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Cryopreservation of Gametes, Embryos and Ovarian Tissue as a Method for Fertility Preservation in Oncological Patients

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Cancer is the second most common cause of death after the diseases of the cardio-vascular system. Due to the modern complex treatment methods, there are increasing survival rates of oncological patients. That puts forward the question for the quality of life of the recovered and respectively, their ability to have children. In large number of cases, the chemo-/radiotherapy leads to damage to oogenesis and spermatogenesis. For that reason, the patients are offered fertility preservation solutions before the start of the anti-tumour therapy. The basic approaches to fertility preservation in patients with pending chemo- or radiotherapy are presented in the current review.

Key words: fertility preservation, cryopreservation, gametes, embryos, ovarian tissue

Introduction

Worldwide, cancer diseases are the second most common reason for death after the disorders of the cardio-vascular system. At the same time, mortality is constantly decreasing, due to the contemporary complex treatment methods, and in a number of cases there is full recovery. For example, the five-year survival rates of the patients with haematological cancers, breast carcinoma, etc. is often above 90%. That brings up the question about the quality of life of the recovered and respectively, their ability to have children. It is known, that in many cases the chemo-/radiotherapy leads to damage of the gonadal functions – the oo- and spermatogenesis. Therefore, it is important that patients are offered options for their fertility preservation prior to the start of the anti-tumour treatment. The advances in the modern science of cryobiology provide opportunities for successful freezing of reproductive cells and tissues, which later can be used for transplantation or in the assisted reproductive technologies (ART).

Approaches for fertility preservation in male patients

Spermatogenesis starts in puberty and continues almost through the whole life of males. The duration of 1 complete cycle is a little more than 2 months, with 90 – 200 mln spermatozoa forming each day [3, 14]. Different disorders and exogenic factors influence the process and the quality of the resulting gametes. It is known that chemo- and radiotherapy in oncology have a negative effect: the quality of the semen deteriorates; the morphology worsens; DNA-fragmentation increases; the number of the spermatozoa is reduced and in some cases azoospermia is observed [11]. Surgery is also applied in certain types of cancer, sometimes with removal of the reproductive organs: orchiectomy, prostatectomy, radical cystectomy. The hormonal medication for prostate tumours effects the production of spermatozoa as well [2]. After treatment, many patients show remission or full recovery, but have lost or impaired reproductive abilities [7]. That suggests that fertility preservation opportunities should be offered to them before the beginning of the treatment. The cryopreservation of semen is most often used. The technology is well-established and routinely applied in the ART clinics. The semen is obtained by masturbation after 2 to 5 days of abstinence from sexual intercourse, and after liquification is diluted with cryopreservation medium and frozen with programme freezer or on liquid nitrogen vapour. Ready-to-use media are available on the market, with proven efficacy. The introduction of the vitrification method in clinical practice allows for the successful cryopreservation of low quality samples (insufficient number of spermatozoa) and gametes directly aspirated from the testes [13]. The storage period of the frozen semen (in liquid nitrogen, or its vapour or in low-temperature freezers) is practically indefinite.

The cases of pre-pubertal oncological patients, in which the spermatogenesis has not been initiated, are more challenging. The only available option for them is the cryopreservation of testicular tissue. Research shows that it contains spermatogonial stem cells [8]. Data exists, that after autologous transplantation of the frozen tissue the spermatogenesis can be recovered [6]. It is important to be noted that the method is still experimental and far from introduction into clinical practice. Protocols for collecting and cryopreservation of testicular tissue have been developed, but the techniques for the subsequent obtaining of spermatozoa from the thawed tissue are at research stage [5, 14].

Methods for fertility preservation of female patients

Different fertility preservation approaches are applied in women: selection of less invasive chemotherapeutics, ovary transposition, cryopreservation of reproductive cells and tissues. The strategy in every patient is individual and depends on her age, type and stage of the cancer, therapeutic plan, expected long-term results of the treatment, possibility to postpone the start of the therapy, presence of a partner of the patient, biology of the tumour and the potential risk of metastasis in the ovaries, etc [6]. In major part of the cases, cryopreservation of oocytes or zygotes/embryos is offered to the patients, and to lesser extent – of ovarian tissue. The main advantages and drawbacks of the methods are presented on **Table 1.**

Table **1.** Main strategies for fertility preservation in female patients.

Strategy	Methodology description	Applicable in young children	Hormonal stimulation needed	Preservation of the ovarian functions	Limitating conditions
Embryo cryopreservation	Collection of oocytes, in vitro fertilization and freezing of the obtained embryos	No	Yes	No	10 – 14 days of stimulation; presence of a partner or donor sperm; price of the procedure; ethical problems in case of deceased patient
Oocyte cryopreservation	Pick-up and cryopreservation of unfertilized oocytes	No	Yes	No	10 – 14 days of stimulation; price of the procedure
Ovarian tissue cryopreservation	Freezing of ovarian tissue and transplantation post-treatment	Yes	No	Yes	Invasive procedure; risk of tumour cells transmission; price of the procedure

Some authors suggest a combination of approaches to preserve fertility in certain groups of patients, for example the simultaneous freezing of oocytes and ovarian tissue [1].

Oocyte Cryopreservation

The oocytes are a comparatively difficult object for cryopreservation due to their biological characteristics (size of the cell, high cumulative mass, presence of zona pellucida, membrane structure, the presence of the meiotic spindle, structure of the cytoskeleton) [6]. Unlike the freezing of spermatozoa and cell cultures, where the loss of certain quantity does not affect the outcome, the oocytes' number is limited, each of them is cryopreserved individually and the process should be approached carefully. It can be said that it is "all or nothing" principle. On the other hand, the recent developments in modern cryobiology and ART provide the techniques to successfully freeze and fertilize the female gametes. After the birth of the first baby in 1986 [4], worldwide at the moment hundreds of thousands of children have been born from cryopreserved oocytes [15]. On the market, cryobanks with frozen donor female gametes are functioning and there are ready-to-use media and devices for their cryopreservation. The freezing is performed by vitrification, and the fertilization with intracytoplasmic sperm injection (ICSI). The results in terms of fertilization and pregnancy rates after use of thawed oocytes practically do not differ significantly from fresh ones. Unfortunately, in certain groups of women – pre-pubertal girls, oncopatients to whom the hormonal stimulation is contraindicated etc. this approach is unsuited (**Table 1**).

Cryopreservation of pre-implantation embryos

In cases when the hormonal stimulation can be safely performed, the most successful method for fertility preservation is the freezing of pre-implantation embryos. It has to be noted, however, that it is applicable if the patient already has a partner or is agreeing to the use of donor sperm. The technology consists of the collection of oocytes, their in-vitro fertilization (IVF) and cryopreservation of the obtained embryos. They can be stored and after the finishing of the anti-tumour treatment thawed and transferred in the patient's uterus to achieve pregnancy. The method is well-established and routinely used in ART procedures. Two cryopreservation techniques are applied – programme freezing and vitrification, both showing good results. Again, this method cannot be used in young girls. Also, in certain countries, legal restrictions are forbidding the cryopreservation of embryos [12].

Cryopreservation of ovarian tissue

This technique can be applied in pre-pubertal girls or in patients with contraindication for hormonal stimulation. The establishment of ovarian tissue cryobanks is based on the principle of its cryoresistance. The ovarian cortex contains thousands of follicles, which unlike oocytes, can be frozen comparatively easy. Their relatively slow metabolism rate, the absence of zona pellucida and meiotic spindle are in the basis of the higher cryoresistance of primordial, compared to the growing follicles. Additionally, their small size facilitates the faster penetration of the cryoprotective agents.

The methodology includes laparoscopic collection of the ovarian tissue, which is cut into small fragments and cryopreserved most often with programme freezing with slow cooling rates. After thawing, the ovarian pieces are transplanted (ortho- or heterotopicly). When the aim is to have a natural ovulation and conceptus, orthotropic transplantation is performed. The fragments are placed abdominally, close to the fallopian tube or to the remaining part of the ovary. In the heterotopic, the ovarian tissue is transplanted subcutaneously in the abdomen, in the hand between the elbow and the wrist, or other well-vascularized places. Practically, this is a less demanding procedure, but pregnancy can be achieved only after follicle puncture and IVF. Also critical is the fact that the women regain not only their reproductive functions, but their hormonal status as well [9]. Before cryopreservation, it is important to examine the tissue for the presence of tumour cells, to avoid their possible retransmission. Another approach is to culture the thawed primordial follicles, and fertilize *in vitro* the developed oocytes. Besides, the hypothesis for the presence of ovarian stem cells in the ovary is gaining further confirmation among scientists. Their possible differentiation into oocytes in the post-natal period is another consideration for freezing of ovarian tissue [10]. Up until now, over 300 babies have been born worldwide after cryopreservation of ovarian tissue.

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