

# Cryopreservation and Transplantation of Ovarian Tissue

JACQUES DONNEZ, MD, PhD,  
and MARIE-MADELEINE DOLMANS, MD, PhD

*Department of Gynecology, Université Catholique de Louvain,  
Brussels, Belgium*

**Abstract:** Cryopreservation and autotransplantation of ovarian tissue for fertility preservation is considered an experimental technique. However, in some clinical situations it remains the only option. Eleven pregnancies have been reported worldwide from this procedure. Transplantation of ovarian tissue pieces without a vascular pedicle requires the establishment of a new blood supply that takes 5 days. This leads to a substantial loss of follicles in the graft that may limit the longevity of the graft.

**Key words:** ovarian tissue transplantation, ovarian tissue cryopreservation, fertility preservation, gonadotoxicity

## Introduction

Advances in the diagnosis and treatment of childhood, adolescent, and adult cancer have greatly increased the life expectancy of premenopausal women with cancer. Indeed, aggressive chemotherapy and radiotherapy, and bone marrow transplantation, can cure more than 90% of girls affected by childhood malignancies, but have resulted in a growing population of adolescent and adult long-term survi-

vors of childhood malignancies who may experience infertility problems because of induced premature ovarian failure (POF).<sup>1</sup>

## GONADOTOXICITY OF CHEMOTHERAPY

The ovaries are very sensitive to cytotoxic treatment, especially to alkylating agents, which are classified as high risk for gonadal dysfunction (eg, cyclophosphamide, busulfan, melphalan, chlorambucil, dacarbazine, procarbazine, ifosfamide, thiotepa, and nitrogen mustard).<sup>2</sup> Doxorubicin and the alkylating-like agents, cisplatin and carboplatin, fall into the medium-risk category, whereas methotrexate, bleomycin, 5-fluorouracil, actinomycin-D, mercaptopurine, and vincristine are considered as low risk for gonadal dysfunction (Table 1).

Cyclophosphamide is the agent most commonly implicated in causing damage to oocytes and granulosa cells in a dose-dependent manner.<sup>3</sup>

This follicular destruction generally results in loss of both endocrine and reproductive function, depending on the dose and the age of the patient. Indeed, Larsen et al<sup>4</sup>

*Correspondence:* Jacques Donnez, MD, PhD, Department of Gynecology, Université Catholique de Louvain, Cliniques Universitaires St Luc, Brussels, Belgium.  
E-mail: jacques.donnez@uclouvain.be

**TABLE 1. Cytotoxic Agents According to Degree of Gonadotoxicity**

High Risk	Intermediate Risk	Low/No Risk
Cyclophosphamide	Doxorubicin	Methotrexate
Busulfan	Cisplatin	Bleomycin
Melphalan	Carboplatin	5-Fluorouracil
Chlorambucil		Actinomycin D
Dacarbazine		Mercaptopurine
Procarbazine		Vincristine
Ifosfamide		
Thiotepa		
Nitrogen mustard		

reported a 4-fold increased risk of POF in teenagers treated for cancer, and a risk increased by a factor of 27 in women between 21 and 25 years of age. Complete amenorrhea was reported after a dose of 5 g of cyclophosphamide in women above 40 years of age, and after doses of 9 and 20 g in women of 30 to 40 and 20 to 30 years of age, respectively. A combination of various chemotherapeutic agents further increases gonadal toxicity. After chemotherapy with mechlorethamine, oncovin, procarbazine, prednisone/adriamycin, bleomycin, vinblastine hybrid chemotherapy, amenorrhea developed in 89% and 20% of patients above and below 25 years of age at the time of treatment, respectively. The median age of patients who became amenorrheic after therapy was significantly higher than that of patients who maintained normal menses (26 y vs. 20 y;  $P = 0.008$ ).

#### **GONADOTOXICITY OF RADIOTHERAPY**

Abdominal ionizing radiation associated with alkylating agents often induces POF, rendering patients infertile in almost 100% of cases. Indeed, for radiotherapy, it has been stated that a dose of 5 to 20 Gy administered to the ovary is sufficient to completely impair gonadal function, whatever the age of the patient.<sup>5</sup> The dose of radiation required to destroy 50% of the oocyte reserve has been found to be

approximately 2 Gy. Moreover, uterine irradiation at a young age reduces adult uterine volume. Radiation doses between 14 and 30 Gy have been reported to result in uterine dysfunction. The practitioner should be aware of this effect of radiotherapy on the uterus, which could interfere with the implantation capacity of embryos.

Intensive chemotherapy and/or total body irradiation required before bone marrow transplantation constitute the treatment combination presenting the greatest risk of POF (nearly 100%). Indeed, the high doses of chemotherapy (commonly using the highly cytotoxic cyclophosphamide/busulfan regimen) and/or radiotherapy lead to subsequent ovarian failure in almost all cases, children and adults alike.

A large retrospective survey of pregnancy outcomes after hematopoietic stem cell transplantation (peripheral blood or bone marrow transplantation) involving 37,362 patients revealed that only 0.6% of patients conceived after autologous or allogenic stem cell transplantation.<sup>6</sup> It is thus obvious that high doses of alkylating agents, irradiation, and advancing age increase the risk of gonadal damage.

#### **Indications for Ovarian Tissue Cryopreservation**

Oncologic indications for ovarian tissue cryopreservation are summarized in

**TABLE 2. Indications for Cryopreservation of Ovarian Tissue in Case of Malignant and Nonmalignant Diseases**


---

A. Malignant
(a) Extrapelvic diseases
Bone cancer (osteosarcoma and Ewing sarcoma)
Breast cancer
Melanoma
Neuroblastoma
Bowel malignancy
(b) Pelvic diseases
Nongynecologic malignancy
Pelvic sarcoma
Rhabdomyosarcoma
Sacral tumors
Rectosigmoid tumors
Gynecologic malignancy
Early cervical carcinoma
Early vaginal carcinoma
Early vulvar carcinoma
Selected cases of ovarian carcinoma (stage IA)
Borderline ovarian tumors
(c) Systemic diseases
Hodgkin disease
Non-Hodgkin lymphoma
Leukemia
Medulloblastoma
B. Nonmalignant
(a) Uni/bilateral oophorectomy
Benign ovarian tumors
Severe and recurrent endometriosis
BRCA1 or BRCA2 mutation carriers
(b) Risk of premature menopause
Turner syndrome
Family history
Benign diseases requiring chemotherapy:
autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis, Behçet disease, and Wegener granulomatosis)
(c) Bone marrow transplantation
Benign hematologic diseases: sickle cell anemia, thalassemia major, and aplastic anemia
Autoimmune diseases unresponsive to immunosuppressive therapy

---

Table 2. In case of gynecologic malignancy, a conservative fertility approach is only valuable if the uterus can be spared during surgery. This includes cases of early cervical carcinoma, early vaginal carcinoma, early endometrial adenocarcinoma, ovarian tumors of low malignancy, and some selected cases of unilateral ovar-

ian carcinoma (stage IA). The choice of a possible conservative surgical approach in these patients, and the question of implementing such treatment alone, remains controversial, and all the published results were obtained on the basis of retrospective studies and/or case reports. The fertility outcome is conditioned by the adjuvant therapy, that is, local radiotherapy and/or chemotherapy.

Cryopreservation should not be reserved solely for women with malignant disease.<sup>7</sup> Indeed, hematopoietic stem cell transplantation has been increasingly used in recent decades for noncancerous diseases, such as benign hematologic disease (sickle-cell anemia, thalassemia major, and aplastic anemia), and autoimmune diseases previously unresponsive to immunosuppressive therapy (systemic lupus erythematosus and autoimmune thrombocytopenia). Other benign diseases, such as recurrent ovarian endometriosis or recurrent ovarian mucinous cysts, are also indications for ovarian cryopreservation. Patients undergoing oophorectomy for prophylaxis may potentially benefit from ovarian cryopreservation too. The indications for cryopreservation of ovarian tissue in case of nonmalignant disease are summarized in Table 2.

### ***Fertility Preservation in Cancer Patients: Different Cryopreservation Options***

The only established method of fertility preservation is embryo cryopreservation according to the Ethics Committee of the American Society for Reproductive Medicine,<sup>8</sup> but this option requires the patient to be of pubertal age, have a partner or use donor sperm, and be able to undergo a cycle of ovarian stimulation, which is not possible when chemotherapy has to be initiated immediately or when stimulation is contraindicated according to the type of cancer.

Cryopreservation of oocytes can be performed in postpubertal patients who are able to undergo a stimulation cycle, but the effectiveness of this technique is still low, with delivery rates from 1% to 5% for frozen-thawed oocyte with slow cooling techniques. Nevertheless, since the recent introduction of oocyte vitrification, delivery rates have almost doubled per thawed oocyte.

#### **OVARIAN TISSUE CRYOPRESERVATION**

For patients who need immediate chemotherapy, cryopreservation of ovarian tissue is the only possible alternative. The main aim of this strategy is to reimplant cortical ovarian tissue into the pelvic cavity (orthotopic site) or a heterotopic site like the forearm or abdominal wall once treatment is completed and the patient is disease-free (for review, see Donnez et al<sup>3</sup>).

#### ***Lessons Learned From Xenografting Cryopreserved Human Ovarian Tissue***

Human ovarian tissue can be successfully cryopreserved, showing good survival and function after thawing. Hovatta<sup>9</sup> arrived at this conclusion after reviewing all relevant studies since 1996. The choice of cryoprotectant with maximum permeation capacity but minimum toxicity and ice crystal formation potential is specific to each cell and tissue type. Thus, in the ovary, it is a compromise between the stroma, follicular cells, and oocytes. On the basis of current knowledge, the standard method for human ovarian cryopreservation is slow-programmed freezing, using human serum albumin-containing medium, and propanediol, dimethylsulphoxide, or ethylene glycol as a cryoprotectant, combined or not with sucrose.

All reports on human ovarian tissue grafting to mice (for review see<sup>2,10</sup>) that have studied the implantation site have shown peritoneal transplantation, either under the peritoneum or under the kidney

capsule, to be better than subcutaneous transplantation in terms of follicular survival and development. The "ovulatory" capacity of frozen-thawed human follicles in xenografts was evidenced by the development of follicles up to the antral stage the formation of morphologically normal corpora lutea and elevated progesterone levels in immunodeficient mice. Very few data are available on the final maturation of follicles in xenografts and the quality of oocytes obtained. It has therefore not been established whether human oocytes matured in xenografts are ultrastructurally normal and functionally competent.

Experimental studies have indicated a fall in the number of primordial follicles in grafted tissue that could be because of hypoxia and the delay before reimplanted cortical tissue becomes revascularized. The loss of primordial follicles in cryopreserved ovarian tissue after transplantation is estimated to be at least 50% to 65% in some studies.

#### **THE CRUCIAL ISSUE OF REVASCULARIZATION**

Van Eyck et al<sup>11,12</sup> recently characterized the oxygen environment in human ovarian xenografts in the early postgrafting period (until day 21) using electron paramagnetic resonance oximetry. This technique allows sensitive, noninvasive, and repeated measurement of partial pressure of O<sub>2</sub> in vivo. Before day 5, grafts were exposed to hypoxia. From day 5 to day 10, progressive reoxygenation was observed, suggesting an active process of graft revascularization. Using a combined method of perfusion study and double immunohistochemical staining of human and murine vessels, the same team evaluated the revascularization process of human ovarian tissue in this model.

On day 5, reperfusion of ovarian grafts was initiated by host angiogenesis, as evidenced by the appearance of murine neovessels penetrating from the periphery and colocalized with perfused areas. By

day 10, the center of the fragments was perfused and ovarian graft angiogenesis contributed to the vascular pattern of the ovarian transplants.

Host and graft angiogenesis thus both seems to contribute to posttransplantation vascular behavior and could be potential targets to improve the mechanisms leading to perfusion of grafts with the aim of reducing the avascular period.

### ***Autotransplantation of Cryopreserved Human Ovarian Tissue***

There have been numerous reported cases of autotransplantation of cryopreserved ovarian tissue, either to an orthotopic or heterotopic site.

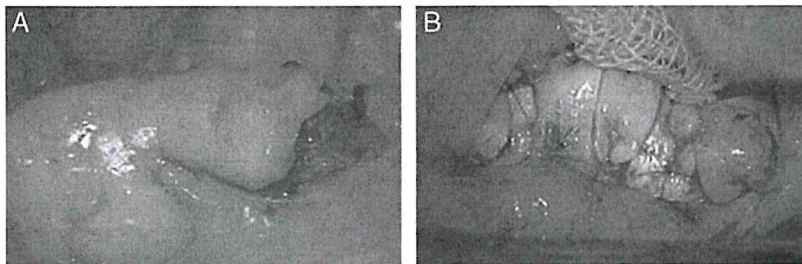
#### **ORTHOTOPIC AUTOTRANSPLANTATION OF CRYOPRESERVED HUMAN OVARIAN TISSUE**

In theory, natural pregnancy may be achieved through orthotopic tissue transplantation if the fallopian tubes remain intact. Figure 1 shows transplantation of ovarian cortical fragments to the site of the remaining ovary.

In 2000, Oktay and Karlikaya<sup>13</sup> reported laparoscopic transplantation of

frozen-thawed ovarian tissue in a 29-year-old patient, who had undergone bilateral oophorectomy for a nonmalignant disease. Follicular development was showed only once by ultrasonography after the patient had been stimulated by gonadotropin 15 weeks after transplantation.

We reported the first successful transplantation of cryopreserved ovarian tissue resulting in a pregnancy and live birth.<sup>14</sup> In 1997, a 25-year-old woman presented with clinical stage IV Hodgkin lymphoma. According to Schilsky et al, the risk of POF after such a regimen in a woman of 26 years of age is more than 90%, whereas according to Wallace et al and Lobo et al, the risk of subfertility after Hodgkin treatment with alkylating agents is more than 80%.<sup>7,15,16</sup> Ovarian tissue cryopreservation was undertaken before chemotherapy. After laparoscopy, the patient received hybrid chemotherapy from August 1997 to February 1998, followed by supradiaphragmatic radiotherapy (38 Gy). In 2003, once the patient had been declared completely disease-free, reimplantation was carried out in orthotopic sites (see Donnez et al for techniques<sup>2</sup>). From 5 to 9 months after reimplantation, concentrations of follicle-stimulating hormone (FSH), 17  $\beta$ -estradiol, and progesterone showed the occurrence of ovulatory cycles. At 11 months, the patient



**FIGURE 1.** This patient had the cortex of 1 ovary removed and cryopreserved before chemotherapy. The other ovary was left intact. At the transplantation process, the cortex of the remaining ovary is removed (A). Ovarian cortical pieces measuring 4 to 5 mm to 1 cm in size were grafted onto this ovary and sutured with 7-0 stitches (B).

became pregnant and subsequently delivered a healthy baby.<sup>14</sup>

In 2005, Meirow et al<sup>17</sup> also published a live birth after orthotopic autotransplantation of cryopreserved ovarian tissue in a patient with POF after chemotherapy. Eight months after orthotopic transplantation, the patient spontaneously menstruated. Nine months after transplantation, she experienced a second spontaneous menstrual period. After a modified natural cycle, a single mature oocyte was retrieved and fertilized. Two days later, a 4-cell embryo was transferred. The patient became pregnant from this embryo transfer and delivered a healthy infant weighing 3000 g.

Demeestere et al<sup>18</sup> reported a pregnancy after natural conception in a woman who had undergone orthotopic and heterotopic transplantation of cryopreserved ovarian tissue. Unfortunately, this pregnancy, obtained by natural conception, ended in miscarriage at 7 weeks because of aneuploidy. The same team performed a second reimplantation to an orthotopic site in the same patient after cessation of graft secretion was evidenced.<sup>19</sup> The patient became pregnant and delivered a healthy baby. She recently gave birth to a healthy child for the second time.

Silber et al<sup>20</sup> reported a pregnancy after reimplantation of cryopreserved ovarian tissue between monozygotic twins. It should be noted, however, that the same woman had already delivered a first healthy baby after reimplantation of fresh tissue.

In 2008, Andersen et al<sup>21</sup> described a series of 6 orthotopic reimplantations of cryopreserved ovarian cortex. In this series, 2 women became pregnant after IVF procedure and delivered healthy babies. Single mature oocytes were retrieved during optimized cycles, fertilized and transferred on day 3. One of these women subsequently became pregnant naturally and gave birth to a second healthy infant.<sup>22</sup>

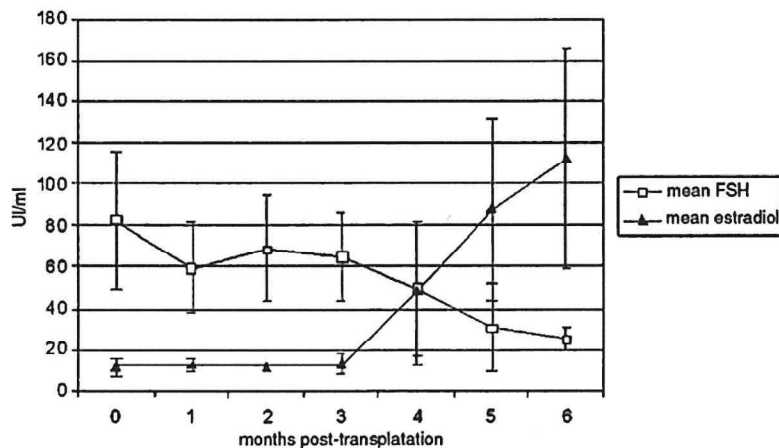
Piver et al<sup>23</sup> reported a pregnancy in 2009 after orthotopic reimplantation of cryopreserved ovarian tissue. The patient became pregnant after an IVF procedure and delivered a healthy baby. The same year, Sánchez-Serrano et al<sup>24</sup> reported the birth of twins (European Society of Human Reproduction and Embryology 2009) after orthotopic reimplantation of cryopreserved tissue and IVF.

In total, 11 live births have been achieved after orthotopic reimplantation of cryopreserved ovarian tissue.

### ***Lessons Learned From Ovarian Orthotopic Transplantation***

We recently published a series of 9 orthotopic transplantations of ovarian tissue in 6 women, proving restoration of ovarian function in all cases. Analysis of these cases raises some important points for discussion.

1. First of all, in all 3 cases, it took between 4 and 6 months after reimplantation before a follicle could be seen by ultrasonography (Fig. 2). The process of folliculogenesis takes around approximately 4 to 6 months, during which time the oocyte and surrounding somatic cells undergo a series of changes that eventually result in the development of a large antral follicle, capable of producing a mature oocyte. Thus, the appearance of the first follicle originating from grafted tissue 5 months after reimplantation, proved by laparoscopy in 1 case, is totally consistent with the expected time course. This time interval between implantation of cortical tissue and the first estradiol peak is also consistent with data obtained from sheep and human beings, although some variations may be observed. Indeed, the delay between transplantation and follicular development was found varying from 6 weeks to 8 months. Such a variation could be explained by a difference in follicular reserve at the time of cryopreservation.
2. Another very interesting finding in our series was the persistence of relatively high FSH levels during the follicular phase. FSH levels remained as high as 25 mIU/mL during the



**FIGURE 2.** Mean FSH and 17 $\beta$ -estradiol levels ( $\pm$  SD) after frozen-thawed ovarian tissue transplantation. It took between 4 and 6½ months after transplantation before a rise in estradiol and a drop in FSH were observed. FSH indicates follicle-stimulating hormone.

follicular phase until ovulation, and then decreased to less than 15 mIU/mL during the luteal phase. This may constitute an argument against the use of gonadotropin injections. The relatively high FSH levels may be explained by the relatively low number of surviving primordial follicles in the graft. The patient should be considered a poor responder, with reduced inhibin-B secretion.

3. A further significant observation was the return to an FSH level of greater than 25 mIU/mL immediately after each menstrual bleed supporting the concept that hormones such as inhibin-B or anti-Müllerian hormones normally produced by developing follicles in intact human ovaries are probably almost greatly reduced in transplanted tissue. After transplantation, the patient would have been regarded a poor responder because, of the 500 to 1000 primordial follicles that would have been transplanted, more than 50% would have been lost because of hypoxia.

#### HETEROTOPIC AUTOTRANSPLANTATION OF CRYOPRESERVED HUMAN OVARIAN TISSUE

There are only a few existing reports on this subject. Kim et al<sup>25</sup> reported a case of

a 37-year-old woman who underwent heterotopic (rectus and pectoralis muscle) transplantation of cryopreserved ovarian tissue. By 14 weeks of transplantation, restoration of endocrine function was shown but, approximately 28 weeks after transplantation, cessation of ovarian function was evidenced by very high FSH levels (62 to 99 mIU/L) and very low estradiol levels.

The same year, Oktay et al<sup>26</sup> reported transplantation of frozen-thawed ovarian tissue beneath the skin of the abdomen. A 4-cell embryo was obtained from 20 oocytes retrieved from an ovarian graft, but no pregnancy occurred after transfer. Oocyte quality might have been compromised by transplantation to a heterotopic site.

Kim et al<sup>27</sup> reported heterotopic transplantation of cryopreserved ovarian tissue in a patient cured of squamous cell carcinoma of cervix. Tissue was transplanted to 2 heterotopic sites: abdominal (rectus muscle) and breast site (pectoralis muscle). Growing follicles were seen in the abdominal site from 14 weeks after transplantation, but ovarian function ceased around 28 weeks after transplantation.

Recently, Kim et al<sup>27</sup> reported heterotopic autotransplantation of cryopreserved ovarian tissue in 4 patients (3 with cervical cancer and one with breast cancer). Thawed ovarian fragments were transplanted into a space between the rectus muscle and the rectus sheath. Recovery of ovarian function was evidenced in 3 patients by hormone profiles obtained between 12 and 20 weeks after transplantation, but only lasted 3 to 5 months. These 3 patients subsequently underwent a second transplantation. Long-term ovarian function (15 to 36 mo) was then established. Ovarian grafts were stimulated daily with FSH, until a dominant follicle size of 14 to 16 mm was reached. During a 27-month follow-up period in 2 patients, 6 oocytes were retrieved (1 germinal vesicle, 4 metaphase I, and 1 metaphase II). The MI oocytes were subjected to in-vitro maturation. All 4 MII oocytes then fertilized and developed to cleavage-stage embryos (up to 6 cells on day 3) before being frozen for transfer to a surrogate.

Articles describing heterotopic transplantation have all reported follicular development, but with follicles always less than 16 mm in size. In our opinion, differences in temperature and pressure could interfere with follicular development in heterotopic sites. As orthotopic transplantation was shown to be efficacious, there is, in our opinion, no indication for heterotopic transplantation if the goal is to restore fertility.

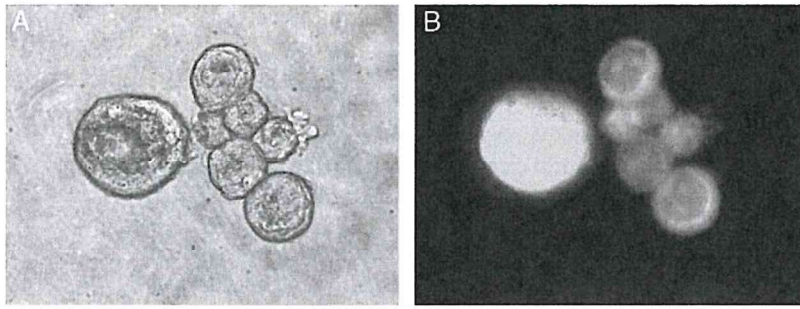
#### **REIMPLANTATION OF ISOLATED PRIMORDIAL FOLLICLES<sup>28,29</sup>**

Although safe transplantation of ovarian tissue from lymphoma patients has been reported in severe combined immunodeficient mice, the possibility of reintroducing tumor cells into cancer patients by autografting of ovarian tissue cannot be excluded. Screening methods must be developed to eliminate the risk of cancer cell transmission with reimplantation. In

some diseases, other options must be considered.

To avoid transferring malignant cells, ovarian tissue culture with in-vitro follicle maturation could be performed.<sup>28,29</sup> Culturing isolated follicles from the primordial stage is another particularly attractive proposition, as they represent greater than 90% of the total follicular reserve and show high cryotolerance. However, isolated primordial follicles do not grow properly in culture and further studies are clearly needed to identify factors sustaining follicular growth and maturation in humans, and to assess the contribution of stromal cells to these processes. Encouraging results were achieved when human primordial follicles were grown in organ culture. Follicle isolation, or partial follicle isolation, can severely impair follicular viability and, after isolation, only more advanced multilaminar preantral follicular stages can survive in short-term culture, a few reaching the early antral stage.

Another approach could be to transplant a suspension of isolated follicles. As the follicular basal lamina encapsulating the membrana granulosa excludes capillaries, white blood cells, and nerve processes from the granulosa compartment, grafting fully isolated follicles could be considered safer. Transplantation of frozen-thawed isolated primordial follicles has indeed been successfully achieved in mice, yielding normal offspring. For human primordial follicles, however, mechanical isolation is not possible because of their size (30 to 40  $\mu$ m) and their fibrous and dense ovarian stroma, and therefore, enzymatic digestion has to be used (Fig. 3). In our group, recent xenotransplantation experiments show encouraging results with development of isolated human follicles to the antral stage. To enhance the chances of follicular survival and reproductive function restoration, enzymatic digestion procedures for human ovarian tissue need to be optimized and standardized.



**FIGURE 3.** Enzymatically isolated follicles (between 30 and 110  $\mu\text{m}$ ) visible under an inverted fluorescence microscope after fluorescent viability staining (calcein-AM and ethidium homodimer-1). Follicles are visible on light microscopy (A) and fluorescence microscopy (B), which show all of them to be viable.

## Conclusions

Approximately one third of young women exposed to chemotherapy develop ovarian failure. In 2010, we believe it is our ethical responsibility to propose cryopreservation of ovarian tissue to all adolescents and young women under institutional review board protocols having to undergo chemotherapy with alkylating agents.

The age of the patient should be taken into consideration, as the follicular reserve of the ovary is age dependent. As a decline in fertility is now well-documented after the age of 38 years, the procedure should probably be restricted to patients below this limit. In any case, irradiation and chemotherapy seem to be less harmful to the gonads of prepubertal than postpubertal women.

We accept that ovarian tissue cryopreservation is a more innovative and invasive procedure than sperm cryopreservation and that all possible applications in adolescents are ethically complex. However, we believe that ovarian cortex banking should be offered before chemotherapy in all cases where emergency IVF is not possible.

In conclusion, the 11 live births obtained after transplantation of frozen-thawed ovarian tissue in humans give a real hope to young cancer patients.

## References

1. Wallace WH, Anderson RA, Irvine DS. Fertility preservation for young patients with cancer: who is at risk and what can be offered? *Lancet Oncol.* 2005;6:209–218.
2. Donnez J, Martinez-Madrid B, Jadoul P, et al. Ovarian tissue cryopreservation and transplantation: a review. *Hum Reprod Update.* 2006;12:519–535.
3. Meirrow D, Lewis H, Nugent D, et al. Subclinical depletion of primordial follicular reserve in mice treated with cyclophosphamide: clinical importance and proposed accurate investigative tool. *Hum Reprod.* 1999;14:1903–1907.
4. Larsen EC, Muller J, Schiegelow K, et al. Reduced ovarian function in long term survivors of radiation and chemotherapy treated childhood cancer. *J Clin Endocrinol Metab.* 2003;88:5307–5314.
5. Wallace WH, Thomson AB, Saran F, et al. Predicting age of ovarian failure after radiation to a field that includes the ovaries. *Int J Radiat Oncol Biol Phys.* 2005;62:738–744.
6. Salooja N, Szydlo RM, Socie G, et al.; the Late Effects Working Party of the European Group for Blood and Marrow Transplantation. Pregnancy outcomes after peripheral blood or bone marrow transplantation: a retrospective survey. *Lancet.* 2001;358:271–276.
7. Donnez J, Dolmans MM, Demylle D, et al. Restoration of ovarian function after orthotopic (intraovarian and periovarian)

- transplantation of cryopreserved ovarian tissue in a woman treated by bone marrow transplantation for sickle cell anaemia: case report. *Hum Reprod*. 2006;21:183–188.
8. Ethics Committee of the American Society for Reproductive Medicine. Fertility preservation and reproduction in cancer patients. *Fertil Steril*. 2005;83:1622–1628.
  9. Hovatta O. Methods for cryopreservation of human ovarian tissue. *Reprod Biomed Online*. 2005;10:729–734.
  10. Aubard Y. Ovarian tissue xenografting. *Eur J Obstet Gynecol Reprod Biol*. 2003;108:14–18.
  11. Van Eyck AS, Jordan BF, Gallez B, et al. Electron paramagnetic resonance as a tool to evaluate human ovarian tissue reoxygenation after xenografting. *Fertil Steril*. 2009;92:374–381.
  12. Van Eyck AS, Bouzin C, Feron O, et al. Both host and graft vessels contribute to revascularization of xenografted human ovarian tissue in a murine model. *Fertil Steril*. 2010;93:1676–1685.
  13. Oktay K, Karlikaya G. Ovarian function after transplantation of frozen, banked autologous ovarian tissue. *N Engl J Med*. 2000;342:1919.
  14. Donnez J, Dolmans MM, Demylle D, et al. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. *Lancet*. 2004;364:1405–1410.
  15. Schilsky RL, Sherins RJ, Hubbard SM, et al. Long-term follow up of ovarian function in women treated with MOPP chemotherapy for Hodgkin's disease. *Am J Med*. 1981;71:552–556.
  16. Lobo RA. Potential options for preservation of fertility in women. *N Engl J Med*. 2005;353:64–73.
  17. Meirow D, Levron J, Eldar-Geva T, et al. Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. *N Engl J Med*. 2005;353:318–321.
  18. Demeestere I, Simon P, Buxant F, et al. Ovarian function and spontaneous pregnancy after combined heterotopic and orthotopic cryopreserved ovarian tissue transplantation in a patient previously treated with bone marrow transplantation: case report. *Hum Reprod*. 2006;21:2010–2014.
  19. Demeestere I, Simon P, Emiliani S, et al. Fertility preservation: successful transplantation of cryopreserved ovarian tissue in a young patient previously treated for Hodgkin's disease. *Oncologist*. 2007;12:1437–1442.
  20. Silber SJ, DeRosa M, Pineda J, et al. Series of monozygotic twins discordant for ovarian failure: ovary transplantation (cortical versus microvascular) and cryopreservation. *Hum Reprod*. 2008;23:1531–1537.
  21. Andersen CY, Rosendahl M, Byskov AG, et al. Two successful pregnancies following autotransplantation of frozen/thawed ovarian tissue. *Hum Reprod*. 2008;23:2266–2272.
  22. Ernst E, Bergholdt S, Jorgensen JS, et al. The first woman to give birth to two children following transplantation of frozen/thawed ovarian tissue. *Hum Reprod*. 2010;25:1280–1281.
  23. Piver P, Amiot C, Agnani G, et al. Two pregnancies obtained after a new technique of autotransplantation of cryopreserved ovarian tissue. In: 25th Annual Meeting of ESHRE, 28 June–1 July 2009. Amsterdam, the Netherlands: Oxford University Press, Hum Reprod 2009:i15.
  24. Sánchez-Serrano M, Crespo J, Mirabet V, et al. Twins born after transplantation of ovarian cortical tissue and oocyte vitrification. *Fertil Steril*. 2010;93:268.e11–268.e13.
  25. Kim SS, Hwang IT, Lee HC. Heterotopic autotransplantation of cryobanked human ovarian tissue as a strategy to restore ovarian function. *Fertil Steril*. 2004;82:930–932.
  26. Oktay K, Buyuk E, Veeck L, et al. Embryo development after heterotopic transplantation of cryopreserved ovarian tissue. *Lancet*. 2004;363:837–840.
  27. Kim SS, Lee WS, Chung MK, et al. Long-term ovarian function and fertility after heterotopic autotransplantation of cryobanked human ovarian tissue: 8-year experience in cancer patients. *Fertil Steril*. 2009;91:2349–2354.
  28. Dolmans MM, Michaux N, Camboni A, et al. Evaluation of Liberase, a purified enzyme blend, for the isolation of human primordial and primary ovarian follicles. *Hum Reprod*. 2006;21:413–420.
  29. Dolmans MM, Yuan WY, Camboni A, et al. Development of antral follicles after xenografting of isolated small human pre-antral follicles. *Reprod Biomed Online*. 2008;16:705–711.