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REVIEW ARTICLE

Current strategies and future perspectives in fertility preservation for cancer patients

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Summary

Nowadays, cancer is being detected at younger ages. Health care providers should consider cancer patients' desire towards fertility preservation before the initiation of possibly sterilizing treatments. The aim of the current review was to register the current state of fertility preservation procedures available for male and female cancer patients.

Key words: *cancer patients, fertility preservation*

Introduction

There is an ongoing trend to detect cancer at earlier stages and in younger patients. Therefore, lately, preservation of fertility in this population has become a matter of great interest.

Furthermore, we are witnessing an increased pressure on health care providers to offer fertility preservation to cancer survivors which are at reproductive age [1,2].

Almost half of cancer patients are able to bear children or want to do so at the time of diagnosis [3]. Additionally, health care providers should not presume that older men may not be as interested in fathering children, as some may desire children later in life or with a different spouse. However, survivors have lower pregnancy rates than the general population. The impact on fertility depends on many factors, with survivors of leukemia showing lowest rates for pregnancy, and testicular cancer and Hodgkin lymphoma showing increased rates over time [4].

The aim of the current review was to establish the current state of fertility preservation procedures. A review of the literature was completed using advanced search on PubMed with the following terms "ovarian tissue cryopreservation", "embryo cryopreservation", "oocyte cryopreservation", "ovarian transposition", "live birth", "pregnancy", "male infertility", and "cancer". We included all relevant articles from 2004-2016.

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Fertility preservation in female patients

Chemotherapeutic agents affect severely the reproductive and endocrine function of the ovaries, and most women developing amenorrhea and never regaining menstrual cycles [5]. Studies show that premature ovarian failure increases with age and varies with regimen, duration and total cumulative dose of chemotherapy [6]. It has been estimated that 60-80% of women who are treated with cyclophosphamide, methotrexate and 5-fluorouracil will develop premature ovarian failure (POF) [7,8]. Several studies have observed that a significant number of younger patients who did regain ovarian function after chemotherapy were at risk of undergoing premature menopause a number of years after treatment. The beneficial effects of adjuvant chemotherapy for breast cancer may result, in part, from suppression of the ovarian function [9,10].

Radiation therapy also has severe adverse impact on endocrine and reproductive function depending on patient age, administered dose and the irradiation field [11]. POF can be induced directly or by affecting the hypothalamic-pituitary axis [12,13].

The choice of fertility preservation method in a practical manner depends on patient age, possibility to delay chemotherapy or radiation, presence of male partner or willingness to use a sperm donor and whether patient malignancy permits ovarian stimulation. According to Chian et al. strategy, we present an update of the current fertility preservation methods in female patients [14].

Embryo and oocyte cryopreservation

For reproductive age women with available male partner (or a willing to use a sperm donor), and for whom health care providers (oncologist, fertility specialist) decide that treatment can be delayed to perform ovarian stimulation; embryo cryopreservation is the current option. Oocyte conservation is suitable for women without a partner, or for those who do not accept embryo freezing [14].

Both options require ovarian stimulation, transvaginal oocyte retrieval, thus making both techniques available only for post pubescent girls who have sufficient time to undergo ovarian stimulation, before starting oncological treatment.

The conventional ovarian stimulation protocols can increase estrogen levels up to twenty times, raising concerns about the estrogen sensitive tumors growth. To avoid the undesirable effects of estrogen, new protocols that use Letrozole and FSH have been developed. Some authors who used the Letrozole and FSH protocol in patients with estrogen sensitive breast cancer reported no significant increase risk in short-term recurrence and number of embryos obtained comparable with other ovarian stimulation protocols. [15-17].

Embryos and oocytes can be cryopreserved using: slow freezing and vitrification. Until recently, efforts to freeze oocytes with the slow freezing protocol remained one of the biggest weaknesses of assisted reproduction, as survival rates were much lower than those of cryopreserved-thawed embryos. Nowadays, the vitrification technique has revolutionized cryopreservation techniques and has now become the standard procedure for embryo and oocyte conservation with high survival and pregnancy rates. A study published by Levi-Setti et al., conducted over a period of 5 years showed that cryopreservation of oocyte by vitrification had a higher survival rate than cryopreservation of oocytes by slow freezing. Pregnancy chances were significantly higher when using fresh or cryopreserved embryos compared to using embryos obtained from cryopreserved oocytes [18].

In case of patients for whom delaying treatment is not an option or for whom ovarian stimulation is not indicated by health care providers, immature oocyte retrieval followed by *in vitro* maturation could be considered. Immature oocytes are extracted from the antral follicles and matured *in vitro*, in order to produce embryos that increase the likelihood of conceiving for these patients. *In vitro* maturation (IVM) can be performed regardless of the patients' menstrual cycle phase, without affecting oocyte quality. Maturation and fertilization rates are comparable after luteal phase and follicular phase retrieval [19].

In 2014 Prasath et al. reported the first case of live birth after IVM in a patient with bilateral borderline serous carcinoma of the ovary [20].

There is an ongoing concern about the timing of cryopreservation of the immature oocytes, since performing it at the germinal vesicle stage may cause certain damage in its quality [21].

IVM can be combined with ovarian tissue cryopreservation (OTC). According to a study published by Hourvitz et al. in which 255 cancer patients were included in fertility conservation programs, employing a combination of OTC, oocyte aspiration and *in vitro* maturation (AIVM), and with oocyte retrieval from ovarian tissue (OTIVM) resulted in more oocytes ($p<0.001$), more metaphase II oocytes ($p<0.001$), better maturation rate ($p<0.01$) and more cryopreserved oocytes ($p<0.05$) than by employing just OTIVM or OTC. Also, the same study found that compared to using just ovarian tissue oocyte cryopreservation,

more oocytes with better maturation rate are obtained if oocyte aspiration is performed right before ovarian tissue cryopreservation [22]. Ovarian tissue cryopreservation

Ovarian cortex biobanking, as a method of preserving fertility, is considered as an option for women, in whom need of chemotherapy is immediate or for pre pubertal girls for whom ovarian stimulation and *in vitro* fertilization (IVF) can't be applied [23].

The main advantage of this method applied for ovarian tissue resides in the fact that the ovarian cortex is the source of primordial and primary follicles [24], assuring a high amount of female gametes. Also, retrieval can be performed without delay in a minimally invasive manner. While the structure of the tissue can remain unaltered, its function can be destroyed irreversibly [25]. The method needs the use of cryoprotective agents because of the risk of ice crystal formation. Toxicity of these agents is another problem limiting the technique's success [26] and it depends on the chemical properties of each agent, duration of exposure, and temperature [27]. Several studies suggest that primordial follicles are more resistant to cryoinjury and to cryoprotectants due to their dormant metabolic state [28].

The strategy underlying this procedure is to harvest and store the ovarian tissue fragments until the patient is ready for transplantation, aiming at the restoration of endocrine and reproductive functions, otherwise destroyed by chemotherapy. The ovarian tissue grafts can be transplanted to the pelvis, near the original ovary sites blood supply (orthotopic transplantation) or to other sites such as abdomen or forearm (heterotopic transplantation). Orthotopic transplantation is the most used method of transplantation and with this technique several pregnancies were obtained; meanwhile, heterotopic transplantation offers a series of advantages regarding its monitoring, but there have been no pregnancies reported yet using this method [29].

The endocrine function duration after transplantation varies between 9 months and 3 years. An important amount of follicles are lost right after transplantation due to local ischemia leading to repeated grafting procedures [30,31].

OTC is an invasive procedure and still considered experimental for young patients, although in some countries, such as Israel, efforts were made to reconsider this [32]. The American Society for Reproduction Medicine guidelines classifies OTC as experimental and recommends applying it on carefully selected patients [33]. The American Society of Clinical Oncology Practice Update also labels OTC as experimental and recommends this procedure only in experienced centers [34]. Both publications raise the theoretical problem concerning transplantation of cancer cells with the graft.

In 2004 Donnez et al. reported the first live-birth after cryopreservation and orthotopic transplantation of ovarian tissue in a woman with stage IV Hodgkin lymphoma. Five months after transplantation, hormone levels and ultrasound findings were consistent with ovulatory cycles and after 11 months pregnancy was confirmed by ultrasound. The patient delivered at term. Concerning the theoretical aspect, regarding the transplantation of malignant cells, histological assessment was completed showing no such findings [35]. Several pregnancies followed this success (Table 1).

Table 1. Ovarian tissue cryopreservation outcomes (2004-2016)

Year of publication/ authors [Ref]	*Orthotopic / heterotopic transplantation*	*Endocrine function restoration (months)*	*Pregnancy / live birth*
2004, *Donez et al* [35]	+/-	5	1/1
2005, *Meirow et al* [36]	+	8	1/1
2007, *Demeestere et al* [37]	+/+	5	1/0
2010, *Demestere et al* [38]	+/+	33	1/1
2008, *Andersen et al* [39]	+/-	4	2/2
2010, *Ernst et al* [40]	+/-	4	1/2
2011, *Donez et al* [41]	+/-	2-5-6	13/13
2010, *Roux et al* [42]	+/-	4	1/1
2010, *Sanchez-Serrano et al* [43]	+/-	2	1/2
2012, *Muller et al* [44]	+/-	3	1/1
2014, *Macklon et al* [45]	+/-	1	1!
2015, *Tanbo et al* [46]	+/-	?	2/2
2016, *Dunlop et al* [47]	+/-	3,5	1
2016, *Meirow et al* [32]	+/-	1-6	16/10

In 2006 a cooperation network to aid fertility preservation for oncologic patients (both women and men) was founded in Germany. Since then, it has extended to more than 100 institutions across the country and in Switzerland and Austria. In 2016, the largest case series published by Fertiprotekt network reports 21 pregnancies and 17 live-births after orthotopic ovarian cortex transplantation [35]. For the purpose of improving some aspects of the procedure Oktay et al. performed ovarian tissue transplantation using a human extracellular tissue matrix scaffold in two patients diagnosed 12 and 7 years respectively before, with hemophagocytic lymphohistiocytosis and non-Hodgkin lymphoma. The transplantation performed was minimally invasive. One pregnancy was ongoing at the time of publishing and one patient delivered a healthy baby [36]. Even though official peers consider OTC as an experimental method of preserving fertility, efforts should be made to offer this for whom other options are not available.

Ovarian transposition

Ovarian transposition is a surgical procedure that should be considered in order to preserve fertility in female patients with genital (cervical cancer, vaginal cancer), urinary (rhabdomyosarcoma of the bladder) and hematologic (Hodgkin's disease) malignancies as well as sarcomas of the pelvic region (Ewing's sarcoma), anorectal cancer or neurologic malignancies that are treated with pelvic radiation. Oophoropexy reduces ovarian exposure to only 5-10% [37-40].

Pelvic radiotherapy may cause ovarian and uterine damage. Radiation tolerance of the uterus and the ovaries depends on the total radiation dose, the fractionation schedule, the volume of the tissue which is irradiated and the patient's age. The more younger the patient, the higher the chance she has to preserve residual ovarian function. The dose of irradiation at which ovarian failure occurs in 97.5% (ESD – the effective sterilizing dose) of patients after treatment is 20.3Gy at birth, 18.4Gy at 10 years, 16.5Gy at 20 years and 14.3Gy at 30 years. Fractionated doses of radiation are less toxic than a single dose [41,42].

Radiation damages the DNA of the ovarian follicle which might lead to decreased follicular reserve. Mature follicles are more radiosensitive than primordial follicles. To destroy half of the follicular reserve, less than 2Gy is needed. Ovarian failure is produced by a dose of irradiation of 24Gy, if it is applied conventionally [40,43]. Ovarian transposition, also known as oophoropexy is a procedure in which one or both ovaries are moved from the irradiation field. Ovaries can be moved to the parabolic gutters, above the pelvic brim, in line with the iliac crests or anterior the psoas muscle, depending on the radiation field, by open surgery, laparoscopy or robotic surgery. Metal clips are placed in order to identify and confirm that the ovary is out of the irradiation field. The procedure should be done as close as possible to the beginning of radiotherapy, and remains the standard of care for patients treated with pelvic radiation. It may be combined with oocyte, embryo or ovarian tissue cryopreservation. Sometimes, to achieve pregnancy, re-transposition is necessary. A recent approach is to transpose one ovary and remove the other one for cryopreservation [37,41,44].

Complications regarding this procedure are relatively rare, but sometimes chronic pelvic pain, adhesions, fallopian tube infarction, ovarian migration and metastasis to the transposed ovaries can occur. The hormonal function is preserved in 70-93% with ovarian transposition before radiotherapy in patients < 40 years. Thibaud et al. reported the first results of 18 children born after ovarian transposition – 2 of them became amenorreheic, 16 had menstruated and 2 pregnancies occurred on a follow-up of 8.6 years [45].

Terenziani et al. reported a number of 14 pregnancies – 12 live births (1 twin) and 3 miscarriages after ovarian transposition in 11 patients with Hodgkin's lymphoma, after a follow-up of 14 years. None of these patients needed artificial insemination or ovarian de-transposition [46].

Ovarian transposition in pediatric patients is still inadequately studied, but the success rate seems to be 60-83%. In adults' long-term outcome studies, only a few pregnancies have been reported – 5 pregnancies in 10 patients with Hodgkin's lymphoma [47] – 3 pregnancies in 107 cervical cancer patients [48] and 3 pregnancies in 12 patients (9 Hodgkin's lymphoma, 3 rectal cancer) [49], but the ovarian hormonal function was well preserved [38].

A surrogate pregnancy may be a valid option for women with cervical cancer treated with radical hysterectomy, lymphadenectomy and oophoropexy, followed by ovarian stimulation, oocyte retrieval from the genetic mother, IVF and embryo transfer to the surrogate mother. Legislation in many countries forbids this approach [50].

Köhler et al. described in a study published in 2016 a successful delivery after ovarian transposition and uterus fixation in a patient with anal cancer followed by chemo-radiation and recto-anal resection [51].

Fertility preservation in male patients

Cancer, treatment and fertility

Cancer itself may influence spermatogenesis, though the mechanisms are not well understood. Preexisting poor quality of germ cells, systemic effects of cancer, endocrinological or immunological effects probably exert some effect [52,53]. All cancer therapies - radiotherapy, chemotherapy, stem cell transplantation, surgery - can impact fertility, either directly affecting spermatogenesis or hormone production. It is important to explain their different risks and benefits, as these may influence patients' treatment decision [54].

Unfortunately, there are no available options for the protection of the gonadal epithelium. Prepubertal age is not a protective factor from gonadotoxic injury, as the cytotoxic treatment directly affects the early germ cells that undergo spontaneous degeneration before the haploid stage [55].

Radiotherapy

Radiotherapy has been utilized in the treatment of prostate, bladder, penile, testicular and rectal cancer. The initial modalities for radiation delivery have evolved from conventional external beam radiotherapy to fractionated intensity modulated radiotherapy. However, it may still have irreversible detrimental effects on fertility and spermatogenesis. The gonadal epithelium is very sensitive to radiation because of its rapid division rate. While Leydig cells can resist to doses up to 20Gy in prepubescent males (and 30Gy in adult males), immature spermatogonia are more sensitive, with doses of 0.1Gy able to influence their shape and number. Radiation doses under 0.8Gy lead to oligozoospermia, while doses up to 2Gy can lead to temporary azoospermia. Higher doses can lead to permanent sterility [56].

The radiation to the testis may result either from direct exposure or scattered radiation, with some authors mentioning that 18.7% of radiation administered in pelvic cancers is received by the testis [57]. It may take 10 to 24 months for the sperm to return to pretreatment levels. The impact of radiation therapy on sperm DNA integrity is not yet known [58].

Chemotherapy

Similarly to radiotherapy, chemotherapy may alter the function of Leydig cells and cause hypogonadism. The amount of damage is also dependent on the type of regimen, the age of the patient and the total dose administered. However, it has been proved that hormonal therapies do not lead to faster recovery of spermatogenesis, nor do gonadotropin releasing hormone antagonists prevent long term infertility when high doses of chemotherapy are used [59].

Combination chemotherapies have been developed to reduce the negative effects and potentiate the efficacy of the agents used, and have become the golden standard in the treatment of several cancers. Although this synergy is desirable against cancer cells, it also has a detrimental impact on fertility, with the possibility of incurring permanent azoospermia. For instance MOPP (mechlorethamine, oncovin, procarbazine and prednisone) used for Hodgkin's lymphoma can cause azoospermia in 90% of men for up to 4 years after treatment. The combination of bleomycin, etoposide and cisplatin used in some testicular cancers has been associated with increased sperm DNA abnormalities [60]. A recent animal study has described a protective effect of the humanin analogue against spermatotoxic effects during chemotherapy, but no human studies are available [61].

Surgery

Surgery may also affect fertility, either directly by affecting the integrity of the genitourinary tract (for instance in bladder or prostate surgery) or its function (for instance, causing retrograde or anejaculation by affecting the lumbar sympathetic plexus or hypogastric plexus during retroperitoneal lymphadenectomy for testicular cancer). Many pelvic operations may also affect the erectile function. As such, even though sperm quality may not be changed, there is a possible permanent loss of fertility, which the patient should be aware of. Currently, there is an effort to develop and deploy nerve sparing techniques whenever possible to maintain erectile and ejaculatory function, with good results.

Surgical approaches to fertility preservation

Several surgical methods for preserving fertility have been described - testis sparing surgery, testis transposition, sperm retrieval, testicular stem cell transplantation.

Although rare, synchronous and metachronous bilateral germ cell tumors occur in 2-5% of the patients, and bilateral orchiectomy will lead to permanent infertility as well as cardiovascular, metabolic and psychological problems. Thus, in extremely selected cases, there is an option for organ sparing surgery, provided the tumor is limited to the testis and of small size (less than 2 cm) [62]. Because of an increased risk of local recur-

rence or adjacent testicular intraepithelial neoplasia, close follow-up is required in all patients undergoing enucleation. Ideally, semen collection is performed prior to surgery, or at least before definitive radiotherapy. Organ sparing surgery may also be an option in the rare cases of Leydig cell tumors, which account for 0.8-3% of all testicular tumors [63].

Testicular transposition is a rarely used technique, that may allow for fertility preservation in patients requiring radiotherapy in the pelvic region (for instance, for rhabdomyosarcoma of the bladder, prostate, or paratesticular tumors). The testicle to be preserved is transposed to the thigh or anterior abdominal wall, and later replaced in the scrotum following completion of local irradiation [64,65]. Due to the rarity of the procedure, its role in normal practice has yet to be defined.

Sperm extraction and preservation

In a study on adolescent patients, Bahadur et al. reported that adequate semen samples were obtainable in the majority of patients regardless of cancer type (86.1%) [66]. If there is no ejaculation or the patient has a history of retrograde ejaculation, a urine analysis following masturbation should be performed to assess the presence of sperm in urine. If positive, alpha agonists may be tried to direct the flow of sperm forward. Failing that, a trial of urine alkalization, collection post ejaculation and isolation of viable sperm may be performed. If no ejaculation can be achieved, vibrostimulation or electroejaculation can be performed, usually under general anesthesia [67]. In azoospermic men surgical means of sperm extraction may be required, such as microsurgical sperm extraction (microTESE), testicular sperm extraction or microsurgical epididymal sperm aspiration (MESA). In men with azoospermia prior to chemotherapy due to their cancer pathophysiology, oncological testicular sperm extraction may be performed, as described by Schrader et al., which uses microsurgical dissection and extraction of seminiferous tubules during the initial gonadal surgery [68].

For prepubertal males there are limited options for fertility preservation, focusing on *in vitro* generation of sperm from harvested spermatogonial stem cells or preservation of immature testicular tissue [69]. Once collected, cryopreservation allows the sperm to remain in a suspended animation state and able to be stored for up to 15 years [70]. Current assisted reproduction techniques - ARTs (*in vitro* fertilization - IVF - and intracytoplasmic sperm injection - ICSI) allows for conception using testicular and epididymal sperm, or sperm with suboptimal motility or morphology.

Fertilization methods

After treatment, many men may need artificial reproductive techniques to procreate as IVF/ICSI. Of these, 15% will require the use of cryopreserved semen due to persistent azoospermia. No studies have so far proven any increase in the rate of malignancy or congenital abnormalities in children born fathered by cancer survivors, but close follow up is recommended. In a large cohort study published by Boice et al. the incidence of anomalies in children of cancer survivors was the same as in the general population. This held true for patients who fathered children after undergoing radiation therapy or alkylating agent therapy [71]. A recent Swedish and Danish study has shown a modest but statistically significant increase in the risk of congenital abnormalities in children of males who had undergone cancer treatment, irrespective of means of conception (natural or ART). The study involved the analysis of a cohort of 8670 children with paternal history of cancer treatment. Of these, 508 children were conceived using ARTs. The children of male cancer survivors were more likely to have major congenital defects than the control group, (RR = 1.17, 95% CI=1.05-1.31, p=0.0043, 3.7 vs 3.2%). Interestingly, the data available allowed the comparison of incidence of congenital abnormalities in children born from semen preserved pre-treatment versus post-treatment, and proved to be equal in both groups (4.4%), indicating that factors other than anticancer therapies may be causing this trend [58].

ART in cancer survivors seems to be as effective as in the general population. Garcia et al. reported that the use of cryopreserved semen is about 10% and the success rate for live births is comparable to that of non-cancer patients [72].

Conclusions

Embryo and sperm cryopreservation are established methods of fertility preservation. Other options, such as ovarian or immature testicular tissue cryopreservation are still in their infancy, but regarded with high hope. More research and funding are needed to embrace the need of reinstating and maintaining cancer patients' fertility.

Conflict of interests

The authors declare no confict of interests.

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