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Review

Cryopreservation and autotransplantation of human ovarian tissue prior to cytotoxic therapy – A technique in its infancy but already successful in fertility preservation ☆

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ABSTRACT

Increasing survival rates in young cancer patients, new reproductive techniques and the growing interest in quality of life after gonadotoxic cancer therapies have placed fertility preservation as an important issue to oncologists, fertility specialists and patients.

Several techniques are now available for fertility preservation in these patients. A new promising method is cryopreservation and transplantation of ovarian cortex. Ovarian tissue can be extracted by laparoscopy without any significant delay of gonadotoxic therapy. The tissue can be cryopreserved by specialised centres of reproductive medicine and transplanted in case the women experience premature ovarian failure (POF).

This review summarises the European expertise on cryopreservation and transplantation of ovarian tissue, following around 30 reported transplantations globally, resulting in six live births and several ongoing pregnancies. It emphasises that fertility preservation by the cryopreservation of ovarian tissue is a new but already a successful clinical option, which can be considered for selected cancer patients.

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1. Introduction

A report released by US president Bush's cancer panel in 2003 'Living beyond cancer' highlighted[41] that fertility preservation is becoming an important quality of life issue to the growing population of cancer survivors treated during their fertile years. The American society of clinical oncology responded by issuing recommendations advising oncologists to address the possibility of infertility and options for fertility preservation with patients treated during the fertile years. [22]

The cryopreservation of oocytes and embryos is now becoming a standard part of infertility treatment. However, the delay which is often required for ovarian stimulation prior to egg collection excludes this option in some cases, since it may postpone the start of chemotherapy/radiotherapy treatment regimes. Further, the number of oocytes is limited, which can have a direct effect on reduced conception rates.

Cryopreservation of ovarian cortex opens new perspectives. Ovarian tissue can easily be extracted by laparoscopy without any significant delay of potentially 'gonadotoxic' therapy. The tissue can be cryopreserved by specialised centres of reproductive medicine and transplanted in case the women experience premature ovarian failure (POF).

In approximately 30 women, ovarian tissue has already been transplanted successfully globally, resulting in the birth of six children, mainly in Europe and Israel.

The present review summarises the European expertise in the cryopreservation and transplantation of ovarian tissue, following an Exploratory workshop of the European Science Foundation in May 2008 (http://www.esf.org/activities/exploratory-workshops/workshops-list.html?year=2008). It emphasises that fertility preservation by the cryopreservation of ovarian tissue is a new but a promising option with a limited clinical experience. As it has been proven to be successful, it can be considered in selected patients with a high risk of ovarian failure due to chemotherapy or radiotherapy.

2. Extraction of ovarian tissue

Extraction of ovarian tissue can easily be performed by laparoscopy and takes around 30 min. It is possible to conduct the procedure as day surgery. Two techniques have been reported.

The first option is to extract around 50% of one ovary by removing a block of cortical tissue (Fig. 1) or by removing 5–10 ovarian cortex biopsies with an average volume of each biopsy of around 5 mm^3. Electrocoagulation should be avoided as histological analysis revealed the destruction of primordial follicles along the incision of the tissue (personal communication, M. Montag). Operation complications such as bleeding or infections are rare according to the national register of the network FertiPROTEKT[12]. None complication were reported in 116 patients, in which ovarian tissue was removed by laparoscopy.

Alternatively an entire ovarectomy can be performed. However, total ovarectomy may only be indicated in cases with radiation of the pelvis, bone marrow transplantation or high dosage chemotherapy, imposing a very high risk of complete ovarian destruction. If the chance of secondary amenorrhoea following chemotherapy is <50%, partial ovarectomy should be preferred as total ovarectomy could possibly increase the risk of secondary amenorrhea due to the decrease of the ovarian reserve.

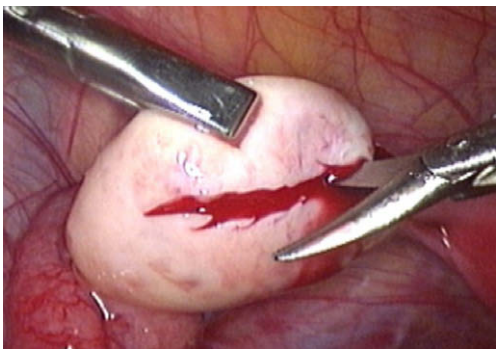

Fig. 1 – Extraction of ovarian tissue by laparoscopy. If the chance to regain ovarian function after gonadotoxic chemotherapy is >50%, partial ovarectomy should be preferred in order not to reduce the chance of spontaneous pregnancies.

3. Cryopreservation of ovarian tissue

Cryopreservation procedures must ensure follicular viability and integrity of tissue compartments and cell-to-cell contacts.[13,18]

Currently, the so-called slow cooling method is mostly used and employed in clinical programmes. An additional technique, vitrification of the ovarian cortex, is currently being developed and receives considerable research interest.[24]

With the slow freezing method, the ovarian cortex is isolated to the thickness of one to 2 mm. This secures preservation of the majority of primordial follicles (i.e. the non-growing follicular reserve) which is exclusively located in the cortex. Individual pieces measuring 5×5 mm are normally prepared and cryopreserved in individual tubes. Different classical permeating cryoprotectants such as dimethyl sulphoxide, 1,2-propanediol and ethylene glycol are most often used in combination with non-permeating substances such as sucrose and human serum albumin.[17,37,8,28] The actual cooling requires a programmable freezer that stepwise and slowly can lower temperatures to at least –40 °C from which the tubes can be plunged into liquid nitrogen for storage. Ovarian tissue maintains viability for many years at –196 °C, as demonstrated by the birth of children after the transplantation of tissue which was stored for up to 6 years.[8] Vitrification is a promising new cryopreservation technique, in which the tissue is exposed to high concentrations of permeating cryoprotectants for a short while (i.e. minutes) and is plunged directly into liquid nitrogen. This induces a glass-like state in the cells and avoids the formation of destructive ice-crystals.[14] Although transmission electron microscopy revealed successful survival of follicles after vitrification/warming (personal communication, V. Keros) and ovarian stroma and vessels appear to be well preserved, the value of this technique in a clinical setting requires further investigations.

4. Centralised cryobanking of ovarian tissue

The increasing number of patients, who are offered the cryopreservation of ovarian tissue,[12] and the necessity for proper quality control in view of future transplantation require appropriate structures as well as proper equipment and personnel to fulfil clinical, legal and scientific standards. Within the European Union all relevant interventions (retrieval, transportation, processing, cryopreservation, storage and transplantation) must follow the EU Tissue Directive 2004/23/EG[11] and the accompanying commission directives. Centralised cryobanking is an economic and effective way of introducing this relatively a new treatment option to the benefit of as many patients as possible. The feasibility of centralised cryobanking has been proven by the Danish experience of transporting ovarian tissue prior to freezing[1] and has also been introduced in Germany.[42]

Centralised cryobanking is a rational way of providing a nationwide cryopreservation service. It allows local hospitals to perform the laparoscopy and to extract the ovarian tissue. The tissue is transported in thermoboxes – that maintain 2–8 °C for >24 h – to the cryobank by commercial transportation companies within 6–24 h for final processing and cryopreservation.[42]

The centralised cryobanks should be highly specialised and capable of performing investigations which are not readily performed in local centres. For example, histopathological investigation of small tissue biopsies taken from the fresh ovary can provide information on the presence and density of primordial follicles. Quality control in the form of follicular viability staining should be assessed. In order to prevent transmission of the underlying disease in oncological or haematological cases, small parts of ovarian tissue from different sites should be frozen separately for later molecular genetic and/or immunohistochemical analysis of candidate genes and gene-products associated with the malignant disease.

Centralised documentation of these and all other relevant data within the cryobank will also benefit the patients as adequate information will be available in the course of the decision making process prior to transplantation.

5. Orthotopic re-transplantation of ovarian tissue (Table 1)

Two different sites can be considered for orthotopic transplantation and both have proved to be successful. The tissue is either transplanted in or onto the remaining ovary or into a peritoneal pocket in the pelvic peritoneum of the fossa ovarica. In many cases, transplantation in or onto the ovaries was preferred as natural conception can occur. In all other cases, in vitro fertilisation (IVF) was performed as transportation of the oocytes/embryos via the tubes into the uterine cavity was not possible (Table 1).

Donnez et al.[8] reported the first successful transplantation of cryopreserved ovarian tissue resulting in a pregnancy and live birth. Ovarian transplantation was done in a peritoneal pocket in two steps. At initial laparoscopy, a peritoneal window was created to induce angiogenesis and neovascularisation in this area. Seven days later, the second laparoscopy was performed to reimplant the frozen–thawed ovarian tissue.

Table 1 – Published cases, in which cryopreserved human ovarian tissue was transplanted.

Reference	Storage ≈ years	Number of cases	Indication[a]	Age before freezing	Location of tissue[b]	Outcome[c]
[30]	0.5	1	Benign disease	29	P	E2 production
[34]	2	1	HL	36	O	E2 production
[4]		1	Benign disease	47	A	E2 production
[21]		1	Cancer of the cervix	37	A	E2 production
[31]	6	1	Breast cancer	30	F	IVF (1 ET)
[8]	6	1	HL	25	P	Birth (sp.); LSK
[46]	4,5	1	Red cell aplasia	30	F	E2 production
[28]	2	1	NHL	28	O	Birth (IVF, 1 E); Lap
[9]	7	1	Sickle cell anaemia	21	O, P	E2 production
[33]	4	2	HL	29	A	Miscarriage (x1); birth (sp.)[d]
[6]	2	2	HL	24	A, O, P	Miscarriage (x1); birth (sp.); LSK
[1]	2	6	HL(x3), NHL,	25, 26, 27	A, O, P	Birth (x2),
[35]			Breast cancer,	28, 32, 36		Miscarriage (x2) (IVF, 11 E);
			Ewing Sarcoma			LSK
[39]	3	1	Ovarian failure	28	O	Birth (sp.); Lap[e]
[7]	2	1	Carcinoma of the anus	28	P	E2 production
[36]		4	Breast cancer, HL (x3)	32,33,34,39	O	E2 production (x2)
[11]	5–8	3	HL, NHL, Wegener's Granulomatosis	22, 28,22	O	E2 production

a HL = Hodgkin's lymphoma; NHL = non-Hodgkin's lymphoma.

b Location of transplanted tissue: A = abdominal wall; F = forearm; O = ovary; P = pelvic peritoneum.

c ET = transfer of embryos (E = embryo) following in vitro fertilisation or intracytoplasmatic sperm injection (IVF); sp. = spontaneous pregnancy; Transplantation of tissue by LSK (laparoscopy) or laparotomy (Lap).

d Spontaneous pregnancy and birth occurred after heterotopic transplantation into the abdominal wall, not into the ovaries or peritoneum (see text).

e Spontaneous pregnancy and birth occurred after transplantation of tissue from a monozygotic twin, not from the patient herself.

From 5–9 months after reimplantation, concentrations of FSH, 17β-estradiol and progesterone showed the occurrence of ovulatory cycles. At 11 months, the patient became pregnant and subsequently delivered a healthy baby.

The same team performed five other cases of reimplantation by grafting the ovarian cortical pieces onto the remaining ovary after the cortex of this ovary had been removed[9,10] (Fig. 2a and b). This procedure was done by laparotomy for the first patients (Fig. 2a), and by laparoscopy (Fig. 2b) for the latest,[10] with the same results in terms of ovarian function recovery. Large ovarian cortical strips were attached to the medulla with two stitches of 7/0 suture (Prolene). Small cubes were placed on the medulla and held in place with absorbable adhesion barrier (Interceed), itself sutured to the remaining cortex of the native ovary. In all the cases, it took between 4 and 5 months after reimplantation before a follicle could be seen.

In 2005, Meirow et al.[29] published a live birth after orthotopic autotransplantation of cryopreserved ovarian tissue. Ovarian strips were sutured onto the ovary. The ovarian tissue developed follicles, and a single mature oocyte was retrieved and fertilised. The patient became pregnant from this embryo transfer and delivered a healthy infant.

Demeestere et al.[5] reported a pregnancy after natural conception in a woman who had undergone orthotopic (tissue fragments were inserted into the ovary) and heterotopic transplantation of cryopreserved ovarian tissue. Unfortunately, this pregnancy, obtained by natural conception, ended in miscarriage at 7 weeks, due to aneuploidy. Thereafter, the patient underwent a second orthotopic transplantation (tissue fragments were sutured onto the ovary) and became pregnant spontaneously.[6] She delivered a healthy baby.

Andersen et al.[1] reported a series of six women who underwent orthotopic and heterotopic autotransplantation (tissue fragments into two pockets created on either side of the ovary or into subperitoneal pockets), two of them receiving additional heterotopic transplants. They observed also a restoration of ovarian function in all women. After ovum pick-up during natural cycle-IVF, two women became pregnant and delivered a healthy baby.

Silber et al.[39] also reported a birth after the transplantation of cryopreserved ovarian tissue (tissue fragments were sutured onto the ovary). The patient who suffered from idiopathic premature ovarian failure received tissue from her monozygotic twin and conceived spontaneously.

So far, nine pregnancies occurred, three resulted in miscarriages and six children have been born, following orthotopic transplantation (Table 1). The tissue in these cases had been stored for up to 6 years. Two of these patients required laparotomy and the others required laparoscopy for transplantation. Five pregnancies occurred spontaneously and five following assisted reproduction.

Summarising orthotopic and heterotopic transplantations, followed by assisted reproduction, 13 embryos were transferred following follicle aspiration. Five pregnancies occurred, two resulted in miscarriages and three babies were born. The implantation rate of these embryos was 38% and thereby higher than that in normal IVF-treatments (15%; German IVF Register[15]). The reason for performing assisted reproduction instead of awaiting spontaneous pregnancy was either transplantation to heterotopic sites or to better control the fertilisation process.

Maximum age of those patients who delivered a baby was 28 years at the time of cryopreservation of ovarian tissue. It has been suggested to define an upper age limit for the cryopreservation of ovarian tissue as the density of primordial follicles and the chance to conceive declines significantly with age.[15] An upper age limit of around 35 years is suggested by the authors of the manuscript.

6. Heterotopic transplantation of ovarian tissue (Table 1)

In heterotopic transplantation, ovarian cortical fragments are not transplanted to the remaining ovary or into the pelvic wall but to any other site such as subcutaneous space of the forearm or the abdominal wall.

The first embryo after heterotopic transplantation of frozen–thawed ovarian tissue to the forearm of a breast cancer patient was generated in 2004.[31] In 2006, Oktay reported about a pregnancy after heterotopic transplantation to the suprapubic region.[32] The patient developed a spontaneous pregnancy following ovulations from those ovaries, previously destructed by the chemotherapy. As premature ovarian failure had been diagnosed by high FSH levels, the occurrence of this pregnancy cannot readily be explained. Following experiments in mice,[23] it has been speculated that the transplanted tissue can induce growth of remaining ovarian follicles.

In the same year, Rosendahl and colleagues reported the generation of an embryo using Intra cytoplasmatic sperm

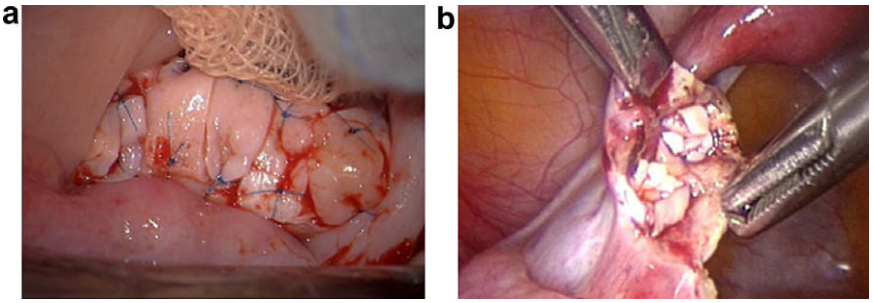

Fig. 2 – (a) Orthotopic ovarian tissue transplantation by laparotomy. Large ovarian cortical strips are attached to the medulla with two stitches. (b) Orthotopic ovarian tissue transplantation by laparoscopy. Small cubes are placed on the medulla. They are then held in place with absorbable adhesion barrier.

injection (ICSI)[1,35] following the aspiration of mature oocytes from follicles that developed in tissue, transplanted to the subperitoneally space in the abdomen.[35] The IVF cycle resulted only in a biochemical pregnancy (increase of HCG).

While orthotopic transplantation is usually preferred as it offers the possibility of natural conception, heterotopic transplantation may be indicated if the pelvis is not suitable for transplantation due to previous radiation or severe scar formation.

However, there are still a number of challenges to perfecting the heterotopic ovarian transplants as the oocyte maturation process appears to occur different than in the orthotopic environment. It remains to be evaluated if the different pressure conditions at the heterotopic transplantation site or different temperature conditions are responsible for the failure of heterotopic transplantation.

7. Risks of ovarian metastasis

If ovarian tissue is cryopreserved and transplanted, metastasis must be definitely excluded to avoid reintroducing malignant cells. Cryopreservation of ovarian tissue should therefore be limited to those patients and diseases, in which the risk of ovarian metastasis is very low.

In Hodgkin's disease, no ovarian metastases have been identified, and transplantation can be considered safe.[26,45,38] Currently, >10 women who had Hodgkin's lymphoma have received transplantation with frozen/thawed tissue without any signs of relapse.

Sarcomas are among the most common malignancies at young age, and also there the risk can be considered low as ovarian metastasis is very rare.

As regards breast cancer, a possibility for ovarian metastases exists in cases with metastatic breast cancer. Cryopreservation in patients with metastasis in lymph nodes or other organs should therefore only be performed with great care and after careful counselling.

In Non-Hodgkin's lymphomas, there is a low but known risk, and one case with ovarian metastasis of Burkitt's lymphoma was identified.[29]

8. Techniques to exclude ovarian metastasis

If cryopreservation of ovarian tissue was performed in cases in which ovaries are at risk to contain malignant cells, sophisticated techniques are required to exclude ovarian metastasis and malignant cells.

Three different approaches have been suggested:

First, imaging (sonography, CT scan, etc.) should be performed in all patients before ovarian tissue collection in order to exclude macroscopic ovarian pathology. In addition, a small piece of fresh ovarian tissue should be evaluated histologically for the presence of sufficient follicles and/or malignant cells.

Second, techniques such as immunohistochemistry and polymerase chain reaction (PCR) can be applied to exclude single malignant cells, especially in haematological malignancies. Meirow et al.[29] applied different techniques in patients treated for chronic myelogenous leukaemia. He identified minimal residual disease (MRD) by highly sensitive RT-PCR in ovarian tissue samples from one patient requiring tissue transplantation and consequently rejected the transplantation of the tissue. Ovarian from another patient was free of MRD.[29]

Third, xenotransplantation of small pieces of frozen/thawed ovarian tissue in SCID (severe combined immunodeficient) mice[20] is a very effective method for the detection of remaining cancer cells. This method also contributes to the assessment of the development potential of stored ovarian tissue before a possible transplantation, which can either be analysed by cell culture[19] or by xenotransplantation.[25]

9. When should cryopreservation of ovarian tissue be offered? (Table 2)

As the transplantation of ovarian tissue is still experimental, it is difficult to define clear recommendations which patients should be offered this technique. Table 2 contains several recommendations which are suggested by the authors who are all involved in large fertility preservation programmes.

Table 2 – Maximum patient's age, types of cancer diseases and kinds of gonadotoxic therapies, in which – according to the authors – the cryopreservation of ovarian tissue should be considered. The list is limited to those conditions, in which the risk of ovarian destruction by gonadotoxic therapies is > ~30% and the chance to generate pregnancy after re-transplantation can be expected to be high and the risk for the patients to spread metastasis by ovarian re-transplantation has been shown to be very low.

Patient's age:
- ○ Maximum age of the patient: 35–(38) years

Cancer diseases:
- ○ Hodgkin lymphoma
- ○ Sarcoma
- ○ Breast cancer

Gonadotoxic therapies:
- ○ External radiation of the pelvis >5–10 Gray
- ○ Hematopoietic stem cell transplantation
- ○ Chemotherapies involving alkylating agents such as cyclophosphamide

In all other cases, different techniques of fertility preservation such as ovarian stimulation[43] and cryopreservation of unfertilised and fertilised oocytes, [33] gonadotropin releasing hormone analogues (GnRH-a)[3] or surgical interventions need to be discussed.[40]

According to the authors, cryopreservation should always be offered to patients, with a high (>30–50%) risk of POF due to the gonadotoxic therapy and a low risk of ovarian metastasis. If the risk is lower, cryopreservation can also be offered if the patient is very frightened to loose her ovarian function.

Cryopreservation can and has been performed in young pre-pubertal girls. The maximum age has not yet been determined but most centres currently limit service to women in their mid-thirties. In women around 40 years of age, the number of follicles is presently considered to be too low to generate pregnancies following transplantation. In patients aged 35–38 years the ovarian reserve can be evaluated using ultrasound measurements and levels of follicle stimulating hormone (FSH), inhibin-B and anti mullerian hormone (AMH) to individually assess the remaining ovarian.[16]

The risk to develop POF is related to the age of the patient and the agents being used. The younger the patient, the lower the risk to develop POF. Patients with hodgkin's lymphoma who received a chemotherapy with an escalated dosage of BEACOPP (Cyclophosphamide, Doxorubicin, Etoposide, Procarbazine, Prednisone, Vincristine, Bleomycin) chemotherapy developed secondary amenorrhea in 40.4% at the age of <30 years and in 70.4% at the age of $\geqslant$30 years.[2] The risk of POF increases with the total dose of chemotherapy agents applied, especially if alkylating agents are used.

The risk to develop secondary amenorrhea is also very high in the case of pelvic radiation. According to Wallace et al.,[44] 50% of oocytes are destroyed if two Gray are applied. If around 10 Gray are applied the risk of amenorrhoea increases to >50%.[27]

The complexity of criteria that need to be considered and the spectrum of alternative fertility preserving techniques that can possibly be offered, clearly indicate that the counselling of patients should be performed by a specialist in reproductive medicine and fertility preservation.

10. Outlook

Fertility preservation by the cryopreservation of ovarian tissue is a new but increasingly successful clinical option. The extraction of ovarian tissue can easily be performed by laparoscopy, and the risk of transplantation of cancer cells remains very low in a number of diseases. This technique should therefore considered in women in her mid-thirties or younger receiving highly gonadotoxic chemotherapy or pelvic radiation. The cryopreservation of ovarian tissue requires the establishment of centralised and highly specialist cryobanks as well as sophisticated networks to integrate oncologists and reproductive specialists in order to provide this procedure to all patients at need.

Conflict of interest statement

None declared.

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