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RESUMEN

La tasa de supervivencia de los pacientes con cáncer está aumentando en los últimos años debido a tratamientos como la radioterapia y la quimioterapia. Este tratamiento también está afectando la capacidad reproductiva de las mujeres, provocando una falla ovárica prematura que lleva a una infertilidad temprana. La preservación de la fertilidad en las mujeres parece ser un tema importante, pero con el avance de las tecnologías de reproducción asistida existe la posibilidad de preservar la fertilidad en pacientes con y sin cáncer. Las opciones actuales de preservación de la fertilidad son métodos tradicionales como la criopreservación de embriones y ovocitos con nuevas tecnologías como la criopreservación y el trasplante de tejido ovárico. La criopreservación de ovocitos se utiliza para mujeres con enfermedades benignas, mujeres que buscan la fertilidad por motivos personales, religiosos y legales. Para las niñas prepúberes y las pacientes que no pueden retrasar los tratamientos contra el cáncer para preservar la fertilidad y la función endocrina ovárica, la criopreservación de tejido ovárico es la única opción. Este documento resumirá los principales métodos y avances en la protección y preservación de la fertilidad femenina.

PALABRAS CLAVE

Infertilidad femenina, preservación de la fertilidad, criopreservación de embriones, criopreservación de ovocitos, criopreservación y trasplante de tejido ovárico.

ABSTRACT

Survival rate of cancer patients is increasing in recent years due to treatments like radiotherapy and chemotherapy. This treatment is also impairing the reproductive ability of women, causing premature ovarian failure leading to early infertility. Fertility preservation in women has appeared to be an important issue but with advancement in assisted reproductive technologies there is a possibility of preserving fertility in cancer and non-cancer patients. Current fertility preservation options are traditional methods like embryo and oocyte cryopreservation with new technology like ovarian tissue cryopreservation and transplantation. Oocyte cryopreservation is used for women with benign disease, women seeking fertility for personal, religious and legal reasons. For prepubertal girls and patients who cannot delay anticancer treatments to preserve fertility and ovarian endocrine function, ovarian tissue cryopreservation is the one and only option. This paper will summarize the main methods and progress of female fertility protection and preservation.

KEYWORDS

Female infertility, fertility preservation, embryo cryopreservation, oocyte cryopreservation, ovarian tissue cryopreservation and transplantation.

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Introduction

With the continuous development of cancer treatment methods like chemotherapy, radiotherapy and hematopoietic stem cell transplantation (HSCT), the survival rate of cancer patients has improved. Radiotherapy and chemotherapy are like a double-edged sword, while killing cancer cells and saving patients' lives, it also irreversibly damages oocytes, resulting in infertility and early menopause among girls and women of childbearing age. Some benign diseases requiring radiotherapy and chemotherapy will also lead to the female infertility. It is predicted that the number of cancer cases in the world will increase to 28.4 million in 2040, and approximately 5.28 million new cancer cases will be diagnosed in China (1), and it is estimated that more than 2 million female patients need fertility protection and preservation every year in China. It is very important to provide timely fertility protection (FP) and preservation consultation for these patients and to select appropriate treatment methods (2). At the same time, fertility protection and preservation methods and technologies are developing rapidly, from traditional embryo cryopreservation and oocyte cryopreservation to new technologies like ovarian tissue cryopreservation (OTC) and oocyte in vitro maturation, hence bringing more choices and hopes to the patients. Cryopreservation is freezing cells and tissues at very low temperatures (–196°C to -170°C) for extended periods, using cryoprotective additives to prevent the ice formation (3). When the patient's disease treatment has an impact on fertility, the doctor has the responsibility to inform the patient about the method of fertility protection.

Fertility protection and preservation indications

- Malignant diseases: Women of reproductive age who need gonadotoxic treatment and HSCT, who have cancers such as hematological malignancies (Hodgkin lymphoma, non-Hodgkin lymphoma and leukemia), breast cancer and pelvic tumors. Malignant tumor is the most common indication for fertility preservation. Chemotherapy, radiotherapy, and surgery in tumor treatment can lead to iatrogenic premature ovarian insufficiency (POI). Ovaries are very sensitive to cytotoxic drugs, especially alkylating agents such as cyclophosphamide, which can lead to single strand and double strand DNA breaks, which damage oocytes (4). Pelvic radiotherapy is also an important cause of ovarian function damage. A dose less than 2 Gy is capable of destroying 50% of primordial follicles and a dose higher than 25 Gy can cause irreversible damage to the uterus.
- Benign diseases: Fertility protection counseling is very important for women of childbearing age who need gonadal toxicity treatment or have premature loss of fertility, such as patients with severe thalassemia, aplastic anemia, sickle cell anemia and myelodysplastic syndrome, because these patients may need HSCT, resulting in a high risk of POI (85% - 100%) (5). Many autoimmune diseases and hematological diseases also need chemotherapy, radiotherapy, and sometimes even bone marrow transplantation. In other cases, such as severe or recurrent endometriosis, recurrent ovarian torsion will lead to reduced ovarian reserve, which is characterized by activated follicular recruitment and subsequent atresia, and local inflammation induces follicular "depletion". Ovarian endometrial cyst resection may cause damage to the ovarian reserve. Therefore, fertility preservation should be further considered in the case of postoperative recurrence (6).
- POI high risk population: POI is a gynecological endocrine disease caused by ovarian follicle depletion or iatrogenic injury before the age of 40. Traditionally, it was affecting approximately 1% of women but recently, it has been reported that the morbidity rate of POI has significantly increased, and it can reach 3.7% ~ 10% (7). For example, patients with Turner syndrome (TS), fragile X mental retardation gene 1 mutation, galactosemia and other patients with decline ovarian function, who have a family history of POI, or those with high risk of POI according to the gene test, should have fertility protection. Girls with TS have increased risk of POI and infertility and their ovarian reserve runs out before and around puberty, so to benefit from fertility preservation, patients with TS should be evaluated (8). The prevalence of iatrogenic POI is growing with increasing survival rates of cancer patients, accounting for 65% of the identified causes of POI (9).
- Age-related infertility: Women are marrying late and planning their 1st pregnancy later and later in life for a variety of personal reasons like career choices, lack of stable life partner or financial issues which is increasing infertility in women due to in-

creasing in age. According to data from the Seventh National Census, the total fertility rate reached a low rate of 1.3 in 2020 in China (10). The average age of first childbearing age for Chinese women has increased from 24.3 years to 27.3 years from 2006 to 2017.

Evaluation of ovarian function

- Ovarian function should be evaluated before fertility protection. Ovarian reserve detection methods include imaging techniques such as pelvic ultrasound, ovarian volume and antral follicle counting (AFC), and biochemical tests such as follicle stimulating hormone (FSH) and anti-Mullerian hormone (AMH). These results are helpful in comprehensively evaluating the type and dose of treatment, assessing the risk of POI after treatment, and helping patients and doctors to decide whether to choose fertility protection or not and to select the specific fertility protection method at the time of diagnosis and before the treatment of the disease.

Female fertility protection and preservation method

- Embryo cryopreservation and oocyte cryopreservation: Embryo cryopreservation and oocyte cryopreservation are used in post puberty women. Embryo freezing in assisted reproductive technology (ART) is a very mature technology with a history of more than 40 years. It is mainly suitable for married women with fixed partners. A retrospective cohort study has suggested that cryopreserved embryos have fewer live births and pregnancy rates than the transfer of fresh embryos (11); however, the data of embryo cryopreservation for fertility protection are still limited. For women who do not want to use sperm donor and/or have no partner, and women who are unable to cryopreserve embryos due to ethical, religious or legal concerns, oocyte cryopreservation can be selected. For many women facing gonadotoxic treatment, oocyte cryopreservation is their best or only option. In 2013, the American Society of Reproductive Medicine reached an international consensus that this technology can be used as one of the protection/preservation methods of clinical female fertility. Although this technology has become more and more mature, due to the particularity of oocytes, it has poor tolerance to cryopreservation, and its success rate is lower than embryo cryopreservation. At present, most research data are related to age factors, because the utilization rate of fertility protection after cryopreservation for malignant or benign diseases is less than 5% (12). In most cases, it takes 10 ~ 12 days to obtain oocytes through ovarian stimulation (13). They are not suitable for patients whose primary treatment cannot be delayed and who have ovarian stimulation and egg retrieval contraindications. There are also some risks due to changes in hormone levels. Estradiol may exceed the physiological level due to ovarian stimulation, which may bring potential harm to hormone sensitive tumors. It can be considered to use anti-estrogen therapy in ovarian stimulation, such as aromatase inhibitor or letrozole, tamoxifen or GnRH agonists or choose other fertility protection methods. Oocyte cryopreservation is still the best and the only one option established along with embryo cryopreservation in ovarian cancer patients (14).
- Ovarian tissue cryopreservation: It is specifically indicated for pre-pubertal girls and women who require immediate cancer treatment. OTC involves surgical removal of the entire ovarian cortex or strips of tissue before the treatment of cancer by radiotherapy or chemotherapy or open surgery and the tissue rich in primordial follicles are fragmented and examined histopathologically, in order to avoid the presence of any malignant cells. The slow-freeze method is used on multiple ovarian biopsies because this protocol has yielded almost all live births after reimplantation (15). After the primary disease is cured the ovarian tissue is transplanted back into the pelvis of the patients by laparoscopy or mini-laparotomy to restore fertility and endocrine function (16). Ovarian tissue biopsy/sampling can be performed at any time of the menstrual cycle through laparoscopic surgery. It is recommended to take more than half of the volume of one or both ovaries (individualized quantification). The removed ovarian tissue is quickly transported to the ovarian tissue cryopreservation center at low temperature (4-8°C). The medulla is removed and the intact cortex (where the original follicle pool is located) is retained. Then the cortex is cryopreserved in size 4 mm × 8 mm tissue slices and stored in liquid nitrogen. Studies have shown that long-term preser-

vation does not affect the morphology and survival rate of the follicles. Ovarian tissue transplantation preserved by slow freezing method for 14 years has been reported. The cryopreservation methods are divided into slow cryopreservation and vitrification cryopreservation. Slow cryopreservation is a widely used, standard and effective method for OTC, which is internationally recognized as the gold standard procedure for OTC (17). This technology has developed rapidly since the world's first live birth after frozen ovarian tissue auto transplantation was reported in 2004 (18). By 2020, there were more than 200 babies born through this technology worldwide (19). Fertility protection has become a clinical routine in some European countries, and more mature fertility protection networks have been established, such as the fertiPROTEK network. A review of 5 leading European centers demonstrates that chemotherapy using low gonadotoxic agents before OTC does not impair the chances of success in ovarian function restoration, pregnancy and live birth after transplantation (16). The first human OTC Bank of China was established at Beijing Obstetrics and Gynecology Hospital, Capital Medical University in 2012 (20), in which more than 400 cases of cryopreservation and 10 cases of adult ovarian tissue transplantation have been successfully performed with restoration of ovarian functions within 3 to 4 months after surgery (21). In 2016, the first successful frozen ovarian tissue transplantation in our institution was also performed on a cervical cancer patient whose ovarian function recovered 3 months after surgery and was maintained for more than 6 years (21). Before starting OTC in patients, our team conducted preclinical and xenotransplantation experiments to confirm the technique's safety and efficacy (22). Our institution also successfully preserved fertility by OTC in patients with breast cancer during pregnancy (PrBC) without delaying her treatment. This method was used for the first time worldwide in a patient with PrBC in our institution (23). The first live birth after OTC transplantation was born on 31 August 2021 in a patient diagnosed with myelodysplastic syndrome (24) which marked a breakthrough in the use of this technology in China. The average age of girls under the age of 14 who have had cryopreserved ovarian tissue in our institute is 7 years, and the youngest is only 1-year-old (25). OTC transplantation is no longer considered experimental with increasing evidence of live births and return of endocrine functions and is recommended by the American Society for Reproductive Medicine (ASRM), the European Society of Human Reproduction and Embryology (ESHRE), and others. In a recent meta-analysis including 20 studies, has shown that after OTCT the pregnancy rate and the live birth rate were 37% and 28% respectively (26). The transplantation time of frozen ovarian tissue is determined according to the multidisciplinary consultation and evaluation of the primary disease (27). Many studies have shown that cryopreservation of ovarian tissue after the remission of primary disease significantly reduces the risk of relapse. OTC is done on orthotopic or heterotopic sites. Orthotopic transplantation of ovarian tissue is on the original ovary site and heterotopic transplantation of the ovarian tissue is on a foreign site. Orthotopic transplantation is better at restoring fertility. The success rate of frozen ovarian tissue transplantation is 85% ~ 100%, and the ovarian function is restored within 4 months on average. The maintenance time of transplanted ovarian tissue is 24.9 months (4 ~ 144 months) on average due to great individual differences in ovarian reserve and transplantation quantity. The advantage of OTC is that it does not need drugs to induce ovulation and avoids the occurrence of ovarian hyperstimulation syndrome. On the other hand, 2 - 3 days is enough for the collection of samples before starting the cancer treatment, each piece of tissue can store hundreds or thousands of follicles, and the fertility reserve is huge. It is the only choice for female fertility protection in prepubertal girls and patients who cannot delay anticancer treatments to preserve fertility and ovarian endocrine functions (28). However, for patients over 36 years of age and AMH < 0.5 ng/mL, individualized discussion and analysis are needed.

- Gonadotropin releasing hormone antagonist (GnRH-a): There is limited evidence for the protective effects of GnRH-a on ovarian reserve and future pregnancy. This is an approved alternative method and accessible fertility preservation option to protect ovarian function in premenopausal women with concomitant use of GnRH analogs like triptorelin, goserelin, leuprolide during the

course of chemotherapy (29). This less expensive approach could reduce chemotherapy induced POI and improve future fertility in premenopausal patients with early breast cancer undergoing chemotherapy. Treatment with GnRH-a is a non-invasive and well-tolerated alternative, which can be offered to all patients if cryopreservation is not feasible due to clinical or logistic issues (30). But we should remember that, for patients interested in fertility preservation, temporary ovarian suppression with a GnRH-a during chemotherapy is not an alternative to cryopreservation techniques (31).

- Ovarian displacement: Before radiotherapy, the ovary is moved away from the radiation position by surgery. For example, for patients with cervical cancer who need pelvic radiotherapy, do not need chemotherapy, and have no ovarian dysfunction, ovarian displacement can be considered. In some patients this technique may have some certain curative effect, but in most there is a poor effect, and the long-term effect is uncertain. It is not suitable for patients who need systemic radiotherapy.
- Oocyte in vitro maturation (OIVM): OIVM is a new fertility protection method combined with OTC. In this technique, immature oocytes are obtained and cultured in vitro for 24-48 hours, followed by vitrification cryopreservation or embryo cryopreservation after fertilization. Data on the effectiveness of OIVM in preserving fertility are limited to oocyte recovery and maturation, and there is little information on subsequent fertilization and embryo implantation. Therefore, this method is currently used as an experimental method.

Conclusion

For patients who need gonadal toxicity treatment and those at risk of POI, the choice of fertility protection methods should be discussed as soon as possible. Embryo cryopreservation is relatively mature and only suitable for married women. With the 1st live birth after OTC transplantation in China, it is proved that this method is no longer experimental and can be added into the routine of reproductive medicine options to preserve ovarian function and also fertility. They are suitable for pre-adolescent children and girls. Ovarian transposition before radiotherapy for cervical cancer has proved to have limited effect. GnRH-a lack evidence of fertility and ovarian function protection, so for patients interested in fertility preservation, temporary ovarian suppression with GnRH-a during chemotherapy is not an alternative to cryopreservation techniques. OIVM is a technology to obtain immature oocytes and freeze them after maturation in vitro. However, it is still in the research stage. OTC has become the most promising method for fertility and ovarian function protection in China and in other countries. International and domestic consensus and guidelines unanimously suggest that OTC is the only fertility protection method for pre-adolescent women. With the continuous development of fertility protection, the in vitro maturation of artificial ovary and ovarian cortical isolated primordial follicles may be the developmental direction in the future.

Author contributions

All authors have certified the author list and the contribution description. All authors have read and approved the submitted manuscript and any substantially modified version of the manuscript.

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