FERTILITY PRESERVATION OPTIONS FOR CANCER PATIENTS

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ABSTRACT

Survival rates for cancer patients have increased in the last few years due to improvements achieved in cancer therapies. But, these treatments produce some adverse effects. One significant effect in prepubertal and young women is premature ovarian failure which impacts on reproductive capacity. For this reason, fertility preservation techniques appear. For the last few years there has been research into new procedures that will allow women to preserve their reproductive function. Specialised groups have appeared who advise women on the best fertility preservation option, always with a personalised approach. Currently, established fertility preservation techniques are embryo cryopreservation and oocyte cryopreservation; these two procedures can be offered widely to these women. However, there are some limitations: they cannot be offered to prepubertal women, they require a time interval to carry out (not always possible in cancer treatments), and they provide a restricted number of embryos or oocytes. On account of this, some specialised centres offer other experimental techniques such as ovarian tissue cryopreservation, which can be useful in this group of patients. We also have to take into account other procedures such as *in vitro* maturation of follicles, oophoropexy or trachelectomy. Gonadotropin-releasing hormone agonists should not be offered to these women because there is no evidence of their usefulness. We must not forget that we can recommend a combination of techniques in order to optimise their fertility options. More research is still needed to find an ideal procedure that will allow a considerable number of metaphase II oocytes to be obtained to ensure a pregnancy avoiding the problems that exist with current techniques.

Keywords: Fertility preservation, embryo cryopreservation, oocyte cryopreservation, ovarian tissue transplantation, IVM, gonadotropin-releasing hormone agonists, oophoropexy, trachelectomy.

INTRODUCTION

It was in 1953 when Jerome K. Sherman, one of the American pioneers of sperm congelation, showed for the first time the capacity of frozen-thawed sperm to fertilise an oocyte and to induce its normal embryo development. During the following years there was a growing interest in the possibility of creating sperm banks, but it was not until the early 70's that the first sperm bank was inaugurated. In 1977, through California Cryobank, Cappy Rothman and Charles Sims offered for the first time the possibility of cryopreserving the sperm in male patients who were in need of a medical castration, to mitigate the negative impact on their procreation capacity. This way if they decided in the future to have children, they could retrieve their sperm to do an artificial insemination. It is at this time that we can start to discuss fertility preservation.[1]

Due to the positive results in oncologic treatments obtaining cure rates of 70-80% and the high number of female survivors after oncologic treatments in reproductive age (around 25%)[2] who suffered from the adverse effects of these treatments (premature ovarian failure [POF]), it

was considered a possibility to offer to these women a chance to preserve their fertility by the mid 90's. In this context, the early experimental studies appeared which were headed towards the cryopreservation of ovarian tissue for its subsequent implant. We expose in this paper current available updated fertility preservation techniques and some notes about future prospects in this field.

EPIDEMIOLOGY

Currently, it is estimated that cancer incidence in women of childbearing age (under 35 years) is around 4% of all malignancies diagnosed in a year, according to American statistics.[3] The most frequently diagnosed cancers in this group are breast cancer, melanoma, cervical cancer, non-Hodgkin lymphoma, and leukaemia.[4] During the last 30 years there has been an increase in the incidence of cancer of 1% in the child population and 2% in teens, especially carcinomas, lymphomas and germ cell tumours.[5] The overall increase in incidence has been accompanied fortunately by the increase in survival, due to improvements in early diagnosis and treatments. According to SEER-NCI (Surveillance Epidemiology and End Results, National Cancer Institute-US), 5-year survival of cancer patients younger than 20-years-old was 55.8% in 1975 and 83.3% in 2005.[6] European statistics show similar figures with 5-year survival rates of 73%.[7] In relation to cancer in the paediatric population, it is considered that 1 in every 640 people aged 20-39 years is a survivor of childhood cancer,[8] which means that in the future, the reproductive problems in cancer survivors will increase progressively.

EFFECTS OF GONADOTOXIC TREATMENTS ON FERTILITY

Ovarian damage is an important and undesirable effect of current cancer treatments in women. At the same time, the reduction or loss of fertility is one of the worst tolerated aspects in these patients. The impact on the ovarian reserve is related to the accelerated depletion of the primordial germ cell pool resulting from the therapies.[9] Hence the importance of quantifying and trying to predict ovarian involvement of these treatments.

The ovarian tissue, due to its characteristics, is one of the organs most sensitive to the effects of radiotherapy, and POF is the main result.[10] Ovarian damage will have a reversible or irreversible effect depending on whether the injury is complete or partial. Although the long-term result will always be POF, its clinical translation will be different depending on the time at which it occurs;[10,11] in prepubertal patients it will manifest as absent pubertal development and those patients who have already had menarche as POF. Ovarian damage and eventual POF caused by radiotherapy will depend essentially on: the age of the patient, total radiation dose received, type of radiation, fractionation of treatment, adjuvant therapy; and depending on the idiosyncrasy of the patient, the ovarian reserve will look more or less affected.[12] When applied conventionally, radiation doses of 24 Gy result in ovarian failure.[13]

Anticancer drugs may diminish the primordial follicle pool, cause ovarian atrophy and harm the ovarian blood vasculature.[14] The extent of damage is related to the patient's age, chemotherapeutic agent, and drug regimen used. Alkylating agents, which are not cell cycle-specific, confer their deleterious effects on the vast supply of primordial germ cells and carry the highest risk of ovarian failure. Antimetabolites impact the cells (granulose and oocytes) of the metabolically active ovarian follicles and are considered to be low-risk for gonadal dysfunction, whereas cisplatin appears to carry intermediate-risk between the antimetabolites and alkylating agents.[15] On the other hand, patients who undergo bone marrow transplantation have extremely high ovarian failure rates, ranging from 72% to 100%.[12] Women over 40-years-old have a 90% chance of amenorrhea subsequent to multiagent chemotherapy, whereas the potential for POF in younger patients varies between 20% and 90%.[16]

FERTILITY PRESERVATION

The American Society of Clinical Oncology (ASCO) recommendation on fertility preservation in cancer patients indicates that all patients susceptible to treatment with radiotherapy or chemotherapy should be informed and provided with counselling depending on age, disease, prognosis, and interval time for freezing. The urgency to begin cancer treatment should not be an excuse to approach fertility preservation options.[3] Currently, embryo and oocyte cryopreservation are considered standard

Table 1. Fertility preservation techniques.

Chemoprophylaxis	Surgical procedures	Cryopreservation
GnRHa*	Oophoropexy	Embryo cryopreservation
	Trachelectomy	Oocyte cryopreservation
		Ovarian tissue cryopreservation
		IVM

*Currently, no evidence to recommend as a fertility preservation method.
GnRHa: Gonadotropin-releasing hormone agonists; IVM: *in vitro* maturation.

practice and are widely available; other fertility preservation methods should be considered experimental and only performed by providers with the necessary expertise.[17]

Gonadotropin-Releasing Hormone Agonists (GnRHa)

Assuming that turning off the reproductive axis would make the ovary less vulnerable to cytotoxic damage; it had been proposed that GnRHa could be used as ovarian protectors during gonadotoxic therapies.[18] There are several possible mechanisms thorough which GnRHa may protect the ovary during chemotherapy: reduced levels of gonadotropins, a direct influence of GnRHa on the ovary, and reduced blood flow to the ovary.

In a recent review including a total of 579 women from 12 studies, among 345 women that reported an ovarian function after the administration of GnRHa concomitant with chemotherapy, Beck-Fruchter et al.[19] found insufficient evidence to show that GnRHa co-treatment is effective in protecting the ovary from the damage of chemotherapy.

Behringer et al.[20] had to prematurely close a study on a group of women aged 18-40 years who were affected by Hodgkin disease because they observed no protection of the ovarian reserve with hormonal co-treatment with GnRHa during BEACOPP. Therefore, today there is not enough evidence to apply the GnRHa as a fertility preservation method.

Oophoropexy

Oophoropexy, defined as the action of removing the ovaries surgically from the radiation field, can be offered to oncological patients before initiating treatment with radiotherapy. The procedure can be performed by laparoscopy, because it is simple, safe and effective, unless laparotomy is necessary for the primary treatment of the tumour.[21] Scattered radiation and altered ovarian blood supply appear to be the main factors causing the failure of the technique.[22] The irradiation dose and the total dose received by the less irradiated ovary also affect the result.[23]

When performing oophoropexy, we have to take into account the difficulties of oocyte retrieval in *in vitro* fertilisation (IVF), possible complications in future pregnancies due to uterine irradiation, and ovarian cysts caused by ovarian dysfunction. This technique has shown variable results in the endocrine function recovery (60-90%) according to the treatment used - brachytherapy versus external radiotherapy[24] - and depending on extension, dose and possible vascular involvement. We must not forget the risk of metastasis reported by some researchers when performing this technique in pelvic malignancies, so they advise only recommending this option to those patients with cervical invasive squamous carcinoma without risk factors for ovarian metastases (when there is no vascular invasion) who have to receive radiotherapy.[25] We must wait until we know the real possibilities of this option. Today it is still considered an experimental technique for those patients who have to start a treatment with radiotherapy because of its variable results.[23]

Trachelectomy

Surgical removal of the uterine cervix, called trachelectomy, can be offered to those patients who are affected by a cervical malignancy. In 1994, Daniel Dargent described trachelectomy for the first time, and in 2000 he presented the first results in combination with pelvic

lymphadenectomy.[26] Currently, there are more than 600 published cases of radical trachelectomy with similar survival rates to those with radical hysterectomy. However, we note that their indications are limited to cervical cancer in the early stages.[27] According to ASCO, radical trachelectomy should be restricted to Stage IA2 to IB cervical cancer with diameter <2 cm and invasion <10 mm.[28] The main disadvantages of this technique are posterior infertility problems (usually due to cervical factors), the rate of second trimester miscarriage which is twice that of the general population, and also the higher rate of preterm deliveries.[13]

Embryo Cryopreservation

Embryo cryopreservation, routinely performed in patients undergoing IVF techniques, affords the patient an optimal chance to preserve her fertility, with pregnancy rates of 20-50% per transfer of two to three thawed embryos, depending on the age of the patient at the time her oocyte was retrieved.[29] However, there are some disadvantages to this technique: it cannot be offered to prepubertal patients or adolescents, the patients have to delay their oncologic treatment to carry out the ovarian stimulation (from 2 to 6 weeks, excessive in some cancers), it requires having a partner or accepting a sperm donor, and the supraphysiological levels of gonadotropins and oestradiol resulting from IVF on oestrogen-dependent neoplasms may decrease the usefulness of this treatment for certain patients.[13] Protocols for controlled ovarian hyperstimulation that include agents such as letrozole (aromatase inhibitor) and tamoxifen (selective oestrogen receptor modulator) appear to yield high-quality embryos and counteract the potential impact of high oestradiol levels.[30]

Oocyte Cryopreservation

Oocyte cryopreservation is currently a widely used fertility preservation technique. The oocyte is particularly susceptible to damage during cryopreservation. Recently, vitrification has been shown to give better results in terms of survival, pregnancy and implantation rates than slow freezing.[14]

Cumulative ongoing pregnancy rates with oocyte vitrification without embryo selection in a standard infertility program are comparable to what is obtained with embryo cryopreservation, although female age significantly affects outcomes in this system.[31] The record of Spanish Fertility Society (SEF) in 2010 reports that 12 metaphase II oocytes (MII) are needed to obtain a 59% chance of pregnancy and about 20 MII to reach 80% chance of pregnancy. Further to this, the chance of pregnancy per oocyte thawed (devitrified) is 4-6%. Approximately only one from every five oocytes obtained (20.09%) gives rise to a pregnancy. We must also take into account that the mean number of oocytes obtained in stimulation cycles in the oncology patient is approximately 10.[32] The available evidence indicates that obstetric and perinatal outcomes in infants conceived from vitrified oocytes do not appear to be associated with adverse outcomes.[33]

This technique has similar disadvantages to embryo cryopreservation: it cannot be offered to prepubertal patients or adolescents, the oncologic treatment has to be deferred some weeks and, in hormone-dependent cancers, the ovarian stimulation protocol should be done with letrozole or tamoxifen to avoid the supraphysiological levels of oestradiol. On the other hand, it does not require the patient to have a partner or access to a sperm donor in the moment of the treatment.

Ovarian Tissue Cryopreservation

In prepubertal patients or those patients who require immediate establishment of chemotherapy treatment, the only way to preserve their fertility is cryopreservation of ovarian tissue. The main objective of this strategy is to obtain ovarian tissue for cryopreservation and subsequent thawing and autografting once the patient has recovered from her oncological disease. The ovarian tissue is obtained, as long as possible, by laparoscopy.[34] The standard method of ovarian tissue cryopreservation is slow freezing using propanediol or albumin and dimethyl sulfoxide (DMSO) as cryoprotectants, usually in combination with sucrose,[35] although researchers are working on vitrification protocols to cryopreserve the ovarian tissue. The tissue is cryopreserved in the form of thin cortical strips (about 1-2 mm^3 thickness) to allow penetration of cryoprotectant agents.

Transplantation of ovarian cortical fragments can either be done orthotopically (in the peritoneal cavity or on the contralateral ovary) or heterotopically (forearm or anterior abdominal wall). It is usually performed orthotopically because it allows spontaneous pregnancies. Currently, we know of the existence of 24 newborns from pregnancies achieved in oncological patients who received orthotopic transplantation from their cryopreserved ovarian tissue.[36]

The main problem of this method is the follicular loss, which is due to the cryopreservation procedure and mainly by the ischaemia produced while the graft is revascularised (it is estimated to be responsible for around 60% of the total follicular loss).[14] Currently, the duration of the graft is from 6 to 88 months,[37,38] with a main duration of 4-5 years.[36] In this way, there are some lines of research to accelerate the process of neoangiogenesis, through the use of growth factors combined with the reimplantation of ovarian tissue in a patient with a double oophorectomy.[39] After the transplantation, the assisted reproductive techniques may be used in order to improve chances of pregnancy and these patients should be considered poor responders. On the other hand, another way to improve pregnancy rates is to combine ovarian tissue cryopreservation with embryo or oocyte cryopreservation.

In vitro Maturation

In vitro maturation (IVM) and vitrification of oocytes retrieved from unstimulated ovaries is considered an experimental technique that can be offered to those women that cannot delay their oncological treatment or to adolescents and prepubertal patients. Maturation rates of 79% in oncological patients[40] and clinical pregnancy rates of 18-30% in patients with normal ovulatory cycles have been recorded. Usually multiple embryo transfers are performed because of low implantation rates. On the other hand the high abortion rate is today one of the biggest obstacles to practicing IVM more widely.[41] Currently, IVM must be considered an experimental technique that should not be offered to oncological patients because the experience is still very limited and pregnancy rates are lower than IVF. Huang et al.[40] suggested that IVM can be offered in combination with ovarian tissue cryobanking to increase chances of future pregnancies.

FUTURE TECHNIQUES

More research is still needed to find a procedure that provides the sufficient MII oocytes to ensure a pregnancy that is without risk of reinsertion of malignant cells, without interacting with oncologic treatment, and that provides comparable results to those obtained in healthy women under 35 years of age. In this way, Oktay's theory proposed oocyte regeneration after the case of a 32-year-old patient with a history of Hodgkin's lymphoma was known. The patient underwent ovarian tissue cryopreservation prior to treatment with chemotherapy, radiotherapy and later, bone marrow transplantation. After heterotopic transplantation of ovarian tissue she became pregnant repeatedly in a spontaneous way and three healthy babies were born. According to the author, this could be due to a connection between the ovary and bone marrow. Bone marrow reserve would act as peripheral germline stem cells and non-steroidal factors required for the differentiation of germ cells would come from the ovarian tissue.[42,43]

Another interesting theory is one which deals with the maturation of primordial follicles in ovarian tissue fragments. So we have a significant reserve of primordial follicles and IVM would allow us to obtain MII oocytes. After the initial work of Picton et al.[44] on IVM from primordial follicle to the MII oocyte, Krotz et al.[45] proposed the artificial ovary. They used the three-dimensional cultures, achieving maturation from early antral follicles (under 10 mm) to MII oocytes. They achieved interaction, growth and functionalism of the ovarian three cell lines: oocytes, granulosa and theca. These options are only two theories and, currently, these must not be taken as options for fertility preservation as studies in these fields need to be furthered.

CONCLUSION

Recent advances in oncological treatment and early diagnosis of cancer diseases have led to an increase in the survival rates of cancer patients. Due to this, more and more women suffer side-effects of oncological therapies such as premature ovarian failure. Clinicians must ensure that these women are referred to fertility specialists to obtain the best advice in fertility

preservation techniques. Embryo and oocyte cryopreservation are well-established techniques that can be useful in adult women who are able to delay their oncologic treatment by a few weeks. Ovarian cryopreservation is an experimental procedure, and the only one that can be offered to prepubertal patients or to those patients who have to start the oncological therapy immediately. The characteristics of the patient must always be assessed and each case individualised to advise the best fertility preservation option. Finally, we must not forget that we can recommend a combination of techniques to optimise fertility options.

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