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Modern aspects of fertility preservation in cancer patients

Achievements of modern oncology have increased the number of young cancer patients of fertile age who able to be cured completely from cancer. Aggressive chemotherapy and radiotherapy increases the long-term survival rate but a result is an ovarian failure and subsequent loss of fertility. When cancer is diagnosed, patients and oncologists generally focus on treating the disease. As a result, the critical question of fertility preservation often remains unasked or unanswered. If it happens, the cancer patients may miss the only opportunity to preserve their ability to have a biological child. Fertility preservation should be an integral part of improving the quality of life of cancer survivors.

Keywords: *cryopreservation, in-vitro fertilization, oocysts, intracytoplasmic spermatozoon injection, cryoprotectant.*

The recent success in the treatment of cancer has led to a significant increase in the long-term survival of patients and, therefore, to an increased attention to the problem of improving the quality of life after the successful completion of cancer treatment. In this regard, an important aspect is the preservation of fertility. Taking into account the known reproductive risks of cancer treatment, there is a growing interest in the field of fertility preservation (also referred to as onco-fertility) [1].

Cryopreservation of immature oocyte or ovarian tissue is no longer considered an experimental direction of medicine and becomes a reality for women of reproductive age before beginning treatment of cancer. In addition, social cryopreservation of oocytes of healthy women without cancer, which wants to delay childbearing, is becoming more common. Currently, the number of patients who have had any cancer is more than 10 million. The proportion of young people with newly diagnosed cancer is increasing annually. Among all oncological diagnoses, 15% falls on patients younger than 40 years.

The use of aggressive chemotherapy (CT) and radiotherapy in young cancer patients led to an increase in the duration of their life, but on the other hand, this treatment often causes infertility due to massive damage to germ cells, which leads to the depletion of the ovarian reserve and premature menopause in women [2] and the violation of spermatogenesis in men. According to statistics, 60% of patients would like to have their own children in the future [3].

Ionizing radiation has a negative effect on the function of the sex glands, regardless of the age of the patients. The degree and prevalence of the defeat of the reproductive system depends on the dose received, the area of irradiation and the age of the patient. It is known that the older woman has the higher the risk of damage to the follicles [4]. This is probably due to the fact that young patients have a larger ovarian reserve, which decreases with age due to atresia of primordial follicles. The effect of all CT drugs is based on interrupting the life processes of the cell and delaying normal cell proliferation. Often, CT agents are used in combination because of their synergistic effects, but this also leads to an additional increase in their adverse effects on the body, in particular, the follicular apparatus of the ovaries. In animal experiments, D. Meir and co-authors [5] found that the presence of regular menstruation and pregnancy after CHT is not always an indicator of a preserved ovarian-follicular reserve. The authors also found that patients with ovarian malnutrition due to the use of high-dose CT or radiation therapy (RT) should not postpone the birth of the child to the distant future. Such patients should think about the possibility of pregnancy within a few years after treatment during the stabilization process, but not earlier than 6-12 months because of possible toxic effects of therapy on growing oocytes [6].

Currently, assisted reproductive technologies (ART) allow the patient to save fertility. In this regard, the fundamental importance is the professional cooperation of oncologists, gynecologists, pediatricians, surgeons, specialists in ART, immunologists and endocrinologists. Joint work is necessary to select a personalized option for preserving the reproductive function for a particular patient before starting treatment for the underlying disease. There are several ways of maintaining reproductive function in women: transposition of the ovaries from the irradiation zone, hormonal suppression of ovarian function using gonadotropin-releasing hormone (GnRH-a) agonists, use of apoptosis inhibitors, cryopreservation of embryos, cryopreservation of oocytes with stimulation or in the natural cycle, cryopreservation of tissue ovary, as well as the creation of artificial oocytes by cloning, i. e., transplantation of the somatic cell nucleus into the donor oocyte [7, 8].

Patients undergoing gonadotoxic radiotherapy, transposition of the ovaries from the irradiation region may partially support the ovarian function. This is indicated with Hodgkin's lymphomas, cervical cancer, vagina, rectum and small pelvis sarcoma. The dose of irradiation attributable to the ovaries after transposition is reduced by approximately 5-10% compare obtained for the ovaries in situ [9].

In experiments, it was found that the administration of a known apoptosis inhibitor-sphingosine-1-phosphate to mice treated with doxorubicin resulted in protection of oocytes from apoptosis [10], and also maintained fertility in irradiated female mice and did not cause any genome damage in the offspring [11]. Studies show that apoptosis inhibitors are promising agents, but they are still in a very early experimental stage. It is also known that most cytotoxic drugs act by activating apoptosis in tumor cells, therefore, the use of inhibitors of apoptosis can adversely affect the reduction of tumor mass. Further studies should explain the negative effect of the use of apoptosis inhibitors in combination with traditional chemotherapy drugs.

At present cryopreservation of embryos is the most promising and well-developed method of preserving fertility. The human embryo is very resistant to the damaging effects of cryoprotectants. The normal survival rates of embryos after thawing are in the range of 35-90% while the normal implantation levels are between 8% and 30%. If several embryos undergo cryopreservation, the cumulative index of pregnancies may exceed 60% [12]. The frequency of transplantation of a frozen embryo on average is estimated as successful only in 18-20% of observations [12]. If one patient managed to obtain a large number of mature oocytes, then it is possible to perform several attempts to transfer embryos. However, this approach requires the presence of a partner for the in vitro fertilization of the ovum. Cryopreservation of embryos is not acceptable for young girls and is contraindicated in patients with hormone-dependent forms of cancer, since hormonal stimulation of the ovaries is required to induce ovulation, maturation and subsequent collection of oocytes. Due to the use of modern ART methods, the male infertility factor remains an irresolvable problem only in very rare cases, when there is complete non-obstructive azoospermia. In such cases, frozen donor sperm can be used.

Collection and cryopreservation of oocytes are more problematic than cryopreservation of sperm or embryos. The first obstacle is the high sensitivity of oocytes to cooling, probably due to the high content of lipids in the cytoplasm and the vulnerable chromosome apparatus. The second: cooling, the formation of intracytoplasmic ice crystals and the addition of cryoprotectants damage the oocyte cytoskeleton and may increase the number of aneuploidies in human oocytes [13]. Incubation with cryoprotectants also causes solidification of the glossy envelope of the oocyte, so all cryopreservation and thawing protocols include the subsequent introduction of a single spermatozoon into the egg, i.e. intracytoplasmic sperm injection, or the so-called ICSI (intracytoplasmic sperm injection) procedure as a measure of precaution and reinsurance. Fertilization in these cases should be performed in the range of 3 to 5 hours after thawing, while the oocyte remains fertile. The drawbacks of this method are that cancer patients have only a single opportunity to take oocytes before starting a potentially sterilizing treatment, as the cycle of induction of ovarian stimulation requires several weeks and delays the beginning of treatment of the underlying disease for several months, which is often critical. The success of the method also depends on the total number of collected oocytes. If the number of oocytes obtained is less than 10, then the probability of pregnancy significantly decreases [14].

In recent years a certain success has been achieved in the field of cryopreservation of human oocytes despite all the difficulties in fetching, freezing and fertilization of oocytes. Survival of oocytes after the freezing / thawing process is within 70-95%, which is comparable to cryopreservation of embryos and depends on the methods used. The results of cryopreservation of oocytes were improved due to a change in the environment. After the progressive method of instantaneous freezing of biological material was developed - vitrification, scientists began to obtain much better results of oocyte survival without the need for complex and expensive equipment [15].

The frequency of fertilization of frozen oocytes using ICSI is 70-90%, which is comparable to the percentage of fertilization of fresh oocytes. The incidence of pregnancy, obtained from frozen oocytes, varies from 10 to 40%. The main causes of variable success are differences in the cryopreservation technique and the use of toxic cryoprotectants. Clinically, cryopreservation of oocytes can be used to preserve fertility in women who have had cancer, in order to postpone the birth of a child and save oocytes for further use in IVF programs. Cryopreservation of oocytes can also be performed as a method alternative to cryopreservation of embryos, due to ethical prerequisites. However, not all cancer patients are shown hormonal stimulation in connection with the possibility of progressing tumor growth, and the fence of a single oocyte in the natural cycle is absurd. Thus, an alternative method of maintaining fertility is cryopreservation of ovarian tissue. The method of cryopreservation of ovarian tissue appeared relatively recently, in the early 1950s, and described as technically simple, quickly feasible and inexpensive. The idea of cryopreservation of ovarian tissue is based on the fact that the cortical layer of the ovary containing a large number of follicles is a protective coating for immature oocytes, and they are more resistant to cryoprotectants compared to mature cells, since they have an inactive metabolism [16]. The medical indications for cryopreservation of ovarian

tissue are practically the same as those for cryopreservation of oocytes and at the same time have fewer restrictions (any phase of the menstrual cycle, children's age) and greater potential for maintaining fertility. Ovarian tissue contains about 1000 primordial follicles (in girls up to 10 years old - in 3 mm3, at the age of 10-15 years - in 15 mm3, from 15 to 34 years - in 50 mm3) and the viability of follicles after freezing was demonstrated and thawing [18]. At the same time, there are many unresolved issues related to the choice as a cryoprotectant of a drug with minimal toxicity for immature oocytes, the choice of conditions for freezing and thawing of germ cells. A serious problem is also the choice of the technique of autologous ovarian tissue autotransplantation and an assessment of the risk of hormonal stimulation of ovulation in patients who have had a malignant oncological disease. The issues of further investigation of the technique of culturing immature oocytes in vitro after thawing of ovarian tissue remain topical. Currently, at least 100 births of children are known in the world as a result of the use of cryopreserved ovarian tissue, but the cryopreservation technique is still experimental and requires further testing in animal models.

Nao Suzuki and co-authors [19] analyzed the results of cryopreservation of ovarian tissue in 37 patients. In 54% of cases histological examination revealed residual follicles. In 9 out of 20 patients with the detected follicles, their growth was observed. After IVF, embryos were obtained in four cases, three were diagnosed as pregnant, one of which was spontaneously interrupted, and two women gave birth.

The method of cryopreservation of ovarian tissue is available for both mature patients and children. Ovarian tissue can also be obtained during any other surgical intervention, regardless of the menstrual cycle. The use of a progressive and highly effective method of vitrification of ovarian tissue, i.e. direct and instantaneous immersion of tissue in liquid nitrogen after impregnating it with cryo-solution, allows maximizing the freezing and preventing the formation of ice crystals that damage cells and tissues during slow freezing. The choice of a low proportion of the cryoprotectant in the freezing solution minimizes the toxic effect of this chemical agent on the follicles and surrounding cells and tissues.

Currently, the use of ART, in particular, cryopreservation of ovarian tissue, gives cancer patients an opportunity to maintain fertility, which is also extremely important for patients for psychological reasons [20, 21].

In connection with the obvious progress in the treatment of malignant diseases, the life expectancy of cancer patients is increasing. In these conditions, the main mission of doctors is to improve the quality of life of these patients, especially for cancer patients of reproductive age. It is well known that the complex treatment of cancer - a combination of aggressive CT and RT - leads to sterilization of both the female and male body. Therefore, the primary task is to preserve the gametal cells and tissues containing them before the treatment, at the stage of diagnosis. Before CT and RT, which can potentially lead to loss of fertility, it is necessary to take, cryopreserve and store material (ovules, spermatozoa, ovarian tissue) to use it in the future when the stage of remission occurs. Special equipment allows storing frozen material for 15-25 years at a temperature of -196 °C, which cannot be achieved in normal life. For cancer patients of reproductive age, it is necessary to create actively cans of germ cells and tissues. The use of modern ART methods will allow patients to find the joy of motherhood and paternity. However, the primary goal of the treating oncologist should remain an increase in the survival rate of cancer patients, and, secondly, the improvement of the quality of life of patients and care for their fertility. Decisions related to the preservation of fertility in children, adolescents and adults can be hampered by the difficulties in predicting cancer treatment, the time constraints for decision-making, and the lack of data on the effectiveness of various therapies. The most unique ethical problems arise in the pediatric and adolescent population. The decision to preserve fertility is difficult to accept for adult patients, but it is even more dramatic and contradictory for parents who make this decision with their children [22]. Young women and men with newly diagnosed cancer, as well as parents of children who will be exposed to CT or radiation for the treatment of cancer, should be aware of the possible negative impact of therapy on reproductive function. Informing patients is necessary so that each patient has the opportunity to maintain the function of gonads or gametes (spermatozoa, eggs or embryos) before the onset of aggressive therapy. It should also be admitted that most oncologists are not familiar with these issues or raise them in the treatment of patients. In such cases, it is advisable to consult with specialists in the field of reproductive medicine - reproductologists and andrologists.

Preservation of fertility should be an integral part of improving the quality of life of patients who have undergone oncological disease. Nevertheless, for many reason it is impossible and unethical to instruct all patients the same method of preserving fertility. Undoubtedly, the first and primary goal of any doctor is to cure cancer, even if this treatment causes infertility.

In April 2015, physicians from several European countries with experience in maintaining fertility in cancer patients were invited to Genoa (Italy) to participate in a seminar on "Cancer and the preservation of fertility." A total of ten controversial issues were discussed at the conference. The experts were invited to up-to-date literature reviews on the topics under discussion. It was announced that they would be encouraged to present their own unpublished data. For physicians recommendations were prepared, based on the data presented, as well as the experience of invited speakers to advise young patients interested in maintaining fertility.

International guidelines recommend that the risk of reproductive and infertility disorders from the disease and / or necessary treatment should be assessed as soon as possible in all patients of reproductive age. It is necessary to understand before the beginning of treatment whether patients have an interest in having children after curing cancer and help them in preserving reproductive function [22-24]. In accordance with the recommendations of the American Society of Clinical Oncology (ASCO) and the European Society of Medical Oncology (ESMO), cryopreservation of embryos / oocytes and semen cryopreservation are standard strategies for maintaining fertility in men and women after treatment for cancer has been treated [23, 24].

Thus, the problem of oncological fertility is becoming more urgent which undoubtedly requires special attention in cancer patients of reproductive age. The development of ART in the world is the best opportunity to solve the reproductive problems of patients who cured of cancer.

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