REVIEW ARTICLE

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Fertility Preservation in Women

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In recent years, the demand for fertility preservation for oncologic and nononcologic reasons, as well as personal reasons, has increased dramatically,[1] and meeting this demand will prove a major challenge in the coming years.[1] Currently, embryo cryopreservation and mature-oocyte cryopreservation after ovarian stimulation are the only methods of fertility preservation endorsed by the American Society for Reproductive Medicine.[2] However, many experts believe that there is now enough evidence to support the use of ovarian-tissue cryopreservation as a valid and effective technique rather than as an experimental approach.[1,3]

Of all the available means of fertility preservation,[1] oocyte cryopreservation by means of vitrification (very rapid freezing) provides the highest yield, not only for women with benign diseases or those seeking fertility preservation for personal reasons but also for women with cancer (if treatment can be postponed). Ovarian-tissue cryopreservation is specifically indicated for adolescents and women in whom cancer treatment cannot be postponed.[1,3] This review focuses on the indications for and results of these two techniques of fertility preservation.

Ovarian Reserve

The term "ovarian reserve" is typically used to refer to the population of primordial follicles.[4] Initiation of the resting primordial-follicle reserve begins in the fetus, when 100 to 2000 primordial germ cells colonize the genital ridges and enter a massive proliferation process that results in 7 million potential oocytes at midgestation. In the human ovary, approximately 85% of these potential oocytes are lost before birth.[4] The decline in the number of follicles continues throughout reproductive life, during which time approximately 450 ovulatory cycles occur, with the majority of follicles undergoing atresia during their growth phase.[4] The serum level of antimüllerian hormone, which is correlated with the number of primordial follicles but is not a direct product of these follicles, can be used to estimate the reproductive life span.[5]

Indications for Fertility Preservation

Malignant Diseases

Fertility preservation remains a challenge, particularly in the case of hematologic cancers (Hodgkin's lymphoma, non-Hodgkin's lymphoma, and leukemia) and breast cancer. These cancers constitute the most frequent indications for fertility preservation, since chemotherapy (especially with alkylating agents), radiotherapy, surgery, or a combination of these treatments can induce premature ovarian insufficiency in some circumstances[1,6-10] (Table 1). The ovaries are very sensitive to cytotoxic drugs, especially alkylating agents, which are likely to cause gonadal dysfunc-

Table 1. Indications for Fertility Preservation.

Malignant diseases requiring gonadotoxic chemotherapy, radiotherapy, or bone marrow transplantation
Hematologic diseases (leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma)
Breast cancer
Sarcoma
Some pelvic cancers
Nonmalignant conditions
Systemic diseases requiring chemotherapy, radiotherapy, or bone marrow transplantation
Ovarian diseases
Bilateral benign ovarian tumors
Severe and recurrent ovarian endometriosis
Possible ovarian torsion
Risk of premature ovarian insufficiency
Family history
Turner's syndrome
Personal reasons
Age
Childbearing postponed until later in life

tion.[6,7,11,12] Cyclophosphamide is the alkylating agent that causes the most damage to oocytes and granulosa cells, and it does so in a dose-dependent manner. In a recent review,[13] it was reported that the North American Children's Oncology Group considers the risk of premature ovarian insufficiency to be highest with busulfan administered at a dose of at least 600 mg per square meter of body-surface area, cyclophosphamide at a dose of at least 7.5 g per square meter, or ifosfamide at a dose of at least 60 g per square meter, but an international multidisciplinary panel reached no consensus on this matter.

Pelvic radiotherapy is also known to cause premature ovarian insufficiency, since exposure to 5 to 10 Gy is toxic to oocytes. Indeed, the human oocyte is very sensitive to radiation — a dose of less than 2 Gy is estimated to be sufficient to destroy 50% of primordial follicles.[14,15]

Ultimately, the probability that premature ovarian insufficiency will develop after chemotherapy or radiotherapy is related to the ovarian reserve. This reserve (the population of primordial follicles) can vary enormously from one woman to the next.[7]

BENIGN CONDITIONS

Fertility preservation should also be offered to women with certain benign conditions that carry the risk of premature ovarian insufficiency. Many autoimmune and hematologic conditions sometimes require chemotherapy, radiotherapy, or both and sometimes even bone marrow transplantation (Table 1). Other conditions can also impair future fertility, such as the presence of bilateral ovarian tumors, severe or recurrent ovarian endometriosis, and recurrent ovarian torsion.

Ovarian endometriomas lead to reduced ovarian reserve.[16] Local intraovarian inflammation induced by the presence of endometriomas has been shown to trigger follicle "burnout," characterized by activated follicular recruitment with subsequent atresia.[17] Moreover, increasing evidence shows that performing cystectomy on endometriomas may cause considerable damage to the ovarian reserve,[18-21] so fertility preservation should certainly be contemplated in case of recurrence after surgery.[22]

Turner's syndrome and a family history of premature ovarian insufficiency are additional indications for fertility preservation[1] (Table 1). There is compelling evidence that certain forms of premature ovarian insufficiency have a genetic cause.[23,24]

AGE-RELATED FERTILITY DECLINE

The largest group of women seeking fertility preservation consists of those who wish to postpone childbearing for various personal reasons; the biggest threat to their fertility is age. Women are increasingly seeking "time out" until they reach the right stage in their life to have a baby, often postponing childbearing because of the lack of a partner, the lack of a stable partner, or career or financial issues.[25-27] The age at which women attempt their first pregnancy has been steadily rising during the past 40 years.

EMBRYO AND OOCYTE CRYOPRESERVATION

Embryo cryopreservation has been carried out since the early years of assisted reproductive technology, more than 30 years ago. Nowadays, transfer of vitrified and warmed embryos in an artificial endometrial-priming cycle is as efficient as fresh-embryo transfer, and embryo storage

time after thawing does not affect live-birth rates.[26,27] However, embryo cryopreservation requires a male partner or use of donor sperm, which opens the door to ethical and legal concerns about the fate of orphan embryos if the patient dies or if she and her partner separate. Cryopreservation of mature oocytes can circumvent these concerns (Fig. 1),[28] preserving a woman's ability to procreate with a chosen partner in the future.

Data from a recent review[27] suggest that the strategy of oocyte vitrification and warming is superior to slow freezing and thawing in terms of clinical outcomes. On the basis of this evidence, laboratories that continue to use slow freezing should consider transitioning to vitrification techniques for purposes of cryopreservation.[27]

When fertility preservation is carried out for benign indications or personal reasons, oocyte cryopreservation is clearly the highest-yield strategy.[26,27] Moreover, it gives women the possibility of reproductive autonomy[28] (i.e., they do not need a male partner or donor sperm to create embryos). For women of advanced childbearing age who do not yet wish to conceive, this technique may be used to extend their fertility potential, in view of the decline in oocyte quality with age.[25,26]

Cobo et al.[26] recently reported outcomes for 137 women who had undergone fertility preservation by means of oocyte vitrification for nononcologic reasons and subsequently returned to use their oocytes. A total of 120 women had undergone the procedure to circumvent age-related fertility decline. Among women who were 35 years of age or younger at the time of oocyte vitrification, the cumulative live-birth rate was much lower when only 5 oocytes were used (15.4%) than when 8 or 10 oocytes were used (40.8% and 60.5%, respectively) (Fig. 2). Among women who were older than 35 years of age at the time of the procedure, the cumulative live-birth rates were 5.1%, 19.9%, and 29.7% with 5, 8, and 10 oocytes, respectively. Hence, with 10 oocytes, the cumulative live-birth rate was twice as high in the group of women who were 35 years of age or younger (60.5%) as in the group of older women (29.7%). These data suggest that women should be encouraged to freeze their eggs at a younger age for the best chance of having a biologic child.[26,27]

Stoop,[29] elaborating on the report by Cobo et al., stressed the importance of providing patients with center-specific information about experience with fertility preservation. Only programs achieving the highest pregnancy rates publish their outcome data, but these results cannot be generalized and used by centers with less experience to counsel candidates for oocyte cryopreservation.

When fertility preservation in women with cancer is contemplated, there are five important points to bear in mind. First, to allow time for oocyte vitrification, chemotherapy should be delayed by at least 10 to 12 days.[1,10,26] Second, the patient must be postpubertal.[1,6-8] Third, specific protocols for controlled ovarian stimulation should be followed according to the steroid sensitivity of the specific cancer. Fourth, information on oocyte quality in women with cancer is lacking because the priority for such women is achieving complete disease remission and because the option of collecting oocytes for vitrification is relatively new.[25] Finally, the excellent results obtained in egg-donation programs[26,28-30] cannot be extrapolated to women who have been treated for cancer,[1] as shown by Pellicer, who reported a lower live-birth rate after oocyte vitrification in this population than in the population of patients who have not had cancer.[31]

OVARIAN-TISSUE CRYOPRESERVATION

Cryopreservation of ovarian tissue (Fig. 1) is the only option for fertility preservation in prepubertal girls and women who cannot delay the start of chemotherapy.[1,6-8,32] The technique is still considered experimental,[2] but it may move toward broader clinical implementation with the use of strict selection criteria.[3]

NEED FOR SELECTION CRITERIA

Gonadotoxicity is age-dependent. First-line cancer treatment does not compromise the ovarian reserve by more than 10% in girls under 10 years of age,[7,32-35] whereas girls who are 11 or 12 years of age have an estimated 30% decline in their ovarian reserve. There is a marked association between the intensity of the treatment received and the likelihood of premature ovarian insufficiency, even in young girls,[13,34,35] but it is impossible to predict exactly who will have premature

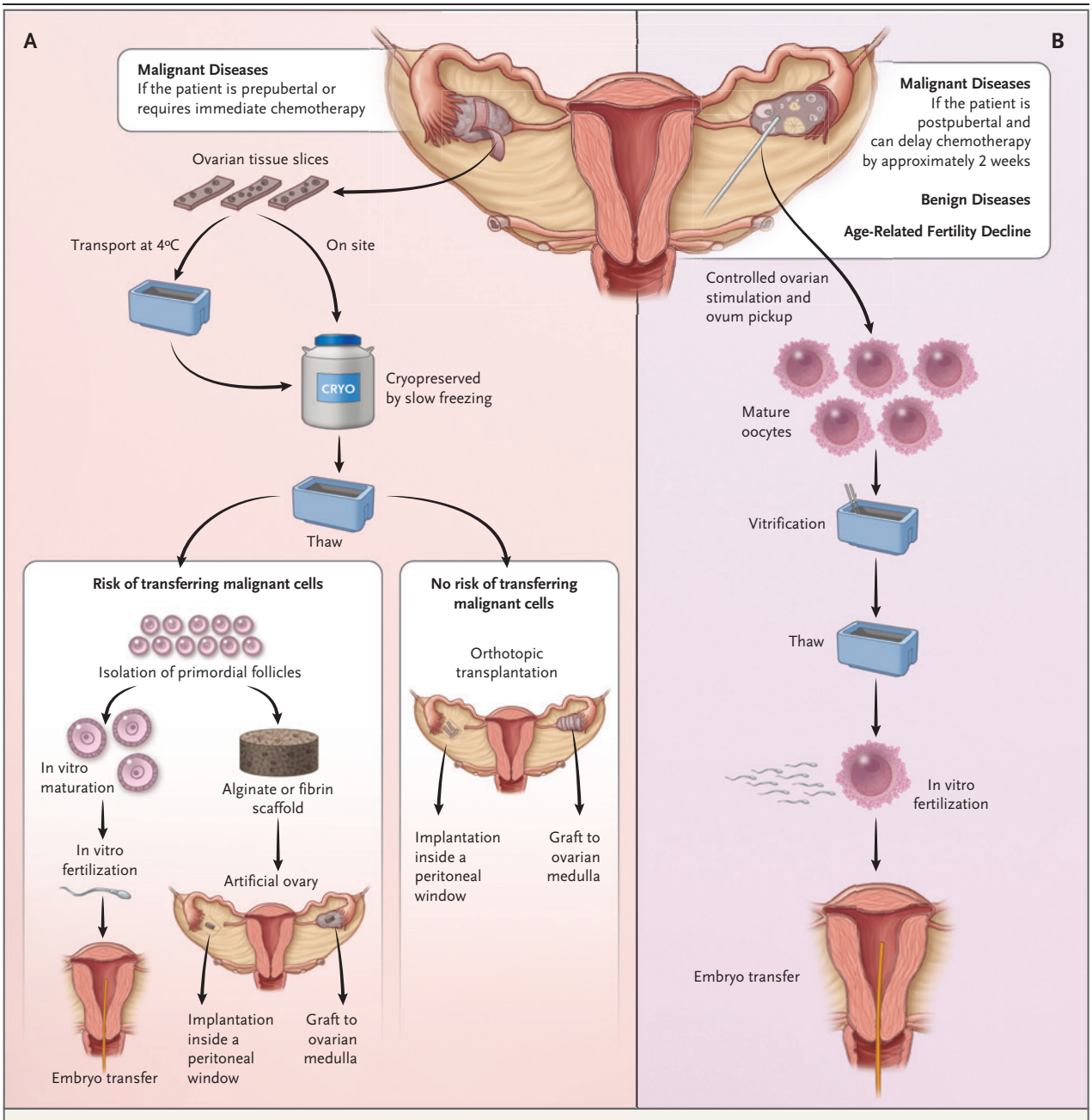

Figure 1. Options for Fertility Preservation.

If the patient is prepubertal or requires immediate chemotherapy (Panel A), ovarian tissue is removed in the form of multiple biopsy specimens (or an entire organ) and cut into cortical strips. The tissue is then cryopreserved by slow freezing on site (or transported to a processing site at a temperature of 4°C). After thawing, if there is no risk of transmitting malignant cells, the ovarian tissue can be grafted to the ovarian medulla (if at least one ovary is still present) or reimplanted inside a specially created peritoneal window. If there is a risk of transmitting malignant cells, ovarian follicles can be isolated and grown in vitro to obtain mature eggs, which can then be fertilized and transferred to the uterine cavity. Isolated follicles may be placed inside a scaffold (alginate or fibrin), creating an artificial ovary that can be grafted to the ovarian medulla or peritoneal window. If the patient is postpubertal and chemotherapy can be delayed for approximately 2 weeks (Panel B), mature oocytes can be removed after ovarian stimulation and vitrified on site. After thawing, they can be inseminated and transferred to the uterine cavity in the form of embryos. This technique can also be used in women with benign diseases or in those with age-related fertility decline. The techniques in Panels A and B can also be combined, with ovarian-tissue cryopreservation followed by controlled ovarian stimulation and vitrification of oocytes. The combined technique theoretically yields a 50 to 60% chance of a live birth.

ovarian insufficiency after aggressive chemotherapy. Selection criteria clearly need to be applied, the most important being an age of less than 35 years (when the ovarian reserve is still relatively high), a realistic chance of surviving for 5 years, and at least a 50% risk of premature ovarian insufficiency.[1,7,32]

EFFECT OF OVARIAN BIOPSIES ON HORMONE PRODUCTION

Obtaining multiple biopsy samples from one ovary has not been shown to compromise future hormone production.[36] Removal of a single ovary has been shown to shorten the time to menopause by 1 to 2 years.[37,38]

REIMPLANTATION OF OVARIAN TISSUE AND RATES OF PREGNANCY AND LIVE BIRTH

After reimplantation of ovarian tissue in the pelvic cavity (Fig. 1),[36,39-42] ovarian activity is restored in more than 95% of cases.[3,43] The mean duration of ovarian function after reimplantation is 4 to 5 years, but the function can persist for up to 7 years, depending on the follicular density at the time of ovarian-tissue cryopreservation[43] (see Fig. S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org).

The first pregnancy after this procedure was reported in 2004,[42] and the second in 2005.[44] Rates of pregnancy and live birth have continued to climb steadily, showing an exponential increase (Fig. 3). Indeed, taking into account the latest published series,[45-50] the number of live births as of June 2017 exceeded 130.

Since the denominator (the number of reimplantations performed worldwide) is not known, the calculation was based on patients from five major centers (a total of 111 patients), yielding a pregnancy rate of 29% and a live-birth rate of 23%.[3] These rates were subsequently confirmed in a case series of 74 women, with pregnancy and live-birth rates of 33% and 25%, respectively.[46] In our series of 22 women who underwent ovarian-tissue reimplantation, pregnancy and live-birth rates were respectively 41% (9 of 22) and 36% (8 of 22), with a total of three ongoing pregnancies and 12 live births.[3,43,45] One woman in this series delivered three times, making her 1 of 2 patients worldwide to have three pregnancies and births resulting from a single ovarian-tissue reimplantation procedure.[45,50] Transplanting ovarian tissue to heterotopic sites remains somewhat questionable, however, and only one pregnancy has been reported in a woman who underwent this procedure.[51]

To improve the results, loss of follicles after reimplantation needs to be addressed. One way of enhancing graft revascularization is by delivering (locally) both angiogenic and antiapoptotic factors.[1] As far as the freezing procedure is concerned, there is no evidence that vitrification of

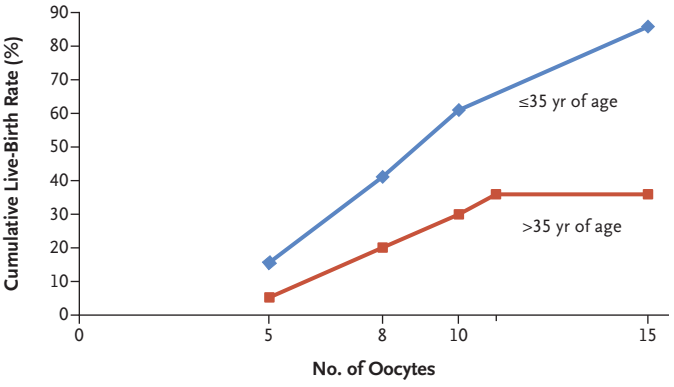

Figure 2. Cumulative Live-Birth Rates with 5 to 15 Oocytes, According to Age.
The cumulative live-birth rate increases with the number of oocytes and is higher among younger women (≤35 years of age) than among older women (>35 years of age). Data are from Cobo et al.[26]

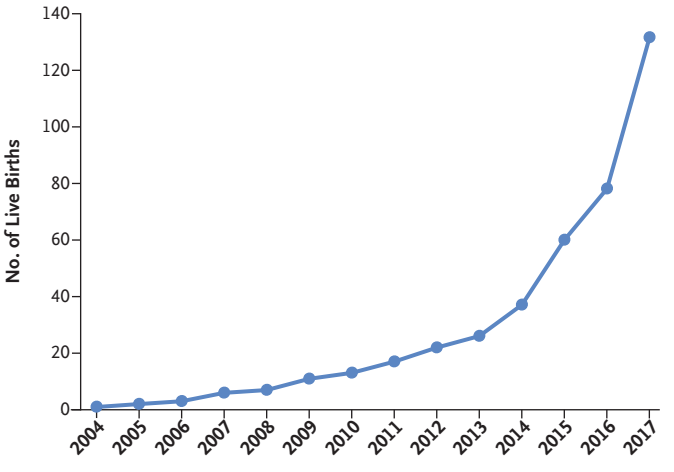

Figure 3. Reimplantation in an Orthotopic Site.
Since 2004, when the first pregnancy after reimplantation in an orthotopic site (namely, a site in the pelvic cavity) was reported, the number of live births has reached more than 130, showing a logarithmic increase during the past 2 years and highlighting the need to move from experimental studies to widespread clinical application.

ovarian tissue is superior to slow freezing, since vitrification has resulted in only two live births so far.[52] For women with acute leukemia, the risk of reimplantation of malignant cells along with grafted tissue is high (Table S1 in the Supplementary Appendix)[53-55]; alternative approaches, such as in vitro maturation of primordial follicles or an artificial ovary, are needed (Fig. 1A).

OVARIAN-TISSUE CRYOPRESERVATION AND SUBSEQUENT OOCYTE VITRIFICATION

Cryopreservation of ovarian tissue, followed immediately by ovarian stimulation and oocyte retrieval (with a view to vitrifying mature oocytes), does not impair oocyte number or quality. It may actually increase the efficacy of the procedure by giving young patients with cancer more chances of success.[56]

Vitrification of oocytes for age-related fertility decline or other nononcologic reasons is the best strategy for fertility preservation, yielding a cumulative live-birth rate of 60.5% among healthy women who are 35 years of age or younger.[26] Among women with cancer, however, the cumulative live-birth rate after vitrification of oocytes is 34%,[31] probably because of inferior oocyte quality in women affected by the disease.[1,31]

By combining vitrification of oocytes and cryopreservation of ovarian tissue in patients with cancer, a live-birth rate of 50 to 60% might be possible (Fig. 1). We therefore suggest that this combined technique be offered to postpubertal patients who are at high risk for premature ovarian insufficiency, as long as chemotherapy can be delayed without jeopardizing cancer treatment, in order to make the most of fertility preservation in terms of pregnancies achieved.

IMPORTANCE OF APPROPRIATE COUNSELING

At diagnosis, all women with cancer who wish to retain fertility options are entitled to a consultation during which they are informed that there is a risk that first-line treatment will compromise their fertility. However, because of the emotional shock at the cancer diagnosis, coupled with multiple investigations and procedures (which sometimes involve enrollment in complex clinical studies, requiring informed consent from participants) and the fact that health care workers are likely to be unfamiliar with the current options for fertility preservation,[1,6] only a small fraction of patients are referred to a specialist to discuss fertility preservation before they undergo cancer treatment.[1,6,7,32] Not only gynecologists but also pediatricians and oncologists need to know when to refer patients for possible fertility preservation.

THE FUTURE

ARTIFICIAL OVARY

One alternative to obtaining mature oocytes would be using the so-called transplantable artificial ovary (Fig. 1). Isolating primordial follicles and transferring them onto a scaffold to create this artificial organ would serve to eliminate the risk of transmission of malignant cells.[57,58] Recent developments in the isolation technique, involving washing the follicles three times, have proved successful.[59] Growing antral follicles were observed after autografting primordial follicles inside a fibrin scaffold in a mouse model[57,58] and after xenografting human primordial follicles in mice with severe combined immunodeficiency.[60]

IN VITRO DEVELOPMENT OF PRIMORDIAL FOLLICLES

A dynamic multistep culture system is needed to support each of the transitional stages of follicles[61] (Fig. 1A). This multistep approach to in vitro follicle growth must meet the changing requirements of the developing oocyte and its surrounding somatic (granulosa) cells in order to maintain interactions between these cells.[61,62] The challenges, such as acquisition of meiotic and developmental competence as well as genome imprinting, are numerous.

OVARIAN STEM CELLS

The discovery of ovarian stem cells has challenged the theory that production of germ cells in female mammals ceases before birth.[63] However, in vitro derivation from ovarian stem cells[64] might interfere with the complex genomic imprinting and epigenetic mechanisms required for the development of fully competent oocytes.

NEW AVENUES OF RESEARCH

Preventive Strategies

The possibility of administering gonadotropin-releasing hormone agonists that can minimize gonadal damage caused by gonadotoxic agents is an attractive option.[65,66] However, a meta-

analysis of 28 randomized, controlled trials,[67] as well as a more recent randomized, controlled trial,[68] confirmed that although there was evidence of the potential benefits of this approach in terms of recovery of menses and ovulation, pregnancy rates did not improve.[3]

According to the recommendations of the American Society of Clinical Oncology[69] and the American Society for Reproductive Medicine,[2] evidence supporting the effectiveness of gonadotropin-releasing hormone agonists for fertility preservation is currently insufficient, which is why other preventive strategies to reduce the effects of gonadotoxic treatment need to be developed. One approach involves nanoencapsulation of chemotherapeutic agents that can target solid tumors, mitigating the effect on the gonads.[70] A second approach relies on protective agents, such as the AS101 immunomodulator, which is used to prevent the "burnout" effect of chemotherapy,[71] and sphingosine-1-phosphate, which is used to inhibit cell apoptosis caused by radiotherapy and chemotherapy.[72] A third strategy is continued research into the efficacy of new, less gonadotoxic drugs or novel combinations in an attempt to reduce the need for alkylating agents.[1,13]

Allografting

The first live birth to occur after ovarian-tissue transplantation between two genetically different sisters was reported in 2011 (Fig. S2 in the Supplementary Appendix).[73] Since this is an acceptable practice with monozygotic twins,[40,74] there is no apparent reason to refrain from using it with genetically different sisters, especially if one of the sisters previously received bone marrow from the other,[73] leading to complete chimerism (HLA compatibility) between the sisters and obviating the need for immunosuppressive treatment. This approach allows for natural conception, which could be important on moral, ethical, or religious grounds.[73]

CONCLUSIONS

Improving freezing techniques, ensuring safe ovarian-tissue transportation (to provide and extend access to fertility preservation in large countries and low-resource areas[75]), and minimizing the risks of fertility-preservation strategies in patients with cancer constitute formidable challenges for the coming decade. In the near future, such strategies will be implemented increasingly frequently among women with benign diseases (e.g., recurrent endometriosis) and those with age-related fertility decline, with vitrification of oocytes emerging as the technique of choice for nononcologic indications.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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