Modern Fertility Preservation Methods in Female Malignancy

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Abstract

Female malignancies can induce severe fertility issues in reproductive age women due to the therapeutic procedures addressing such malignancies (radiotherapy, chemotherapy, surgical intervention). Conservative treatment with medication, while allowing the ovarian tissue to remain intact (antioxidant regimens, gametogenesis-suspending regimens), does not seem to provide any clinical benefits. Ovarian tissue cryopreservation was recently developed in order to overcome the loss of viable follicles (due either to malignancy or to therapeutic side-effects) whenever patient's clinical status permits it; the ovarian tissue is later thawed and used for patient's fertility benefits. There are mainly three methods addressing this issue: autotransplantation (albeit orthotopic - in pelvis - or heterotopic), xenotransplantation (mainly in mice) and in vitro follicular maturation (IVM). The IVM of ovocytes (harvested either by follicular retrieval or by laparoscopic ovarian tissue biopsy) followed by modern rapid cryopreservation method (vitrification) seems to be the most promising method. Cryopreserved tissue is then restored and used (as soon as the clinical status permits it) for in vitro fertilization (IVF) and embryo transfer procedures.

Keywords: *Female malignancy, ovarian tissue cryopreservation, autotransplantation, xenotransplantation, in vitro ovarian maturation, vitrification.*

Introduction

Human oocytes may get destroyed, besides natural menopause, due to various natural or iatrogenic factors (such as ovarian cancer in patients of reproductive age or ovarian tissue damage due to radiotherapy, chemotherapy and surgical interventions).

It was suggested that ovarian tissue cryopreservation technology may overcome this problem, but it still remains a technically difficult issue so far, in contrast with semen cryopreservation (already available for clinical practice for decades). Recent scientific developments are impressive and have lead to the birth of healthy babies following chains of successful procedures like frozen-thawing oocyte, in vitro maturation, lab fertilization and embryo transfer.

Scientific progress has also been achieved in ovarian tissue autotransplantation to another part of the human body (such as the upper limbs or the upper abdomen).

Such scientific achievements may lead to the foundation and development of ovarian tissue or oocyte laboratory deposits for clinical use, similar to the semen laboratory deposits which have already been successfully created.

Ovary-destructive influence of ovarian cancer treatments

A. Radiotherapy

Low-dose X-ray exposure stimulates oocytes. However, when exceeding 1 Gy doses, a gradual primary oocyte destruction dose- dependent effect is noted; estimates are that a 9 Gy dose induces complete and irreversible damage.

We should also note that the X-ray destructive influence varies from one patient to another, this effect being age-dependent.

X-ray can also damage the uterus, as suggested by poor implantation results following embryo transfer (embryos resulting from ovarian donation to the uterus of patients who had already received intracavitary irradiation).

B. Chemotherapy

Most chemotherapy regimens have a detrimental effect on growing oocytes as suggested by post-chemotherapy amenorrhea.

Alkylating agents in particular seem to be highly toxic, directly targeting

primary oocytes and thus depleting the overall oocyte reserve (which obviously leads to premature menopause and definitive infertility).

C. Surgical therapy

It looks like surgical therapy may also have a detrimental effect on fertility due to oophorectomy (albeit total or partial) or to other mutilating surgical procedures performed on patient's genital tract (hysterectomy, salpingectomy, surgically-induced poor blood supply etc.).

Medications preventing the ovarian destruction induced by radio- and chemo-therapy

We distinguish two types of drug regimens: antioxidant regimens and gametogenesis-suspending regimens.

A. Antioxidant regimens

The detrimental effects of X-rays and alkylating agents on ovaries are due to oxygen-free radicals. The antioxidant regimens decrease the toxic effects of oxygen-free radicals.

Although some benefits of antioxidant regimes were noted in animal studies, no such benefits were proven for human ovaries.

However, limiting the detrimental effects of cancer therapies on ovaries may also compromise their required aggressive effects against cancer cells, thus decreasing their therapeutic efficacy.

In conclusion the use of antioxidant regimens may be not only ineffective but also dangerous for patient's health.

B) Gametogenesis-suspending regimens

The theoretical concept for their clinical use was based on two arguments.

a. Most anticancer therapies damage gonadal cells (albeit growing or already differentiated cells), so we could estimate that a primary oocyte is less sensitive than a mature one.

b. The gonads are less sensitive in children than in adults to the deleterious effects of anticancer therapies.

These two theories have already been confirmed by animal experimental models in which the use of Gn RH-analogues or contraceptive pills has actually protected the gonads. Unfortunately no such protective effect was noted for human gonads, although human studies had rather small patient enrollment counts and were mainly retrospective (hence, of limited value).

Nevertheless it was recently confirmed that X-rays and alkylating agents are most detrimental to primary oocytes.

In conclusion, inducing an iatrogenic, temporary and reversible menopause during chemo- and/or radio-therapy does not provide any clinical benefits, although some clinicians still adhere to such recommendations[1,2,3].

Development of ovarian cryopreservation methods

Ovarian tissue cryopreservation is a recently developed procedure in an attempt to overcome the loss of viable follicles due to malignant disorders, therapeutic side-effects or premature menopause. The first relevant reference was published in 1994 by Gosden et al[4] but major concerns about this new technology have quickly arisen.

While semen cryopreservation has already been implemented for clinical use for decades, the development of ovarian tissue cryopreservation was drawn back by difficulties related to preserving the integrity of the cytoplasm and of the nucleus of unfertilized ova during freeze-thaw cycles. Apparently these cells are very sensitive.

Due to advances in assisted reproductive technologies (such as IVF) ovarian freezing was made possible nowadays, since the ova can be recovered following hormone therapy at specific menstrual cycle times.

Besides IVF and ova-freezing procedures, in malignancies the ovarian tissue can be recovered for later freezing either by laparoscopy or by laparotomy. In such situations only one ovary is usually removed, so that normal hormone production can be sustained by the remaining ovary. Follicles are confined in the ovarian cortex, which is later cut in small pieces to be later on cryopreserved at - 196°C using specific cryoprotective agents (such as DMSO). All laboratory equipment requirements are suitably planned so that the whole freezing procedure gets performed slowly, in a controlled way.

Clinical uses of frozen ovarian tissue

The survival rates for frozen ovarian tissue following freeze - thaw cycles are high, especially since the development of modern fast freezing procedures (vitrification). When the clinical status of the patient permits it, tissue can be thawed and used for patient's fertility benefits.

There are three main methods for the clinical use of frozen ovarian tissue.

- Autotransplantation (albeit orthotopic or heterotopic);
- Xenotransplantation;
- In vitro follicular maturation.

Autotransplantation

A. Autotransplantation is the surgical reinsertion of ovarian tissue into patient's body, either into the pelvis (orthotopic transplantation) or into another part of the human body (heterotopic transplantation). The tissue function is restored within months in most cases[5,6,7].

An apparent advantage of the orthotopic autotransplantation is that spontaneous conception can be achieved without using stimulating agents - which may be detrimental for estrogen - dependent tumors (e.g. some breast cancers).

On the other hand the main disadvantage of orthotopic autotransplantation is the quite limited extent of neoangiogenesis is pelvis; therefore, the quite poor blood supply of the autotransplanted ovary may further restrict its viability and function.

In contrast heterotopic autotransplantation (ovary reinsertion into the forearm) provides a more adequate neoangiogenesis (better linked to regional blood vessels) which leads to an earlier and improved function and long-term viability of the tissue[8]. However, since natural conception is impossible in this case, ovarian stimulation and IVF procedures are required for pregnancy (but we already noted that these procedures are contraindicated in estrogen-dependent malignancies).

The recovery of a single oocyte in natural cycle fertilization via IVF and transferring the single embryo into the uterus could be a reliable answer in this specific clinical challenge.

The first birth of a live child following orthotopic autotransplantation of cryopreserved ovarian tissue was accomplished by Donnez et al in Brusells (2004). The ovarian tissue harvested from a 25 year old patient with breast cancer before chemotherapy initiation. The autotransplantation into her pelvis was performed 7 year later. Following natural conception she delivered a healthy female baby weighing 3.72 kg[9].

The first IVF application for creating an embryo after heterotopic autotransplantation was performed by Oktay et al in 2004.

The ovarian tissue was harvested from a 30 year old patient with breast cancer (prior to chemotherapy) and frozen; 6 years later, the tissue was transplanted under her belly skin. Three months later the tissue resumed its normal function, menses restarted and the IVF procedure could be used on an oocyte recovered from natural menstruation. The transfer of the 4 cell embryo was performed, but unfortunately there was no conception[10].

In conclusion, both the endocrine function and the fertility can be restored (following long-term ovarian tissue cryopreservationandautotransplantation) in patients with malignancies whose gonadal function was compromised by chemo-or radio-therapy.

B) Xenotransplantation

Xenotransplantation is defined as the surgical reinsertion of patient's thawed ovarian tissue into immunodeficient animals such as SCID mice. Although satisfactory survival rates for human follicles were achieved, so far no scientific team has been able to maturate human follicles beyond the stage of 5 to 7 mm, although adequate follicular stimulation was administered given to those mice. Moreover, relevant bioethical dilemmas also arise.

In conclusion, xenotransplantation is so far only a matter of theoretic concern and its future application for clinical uses is not yet deemed as possible[11].

B. In vitro follicular maturation (IVM)

In vitro follicular maturation (IVM) is a modern assisted reproductive procedure, the main indication of which is the prevention of in vivo ovarian stimulation complications (such as ovarian hyper stimulation syndrome, mainly in patients with polycystic ovaries).

The latest indication for clinically using this method is for preserving the fertility function in spite of ovarian destruction due to cancer therapies. In these situations the IVM procedure is used in conjunction with freeze-thawing of in vitro matured ovocytes, with in vitro fertilization and later with embryo transfer (whenever the clinical status permits it).

Should the above mentioned technique be improved in the near future, it will definitely become best treatment option in such patients for two reasons:

1. It is, technically speaking, much easier to perform a follicular puncture or ovarian biopsy than to surgically harvest ovarian tissue, later followed by surgical autotransplantation,
2. There is no risk for reinserting into the patient's body of any possible remaining malignancy originating in the ovarian graft[12].

The Copenhagen and Helsinki scientific teams are the worldwide pioneers of IVM research. Nevertheless the Seang Lin Tan and Richeng Chian teams (Mc Gill Reproductive Center, Canada) are the international pioneers of combining the IVM and ovarian cryopreservation.

The first team who announced pregnancy in July 2007 used this method; 20 patients with polycystic ovary disease were chosen and 296 ovocytes were collected with no ovarian stimulation (the diameter ranged from 5 to 9 mm and they were matured in the laboratory for 48 hrs). Subsequently, 215 of them were matured in the lab using specific rapid freezing procedures (Cryoleaf oocyte vitrification, McGill University) and they were thawed a few months later. 148 of them survived (two out of three) and were fertilized in vitro.

64 embryos were received (a 43% fertilization rate) and transferred to the patients. 3 positive pregnancy tests were obtained for the 5 last women enrolled in the study after significant improvements into the consistence of the media used for follicular IVM[13].

Although this study did not enroll malignant patients and in spite of the fact that the freezing lasted only for a few months, it is still obvious that it opens new scientific horizons for preserving fertility in women with malignancies.

Actually, additionally to the above mentioned advantages of joint-using IVM and cryopreservation, a third advantage is soon expected to emerge: high pregnancy rates.

Conclusions

1. Malignant disorders in women of reproductive age induce severe fertility issues, albeit due to ovarian destruction due to the disease, or due to chemo- or radio-therapy side effects.

2. Conservative treatment with drugs expected to preserve fertility has been deemed insufficient.

3. Great progress has been achieved with modern techniques for later ovarian tissue autotransplantation (orthotopic or heterotopic).

4. The in vitro maturation of ovocytes (harvested either by follicular puncture or by ovarian tissue biopsy) followed by rapid freeze-thaw procedures, in vitro fertilization and embryo transfer (when clinical status permits it), seem to yield great promises.

5. Modern rapid freezing procedures (vitrification) lead to high survival rates of frozen ovocytes, which further leads to higher pregnancy rates. ■

References

1) Plante M. Fertility preservation in the management of gynecologic cansers Curr Opin Oncal, 2000 ;12:496-507.
2) Pereyra Pacheco B, et al. Use of Gn RH-analogues for functional protection of the ovary and preservation of fertility during cancer treatment in adolescent: a preliminary report. Gynecol Oncol, 2001; 81:391-397.
3) Clavere P.et al. Methodes non chirurgicales de protection de la fertilite lors des traitements anticancereux, in Fertilite apres traitements anticancereux, Y.Aubard and F.Olivennes, Editors 1999, Masson: Paris, p.25-40.
4) Gosden RG et al. Restoration of fertility to oopherectomized sheep by ovarian autografts stored at-196 degrees C. Hum Reprod.1994 Apr; 9(4): 597-603.
5) Parrott D. The fertility of mice with orthotopic ovarian. Ovarian graft derived from frozen tissue. J Report Fert, 1960;1:230-241.
6) Aubard Y. et al. Greffes et transplantation ovariennes chez la femme: le point Rev fr Gynecol Obstet, 1993; 88:583-590.
7) Oktay K et al. Ovarian function after transplantation of frozen, banked autologens ovarian tissue. N Eng y Med, 2000; 342:1919.
8) Oktay K et al. Restoration of ovarian function after autologous transplantation of human ovarian tissue in the forearm. Fertil Steril, 2000;74 :90-91.
9) Donnez j, et al. Livebirth after cryopreserved ovarian tissue autotranslantation. Lanset, 2004; 364(9451):2092-93.
10) Oktay K et al.Embryo development after heteotopic transplantation of cryopreserved ovarian tissue. Lanset, 2004;363(9412):837-840
11) Nissole et al.Histologic and ultrastructural evaluation of fresh and frozen - thawed human ovarian xenografts in nude mice. Fertil steril 200; 4(1):122-129.
12) Shaw J.M.et al. Fresh and cryopreserved ovarian tissue samples from donors with lymphoma transmit the canser to graft recipients.Hum.Reprod, 1996; 11:1668-1673.
13) Wean-Young Son,Jin-Tae Chung,Ri-Cheng Chian et al.A 38h interval between h (G priming and oocyte retrieval increases in vivo and in vitro oocyte maturation rate in programmed IVM cycles Hum Reprod. 2008;23:2010-2016.