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FERTILITY PRESERVATION

Applicability of adult techniques for ovarian preservation to childhood cancer patients

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Abstract
Purpose To appraise the feasibility of current adult medical and surgical techniques for ovarian preservation in pre-pubertal and adolescent girls with cancer.
Methods Literature search using PubMed and SCOPUS up to February 2012. In addition, the reference lists of selected studies and all identified systematic and narrative reviews were scanned for relevant references. Inclusion criteria were ovarian preservation and cancer. Exclusion criteria were non-English publications, letters, personal communications, and ovarian preservation for conditions other than cancer.
Results Data from the selected publications was interpreted and discussed in the relevant sections. Cryopreservation of ovarian tissue followed by autologous transplant represents the only surgical option available for pre-pubertal girls and adolescents who cannot delay the start of chemotherapy. Few studies report on pre-pubertal and adolescent girls undergoing ovarian preservation surgeries with good harvesting, and no follow-up has been conveyed, to date. Outcomes of ovarian function after ovarian suppression with GnRH-analogs in adults have been controversial and no reports are available for pre-pubertal girls.
Conclusions Autologous transplantation of cryopreserved ovarian cortex probably represents the best option for preservation of fertility and hormonal function in childhood cancer females; however, future research needs to address the safety of this technique, especially in patients with blood-borne cancers. Ovarian suppression with GnRH-analogs at the time of chemotherapy treatment has not proven to be superior to non-suppression for fertility preservation purposes in adults. Not enough evidence is presently available in childhood cancer patients.

Keywords Ovarian preservation · Fertility · Transplant · Cryopreservation · Freezing · Vitrification · Pre-pubertal · Adolescents

Capsule Critical appraisal of adult ovarian preservation tecniques for childhood cancer patients.

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Introduction

In the United States, about 11,210 children under the age of 15 will be diagnosed with cancer in 2011, and about 1,320 children are expected to die from the disease [1]. About 80 % of children will be cancer survivors. Among the major types of childhood cancers, about one-third are leukemias and the most common type in children is acute lymphoblastic leukemia. These are followed by central nervous system tumors (21 %), neuroblastoma (6.9 %), Wilms tumor (4.8 %), lymphomas (8 % combined), and others. Cancer treatments involve the use of chemotherapeutic agents and/or radiotherapy. Among the different chemotherapeutics, alkylating agents are considered the most deleterious for the gonads and the extent of the damage is dose-dependent [2, 3]. Cyclophosphamide was shown to cause follicle damage by apoptosis [4]. Radiation therapy causes gonadal damage, as well [5]. A radiotherapy dose of 5–20 Grays (Gy) administered to the ovary is sufficient to completely impair gonadal function, whatever the age of the patient. However, less than 2 Gy to the gonads is able to destroy 50 % of the oocyte reserve. Moreover, uterine irradiation at a young age hampers the final adult uterine volume [6]. Intensive chemotherapy and/or total body irradiation (TBI) required before bone marrow transplant constitute the treatment combination presenting the greatest risk of primary

ovarian insufficiency. Reduction of the primary follicle reserve in pre-pubertal cancer patients has been shown to result in acute or chronic ovarian insufficiency [7]. Chronic insufficiency has been shown to be associated with early menopause and loss of reproductive capacity [8].

With the progressive increase in pediatric cancer survival, the medical community has become more aware of the far from acceptable reproductive results that the current therapies provide. Even though reproduction requires a functioning hypothalamic-pituitary-ovarian axis, the reproductive potential in childhood cancer survivors is mainly limited by the diminished number of follicles after chemotherapy/radiotherapy. It has been a dogmatic belief that follicle reserve is established in utero in humans, and that there is no regeneration postnatally [9]. However, recent studies claimed 'regeneration' of new follicles from stem cells in adult human ovaries [10]. In support of this new notion is the observation that many women conceive years after experiencing premature menopause as a result of high-dose chemotherapy and or radiotherapy [11, 12], or even after idiopathic primary ovarian insufficiency has been established [13]. No spontaneous or post-bone marrow transplant substantial regeneration of follicle reserve has ever been demonstrated in humans, though. Regardless of residual ovarian function, long-term survival after treatment for cancer during childhood is associated with increased risk of impaired quality-of-life and psychosocial problems. Adolescent cancer survivors have increased concern about body image and dating, and, when adult, they are less likely to marry than matched controls [14, 15]. Fertility after chemotherapy/radiotherapy is another concern: although cancer survivors can become parents by adoption or gamete donation, most would prefer to have biologic parenthood and biologically-related children [8, 16].

This represents a critical review of the literature on the techniques for ovarian preservation in adults and their applicability to childhood cancer patients. We searched Medline and SCOPUS through February 2012 with the key words: ovarian and fertility preservation, childhood cancer, adolescent. Because of the broad implications of our topic, we also scanned the reference lists of selected studies and all identified systematic and narrative reviews for relevant references. No attempt was made to further analyze or discuss the relevance of each study, but just to summarize what has been published.

Ovarian suppression

The rationale of using gonadotropin-releasing hormone (GnRH)-analogs to make the ovaries quiescent during chemotherapy treatment is to salvage those follicles that are follicle stimulating hormone (FSH)-dependent, but their benefit might be extended [17]. GnRH-analogs are believed to act by decreasing utero-ovarian perfusion, resulting in a decreased exposure of the follicles to the chemotherapeutic agents [18, 19], by up-regulating intra-gonadal anti-apoptotic molecules such as sphingosine-1-phosphate, or by directly protecting the developing follicles [18]. Primordial and primary follicles are not believed to be dependent on FSH for their growth and differentiation, even though the evidence is not definitive [20–22]. Primordial and primary follicles are the ones subject to the greatest depletion from apoptosis during cancer treatment at all ages [7].

GnRH-analogs have been used only in post-pubertal women, in most studies affected by breast cancer. There is a limited number of prospective studies and most are flawed by a short-term follow-up, or by lack of a control group. Two different GnRH- agonists have been used, goserelin acetate and triptorelin pamoate. The results are controversial, as some studies found a protective effect on the ovaries and others didn't. However, none of the studies found adverse effects of the GnRH-analog on cancer therapy outcomes. An initial study investigated the protective role of goserelin acetate in 64 premenopausal patients with early breast cancer [23]: at the end of the 55-month follow-up, 86 % had resumed menses and one conceived and had a healthy pregnancy. The authors' conclusion of a positive role of GnRH-analogs towards ovarian protection could not be supported by their results because of lack of a control group. A beneficial role of goserelin was also found when administered for 6 months in a randomized, controlled trial [24]. However, this study had many flaws, including different treatment regimens and a short follow-up, which precluded a true assessment of residual fertility. Again, goserelin was found to be protective when continuously administered for 2 years to women with early breast cancer treated with alkylating agents [25]. In this study, primary outcome was resumption of menses and follow-up was 12 months after completion of the goserelin treatment. At the 36 months mark, 36 % of the women resumed menses in the goserelin group, as opposed to 7 % in the goserelin plus tamoxifen group, 13 % in the tamoxifen group, and 10 % of the controls. The ovarian insufficiency figures in the control groups seem improbably low when compared to other studies, possibly indicating randomization bias. Goserelin was not found to be helpful in another trial in which 60 patients receiving the same adjuvant chemotherapy (anthracycline/cyclophosphamide) for breast cancer were randomized to use the GnRH-analog for ovarian suppression for the duration of chemotherapy [26]. All patients in the trial, but one in the control group, reported regular menses at 24-months of follow-up. In addition, the authors noted that chemotherapy resulted in a similarly decreased ovarian reserve in both groups, as measured by inhibin B and anti-Mullerian hormone (AMH). The same conclusion was reached by another

randomized trial in patients with Hodgkin lymphoma [27]. This study was prematurely closed when a 12-month interim analysis showed goserelin-treated patients having the same degree of acute and chronic insufficiency as women treated with oral contraceptives, as reflected by menstrual patterns and AMH and FSH measurements.

A first controlled randomized trial using triptorelin pamoate was terminated after 18 months of follow up because there was no difference in menstrual cycle prevalence in the triptorelin versus control group [28]. In contrast, a controlled trial using triptorelin pamoate showed protection of ovarian function [29]. The rate of early menopause, defined as no resumption of menses and postmenopausal FSH and estradiol levels for 1 year after the end of chemotherapy, was 25.9 % in the chemotherapy-alone group and 8.9 % in the chemotherapy plus triptorelin group, and the difference was maintained even after controlling for age at time of treatment and use of alkylating agents. There was one pregnancy in the chemotherapy-alone and three in the chemotherapy plus triptorelin groups, respectively. Strengths of the study were the size, 281 women, multicenter design, and a 6-year follow-up. Drawbacks were the non-homogeneous chemotherapy treatments, and the fact that some patients had only one cycle as opposed to multiple cycles of chemotherapy, with the distribution of those patients in the two groups not recorded in the paper. In addition, the number of patients actively seeking fertility in the 2 groups was not reported. The most recent randomized trial that used triptorelin pamoate in reproductive age breast cancer patients was, again, prematurely closed for futility after 30 % of the planned number of patients was recruited [30]. In the 34 months of the study, there was no difference in the resumption of menses (5.0 months in the control and 5.8 months in the triptorelin arms), and two patients in the control arm became pregnant.

Only one prospective cohort study with historic controls was undertaken specifically in adolescents [31]. Twelve girls between 14 and 20 years of age were treated with leuprolide acetate prior and during chemotherapy regimens with alkylating agents and/or bone marrow transplant. All patients resumed menstruation within 45–120 days from cessation of ovarian suppression, as opposed to no resumption in the 4 patients in the historic control group where no leuprolide was administered. Two of 12 patients conceived within the 5-year follow-up. Despite the retrospective nature of the controls, noteworthy in the study was the addition of another historic control group of 5 pre-pubertal girls (range 3–7.5 years old) who did not receive ovarian suppression while undergoing chemotherapy (no radiotherapy was administered in this group). All of them had spontaneous puberty between age 12 and 17.9 years, and 3 of them conceived within the 18 years of follow-up. However, no information on the onset of POI was provided.

A recent murine study tried to elucidate the mechanism by which the GnRH-antagonist cetrorelix acetate would protect from primordial and primary follicle depletion [32]. The authors showed a possible reduction in DNA damage when cetrorelix acetate was administered simultaneously to cyclophosphamide treatment in adult mice. Whether these results could be applied to human subjects remains to be demonstrated, as the only pilot study conducted in Australia has too many flaws to be interpreted, including the lack of data reporting and ambiguous definition of 'normal' values [33]. However, it did report a possible protective effect on ovarian function by cetrorelix acetate without major side effects.

To date, no study on ovarian suppression during cancer treatment has been undertaken in pre-pubertal girls. The explanation for this might be threefold: dishomogeneous results in adults; pre-pubertal ovaries are made of primary and early secondary follicles, which may only partially be under FSH control [34]; the effect of such a hormonal treatment on cancer treatment outcome is currently unknown and could potentially be deleterious, thus precluding study protocol design.

Whole ovary transplant

Thus far, four reports have described fresh whole ovary grafts with vascular anastomoses in adults [35–38]; an additional one involved fresh whole ovarian autotransplantation in the retroperitoneum of the psoas muscles without vascular anastomoses [39]. In this case, resumption of hormonal function occurred 4 months later.

Fresh upper-extremity autologous transplantation was reported in 3 pre-pubertal girls with Wilms tumor [40]. Strips of ovarian cortex were apposed to the triceps and deltoid muscles in 2 patients, whereas a whole ovary was transplanted to the axillary region with end-to-end vascular anastomoses to the thoracodorsal vessels in the third patient. Each subject then received abdominal/pelvic radiation therapy (approximately 30 Gy) and multi-agent chemotherapy (vincristine and actinomycin D). The 3 girls had spontaneous menarche at age 12–15 years. The one who had the axillary ovary transplant underwent a second procedure because of painful follicular development. This time the ovarian vessels were anastomosed to the inferior epigastric vessels underneath the rectus muscle. While the patients who underwent ovarian cortex transplant had regular menses until they went into menopause at age 26 and 30 years, the one who underwent whole ovary transplant experienced only sporadic ovarian function for 12 years after the second transplant procedure and needed hormonal therapy. None of the patients ever conceived. In patients undergoing total

body irradiation, the possibility of performing fresh whole ovary transplant is precluded.

In the past few years, attempts at freezing and grafting whole ovaries in animals have yielded encouraging results. The first case of restoration of fertility in rats after whole frozen–thawed ovary transplantation was described by Wang et al. in 2002 [41]. They described successful vascular transplantation of frozen–thawed rat ovaries and reproductive tract in 4 out of 7 (57 %) transplants. These ovaries survived for 60 days or more, and resulted in one pregnancy. Chen et al. showed that frozen–thawed rabbit ovaries remained functional for at least 7 months after microvascular transplantation in 13 out of 15 (86.7 %) animals [42]. However, rat and rabbit ovaries are smaller than human ovaries and thus easier to evenly freeze and thaw. In sheep studies, 73 % of the frozen/thawed ovaries were lost because of vascular complications after transplantation with vascular anastomoses [43]. In addition, transplantation of cryopreserved autologous ovarian tissue could potentially trigger generation of anti-ovarian antibodies [44]. These antibodies have been implicated in the development of primary ovarian insufficiency similar to the one obtained with chemotherapy. To date, it has not been possible to perform whole ovary transplantation after cryopreservation in humans because of technical difficulties in assuring intact oocytes, follicles, stroma and blood vessels in the whole organ during the freezing/thawing process. Reseeding of cancer cells is another concern of whole ovarian transplant [45].

Ovarian tissue freezing

With the premise that whole ovary cryostorage is not currently feasible, the current method for cryopreservation of ovarian cortical tissue is controlled slow rate freezing. Poor survival of stroma and hindered integrity of vascular endothelium, are the main limitations of this method. These will cause the major follicular loss observed in transplanted tissue. Nonetheless, in vitro and in vivo results are encouraging. Being the majority of follicles primordial, these represent also the greater part surviving the cryopreservation process [46–49].

The recently described technique of vitrification for freezing of ovarian tissue seems to improve viability of all compartments of the cortex with a similar follicular survival rate, but with much improved integrity of ovarian stroma and morphology of blood vessels than the slow-freezing technique [50, 51]. Vitrification involves equilibration of the specimen in one or more cryoprotectants followed by plunging into liquid nitrogen. It requires a very rapid rate of cooling and re-warming to avoid ice nucleation. In the ovarian cortex every cell has a slightly different optimum for cryopreservation and protocols for vitrification have to compromise by focusing on the central tendency [52]. To date, there are no reported live human births using vitrification. However, vitrification holds promising and is becoming the technique of choice for cryopreservation of ovarian tissue.

Ovarian cortical tissue transplant

The aim of this procedure is to reimplant ovarian cortical tissue once the patient is disease-free, to allow a physiologic ovarian function. This represents the most promising technique to preserve ovarian function and fertility in pre-pubertal girls. In this population, ovarian tissue cryopreservation and ortho- or heterotopic autotransplantation could restore normal hormonal function and would allow a physiologic sexual development.

After initial pioneer studies [53], the first ovarian tissue transplant after cryopreservation in a woman was performed by Dr. Oktay and his group in 1999 [54]. The patient had undergone salpingo-oophorectomy of her only ovary for the treatment of intractable menorrhagia. After explant, cortex was obtained from the ovary and was frozen following a slow-freezing protocol. Subsequently, the patient underwent laparoscopic apposition of the cortex pieces to the pelvic peritoneum and resumed ovarian function with gonadotropin stimulation approximately 15 weeks after the transplant. Evaluation of ovarian hormonal production was precluded by the patient being kept on hormone treatment in between ovarian stimulations with gonadotropins. Still, ongoing function was confirmed 6 months after transplant.

A few investigators reported their experience on ovarian tissue cryopreservation in pre-pubertal and adolescent girls [55–60]. While all the studies describe the technique, which involves ovarian biopsy or unilateral oophorectomy followed by tissue or isolated oocyte freezing, no follow-up information is available in regards to undergoing autologous transplantation or ART with autologous gametes, to date. Despite this, the alarming information arising from these studies is that, regardless of diversity of diagnosis and therapeutic regimen used, cancer treatment caused POI in 10–25 % of pre-pubertal patients and in 36 % of post-menarcheal patients [57, 60]. In addition, these figures do not include the chronic ovarian insufficiency, which inevitably derives from those treatments, and the incidence of POI beyond the study follow-up. These outcomes contrast with the Pereyra Pacheco et al. outcomes, possibly because of the small number of patients followed in this last study [31]. The first case report of cryopreserved cortex autograft in a pre-pubertal girl underscores the importance of using this technique in pre-pubertal girls [61]. The patient had previously undergone pre-conditioning therapy for bone marrow transplant for sickle cell disease and, following transplant of three ovarian cortex fragments to the

suprapubic subcutaneous tissue, she was able to undergo natural puberty.

Despite the of follow-up in childhood cancer survivors, the value of surgical ovarian preservation has been validated by the multiple studies performed in adult cancer patients. Different techniques for heterotopic and orthotopic transplantation of ovarian tissue have been described. Heterotopic transplantation has been performed to the subcutaneous tissue or muscles of the distal upper arm, of the lower pelvis, or in the pelvic peritoneum [62, 63]. This approach, possibly with the exception of transplant to the pelvic peritoneum, requires performing in vitro fertilization for fertility purposes. Nevertheless, there have been reports of spontaneous pregnancies after heterotopic transplantation to the subcutaneous and intramuscular tissues, possibly due to reintroduction of ovarian germinal cells with the tissue transplant [10, 64]. Based on this hypothesis, germinal stem cells contained in the transplanted tissue would re-colonize the ovary/ies or would trigger the existing quiescent germinal stem cells to develop into follicles and oocytes capable of producing pregnancies. This hypothesis, however, has been challenged: the mechanism through which the transplanted tissue rescues ovarian function would be by re-activaton of the immunitary self-tolerance mislaid by chemotherapy [65].

Orthotopic transplantation is performed by grafting frozen-thawed cortical strips directly to ovarian stump after 'decortication', or to the peritoneum adjacent to the fimbrial portion of the tube. Orthotopic transplantation of tissue seems to provide an improved ovarian function, both hormonal and for fertility purposes [66]. While ovarian function restoration has been proven to be possible and prolonged after fresh and frozen cortex transplant [51, 67], fertility outcomes are still reported as isolated cases. The current literature reports the number of pregnancies achieved, but there is no mention of how many patients underwent autologous transplants to yield those pregnancies. Up to now, approximately 15 children were conceived from autotransplanted cryopreserved ovarian tissue in the world since the first case was reported in 2004 [68–75]. In most of the cases, assisted reproduction was used. All the cortex specimens were cryopreserved by slow freezing except for 1-2 later cases in which vitrification was used [76]: histological analysis showed a pooled 89 % oocyte survival in the patients that underwent tissue vitrification, even though this might not reflect the actual ovarian function.

In ovarian transplantation studies, the following factors influence graft development: the inhomogeneous distribution of follicles in the ovarian cortex (intra-patient variation), the age-related decline of follicles in the cortex, the inter-patient variation, and the size of the grafts [73]. The normal human ovary contains predominantly primordial follicles with a low proportion (1 %) of secondary or more advanced follicles [46–48, 77]. Compared to fresh ovarian tissue, with slow, controlled freezing techniques, 40 % (39–45 %) of the follicles were intact (oocyte and granulosa cells) after thawing, 45 % (38–47 %) had surviving oocyte and >50 % of granulosa cells, and 15 % (9–20 %) had surviving oocyte and <50 % of granulosa cells. Very few follicles (0–5 %) were dead [78]. Follicular distribution was modified in fresh and frozen/thawed tissue: primordial follicles constituted 99 % of all follicles prior to xenotransplantation in both groups, whereas the primary and secondary follicle proportions were increased after grafting, indicating a tendency to progression of follicular development [79]. After grafting, however, follicular density decreased in frozen/thawed tissue.

It is known that the main reason for the follicular loss after cryopreservation and xenografting is the ischemic effect after transplantation rather than the cryopreservation process itself [80, 81]. The detrimental effect of chemotherapy on the existing vascular network of grafts could also play a role [82]. This may explain not only follicular depletion but also the 2 to 5-day delay that occurs between transplantation and revascularization of the graft. Indeed, recent studies showed that revascularization of grafts in a human xenograft model depended not only on neoangiogenesis from the host but also on existing blood vessels in grafted tissue [83]. The presence of chimeric vessels after 7 days from transplant highlights the crucial role of the pre-existing vascular network in grafts at the time of re-implantation. For this reason, efforts have been made to improve the process of neovascularization after transplant. Gook et al. tried to administer gonadotropins to the recipient in an effort to increase vascular and endothelial development factors [84]. Contrarily to their expectations, they observed a depletion of primordial follicles in xenotransplanted frozen/thawed human ovarian tissue after gonadotropin stimulation. Their results confirmed Richardson and Nelson [85] and Flaws et al. [86] studies, which found that chronically elevated LH levels deplete the primordial follicle pool and thus may hasten ovarian reserve depletion in a mouse model. Relevance of LH treatment to human follicles has not been studied, and treatment with the GnRH-agonist triptorelin pamoate around the transplantation period was not able to prevent primordial follicle depletion after xenografting [78]. Triptorelin actually caused an additional loss of follicles when administered during the critical neovascularization period after transplantation. In this controlled study, it is noteworthy that untreated xenografted animals showed a normal uterine development, whereas those xenografted and treated with gonadotropins, triptorelin, or both, showed underdevelopment [78]. Other substances such as the anti-apoptotic S1P and angiogenic factors, such as VEGF, are currently under scrutiny to establish if they could improve the immediate post-transplant follicular loss [87–89].

Risk of reseeding cancer

To date, no case of cancer reseeding has been reported from ovarian cortex autotransplantation. In many circumstances, the risk of cancerous involvement of the ovary is absent or minimal, and autografting would present little or no danger [90, 91]. However, the risk of ovarian tissue harboring malignant cells, especially from subjects with a blood-borne cancer, cannot be underestimated [45, 92–94].

In adults, the cancers at highest risk of being transmitted to recipients include central nervous system tumors, choriocarcinoma, breast cancer, renal carcinoma, and lung cancer [95]. The overall risk of death from a donor-derived malignancy is estimated to be less than 1 % [96]. Nonetheless, the decision of transplanting ovarian cortex back into young women who had blood-borne malignancies is still unsettling and has to be accurately contemplated. With blood-borne malignancies being the majority of childhood cancers and the ones whose treatment most frequently results in primary ovarian insufficiency (American Cancer Society [1–3]), the risk of reseeding after ovarian cortex transplantation is high, as shown by polymerase chain reaction (PCR) studies [97].

In these instances, other options must be considered, such as intervening with ovarian preservation procedures after the first cycle of chemotherapy, as described by Radford et al. [98]. This approach, albeit not optimal for integrity of ovarian cortex, possibly provides better safety at the time of autotransplant. Transplantation of isolated follicles, as described by Dolmans et al. [99], is a promising possibility; however, while addressing fertility preservation, it would not allow ovarian hormonal production resumption. Isolated follicles may also be cultured in vitro (in-vitro maturation) or reimplanted in a tissue-engineered matrix such as alginate [100, 101]. This last technique has not yet achieved clinical use in the human setting. In vitro maturation of ovarian follicles taken from cryopreserved ovarian cortex with subsequent in vitro fertilization and embryo transfer is a promising technique, but it has not yet achieved standardized performances [102]. In addition, similarly to in vitro follicular maturation, while addressing fertility preservation, it would not allow ovarian hormonal production resumption. Xenografting of ovarian cortex with subsequent in vitro fertilization and embryo transfer back to the patient is not an acceptable option due to the risk of immunologic and infectious contamination. Screening methods should be developed to eliminate the risk of cancer cell transmission with re-implantation. Performing immunohistochemical analysis and/or PCR to evaluate the tissue prior to its transplant should be recommended.

A first step in removing tumor cells from cryopreserved ovarian tissue in vitro was done by Schröder et al. [103]. The authors were able to safely separate ovarian follicles and stroma by mechanical and enzymatic dissection; breast cancer cells were then added to the suspension and successively killed by activated lymphocytes. The procedure seemed promising; however, ovarian tissue was reduced to a suspension, which makes it difficult to be transplanted back to the patient.

Cryopreservation of oocytes

Cryopreservation of oocytes requires ovarian stimulation with gonadotropins and for this reason it can be performed solely in patients who are post-pubertal and whose cancer treatment can be postponed by 2 weeks or longer. The oocytes so obtained are typically arrested in the developmental stage distinctive of ovulated oocytes, metaphase of the second meiotic division, and are considered ‘mature.’ Freezing of single oocytes is more challenging than freezing embryos. What makes the oocyte more vulnerable during the freezing process is the increased volume to surface ratio, and the easy damaging of the various organelles by the ice crystals that form during the process. Despite this, there has been a marked improvement in embryo creation and pregnancy rates from cryopreserved oocytes since its first introduction in 1986 [104, 105]. Freezing of ‘immature’ oocytes, such as oocytes arrested in diplotene of the first meiotic division found in pre-ovulatory follicles, has better survival than freezing ‘mature’ oocytes [106], however, these oocytes have to undergo in vitro maturation, a technique that is still considered experimental and is not broadly performed [107]. The advent of vitrification techniques for cryopreservation has greatly improved the outcomes of oocyte cryopreservation. Two randomized, controlled, trials showed that embryos originated from fertilization of cryopreserved oocytes have equal fertilization and pregnancy rates than embryos from fertilization of fresh oocytes [108, 109]. A recent systematic review and meta-analysis of randomized controlled trials comparing outcomes of vitrified versus fresh and slow-freezed oocytes, showed that rates of ongoing pregnancy (49.1 % in the vitrification and 48.3 % in the fresh oocytes groups), top-quality embryo, embryo cleavage, and fertilization did not differ between the vitrification and the fresh oocyte groups, and were better in the vitrification than the slow-freezing groups [110].

In order to recruit mature oocytes, ovarian stimulation with gonadotropins would need to be started in the early follicular phase (day 2 or 3 of the cycle). Hence, it requires from 2 to 6 weeks of time to perform retrieval of the oocytes, depending on the cycle phase of the patient. In most instances, there is not enough time prior to initiation of cancer treatment to allow for this procedure. However, recent reports have indicated that there are at least 2 or 3 follicle recruitment waves during a normal menstrual cycle [111]. This allowed the development of protocols of

ovulation induction that result in oocyte retrieval within 2 weeks, thus making oocyte cryopreservation a much more flexible and feasible technique for fertility preservation [112–115].

Ethical considerations

The American Academy of Pediatrics states that "patients have a right to know about their health, to know about available diagnostic and treatment options and their risks and probable benefits, and to choose among the alternatives", however, these principles apply only to individuals 18 years of age or older [116]. Children and adolescents can only give 'assent' to acknowledge that they participated in the discussion and that they 'agree' with their parents'/guardians' decisions. Adolescents older than 14, can gain the status of 'emancipated minors' only if they are pregnant or seek treatment for specific disorders, which do not include cancer or ovarian preservation [117]. The decision to undergo cancer treatment is based on the principles of *beneficence* and *non-maleficence*, which means the physician should benefit the patient, and not cause harm to her. Evaluation of the risks and benefits of cancer treatment universally enables the physician to pursue a specific therapy even when cancer treatment could have major adverse consequences. When evaluating the risks and benefits of ovarian preservation in childhood cancer patients, physicians and family members normally think that the risks outweigh the benefits, especially if cancer therapy needs to be delayed to perform ovarian preservation procedures [118]. Because ovarian follicle depletion is generally not emphasized as a risk of cancer therapy, it does not find the same attention as the risk of new malignancies or other organ damage. For this reason, children and adolescents are often not informed about the adverse hormonal and reproductive outcomes such as inability to achieve and/or complete pubertal changes and future infertility, and their parents are misled to believe that, if needed, 'something can be done in the future to fix the problem.'

Ovarian preservation procedures are considered experimental. The Office of Human Subject Research at the National Institute of Health would classify ovarian preservation in children as "research involving greater than minimal risk but presenting the prospect of direct benefit to the individual subjects" [119]. Under these guidelines, parents/guardians have to consent to the procedures and the minors have to give their assent. Physicians should implement a team approach to counsel childhood cancer patients preferably before any therapy is instituted. If time permits, reproductive endocrinologists, oncologists, psychologists, and nurses should discuss ovarian preservation options over several visits. This will allow understanding the family's and patient's perspective, and will establish a relationship in which both parties discuss the risks, benefits, and alternatives of fertility preservation, in addition to long-term prognosis and disposition of tissues.

Final considerations

Irradiation and chemotherapy is believed to be less harmful to the gonads of pre-pubertal than post-pubertal women [120, 121]. However, a big proportion of children will still face complications related to the loss of primordial follicles [2–7, 122].

As it was recognized by the American Society of Clinical Oncology, ASCO, in the clinical guidelines published in 2006, "the Panel recommends that oncologists discuss at the earliest opportunity the possibility of infertility as a risk of cancer treatment. People attempting fertility preservation in the context of cancer treatment are encouraged to enroll in clinical trials that will advance the state of knowledge" [123]. For fertility preservation purposes, ovarian suppression at the time of chemotherapy treatment has not proven to be superior to non-suppression; however, for preservation of the ovarian endocrine function, an argument could be made that this represents a reasonable approach. Enabling prepubertal girls to undergo natural as opposed to iatrogenic puberty and giving them hope for future fertility, would be of outmost importance for their physical, sexual, and psychological development into adulthood. Cryopreservation of ovarian tissue is the only surgical option available for prepubertal girls and women who cannot delay the start of chemotherapy.

Ovarian tissue transplant, whether orthotopic or heterotopic, would allow for ovarian hormonal production and restoration of a normal hormonal milieu. This technology for ovarian preservation is now reproducible and promising and should be offered to pre-pubertal girls. However, our knowledge needs to be expanded on its safety in patients with blood borne cancers. Techniques such as in vitro maturation of isolated oocytes, in vitro maturation of follicles, assessment of sections of ovarian cortex for the presence of malignant cells prior to auto-transplantation, purging of malignant cells from cryopreserved ovarian tissue, and/or replenishment of germinal stem cells, need to be the object of future research.

Case selection should be carried out on the basis of a multidisciplinary staff discussion including oncologists, gynecologists, biologists, psychologists, and pediatricians. Counseling should be given and informed consent obtained from the patient. Cancer treatment takes priority over potential restoration of fertility, but offering the chance to preserve fertility may greatly enhance the quality of life for cancer survivors.

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