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Clinical Centre of Vojvodina, Novi Sad
Department of Obstetrics and Gynecology¹
University of Novi Sad, Faculty of Medicine Novi Sad²

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FERTILITY PRESERVATION IN CANCER PATIENTS

OČUVANJE FERTILITETA KOD ONKOLOŠKIH PACIJENATA

Artur BJELICA^{1,2}, Aleksandra TRNINIĆ PJEVIĆ^{1,2}, Ivana JAGODIĆ¹ and Jelena VUKOSAVLJEVIĆ¹

Summary

Introduction. Progress in the field of cancer treatment has led to increased survival rate of cancer patients. Chemotherapy and surgical treatment may be the consequence of decreased fertility in both genders. **Fertility Preservation.** Some of the prominent techniques for fertility preservation, cryopreservation of gametes and embryos, are efficient and safe techniques in clinical practice, while cryopreservation of gonadal tissue and cells is considered experimental, and it is not used in everyday practice yet. **Conclusion.** Timely and complete information about the effects of cancer treatment on fertility, as well as information about the potential techniques for fertility preservation, should be available to all patients.

Key words: Fertility Preservation; Neoplasms; Cryopreservation; Tissue Preservation; Antineoplastic Agents; Reproductive Techniques, Assisted; Oocytes; Drug Related Side Effects and Adverse Reactions

Introduction

The progress in the field of early diagnosis and cancer treatment in the past few decades has led to a significant decrease in mortality rate of cancer patients, whereas the quality of their lives has significantly deteriorated. Taking into consideration that cancer treatment requires aggressive methods, which are exceptionally difficult for patients and affect their social and emotional lives and behavior, the focus is on preserving the overall integrity of the person, rehabilitation and return to their families and social life [1].

Despite the increase of long-term survival of cancer patients, cancer treatment, including chemotherapy, radiotherapy and surgical treatment methods, can contribute to a decrease of fertility in both genders. Considering the increasing number of surviving young patients, there is a need to introduce the term - oncof-

Sažetak

Uvod. Napredak na polju lečenja karcinoma doveo je do povećanja stope preživljavanja kod onkoloških pacijenata. Posledica primene radio i hemioterapije kao i hiruškog lečenja je smanjenje fertitilne sposobnosti kod oba pola nakon sprovedenog tretmana lečenja.

Očuvanje fertiliteta. Među dostupnim tehnikama za očuvanje fertiliteta izdvajaju se krioprezervacija gameta i embriona kao efikasne i bezbedne za primenu u kliničkoj praksi dok se krioprezervacija tkiva gonada smatra eksperimentalnom metodom i za sada se ne primenjuje u rutinskoj praksi. **Zaključak.** Blagovremene i kompletne informacije o uticaju onkološke terapije na fertilitet, kao i informacije o mogućim tehnikama za očuvanje fertiliteta moraju da budu dostupne svim pacijentima.

Ključne reči: očuvanje fertiliteta; karcinomi; krioprezervacija; prezervacija tkiva; antineoplastički agensi; asistirane reproduktivne tehnike; oociti; nuspojave i neželjene reakcije izazvane lekovima

ertility, which means preservation of fertility in cancer patients after treatment [2].

Infertility induced by cancer treatment decreases the quality of life of surviving patients in the reproductive age, and leads to a long-term emotional stress, especially among patients who did not get relevant information about the possibilities and ways of fertility preservation before treatment initiation [3].

The aim of this paper is to present current options and techniques for fertility preservation in cancer patients.

The Effects of Cancer Treatment on Fertility in Women

Cancer treatment may affect the reproductive potential of women through various mechanisms. In order to get pregnant naturally, women must have a normal ovarian reserve, a functioning hypotha-

Abbreviations

FSH	– follicle-stimulating hormone
DNA	– deoxyribonucleic acid
MOPP	– mechlorethamine, oncovin, procarbazine, prednisone
AC	– adriamycin, cyclophosphamide
GnRH	– gonadotropin-releasing hormone
COS	– controlled ovarian stimulation
OHSS	– ovarian hyperstimulation syndrome
cIVF	– conventional <i>in vitro</i> fertilization
ICSI	– intracytoplasmic sperm injection
ASRM	– American Society for Reproductive Medicine

lamic-pituitary-gonadal axis, a functional uterus capable of fetal implantation and development, as well as the capacity of other organs and the cardiovascular system to respond to the changes caused by pregnancy. A malfunction of any of the mentioned systems after cancer treatment can render natural conception and pregnancy impossible.

An accurate assessment of the effects of cancer and its treatment on fertility in women cannot be determined in a simple manner, considering that it depends on: the type and stage of the disease, treatment modality, and patient age, number of treatments and cumulative dose of radiation and chemotherapy, basal ovarian reserve and many other factors [4].

The Effects of Chemotherapy

The mechanism of impairment of the ovarian function caused by chemotherapy involves the decay of primordial follicles, disruption in the maturation of recruited follicles, or a combination of these two mechanisms, depending on the class of drug used. The degree of impairment of the ovarian function depends on the drug dose, and considering that most agents disrupt cell division, the damage occurs to the oocytes in the stage of maturation, and to somatic granulosa cells in the stage of cell growth and proliferation [5]. The impaired ovarian function is characterized by higher levels of follicle-stimulating hormone (FSH) (≥ 20 IU/L) and estradiol (>75 pg/ml), while the levels of inhibin B and anti-Mullerian hormone are decreased. Ultrasonography shows a decrease in the antral follicle count and ovarian volume itself [6].

Alkylating agents are a class of drugs used in chemotherapy and their gonadotoxicity has been well-studied. Their effect is related to deoxyribonucleic acid (DNA) damage which leads to breaking the process of DNA transcription and replication, later manifested through cell cycle arrest. Alkylating agents also lead to vascular damage and ovarian cortical fibrosis, which is another mechanism that leads to ovarian dysfunction. Most agents used in chemotherapy are not used independently, but as a part of joint protocols, so one of the approaches in assessing the gonadotoxic effect of these agents is to analyze the effect of a comprehensive treatment protocol on the ovarian function. The mechlorethamine, oncovin, procarbazine, prednisone (MOPP) therapy, used in lymphoma treatment, causes amenorrhea in up to 80% of female patients

and acute ovarian failure in 39–46% of young adults [7]. Newer regimens such as doxorubicin, cyclophosphamide (AC) have recorded a significantly lower amenorrhea rates, in 55% patients [8]. The effects of biological agents (tamoxifen, herceptin, etc.) on fertility, as a relatively new class of cancer drugs, which are directed towards specific receptors, growth factors or signalling pathways, are still insufficiently tested.

The Effects of Radiotherapy

The degree of ovarian damage is directly associated with the proximity of the ovary to the radiation field, the basal ovarian reserve, and the dose of radiation. Also, certain high doses of radiation lead to a higher degree of ovarian damage compared to fractionated, lower doses, even when the cumulative radiation dose is the same.

Ovarian failure has been reported in 97% of girls after total body irradiation with 20–30 Gy, while in adults, doses of 10–15.75 Gy cause the same effect in 90% of cases [9]. There are numerous consequences of radiation on the uterus, which can lead to infertility. A direct exposition of the uterus leads to reduced vascularization, endometrial insufficiency, and even fibrosis of the myometrium with consequential disruptions of implantation and gestation. A radiation dose higher than 25 Gy, used in children, induces irreversible damage to the uterus in treated patients. Reulen et al. reported an increased rate of miscarriages, premature births and intrauterine growth restriction after abdominal and pelvic radiation treatment [10]. The use of high doses of cranial radiation can also induce hypogonadism by disrupting the function of the hypothalamus and pituitary gland. Unlike the previously mentioned damage to the ovaries and uterus, which is irreversible, infertility occurring as a consequence of that type of radiation can be successfully treated with hormone substitution therapy [11].

Surgical treatment can jeopardise the anatomic functionality of the female genital tract by injuring or removing the reproductive organs. Lately, there is a tendency to use less radical surgical approaches in patients of reproductive age [12].

Fertility Preservation Techniques in Women

The following techniques of fertility preservation are nowadays used in female cancer patients: cryopreservation of embryos and ova, cryopreservation of ovarian tissue, protective hormonal suppression of ovaries, gonadotropin-releasing hormone (GnRH) analogues and antagonists, and ovarian transposition in radiation therapy. When choosing the technique for fertility preservation, the matters to be considered include the age of patients, whether they have a partner, and the time available before the initiation of therapy.

Cryopreservation of Embryos

Cancer patients of reproductive age who need to be submitted to some treatment procedures which

can lead to early ovarian dysfunction and decline of fertility, are candidates for cryopreservation of ova and embryos.

As a technique routinely used in assisted reproductive technologies, cryopreservation is a widespread method of fertility preservation in cancer patients. After the first successful pregnancy in 1983 [13], the perfection of cryopreservation technology has today approximately equalized the rate of live births after frozen embryo transfer and embryo transfer in fresh *in vitro* fertilization (IVF) cycle [14].

The rate of embryo viability after thawing, the rate of implantation, and the rate of clinical pregnancies after frozen embryo transfers is not significantly different among cancer patients compared to the cycles of patients who are not facing cancer. In a retrospective study, Cardozo et al. compared the pregnancy rate in cancer patients after frozen embryo transfer with the pregnancy rate in patients with tubal factor infertility in the IVF process. The cumulative pregnancy rate after frozen embryo transfer in cancer patients was 37%, and 43% in the control group. The rate of births per embryo transfer also showed no significant deviations, 30% compared to 32% in the control group [15].

The process of cryopreservation of embryos implies the existence of a partner or a sperm donor sample depending on the legislation of the state in question. Also, this procedure is reserved only for the patients whose medical state allows for a safe implementation of ovarian stimulation. Ovarian stimulation requires certain time for follicles to develop, and it is the doctor's duty to assess the safety of postponing the start of treatment of the given patient. The process involves a controlled ovarian stimulation, aspiration of oocytes, IVF and freezing of embryos.

Protocols with GnRH antagonists are most often used for controlled ovarian stimulation (COS) considering that this type of protocol requires less time and bears a lower risk for the development of the ovarian hyper-stimulation syndrome (OHSS). The protocol starts on the second or third day of the menstrual cycle by administration of gonadotropin, while the dose depends on the ovarian reserve. The development of follicles is followed by a series of transvaginal ultrasound folliculometry procedures, and when follicles achieve the desired size, GnRH antagonists are administered in order to prevent a premature peak of the luteinizing hormone. After administration of the human chorionic gonadotropin or synthetic luteinizing hormone, the final maturation of oocytes is initiated, and transvaginal aspiration of oocytes is conducted in 35 – 36 hours [14]. The fertilization of oocytes is conducted in IVF conditions using the method of conventional *in vitro* fertilization (eIVF) or via the intracytoplasmic sperm injection (ICSI). After the embryo formation, it is further cultivated in controlled laboratory conditions, followed by cryopreservation and storage. Embryo cryopreservation is performed using the slow freezing or the vitrification technique.

If the treatment of the primary disease cannot be postponed, modified protocols are used with administration of GnRH antagonists, regardless of the phase of the menstrual cycle [16]. Antiestrogens (tamoxifen and letrozole) are induced to stimulate ovulation in patients with estrogen-dependent cancers, considering that they decrease the risk of exposure to high estrogen concentrations.

Cryopreservation of Oocytes

The cryopreservation of oocytes is a convenient method for fertility preservation in sexually mature women, women without a partner, or women who refuse embryo cryopreservation because of ethical, religious or other reasons.

The first successful pregnancy resulting from an IVF cycle with frozen and then thawed oocytes by using the slow freezing method was achieved in 1986 [17]. In the following decades, achievements in the field of cryobiology have enabled significant improvements in the field of cryopreservation of oocytes. The introduction of the vitrification technique, which includes the process of solidifying liquid to a noncrystalline, "glassy" phase, which is achieved through rapidly lowering the temperature below the temperature of glass transition, while at the same time increasing the viscosity in order to prevent intracellular formation of ice crystals, enables a safe freezing of the very sensitive oocyte structure. A combination of enhanced cryoprotectants and fertilization via the ICSI method has led to more than 1,500 children being born in cycles with cryopreserved oocytes [18].

Studies have shown that there is no increased risk of aneuploid embryos after oocyte cryopreservation, nor an increased rate of children born with congenital anomalies [19, 20]. The oocyte survival rate after thawing is 90–95%, while the rate of pregnancies per embryo transfer is 38% in a cycle with frozen oocytes. The percentage of pregnancies in cycles with fresh embryos is 45%, according to the same study [21]. Owing to this progress, according to the American Society for Reproductive Medicine (ASRM), cryopreservation of oocytes is no longer considered experimental, but used as a standard method of fertility preservation in cancer patients [22]. The protocols of ovary stimulation are no different than protocols used when it comes to embryo cryopreservation, while oocytes in metaphase II stage are subjected to cryopreservation in laboratory conditions.

Cryopreservation of Ovarian Tissue

Cryopreservation of ovarian tissue is a technique of freezing the ovarian cortex, which is rich in primordial follicles. This technique of fertility preservation is advised for prepubertal girls as well as adult patients who need to start their treatment right after being diagnosed with cancer. The advantage of this technique is that it does not require a partner, like embryo cryopreservation, the start of treatment and ovarian stimulation are not postponed, a big pool of primordial follicles can be preserved, and

after the transplantation, ovarian function can be re-established [23].

Ovarian tissue needed for cryopreservation does not require previous ovarian stimulation, and it can be provided soon after cancer has been laparoscopically diagnosed. The obtained tissue is cryopreserved after dissection in small fragments, either through slow freezing technique or the vitrification technique in laboratory conditions [23].

When the cancer treatment is finished, after thawing the ovarian cortex tissue, an orthotopic or heterotopic transplantation is possible. Orthotopic transplantation involves transplantation in the pelvis area, while in heterotopic transplantation the tissue is transplanted outside the pelvis area, most often on the forearm or in the abdominal region. Orthotopic transplantation is a successful way of re-establishing fertility, and numerous cases of live births were recorded after spontaneous pregnancy and after hormonal ovary stimulation and IVF [24]. The first pregnancy after heterotopic transplantation of ovarian tissue was achieved in 2013 by Stern and associates [25].

Apart from the above mentioned advantages, ovarian tissue cryopreservation also bears risks of reintroducing cancer cells after autotransplantation. Many types of cancer do not metastasize in the area of ovaries, but autologous transplantation is contraindicated if there is a possibility of cryopreserved tissue containing cancer cells, as with hematological malignancies.

According to current recommendations of the ASRM, this method of fertility preservation remains experimental, and is not routinely used in practice [26].

Ovarian Transposition

Ovarian transposition is a surgical relocation of the ovary outside the radiation field in case radiotherapy is used directly in the area of the pelvis [4]. The ovarian transposition procedure can be done laparoscopically, or, if needed, through laparotomy. During the selection of patients for this procedure, it should be considered if the uterus will be exposed to high radiation doses (14–30 Gy), because such doses cause high and permanent damage to the uterus function [9]. Since this procedure cannot reduce the gonadotoxic effect of chemotherapy, its use should be limited to patients whose treatment protocols do not require a combination of radiotherapy and chemotherapy. Ovarian transposition may hamper the transvaginal approach during oocyte aspiration in the process of IVF, and if transabdominal oocyte aspiration is not an option, the ovary needs to be repositioned before using some of the methods of assisted reproduction.

Immature Oocyte Retrieval and In-Vitro Oocyte Maturation

This method involves aspiration of immature oocytes without controlled ovarian stimulation, or with minimal stimulation. It is used in patients in whom postponing cancer treatment would negative-

ly affect the success of treatment, and for prepubertal patients. After *in vitro* maturation of oocytes, the mature cells are cryopreserved or fertilized, and then the embryo is cryopreserved through previously described techniques. In addition to transvaginal aspiration, oocytes can also be collected from ovarian tissue, which was removed in order to cryopreserve it. *In vitro* maturation of oocytes is nowadays successfully used in patients who are diagnosed with polycystic ovary syndrome and in patients who are candidates for developing the OHSS in IVF programs with a high rate of clinical pregnancies [27].

Hormonal Ovarian Function Suppression

Ovarian suppression by GnRH analogues or antagonists is used before or during the administration of gonadotoxic chemotherapy in order to reduce the risks of premature ovarian function failure. GnRH-agonists cause a suppression of the pituitary function and secretion of gonadotropins, and decrease utero-ovarian perfusion, so it is considered that these mechanisms can protect the deterioration of ovarian reserve. The ovarian suppression is debatable because there are not enough random studies to confirm the efficiency of this method for the preservation of ovarian reserve and fertility. Even if some studies do show a much higher percentage of establishing the menstrual cycle in patients co-treated with GnRH-agonists during chemotherapy, there is no evidence that the pregnancy rate has increased, which is the ultimate goal of successful fertility preservation after treatment [28].

The results of recent researches on using GnRH analogues in women with cancer are controversial, and there is no definitive consensus on their use.

Surgery and Fertility Preservation

Less radical surgical cancer treatment techniques, intended to preserve reproductive organs, are contemporary and mostly still being developed. In young patients diagnosed with early-stage cervical cancer, a radical trachelectomy can be conducted instead of standard radical hysterectomy. Complications were recorded after this procedure, such as cervical stenosis and premature birth, but increased cancer recurrence rate was not recorded [12]. In early-stage endometrial cancer, a hormonal progestogen treatment is used, but the recurrence rate is high, and the patient requires frequent controls with endometrial biopsy, and definitive treatment after giving birth [29].

The Effects of Cancer Treatment on Fertility in Men

The etiology of male infertility after cancer treatment involves direct and indirect effects of cancer, as well as damages occurring after surgical treatment, radiotherapy and chemotherapy. The treatment itself involves a combination of the mentioned methods, and there is a decrease or complete multifactorial lack of fertility in conjunction with the type, dose and duration of the therapy, as well as the fertile capacity of the patient before the treatment [4].

Affected semen analysis parameters are often found in cancer patients as a primary issue, before therapy is conducted. The tumor secretion of metabolically active substances, such as cytokines, can cause direct damage to the germinal epithelium, while hormonally active tumors can have an indirect effect on spermatogenesis by disrupting the interaction between the hypothalamus and pituitary gland [30]. Among the various types of cancer (leukemia, lymphoma, and testicular cancer) which affect spermatogenesis as the primary illness, the prominent ones are the testicular germinal epithelium tumors, having the greatest negative effects on spermatogenesis and male fertility [31].

The Effects of Chemotherapy

The primary effect of chemotherapy on fertility is the direct damage to the spermatogonia, which is the most sensitive stage of spermatogenesis. Sertoli cells and Leydig cells show a lower degree of sensitivity, and the gonadotoxic effect of chemotherapy is reflected in a low sperm count, low motility, distortion of morphology and DNA integrity, while the production of testosterone may remain preserved after treatment [32]. The extent of damage depends on the type and dose of drugs, manner of administration (oral or intravenous), duration of treatment and the patient's age. Among chemotherapeutics, the drugs with the highest gonadotoxic effect are alkylating agents (cyclophosphamide, chlorambucil, procarbazine, busulfan) and platinum agents (cisplatin and carboplatin) [33].

The Effects of Radiotherapy

The effects of radiotherapy on male fertility also depend on the dose. A dose of 0.15 Gy, used on the testicles, leads to a decrease in the sperm count, while a dose of 2.5 Gy can cause prolonged or permanent azoospermia. If the radiation dose exceeds 20 Gy, the germinal epithelium is completely destroyed and consequently the patient becomes permanently sterile [34]. It should be noted that, in addition to direct radiation of testicular tissue, the indirect exposure of testicles to radiation is also fatal to spermatogenesis. Due to cranial radiation with doses exceeding 35 Gy, the function of the pituitary gland is disrupted, which can lead to secondary hypogonadism [35]. Spermatogenesis arrest can be reversible, but radiotherapy causes DNA damage, which should be considered if the patient will be subjected to some of the assisted reproduction techniques.

Surgical treatment of cancer can lead to damaging the secretory canals of the genital tract, and it can also lead to erectile dysfunction. Unilateral orchiectomy of testicular cancer can decrease sperm production, while bilateral orchiectomy leads to permanent male infertility [4].

Fertility Preservation Techniques in Men

Semen Cryopreservation

Semen cryopreservation is the most commonly used technique for fertility preservation in pubertal and post-pubertal males. Due to the progress in the

field of assisted reproduction and to the introduction of intracytoplasmic sperm injection, sperm cryopreservation, as a method of fertility preservation is especially important for cancer patients because only one sperm is enough to fertilize an egg and the patient may become a biological father.

The manner of collecting the sample for cryopreservation depends on the level of integrity of the reproductive tract of the given patient. If there is no anatomical or neurological disparity, a sample of ejaculate is provided by ejaculating after 2 – 5 days of abstinence. Two or three ejaculate samples are collected, mostly because of low referential values of proper semen caused by the cancer. The samples need to be cryopreserved before the treatment, in order to preserve DNA integrity and sperm quality [36]. Cryopreservation is done by vitrification method, or slow freezing method, after which the samples are stored until further use. Due to the urgency of treatment, after the patient is diagnosed with cancer, cryopreservation can be conducted during the treatment, considering that the lowest sperm count in the ejaculate is recorded 4 – 6 months after the therapy ends [35].

If the patient has anatomical, neurological or other changes which disrupt ejaculation, the sperm sample can be collected via: vibrostimulation, electroejaculation, catheterization and by isolating sperm cells from the urinary bladder in the case of retrograde ejaculation, as well as aspiration or surgical extraction from the testicles or epididymis if spermatogenesis is preserved [37].

Hormonal Suppression

The effects of hormonal gonadal suppression during cancer treatment have not been studied enough. Recent studies have not shown positive effects of hormonal suppression on lowering the risk of infertility after cancer treatment. Improvements in re-establishing spermatogenesis after chemotherapy have also not been recorded [36].

Cryopreservation of Spermatogonia and Testicular Tissue

Currently available fertility preservation techniques are only used in pubertal and postpubertal young men in whom spermatogenesis has started. When it comes to boys in whom there was no initiation of spermatogenesis, the possibilities of fertility preservation are limited to cryopreservation of testicular tissue. The cryopreservation of testicular tissue is still considered experimental, considering that cryopreserved testicular tissue cannot be used in clinical practice at present [4]. Initial studies on animals showed encouraging results, and there is hope that in the future, as technology in the field of reproductive medicine advances, cryopreserved testicular tissue will find its use in assisted reproductive technologies [38]. Excluding the technical limitations for obtaining mature, functional sperm cells from cryopreserved, immature testicular tissue, there is also fear of reintroducing malignant cells to the body after the transplantation.

Conditions for Implementing Fertility Preservation Programs in Cancer Patients

According to the ASRM, a comprehensive program for fertility preservation includes:

- *Quick access* of patients to doctors.
- *Interdisciplinary medical team* comprising an oncologist, a reproductive endocrinologist, urologist, and a specially trained surgeon for conducting surgical techniques for fertility preservation.
- *Laboratory requirements* in fertility preservation programs include all techniques for fertility preservation, with an emphasis on successfully established programs of cryopreservation of gametes and embryos, which are constantly available. In addition, the program should provide adequate counselling for prepubertal patients, and offer the possibility of performing testicular and ovarian tissue cryopreservation.
- *A clinic* with experts in the field of genetics, mental health and finance, considering that many fertility preservation techniques are not covered by health insurance.
- *Interdisciplinary cooperation* between oncologists, surgeons, reproductive endocrinologists and urologists is of crucial importance. The oncologist is obliged to inform the patient about the risks cancer treatment poses to future fertility, while the reproductive endocrinologist and urologist should provide information about the options for fertility preservation. Interdisciplinary cooperation is crucial for determining the optimal strategy and the length of using fertility preservation techniques, with mandatory individual approach to each patient.

Patients in the fertility preservation programs should be informed about all available techniques for preserving their own gametes and embryos, about programs of donating gametes, embryos, surrogacy and adopting children in accordance with the legislation in cases when the use of mentioned techniques is not possible due to the primary disease.

In patients who decide to cryopreserve gametes, embryos and gonadal tissue, options of further use or destruction of the reproductive material should be considered in case of death, and these decisions should be appropriately documented.

Ideally, if the time from making the diagnosis to cancer treatment allows, the patient should talk to and get advice from medical experts in the mentioned fields on several occasions in order to choose the appropriate technique for fertility preservation. This approach allows a comprehensive evaluation and understanding of psychosocial and medical needs of each patient [4].

Conclusion

Progress in the field of cancer treatment has led to a long-term survival of patients, and fertility preservation in such patients is an imperative. Considering the influence of the possibility of having children on the quality of life of reproductive age patients, all cancer patients should be informed about the available techniques for fertility preservation and about the potential effects of cancer treatment on their fertility before the treatment protocol starts. Raising the awareness about the importance of preserving fertility requires a multidisciplinary approach.

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