Embryologists perspective in handling gametes of cancer survivors in ART

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Summary

Improvements in diagnosis and treatment of cancer patients have lead to increase in their survival rates. An increasing number of people are now cured of cancer and are concerned about their options of bearing children with their own gametes. Chemo and radiotherapy are known to be gonadotoxic. The major concerns regarding handling of gametes of cancer survivors is related to their quantity and quality, non availability of any gamete in future, freezing and storage issues. Sperm cryopreservation is a well established technique. Though, embryo freezing is much more an effective technique for female fertility preservation, this option cannot be offered to patients who do not have a partner and are not in consent to accepting donor sperm. Hence, oocyte cryopreservation can be offered to these patients.

KEY WORDS: sperm, oocyte cryopreservation, fertility preservation, cancer survivors.

Introduction

There have been positive and dramatic innovations and awareness in the overall diagnosis and treatment of cancer patients. The impact of these has been on the survival rates of cancer patients. Growing knowledge of oncological pathophysiology, individualizing of cancer therapies, less radical and more conservative approach, fertility sparing surgeries where ever possible and advances in ART have all contributed in fulfilling the desire of cancer patients towards leading a normal life with the possibility of parenting their own children. An increasing number of people is now cured of cancer and is concerned about their options of bearing children with their own gametes.

The introduction of children's cancer centers and multicentre protocols have dramatically increased the survival to 60-65% and for specific cancers like leukemia, lymphomas and germ cell tumors it is almost 100%. The multidisciplinary working group conveyed by the British Fertility Society estimates that a significant number of these (15%) children will have compromised reproductive function. In women under 35 years breast cancer is one of the most common cancers in UK (1) and represents 30% of malignant tumors of childbearing age (2). The mortality rate for early breast cancer has reduced by 38% in women less than 50 years at the time of diagnosis (3).

However these improvements in survival come with a heavy price. Some of the treatment options which may involve surgery, chemotherapy or radiotherapy can have potentially sterilizing effects. Fertility issues after cancer treatment is a major issue in cancer survivors (4). The ASCO recommends fertility preservation to be of great importance to many patients diagnosed with cancer. Infertility resulting from cancer treatment may be associated with psychological stress and fertility preservation option can be of psychological benefit to the patient (5). There is no evidence that these techniques lead to reduced success rates in treatment of cancer (6). So far there seems little evidence to support any increase in risk of malformations, cancer or impaired psychological development in the offspring of cancer survivors (7).

There also seems to be lack of knowledge and awareness among the health care providers re-

garding fertility preservation. In a survey of oncologists of 2 cancer centers in the US, 48% of oncologists either never discuss the topic of sperm banking or mention it to less than a quarter of those who would be eligible for it. 91% of the responders agreed that sperm banking should be offered to as a means of fertility preservation in patients with cancer (8). A Swedish study showed that 48% of the female cancer survivors were counseled regarding fertility risks involved and 14% recalled being offered preservation options, only 2% of them used fertility preservation options (9). The health providers and the cancer survivors are now facing a dual challenge of optimizing survival probability and ensuring quality of life which mainly included the fertility potential of the survivor (10).

Embryologists concerns

Chemotherapy and radiotherapy can affect the fertility of the individual. Some of the contributing factors here are drug, dose, size and location of the radiation field, intensity, method of administration (oral or intravenous), etc. The risk of fertility compromise is highest with total body irradiation and localized radiation to the pelvis and testicular region. Chemotherapeutic agents like the alkylating agents, Vinca alkaloids and anti metabolites are proven to be gonadotoxic (11).

It is extremely necessary that the adverse reactions of the cancer therapy on the gametes be well understood by the embryologists. Uncertainty regarding both the quantity and quality of gametes post therapy makes cryopreservation an important tool for future fertility management. The major limitations arise due to the concern pertaining to non availability of any gamete in future. Hence the two major concerns are regarding the freezing and storage of these gametes. It is imperative to presume that there would be no second sample or no more gametes available in the future. Hence at least in the case of the semen sample adequate quantity needs to be frozen. The decision as to what could be adequate depends on the future course of fertility treatment that is envisaged for the patient. There is less or in certain cases no choice as far as the quantity of oocytes frozen are concerned. Whatever available, the sample needs to be frozen irrespective of quality and quantity.

The dilemma is when these are thawed how do we select or deselect the gamete to be used for fertilization. The recovery and efficacy of the freezing program also needs to be accessed in terms of those that over time will give consistent and good clinical outcome. The SCAAC committee found that some techniques were more advanced than the others; it also noted that the success rate of egg freezing technique varied and it was difficult to identify which technique was better than the other (12). It has been estimated that 1 in 250 adults under the age of 45 will be a long term survivor of childhood cancer by the end of this decade (13). In adolescent male patients the semen sample may have to be kept for much longer periods than their adult counterparts, as post treatment adolescents can expect many decades of life ahead of them (14). Hence we should be considering that these gametes are for long years of storage. The HFEA permits the sperm and oocytes to be stored for up to 10 years which can be extended to 55 years (HFEA). During storage the common concerns regarding storage like security and contamination risks need to be addressed. As the duration of storage here is unknown and would be longer than the storage duration in regular ART cycles, practical aspects like procedural changes, changes in management and staff that can happen over time need to be considered. Ethical and legal issues of storage of gametes both in children and adults are challenges that continue to pose a dilemma. This dilemma is more pronounced when dealing with patients who have their gametes frozen but have not survived cancer. Posthumous donation can be an emotional issue with the surviving partner or with the parents in case of no partner.

Male gamete

Effects of chemotherapy and radiotherapy

Though semen abnormalities are common in other cancers those with testicular cancer and Hodgkins lymphoma seem to show poorer sperm quality than those with other type of cancers (15-18). A basic semen analysis can be done to evaluate the compromise in the male fertility potential, which will show a reduction in the count, motility and morphology. The DNA integrity may also be compromised.

Table 1 - Freezing and storage concerns.

1. No sample may be available in future.
2. Adequacy of sample
3. Criteria to select/deselect gametes prior to freezing and post thawing
4. Optimal strategy for good and consistent clinical outcome
5. Long term storage issues
• Validity of the sample
• Security
• Procedural changes, management/staff changes
• Contamination Risk
• Ethical and legal Issues
• Posthumous donation

Chemotherapy and radiotherapy target the rapidly dividing cells and hence makes the germinal epithelium extremely vulnerable to its gametotoxic effects (19). The Leydig cells seem more resistant. During radiation the fractionation delivered in single dose seems to have less effect on the testicular germ cells in comparison to the fractioned regimes (20). A total gonadal failure is seen in up to 80% of the patients undergoing total body irradiation for stem cell transplantation (21).

The mechanisms by which spermatogenesis is adversely affected by these treatment options are, dose dependent radiation damage which can be permanent (22), depletion of both stem cells and differentiating spermatogonia (22, 23) or germ cell defect (24). The regime, type of agent and cumulative dose of the chemo therapeutic drug determines the impact on spermatogenesis (25). The short term DNA integrity is damaged following cancer treatment (26, 27) but similar effect on long term survivors is yet to be proved (28). In testicular tumors along with the paracrine and endocrine it could also be the systemic effects of the tumor that aggravates the reduction in the sperm quality (29). The disruption of the blood/testis barrier by the tumor leads to development of antisperm antibodies. 3-6 months after radiation the sperm counts are at their lowest and usually recover to their pretreatment levels 10-24 months post radiation (30, 31).

High doses of alkylating agents are most gonadotoxic with 90% of the recipients turning azoospermic for a prolonged duration (32). Regimens without high doses of alkylating agents or platinum compounds are unlikely to cause prolonged azoospermia (33). The sperm counts recover after an adequate time off of therapy. In a study, 2 years after treatment 97% patients on chemotherapy and 94% after radiotherapy (with shielding of contralateral testicle) showed good recovery of spermatogenesis (30). However in lymphoma there could be a long recovery time of almost 45 months (34). Hence though to some extent the adverse effect of the cancer treatment options on spermatogenesis could be reversible the extent and duration when this would recover seems to be variable. A high incidence of aneuploidy in sperm has been reported following some chemotherapy regimens (35). However there is no evidence till date to suggest any increased risk of congenital abnormalities among the male cancer survivors (36, 37).

Sperm cryopreservation

Sperm cryopreservation has undergone many improvements since the first report of a successful pregnancy from frozen spermatozoa (38). Sperm cryopreservation is now a well established technique for either ejaculated or surgically retrieved sperm, even in samples with reduced count and motility. Sperm banking is recommended before initiation of the treatment. It is essential that the patient has access to independent counseling and written information about sperm banking. If there are any accompanying persons specially parents they need to be privately sectioned off. This will psychologically help the patient to successfully produce a sample (39).

The feasibility depends upon the sexual maturity of the patient. Majority of adolescent cancer sufferers are able to produce semen, including boys of 12 years of age (16). Even the childhood testis does not appear quiescent and therefore

fertility preservation even in children before treatment should be considered (40). When it is not possible to obtain an ejaculate, surgically it can be retrieved by epipidymal aspiration or testicular biopsy in sexually mature patients. Sample produced by boys of 12 years of age have been found suitable for ART, and hence sperm banking should be recommended to all adolescent patients (16). There have also been case reports of sperm collection from the urine sample post masturbation and by recto ejaculation under sedation or anesthesia. Advancements in ART in the form of ICSI largely circumvent the challenges posed by low counts and motility which are not rare in cancer survivors. Hence sperm cryopreservation is recommended as a simple and low cost method of fertility preservation in male cancer patients. It is unfortunate that very few survivors actually use their sperm to achieve a pregnancy.

Sperm cryopreservation is simple, effective and non invasive and hence one should not hesitate to obtain adequate number of ejaculates for freezing. When using 1 ml vials for freezing containing equi volume semen and cryoprotectant, it would be reasonable to obtain 3-4 vials per ejaculate for an adolescent patient. This means obtaining approximately 2-3 ejaculates. This can be offered as a broad policy, however in circumstances like inability to provide second or third sample or poor semen sample quality the number of vials can be increased (16).

ICSI offers a very promising option for these patients to father their own children (41). In a small study of 30 patients Meseguer et al. (42) reported an identical success rate with cryopreserved sperm in cancer patients with the procedure of ICSI. Agarwal et al. (43) reported a pregnancy rate of 18.3% in 87 ART cycles of which 42 were IUI, 26 IVF and 19 with ICSI. Of these pregnancies 75% resulted in a live-birth. Amnon Botchan et al. (44) reported a 62.1% fertilization rate, 13.8% implantation rate and 37.4% pregnancy rate per cycle with ICSI using thawed sperm in cancer patients.

Freezing of testicular tissue or germ cells and reimplantation of this tissue post cancer treatment has been successfully applied in animal models (45) but have not been attempted in humans. Testicular tissue freezing and transplantation is considered an emerging technique for the future. The concern here being of reintroducing cancer cells (HFEA).

Female gamete

Effects of chemotherapy and radiotherapy

The outer cortex of the ovary contains the oocytes which is also the site of hormone production. From a peak number of 6.8 million at 5 months gestation the store of oocytes reduces to approximately 2 million at birth with only 300,000 left at puberty. During her reproductive life span a women will ovulate 300-500 mature eggs while the rest become atretic (46).

There seems no direct effect of the cancer on the female reproductive system; however the treatment options adversely affect different sites in the reproductive tract (47). Damage induced by radiation and chemotherapy to the ovary, is progressive and irreversible resulting in amenorrhea and infertility (48, 49). Follicular damage can affect both endocrine and reproductive functions. As the ovarian follicular pool is pre-defined lower doses of radiation can cause premature ovarian failure and higher doses can lead to total depletion of ovarian reserve (31, 50). As the ovarian pool reduces with age, the likelihood of premature ovarian failure secondary to the treatment modalities increases.

Persistent amenorrhea is used as a surrogate marker of ovarian failure.AMH levels pre and post treatment can help evaluate the extent of ovarian damage. High pre-treatment AMH concentrations are predictive of higher post treatment AMH (51). A long term sequel of radiation on the uterus can lead to obstetric complications like spontaneous abortions, preterm labor, IUGR and low birth weight infants. This is due to the irreversible changes on the uterine musculature, reduced elasticity of the myometrium and vascular damage (52).

Oocyte cryopreservation

Embryo cryopreservation is considered the best and most successful means of fertility preservation in females (41). It is now a routine and mandatory procedure in ART centers. However the option depends on the patient's age, time available to initiate cancer therapy and whether she has a partner available at the time of treatment. It cannot be offered to patients who do not have a partner or who do not wish to use donor sperm to create embryos (50). It also involves ethical issues. Oocyte cryopreservation is an alternative to embryo storage in these scenarios. Some cancers like estrogen positive breast can-

cer are hormone dependent. Oocyte or embryo cryopreservation in these patients through controlled ovarian stimulation and IVf may be contraindicated.

Oocyte cryopreservation techniques have undergone significant improvements. The use of vitrification method for freezing oocytes has increased the survival rates to 80% (53-55) as compared to 50-65% with slow freeze method (56, 57). The success results with frozen thawed oocytes in young patients and donor oocytes has been encouraging (58, 59). Based on these current states of evidence, modern procedures to cryopreserve oocytes have fuelled in a lot of confidence in oocyte cryopreservation. In 2013, the American Society for Reproductive Medicine (ASRM) and the Society for Assisted Reproductive Technology (SART) published a joint document, *Mature Oocyte Cryopreservation: a Guideline*, wherein mature oocyte cryopreservation is no longer considered experimental. The American College of Obstetricians and Gynecologists' Committee on Gynecologic Practice has also endorsed the ASRM-SART document (ACOG) (60, 87).

Comparison of slow freeze and vitrification procedure for oocyte cryopreservation suggests that vitrification is more effective a procedure as it gives a higher survival rate without compromising on the DNA integrity despite a higher misalignment between the meiotic spindle and the polar body (61). In a meta-analysis of randomized control trials vitrification seems superior in terms of not only oocyte survival but also fertilization, embryo cleavage rate and formation of top quality embryos (62).

There is an attrition involved with oocyte cryopreservation from survival to fertilization and embryo formation. However, Cobo et al. (63) in their study were able to get a survival rate of 96.9%. There was no difference in fertilization rates (76.3 and 82.2%), day 2 cleavage (94.2 and 97.8%), day 3 cleavage (80.8 and 80.5%), and blastocyst formation (48.7 and 47.5%) for vitrified and fresh oocytes, respectively. Embryo qualities on day 3 and on day 5-6 were similar for vitrified and fresh oocyte group (80.8 *vs* 80.5% and 81.1 *vs* 70%, respectively). The metabolomic profiles of the embryos generated from vitrified oocytes does not seem to be disturbed (64). A study comparing the 1,024 babies born after use of vitrified oocytes did not show any increase in adverse obstetric nor perinatal outcome (65). Chian et al. in their study of 200 suggested no increase in chromosomal abnormalities, birth defects or developmental deficits in children born from cryopreserved oocytes (56).

In fact, there have been few reports of live births in patients with cancer who opted for oocyte cryopreservation (66-70). In four pregnancies no major or minor malformations were noted in the babies born from cryopreserved oocytes prior to cancer therapy (71). Large well controlled studies with adequate duration of follow up on long term safety of oocyte freezing are much needed.

The oocytes can be obtained by ovarian stimulation and egg retrieval. Conventional ovarian stimulation needs 8-10 days with exogenous gonadotrophins. As it leads to supraphysiological serum levels of FSH and oestradiol ovarian it cannot be advocated in women where the tumor is hormone sensitive or the cancer treatment has to be initiated urgently. Modified ovarian stimulation has been advocated for mature oocyte collection (72).

Immature oocyte aspiration and *in vitro* maturation of oocytes can also be considered in patients where ovarian stimulation cannot be offered. IVM in a natural cycle avoids use of hormones for ovarian stimulation, prevents OHSS and the oestradiol concentrations are also within the natural range (73, 74). In a natural cycle usually only a single follicle develops to ovulate. However during the same phase many small follicles develop simultaneously. The immature oocytes from these non dominant follicles can be harvested matured *in vitro* and fertilized. They have resulted in several pregnancies and healthy live births (75, 76). Immature oocytes can be aspirated in late follicular phase (54, 77) or during the luteal phase in an emergency (78, 79). Irrespective of the phase of the menstrual cycle, there appears no statistically significant difference in their oocyte retrieval rate, maturation rate, fertilization rate or total number of oocytes or embryos cryopreserved (80). Thus in cancer patients with severe time constraints IVM followed by oocyte or embryo cryopreservation can be considered.

This can be followed by embryo freezing or oocyte freezing.

The number of births from IVM oocytes though

on a rise are still few. There have been concerns regarding the obstetric, perinatal and long term well being of the offspring. The recent reports comparing IVM births with natural and IVF conceptions following COH have been encouraging (81, 82).

Mature oocyte freezing poses a technical challenge as it is extremely sensitive to temperature changes with a limited capacity to repair the cytoplasmic damage. Secondly depolarization of the meiotic spindle due to the cryoprotectants or ice crystal formation can lead to aneuploidy (83, 84). In the GV stage the chromosomes are protected in a nuclear membrane and this may offer protection against damage to the meiotic spindle. However their survival and maturation post warming is compromised in comparison to oocytes which were matured *in vitro* prior to freezing (85). Thus freezing metaphase II oocytes is a better option.

Ovarian tissue cryopreservation has been considered primary method of fertility preservation in prepubertal girls along with transplantation later after puberty. There has been a recent case report of spontaneous conception and delivery in a 27-year-old patient following ovarian tissue freezing prior to menarche at the age of 13 years and 11 months (86). This patient was diagnosed as Sickle cell anemia at the age of 5. Ovarian tissue cryopreservation along with immature oocyte collection followed by IVM-vitrification can also be considered prior to gonadotoxic treatment.

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