment within a single interovulatory period [28]. Moreover, this approach is possible because the purpose of the protocol is to retrieve mature eggs from the ovary and does not require the full maturation of the uterus. Several case reports and comparative studies have shown that the number of total and mature oocytes retrieved, oocyte maturity rates, and fertilization rates were not compromised in random-start COS cycles compared to conventional cycles [29,30]. This protocol minimizes delays in treatment and enables more patients to pursue fertility preservation with egg or embryo cryopreservation prior to chemo- or radiation therapy.

2) The use aromatase inhibitors or tamoxifen in patients with hormone-sensitive cancer
 For women who have hormone-sensitive cancers, such as breast cancer, mature oocyte retrieval can be attempted during a natural cycle to avoid exposure to supraphysiological levels of estrogen during COS. However, the number of oocytes collected is reported to be low. Recently, daily letrozole administration during COS has been used to prevent high levels of circulating estradiol in patients with estrogen-sensitive tumors. A commonly used protocol involves the use of 5 mg of letrozole from the 2nd to 3rd day of the cycle, followed by initiation of gonadotropins. The use of letrozole did not affect the number of oocytes retrieved, but a lower oocyte maturity rate in letrozole cycles was reported in some studies [30,31]. This protocol is used only to obtain oocytes for cryopreservation, after which patients can then proceed to their planned cancer treatment. Some studies have examined the use of letrozole immediately prior to embryo transfer; in a study of 911 infants, there was no difference in the incidence of birth defects in children when letrozole versus clomiphene was used during ovulation stimulation [32]. Thus far, prospective data regarding the risk of breast cancer recurrence in patients undergoing COS with the letrozole protocol are reassuring when compared to the risk of recurrence in women with breast cancer who did not undergo COS [33].

4. Ovarian tissue cryopreservation

Ovarian tissue cryopreservation—as opposed to oocyte or embryo cryopreservation—does not require COS or a delay the start of cancer therapy, and there is no need for a partner or sperm donor. Ovarian tissue cryopreservation may be the best option for preserving fertility for single women who do not wish to use donated sperm and prepubertal girls. However, this procedure is invasive, and requires general anesthesia and surgical removal of ovarian tissue. In addition, there are relatively few medical centers with experience in this procedure. Because the ovarian reserve—the number of immature oocytes enclosed in follicles within the ovary—is age-dependent, the procedure should not be offered to women after the age of 39 years (Oncofertility Consortium) [34,35]. Once removed, the ovarian tissue, which contains multiple follicles, is cryopreserved, and can potentially be used later to restore fertility in two main ways: (1) transplantation of the thawed tissue back into the cancer survivor or (2) isolation of follicles from the thawed tissue for *in vitro* growth, maturation, and fertilization (Fig. 2C). The main causes of low follicular survival rate after transplantation are cryo-induced injury and ischemic damage. Preventing cryo-injury and reducing the duration of ischemia from transplantation to angiogenesis is essential for preservation of follicles and extension of function and lifespan of the transplanted tissue [36].

1) Cryopreservation of ovarian cortical tissue

In 2004, Donnez et al. [37] reported the first successful pregnancy and birth from transplantation of strips of cryopreserved ovarian cortex (Fig. 2C). To date, at least 60 live births from either natural conception or IVF have been achieved after re-implantation of cryopreserved ovarian tissue [38]. Women who receive an ovarian tissue transplant are generally poor responders to COS, since of the 500 to 1,000 primordial follicles that are present in the transplanted ovarian tissue, more than 50% are lost [37]. Restoration of ovarian activity is higher if primordial follicles are present in the transplanted tissue and the chance of live birth after replacement of ovarian tissue is roughly 20% [39]. Recently, the first case was reported of successful fertility restoration after transplantation of ovarian tissue cryopreserved before menarche [40]. According to studies published to date, it takes approximately 4 to 6 months after transplantation of ovarian cortex tissue for follicular development to resume, with a rise in estradiol [41]. This is consistent with the 4- to 6-month period required for a primordial follicle to progress to the large antral follicle stage with a fully mature oocyte. Follicle-stimulating hormone values during the follicular phase were relatively higher after transplantation, which can be explained by the small number of primordial follicles that survived in the transplanted tissues. Following transplantation, ovarian function continues for approximately 5 years on average [38,39].

2) Cryopreservation of whole ovary

Theoretically, whole ovary transplantation with vascular anastomosis is the most ideal method for minimizing duration of ischemia from transplantation to angiogenesis because it allows immediate blood circulation after the transplantation. However, adequate dispersion of cryoprotectant throughout the ovary is difficult in large mammals or humans due to the size of the tissue. Vascular damage from formation of ice crystals in the blood vessels can also occur. Recently, Martinez-Madrid et al. [42] proposed a method involving cryopreservation of the whole ovary together with blood vessels, which showed high post-thawing survival rate (75.1%) of follicles, blood vessels, and stromal structures. Bedaiwy et al. [43] also achieved a high follicle survival rate after freezing and thawing of the whole ovary, using a method that involved dispersing cryoprotectant into the ovary parenchyma by injection through the ovarian artery. Further studies on whole-ovary cryopreservation and transplantation are necessary for this technique to be applied in clinical practice.

3) Aspiration of immature oocytes during ovarian tissue cryopreservation

Oocytes collected from ovarian tissue prior to ovarian tissue cryopreservation could be cryopreserved or undergo IVM to produce mature oocytes for cryopreservation. Oocytes can be collected from antral follicles within the ovarian tissue or from the dissection medium. There is only one report of a live birth resulting from cryopreserved embryos that were created using IVM oocytes retrieved from ovarian tissue after oophorectomy [44].

4) Practical considerations with ovarian tissue cryopreservation

(1) Freezing methods

Rapid advances in vitrification methods have led to successful cryopreservation of embryos and mature oocytes. However, unlike embryo and oocyte cryopreservation, studies comparing slow freezing and vitrification of ovarian tissue have presented conflicting results [45-47]. Most reported live human births were achieved after transplantation of ovarian tissue that had been slow-frozen [39] and there are only a few reports of live births from transplantation

of vitrified human ovarian tissues [48,49]. An optimal protocol for the vitrification of ovarian tissue has been investigated [36,50,51]. The results of recent studies show that both vitrification and slow freezing are able to preserve follicle and stromal morphology.

(2) Transplantation sites

Orthotopic reimplantation of cryopreserved ovarian tissue onto the ovary has the advantage of allowing for resumption of natural cycling within an appropriate environment for follicular development. When the ovary remains in the pelvic cavity, the ovarian tissue is transplanted below the cortical capsule. When no ovary remains on which to transplant, the tissue is instead transplanted into the peritoneal window. Heterotopic transplantation, in which the ovarian tissue is transplanted to sites other than the pelvic cavity, includes the rectum, pectoralis muscle, abdominal wall, and forearm [52-56]. Most reported live births using cryopreserved ovarian tissue were from orthotopic transplantation, with only one published case report of a live birth after heterotopic transplantation [57].

Recurrence of the primary cancer or malignant transformation of transplanted ovarian tissue are possible outcomes of ovarian tissue transplantation. Although most cancers that occur in reproductive-age women rarely metastasize to the ovary, lobular tumors in the breast, leukemia, and Burkitt's lymphoma are known to frequently metastasize to the ovary. Moreover, the risk of malignant transformation of the ovarian tissue may be increased by the rapid temperature changes that occur during the cryopreservation process, exposure to cryoprotectant, and incomplete methylation during the IVM process [58-61]. Therefore, thorough examination of the ovarian tissue to detect residual malignant disease needs to be performed prior to both cryopreservation and transplantation.

5. Fertoprotective adjuvant therapy

Another approach to preventing ovarian damage by cancer treatment is to mitigate gonadotoxicity by administering fertoprotective agents (Fig. 2B). Recently, gonadotropin-releasing hormone (GnRH) agonists have been used during chemotherapy as a way to reduce the toxic effects of cancer treatment on growing ovarian follicles and their enclosed oocytes. GnRH agonists interfere with the hypothalamic-pituitary-gonadal axis and block ovarian function by suppressing gonadotropin levels to prepubertal levels. GnRH agonists are administered at least 10 days before the beginning of chemotherapy because of the initial flare-up effect and treatment continues to 2 weeks after the end of chemotherapy. It is still unclear how GnRH agonists protect the ovary from cancer treatment-induced damage or preserve fertility after treatment [62]. Nevertheless, recent studies have demonstrated protective effects of GnRH agonists, with a highly significant reduction in amenorrhea and ovarian insufficiency [63,64].

EMERGING TECHNOLOGIES

1. Ovarian follicle culture *in vitro*

Some cancer patients, including those who have been diagnosed with acute lymphoblastic leukemia or acute myeloblastic leukemia (AML), cannot delay cancer treatment and thus cannot utilize embryo or oocyte cryopreservation methods to preserve fertility. Ovarian tissue cryopreservation, as described above, has been investigated as an alternative option; however, the cryopreserved ovarian tissue might contain lingering malignant tumor cells such as

leukemic cells [65], which could be reintroduced into patients when the tissue is transplanted [66,67]. Cancer recurrence after transplantation of cryopreserved ovarian tissue was recently reported [68]. Some studies in male mice have demonstrated that re-introduction of as few as 10 AML cells was able to induce leukemia [69]. Therefore, careful consideration should be given before the transplantation of frozen-thawed ovarian tissues. Moreover, breast cancer patients who carry BRAC1 and BRAC2 mutations have an increased potential of developing ovarian cancer [70]. To mitigate the risks of reintroducing cancer cells with transplanted ovarian tissue, methods to isolate and culture individual follicles from banked tissue are practical approaches. The goal of these methods are to be able to retrieve fully developed oocytes from cultured follicles that could be matured *in vitro* and fertilized, producing viable embryos for transfer to the uterus (Fig. 2C).

Techniques to mechanically isolate ovarian follicles were first established in the 1990s and have greatly improved since then. Isolated single follicles can be cultured in two-dimensional (2D) [71] or three-dimensional (3D) systems (Fig. 2A, C) [9,72,73]. For example, bovine follicles in 2D culture successfully produce estradiol and form antral cavities, suggesting that 2D culture is capable of supporting follicular viability and function [74]. Recent reports using murine follicles have found that 3D culture systems are more appropriate than 2D culture systems with regard to maintaining the spatial morphology, growth rate, and gene expression patterns associated with normal oocyte development [75]. Three-dimensional *in vitro* culture methods have been developed that mimic the native physiological environment of the ovary *in vivo* by maintaining a spherical morphology and the cell-cell and cell-matrix interactions between the oocyte and somatic cells of the follicle. These 3D systems have been shown to achieve greater follicular viability, follicle and oocyte diameters, and steroid hormone production [76-81], and have been successfully used to culture follicles from mice [76], buffalo [82], rhesus monkeys [83], and humans [77,84,85].

2. Ovarian follicle transplantation (artificial ovary)

While *in vitro* follicle culture presents an alternative to ovarian tissue transplantation that avoids the risk of reintroducing cancer cells, researchers have also been working on the development of an engineered ‘artificial ovary’ for transplantation. This artificial ovary comprises follicles isolated from a patient’s cryopreserved ovarian tissue, along with other ovarian cells, which are assembled on a 3D matrix scaffold; the resulting structure would allow follicles to grow within an ovary-like environment and would potentially restore both fertility and endocrine function once transplanted into the patient [86-89]. Researchers have successfully grown ovarian follicles on a bioplotting scaffold seeded with isolated mouse follicles [89]. Evidence of vascularization has been observed in fibrin clots containing ovarian cells grafted into mice [90]. Recent studies have shown that an artificial ovary using alginate microcapsules with granulosa and theca cells from rats was able to produce the sex hormones estrogen and progesterone [91] and that primary ovarian cells seeded onto decellularized scaffolds successfully produced estradiol [92]. These studies reflect promising progress in the development of an artificial ovary, not only for applications in restoring fertility of cancer patients, but also for providing a possible alternative to hormone replacement therapy, since artificial ovaries can potentially restore a functional endocrine system.

3. Oogonial stem cells

When healthy follicles—for either *in vitro* follicle growth or for building an artificial ovary—are not able to be retrieved from the ovarian tissue of cancer patients, other sources of

cells carrying the patient's genetic material may be considered, including oogonial stem cells (OSCs) (Fig. 2D) [93]. The existence of OSCs remains controversial, however [94]. Another potential source may be oocytes derived from embryonic stem (ES) cells or induced pluripotent stem (iPS) cells. This method has been successful in male mice, in which normal sperm was produced from ES cells [95]. A recent study reported successful production of oocytes from ES cells, also in mice [96]. These findings provide proof-of-concept support for future methods that may be able to produce new oocytes for cancer patients who have lost all of their oocytes as a result of chemo- or radiation therapy. These methods might also have applications in the treatment of POF.

4. *In vitro* activation of ovarian follicles

Ovarian tissues collected from prepubertal cancer patients contain immature primordial follicles that must be activated in order to enter the growing pool and produce mature oocytes that can be fertilized. The same is true for ovarian tissues from patients with primary ovarian insufficiency who have a reduced ovarian reserve [97]. Researchers have been successful in inducing primordial follicle activation using ovarian fragmentation, drilling, and laser techniques [98]. These approaches disrupt the ovarian Hippo signaling pathway and promote actin polymerization in somatic and granulosa cells, which activates the growth of primordial follicles in the ovarian reserve [48,99]. This work supports the hypothesis that primordial ovarian follicles are held by surrounding cells in a state of restricted growth, and that local Hippo signaling regulates the location of growing follicles and interfollicle communication [100]. Once activated, the follicles are treated with Akt stimulators, PI3K activators, and PTEN inhibitors before used in auto-transplantation [49,101-103]. This approach has resulted in a successful live birth.

5. Administration of drugs for Specific target tissues

1) Use of nanoparticles

Researchers are continuing to focus on finding better ways to protect oocytes from the toxic effects of cancer treatments. One approach is to encapsulate chemotherapeutic reagents inside nanoparticles that specifically target cancer cells [104,105], thereby decreasing the plasma levels and toxicity of these drugs into non-target tissues like ovary. This strategy can be applied to other chemotherapeutics to more specifically target cancer cells while also protecting the gonads.

2) Use of fertoprotective reagents

Other studies aim to develop fertoprotective agents that can kill cancer cells but also protect oocytes against chemo- and radiation treatment (Fig. 2B). Several studies have explored the development of such fertoprotective reagents, including the apoptosis inhibitors imatinib, AS101, T3, S1P, G-CSF, and tamoxifen. It has been suggested that imatinib mesylate (Gleevec, Novartis, Basel, Switzerland) protects oocytes through binding to c-Abl [106,107], a protein tyrosine kinase. It was also proposed that c-Abl is upregulated by DNA damage induced by radiation or cisplatin and stimulates apoptotic pathways. TAp63 lies downstream of c-Abl and is a main factor involved in primordial oocyte death [108], indicating that c-Abl may be a target for blocking apoptotic pathways in oocytes using imatinib mesylate. Although c-Abl is upregulated by DNA damage [109], further studies are needed to understand the relevance of c-Abl in oocyte death or for the direct regulatory relationship between c-Abl and TAp63.

AS101 has been identified as an immunomodulator that protects oocytes in large growing follicles co-treated with Cy, through reduction of apoptosis and down-regulation of the PI3K/PTEN/AKT pathway, resulting in a significantly higher numbers of pups from mice treated with Cy and AS101 than with Cy alone [110]. This finding suggests that AS101 can block the abnormal activation and growth of follicles induced by Cy treatment. More importantly, clinical trials have shown that AS101 is not harmful to humans. However, further research is needed to determine how Cy activates primordial follicles without inducing apoptosis.

3,5,3'-Triiodothyronine (thyroid hormone, T3) has anti-apoptotic effects in various cell lines and protects granulosa cells against paclitaxel-induced apoptosis [111]. However, there is no clear evidence of a fertoprotective effect of T3 against chemotherapeutics, requiring further investigation. Sphingosine-1-phosphate (S1P) is an inhibitor of sphingomyelin hydrolysis-induced apoptosis in cells. S1P (FTY720) pretreatment has protective effects against both radiation treatment and chemotherapy agents including dacarbazine (DIC or DTIC) [112]. This effect was confirmed in human ovarian tissue with S1P pretreatment followed by Cy and doxorubicin (DXR) treatment [113,114]. These promising results were replicated in macaques, which had live births after co-treatment of S1P with radiation exposure [115]. However, the mechanism by which S1P protects primate ovaries from cytotoxic drug-induced damage *in vivo* still remains to be determined. Moreover, S1P must be administered directly into the ovary, making clinical translation a challenge. Granulocyte colony-stimulating factor (G-CSF) has shown to reduce the loss of primordial follicles induced by Cy, busulfan, and cisplatin through protection of the ovarian blood vessels [116]. Despite optimistic results showing a protective effect against chemotherapy, the mechanism by which G-CSF protects blood vessels is still not known. Finally, tamoxifen also showed a protective effect against follicular loss caused by Cy, 7,12-dimethylbenzanthracene, and DXR in rat ovaries [117] and against radiation in oocytes [118]. In summary, these data suggest several feasible fertoprotective agents with the potential to protect oocytes against chemo- or radiation therapy may be available in the near future.

CONCLUSIONS

As the number of cancer patients who survive from their disease increases, concerns about quality of life are becoming more important. Young women diagnosed with cancer may be particularly concerned about their ability to have a family in the future as a part of good quality of life as well as to maintain endocrine function for overall their health. There are many options to help patients confront the threat that cancer treatment poses to their fertility, using both existing technologies that are widely available, as well as investigational techniques. New techniques for mitigating the negative effect of cancer treatments, protecting ovarian function, and preserving fertility are constantly being developed. A 'precision medicine' approach should be taken with regard to addressing the fertility concerns of women diagnosed with cancer, with the risks of cancer treatment, and with the best and the most appropriate options for fertility preservation communicated to each patient in a collaborative discussion that includes patients, family members, counselors, and health care providers.

ACKNOWLEDGMENTS

We thank Marilia H. Cordeiro for contribution in the figures and comments on the manuscript and Stacey C. Tobin, Maxwell E. Edmonds, and Megan M. Romero for editing the manuscript.

REFERENCES

1. Baker TG. A quantitative and cytological study of germ cells in human ovaries. *Proc R Soc Lond B Biol Sci* 1963;158:417-33.
[PUBMED](#) | [CROSSREF](#)
2. Mamsen LS, Lutterodt MC, Andersen EW, Byskov AG, Andersen CY. Germ cell numbers in human embryonic and fetal gonads during the first two trimesters of pregnancy: analysis of six published studies. *Hum Reprod* 2011;26:2140-5.
[PUBMED](#) | [CROSSREF](#)
3. Faddy MJ, Gosden RG. A model conforming the decline in follicle numbers to the age of menopause in women. *Hum Reprod* 1996;11:1484-6.
[PUBMED](#) | [CROSSREF](#)
4. Wallace WH, Kelsey TW. Human ovarian reserve from conception to the menopause. *PLoS One* 2010;5:e8772.
[PUBMED](#) | [CROSSREF](#)
5. Morgan S, Anderson RA, Gourley C, Wallace WH, Spears N. How do chemotherapeutic agents damage the ovary? *Hum Reprod Update* 2012;18:525-35.
[PUBMED](#) | [CROSSREF](#)
6. Howell SJ, Shalet SM. Testicular function following chemotherapy. *Hum Reprod Update* 2001;7:363-9.
[PUBMED](#) | [CROSSREF](#)
7. Meirow D, Biederman H, Anderson RA, Wallace WH. Toxicity of chemotherapy and radiation on female reproduction. *Clin Obstet Gynecol* 2010;53:727-39.
[PUBMED](#) | [CROSSREF](#)
8. Wallace WH, Thomson AB, Saran F, Kelsey TW. Predicting age of ovarian failure after radiation to a field that includes the ovaries. *Int J Radiat Oncol Biol Phys* 2005;62:738-44.
[PUBMED](#) | [CROSSREF](#)
9. De Vos M, Smits J, Woodruff TK. Fertility preservation in women with cancer. *Lancet* 2014;384:1302-10.
[PUBMED](#) | [CROSSREF](#)
10. Dursun P, Doğan NU, Ayhan A. Oncofertility for gynecologic and non-gynecologic cancers: fertility sparing in young women of reproductive age. *Crit Rev Oncol Hematol* 2014;92:258-67.
[PUBMED](#) | [CROSSREF](#)
11. Wallace WH, Shalet SM, Crowne EC, Morris-Jones PH, Gattamaneni HR. Ovarian failure following abdominal irradiation in childhood: natural history and prognosis. *Clin Oncol (R Coll Radiol)* 1989;1:75-9.
[PUBMED](#) | [CROSSREF](#)
12. Wo JY, Viswanathan AN. Impact of radiotherapy on fertility, pregnancy, and neonatal outcomes in female cancer patients. *Int J Radiat Oncol Biol Phys* 2009;73:1304-12.
[PUBMED](#) | [CROSSREF](#)
13. Jeruss JS, Woodruff TK. Preservation of fertility in patients with cancer. *N Engl J Med* 2009;360:902-11.
[PUBMED](#) | [CROSSREF](#)
14. Mandelbaum J, Belaisch-Allart J, Junca AM, Antoine JM, Plachot M, Alvarez S, et al. Cryopreservation in human assisted reproduction is now routine for embryos but remains a research procedure for oocytes. *Hum Reprod* 1998;13 Suppl 3:161-74.
[PUBMED](#) | [CROSSREF](#)
15. Senn A, Vozzi C, Chanson A, De Grandi P, Germond M. Prospective randomized study of two cryopreservation policies avoiding embryo selection: the pronucleate stage leads to a higher cumulative delivery rate than the early cleavage stage. *Fertil Steril* 2000;74:946-52.
[PUBMED](#) | [CROSSREF](#)
16. Practice Committees of American Society for Reproductive Medicine; Society for Assisted Reproductive Technology. Mature oocyte cryopreservation: a guideline. *Fertil Steril* 2013;99:37-43.
[PUBMED](#) | [CROSSREF](#)

17. Chen C. Pregnancy after human oocyte cryopreservation. *Lancet* 1986;1:884-6.
[PUBMED](#) | [CROSSREF](#)
18. Cobo A, Garcia-Velasco JA, Domingo J, Remohí J, Pellicer A. Is vitrification of oocytes useful for fertility preservation for age-related fertility decline and in cancer patients? *Fertil Steril* 2013;99:1485-95.
[PUBMED](#) | [CROSSREF](#)
19. Forman EJ, Li X, Ferry KM, Scott K, Treff NR, Scott RT. Oocyte vitrification does not increase the risk of embryonic aneuploidy or diminish the implantation potential of blastocysts created after intracytoplasmic sperm injection: a novel, paired randomized controlled trial using DNA fingerprinting. *Fertil Steril* 2012;98:644-9.
[PUBMED](#) | [CROSSREF](#)
20. Goldman KN, Kramer Y, Hodes-Wertz B, Noyes N, McCaffrey C, Grifo JA. Long-term cryopreservation of human oocytes does not increase embryonic aneuploidy. *Fertil Steril* 2015;103:662-8.
[PUBMED](#) | [CROSSREF](#)
21. Rienzi L, Romano S, Albricci L, Maggiulli R, Capalbo A, Baroni E, et al. Embryo development of fresh 'versus' vitrified metaphase II oocytes after ICSI: a prospective randomized sibling-oocyte study. *Hum Reprod* 2010;25:66-73.
[PUBMED](#) | [CROSSREF](#)
22. Oktay K, Buyuk E, Rodriguez-Wallberg KA, Sahin G. In vitro maturation improves oocyte or embryo cryopreservation outcome in breast cancer patients undergoing ovarian stimulation for fertility preservation. *Reprod Biomed Online* 2010;20:634-8.
[PUBMED](#) | [CROSSREF](#)
23. Son WY, Park SE, Lee KA, Lee WS, Ko JJ, Yoon TK, et al. Effects of 1,2-propanediol and freezing-thawing on the in vitro developmental capacity of human immature oocytes. *Fertil Steril* 1996;66:995-9.
[PUBMED](#)
24. Chen H, Lv JQ, Ge HS, Wu XM, Xi HT, Chi HH, et al. Live birth following vitrification of in vitro matured oocytes derived from sibling smaller follicles at follicle selection phase in the context of in vitro fertilization. *Gynecol Endocrinol* 2014;30:624-6.
[PUBMED](#) | [CROSSREF](#)
25. Bedoschi GM, de Albuquerque FO, Ferriani RA, Navarro PA. Ovarian stimulation during the luteal phase for fertility preservation of cancer patients: case reports and review of the literature. *J Assist Reprod Genet* 2010;27:491-4.
[PUBMED](#) | [CROSSREF](#)
26. Nayak SR, Wakim AN. Random-start gonadotropin-releasing hormone (GnRH) antagonist-treated cycles with GnRH agonist trigger for fertility preservation. *Fertil Steril* 2011;96:e51-4.
[PUBMED](#) | [CROSSREF](#)
27. Sönmez M, Türkçüoğlu I, Coşkun U, Oktay K. Random-start controlled ovarian hyperstimulation for emergency fertility preservation in letrozole cycles. *Fertil Steril* 2011;95:2125.e9-11.
[PUBMED](#) | [CROSSREF](#)
28. de Mello Bianchi PH, Serafini P, Monteiro da Rocha A, Assad Hassun P, Alves da Motta EL, Sampaio Baruselli P, et al. Review: follicular waves in the human ovary: a new physiological paradigm for novel ovarian stimulation protocols. *Reprod Sci* 2010;17:1067-76.
[PUBMED](#) | [CROSSREF](#)
29. Cakmak H, Katz A, Cedars MI, Rosen MP. Effective method for emergency fertility preservation: random-start controlled ovarian stimulation. *Fertil Steril* 2013;100:1673-80.
[PUBMED](#) | [CROSSREF](#)
30. Kim JH, Kim SK, Lee HJ, Lee JR, Jee BC, Suh CS, et al. Efficacy of random-start controlled ovarian stimulation in cancer patients. *J Korean Med Sci* 2015;30:290-5.
[PUBMED](#) | [CROSSREF](#)
31. Oktay K, Hourvitz A, Sahin G, Oktem O, Safro B, Cil A, et al. Letrozole reduces estrogen and gonadotropin exposure in women with breast cancer undergoing ovarian stimulation before chemotherapy. *J Clin Endocrinol Metab* 2006;91:3885-90.
[PUBMED](#) | [CROSSREF](#)
32. Tulandi T, Martin J, Al-Fadhli R, Kabli N, Forman R, Hitkari J, et al. Congenital malformations among 911 newborns conceived after infertility treatment with letrozole or clomiphene citrate. *Fertil Steril* 2006;85:1761-5.
[PUBMED](#) | [CROSSREF](#)
33. Azim AA, Costantini-Ferrando M, Oktay K. Safety of fertility preservation by ovarian stimulation with letrozole and gonadotropins in patients with breast cancer: a prospective controlled study. *J Clin Oncol* 2008;26:2630-5.
[PUBMED](#) | [CROSSREF](#)

34. Donnez J, Dolmans MM. Fertility preservation in women. *Nat Rev Endocrinol* 2013;9:735-49.
[PUBMED](#) | [CROSSREF](#)
35. Waimey KE, Duncan FE, Su HI, Smith K, Wallach H, Jona K, et al. Future directions in oncofertility and fertility preservation: a report from the 2011 oncofertility consortium conference. *J Adolesc Young Adult Oncol* 2013;2:25-30.
[PUBMED](#) | [CROSSREF](#)
36. Ting AY, Yeoman RR, Lawson MS, Zelinski MB. In vitro development of secondary follicles from cryopreserved rhesus macaque ovarian tissue after slow-rate freeze or vitrification. *Hum Reprod* 2011;26:2461-72.
[PUBMED](#) | [CROSSREF](#)
37. 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.
[PUBMED](#) | [CROSSREF](#)
38. Donnez J, Dolmans MM. Ovarian cortex transplantation: 60 reported live births brings the success and worldwide expansion of the technique towards routine clinical practice. *J Assist Reprod Genet* 2015;32:1167-70.
[PUBMED](#) | [CROSSREF](#)
39. Donnez J, Dolmans MM, Pellicer A, Diaz-Garcia C, Sanchez Serrano M, Schmidt KT, et al. Restoration of ovarian activity and pregnancy after transplantation of cryopreserved ovarian tissue: a review of 60 cases of reimplantation. *Fertil Steril* 2013;99:1503-13.
[PUBMED](#) | [CROSSREF](#)
40. Demeestere I, Simon P, Dedeken L, Moffa F, Tsépélidis S, Brachet C, et al. Live birth after autograft of ovarian tissue cryopreserved during childhood. *Hum Reprod* 2015;30:2107-9.
[PUBMED](#) | [CROSSREF](#)
41. Donnez J, Jadoul P, Squifflet J, Van Langendonck A, Donnez O, Van Eyck AS, et al. Ovarian tissue cryopreservation and transplantation in cancer patients. *Best Pract Res Clin Obstet Gynaecol* 2010;24:87-100.
[PUBMED](#) | [CROSSREF](#)
42. Martinez-Madrid B, Camboni A, Dolmans MM, Nottola S, Van Langendonck A, Donnez J. Apoptosis and ultrastructural assessment after cryopreservation of whole human ovaries with their vascular pedicle. *Fertil Steril* 2007;87:1153-65.
[PUBMED](#) | [CROSSREF](#)
43. Bedaiwy MA, Hussein MR, Biscotti C, Falcone T. Cryopreservation of intact human ovary with its vascular pedicle. *Hum Reprod* 2006;21:3258-69.
[PUBMED](#) | [CROSSREF](#)
44. Prasath EB, Chan ML, Wong WH, Lim CJ, Tharmalingam MD, Hendricks M, et al. First pregnancy and live birth resulting from cryopreserved embryos obtained from in vitro matured oocytes after oophorectomy in an ovarian cancer patient. *Hum Reprod* 2014;29:276-8.
[PUBMED](#) | [CROSSREF](#)
45. Isachenko V, Lapidus I, Isachenko E, Krivokharchenko A, Kreienberg R, Woriedh M, et al. Human ovarian tissue vitrification versus conventional freezing: morphological, endocrinological, and molecular biological evaluation. *Reproduction* 2009;138:319-27.
[PUBMED](#) | [CROSSREF](#)
46. Isachenko V, Isachenko E, Kreienberg R, Woriedh M, Weiss J. Human ovarian tissue cryopreservation: quality of follicles as a criteria of effectiveness. *Reprod Biomed Online* 2010;20:441-2.
[PUBMED](#) | [CROSSREF](#)
47. Sheikhi M, Hultenby K, Niklasson B, Lundqvist M, Hovatta O. Clinical grade vitrification of human ovarian tissue: an ultrastructural analysis of follicles and stroma in vitrified tissue. *Hum Reprod* 2011;26:594-603.
[PUBMED](#) | [CROSSREF](#)
48. Kawamura K, Cheng Y, Suzuki N, Deguchi M, Sato Y, Takae S, et al. Hippo signaling disruption and Akt stimulation of ovarian follicles for infertility treatment. *Proc Natl Acad Sci U S A* 2013;110:17474-9.
[PUBMED](#) | [CROSSREF](#)
49. Suzuki N, Yoshioka N, Takae S, Sugishita Y, Tamura M, Hashimoto S, et al. Successful fertility preservation following ovarian tissue vitrification in patients with primary ovarian insufficiency. *Hum Reprod* 2015;30:608-15.
[PUBMED](#) | [CROSSREF](#)

50. Youm HW, Lee JR, Lee J, Jee BC, Suh CS, Kim SH. Optimal vitrification protocol for mouse ovarian tissue cryopreservation: effect of cryoprotective agents and in vitro culture on vitrified-warmed ovarian tissue survival. *Hum Reprod* 2014;29:720-30.
[PUBMED](#) | [CROSSREF](#)
51. Ting AY, Yeoman RR, Campos JR, Lawson MS, Mullen SF, Fahy GM, et al. Morphological and functional preservation of pre-antral follicles after vitrification of macaque ovarian tissue in a closed system. *Hum Reprod* 2013;28:1267-79.
[PUBMED](#) | [CROSSREF](#)
52. Demeestere I, Simon P, Buxant F, Robin V, Fernandez SA, Centner J, et al. Ovarian function and spontaneous pregnancy after combined heterotopic and orthotopic cryopreserved ovarian tissue transplantation in a patient previously treated with bone marrow transplantation: case report. *Hum Reprod* 2006;21:2010-4.
[PUBMED](#) | [CROSSREF](#)
53. Oktay K, Buyuk E, Veeck L, Zaninovic N, Xu K, Takeuchi T, et al. Embryo development after heterotopic transplantation of cryopreserved ovarian tissue. *Lancet* 2004;363:837-40.
[PUBMED](#) | [CROSSREF](#)
54. Rosendahl M, Loft A, Byskov AG, Ziebe S, Schmidt KT, Andersen AN, et al. Biochemical pregnancy after fertilization of an oocyte aspirated from a heterotopic autotransplant of cryopreserved ovarian tissue: case report. *Hum Reprod* 2006;21:2006-9.
[PUBMED](#) | [CROSSREF](#)
55. Callejo J, Salvador C, Miralles A, Vilaseca S, Lailla JM, Balasch J. Long-term ovarian function evaluation after autografting by implantation with fresh and frozen-thawed human ovarian tissue. *J Clin Endocrinol Metab* 2001;86:4489-94.
[PUBMED](#) | [CROSSREF](#)
56. Dittrich R, Mueller A, Binder H, Oppelt PG, Renner SP, Goecke T, et al. First retransplantation of cryopreserved ovarian tissue following cancer therapy in Germany. *Dtsch Arztebl Int* 2008;105:274-8.
[PUBMED](#) | [CROSSREF](#)
57. Stern CJ, Gook D, Hale LG, Agresta F, Oldham J, Rozen G, et al. First reported clinical pregnancy following heterotopic grafting of cryopreserved ovarian tissue in a woman after a bilateral oophorectomy. *Hum Reprod* 2013;28:2996-9.
[PUBMED](#) | [CROSSREF](#)
58. Lucifero D, Mertineit C, Clarke HJ, Bestor TH, Trasler JM. Methylation dynamics of imprinted genes in mouse germ cells. *Genomics* 2002;79:530-8.
[PUBMED](#) | [CROSSREF](#)
59. Shaw JM, Bowles J, Koopman P, Wood EC, Trounson AO. Fresh and cryopreserved ovarian tissue samples from donors with lymphoma transmit the cancer to graft recipients. *Hum Reprod* 1996;11:1668-73.
[PUBMED](#) | [CROSSREF](#)
60. Dudzinski DM. Ethical issues in fertility preservation for adolescent cancer survivors: oocyte and ovarian tissue cryopreservation. *J Pediatr Adolesc Gynecol* 2004;17:97-102.
[PUBMED](#) | [CROSSREF](#)
61. Dolmans MM, Luyckx V, Donnez J, Andersen CY, Greve T. Risk of transferring malignant cells with transplanted frozen-thawed ovarian tissue. *Fertil Steril* 2013;99:1514-22.
[PUBMED](#) | [CROSSREF](#)
62. Xu M, Pavone ME, Woodruff T. Fruitful progress to fertility: preserving oocytes from chemodestruction. *Nat Med* 2011;17:1562-3.
[PUBMED](#) | [CROSSREF](#)
63. Moore HC, Unger JM, Phillips KA, Boyle F, Hitre E, Porter D, et al. Goserelin for ovarian protection during breast-cancer adjuvant chemotherapy. *N Engl J Med* 2015;372:923-32.
[PUBMED](#) | [CROSSREF](#)
64. Del Mastro L, Ceppi M, Poggio F, Bighin C, Peccatori F, Demeestere I, et al. Gonadotropin-releasing hormone analogues for the prevention of chemotherapy-induced premature ovarian failure in cancer women: systematic review and meta-analysis of randomized trials. *Cancer Treat Rev* 2014;40:675-83.
[PUBMED](#) | [CROSSREF](#)
65. Meirou D, Hardan I, Dor J, Fridman E, Elizur S, Ra'anani H, et al. Searching for evidence of disease and malignant cell contamination in ovarian tissue stored from hematologic cancer patients. *Hum Reprod* 2008;23:1007-13.
[PUBMED](#) | [CROSSREF](#)

66. Dolmans MM, Jadoul P, Gilliaux S, Amorim CA, Luyckx V, Squifflet J, et al. A review of 15 years of ovarian tissue bank activities. *J Assist Reprod Genet* 2013;30:305-14.
[PUBMED](#) | [CROSSREF](#)
67. Rosendahl M, Greve T, Andersen CY. The safety of transplanting cryopreserved ovarian tissue in cancer patients: a review of the literature. *J Assist Reprod Genet* 2013;30:11-24.
[PUBMED](#) | [CROSSREF](#)
68. Ernst EH, Offersen BV, Andersen CY, Ernst E. Legal termination of a pregnancy resulting from transplanted cryopreserved ovarian tissue due to cancer recurrence. *J Assist Reprod Genet* 2013;30:975-8.
[PUBMED](#) | [CROSSREF](#)
69. Hermann BP, Sukhwani M, Salati J, Sheng Y, Chu T, Orwig KE. Separating spermatogonia from cancer cells in contaminated prepubertal primate testis cell suspensions. *Hum Reprod* 2011;26:3222-31.
[PUBMED](#) | [CROSSREF](#)
70. Colgan TJ, Murphy J, Cole DE, Narod S, Rosen B. Occult carcinoma in prophylactic oophorectomy specimens: prevalence and association with BRCA germline mutation status. *Am J Surg Pathol* 2001;25:1283-9.
[PUBMED](#) | [CROSSREF](#)
71. Cortvrindt R, Smitz J, Van Steirteghem AC. In-vitro maturation, fertilization and embryo development of immature oocytes from early preantral follicles from prepuberal mice in a simplified culture system. *Hum Reprod* 1996;11:2656-66.
[PUBMED](#) | [CROSSREF](#)
72. West ER, Shea LD, Woodruff TK. Engineering the follicle microenvironment. *Semin Reprod Med* 2007;25:287-99.
[PUBMED](#) | [CROSSREF](#)
73. Pangas SA, Saudye H, Shea LD, Woodruff TK. Novel approach for the three-dimensional culture of granulosa cell-oocyte complexes. *Tissue Eng* 2003;9:1013-21.
[PUBMED](#) | [CROSSREF](#)
74. McLaughlin M, Bromfield JJ, Albertini DF, Telfer EE. Activin promotes follicular integrity and oogenesis in cultured pre-antral bovine follicles. *Mol Hum Reprod* 2010;16:644-53.
[PUBMED](#) | [CROSSREF](#)
75. Sadr SZ, Ebrahimi B, Shahhoseini M, Fatehi R, Favaedi R. Mouse preantral follicle development in two-dimensional and three-dimensional culture systems after ovarian tissue vitrification. *Eur J Obstet Gynecol Reprod Biol* 2015;194:206-11.
[PUBMED](#) | [CROSSREF](#)
76. Xu M, Kreeger PK, Shea LD, Woodruff TK. Tissue-engineered follicles produce live, fertile offspring. *Tissue Eng* 2006;12:2739-46.
[PUBMED](#) | [CROSSREF](#)
77. Xu M, Barrett SL, West-Farrell E, Kondapalli LA, Kiesewetter SE, Shea LD, et al. In vitro grown human ovarian follicles from cancer patients support oocyte growth. *Hum Reprod* 2009;24:2531-40.
[PUBMED](#) | [CROSSREF](#)
78. Xu M, West-Farrell ER, Stouffer RL, Shea LD, Woodruff TK, Zelinski MB. Encapsulated three-dimensional culture supports development of nonhuman primate secondary follicles. *Biol Reprod* 2009;81:587-94.
[PUBMED](#) | [CROSSREF](#)
79. Xu M, Fazleabas AT, Shikanov A, Jackson E, Barrett SL, Hirshfeld-Cytron J, et al. In vitro oocyte maturation and preantral follicle culture from the luteal-phase baboon ovary produce mature oocytes. *Biol Reprod* 2011;84:689-97.
[PUBMED](#) | [CROSSREF](#)
80. Tagler D, Makanji Y, Anderson NR, Woodruff TK, Shea LD. Supplemented α MEM/F12-based medium enables the survival and growth of primary ovarian follicles encapsulated in alginate hydrogels. *Biotechnol Bioeng* 2013;110:3258-68.
[PUBMED](#) | [CROSSREF](#)
81. Brito IR, Lima IM, Xu M, Shea LD, Woodruff TK, Figueiredo JR. Three-dimensional systems for in vitro follicular culture: overview of alginate-based matrices. *Reprod Fertil Dev* 2014;26:915-30.
[PUBMED](#) | [CROSSREF](#)
82. Sharma GT, Dubey PK, Meur SK. Survival and developmental competence of buffalo preantral follicles using three-dimensional collagen gel culture system. *Anim Reprod Sci* 2009;114:115-24.
[PUBMED](#) | [CROSSREF](#)
83. Xu J, Lawson MS, Yeoman RR, Pau KY, Barrett SL, Zelinski MB, et al. Secondary follicle growth and oocyte maturation during encapsulated three-dimensional culture in rhesus monkeys: effects of gonadotrophins, oxygen and fetuin. *Hum Reprod* 2011;26:1061-72.
[PUBMED](#) | [CROSSREF](#)

84. Barrett SL, Shea LD, Woodruff TK. Noninvasive index of cryorecovery and growth potential for human follicles in vitro. *Biol Reprod* 2010;82:1180-9.
[PUBMED](#) | [CROSSREF](#)
85. Skory RM, Xu Y, Shea LD, Woodruff TK. Engineering the ovarian cycle using in vitro follicle culture. *Hum Reprod* 2015;30:1386-95.
[PUBMED](#) | [CROSSREF](#)
86. Soares M, Sahrari K, Chiti MC, Amorim CA, Ambroise J, Donnez J, et al. The best source of isolated stromal cells for the artificial ovary: medulla or cortex, cryopreserved or fresh? *Hum Reprod* 2015;30:1589-98.
[PUBMED](#) | [CROSSREF](#)
87. Vanacker J, Luyckx V, Dolmans MM, Des Rieux A, Jaeger J, Van Langendonck A, et al. Transplantation of an alginate-matrigel matrix containing isolated ovarian cells: first step in developing a biodegradable scaffold to transplant isolated preantral follicles and ovarian cells. *Biomaterials* 2012;33:6079-85.
[PUBMED](#) | [CROSSREF](#)
88. Luyckx V, Dolmans MM, Vanacker J, Scalercio SR, Donnez J, Amorim CA. First step in developing a 3D biodegradable fibrin scaffold for an artificial ovary. *J Ovarian Res* 2013;6:83.
[PUBMED](#) | [CROSSREF](#)
89. Luyckx V, Dolmans MM, Vanacker J, Legat C, Fortuño Moya C, Donnez J, et al. A new step toward the artificial ovary: survival and proliferation of isolated murine follicles after autologous transplantation in a fibrin scaffold. *Fertil Steril* 2014;101:1149-56.
[PUBMED](#) | [CROSSREF](#)
90. Dath C, Dethy A, Van Langendonck A, Van Eyck AS, Amorim CA, Luyckx V, et al. Endothelial cells are essential for ovarian stromal tissue restructuring after xenotransplantation of isolated ovarian stromal cells. *Hum Reprod* 2011;26:1431-9.
[PUBMED](#) | [CROSSREF](#)
91. Sittadjody S, Saul JM, Joo S, Yoo JJ, Atala A, Opara EC. Engineered multilayer ovarian tissue that secretes sex steroids and peptide hormones in response to gonadotropins. *Biomaterials* 2013;34:2412-20.
[PUBMED](#) | [CROSSREF](#)
92. Laronda MM, Jakus AE, Whelan KA, Wertheim JA, Shah RN, Woodruff TK. Initiation of puberty in mice following decellularized ovary transplant. *Biomaterials* 2015;50:20-9.
[PUBMED](#) | [CROSSREF](#)
93. 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.
[PUBMED](#) | [CROSSREF](#)
94. Zhang H, Panula S, Petropoulos S, Edsgård D, Busayavalasa K, Liu L, et al. Adult human and mouse ovaries lack DDX4-expressing functional oogonial stem cells. *Nat Med* 2015;21:1116-8.
[PUBMED](#) | [CROSSREF](#)
95. Hayashi K, Ohta H, Kurimoto K, Aramaki S, Saitou M. Reconstitution of the mouse germ cell specification pathway in culture by pluripotent stem cells. *Cell* 2011;146:519-32.
[PUBMED](#) | [CROSSREF](#)
96. Hayashi K, Ogushi S, Kurimoto K, Shimamoto S, Ohta H, Saitou M. Offspring from oocytes derived from in vitro primordial germ cell-like cells in mice. *Science* 2012;338:971-5.
[PUBMED](#) | [CROSSREF](#)
97. Nelson LM. Clinical practice. Primary ovarian insufficiency. *N Engl J Med* 2009;360:606-14.
[PUBMED](#) | [CROSSREF](#)
98. Farquhar C, Brown J, Marjoribanks J. Laparoscopic drilling by diathermy or laser for ovulation induction in anovulatory polycystic ovary syndrome. *Cochrane Database Syst Rev* 2012;6:CD001122.
[PUBMED](#) | [CROSSREF](#)
99. Hsueh AJ, Kawamura K, Cheng Y, Fauser BC. Intraovarian control of early folliculogenesis. *Endocr Rev* 2015;36:1-24.
[PUBMED](#) | [CROSSREF](#)
100. Baker SJ, Spears N. The role of intra-ovarian interactions in the regulation of follicle dominance. *Hum Reprod Update* 1999;5:153-65.
[PUBMED](#) | [CROSSREF](#)
101. Li J, Kawamura K, Cheng Y, Liu S, Klein C, Liu S, et al. Activation of dormant ovarian follicles to generate mature eggs. *Proc Natl Acad Sci U S A* 2010;107:10280-4.
[PUBMED](#) | [CROSSREF](#)
102. Cheng Y, Kim J, Li XX, Hsueh AJ. Promotion of ovarian follicle growth following mTOR activation: synergistic effects of AKT stimulators. *PLoS One* 2015;10:e0117769.
[PUBMED](#) | [CROSSREF](#)

103. Adhikari D, Gorre N, Risal S, Zhao Z, Zhang H, Shen Y, et al. The safe use of a PTEN inhibitor for the activation of dormant mouse primordial follicles and generation of fertilizable eggs. *PLoS One* 2012;7:e39034.
[PUBMED](#) | [CROSSREF](#)
104. Ahn RW, Chen F, Chen H, Stern ST, Clogston JD, Patri AK, et al. A novel nanoparticulate formulation of arsenic trioxide with enhanced therapeutic efficacy in a murine model of breast cancer. *Clin Cancer Res* 2010;16:3607-17.
[PUBMED](#) | [CROSSREF](#)
105. Ahn RW, Barrett SL, Raja MR, Jozefik JK, Spaho L, Chen H, et al. Nano-encapsulation of arsenic trioxide enhances efficacy against murine lymphoma model while minimizing its impact on ovarian reserve in vitro and in vivo. *PLoS One* 2013;8:e58491.
[PUBMED](#) | [CROSSREF](#)
106. Gonfloni S, Di Tella L, Caldarola S, Cannata SM, Klinger FG, Di Bartolomeo C, et al. Inhibition of the c-Abl-TAp63 pathway protects mouse oocytes from chemotherapy-induced death. *Nat Med* 2009;15:1179-85.
[PUBMED](#) | [CROSSREF](#)
107. Woodruff TK. Preserving fertility during cancer treatment. *Nat Med* 2009;15:1124-5.
[PUBMED](#) | [CROSSREF](#)
108. Suh EK, Yang A, Kettenbach A, Bamberger C, Michaelis AH, Zhu Z, et al. p63 protects the female germ line during meiotic arrest. *Nature* 2006;444:624-8.
[PUBMED](#) | [CROSSREF](#)
109. Kim SY, Cordeiro MH, Serna VA, Ebbert K, Butler LM, Sinha S, et al. Rescue of platinum-damaged oocytes from programmed cell death through inactivation of the p53 family signaling network. *Cell Death Differ* 2013;20:987-97.
[PUBMED](#) | [CROSSREF](#)
110. Kalich-Philosoph L, Roness H, Carmely A, Fishel-Bartal M, Ligumsky H, Paglin S, et al. Cyclophosphamide triggers follicle activation and “burnout”; AS101 prevents follicle loss and preserves fertility. *Sci Transl Med* 2013;5:185ra62.
[PUBMED](#) | [CROSSREF](#)
111. Verga Falzacappa C, Timperi E, Bucci B, Amendola D, Piergrossi P, D’Amico D, et al. T(3) preserves ovarian granulosa cells from chemotherapy-induced apoptosis. *J Endocrinol* 2012;215:281-9.
[PUBMED](#) | [CROSSREF](#)
112. Hancke K, Strauch O, Kissel C, Göbel H, Schäfer W, Denschlag D. Sphingosine 1-phosphate protects ovaries from chemotherapy-induced damage in vivo. *Fertil Steril* 2007;87:172-7.
[PUBMED](#) | [CROSSREF](#)
113. Li F, Turan V, Lierman S, Cuvelier C, De Sutter P, Oktay K. Sphingosine-1-phosphate prevents chemotherapy-induced human primordial follicle death. *Hum Reprod* 2014;29:107-13.
[PUBMED](#) | [CROSSREF](#)
114. Perez GI, Knudson CM, Leykin L, Korsmeyer SJ, Tilly JL. Apoptosis-associated signaling pathways are required for chemotherapy-mediated female germ cell destruction. *Nat Med* 1997;3:1228-32.
[PUBMED](#) | [CROSSREF](#)
115. Zelinski MB, Murphy MK, Lawson MS, Jurisicova A, Pau KY, Toscano NP, et al. In vivo delivery of FTY720 prevents radiation-induced ovarian failure and infertility in adult female nonhuman primates. *Fertil Steril* 2011;95:1440-5.e1-7.
[PUBMED](#) | [CROSSREF](#)
116. Skaznik-Wikiel ME, McGuire MM, Sukhwani M, Donohue J, Chu T, Krivak TC, et al. Granulocyte colony-stimulating factor with or without stem cell factor extends time to premature ovarian insufficiency in female mice treated with alkylating chemotherapy. *Fertil Steril* 2013;99:2045-54.e3.
[PUBMED](#) | [CROSSREF](#)
117. Ting AY, Petroff BK. Tamoxifen decreases ovarian follicular loss from experimental toxicant DMBA and chemotherapy agents cyclophosphamide and doxorubicin in the rat. *J Assist Reprod Genet* 2010;27:591-7.
[PUBMED](#) | [CROSSREF](#)
118. Mahran YF, El-Demerdash E, Nada AS, Ali AA, Abdel-Naim AB. Insights into the protective mechanisms of tamoxifen in radiotherapy-induced ovarian follicular loss: impact on insulin-like growth factor 1. *Endocrinology* 2013;154:3888-99.
[PUBMED](#) | [CROSSREF](#)