Oncofertility: A multi-disciplinary approach to fertility preservation

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Cancer treatment and assisted reproductive technology have both made impressive advancements over the last decade. Improved cancer treatment and survival rates have increased the number of cancer survivors, some of whom may benefit from emerging approaches to fertility preservation. It is estimated that today one in every 250 people in the adult population are childhood cancer survivors [1]. Life-saving cancer treatments may affect the future fertility of these individuals by damaging or destroying the gamete cells responsible for the production of sperm and oocytes. In most instances the aggressive chemotherapy and/or radiation treatments used to cure the cancer elicits non-reversible effects on the reproductive system. Therefore, initiation of strategies prior to the potentially gonadotoxic treatment is an important first step towards fertility preservation.

Assisted Reproductive Technologies to Preserve Fertility

Since the successful birth of Louise Brown in 1978 the field of *in vitro* fertilization (IVF) has rapidly evolved. As this field has expanded there have been several technological advancements that have positioned reproductive specialists to team up with oncologists to provide several options to women for the purpose of preserving their fertility after cancer treatment. Several techniques are currently available including embryo, oocyte or ovarian tissue cryopreservation and are discussed in detail below. Fertility preservation options depend on the patient's age, type of cancer treatment, whether she has a partner, the time period between diagnosis and treatment, and the potential that cancer has metastasized to the ovary.

Embryo Cryopreservation

The first successful human birth from a frozen embryo was reported in 1984 [19] and has since become a routine technique in IVF centers world-wide with proven efficiency in terms of pregnancy success. This approach requires a period of time for ovarian stimulation, with follicle stimulating hormone (FSH) injections to recruit and optimize follicle development. Follicle development is monitored by serial ultrasounds and blood tests, and at the appropriate time, human chorionic gonadotropin (HCG) is administered to aid in the final maturation of the growing cohort of follicles. Oocytes are collected by ultrasound guided trans-vaginal needle aspiration under intravenous sedation. Oocytes are fertilized *in vitro* and may be cryopreserved at the zygote or early cleavage stages of pre-implantation embryo development.

While embryo freezing is an established method of fertility preservation there are significant drawbacks to its' use that need to be considered. Firstly and most importantly, medical reasons might hinder this avenue if: (i) chemotherapy needs to be initiated immediately there is no time to undergo the ovarian stimulation required to get several oocytes for the IVF procedure, or (ii) the hormone stimulation associated with IVF is deemed harmful to patients with a hormone sensitive tumor, such as breast cancer. To overcome these problems it has been proposed that women could proceed with IVF on a spontaneous, unstimulated or natural cycle. However, the small number of attainable oocytes with this method and subsequently few viable embryos for transfer makes successful pregnancy unlikely. Women who undergo IVF after chemotherapy have a poor response to stimulating gonadotropins [20] and more recently, Dolmans et al., [21] demonstrated poor outcomes of IVF and embryo freezing, even if the attempt is carried out after only one or two regimens of chemotherapy. Another strategy that has been recently used successfully involves ovarian stimulation with tamoxifen and letrozole which may provide a suitable alternative to traditional IVF stimulation protocols and reduce the exposure to estrogen [22]. Only a small number of cancer survivors have used this method and many have yet to return to use their frozen embryos, but the initial pregnancy rates are encouraging and the efficacy of this approach

warrants further investigation [22]. The second major drawback to embryo cryopreservation is partner status. If the patient does not have a partner or is an adolescent, the only available option is to use donor sperm for the fertilization of her oocytes. This technique is not appropriate for children who have not reached puberty. Finally, the cost of the IVF procedures are expensive and may be limiting to some women.

In summary, embryo cryopreservation is an efficient technique offering a reasonable opportunity for success for women who have a partner and can undergo ovarian stimulation regimes to obtain several mature oocytes for IVF.

Oocyte Cryopreservation

The ability to cyropreserve oocytes would be the most desirable method of preserving fertility since it would overcome several of the problems discussed above. Oocyte freezing provides an alternative option for patients with the same characteristics as those who would be candidates for embryo freezing, but who don't have a partner or do not wish to use donor sperm. Therefore the ability to freeze the female gamete prior to fertilization theoretically offers a viable option to young women. The frozen oocytes are thawed later and fertilized *in vitro*. Ovarian stimulation is still required to harvest a suitable number of oocytes, and thus this technique is also subject to similar concerns regarding delays in cancer treatment and potential risks of short-term exposure to high exogenous hormonal levels. As previously discussed with embryo cryopreservation the use of tamoxifen or letrozole may potentially alleviate this problem [22].

Human oocyte cryopreservation has been successfully incorporated into several clinical IVF practices [23, 24]. Cryopreservation involves two main steps: (i) chilling and freezing. Chilling involves lowering the temperature from the physiological temperature to the point of freezing. Chilling injury is temperature dependant and can modigy the structure and integrity of the cell membrane [25]. Inappropriate handling of oocytes at room temperature posed the greatest threat to the oocyte [26]. Freezing further reduces the temperature to the storage temperature (liquid nitrogen at -196^0C). Since oocyte recovery results in oocytes at different stages of maturity there is the opportunity to be frozen as mature or immature oocytes.

<u>Cryopreservation of mature oocytes</u>

Freezing mature oocytes appears, at least in theory, to be the most logical way of storing female germ cells. It would be comparable to routinely banked sperm samples, and is an attractive option for women who do not have a partner to provide the male gametes required for fertilization. Unfortunately, the success of oocyte freezing has been largely disappointing. Problems associated with oocyte cryopreservation relate to the fact that the mature, metaphase II (MII) oocyte is a large and highly specialized cell that is extremely fragile and sensitive to chilling. During the freezing process the oocyte is prone to various types of cell injury which may explain the low survival and fertilization rates [27]. Two main reasons have been implicated for this poor outcome. Firstly, the freezing process results in the premature exocytosis of cortical granules that causes a hardening of the zona pellucida impairing sperm penetration and normal fertilization. However, intracytoplasmic sperm injection (ICSI) has been recently proven to successfully overcome this barrier [28]. The second challenge associated with freezing mature oocytes is that the metaphase chromosomes are aligned by the meiotic spindle along the equatorial plate, and the spindle apparatus is easily disrupted by intracellular ice crystal formation during freezing or thawing [26]. The cellular cooling process induces depolymerization of the meiotic spindle, which is a dynamic structure with microtubules being continually assembled at one of its ends and separated at the other. The oocyte is therefore sensitive to chilling, often doesn't survive the freeze-thaw process and is vulnerable to chromosome loss resulting in aneuploidy. Vitrification, or ultra-rapid freezing may improve post-thawing oocyte recovery, but requires further study [29, 30].

<u>Cryopreservation of immature oocytes</u>

Oocytes at the diplotene stage of prophase I, or germinal vesicle (GV) stage, survive the cyropreservation procedure better than those frozen at the MII stage [31]. GV stage oocytes have reached full size and meiotic competence but have not yet resumed their maturation process and initiated the second metaphase. Although the risk of hardening the zona pellucida or damage to the cytoskeleton cannot be

avoided, it is probable that the absence of a meiotic spindle and the presence of a nuclear membrane protecting the chromatin reduces the likelihood of cytogenetic anomalies during further cell divisons. Therefore, freezing oocyted followed by *in vitro* maturation offers practical and theoretical advantages. But this method is still sub-optimal. Frozen-thawed immature oocytes must go through *in vitro* maturation in order to reach nuclear maturation so that they can be successfully fertilized. Oocyte maturation is considered as the re-initiation and completion of the first meiotic division from the GV stage to the MII stage. Cytoplasmic maturation that occurs during this time is crucial for fertilization and early embryonic development [32]. Co-ordination of both nuclear and cytoplasmic maturation *in vitro* has been very difficult to achieve. Although there are several reports of pregnancies achieved after *in vitro* maturation of fresh GV-stage oocytes, only a few births have resulted from an immature oocyte cryopreserved at the GV-stage, with subsequent *in vitro* maturation [33, 34]. Therefore, until *in vitro* maturation of these oocytes becomes more reliable the cryopreservation of GV-stage oocytes will not be a practical strategy [35].

Ovarian Tissue Cryopreservation

For patients that require immediate chemotherapy, preservation of ovarian tissue is the only option. Ovarian tissue cryopreservation is an investigational method of fertility preservation but offers the advantage of requiring neither a sperm source (partner or donor) nor ovarian stimulation. Ovarian tissue is removed laproscopically and frozen in thin slices. The ovarian cortex contains primordial follicles with ooctyes arrested in the diplotene stage of prophase of the first meiotic division. It has been proposed that the relatively high surface to volume ratio, low metabolic rate and the absence of the zona pellucida make primordial follicles less susceptible to cryodamage. The main aim of behind the strategy of oocyte freezing is to re-implant cortical ovarian tissue into the pelvic cavity (orthotropic) or to a heterotrophic site like the forearm or the abdominal wall once cancer treatment is completed and the patient is disease free. One problem associated with ovarian freezing occurs at the time of re-implantation. Primordial follicles survive the freezing and thawing well, but because of the initial ischemia encountered after transplantation, a quarter or more of the follicles might be lost. To offset this relatively large loss, typically the whole cortex from an ovary is sectioned and frozen in adults.

In humans resumption of endocrine function has been reported after orthotropic and heterotropic transplantation of frozen-thawed ovarian cortical strips [36]. Recently, an embryo was created from oocytes retrieved from sub cutaneous transplanted ovarian tissue in a breast cancer survivor [37]. More recently, two live births were reported after orthotropic transplantation of frozen-banked ovarian tissue in lymphoma survivors [38].

A major concern associated with re-implanting ovarian tissue is the potential for reintroduction of cancer cells. In patients without evidence of system metastasis to other organs, the likelihood of occult ovarian metastasis appears to be low in the majority of cancers seen in young women. Ovarian tissue screening to detect malignant cells should be performed to minimize the risk of inadvertent transfer with the ovarian tissue(s). In patients with a high risk of ovarian involvement, xenografting and *ex vivo* follicle growth are experimental but not yet practical possibilities [38]. To avoid transferring malignant cells, ovarian tissue culture with *in vitro* follicle maturation would be a desirable option. Culturing isolated follicles from the primordial stage is an attractive proposition because they represent > 90% of the total follicular reserve and demonstrate high cryotolerence [39]. Unfortunately, isolated primordial follicles do not grow properly in culture [40] and further studies are needed to identify the factors required to sustain proper follicular growth and maturation [39] and the role of supporting theca and granulosa cells to these processes.

Donor Eggs and Surrogacy

IVF with donor eggs is another alternative for women when a cancer survivor suffers from premature ovarian failure. Gestational surrogacy can be a viable solution in patients that have undergone a hysterectomy, or have received pelvic radiation for cervical cancer. Success rates with these types of assisted conception techniques are very good. However, laws and regulations in different countries may be prohibitive to these options.

The challenge becomes bridging the gap between these two separate disciplines to improve the quality of life for cancer survivors. Effective multi-disciplinary teams of oncologists, nurses in the specialties of oncology and infertility, social workers, reproductive endocrinology and infertility specialists, andrologists, and embryologists are required to work together in order to achieve success.

Changing practices and attitudes towards fertility preservation requires a contribution at a number of different levels amongst a multi-disciplinary team. A schematic is of this framework is represented in Figure 1. Briefly, the oncology patient is at the centre of this schematic and has their original contact with an oncologist and oncology nurses. But as depicted the referral to a Reproductive Endocrinology and Infertility Specialist brings along the expertise of fertility nurses and the special skills of assisted conception technologies. Counseling becomes an important aspect from both the oncology and fertility side of the equation. Basic science research contributes to both the improved success of cancer treatment along with emerging advances in fertility preservation options for both men and women. Of equal importance is the patient centered quality of care research. A group with representatives of all relevant health care professions in this web was brought together to assess patients' needs and plan a strategy for improving the system which includes extensive improvement of both staff and patient information and awareness.

Adolescents and young adults with cancer are a unique group. Due to many external factors and changes that take place during this time in their lives the diagnosis of cancer can be overwhelming. Providing oncology patients with evidence-based information about fertility preservation by staff trained to impart this information allows them to make informed decisions about their fertility preservation options. While the primary focus to date has been on the male, emerging assisted conception technology is allowing parallel fertility preservation options for women. As a result it is imperative that health care professionals caring for patients with cancer become familiar with local options for their patients. The framework for coordinating efforts in providing fertility preservation options to patients undergoing treatment for cancer encourages the use of effective multi-disciplinary teams that include: oncologists; nurses in both specialties of oncology and infertility, social work, reproductive endocrinology and infertility specialists, andrologists, and embryologists are required to work together in order to achieve success. The result of this unique team approach is not only a cancer survivor but one that is able to round out their quality of life by being able to have a family of their own.

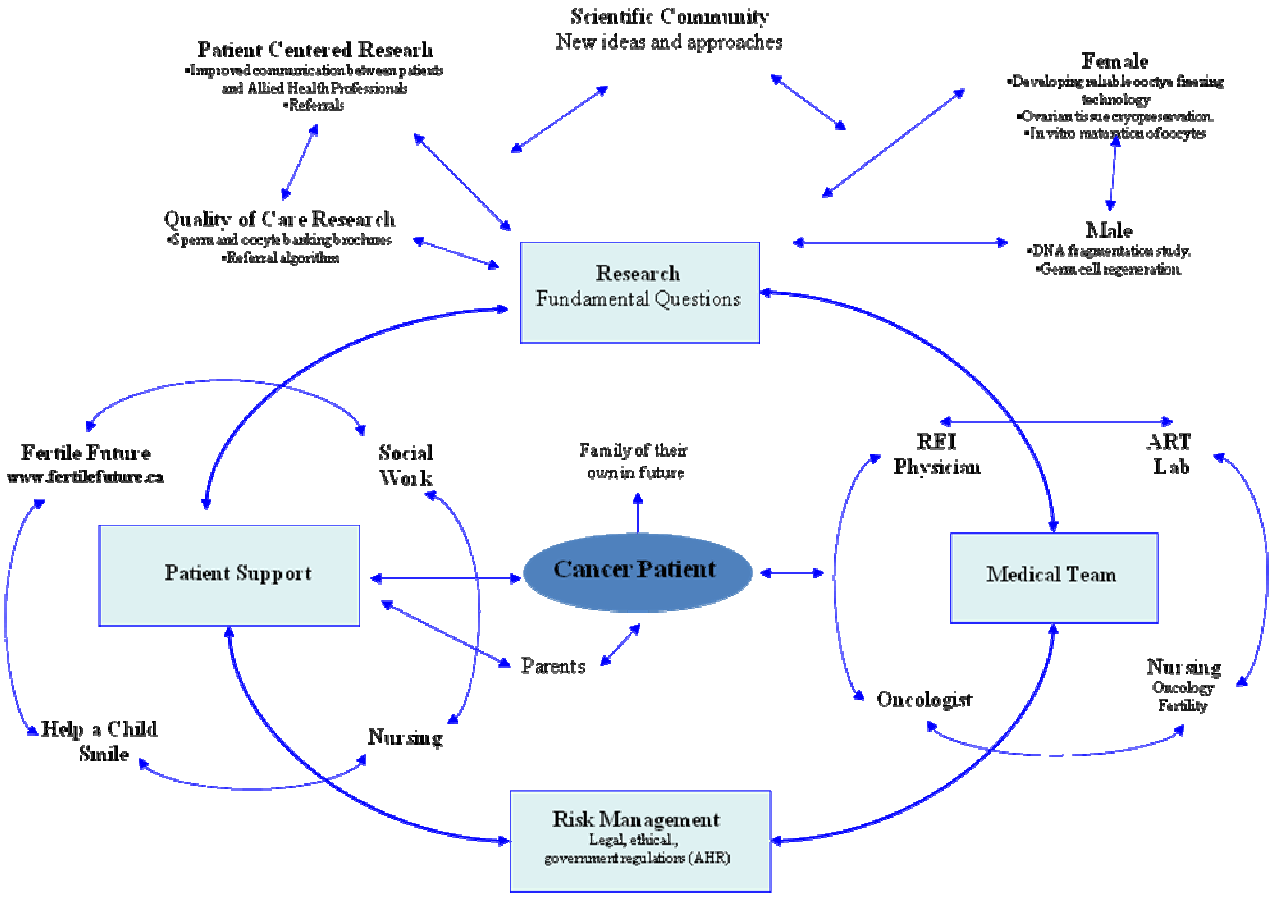

Figure 1: Multi-disciplinary Team Approach to preserving the fertility of cancer patients.

References

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