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Fertility Preservation for Prepubertal Girls: Update and Current Challenges

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Abstract With increasing rates of diagnosis of childhood cancers and the evolution of more effective treatment options resulting in prolonged life spans, fertility preservation counseling is an integral component of the discussion at the time of diagnosis of childhood cancers. The primary fertility preservation option that exists for prepubertal girls is ovarian tissue cryopreservation. Although ovarian tissue cryopreservation is still considered to be experimental in nature, live births have resulted from orthotopic tissue transplantation. Fertility preservation should be offered to all prepubertal girls at high-risk for premature ovarian failure as a result of gonadotoxic treatment. Ethical and legal questions surrounding these issues must be considered as more and more pediatric patients pursue fertility preservation.

Keywords Pediatric cancer · Fertility preservation · Ovarian tissue cryopreservation · Cancer survivorship · Oncofertility

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Introduction

While childhood cancer represents 1 % of all malignancies, increasing rates of early diagnosis and the evolution of more effective treatment options are resulting in high survival rates [1]. Although childhood cancer rates have been rising slightly for the past few decades, survival has increased as well. The 5-year overall survival rate for childhood cancer has improved from 58 % in patients diagnosed between 1975 and 1977 to 83 % in those diagnosed between 2002 and 2008 [2]. With the increasing number of childhood cancer survivors, fertility preservation is becoming a key issue for quality of life and survivorship. The American Society of Clinical Oncology (ASCO) recognizes fertility preservation as a key survivorship issue, and has also used fertility care as a key measure of quality of care [3, 4•]. The American Society of Reproductive Medicine (ASRM) also endorses early referral to the fertility specialist as an essential element in the cancer treatment plan [5]. Although cancer survivors could become parents in the future via adoption or egg donation, most would prefer to have biologically related children [6, 7].

While embryo and oocyte cryopreservation are widely used to preserve fertility in postpubertal women undergoing gonadotoxic treatment, these modalities are not an option for younger patients [8, 9]. Prepubertal females are a unique subgroup of the fertility preservation population in that they are commonly too young to understand the full scope of their disease process and its impact on their future fertility. Since they have not yet commenced the hormonal changes concurrent with puberty and maturation of the hypothalamic–pituitary axis, treatment options are usually limited to ovarian tissue cryopreservation. The objective of this paper is to review the epidemiology of cancers affecting young girls and describe the fertility preservation options available for this group along with future advancements. We will also touch

upon the ethical and social implications of fertility preservation in this population.

Background/Epidemiology

Childhood cancers represent the second leading cause of death in the United States under the age of 15 years old, second only to accidents [10]. Constituting about 34 % of childhood cancers are leukemias, with the most common childhood leukemia being ALL (acute lymphoblastic leukemia) and a minority of cases being AML (acute myeloid leukemia). Cancers of the brain and spinal cord comprise 25 % of childhood cancers. Less common childhood cancers include soft tissue sarcomas (7 %), neuroblastoma (6 %), renal tumors (5 %), and Hodgkin and non-Hodgkin lymphomas (4 % each) [11]. While the overall incidence has continued a trend of slight increase every year over the past 40 years, the death rate for childhood cancer has decreased by more than half during the same period. With the growing population of long-term survivors of childhood cancer, there has been increased interest in survivorship care and decreasing the long term toxicity of upfront therapy. The childhood cancer survivorship study (CCSS) is a large retrospective cohort study following the outcomes and late effects of childhood cancer in 5,149 females. The study found that compared to siblings, childhood cancer survivors were less likely to ever become pregnant with a relative risk (RR) of 0.81 (95 % CI, 0.73–0.90) [12].

Normal Physiology of the Ovary

Oogenesis is a process that occurs during fetal development, with a human female's peak number of oocytes occurring at approximately 20 weeks gestational age. This number is reduced with follicular assembly; by the time of delivery only about 1–2 million oocytes, arrested in prophase I of meiosis, remain. The majority of oocytes die secondary to atresia and by puberty oocyte numbers are reduced approximately 25 %. At the time of ovulation, an oocyte will complete meiosis through metaphase II arrest. In the prepubertal state, ovaries are functionally suppressed due to low gonadotropin releasing hormone (GnRH) levels. Pulsatile GnRH activity during puberty results in the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) and ovarian activity ensues [13].

Effect of Cancer Treatment on Ovarian Function

The two main categories of gonadotoxic cancer therapy include chemotherapy and radiation therapy. The ultimate effect of a cancer therapy on ovarian function and future fertility depend on many factors, including the patient's age, type and stage of cancer being treated, drug selection, method of administration, size and location of the radiation field, and the selected dose-intensity of the radiation regimen [13]. With the physiologic decline of oocytes, older patients require much less gonadotoxic therapy than younger patients to become infertile. Even if the initial gonadotoxic therapy does not make the patient completely infertile, the effect on the reduction of the overall oocyte reserve can affect many patients. Due to this effect, the clinical presentation of the long term sequelae of gonadotoxic therapy can vary greatly according to age of treatment. For prepubertal patients, this could be delayed puberty followed by ovarian failure and infertility. Patients who received therapy after puberty can present with primary ovarian failure and subsequent infertility, or can present with premature ovarian failure after temporarily resuming normal ovarian function.

Chemotherapy

Chemotherapeutic regimens are commonly first-line therapies for many childhood cancers, and the dose intensification through clinical trials over the past 30 years has led to a remarkable improvement on overall cure rates. Although many chemotherapeutic agents can be temporarily gonadotoxic, alkylating agents are the drug group with the highest risk of associated permanent infertility. Commonly used alkylating agents such as cyclophosphamide, ifosfamide, and procarbazine have been shown to cause primary ovarian failure in patients in a dose-dependent manner [11, 14]. The gonadotoxicity from alkylating agents seem to be through induction of apoptosis in primordial follicles. Histologic evaluation of demonstrates a wide range of findings, including stromal fibrosis to absent follicles [15–17]. While alkylating agents are used in virtually every childhood cancer chemotherapy regimen to some degree, the treatment regimens with the highest risk of fertility loss are seen in sarcoma and CNS tumor regimens as well as some high-risk leukemia and lymphoma regimens [18, 19].

Radiation

Some pediatric cancer regimens combine chemotherapy with radiation, or employ radiation alone. Radiotherapy may affect oocytes when ovaries are in the direct field of radiation, but also through scattered radiation. The human oocyte is extremely sensitive to ionizing radiation; direct radiation causes a dose and age-related reduction in the ovarian follicular pool. Doses as low as 1,000–2,000 cGy in children can effect ovarian function [20] . Additionally, radiation changes of the uterus may produce scarring and decreased blood supply, which can increase rates of subfertility as well as result in poor obstetrical outcomes such as miscarriage and preterm birth [11, 21, 22]. Cranial radiation can also affect the hypothalamic–pituitary

axis and thus impact the hormonal surges that are necessary for puberty to occur [23].

In restrospective reviews including both prepubertal and pubertal females, pelvic radiation doses greater than 15 Gy were nearly always associated with primary ovarian dysfunction [24]. In the childhood cancer survivor study, an abdominal radiation dose greater than 5 Gy was a significant risk factor for fertility loss, with even higher risk with doses over 10 Gy [22, 25].

Stem Cell Transplantation

Autologous or allogeneic stem cell transplantation has been increasingly used in many pediatric conditions, both malignant and benign. While the preparative intensity differs greatly among conditions being treated with stem cell transplantation, most regimens include high doses of combination chemotherapy with alkylating agents with or without radiation [26]. Total body irradiation is typically performed as part of the preparative process for BMT, serving to suppress the immune system. Radiation doses utilized usually range from 8 to >12 Gy [27]. Ovarian failure after stem cell transplantation occurs commonly, being observed in 65–84 % of patients. While all patients undergoing hematopoietic stem cell transplantation are at high risk for fertility loss, combination therapy and inclusion of radiation to the conditioning regimen seems to raise the risk of infertility greater than cyclophosphamide alone [28]. Counseling for fertility loss and consultation for potential fertility preservation is essential to this population.

Fertility Preservation Options in Prepubertal Females

Mainstay fertility preservation options for females undergoing gonadotoxic treatment typically include embryo cryopreservation and oocyte cryopreservation. However, prepubertal young girls are not candidates for these treatments given their immature hypothalamic–pituitary axis. Instead, the primary fertility preservation option for this age group is ovarian tissue cryopreservation. Ovarian tissue cryopreservation may be the only option for even some postpubertal females, particularly in cases where urgent initiation of chemotherapy is essential and the patient cannot wait the 2–4 weeks necessary for oocyte and embryo cryopreservation.

Ovarian Tissue Cryopreservation

Ovarian tissue cryopreservation is considered an experimental technique, but it is the mainstay infertility treatment for young patients undergoing gonadotoxic cancer treatment regimens. It also serves as the preferred method for postpubertal patients and women who cannot delay their therapies or may not be able to tolerate the hormonal stimulation required to harvest oocytes. Currently about 100 centers worldwide perform ovarian tissue cryopreservation, most under the umbrella of an institutional review board (IRB) approved protocol.

The typical approach utilized requires removal of ovarian cortical tissue through a laparoscopic approach. A segment of ovarian cortex, which contains primordial follicles, is surgically excised. Usually, less than half of the native ovary is resected as a wedge, though this fraction may be modified based on ovarian volume. Pediatric gonadal tissue cryopreservation can be combined with other medically indicated procedures such as central line placement to minimize the potential inconvenience, additional anesthetic risks, and costs [29–31]. In one study involving 28 female patients, 42 % were able have ovarian cryopreservation performed concurrently with another procedure [32•].

There are two primary methods to cryopreserve ovarian tissue: slow freezing and vitrification. Disadvantages of the slow freezing method include poor survival of ovarian stroma and damage to the vascular endothelium. However, a percentage of primordial follicles survive the slow-freezing process and offer promise for fertility preservation [33–35]. Evolving data suggests that vitrification methods may be advantageous due to decreased formation of ice crystals which may be destructive to cells in the thawing process [36]. Vitrification involves rapid rate cooling and re-warming of tissue by quickly placing tissue in liquid nitrogen following cryoprotectant exposure [37]. A recent study evaluated the impact of slow freezing and vitrification procedures on oocyte survival rates in 16 cancer patients. The viability of oocytes from slow-freezing specimens was 42 % compared to 92 % of oocyte survival from vitrified specimens [38]. Possible advantages of vitrification over slow-freezing include improved viability of ovarian cortex viability, similar follicular survival rate and less damage to ovarian stroma and vasculature [38, 39•]. Animal models have been instrumental in the development of vitrification methods. Recently published macaque models studied by Ting et al. suggest that tissue vitrification is an acceptable method to fresh tissue. Antral follicle development following ovarian tissue vitrification using primate ovarian tissue showed diminished antrum formation and slower growth, but demonstrated continued development of ovarian tissue [40•].

Once the patient has decided to pursue fertility goals, the ovarian tissue is thawed and reimplanted. Two different surgical approaches for autotransplantation are available: orthotopic and heterotopic [41]. Orthotopic sites include the ovarian fossa, pelvic cavity, or peritoneal window. Heterotopic locations include the forearm, abdominal wall, or rectus muscle [42]. While both orthotopic and heterotopic sites are options for transplantation, no successful live births have resulted from heterotopic transplantation. The first ovarian

tissue transplant was performed in 1999 and the first pregnancy from this technique was reported in 2004 [43, 44]. To date, there have been 24 successful pregnancies after greater than 60 orthotopic ovarian tissue transplants [45•]. All of these pregnancies were achieved using slow freezing techniques [26]. Ischemia following transplantation is the primary reason for follicular loss after cryopreservation [46, 47]. To date, there have been no reports of live births from ovarian tissue harvested prepubertally, due primarily to the young age of the patients at the time of treatment [9, 29]. Research is underway in xenograft models to improve follicular loss. Factors being evaluated include sphingosine-1-phosphate, thought to prevent apoptosis, and VEGF, an angiogenic factor [48, 49].

There are several risks involved with undergoing ovarian tissue cryopreservation which must be considered and reviewed during the consent process. Innately, there are risks involved with the laparoscopic procedure, including bleeding, infection, surgical injury and risks of anesthesia. The risk of these complications seems to be very small with one report of 476 patients having no complications with laparoscopic ovarian tissue harvesting [50]. Another risk that must be carefully considered is the risk of reseeding cancer in the future following autotransplantation [51]. While there have been, to date, no reported cases of ovarian tissue exposing a patient to malignancy after transplantation, the risk is inherent with certain cancer types such as hematologic malignancies [52, 53, 54•]. One study that evaluated 391 cryopreserved ovarian tissue specimens found 1.3 % of samples were positive for malignancy using light microscopy [50].

Since it is still considered experimental, this procedure is not covered by standard insurance. Cost to the patient may be reduced by combining the laparoscopic ovarian tissue collection with a procedure requiring general anesthesia in the operating room setting such as central venous line placement. Studies are available which may support or fully sponsor the cost of procedure itself, but the patient's family may be responsible for paying for some portion of tissue storage costs [21, 55].

Future Directions

The risk of malignancy from autotransplantation is guiding research into new techniques to obtain mature oocytes from cryopreserved ovarian tissue. Improving in vitro maturation techniques is an active area of research to bypass autotransplantation while still obtaining oocytes. The concept is to isolate primordial follicles and then proceed with in vitro follicle growth to obtain mature oocytes capable of fertilization [56]. An alternative method would be to isolate primordial follicles and then transplant them on an alginate matrigel matrix with isolated ovarian cells to ultimately obtain oocytes [57].

Patients with Secondary Sexual Characteristics

By definition, prepubertal girls have not completed puberty, but some may be in early stages of pubertal development and thus benefit treatments mainly targeted from those who have completed puberty. For those patients who are beginning to develop the secondary sexual traits of thelarche, there may be support for another option. These signs indicate the start of ovarian maturation by stimulation of the hypothalamic–pituitary axis. Ovarian suppression with gonadotropin-releasing hormone agonists during chemotherapy is an active area of research. It has been postulated that some of the proposed protective mechanisms of GnRH suppression could theoretically protect the prepubertal ovary, as it has been theorized in many of the studies on reproductive age women [58]. Although the exact mechanism is unknown, the overarching hypothesis suggests that GnRH analog administration reverts the ovaries to an endocrine prepubertal state, thus making them less susceptible to cancer treatments. Other theories involve the possibility of decreased perfusion to the uteroovarian system leading to a decreased chemotherapeutic exposure and protection of undifferentiated stem cells [59]. The overall consensus indicates that GnRH analogs should be considered in women of reproductive age receiving chemotherapy. The use of GnRH analogs is effective in restoring menses, but evidence for protection of downstream fertility is lacking [60]. At our institution, we routinely offer GnRH agonists to premenarchal girls with signs of early puberty.

Determining Candidates for Fertility Preservation

Table 1 stratifies cancer treatment into risks for future infertility: high risk and intermediate risk [11, 14]. Those patients who will be receiving high-risk treatment should be offered ovarian tissue cryopreservation if time allows. Those in the intermediate group can be offered GnRH analogs if Tanner Stage 2 or above, but otherwise they do not necessarily require ovarian tissue cryopreservation. Patients whose chemotherapy and/or radiation therapy protocols do not meet requirements for high- or intermediate-risk treatments are considered low-risk for future infertility.

Role of Medical Teams

Qualitative studies surveying pediatric oncologists have determined that there may be a knowledge gap regarding the topic of fertility preservation. Training on how to address parents' and patients' emotions, culture, and other complex factors may promote discussion regarding fertility preservation [61]. Notable barriers to discussion of these topics include being too ill to discuss therapy, concerns of costs, time constraints, and lack of

Table 1 Stratification guidelines for female infertility risk assessment [11, 14]

A. High risk
1. Moderate- to high-dose alkylating agents
   a. Cyclophosphamide >7.5 gm/m^2 or as part of bone marrow transplant conditioning
   b. High-dose carboplatin therapy requiring stem cell rescue
   c. Stem cell transplant therapy with high-dose chemotherapy total-body irradiation
   d. Alkylating score ≥3rd tertile
2. Whole abdominal or pelvic radiation
   a. ≥15 Gy in prepubertal girls
   b. ≥10 Gy in postpubertal girls
3. Any alkylating agent combined with pelvic irradiation

B. Intermediate risk
1. Whole abdominal or pelvic radiation
   a. 10-<15 Gy in prepubertal girls
   b. 5-<10 Gy in postpubertal girls
   c. Spinal radiation ≥25 Gy
2. Alkylating score =2rd tertile

C. Alkylating score chart

	Cumulative dose by tertile		
	First	Second	Third
BCNU, mg/m^2	1-300	301-529	530-
Busulfan, mg/m^2	1-317	318-509	510-
CCNU, mg/m^2	1-361	362-610	611-
Chlorambucil, mg/m^2	1-165	166-634	635-
Parental cyclophosphamide, mg/m^2	1-3704	3705-9200	9200-
Oral cyclophosphamide, m^2	1-4722	4723-10636	10637-
Ifosfamide mg/m^2	1-16771	16772-55758	55759-
Melphalan, mg/m^2	1-39	40-137	138-574
Nitrogen mustard, mg/m^2	1-44	45-64	65-
Procarbazine, mg/m^2	1-4200	4201-7000	7001-
Thiotepa, mg/m^2	1-77	78-220	221-
Thiotepa, intrathecal mg/m^2	1-80	81-320	321-

places to refer to. Establishing an easily accessible fertility preservation practice, raising awareness of the patient needs for fertility preservation, and continuing staff education is essential in improving care for this population.

Ethical, Legal and Social Issues

There are unique ethical issues involved in both the fertility preservation of prepubertal girls as well as involving ovarian tissue cryopreservation. The consent process is one issue that must be considered, and it has been suggested that the current consent process for cryopreservation in the setting of cancer diagnosis is inadequate [21]. The consent process is inherently difficult in this patient population. Prepubertal patients are not allowed to consent for the procedure themselves but only to give "assent" that they agree with the decision made by their parents. While it can be presumed that in most cases parents or guardians would act in child's best interest, parents may have varying ideas of what constitutes the best interest for their child [26, 62].

Since ovarian cryopreservation is still largely considered an experimental procedure, it must first be rooted in the principle of "first, do no harm." Although ovarian tissue harvesting can usually be performed simultaneously with a medically required procedure for treatment of the underlying malignancy, it may potentially delay treatment. Fertility preservation may also present challenges when the risks of the harvesting procedure outweigh the potential benefits, for example when the child is too ill to undergo surgical intervention. The ethical principles of beneficence and non-maleficence must guide the team in recommending what most benefits the patient in terms of fertility preservation while not harming the patient [63, 64].

Fertility preservation for pediatric cancer patients also introduces the issue of finances and socioeconomic inequality. Although families may be able to support the initial tissue harvesting, cryopreservation and storage for several years,

they may not be able to afford preservation until the patient desires childbearing [65]. The utility of investing financially into an experimental procedure may be questionable. As insurance does not usually cover expenses involved with ovarian tissue cryopreservation, only patients with wealthier families may be able to afford the procedure while other patients may be denied the opportunity.

Disposition of frozen ovarian tissue in the event of a patient's death is a complex issue. While gametes have been classified as "property" with disputes often settled in the court system, cryopreserved ovarian tissue is technically an "organ" and thus not necessarily considered gametes [66, 67]. Many of these are new issues in the legal system and are yet to be addressed in court on a case to case basis. Issues surrounding the optimal duration of tissue freezing have not yet been fully addressed, and it is certainly plausible that family members could disagree about tissue disposition in cases of a child's death.

Conclusion

Although embryo and oocyte cryopreservation are considered the gold standard methods for fertility preservation, these option are largely not available for prepubertal females. Ovarian tissue cryopreservation, although considered experimental, is a viable option for fertility preservation for this population. In light of increasing survival rates in this group, a discussion regarding fertility preservation options should be initiated with parents and/or guardians early on after diagnosis. Fertility preservation should be an integral part of the treatment planning process, especially for patients facing high-risk treatments such as high-dose alkylating agents, abdominal radiation and hematopoietic stem cell transplantation. Currently, live pregnancies have only been achieved with orthotopic ovarian tissue transplants.

There are many ethical and legal situations which may be incurred surrounding the topic of posthumous conception. This is a delicate issue and must be addressed as more pediatric patients undergo fertility preservation methods. Provider education and care provision by multidisciplinary teams is pivotal in the management and support of pediatric patients and their families.

Compliance with Ethics Guidelines

Conflict of Interest Nina Resetkova, Masanori Hayashi, Lisa A. Kolp, and Mindy S. Christianson declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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