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Review Article

Fertility Preservation Options for Female Cancer Patients: A Systematic Review

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Abstract

Advances in cancer detection and treatment have resulted in an increasing amount of long-term survivors who are left to deal with the adverse effects of their treatments. Fortunately, progress in fertility preservation technologies has been paralleling the trend in improving cancer outcomes.

Because of the variations in type and dose of chemotherapy or radiation, the type of cancer, the time available before treatment initiation, and the patient's age and partner status, each case is unique and requires a different strategy for fertility preservation.

The field of fertility preservation is growing rapidly. It is the goal of this paper to review some of the options, their success rates, and their limitations, so physicians can provide appropriately counsel and expeditiously refer their pre-menopausal patients diagnosed with cancer to a fertility specialist.

Introduction

In 2010, it was estimated that almost 740,000 women were newly diagnosed with cancer in the United States [1]. Fortunately, advances in cancer therapy have resulted in improvements in both mortality rates and long-term survival rates. Between 2002 and 2006, death rates for all cancer sites in women decreased by 1.5% per year, almost double the decline of 0.8% per year from 1994 to 2002 [1]. The 5-year relative survival rate for all cancer sites in American women aged 20 to 49 years increased from 71.1% in 1977 to 82.1% in 2002 [2]. In children, the 5-year relative survival rate for all cancer sites improved from 58% for patients diagnosed between 1975 and 1977 to 81% for those diagnosed between 1999 and 2005 [1]. It was estimated that by 2010, 1 in every 250 young adults (15 to 45 years old) would be a childhood cancer survivor [3].

Meanwhile, women in the Western hemisphere continue to delay the start of childbearing to later in life. Since 1990, national first birth rates have fallen for women under 30 years of age while rising for those over 30 years of age for all population groups [4]. Between 1990 and 2008, the national first birth rate among all races decreased 30% for teenage women (15 to 19 years old) but increased 33% for women 30 to 34 years old, 58% for women 35 to 39 years old, and more than doubled for women 40 to 44 years old [5]. This trend, along with improving survival rates and the fact that the incidence of most cancers increases with age, means that an increasing number of female cancer survivors will either not have started or not yet completed childbearing and will be interested in future fertility [2].

However, the advances in surgery, chemotherapy, and radiation that are responsible for improved cancer outcomes are not without long-term side-effects. These include growth disorders, cardiovascular problems, neurocognitive abnormalities, secondary malignant tumors, and reproductive failure, all of which negatively impact cancer survivors' quality of life [6]. A recent literature review of the fertility-related psychosocial needs and concerns of younger women with breast cancer revealed that a substantial proportion of these women are concerned about the impact of premature menopause and, in particular, the potential for infertility [7]. In June 2006, the American Society of Clinical Oncology published new guidelines recommending that oncologists address the possibility of infertility with patients treated during their reproductive years and be prepared to discuss possible fertility preservation options or refer appropriate and interested patients to reproductive specialists [8]. Despite these guidelines, several national surveys have shown that oncologists are still not discussing treatment-related fertility risks with patients nor referring patients to reproductive specialists. A 2009 national survey reported that only 47% of responding oncologists routinely referred their cancer patients of childbearing age to a reproductive endocrinologist [9]. In another survey of academic medical centers, 95% of oncologists reported that they routinely discuss the effect that treatment may have on patients' fertility, but only 39% routinely referred patients to a specialist in reproductive medicine [10]. These studies also show that oncologists have limited personal experience with fertility preservation techniques as well as gaps in their knowledge of gonadotoxicity from specific treatment regimens [10]. Other barriers to discussion and referral included limited knowledge of fertility preservation methods and resources [11-13]. This review will provide updated information on the options for fertility preservation in female cancer patients, with an emphasis on oocyte cryopreservation.

Fertility preservation options

Advancements in assisted reproduction and cryobiology techniques have resulted in several options for the growing number of female cancer patients interested in fertility preservation. Some of the possibilities, and those that will be discussed in this review, include: (1) embryo cryopreservation, (2) ovarian transposition (oophoropexy), (3) medical strategies such as ovarian suppression with GnRH analogs or antagonists, (4) ovarian tissue cryopreservation, and (5) oocyte cryopreservation. Of these options, only embryo cryopreservation and ovarian transposition are considered standard interventions for the preservation of fertility in women undergoing potentially gonadotoxic cancer treatment [8]. The choice of which option to pursue depends on the type of cancer, the treatment protocol (i.e., radiation and/or

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chemotherapy), the amount of time available before treatment begins, the patient's age, and whether the patient has a partner [14].

Embryo cryopreservation: In Vitro Fertilization (IVF) followed by embryo cryopreservation has been a reliable method for fertility preservation since 1983 [15] and is currently the only established strategy for fertility preservation in female cancer patients according to the American Society of Clinical Oncology and the Ethics Committee of the American Society for Reproductive Medicine [8,16]. Embryo cryopreservation involves ovarian stimulation for multifollicular development, oocyte retrieval, embryo generation through IVF, and freezing of embryos for future implantation. Survival rates for thawed embryos range from 35 to 90%, implantation rates from 8 to 30%, and cumulative pregnancy rates can exceed 60% [17]. In 2008, the most recent year for IVF success rate data, the Society for Assisted Reproductive Technologies reported an overall live birth rate of 30.6% per frozen-thawed embryo transfer compared to a 36.7% live birth rate per fresh embryo transfer [18].

Despite respectable success rates, this approach has several major drawbacks [19]. First, the patient must have a male partner or a willingness to use a sperm donor. Second, there must be a delay of 2 to 3 weeks before the start of chemotherapy or radiation to allow for ovarian stimulation and oocyte retrieval, as the effectiveness of IVF has been found to decrease dramatically after even one round of chemotherapy [20]. Oocyte retrieval can be performed without ovarian stimulation ("natural cycle IVF"), but efficacy is hampered by high cancellation rates, low oocyte yield, and fewer embryos generated per cycle [21,22]. Third, exposure to supraphysiologic levels of estrogen during ovarian stimulation with gonadotropins may not be safe for women with estrogen-sensitive tumors such as breast cancer and endometrial cancer. Studies of transgenic mice that overexpress Luteinizing Hormone (LH) and consequently overproduce estradiol, progesterone, and prolactin develop metastasizing mammary cancers that exhibit substantial levels of aneuploidy [23,24]. In humans, it is well known that high estrogen levels are associated with a greater risk of breast cancer [25,26], even in Estrogen Receptor negative (ER-) tumors [27], likely by enhancing the mitotic rate of breast cells [28] and increasing host angiogenesis [27]. Finally, patients who choose to pursue embryo cryopreservation must decide whether the embryos should be discarded, donated to another couple, or used for research in case of death [14].

Ovarian transposition/Oophoropexy: The American Society of Clinical Oncology considers ovarian transposition, or oophoropexy, a standard intervention for the preservation of fertility in women undergoing abdominal or pelvic radiation for cancer treatment [8]. McCall et al. first described this procedure in 1958 for patients with cervical carcinoma [29]. Since then, ovarian transposition has been used in young, premenopausal women diagnosed with several other cancers, in addition to cervical carcinoma, that can be cured by radiation therapy, such as: vaginal and anorectal carcinomas, dysgerminoma, Hodgkin's disease, and central nervous system tumors [30,31]. The procedure is of limited value in patients older than 40 years of age because of their intrinsically reduced fertilization potential, as well as a much higher risk for ovarian failure despite the transposition [32]. Furthermore, when gonadotoxic chemotherapy is used along with radiation, there is no strong rationale to perform this procedure [17].

Ovarian transposition before radiation is based on the well-recognized adverse effects of ionizing radiation on gonadal function, including Premature Ovarian Failure (POF) and permanent infertility. The degree and persistence of ovarian damage is influenced by the age at time of exposure, dose, fractionation schedule, and extent and type of radiation therapy (e.g. abdominal, pelvic external beam irradiation, intracavitary brachytherapy) [17]. Radiation causes a dose-related reduction in the primordial follicle pool, inducing dose-dependent increases in DNA damage of somatic and germ cells [33,34]. By constructing a mathematical model of ovarian follicle decay, Wallace et al. [35] estimated the LD_{50}, the dose of radiation required to destroy 50% of primordial follicles, to be <2 Gy (200 cGy), yet patients with Hodgkin's disease typically receive 2,000 to 4,000 cGy to the ovaries during total lymph node irradiation, invariably resulting in POF [36]. Surgically transposing the ovaries out of the radiation field before abdominal or pelvic radiation reduces radiation exposure to the ovaries to approximately 5 to 10% of non-transposed ovaries [37-39].

The success rate of ovarian transposition before radiation in preserving fertility varies widely in the literature, from 16 to 90%, owing to the fact that the outcome depends on many factors, such as: the degree of scatter radiation; vascular compromise to the ovaries; ovarian shielding; the use of concomitant chemotherapy, vaginal brachytherapy, or pelvic external beam irradiation plus brachytherapy; and, most importantly, the age of the patient at the time of treatment and the dose of radiation used [17,40]. The most debatable of these variables seems to be whether to do a laparotic or laparoscopic procedure and where to fix the transposed the ovaries.

The decision to transpose the ovaries by laparotomy or laparoscopy depends on whether the patient needs abdominal surgery (ovaries can be transposed simultaneously via laparotomy) or whether she can be treated non-surgically (ovaries can be transposed via laparoscopy). In a review of 44 cases, Bisharah and Tulandi [41] reported that laparoscopic ovarian transposition in women younger than 40 years is associated with preservation of ovarian function in 88.6% of cases, similar to the rate of 83% for ovarian transposition by laparotomy reported by Husseinzadeh et al. [42]. However, laparotomy is associated with a large abdominal incision, a long hospital stay, and an increased risk of adhesions formation and intestinal obstruction or ileus [41,43]. More importantly, laparotomy requires a delay of several months to allow the incision to heal before radiation, during which time the ovaries can migrate back to their original position, thereby explaining many cases of ovarian failure [36]. In a follow-up of 54 female patients under 45 years of age for assessment of ovarian function after treatment for Hodgkin's disease, Hunter et al. [44] found that only 28 of the 46 patients (60.8%) who underwent laparotic oophoropexy performed had ovaries sufficiently displaced from their normal positions, such that if they had received radiation, their ovaries would have been spared. In the twelve patients who were treated with radiation after laparotic oophoropexy, only one patient maintained normal ovarian function. Similarly, in an analysis of the ovarian function after laparoscopic oophoropexy in 10 women under age 40 with Hodgkin's disease, Williams et al. [36] reported on two patients who had undergone initial oophoropexy at the time of staging laparotomy five and six months, respectively, before laparoscopy and pelvic irradiation. In both those patients, they found that the ovaries had migrated back to their original positions, and their therapy would have resulted in ovarian failure if the laparoscopic oophoropexy had not been performed. In contrast, with laparoscopic ovarian transposition, radiation therapy can be started immediately after surgery, thereby avoiding ovarian migration and failure. These reports, and others [30,32,41] asserting the safety, efficacy, and simplicity of laparoscopic ovarian transposition, have led surgeons to prefer laparoscopy over laparotomy in recent years.

The proper site to fix the transposed ovaries depends on the shape, size, and location of the radiation field [45]. Sites of fixation vary in the literature from the base of the round ligament [46] to the lower

kidney pole [47]. Traditionally, the ovaries were transposed medially behind the uterus and shielded with a lead block for pelvic lymph node irradiation, as in Hodgkin's disease, and high and laterally above the pelvic brim for cervical cancer. However, newer reports assert that the ovaries must be mobilized laterally and at least 3 cm from the upper border of the radiation field in order to survive radiation [48]. A review of the outcomes of lateral versus medial transposition among ten case reports and small series found significantly better preservation of ovarian function with lateral transposition (86%) than medial transposition (50%) [49]. With midline oophoropexy, a large amount of scattered radiation may still reach the ovaries despite shielding of the median area [50]. Grabenbauer et al. [51] reported on 15 patients who underwent bilateral oophoropexy during staging laparotomy for Hodgkin's disease before total lymphoid irradiation, including pelvic and inguinal nodes: ten had lateral, five had midline ovarian transposition. The authors determined that the median calculated dose was 325 cGy (range 260 to 500 cGy) to the laterally fixed ovaries compared to 490 cGy (range 390 to 500 cGy) for midline transposition. Normal cyclic ovarian activity was found in seven out of nine (77.8%) patients following lateral oophoropexy (including one pregnancy), but only in one out of four (25%) cases after midline fixation. Furthermore, lead blocks used to shield medially transposed ovaries may also shield affected nodes [41]. It has also been suggested that the higher failure rate with medial oophoropexy before pelvic irradiation might be due to relocation of the ovaries back to their normal, unshielded, anatomic position before radiation is completed rather than simply more radiation exposure in the medial position [49]. Indeed, in CT scans of seven patients with cervical cancer who underwent Lateral Ovarian Transposition (LOT) and nine patients with Hodgkin's disease who underwent Medial Ovarian Transposition (MOT), Hadar et al. [52] found that 11 of the 13 ovaries (85%) that underwent LOT were located outside the radiation field, while only three of the 13 identified ovaries (23%) in the MOT group were completely outside the radiation field ($P = 0.005$). The former group received 100 to 300 cGy of radiation, while the latter group received approximately 300 cGy. These results suggest an improved likelihood of ovarian protection from direct radiation when LOT is performed, as opposed to MOT.

Laparoscopic LOT is usually performed by dividing the utero-ovarian ligaments and the mesovarium to separate the ovary from the uterus and tubes [14]. The ovarian vessels are mobilized to transpose the ovaries laterally above the pelvic brim without tension. The ovaries are brought through a peritoneal tunnel such that the ovaries remain intraperitoneal, but the vessels are kept retroperitoneal to reduce the risk of kinking or torsion, which could compromise the ovarian blood supply. Permanent suture is used to secure the ovaries to the peritoneum and surgical clips are applied to the ovaries so that they can be located on x-ray before radiation treatment. Recently, a new laparoscopic technique was described in which the ovaries are percutaneously fixed to the anterior abdominal wall at the level of the anterior superior iliac spine and then repositioned after radiation by cutting the subcutaneous suture with local anesthesia in an outpatient facility [43]. Follow-up of the 12 patients (nine with rectal cancer and three with Hodgkin's disease) who underwent this procedure, all under 40 years old, showed evidence of normal ovarian function in 11 patients (91.7%), and three of them (27.3%) who desired children achieved pregnancy. These results are similar to the 88.6% rate of ovarian preservation in women younger than 40 years old with traditional laparoscopic transposition, as reported by Bisharah and Tulandi [41], however without the complications of a longer, more complex surgery.

Despite improving success rates of ovarian transposition, this procedure is not without complications: fallopian tube infarction, chronic ovarian pain, and ovarian cyst formation have been reported, some of which may require additional gynecological surgeries [17]. Furthermore, even after transposition and shielding, ovaries are still subjected to scatter radiation, which may amount to as much as 8 to 15% of the total pelvic radiation dose [53]. Other concerns include the possibility of ovarian metastasis and radiation-induced cancer in the transposed ovaries. Morice et al. [54] reported on ovarian metastases in only 2 of 107 patients treated with ovarian transposition and radiation for cervical carcinoma. Both patients had stage IB squamous cell cervical cancer and no nodal involvement or distant metastases, but had uterine corpus and lymphovascular space involvement in the cervix or paracervix. After reviewing these cases and the literature, Morice et al. noted that the risk of ovarian metastasis in transposed ovaries is increased among patients who have a bulky tumor. They concluded that ovarian transposition should be performed only in women under 40 years old with small tumors (<3 cm; Stage IB1 according to the FIGO classification) who are also devoid of extrauterine disease, uterine involvement, and lymphovascular space involvement. In another study of 2,068 women who received 500-1,000 cGy to the ovaries for treatment of menorrhagia and who were followed up for a mean of 19 years, no excess cases of ovarian cancer were observed [55].

Successful, term pregnancies have been reported after ovarian transposition and radiation. In a study by Morice et al. [40] of 37 patients who underwent ovarian transposition and radiation for various cancers, 12 patients (32%) produced 18 pregnancies, of which 16 (89%) were spontaneous, two (11%) followed IVF, and 12 (67%) were from patients whose ovaries remained in the abdominal position. The ovaries were repositioned only in cases of persistent infertility. Oocyte retrieval may be more complicated if IVF is needed and the ovaries remain in their transposed position [17,31]. Reports on pregnancy outcomes after pelvic irradiation have been more equivocal. Swerdlow et al. [56] found no excess cases of stillbirths, low birth weight, congenital malformations, abnormal karyotypes, or cancer in the offspring of women treated for Hodgkin's disease. However, other studies report an increase in stillborn, premature, and small-for-date infants, especially if conception occurs less than a year after radiation exposure, leading to the recommendation by some to defer conception for at least a year after the cessation of radiation therapy [57,58]. Despite the complications listed above, ovarian transposition is a simple, effective, minimally invasive, but grossly underused means of preserving ovarian function and enabling future pregnancy for girls and women younger than age 40 who will be undergoing irradiation without chemotherapy [41,59]. For women in this category who do not wish to undergo some of the more experimental options of fertility preservation, ovarian transposition represents a good choice.

Medical strategies

Novel stimulation regimens: Alternative strategies using tamoxifen and letrozole for ovarian stimulation before IVF have been developed for women with breast cancer, a unique group of cancer patients due to the concerns listed above and the presence of a 6-week hiatus between surgery and chemotherapy in most treatment protocols that may be adequate for ovarian stimulation and IVF [19]. Tamoxifen, a Selective Estrogen Receptor Modulator (SERM), is used to treat and prevent ER+ breast cancers by blocking the action of estrogen on breast tissue. However, it is also effective as an ovarian-stimulation agent given that its use results in an increase in estradiol levels via inhibition of estrogen's negative feedback mechanism [60]. Oktay et al. first studied tamoxifen as an ovarian stimulating agent for IVF in 12 women with breast cancer patients and found that its use resulted in a greater number of mature oocytes (1.6 ± 0.3 versus 0.7 ± 0.2, $P = 0.03$) and embryos (1.6 ± 0.3

versus 0.6 ± 0.2, P = 0.02) per initiated cycle compared to a retrospective control group consisting of breast cancer patients attempting natural cycle IVF [22]. However, the mean peak estradiol level in the tamoxifen group was significantly higher than in the natural cycle IVF patients (442.4 ± 32.6 versus 278 ± 39.9, P = 0.006).

More recent studies have focused on the aromatase inhibitor letrozole as an ovulation induction agent. Aromatase inhibitors prevent the aromatase enzyme from catalyzing the reaction that produces estrogen from androgens and have become common therapy in postmenopausal breast cancer patients. They can also be used for ovulation induction due to their inhibition of estrogen's negative feedback mechanism on the hypothalamus and pituitary, as well as their sensitization of ovarian follicles to gonadotropins [60]. Letrozole combined with FSH for ovarian stimulation resulted in an improved oocyte response to gonadotropins, an increased numbers of preovulatory follicles, and up to a 44% decrease in the amount of gonadotropin required per IVF cycle [61-63]. The main advantage of ovulation induction with aromatase inhibitors, however, is that the peak estradiol levels are lower than in standard regimens and closer to that observed in natural cycles. A subsequent prospective controlled study, also by Oktay et al., showed that compared to the use of tamoxifen alone for ovarian stimulation in IVF, the combination of tamoxifen plus low-dose FSH or letrozole plus low-dose FSH generated greater numbers of follicles (2 ± 0.3 versus 6 ± 1 and 7.8 ± 0.9, respectively; P < 0.0001), mature oocytes (1.5 ± 0.3 versus 5.1 ± 1.1 and 8.5 ± 1.6, respectively; P < .001), and embryos (1.3 ± 0.2 versus 3.8 ± 0.8 and 5.3 ± 0.8, respectively; P < .001) in 60 women with breast cancer [64]. When compared with standard IVF cycles in non-cancer patients, the number of oocytes and embryos were lower in the tamoxifen group and tamoxifen with FSH group, but were similar in the letrozole with FSH group. Additionally, peak estradiol levels in the letrozole with FSH group were found to be lower than those in standard IVF cycles and only minimally higher than those in an unstimulated cycle. They also reported the first pregnancy from cryopreserved embryos generated after tamoxifen stimulation. A follow-up study by Oktay et al. comparing the efficiency of the letrozole-FSH protocol to standard IVF protocols used in patients without breast cancer showed that the length of stimulation, number of embryos obtained, and fertilization rates were similar between the breast cancer patients and age-matched retrospective controls composed of women who underwent IVF for tubal disease [63].

More recently, Azim et al. investigated the effect of ovarian stimulation with letrozole and gonadotropins on recurrence rate and disease-free survival in breast cancer patients undergoing embryo or oocyte cryopreservation before adjuvant chemotherapy [65]. During a median follow-up period after surgery of 23.4 months in the study group (patients who underwent ovarian stimulation) and 33.05 months in the control group (patients who elected not to undergo ovarian stimulation), there were 3.8% recurrences or contralateral breast cancers in the letrozole group and 8.1% in the control group (P = 0.26). There was no significant difference in relapse-free survival between the groups (P = 0.36; hazard ratio = 0.56; 95% CI, 0.17 to 1.9). This study suggests that the use of letrozole and gonadotropins for ovarian stimulation before embryo or oocyte cryopreservation may be a safe option for women with breast cancer and is unlikely to have any significant effects on relapse or recurrence, at least in the short term.

Although tamoxifen and letrozole are both contraindicated during pregnancy, multiple studies show that their use prior to conception poses no risk to the oocyte, embryo, or fetus [66-69]. Moreover, embryos that are cryopreserved are never even exposed to these drugs because the fertilization occurs *in vitro*, and they are not transferred to the uterus until after completion of therapy. Oktay et al. has reported healthy live births after tamoxifen stimulation, IVF, and fresh embryo transfer [22], and in the study by Azim et al., no deleterious effects of letrozole were noted on embryo quality [65]. These findings are very encouraging for women diagnosed with breast cancer who wish to preserve their fertility.

Medical ovarian suppression with GnRH analogs or antagonists: Unlike the options discussed above, Gonadotropin-Releasing Hormone Agonists (GnRH-a) or Antagonists (GnRH-antag) offer a potentially simpler and non-invasive alternative for women interested in preserving their fertility before chemotherapy. The theoretical basis behind this approach is the observation that ovarian function is less likely to be destroyed when chemotherapy is given before puberty [70-72]. Although several investigators have demonstrated that GnRH-a may inhibit chemotherapy-induced ovarian follicle depletion in rodents and primates, its use for gonadal protection in humans is still considered experimental [8], and its efficacy is widely debated due to its unclear mechanism of action and a lack of well-controlled, randomized studies [8,73-77].

Briefly, the proposed, yet controversial, mechanisms of gonadotoxic protection by GnRH-a include [78-80]: (1) simulating the pre-pubertal, hypogonadotropic milieu to delay follicle maturation and chemotherapy-induced destruction; (2) decreasing utero-ovarian perfusion via a hypo-estrogenic state with resultant lower total cumulative exposure of the ovaries to chemotherapeutic agents; (3) directly activating ovarian GnRH receptors and thereby decreasing cellular apoptosis; (4) upregulating a gonadal protective molecule, such as sphingosine-1-phosphate, which may prevent germ cell or follicular apoptosis; and (5) protecting undifferentiated germ line stem cells, which may ultimately replenish the primordial follicle pool. Opponents of these theories argue against the presence of receptors for FSH or GnRH on primordial follicles [81-84]; challenge the proposal of neo-folliculogenesis in the adult mammalian ovary [85,86]; assert that because alkylating agents are not cell-cycle specific, primordial follicles held in a resting state by a hypogonadotropic milieu could still be damaged [77]; and question how decreased blood flow could affect only the ovary but not other organ systems or even the tumor itself [77]. Glode et al. [73] were the first to test the hypothesis of fertility preservation by GnRH-a in a murine model and concluded that GnRH-a appeared to protect mice from the gonadal damage produced by cyclophosphamide. Their findings suggested that primordial germ cells fare better when exposed to cyclophospamide than germ cells that are part of an active cell cycle [73]. Subsequently, a long-term follow-up study of 240 children treated with Mustine, Vincristine, Procarbazine, And Prednisone (MOPP) for Hodgkin lymphoma showed azoospermia in 83% of the boys but POF in only 13% of the girls [71]. Because ovarian function was preserved in most long-term survivors who were treated pre-pubertally for lymphoma but only in about half of similarly treated adult patients [71], it seemed logical that by creating a temporary pre-pubertal milieu in reproductive-aged women before and during chemotherapy, their gonads, and hence their fertility, could be spared from toxicity and premature failure [78]. Ataya et al. [76], in the only prospective randomized study in primates, found that GnRH-a protected the ovary against cyclophosphamide-induced damage in a small group of Rhesus monkeys by significantly decreasing the number of primordial follicles lost (64.6 ± 2.8% in the cyclophosphamide group versus 28.9 ± 9.1% in the GnRH-a + cyclophosphamide group, P < 0.05) and by decreasing the daily rate of follicular decline (0.120 ± 0.012 for the cyclophosphamide group versus 0.057 +/- 0.019 in the GnRH-a + cyclophosphamide group, P < 0.05) during chemotherapy. Numerous studies in women with Hodgkin lymphoma [78-80,87-

89] and breast cancer [90-96], as well as those with systemic lupus erythematosus (SLE) treated with cyclophosphamide [97-99], have subsequently been published. In a prospective non-randomized study of 115 female patients with Hodgkin lymphoma, Blumenfeld et al. [79] compared rates of POF (defined as FSH > 40 U/L on at least two occasions and low menopausal E_2 levels) and cyclic ovarian function (defined as regular spontaneous menstrual cycles, normal gonadotropins and E_2 levels, ovulatory progesterone, and visualization of ovarian follicles or corpora lutea and/or spontaneous ovulation) in 65 patients receiving a monthly injection of GnRH-a, administered before starting chemotherapy until its conclusion for a maximum of 6 months versus 46 patients treated with similar chemotherapy protocols but without GnRH-a either concurrently or historically. There were no significant differences in any of the clinical parameters (epidemiologic or treatment) between the study (GnRH-a + chemotherapy) and control (chemotherapy alone) groups. However, there was a significant difference between the study and control groups in the rates of POF (3.1% versus 37%, respectively; $P < .001$) and COF (96.9% versus 63%, respectively; $P < .001$). Similar case-control studies by Huser et al. [87], Pereyra Pacheco [88], and Castelo-Branco [89] also reported higher rates of POF among women treated with chemotherapy alone compared with women treated with both GnRH-a and chemotherapy. A recent summary of the nine human-controlled GnRH-a studies published between 1980 and 2008 yielded a POF rate of 11.1% in women treated for hematologic malignancies or SLE with chemotherapy and GnRH-a versus 55.5% in controls of similar age receiving identical chemotherapy without GnRH-a [80].

Similarly, GnRH-a has been shown to prevent chemotherapy-associated POF in premenopausal breast cancer patients [90-95]. A recent summary by Maltaris et al. [90] of the four phase II studies [91,92,94,95] on GnRH-a co-treatment in premenopausal breast cancer patients suggested that receiving GnRH analogue throughout treatment may increase a woman's likelihood of remaining premenopausal after chemotherapy and enable the resumption of ovarian function in a high percentage of treated patients, in the range of 83%-96% [95]. All 13 patients in one study [91], aged 26-39 years, resumed normal ovarian function after a mean of 4.9 months post-chemotherapy, while in another study [92], 86% of the 64 premenopausal patients, aged 27-50 years, resumed cyclic menstruation despite a relatively advanced median age of 42 years. In an update of this latter study [92], Recchia et al. [93] found that all their breast cancer patients younger than 40 who received GnRH-a in addition to chemotherapy resumed cyclic ovarian function, with excellent 5- and 10-year survival rates. Del Mastro et al. [94] also reported the resumption of normal menses in 94% of patients under the age of 40 years and by 42% of patients older than 40 years treated with both GnRH-a and chemotherapy. A recent American Society of Clinical Oncology (ASCO) educational book [96] summarized the overall published rates of chemotherapy-induced POF in female breast cancer patients, finding 32-47% for Cyclophosphamide, Methotrexate, And Fluorouracil (CMF) or Cyclophosphamide, Epirubicin, And Fluorouracil (CEF) combinations in patients younger than 40, whereas the addition of GnRH-a to these chemotherapeutic protocols significantly reduced the POF rate to only 0-6%. Despite the above findings, these and other studies have been criticized for their lack of randomization and/or control groups, different follow-up periods for study and control groups, small sample sizes, poor matching between study and control groups, and the use of menstrual status as an index of residual fertility [17,77]. Moreover, the original Randomized Controlled Trial (RCT), conducted by Waxman et al. [100] in 1987, showed no additional protective effect in women with advanced Hodgkin disease randomized to buserelin co-treatment, although the study was later criticized for its small sample size and incomplete pituitary-ovarian suppression [101]. Twenty years after this study, investigators are finally conducting RCTs in response to the aforementioned critiques. A 2011 meta-analysis by Bedaiwy et al. [101] looked at rates of POF, spontaneous ovulation, and spontaneous pregnancy among women co-treated with GnRH-a versus chemotherapy alone in the published, unpublished, and ongoing prospective RCTs from 1960 to January 2010. Of the 28 reports identified, only six trials met the inclusion criteria [100,102-106]. Data from all six studies showed a statistically significant difference in the incidence of women with spontaneous menstruation in favor of the use of GnRH-a (OR 3.46; 95% CI, 1.13-10.57). Data from two studies [100,104] showed a significantly greater incidence of spontaneous ovulation in women co-treated with GnRHa (OR 5.70; 95% CI, 2.29-14.20). Data from three studies [100,102,106] showed no statistically significant difference between the study and control groups in the incidence of spontaneous pregnancy (OR .26; 95% CI, 0.03-2.52). Despite randomization, however, many of these studies still have the same flaws as the non-randomized studies [101,107,108]. These results await confirmation by larger, multicenter, prospective RCTs, several of which are already ongoing in Italy [109], the U.S. [110], and Germany [99]. The safety of GnRH-a has also been called into question. Challengers of GnRH-a argue that not only are they expensive and the cause of severe menopausal symptoms (hot flushes and potential bone loss), but that their direct effects on human cancer cells are not sufficiently understood [77]. They propose that GnRH-a may decrease the effectiveness of chemotherapy via anti-proliferative and anti-apoptotic activity in tumor cells, specifically among hormone-sensitive malignancies such as ER+ breast cancer, [111] or increase the gonadotoxicity of chemotherapy via reduction of detoxifying enzymes in the granulosa cells [112,113]. A recent a Lancet meta-analysis [114], based on data from 11,906 premenopausal women with early breast cancer randomized in 16 trials, has concluded that the addition of GnRH-a reduced the recurrence rate by 12.7% (95% CI, 2.4-21.9%; $P < 0.02$) and death after recurrence by 15.1% (95% CI, 1.8-26.7%; $P < 0.03$), clearly contraindicating some of these concerns. Supporters of GnRH-a also argue that its use is highly effective for the prevention of thrombocytopenia-associated menometrorrhagia in hematologic malignancies [115]. There is also concern related to the delay in chemotherapy initiation when using GnRH-a because it must be started in the luteal phase and administered for several weeks until pituitary-ovarian down-regulation is achieved. The use of GnRH-antag, which do not have the initial increase in gonadal activity ("flare") caused by GnRH-a, has been proposed because down-regulation occurs within a matter of days, regardless of the point in the menstrual cycle [101]. Although Meirow et al. [116] found that a GnRH-antag decreased ovarian damage induced by cyclophosphamide in rats, other rodent studies [117,118] have found that GnRH-antag do not protect the ovary from the damaging effects of cyclophosphamide and may even cause a significant reduction in the number of primordial follicles without cyclophosphamide [117]. To achieve faster down-regulation without the damaging effects of GnRH-antag alone, Mardesic et al. [119] that the combination of GnRH-a and GnRH-antag induced a reliable and long-lasting suppression of gonadotropin secretion within 96 hours in all patients, allowing cytotoxic therapy to be started without any delay. At this time, the American Society of Clinical Oncology states: "Since there is insufficient evidence regarding the safety and effectiveness of GnRH analogs and other means of ovarian suppression on female fertility preservation, women interested in ovarian suppression for this purpose are encouraged to participate in clinical trials" [8]. Although the outcomes of recent RCTs [100,102-106] are promising, the results of

additional multicenter, prospective RCTs are needed before unequivocal support for GnRH-a can be made.

Ovarian tissue cryopreservation

Ovarian tissue cryopreservation is another experimental option for female cancer patients, especially those who need treatment without delay, as in the case of a rapidly growing tumor; who are unwilling to undergo ovarian stimulation, as in the case of breast cancer patients; or who do not have a male partner and do not want to use donated sperm. If successful, ovarian tissue cryopreservation can restore ovarian endocrine function, allowing the possibility of spontaneous conception, which cannot be provided by embryo or egg freezing. Moreover, because ovarian stimulation is not ethically or technically practical in children, ovarian tissue freezing is currently the only available option for pre-pubertal girls [6,120,121]. The drawbacks of this technique include the necessity for surgery to harvest and then transfer the tissue, the decreased efficacy in women over age 40 due to age-related declines in primordial follicle counts [122], and the concern for reseeding occult cancer cells in the cryopreserved ovarian tissue during transfer [123]. Ovarian tissue cryopreservation is not an option for women with ovarian cancer.The procedure generally entails harvesting ovarian cortical tissue by laparoscopy, which allows the patient to be discharged the same day as the procedure, or laparotomy, followed by cutting the tissue into strips around 1-3 mm in thickness and up to 1 cm^2 in total area to ensure adequate penetration of Cryoprotectant Agents (CPAs), and freezing the strips for future use [124]. Freezing the ovarian cortex has an advantage over freezing antral follicle oocytes, in that the tissue conserves a significant number of primordial follicles (the most abundant follicles in the ovary) - approximately 120 small follicles contained in one 4 mm ovarian disk in a 30 year old patient - that are relatively resistant to freeze-thaw injury (about 70%-80% survival) [125]. The oocytes within the primordial follicles are arrested in the diplotene stage of prophase of the first meiotic division and are less susceptible to cryodamage than both mature and immature oocytes due to their smaller size, slower metabolic rate, absence of a zona pellucida, and lack of metaphase spindles [19]. Tissue samples can be frozen by either a slow-freeze/rapid-thaw or vitrification method, although it is as yet unclear which technique is superior. Whereas Gandolfi et al. [126] and Isachenko et al. [127,128] have reported better preservation of all types of follicles and higher follicular developmental potential with slow-freezing than vitrification, Li et al. [129], Keros et al. [130], and Silber et al. [131] have reported more morphologically intact primordial follicles, greater morphological integrity of the ovarian stroma, and better oocyte survival with vitrification than slow-freezing. Because most of the published studies on human ovarian tissue freezing utilized slow freezing methods, the data on the success rates of grafts frozen with newly emerging vitrification methods are still lacking.

There are, in theory, three options for re-implantation of the ovarian tissue: (1) auto-transplantation orthotopically or heterotopically, (2) xenograft transplantation, (3) cryopreservation of the whole ovary with vascular transplantation after thawing.

Orthotopic transplantation involves replacing the thawed ovarian tissue back in the pelvis, at close proximity to the infundibulo-pelvic ligament, either onto the remaining ovary in the case of a unilateral oophorectomy or into the peritoneal pocket of the ovarian fossa. Although this procedure requires abdominal surgery and general anesthesia, the advantage is that a natural pregnancy can potentially occur. The resumption of ovarian endocrine function has been demonstrated in women receiving orthotopic auto-transplantations of their previously cryopreserved ovarian tissue [132,133], and to date, 13 healthy babies born to 10 women after orthotopic re-implantation have been reported [134-142]. In all instances, it took between 3 ½ and 6 ½ months after transplantation before ovarian function was restored, as detected by a rise in E_2 and a fall in FSH, and the duration of restored ovarian activity ranged from one to five years [143]. It is also notable that all patients except one were under 30 years of age (17-28 years), and six of the ten patients were less than 25 years old (mean 23 years; range 19-25 years) [143]. This makes the procedure unproven in older women, many of whom are also at risk of treatment-induced POF.

Meanwhile, heterotopic transplantation has had less encouraging outcomes. Although heterotopic transplantation avoids abdominal surgery and general anesthesia, makes the monitoring of follicular development and the recovery of oocytes easy, and offers a practical and cost-effective solution when repeated transplantation or removal of the graft is required, to date, there have been no live births reported with this procedure [17]. Nevertheless, heterotopic transplantation may be offered to patients whose risk of ovarian metastasis or recurrence is high; whose pelvis is not suitable for transplant due to previous radiation or scarring; and who are concerned about the higher cost and invasiveness of orthotopic transplantation [6]. Ovarian tissue has been heterotopically transplanted into the subcutaneous tissue of the forearm [144], the space between the rectus sheath and rectus muscle [145,146], and the breast tissue [145], but the optimal site for transplantation is still unknown. Several investigators have documented the return of endocrine functions as well as ooctye retrieval after heterotopic transplantation of human ovarian tissue, with ovarian function lasting from two months to three years in various reports [144-148]. Owing to the limited life-span of the grafts in both orthotopic and heterotopic transplants, auto-transplantation should occur only when the patient is ready to conceive. A significant concern associated with auto-transplantation is the risk of reintroducing metastatic cancer cells from the ovarian tissue back into the body. This risk has been estimated to be highest for hematological cancers, such as leukemia and lymphoma [123], although over 30 cases of ovarian transplantation have been performed in women with various cancers, including breast cancer, cervical cancer, non-Hodgkin's lymphoma, Hodgkin's lymphoma, and Ewing sarcoma, without any case of cancer cell reintroduction after ovarian tissue transplantation in humans [149]. Rosendahl et al. [150] recently conducted histological and immunohistochemical analyses on cryopreserved ovarian cortical biopsies from 51 patients with breast cancer, bringing the published total up to more than 160 ovarian cortical biopsies and six entire cortex biopsies from 133 patients with breast cancer in which no evidence of malignant cell contamination was found. Nevertheless, histologic and immunohistochemical analysis may not be sufficiently sensitive to detect infiltration with a small number of cells. In evaluation of 58 hematological cancers referred for ovarian tissue cryopreservation, Meirow et al. [151] found that a sample that had shown to be negative for cancerous cells by conventional histology was actually positive for chronic myeloid leukemia with highly sensitive Real-Time Reverse Transcriptase (RT)-PCR. Another method, with higher certainty, of ruling out cancer cells from the cryopreserved ovarian tissue would be to xenotransplant the ovarian cortex to immune-compromised mice. This method was recently validated as a screening tool when mice transplanted with the ovarian cortex from women with leukemia showed evidence of reactivation of the cancer [152]. Mice with Severe Combined Immunodeficiency (SCID) can easily accept tissues from foreign species without concern for a graft-versus-host response due to a deficiency in both T- and B-cell mediated immunity [153]. Xenotransplantation can also potentially be used to mature the primordial follicles without the possibility of cancer cell transmission and relapse because

cancer cells do not penetrate the zona pellucida [19]. Oktay et al. [154] documented the presence of healthy follicles in a graft when removed 22 weeks after the initial transplantation; Weissman et al. [155] observed follicular growth, including the development of antral follicles, in response to exogenous gonadotropin stimulation after subcutaneous placement of human ovarian cortical tissue into mice; and Kim et al. [156] provided evidence that human primordial follicles after xenotransplantation to a subcutaneous site could be matured to an ovulatory stage and subsequently form functional corpora luteae. Xenotransplantation also allows for convenient monitoring of follicular development and easy access for follicle aspiration [19]. This method also avoids further surgery to transplant the tissue back to the patient and may be a possible option for women in whom hormonal stimulation is contraindicated [14,19]. However, possible transmission of zoonoses to humans is a serious concern [19]. To date, there have been no clinical pregnancies with xenografting. The main threat to the success of ovarian tissue cryopreservation, however, is ischemia-reperfusion injury to tissue after transplantation. It has been reported that more primordial follicles die of ischemia than of freezing injury [157,158]. More than 60% of primordial follicles are lost after transplantation during the ischemic period until revascularization is established according to animal autograft [158] and human xenograft studies [159]. An additional 7% appear to be lost during freezing and thawing [160]. To overcome this obstacle, studies have focused on cryopreservation of the intact human ovary with its vascular pedicle [161-163]. Although restoration of fertility after whole frozen ovary transplantion has been documented in animals [164-166], all human ovarian whole ovary transplantations to date have been performed using fresh ovaries [167-169]. Martinez-Madrid et al. [161,162] have, however, described a protocol for cryopreservation of the intact human ovary with its vascular pedicle in which high survival rates of follicles (75.1%), small vessels, and stromal cells, as well as a normal histological structure in all ovarian components, were achieved after thawing. Moreover, they observed no signs of apoptosis or ultrastructural alterations in any cell types [162]. Nevertheless, the challenge of whole ovary cryopreservation remains the difficulty of adequate CPA diffusion into large tissue masses and the risk of vascular injury caused by intravascular ice formation [163].

Oocyte cryopreservation

For female cancer patients without a partner or who want to avoid surgery, oocyte cryopreservation may represent a more attractive means of fertility preservation than the aforementioned options. Oocyte cryopreservation does not require a male partner or sperm donor, requires no surgery, eliminates the concern for cancer cell contamination, takes advantage of well-tested controlled ovarian hyper-stimulation protocols, and avoids the ethical and legal concerns related to embryo storage and disposal. Although the first birth after human oocyte cryopreservation was reported in 1986 [170], oocyte cryopreservation is still considered experimental by the ASCO, the American Society of Reproductive Medicine (ASRM), and the Society for Assisted Reproductive Technology (SART) owing to historically low success rates [8,171]. Pregnancy rates were as low as 1-2% in the late 1990s due to low oocyte survival rates (25-40%), low fertilization rates after traditional IVF, high incidences of polyploidy, and poor developmental abilities of the embryos [170,172-174]. Due to these inefficiencies, oocyte cryopreservation was largely abandoned as a means of fertility preservation, with only about 100 children born from frozen oocytes by 2005 [172]. However, recent advances in oocyte cryopreservation technology have led to better post-thaw survival, fertilization, and pregnancy rates, resulting in a renewed interest in oocyte cryopreservation. Indeed, by 2009, more than 900 babies were reported to be born from cryopreserved oocytes with reassuring birth defect data [175], and at least three births using cryopreserved oocytes from cancer patients have also been reported [176,177].

Cryopreserved oocytes vs. Fresh oocytes

The 2006 meta-analysis by Oktay et al. [172] reported that in comparison to IVF with slow-frozen/rapid-thawed oocytes, IVF with unfrozen oocytes resulted in significantly better rates of fertilization (OR 2.22; 95% CI 1.80-2.74), live-birth per injected oocyte (OR 1.5; 95% CI 1.26-1.79), live-birth per embryo transfer (OR 6.83; 95% CI 5.76-8.09), and implantation (OR 4.66; 95% CI 3.93-5.52). Although slow-freeze/rapid-thaw success rates were considerably lower than those of IVF with fresh oocytes, vitrification success rates were close to those reported by SART with fresh oocytes (45.5% versus 46.6% clinical pregnancy rate per embryo transfer and 36.6% versus 38.4% live birth rate per embryo transfer, respectively) for the same time period. In a subsequent study, Antinori et al. [178] cryopreserved surplus oocytes of patients who underwent IVF with fresh oocytes and compared the success rates of the same group of patients with vitrified and non-vitrified oocytes. They found that the pregnancy (32.5% versus 28.6%) and implantation (13.2% versus 10.3%) success rates with vitrified oocytes were similar to the rates with fresh oocytes. In a prospective randomized study in which oocytes from a given donor were either inseminated fresh or were frozen by vitrification for a minimum of 1 hour and then thawed, inseminated, and cultured along with the fresh oocytes from that same donor cycle, the authors found no statistical difference in fertilization rates (76.3% versus 82.2%), day 2 cleavage (94.2% versus 97.8%), day 3 cleavage (77.6% versus 84.6%), blastocyst formation (48.7% versus 47.5%), and embryo quality on day 3 (80.8% versus 80.5%) and day 5-6 (81.1% versus 70%) between vitrified and fresh oocytes [179]. In 23 cycles, embryos from vitrified oocytes were transferred resulting in a 65.2% pregnancy, 40.8% implantation and 47.8% ongoing pregnancy rate, similar to what was obtained with fresh oocytes. In a prospective randomized trial on 244 sibling oocytes to compare the *in vitro* performance of fresh and vitrified oocytes post-ICSI, Rienzi et al. [180] found similar rates of oocyte fertilization and embryo development in the vitrified and fresh oocyte groups, concluding that oocyte vitrification followed by ICSI is not inferior to fresh insemination in terms of fertilization and embryo development. Most recently, Grifo and Noyes [181] reported a live-birth/ongoing pregnancy rate of 57% for cryopreserved oocytes, which was not statistically different from cycles performed consecutively in age-matched controls using fresh, non-frozen autologous or donor oocytes during a similar time period.

Oocyte vs. Embryo cryopreservation

Although many studies compare the outcomes of oocyte cryopreservation with those of conventional IVF using fresh oocytes, it may be more appropriate to compare the outcomes of oocyte cryopreservation with those of cryopreserved embryos [182]. The live-birth rate per transfer of 32.4% with slow-freeze between 2002 and 2004 and of 39% with vitrification after June 2005 in the meta-analysis by Oktay et al. [172] compare favorably with those reported for cryopreserved donor embryos (30.1% in 2003 and 32.1% in 2006), which yield the highest pregnancy rates and are most representative of fertile women Similarly, Chen et al. [183] reported a protocol for slow freezing oocytes in 1.5M PrOH plus 0.3 M sucrose and performing ICSI at 3hours post-thaw that allowed for survival, pregnancy, and

implantation rates comparable to those obtained from-thawed pronuclear embryos (75% versus 79%; 33% versus 32%; 11% versus 9%).

Safety

Because of the known effects of cryopreservation on the meiotic spindle of the oocyte, there are concerns regarding the risk of chromosomal aneuploidy. In 2008, the Human Oocyte Preservation Experience (HOPE) Registry, a phase IV, prospective, multi-center, observational oocyte cryopreservation patient registry, was established to prospectively and systematically track the outcome of oocyte cryopreservation cycles in order to validate both the efficacy of oocyte cryopreservation techniques and the safety of these procedures [184]. The study, however, is not estimated to be complete until September 2012. In the meantime, obstetrical and perinatal outcomes of pregnancies from frozen oocytes have been reported in several smaller studies. In 2000, Porcu et al. [185] published the first clinical confirmation that oocyte freezing is associated with the birth of healthy children. They reported no major or minor malformations and normal postnatal growth and physical and intellectual development in 13 children born from slow-frozen/rapid-thawed oocytes. Borini et al. [186] more recently reported on obstetric outcome and anomalies or malformations of 105 babies born from slow-cooled oocytes. The mean gestational age at delivery was 38.9 weeks; the mean weight of a singleton was 3,353 kg and a twin was 2,599 kg. Two malformations were found: choanal atresia and the Rubinstein-Taydi Syndrome. Chian et al. [187] analyzed the outcomes of 165 vitrified oocyte pregnancies totaling 200 infants born and showed no difference in mean birth weight or incidence of congenital anomalies in children born from vitrified oocytes compared to those from either spontaneous conception in fertile women or in women conceiving through fresh IVF. More recently, Cobo et al. [188] compared a cohort of 160 pregnancies (212 live births) following vitrification with another cohort of 262 pregnancies (315 live births) achieved using fresh oocytes (control) and concluded that obstetric and perinatal outcomes in oocyte vitrification were not significantly (NS) different from those achieved using fresh oocytes. Gestational age at delivery was 37.4 ± 2.5 weeks for vitrified oocytes and 38.0 ± 2.3 weeks for fresh oocytes (P = NS). The mean birthweight was 2718 ± 0.7 g for vitrified oocytes and 2896 ± 0.7 g for fresh oocytes (P = NS). There were 4 major birth defects in the vitrification group (1.8%) and 2 in the control group (0.6%) (P = NS). Differences in rates of preterm premature rupture of membranes, gestational diabetes, gestational hypertension, and preterm delivery were also not significant. In the largest report of perinatal outcomes so far, Noyes et al. [175] tabulated data from 58 reports from multiple centers around the world, between 1986 and 2008, using either slow-freezing or vitrification and found that in 936 babies born from frozen oocytes, there was no apparent increase in the rate of congenital anomalies as compared to United States national statistics for natural conceptions as reported by the CDC. Follow-up studies of children conceived by IVM are more limited, but have been reassuring so far. Söderström-Anttila et al. [189] collected data from all deliveries after IVM treatment during 1999-2004 at their infertility clinic in Helsinki, Finland, and reported good obstetric and perinatal outcomes. They assessed the growth and development of the IVM children at six, 12, and 24 months using the Muenchener Funktionelle Entwicklungs Diagnostik and Bayley Scales of Infants and compared the results to well-documented Finnish national standards. The study consisted of 43 women who gave birth to 40 singleton infants and three sets of twins. Adverse events occurred in 15 pregnancies (35%): eight cases of pre-eclampsia, three cases of gestational diabetes, and four cases of pre-term delivery. The mean ± SD duration of pregnancy was 282 ± 11 days in singleton and 257 ± 15 in twin pregnancies. Post-partum complications occurred in 28% of the mothers: three infections, three hemorrhages requiring curettage or operation, five cases of anemia requiring blood transfusion, and one rupture of the anal sphincter. The mean birthweight of the IVM infants (singletons 3550 g and twins 2622 g) was within the normal range for Finnish newborns, with no singleton low-birth weight newborns and only three small for gestational age babies. Only six newborns (13%) were admitted to a neonatal surveillance or an intensive care unit, which was less than the admission rate of Finnish IVF newborns (25%) in general. There were no major malformations found in the children up to 2 years of age. Three children (7%) showed mild developmental delay at the age of six months, but two of them had caught up to normal developmental profile by the age of 12 months. However, at the age of 12 months, eight children (19%) expressed mild developmental problems, which was higher than that of 10% usually reported in the general Finnish population, and one child had considerable developmental delay with nystagmus and visual and motor problems because of an optical glioma. Although minor developmental delays were overexpressed at 12 months, the development of the majority children (97%) was normal at 2 years. All nine children with mild or considerable developmental delay at 12 months of age were re-examined at the age of 2 years; eight of them had normal neuropsychological development compared to the standard population, whereas only one child (3%) continued to show mild delay. William et al. [190] found that IVM is not associated with an increased risk of congenital abnormality compared with IVF, ICSI, or spontaneously conceived controls. More recently, Zhang and Cao [191] found no differences in multi-pregnancy, ectopic pregnancy, miscarriage, and cesarean delivery rates; gestational age; birth weight; Apgar scores; neonatal and obstetric outcomes; and congenital abnormalities in an IVM treatment group compared with a conventional IVF and ICSI treatment group during the same time period. In a study comparing the obstetric and perinatal outcomes following vitrification of oocytes obtained from ovarian stimulation versus IVM cycles, Chian et al. [192] reported for the first time that a series of healthy live births (four) can be achieved from the combination of IVM and oocyte vitrification [193].

Conclusion

Advances in cancer detection and treatment have resulted in an increasing amount of long-term survivors who are left to deal with the adverse effects of their treatments. Fortunately, progress in fertility preservation technologies has been paralleling the trend in improving cancer outcomes.

Because of the variations in type and dose of chemotherapy or radiation, the type of cancer, the time available before treatment initiation, and the patient's age and partner status, each case is unique and requires a different strategy for fertility preservation. If the patient has a partner or is willing to accept donor sperm, embryo cryopreservation should be considered first, since this is a clinically well-established procedure. In breast cancer patients, tamoxifen or letrozole can be used for ovarian stimulation before embryo cryopreservation. When only pelvic radiotherapy is used, ovarian transposition can be performed, although the success rates vary due to scatter radiation, location of the fixed ovaries, and vascular compromise. GnRH-a co-treatment, despite its debatable efficacy, is currently being used for most patients treated with chemotherapy. Despite few reported pregnancies so far, ovarian tissue cryopreservation holds great promise for pre-pubertal cancer patients. Finally, the future of fertility preservation for female cancer patients points towards the more efficient coupling of *in-vitro* matured oocyte cryopreservation

with ovarian tissue cryopreservation to maximize fertility potential. Important issues for the patient to consider before deciding on a method of fertility preservation include: surgical complications, ovarian hyper-stimulation syndrome, delaying cancer treatment, cost, low or variable success rates, the experimental nature of the fertility preservation treatment, and the disposition of embryos in the event that the patient does not survive her cancer.

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