

# Live birth after transplantation of frozen-thawed ovarian tissue after bilateral oophorectomy for benign disease

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**Objective:** To report the restoration of ovarian function and pregnancy in a woman after bilateral oophorectomy for benign disease after autotransplantation of cryopreserved ovarian cortex.

**Design:** Case report.

**Setting:** Gynecology research unit in a university hospital.

**Patient(s):** A 28-year-old woman who underwent bilateral adnexectomy for ovarian abscesses at the age of 18 years.

**Intervention(s):** We performed ovarian cortex autotransplantation to a peritoneal pocket in the broad ligament.

**Main Outcome Measure(s):** Restoration of ovarian activity and pregnancy.

**Result(s):** Restoration of ovarian function began at 20 weeks and was achieved 24 weeks after transplantation. After the fifth stimulation attempt, two mature oocytes were obtained and microinjected. One embryo (seven cells) was obtained and transferred, leading to a normal pregnancy. The patient delivered a healthy baby boy weighing 2,370 g at 38 weeks of gestation.

**Conclusion(s):** Ovarian cortex cryopreservation can be performed at the time of surgery for benign diseases when fertility is impaired. We report the first pregnancy to occur after ovarian tissue cryopreservation for benign ovarian pathology after bilateral oophorectomy. (Fertil Steril® 2012;98:720–5. ©2012 by American Society for Reproductive Medicine.)

**Key Words:** Bilateral oophorectomy, ovarian cortex, cryopreservation, transplantation, fertility restoration

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**P**remature ovarian failure (POF) can result from many different causes. Women with cancer may

face POF owing to high doses of chemotherapy or radiotherapy (1), but there are also benign conditions in which go-

nadal damage can occur (Table 1). For instance, bone marrow transplantation is required for some benign hematological diseases, leading to a risk of POF (2–4). POF may also occur in benign ovarian pathologies such as ovarian endometriomas or dermoid, mucinous, or serous cysts treated by repeated or extensive surgery, especially in the case of recurrence or torsion (5–7).

Several options are available to preserve fertility in patients facing POF. These include immature and mature oocyte cryopreservation, embryo cryopreservation, and ovarian tissue cryopreservation (8). In some instances, like

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J.D. and P.J. contributed equally to this article.

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**TABLE 1****Indications for cryopreservation of ovarian tissue in cases of nonmalignant diseases.****Indication**

Uni/bilateral oophorectomy  
 Benign ovarian tumors  
 Severe and recurrent endometriosis  
 BRCA-1 or BRCA-2 mutation carriers  
 Risk of premature menopause  
 Turner syndrome  
 Family history  
 Benign diseases requiring chemotherapy: autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis, Behçet's disease, Wegener's granulomatosis)  
 Bone marrow transplantation  
 Benign hematological diseases: sickle cell anemia, thalassemia major, aplastic anemia  
 Autoimmune diseases unresponsive to immunosuppressive therapy

Note: From Donnez et al. (8).

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the present case, where no delay allowing oocyte cryopreservation or embryo preservation was possible, cryopreservation of ovarian tissue is the only possible option (7).

We report the first live birth to be achieved after grafting of ovarian cortex cryopreserved at the time of bilateral oophorectomy for severe and recurrent pelvic inflammatory disease (PID). Crucially, in this case, no residual ovarian tissue remained in situ, proving beyond doubt that this pregnancy resulted from the grafted ovarian tissue.

## MATERIALS AND METHODS

In December 2001, the patient, who was 18 years of age at the time, underwent laparoscopy followed by midline laparotomy

for peritonitis, appendectomy, and partial resection of the cecum. In February 2002, she developed clinical features of PID that resolved with antibiotic therapy. In June 2002, laparotomy was carried out for recurrent PID. Because of the presence of large 10-cm in diameter tubo-ovarian abscesses, bilateral salpingo-oophorectomy was carried out. Owing to extensive inflammation and infection, no remnants of ovarian tissue were left behind. Histology revealed numerous ovarian abscesses. No endometriotic tissue was found.

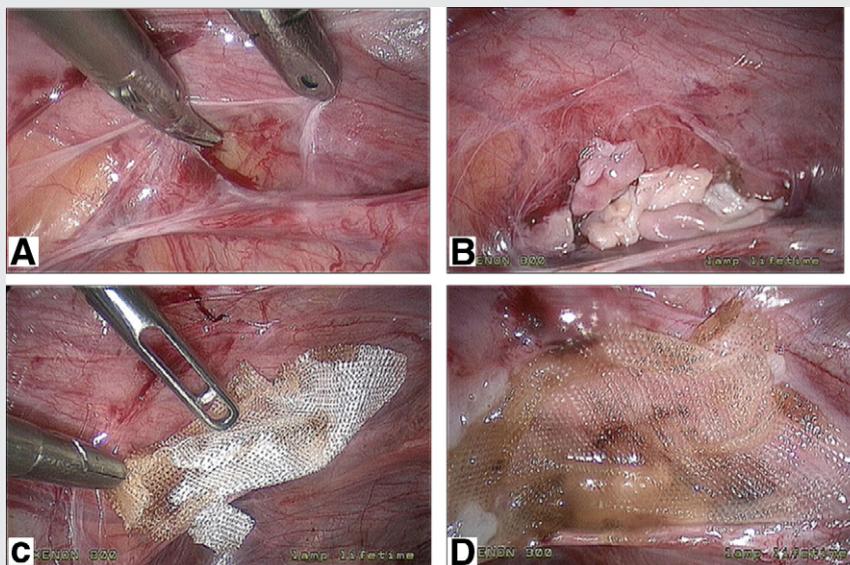
Ovarian tissue cryopreservation was performed in the laboratory of the Free University of Brussels by one of the authors (J.S.). Twenty-four fragments of 7–8 mm by 4 mm in size were obtained. They were 1–2 mm thick. Freezing of ovarian tissue was undertaken according to the protocol described elsewhere (9).

Hormone therapy (HT) was initiated. In 2009, the patient was referred to our center for ovarian transplantation (Cliniques Universitaires St-Luc, Université Catholique de Louvain) to discuss the possibility of ovarian tissue grafting. Before scheduling grafting, one piece of cryopreserved cortex was thawed to evaluate follicular density, which was considered by the pathologist to be normal ( $n > 15\text{--}20$  follicles/mm<sup>2</sup>).

In November 2009, ovarian tissue transplantation was carried out.

## Surgery

Since the patient had undergone bilateral oophorectomy, orthotopic reimplantation to the ovarian medulla, as described by Jadoul et al. and Donnez et al. (6, 9) and Silber et al. (10), was not possible. Instead, the peritoneal window procedure, as described by our group in 2004 and subsequently by others (11), was proposed, having already yielded live births after frozen-thawed ovarian tissue transplantation (9, 11). Reimplantation in subcutaneous fatty

**FIGURE 1**

Surgical procedure. (A) Creation of the peritoneal window in the anterior leaf of the broad ligament. (B) Cryopreserved thawed ovarian fragments are placed in the peritoneal window. (C, D) The ovarian cortex pieces are then covered by Interceed (Johnson and Johnson).

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tissue was not proposed, as only poor-quality embryos (and no pregnancies) have ever been obtained by this procedure.

At laparoscopy, numerous bowel adhesions were present and only the anterior face of the uterus was visible, as well as the anterior leaf of the broad ligament. After adhesiolysis, a peritoneal window (Fig. 1A) was created on the anterior leaf of the left broad ligament, close to the ascending uterine artery.

Six fragments of about 7–8 mm by 4 mm in size were placed in the peritoneal window (Fig. 1B) and covered with Interceed (Johnson and Johnson; Fig. 1C and D).

## Ethics

Our protocol was authorized by the Ethics Committee of the Université Catholique de Louvain, which, back in 1995, approved research protocols including reimplantation of ovarian tissue to preserve or restore fertility in women undergoing procedures at risk of inducing iatrogenic ovarian failure. The patient gave her informed written consent before reimplantation.

## RESULTS

### Ovarian Biopsy after Cryopreservation

Histological examination of a sample of cryopreserved ovarian tissue thawed at the time of transplantation revealed the presence of 12 primordial follicles/mm<sup>2</sup>.

### Ovarian Activity (Fig. 2)

The patient continued HT for 8 weeks after surgery. Twelve weeks later (thus 20 weeks after transplantation), vaginal ultrasound revealed the presence of a follicle of 6 mm in the transplantation area. The E<sub>2</sub> level was 19 pg/mL and FSH had significantly decreased to 25.6 mIU/mL.

Four weeks later (24 weeks post-transplantation), vaginal ultrasound revealed the presence of three growing follicles of 14, 13, and 10 mm in size in the transplantation area. The

endometrium was 9.1 mm thick (triple layered). E<sub>2</sub> and FSH were, respectively, 79 pg/mL and 12.4 mIU/mL.

Mild stimulation by recombinant FSH (Gonal-F, MSD) associated with GnRH antagonist (Cetrorelix, Merck Serono) was initiated. The first four cycles were unsuccessful, because no oocytes were retrieved at pickup, they appeared abnormal, or no fertilization occurred.

At the fifth attempt, the patient underwent controlled ovarian hyperstimulation using HP-hMG (Menopur, Ferring Pharmaceuticals, Inc.) treatment at a dose 375 IU daily, starting on day 1 of the period.

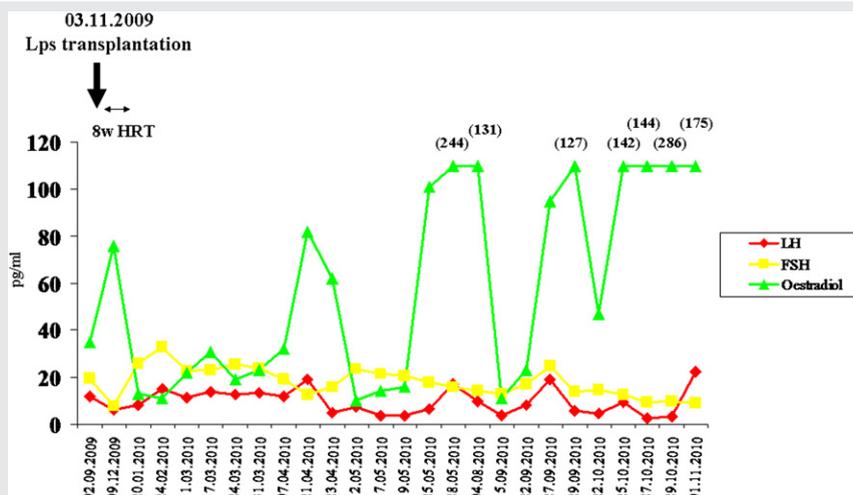
On day 6 of the stimulation the leading follicle was 17 mm, and daily administration of 0.25 mg of a GnRH antagonist (Cetrorelix, Merck Serono) was initiated up to the day before ovulation trigger. On day 13, five follicles were observed (26, 18, 16, 15, and 9 mm in size; Fig. 3), the E<sub>2</sub> level was 568 pg/mL, and ovulation was triggered with hCG 10,000 IU (Pregnyl, Merck, Whitehouse Station) followed by vaginal administration of 200 mg micronized progesterone 3 times daily (Utrogestan, Besins International).

Five oocytes were retrieved (two metaphase II, two metaphase I, and one immature). The two that were on metaphase II stage were injected (intracytoplasmic sperm injection), and one fertilized. The zygote was cultured up to day 3, and an embryo of seven cells was transferred, leading to an ongoing pregnancy (13 months after transplantation).

### Pregnancy

The first trimester was uneventful, with combined screening at 12 weeks' gestational age (GA) revealing the risk of Down's syndrome to be 1 in 15,046. The blood group was A–, and infection serology was negative. Detailed anatomical scans were performed at 17 and 23 weeks, showing a normal male fetus. These and subsequent scans were technically difficult to perform owing to the high body mass index of the patient (33 kg/m<sup>2</sup>) and poor acoustic transmission owing to the

FIGURE 2



FSH, LH, and 17- $\beta$ -estradiol concentrations before and after reimplantation of cryopreserved ovarian tissue.

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FIGURE 3



Ultrasound of the broad ligament during the fifth attempt of ovarian stimulation. Five follicles (26, 18, 16, 15, and 9 mm in size) observed on day 13.

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presence of the small intestine interposed between the uterus and the anterior abdominal wall. Uterine artery Doppler waveforms were pathological (confirmed at 27 weeks' GA), but serial growth scans appeared to show normal growth along the 50th percentile. The patient presented at 38 weeks and 2 days with reduced fetal movements. The amniotic fluid index was down to 2 cm, and recent estimated fetal weight was 2,600 g with cephalic presentation. Blood pressure was 150/84, and there was no proteinuria. The cervix was 1.5 cm long and 1 cm dilated. According to hospital protocol, an oxytocin stress test was performed, which showed no deterioration in fetal heart rate patterns. Induction was planned for the following day. However, as fetal heart rate patterns the following morning revealed the presence of spontaneous decelerations associated with mild uterine contractions, an emergency lower segment cesarean section was performed via a midline incision under regional anesthesia (Graham Hutchings). Access was difficult owing to widespread small bowel adhesions. The uterovesical fold was extremely thickened, and the anterior leaf of the broad ligament (site of ovarian tissue graft) was obliterated by adhesions with the anterior abdominal wall. A small for gestational age (<5th percentile) but otherwise healthy male infant was born weighing 2,370 g, with an Apgar score of 7, 9, and 9 at 1, 5, and 10 minutes. The patient made an uneventful postoperative recovery. The growth of the baby is normal.

## DISCUSSION

This paper reports the first pregnancy to occur after bilateral oophorectomy and ovarian cortex cryopreservation and grafting for benign ovarian pathology (bilateral recurrent abscesses on probable endometriomas). Fertility may indeed be decreased by radical surgery in case of torsion of benign cysts like dermoids or by extensive surgery for endometriomas. Several papers have shown reduced anti-Müllerian hormone levels, a sign of decreased ovarian reserve, after ovarian

cystectomy for endometriomas, especially in larger and bilateral cysts (12–14). In young patients facing such treatments, it may be wise to discuss fertility preservation options. Ovarian cortex cryopreservation can easily be performed during the surgical procedure. In case of endometriosis, one should bear in mind that the ovarian reserve may be reduced owing to structural anomalies. Indeed, one of our studies showed lower follicular density in noninvolved ovarian cortex of endometriotic ovaries (15). In case of severe PID and technically difficult surgery, such as in our patient, thawing of a piece of ovarian cortex before transplantation might be indicated to assess follicular density in the cryopreserved tissue. Despite it possibly being less successful than for oncological indications (because of the presenting ovarian pathology), our case nevertheless shows that it is worthwhile considering ovarian cryopreservation in these patients, especially when it is the only fertility preservation option available. Alternatively, follicle aspiration of immature oocytes, followed by in vitro maturation and cryopreservation, can also be performed at the same time, as proposed by Revel et al. (16), but in cases of severe ovarian abscesses, the technique is not possible.

This was the first time that pregnancy was achieved after ovarian cryopreservation and grafting after bilateral oophorectomy. After the first reports of live births after transplantation of frozen-thawed ovarian cortex (9, 17, 18), doubts were cast on the validity of the procedure, and possible reactivation of the remaining native ovary was suggested (19). In our patient, both ovaries were completely removed at the time of surgery in 2002. Moreover, the frozen-thawed ovarian cortex was transplanted to the anterior leaf of the broad ligament, and follow-up by ultrasound clearly showed follicular development in the area of transplantation. This case therefore provides definitive proof that pregnancy can occur from transplanted ovarian cortex.

As far as the technique of reimplantation is concerned, our case confirms the peritoneal window to be an adequate site of reimplantation. Although some authors consider follicular activity in the peritoneal site to be lower than on the ovarian medulla, others have demonstrated comparable activity in both locations (6, 7, 20–25). Unlike our previous pregnancy after reimplantation to a peritoneal window (9), we did not perform prior laparoscopy to induce neoangiogenesis. This might explain the longer delay observed before restoration of ovarian activity, compared with reimplantation to the atrophic medulla of the remaining ovary, as first described by Donnez et al. (9) and Silber et al. (10).

Restoration of ovarian function confirmed by a rise in E<sub>2</sub> levels and decrease in FSH was seen after 4.5 months, which is in the range observed after other transplantations leading to pregnancy (3.5–6.5 months) (4). The delay in restoration of ovarian function is mostly influenced by the revascularization time of grafted tissue (26, 27), the reoxygenation process taking 4–5 days. However, revascularization not only depends on neoangiogenesis from the host but also on existent blood vessels in the grafted tissue (26, 27). In the present case, infection might have induced a deleterious effect on blood vessels of the remaining ovarian cortex.

TABLE 2

Series of 20 live births after transplantation of frozen-thawed ovarian cortex.

References	Cryopreservation procedure	Graft site	Live birth	
			Spontaneous	IVF
Donnez et al., 2004 (9), 2008 (20), 2011 (4,32)	SF	Peritoneal window (2 steps) Ovarian medulla	+ ++ (+)*	+ (+)*
Meirow et al., 2005 (17)	SF	Beneath the ovarian cortex	—	+
Demeestere et al., 2007 (24)	SF	Ovarian and peritoneal windows (2 steps)	++	—
Andersen et al., 2008 (18); Ernst et al., 2010 (33); Schmidt et al., 2011 (29)	SF	Subcortical ovarian pocket Ovarian medulla	+ +	+ +
Silber et al., 2008 (34), 2010 (35)	SF	Ovarian medulla	+ +	—
Piver et al., 2009 (25); Roux et al., 2010 (11)	SF	Ovarian and peritoneal windows (1 and 2 steps)	+ +	—
Sanchez-Serrano et al., 2010 (36)	SF	Ovarian medulla	—	++ (twins)
Revel et al., 2011 (37)	SF	Peritoneal window	—	+
Dittrich et al., 2012 (38)	SF	Ovarian medulla	—	+
Revelli et al., 2012 (39)	SF	Ovarian medulla	+	+

\* Parentheses indicate ongoing pregnancy at the present time.

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As noted in other patients undergoing transplantation of frozen-thawed ovarian cortex, our patient appeared to have a poor response to ovarian stimulation and poor oocyte quality (9, 17, 18). Indeed, the first stimulation cycle was interrupted because of lack of response (decrease in E<sub>2</sub> levels). During the second cycle, no oocytes were obtained, despite the presence of two follicles of 14 and 15 mm. The third and fourth cycles yielded three oocytes, two of which were abnormal, and no fertilization occurred in the third oocyte. During the last cycle, five oocytes were obtained, two of which were in metaphase II, and only one yielded an embryo after fertilization. These results are in line with previous reports (9, 17, 18). Our first results showed empty follicle rates of 29% per retrieval, immature oocytes in 38% of cases, a 31% fertilization rate, and an embryo transfer (ET) rate of 24% per retrieval (28). These results were confirmed by Andersen's team (29), with 35% of empty follicles, 33% of immature oocytes, fertilization rates of 37%, and a 33% ET rate per retrieval.

Only in Piver's paper were good results obtained (11), with a total of 11 oocytes and nine embryos after one IVF cycle, but it should be emphasized that creation of a peritoneal window and grafting of small ovarian cortex pieces were performed to induce angiogenesis, before grafting numerous pieces of cryopreserved ovarian tissue. This two-step procedure probably favors graft revascularization, as in the two-step procedure used in the first case (9). Even if there are not many cases so far, it nevertheless prompts discussion on the importance of the concept of a "vascular bed" (4, 30).

The mechanisms of these increased empty follicles and decreased oocyte quality and fertilization rates, that is dysfunctional folliculogenesis, whereby early oocyte atresia occurs with an apparently normal hormone response, asynchrony between granulosa cell maturation, and oocyte maturation and damage to the oocyte itself by freezing, thawing, and transplantation resulting in oocyte activation abnormalities, have been discussed elsewhere (28, 31). It remains unclear whether the freezing or the delay in

revascularization after transplantation is the main factor responsible for these abnormalities. On the one hand, several studies have identified oocyte damage by transmission electron microscopy (31) after different cryopreservation protocols, showing that structural anomalies in oocytes may be attributed to the cryopreservation procedure. On the other hand, the high rate of pregnancies obtained in Silber's series after orthotopic transplantation of fresh tissue seems to indicate that freezing of ovarian tissue could negatively influence the success rate. However, transplantation itself and post-transplantation ischemia cannot be excluded as causes of damage (31). This is the 19th pregnancy to be achieved after cryopreservation and reimplantation, the 20th being recently reported by an Italian team (39) (Table 2).

In conclusion, we report the first pregnancy to occur after reimplantation of ovarian cortex after bilateral oophorectomy, confirming that ovarian freezing, thawing, and reimplantation can yield pregnancies and excluding the hypothesis that the so far described pregnancies could have as an origin a remaining native ovary.

We also confirm high rates of empty follicles and abnormal oocytes and fertilization after transplantation of frozen-thawed ovarian cortex. Further research is therefore required to enhance freezing and reimplantation protocols, but ovarian cryopreservation and transplantation should be considered in all women at risk of ovarian failure owing to ovarian surgery, especially when other methods like embryo cryopreservation or oocytes vitrification are impossible or unavailable.

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