Fertility preservation in women with cancer: update and perspectives

Preservação da fertilidade em mulheres com câncer: atualização e perspectivas

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ABSTRACT

With the increased number of cancer diagnoses among young women and the advances in treatment, many patients who may have had their fertility compromised by chemotherapy express desire to become pregnant in the future. Freezing embryos for later IVF so as to preserve fertility is a well established process. Oocyte cryopreservation by vitrification has evolved greatly in recent years and is no longer considered experimental. By 2009 more than 900 children were born from cryopreserved oocytes, without increased risk of congenital anomalies. The preventive use of GnRH analogues for ovarian suppression during chemotherapy to avoid premature ovarian failure has uncertain outcomes. Other techniques such as cryopreservation of ovarian tissue for later autograft are still considered experimental. Although use has already been reported in 24 births, doubts still persist and motivate further study. In vitro maturation of ovarian follicles is a promising alternative for preserving patient fertility and has shown positive results in rodents, monkeys, and humans. Caution should be used with experimental techniques, especially when offered to emotionally fragile patients. Therefore it is important to thoroughly convey information on the chances of pregnancy with existing treatments and the limitations of experimental techniques.

Key words: Fertility Preservation; Cryopreservation; Fertilization in Vitro; Ovary/anatomy & histology; Oocytes; Embryonic Structures; In Vitro Oocyte Maturation Techniques; Gonadotropin-Releasing Hormone/analogs & derivatives.

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RESUMO

Com o aumento do diagnóstico de câncer em mulheres jovens e os avanços no seu tratamento, muitas pacientes que poderão ter sua fertilidade comprometida com a quimioterapia têm manifestado desejo de engravidar futuramente. O congelamento de embriões, após fertilização in vitro, para preservar a fertilidade está bem estabelecido. A criopreservação de oócitos por vitrificação evoluiu bastante nos últimos anos, deixando de ser experimental. Até 2009 nasceram mais de 900 crianças a partir de oócitos criopreservados, sem aumento do risco de anomalias congênitas. O uso de análogos do GnRH para a supressão ovariana durante a quimioterapia na tentativa de prevenir a falência ovariana prematura apresenta resultados incertos. Outras técnicas ainda são consideradas experimentais, como a criopreservação e posterior autotransplante de tecido ovariano. Já foram relatados 24 nascimentos com o seu uso, persistindo, entretanto, dúvidas que motivam o seu estudo. A maturação de folículos ovarianos in vitro é alternativa promissora para preservação da fertilidade nessas pacientes e tem apresentado resultados positivos em roedores, macacos e humanos. Muita cautela deve ser tomada com o uso de técnicas experimentais, especialmente quando oferecidas para pacientes diante de

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fragilidade emocional. Por isso, é importante transmitir corretamente informações sobre chances de gravidez com tratamentos existentes e as limitações das técnicas experimentais.

Palavras-chave: Preservação da Fertilidade; Criopreservação; Fertilização in Vitro;Ovário/antomia & histologia; Estruturas Embrionárias; Oócitos; Técnicas de Maturação in Vitro de Oócitos; Hormônio Liberador de Gonadotropina/análogos & derivados.

INTRODUCTION

Each year a larger number of young women are diagnosed with cancer. In 2008 in Brazil, there were around 30,000 cases of cancer in women under the age of 44, and it is estimated that in 2012 there were 52,680 new cases of breast cancer and 4,450 of non-Hodgkin lymphoma.[1] Thanks to early diagnosis and treatment advances, many women have been cured, many of whom have not yet had any children or have not had all the children they planned on having at the time of treatment and wish to become pregnant in the future. Radiotherapy performed on the pelvis can lead to ovarian failure and some of the drugs and schemes used in chemotherapy can have a toxic effect on the gonads, possibly leading to infertility by reduction of ovarian reserve and even to oophorectomy in some cases.[2-5]

Almost three-quarters of women without children at the time of cancer diagnosis wish to become pregnant in the future and 81% of adolescents and 93% of their parents are interested in preserving their fertility, even with experimental treatments.[6] Some women describe loss of fertility consequent to treatment of cancer as being as dramatic an event as the cancer diagnosis.[5]

Women between 14 and 40 years old and who are chemotherapy candidates with a high chance of depleted ovarian function should be advised by a specialist in Reproductive Medicine who must cooperate with the oncologist in charge regarding methods of preservation of fertility.[7,8] However, since this is a critical moment when many decisions must be made in a short period of time, the conduct is not often followed by oncologists. Besides, patients and doctors also have difficulty in obtaining updated information and accessing procedures for preservation of fertility. Niemasik *et al*.[5] evaluated what women who survived cancer remembered about the risk of infertility related to treatment at the time of their diagnosis in order to identify the barriers to advice on preservation of fertility. Almost half of the women could not remember any advice on reproduction and preservation of fertility. Uncertain prognosis, the risk of recurrence and the doctor-patient communication barrier can lead to a lack of adequate information in this patient population.[9,10]

OPTIONS FOR PRESERVATION OF FERTILITY

Some of the techniques currently used for preservation of fertility are already well established, such as in vitro fertilization and subsequent freezing of embryos. Others are more recent and still deliver uncertain results, such as freezing mature oocytes and using gonadotropin-releasing hormone analogs (GnRH). There are, moreover, those techniques considered experimental, for example cryopreservation and subsequent autotransplantation of ovarian tissue.

Cryopreservation of oocytes or embryos

Cryopreservation of embryos and cryopreservation of oocytes are alternatives for patients who will undergo treatment against cancer and manifest a desire to preserve their fertility. They are aimed at patients in reproductive age who can undergo ovarian stimulation prior to cancer treatment. They include the ovarian stimulation stages with GnRH analogs, urinary or recombinant gonadotropins (follicle-stimulating hormone – FSH), luteinizing hormone (LH) and human chorionic gonadotropin (hCG), ultrasound monitoring and ovarian puncture for follicular aspiration guided by transvaginal ultrasound under analgesia. The oocytes obtained can be frozen on the same day they are collected or fertilized with the partner's sperm. Embryos can be frozen on the third to fifth day of cultivation.

In case of good response to the cancer treatment, with good prognosis and authorization by the oncologist in charge, embryos can be thawed and transferred into the patient's uterus after a brief hormonal preparation. Frozen oocytes must be thawed and fertilized with the partner's sperm. After a brief period of cultivation in a laboratory, the embryos are transferred into the patient's uterus.

Both of these options require 15 to 20 days for the induction and collection of oocytes before cancer treatment. For many oncologists and patients, this wait can obstruct the process because they fear worsening the prognosis, especially in cases of hormone-dependent cancer, through increase in estradiol lev-

els during the induction, which can be avoided when an aromatase inhibitor such as oletrozol is associated with the process of ovarian stimulation.

Cryopreservation of embryos is a well established technique, with pregnancy rates per cycle of thawed embryos similar to those of cycles with fresh embryo transfer.

The chances of a pregnancy in the future will depend on the patient's age at the time of freezing, which may be 20 to 40% [11-14] and 50% for transfer in women with younger than 35 years.[15-17]

Cryopreservation of oocytes by vitrification has evolved considerably in recent years and is no longer considered experimental.[18] By 2009, more than 900 children were born from cryopreserved oocytes without increased risk of congenital anomalies.[19,20]

Although effective, freezing oocytes and embryos requires use of the technology of in vitro fertilization and embryo culture, ovulation induction medication, monitoring of ovulation, sophisticated equipment and laboratory, and a trained staff. They do not restore ovarian function, but provide chances of achieving pregnancies in the future. In the case of freezing of embryos, there are potential ethical issues in the future if the patient dies or the couple separates. They only apply to pre-menopausal women with established ovarian function and may not be used in children. Around 15 to 20 days are required before starting the cancer treatment.

Cryopreservation of ovarian tissue

Freezing of ovarian tissue removed from the patient prior to treatment onset and subsequent autotransplantation is a promising technique under development. It requires further research but has been successfully performed in various animal species.[21] It was first performed in humans in 1996, in a patient who had already undergone chemotherapy. Cases and small series have been reported since then, with reimplantation of the ovarian tissue in the original site (orthotopic)[22,23] or in various sites, such as the abdominal wall or the forearm.[24,25]

Some cases of return of ovarian function have been reported after transplantation of tissue and others in which aspiration of oocytes was possible after stimulation of the ovary implanted in the forearm or the abdomen. In 2004, in Belgium, the first spontaneous pregnancy after an orthotopic transplant was described.[26]

The relation between this pregnancy and transplantation of cryopreserved tissue was called into question soon after its publication.[27] The debate resumed in 2012, after reviewing the medical records and signs of ovulation in the ovarian tissue that remained in the patient. Questions were raised whether the pregnancy had resulted from the transplanted tissue or from this residual ovarian tissue.[28-30]

Residual ovarian function before transplantation, which could lead to spontaneous pregnancy, is a recurring matter in discussions of pregnancy reports.[31,32] Other pregnancy reports, including a birth after reimplantation of ovarian tissue in a patient who underwent bilateral oophorectomy for ovarian abscesses, confirm their effectiveness.[33]

It is estimated that 24 births have occurred after transplantation of cryopreserved ovaries, none yet in Brazil.[32-34] Four more pregnancies are in progression, with more than 60 cases having occurred in three European centers until now.[35] Several centers throughout the world perform this procedure with positive results[36] and there are still some cancer patients who have had fragments of ovarian tissue cryopreserved and are waiting for the end of treatment and clearance from their oncologists to have them placed back into their systems.

There are still several questions regarding the procedure which still motivate further studies in the area, such as: the amount of tissue to be cryopreserved; the best technique of cryopreservation; risks of transplanting malignant cells; best site to transplant; techniques to reduce tissue ischemia and improve the implementation and function of ovarian tissue; for how long the ovarian function can be maintained; how long the tissue can remain cryopreserved; what is the probability of achieving a pregnancy.

The fragments of ovarian tissue are obtained through laparoscopy, a minimally invasive and well established surgery with low morbidity and mortality rates. Postoperative recovery is usually quick and the patient is referred to initiation of chemotherapy. Tissue samples are collected and brought to the laboratory, where the process of cryopreservation is carried out. Collection and freezing of an entire ovary is recommended;[37,38] however, this type of procedure can impair fertility if the cancer treatment does not cause ovarian failure. Moreover, freezing the entire ovary is more difficult because of the number of cells involved and the difficulty for cryoprotectants to penetrate. Currently, what has been done is the cryopreservation of fragments of ovarian cortex, which can be transplant-

ed back into the patient's body after recovery and restoration of health after the cancer treatment.[6,34]

The gold standard for cryopreservation of ovarian tissue has been slow freezing. However, the vitrification method has grown in popularity because of its good results with oocytes and embryos.[39-42] The length of time the ovarian tissue remains cryopreserved does not seem to interfere with the efficacy of the method or its results. Damage caused by the cryopreservation process in up to 30 days seems to remain stable after 180 days or more.[43]

The live births so far were obtained from cryopreservation of ovarian tissue by slow freezing. The main characteristic of slow freezing is the use of a freezing curve that gradually reduces the temperature. A curve of 0.3C per minute is usually used, depending on the material to be cryopreserved. In order to achieve a balance between the various cellular elements, low concentrations of cryoprotectants are used in an attempt to favor non-formation of intracellular ice crystals.[44] Nevertheless, these concentrations may prove unable to eliminate all the water from the inside the material, thus allowing formation ice crystals, which may cause cellular damage.

Formation of ice happens when the efflux of intracellular water does not occur efficiently, making adequate cellular dehydration impossible. In this case, the water that remains inside the cell freezes, forming ice crystals. Formation of these crystals must be prevented by removing the largest quantity of intracellular water possible, which occurs when the cooling process is sufficiently slow to try to prevent cellular injury. Formation of ice crystals, osmotic shock, and the toxicity of cryoprotectants may limit the success of cryopreservation because they affect the survival, and change the functionality, of the ovarian follicles after the procedure of cryopreservation of ovarian tissue.

In order to minimize these issues, the vitrification method has been gaining more attention and being tested as it acts quickly in bitterly cold temperatures, in addition to turning the liquid from inside the cell into a stage similar to glass.

Vitrification is currently widely used for freezing oocytes, embryos, and ovarian tissue. However, it is a technique that requires high concentrations of cryoprotectant agents and high speed during freezing. This high concentration of cryoprotectants can induce cellular toxicity and cause osmotic trauma.[45] For these reasons, there is still no consensus about the most appropriate technique to be used routinely.

As regards ovarian tissue, many studies have attempted to compare slow freezing to vitrification. The results are still controversial as they include various sizes of cryopreserved tissue, use of different types and concentrations of cryoprotectants and heterogeneous follicular tissue, which may facilitate the slow freezing method[46-48] or virtification[49] or consider them to be equivalent in effectiveness.[50-51]

The technique of cryopreservation of ovarian tissue has been regarded as a promising treatment as it has shown good results[52-54] in several animals, such as primates [52] and humans, currently with 24 live births already reported in the world after freezing of ovarian tissue and subsequent autotransplantation.[6,32,34,55]

However, there are still obstacles concerning autotransplantation of cryopreserved ovarian tissue due to factors such as ischemic injury, as well as damage caused by the process during slow freezing. The greatest concern in investing in autotransplantation is the possibility of reinserting malignant cells into patients after cancer treatment.[56] Bearing in mind this situation, maturing the follicles in vitro after cryopreservation of ovarian tissue would be recommendable. This technique, however, is still considered largely experimental. The magnitude of the risk of reinserting malignant cells is unknown, with no reports of cases in humans. Techniques to identify malignant cells in tissue using immunohistochemistry and polymerase chain reaction (PCR) are being developed. It is currently recommended to avoid retransplantations in patients with high risk of ovarian metastasis.[3]

To completely avoid the risk, maturing the follicles in vitro and laboratory fertilization could be good alternatives;[4] moreover, as an alternative to avoid the risk of recurrence, isolated follicles could be transplanted after being thawed and in suspension, which has delivered good results and even live births in experiments with mice.[57] Studies with xenotransplantation show that human follicles isolated after transplantation are able to survive and grow.[58]

There are still many questions regarding the best site for retransplantation. Fragments of ovarian tissue have been placed in the forearm, and abdominal, thoracic or orthotopic, walls. In situations of retransplantation outside the ovarian surface, success has been achieved, through stimulation of gonadotropins, with follicle growth, acquisition of oocytes and production of embryos after in vitro fertilization, but pregnancy has not yet been achieved. There are problems in follicular growth that causes failure to reach the normal pre-ovulatory diameter.[59]

Transplantation of tissue onto the remaining ovarian surface to allow a spontaneous pregnancy or for a peritoneal recess made in pelvic cavity has been the dominant trend, both through laparoscopy. There are, however, important technical difficulties to be studied, including the need for vascular reconstitution of ovarian tissue or stimulation of angiogenesis in the transplant site. Perhaps the biggest obstacle to ovarian function is ischemia occurring until the ovary is properly implanted. The greater part of loss of follicular content happens during this period. Studies have been made with the use of components potentially able to stimulate angiogenesis and facilitate the implant.[60] Even with the difficulties, there are several records of live births after cryopreservation of ovarian tissue and its orthopic retransplantation.

In vitro maturation of follicles and in vitro fertilization

The technique consists in isolating preantral follicles from the cortex of the ovarian tissue and in vitro maturation of isolated follicles until they reach the antral stage and mature oocytes can be obtained from them, which could be fertilized in the laboratory with the partner's semen.

This technique can rule out the theoretical risk of disease recurrence in case of retransplantation, mainly before ovarian cancer,[4] although there are no studies that report this fact.

In vitro follicular maturation is still considered experimental. The development of ideal conditions for growing follicles is one of the greatest challenges of reproductive technology. A better understanding of the physiological requirements of the oocytes, granulosa and Theca cells and even stromal cells is needed. The physiology involving follicular development is complex and still not fully understood.[61]

There have been positive results in experiments with rodents,[60] non-human primates (Rhesus monkeys)[62,63] and humans.[64] Live births have been reported for fertile mice obtained from oocytes which came from follicles matured in vitro,[65] and embryos of non-human primates have been successfully produced.[66]

Extensive studies of folliculogenesis and maturation technique are still required so that the latter can be effectively established and routinely delivered to patients.

Use of GnRH analogs

Temporary ovarian suppression with use of GnRH analogs during chemotherapy has been proposed to prevent premature ovarian failure; however, its value is controversial. The reason for using this technique is the greater resistance of prepubescent ovaries to chemotherapy found in animals and humans.[67,68] The mechanism of action of this drug is not yet fully defined since the main known effect of hypothalamic block would be on the ovulatory cohort and not on the primordial follicles, which are the main components of the ovarian reserve. Different rates of amenorrhea not been demonstrated for patients who used the GnRH analog,[69-70] and its real role awaits further studies.

CONCLUSIONS

Oncofertility (the field of preservation of fertility in patients with cancer) shows great progress and great perspectives. Oncologists, hematologists, mastologists, specialists in reproductive Medicine, urologists, embryologists, and psychologists need to be involved in a multidisciplinary effort to provide quick, efficient, and friendly care to patients. The option of using experimental techniques, especially when offered to patients in situations of emotional fragility, must be cautious. Several factors, especially the patient's age, the tumor's histological type, disease staging, chemotherapy and/or radiotherapy schemes and treatment prognosis must be exhaustively discussed[7] with patients and family members, who must be given adequate psychological support and correct information about the actual chances of obtaining a pregnancy in the future with the established techniques and the limitations of experimental techniques.

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