[Frontiers in Bioscience E4, 1654-1662, January 1, 2012]

Fertility preservation in women with ovarian endometriosis

Jacques Donnez[1], Jean Squifflet[1], Pascale Jadoul[1], Jean-Christophe Lousse[1], Marie-Madeleine Dolmans[1], Olivier Donnez[1]

[1]*Universite Catholique de Louvain, Cliniques Universitaires St. Luc, Department of Gynecology, Institut de Recherche Experimentale et Clinique, Avenue Hippocrate 10, B-1200 Brussels, Belgium*

TABLE OF CONTENTS

1. ABSTRACT

Endometriosis is one of the most frequently encountered benign diseases in gynecology. Complete resolution of endometriosis is not yet possible, but therapy has essentially three main objectives: (1) to preserve and improve fertility, (2) to reduce pain, and (3) to delay recurrence for as long as possible. The aim of this paper is to focus on fertility preservation in women with severe endometriosis. In moderate and severe endometriosis, a medico-surgical approach remains the gold standard, but more and more papers are reporting a low ovarian reserve after laparoscopic cystectomy for endometriomas. Indeed, very frequently, normal ovarian tissue is excised together with the endometrioma wall. Ovarian surgery in endometriosis patients should therefore be performed by experienced surgeons in order to both preserve and improve fertility. Preservation of ovarian tissue should be considered in all patients at serious risk of future fertility impairment, particularly before any treatment likely to result in ovarian endometriosis recurrence and/or premature ovarian failure.

2. INTRODUCTION

Endometriosis is one of the most frequently encountered benign diseases in gynecology. Complete resolution of endometriosis is not yet possible, but therapy has essentially three main objectives: (1) to preserve and improve fertility, (2) to reduce pain, and (3) to delay recurrence for as long as possible. The aim of this paper is to focus on fertility preservation in women with severe endometriosis. Treatment of endometriosis-associated infertility has been investigated with medical and surgical therapeutic modalities, individually and in combination (1). In moderate and severe endometriosis, a medico-surgical approach remains the gold standard (1,2). The most important operative intervention in endometriosis-associated infertility is ovarian surgery (hemorrhagic cysts or endometriomas), but there are some concerns. Indeed, excessive surgery may lead to normal ovarian tissue destruction, while incomplete surgery is associated with a much higher risk of recurrence. More and more papers are reporting a low ovarian reserve after laparoscopic cystectomy for endometriomas (3-10) as, very frequently,

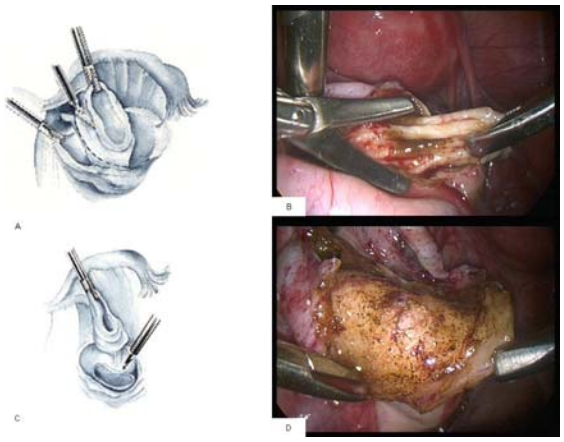

Figure 1. Combined technique: schematic and laparoscopic views. Partial cystectomy of the endometrioma is first carried out (A, B). To avoid excessive surgical damage close to the hilus, vaporization of the residual cyst is then performed (C, D).

normal ovarian tissue is excised together with the endometrioma wall (9). Ovarian surgery in endometriosis patients should therefore be performed by experienced surgeons in order to both preserve and improve fertility. We very recently described a laparoscopic procedure that combines the advantages, while avoiding the corresponding risks, of current techniques used in endometrioma surgery (cystectomy and ablative surgery) (10). In severe pelvic endometriosis and/or recurrent endometriomas, normal residual ovarian tissue and/or ovarian vascularization may be compromised (8-11)**.** Preservation of ovarian tissue should thus be considered in all patients at serious risk of fertility impairment, particularly before any treatment likely to result in ovarian endometriosis recurrence.

3. HOW TO PRESERVE FERTILITY IN ENDOMETRIOSIS

3.1. Laparoscopic management of endometriomas using a combined technique of excisional and ablative surgery

There are two main risks associated with the surgical treatment of endometriomas: 1) the risk of excessive surgery (removal or destruction of normal ovarian cortex together with the endometrioma) (10) and 2) the risk of incomplete surgery (with subsequent early recurrence of endometriomas). Depending on the risk, two techniques are currently used, with both advantages and disadvantages: either cyctectomy involving removal of the endometrioma wall, or ablative surgery that entails opening the endometrioma and destroying the internal cyst wall by laser vaporization or bipolar coagulation.

Ablative surgery may prove difficult because of the thickness and hypervascularization of the cyst wall. Recently, a Cochrane Review reported a higher rate of recurrence after ablative surgery than cystectomy (12). On the other hand, recent data in the literature appear to indicate that excisional surgery of endometriomas may be deleterious for ovarian function, causing ovarian trauma and removal of follicles. According to Muzii *et al*, recognizable ovarian tissue was inadvertently excised together with the endometriotic cyst wall in most cases during stripping for endometrioma excision (9). Close to the ovarian hilus, ovarian tissue removed along the endometrioma wall contained primordial, primary and secondary follicles in 69% of cases. Away from the hilus, the presence of follicles was infrequent (9).

In view of these data, we set out to develop a new approach that combines the techniques of cystectomy and ablative surgery, in order to take the best elements from both, while avoiding the corresponding risks (excessive surgery or incomplete surgery respectively). As illustrated in Figure 1, a large part of the endometrioma is first excised according to the cystectomy technique. The endometrial cyst is opened and washed out with irrigation fluid. After identifying the correct plane of cleavage between the cyst wall and ovarian tissue by applying opposite bimanual traction and countertraction with two grasping forceps, providing strong but non-traumatic force, the inner lining of the cyst is stripped from the normal ovarian tissue. When approaching the hilus, where the ovarian tissue is more functional, partial cystectomy is performed by resecting the excised tissue with scissors (Figures 1A and B). The stripping technique allows removal of 80-90% of the cyst. If the excision provokes bleeding or the plane of cleavage is not clearly visible, the cystectomy is stopped because of the risk of removing normal ovarian tissue containing primordial, primary and secondary follicles along with the endometrioma. After this first step (partial cystectomy), CO2 laser (Lumenis, USA) is used to vaporize the remaining 10-20% of the endometrioma close to the hilus. Care must be taken to vaporize all the residual cyst wall in order to avoid recurrence (Figures 1C and D).

As published in recent papers, this combined technique was applied in 52 patients with ovarian endometriosis (10). Six months after surgery, vaginal ultrasound was carried out in order to evaluate the ovarian volume and antral follicle count (AFC) on day 2-5. In 20 women who had unilateral endometriomas, a comparison was made with the contralateral healthy ovary. Data were also compared with those from women of similar age with normal ovaries and regular ovulatory cycles presenting for IVF because of male factor infertility (Tables 1 and 2). The combined technique was possible in all cases. The volume of the ovary after the combined technique was similar to that of the contralateral normal ovary, as well as that observed in infertile women without endometriosis presenting for male factor infertility. The AFC on day 2-5 showed the same number of antral follicles in all subgroups (Tables 1 and 2). Histopathology of the excised part of the endometrioma revealed the presence of follicles in only one case (2%). According to our present data, this new technique appears to combine the best results of the stripping technique in terms of recurrence outcomes, since most of the cyst wall is excised, and the ablation technique, since the hilus area of the ovary is spared from surgical damage.

Table 1. Ovarian volume and antral follicle count (AFC) six months after surgery

	n	Ovarian volume in cm^3	Antral follicle count
Combined technique	31	7.64 +/- 2.95	6.1 +/- 3.2
Women without endometriosis	20	7.99 +/- 5.33	6.2 +/- 4.8

Performed in women treated for endometriomas by the combined technique and women of similar age with normal ovaries and regular ovulatory cycles presenting for IVF because of male factor infertility.

Table 2. Ovarian volume and antral follicle count (AFC) six months after surgery

	n	Ovarian volume in cm^3	Antral follicle count
Combined technique	20	7.45 +/- 2.93	5.5 +/- 2.4
Contralateral normal ovaries	20	7.82 +/- 3.91	5.7 +/- 1.6

Performed in women with unilateral endometriomas and contralateral normal ovaries serving as controls.Reproduced with permission from (10).

3.2. Autotransplantation of fresh human ovarian tissue in endometriosis patients

Normal residual ovarian tissue and/or ovarian vascularization may be compromised in patients with severe endometriosis and/or recurrent endometriomas. In case of radical treatment (oophorectomy), but also if there is a risk of recurrence after conservative treatment, preservation of ovarian tissue with future autotransplantation should be seriously considered. We report our experience with autotransplantation of human ovarian tissue (fresh or cryopreserved). Current research into isolation of primordial follicles from cryopreserved tissue is also discussed, as transplantation of isolated follicles may prove to be an alternative option in the future. Silber *et al* reported successful reimplantation of fresh ovarian tissue using ovarian cortex donated by a monozygotic twin (13,14). In 2005, we reported the first two cases of orthotopic transplantation of fresh ovarian tissue in humans with ovarian endometriosis (15). Two women (aged 25 and 27 years) were diagnosed with recurrent large left unilateral endometriomas. At laparoscopy, the left part of the pelvis was found to be frozen in both cases. After careful dissection, left ovarian vascularization appeared to be compromised, so left oophorectomy was performed. However, before removal of the ovary, two to four strips of ovarian cortex (measuring 3-4 x 12mm) were taken from residual healthy ovarian tissue. A window was created beneath the healthy right ovarian hilus close to the ovarian blood vessels. One strip of fresh ovarian cortex was placed in the window and fixed (Figure 2A-F). The remaining healthy tissue from both patients was cryopreserved. At second-look laparoscopy, macroscopically viable-looking ovarian tissue of ± 1cm in size was visible in the grafted area of the two patients and biopsies were taken (Figures 3A and B). In one patient, a small cystic structure (follicle) not covered with peritoneum was seen on the grafted ovarian tissue. Biopsies of reimplanted tissue were evaluated by histology and vital fluorescent staining (calcein-AM and ethidium homodimer-1), according to the technique described by Donnez et al (16). In both patients, primordial follicles and active angiogenesis (demonstrated by the presence of numerous small vessels in the grafted tissue) were observed. Viability of the primordial follicles was proved by vital fluorescent staining. Biopsy of the small cystic structure seen in one patient at laparoscopy revealed granulosa cells. Finally, in part of the biopsy from one patient, six viable follicles were detected after collagenase isolation and vital fluorescent staining (Figures 3C and D).

This study provides histological data after orthotopic autotransplantation of fresh ovarian cortex, evidencing survival of primordial follicles and the presence of a neovascular network. This technique could therefore be used to preserve ovarian tissue in case of severe and/or recurrent ovarian endometriosis, when normal residual ovarian tissue is compromised. In some instances, where appropriate, fresh ovarian cortex could also be orthotopically transplanted to the heterolateral ovary, according to the following technique:

1. Normal residual cortex is dissected from the endometriotic tissue (in the peritoneal cavity).

2. As much of the medulla as possible is removed from the cortex.

3. The antihilar region of the heterolateral ovary is opened.

4. Strips of cortical tissue are sutured to the decorticated medulla, as previously described (17).

The goal is to increase the follicular ovarian reserve of the heterolateral ovary.

3.3. How to preserve fertility in women at risk of premature ovarian failure

As already stated, fertility preservation is a priority in the treatment of endometriosis in patients at risk of impaired future fertility.

Several options are currently available to preserve fertility in patients at risk of premature ovarian failure (POF): embryo cryopreservation, oocyte cryopreservation, and ovarian tissue cryopreservation (for review see 17-20). The choice depends on various parameters, such as the type of pathology, the patient's age and the partner status. The only established means of fertility preservation is embryo cryopreservation according to the Ethics Committee of the American Society for Reproductive Medicine (21). This is a very effective method that should be considered as first-line treatment in case of severe endometriosis when there is a risk of POF, but it requires the patient to have a partner. Cryopreservation (vitrification) of oocytes has also proved very effective, especially in egg donation programs (22,23). Freezing of immature (24) and mature (25) oocytes, as well as continuing technological breakthroughs in *in vitro* follicular growth (26,27), are promising developments in the field (19).

Table 3. Indications for cryopreservation of ovarian tissue in case of non-malignant disease

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

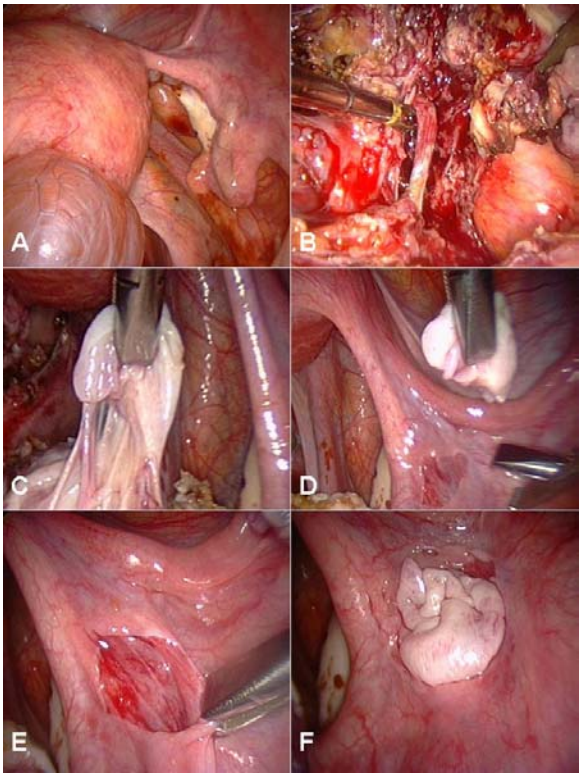

Figure 2. Orthotopic transplantation of fresh ovarian cortex: laparoscopic procedure. At laparoscopy, the left part of the pelvis was found to be frozen, while the right part was free of adhesions (A). After careful dissection, left ovarian vascularization appeared to be compromised (B). Before removal of the ovary, strips of ovarian cortex were taken from residual healthy ovarian tissue (C and D). A window was created beneath the healthy right ovarian hilus close to the ovarian blood vessels (E). One strip of fresh ovarian cortex was placed in the window and fixed (F).

The goal of this manuscript is not to extensively describe the already well known results of embryo and oocyte cryopreservation, but to present a new option, namely cryopreservation of healthy ovarian tissue obtained from women undergoing surgery for severe ovarian endometriosis, especially in case of recurrence. Orthotopic autotransplantation of cryopreserved ovarian cortex has already been shown to be efficient in cancer patients, leading to restoration of ovarian function, pregnancy and 15 live births to date (14,16,17,28-37). In our department, cryopreservation of ovarian tissue is also proposed for benign diseases, including recurrent ovarian endometriosis. The indications for cryopreservation of ovarian tissue in case of both malignant and non-malignant disease are summarized in Table 3. The age of the patient should be taken into consideration, since the follicular reserve of the ovary is age-dependent. Because a decline in fertility is now well documented after the age of 35 years, the procedure should probably be restricted to patients below this limit. The aim of this strategy is to reimplant ovarian cortical tissue into the pelvic cavity (orthotopic site) in case of POF (Figure 4). So far, orthotopic transplantation of cryopreserved ovarian cortical fragments has resulted in pregnancies and 15 live births. However, other strategies, such as transplantation of isolated frozen-thawed primordial follicles, may prove to be alternative options in the future, as discussed below (38).

3.3.1. Cryopreservation of ovarian tissue and transplantation

Follicles are located inside the ovarian cortex, and thus tissue samples collected for cryopreservation have to come from the surface of the organ. Biopsy can be taken during any gynecological procedure, by laparoscopy or laparotomy, and may be composed of one or several cortical fragments. Laparoscopic forceps and scissors are inserted through one of the 5mm trocars placed in the iliac fossa, and are used to grasp the ovary and cut a fragment from its surface. Cortical biopsy can be easily carried out with laparoscopic scissors. The number of biopsies taken varies according to the size of the patient's ovaries and the estimated risk of POF. Freezing of ovarian tissue is undertaken according to the protocol described by Gosden *et al*. (39). The cryotubes are cooled in a programmable freezer (Kryo 10, Series III; Planer, Sunbury-on-Thames, UK) with the following program: cooled from 0°C to -8°C at -2°C/min; seeded manually by touching the cryotubes with forceps pre-chilled in liquid nitrogen; cooled to -40°C at -0.3°C/min; cooled to -150°C at -30°C/min; and transferred to liquid nitrogen (-196°C) immediately for storage. The thawing procedure is as follows: the cryogenic vials are thawed at room temperature (between 21°C and 23°C) for 2 min and immersed in a water bath at 37°C for another 2 min. Ovarian tissue is immediately transferred from the vials to tissue culture dishes (Becton Dickinson, NY, USA) in L-15 medium and subsequently washed three times at room temperature with fresh medium to remove cryoprotectant before further processing. Thawed ovarian cortical tissue is then placed in sterile medium and immediately transferred to the operating theater. Recent experimental data suggest that vitrification may well be considered a viable alternative to slow-freezing in the near future (40).

3.3.2. Orthotopic transplantation techniques

There are two techniques of ovarian cortex reimplantation:

1. The first technique involves creating a peritoneal window before reimplantation in order to induce

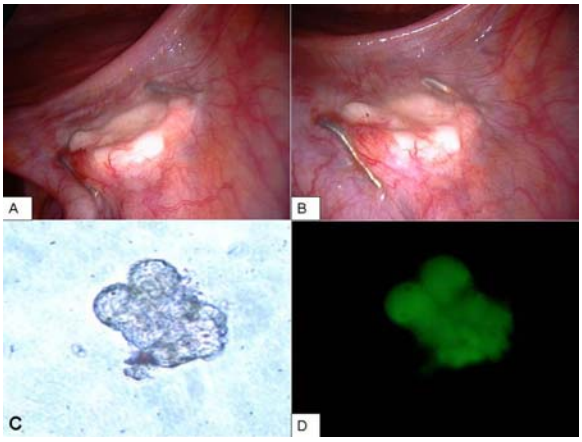

Figure 3. At second look laparoscopy, macroscopically viable-looking ovarian tissue of +/- 1cm in size was visible in the grafted area of the two patients and a biopsy was taken (A and B). In part of the biopsy from one patient, six viable follicles were detected after collagenase isolation and vital fluorescent staining (C and D).

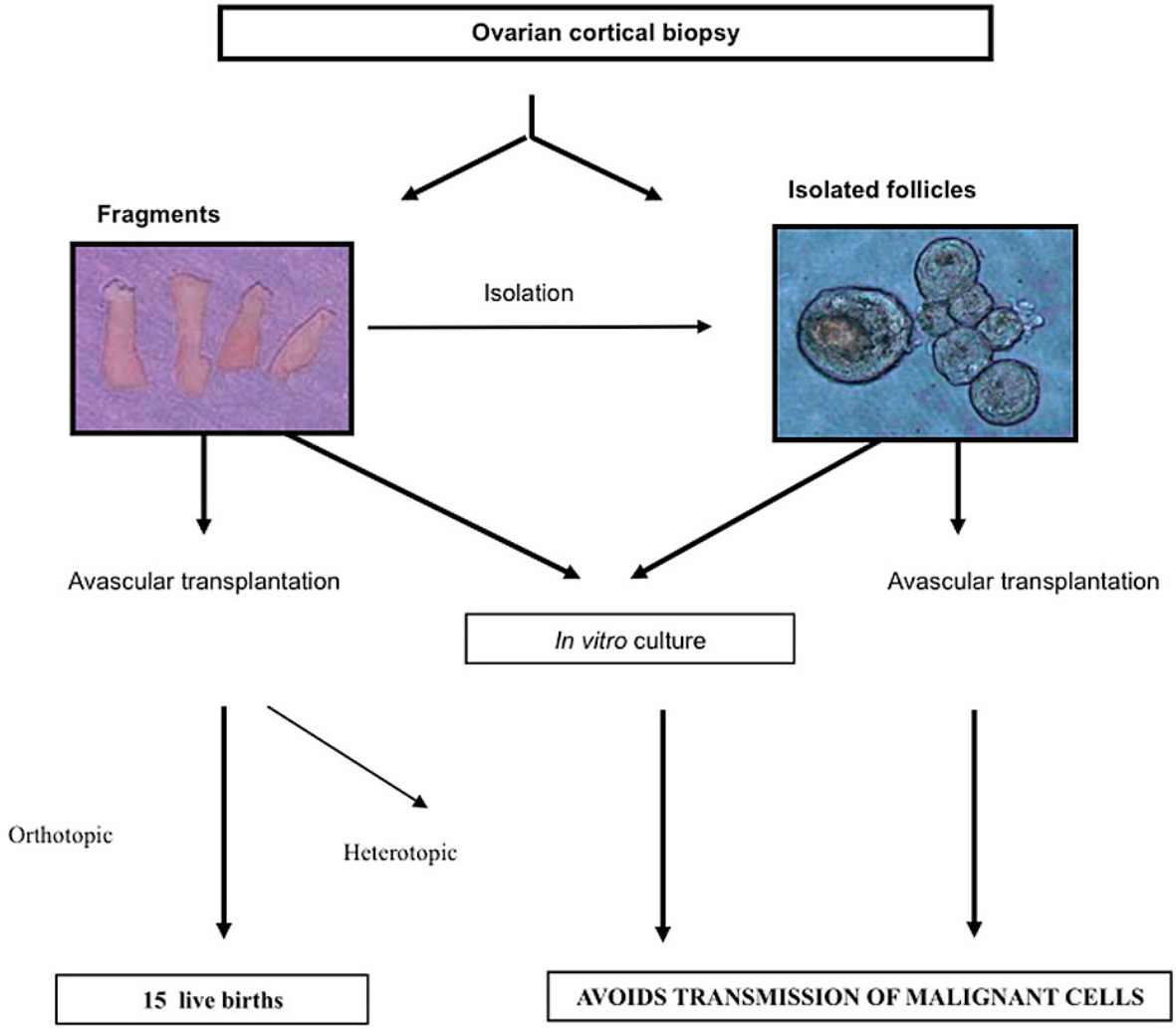

Figure 4. Options for ovarian tissue cryopreservation and reimplantation.

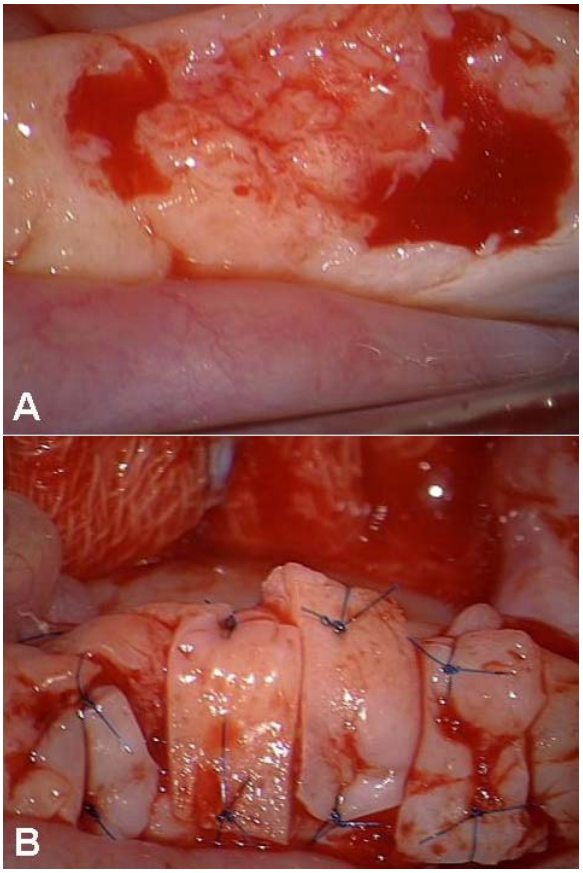

Figure 5. In the other eight cases, ovarian tissue was reimplanted onto the remaining ovary after removal of the native cortex (A). In most cases, large strips of ovarian tissue were attached to the decorticated medulla with stitches (B). Reproduced with permission from (17).

angiogenesis and neovascularization in that area. This procedure was clearly described in our publication reporting the first pregnancy and live birth after orthotopic transplantation of cryopreserved ovarian tissue (16). We performed the first laparoscopy 7 days before reimplantation to create a peritoneal window by means of a large incision just beneath the ovarian hilus, followed by coagulation of the edges of the window. Seven days later, during a second laparoscopy, an extensive neovascular network was clearly visible in this space. Small cubes of frozen-thawed ovarian tissue were pushed into the furrow created by the peritoneal window very close to the ovarian vessels and fimbria.

2. The second technique involves reimplanting ovarian tissue onto the remaining ovary after removal of the native cortex (Figure 5A). Large strips of ovarian tissue can be attached to the decorticated medulla with stitches (Figure 5B), or small ovarian fragments can be placed on the decorticated medulla and an absorbable adhesion barrier used to cover and fix the thawed fragments to the ovary.

The peritoneal window created close to the ovarian hilus, as well as the ovarian medulla, were both found to be equally efficient sites of reimplantation. Large strips (8-10mm x 5mm) or small cubes (2mm) were reimplanted. Both sizes effectively restored ovarian endocrine function (Figure 6). From a microsurgical point of view, however, it is easier to attach large strips to the medulla than small cubes, which cannot be sutured. Since reimplantation of large strips is easier and just as effective as small cube reimplantation, we suggest that large strips be taken from the ovarian cortex for cryopreservation purposes. Revascularization remains the crucial issue and research should be undertaken to find ways of decreasing the period of hypoxia that occurs after graft reimplantation (18,41,42).

3.4. Isolation of primordial follicles from cryopreserved ovarian tissue

In case of microscopic endometriotic foci in apparently normal ovarian tissue, reimplantation of fresh or cryopreserved ovarian tissue may lead to recurrence of the disease, although the risk is probably very low. The same risk is present in cancer patients (particularly in case of breast cancer and leukemia), in whom the possibility of reintroducing malignant cells cannot be excluded. (43). To decrease the risk of transferring endometriotic cells, *in vitro* follicle maturation may be performed after follicle isolation (38,43). Culturing isolated follicles from the primordial stage is an attractive proposition because they represent more than 90% of the total follicular reserve and show high cryotolerance. However, isolated primordial follicles do not grow properly in culture (44). Another approach could be to transplant a suspension of isolated follicles (38). As the follicular basal lamina encapsulating the membrana granulosa excludes capillaries, white blood cells and nerve processes from the granulosa compartment (38,43), grafting fully isolated follicles could be considered safer. Moreover, this would allow the introduction of a high and known number of follicles, obtaining faster angiogenesis and minimizing ischemic and reperfusion damage (41,42). In a murine model, Dolmans *et al* demonstrated the development of antral follicles after xenografting of isolated small human preantral follicles (38) (Figure 7). Human ovarian biopsies were enzymatically dissociated to obtain purified follicles that were xenografted to severe combined immunodeficient (SCID) mice for 5 months. After euthanasia, follicular morphology was assessed by histology, and follicular proliferation by Ki-67 immunohistochemistry. Four grafts containing a total of 84 follicles were recovered. This follicular population was composed of 11 primordial follicles, 38 primary follicles, 31 secondary follicles and four antral follicles. Ki-67 was found to intensively stain granulosa cells in antral follicles.There is no doubt that an artificial ovary model for transplantation of isolated ovarian follicles is one of the key future approaches in the field of cryopreservation.

4. CONCLUSION AND PERSPECTIVES

Ovarian endometriosis is a specific entity and should be treated as such (45). The most important operative intervention in endometriosis-associated infertility is ovarian surgery. Because surgery has to be effective (decrease the risk of recurrence) and protective

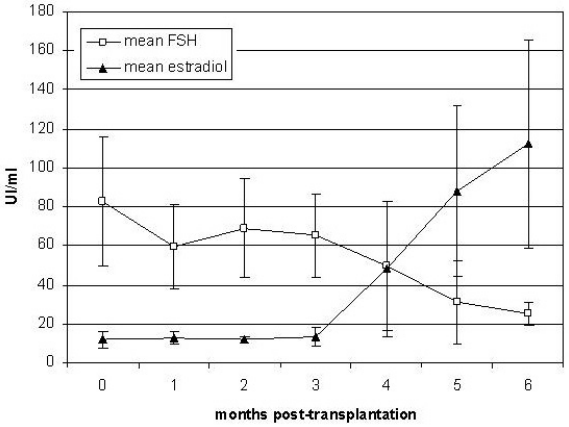

Figure 6. Mean FSH and 17β-estradiol levels (+/- standard deviation) in the 7 cases of frozen-thawed ovarian tissue transplantation. It took between 4 and 6 1/2 months after transplantation before a rise in estradiol and a drop in FSH were observed. (Note that, in the last 4 cases, FSH and estradiol values at 1 month post-transplantation were not taken into account, as the patients were under GnRH agonist downregulation). Reproduced with permission from (46).

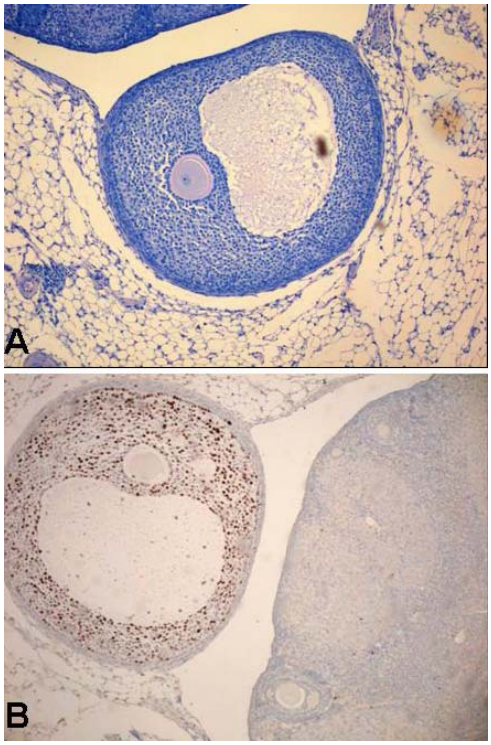

Figure 7. Histological sections of isolated human follicles xenografted for 5 months to severe combined immunodeficient (SCID) mice. (A) Hematoxylin-eosin-stained histological section of a 600-µm antral follicle. H = human ovarian graft, M = mouse ovary. The oocyte with a visible nucleolus is surrounded by an intact zona pellucida. The antral cavity is encapsuled by multiple layers of apparently normal granulosa cells and a few theca cells. (B) Anti-human Ki-67 immunostaining of an antral follicle. Intensive brown staining of the granulosa cells indicates proliferation.

(avoid normal ovarian tissue destruction), we propose a new surgical procedure that combines the best results of the stripping technique in terms of recurrence outcomes, since most of the cyst wall is excised, and the ablation technique, since the hilus area of the ovary is spared from surgical damage. In case of severe endometriosis and/or recurrent endometriomas, normal residual ovarian tissue and/or ovarian vascularization may be compromised. In the event of radical treatment (oophorectomy) in particular, but also conservative treatment as there is a risk of recurrence, preservation of ovarian tissue should be considered with a view to future autotransplantation. Orthotopic autotransplantation of fresh ovarian cortex is one option, as our results provide proof of the survival of primordial follicles and the presence of a neovascular network. Cryopreservation and autotransplantation of frozen-thawed ovarian tissue is another valuable technique, as demonstrated by the live births obtained in cancer patients. Indeed, ovarian cortex cryopreservation should be proposed to all women at high risk of severe recurrent ovarian endometriomas.

5. ACKNOWLEDGEMENTS

The authors thank Mira Hryniuk, BA, for reviewing the English language of the manuscript and Anne Lepage for all administrative support.

6. REFERENCES

1. J. Donnez, F. Chantraine and M. Nisolle: The efficacy of medical and surgical treatment of endometriosis-associated infertility: arguments in favour of a medico-surgical approach. *Hum Reprod Update* 8, 89-94 (2002)

2. J. Donnez , M. Nisolle, N. Gillet, M. Smets, S. Bassil and F. Casanas-Roux. Large ovarian endometriomas. *Hum Reprod* 11, 641-646 (1996)

3. G. Nargund, W. Cheng and J. Parsons. The impact of ovarian cystectomy on ovarian response to stimulation during in-vitro fertilization cycles. *Hum Reprod* 11, 81-83 (1996)

4. F. Loh, A. Tan, J. Kumar J, S. Ng. Ovarian response alter laparoscopic ovarian cystectomy for endometrioci cysts in 132 monitored cycles. *Fertil Steril* 72, 316-21 (1999)

5. H. Ho, R. Lee, Y. Hwu, M. Lin, J. Su and Y. Tsai. Poor response of ovaries with endometrioma previously treated with cystectomy to controlled ovarian hyperstimulation. *J Assist Reprod Genet* 19, 507-511 (2002)

6. S. Geber, D. Ferreira, L. Spyer Prates, L. Sales and M. Samaio. Effects of previous ovarian surgery for endometriosis on the outcome of assisted reproduction treatment. *Reprod Biomed Online* 5, 162-166 (2002)

7. E. Somigliana, G. Ragni, F. Benedetti, R. Borroni, W. Vegettiand P. Crosignani. Does laparoscopic excision of endometriotic ovarian cysts significantly affect ovarian

reserve? Insights from IVF cycles. *Hum Reprod* 18, 2450-2453 (2003)

8. C. Exacoustos, E. Zupi, A. Amadio, B. Szabocs, B. De Vivo, D. Marconi et al. Laparoscopic removal of endometriomas: sonographic evaluation of residual functioning ovarian tissue. *Am J Obstet Gynecol* 191, 68-72 (2004)

9. L. Muzii, F. Bellati, A. Bianchi, I. Palaia, N. Manci, M. Zullo, et al. Laparoscopic stripping of endometriomas: a randomized trial on different surgical techniques. Part II: pathological results. *Hum Reprod* 20, 1987-1992 (2005)

10. J. Donnez, JC Lousse, P. Jadoul, O. Donnez and J. Squifflet. Laparoscopic management of endometriomas using a combined technique of excisional (cystectomy) and ablative surgery. *Fertil Steril* 94, 28-32 (2010)

11. J. Donnez. Reply of the authors to I. Brosens. *Fertil Steril* 94:e74 (2010)

12. R. Hart, M. Hickey, P. Maouris and W. Buckett. Excisional surgery versus ablative surgery for ovarian endometriomata. *Cochrane Database Syst Rev* 16, CD004992 (2008)

13. S. Silber, K. Lenahan, D. Levine, J. Pineda, K. Gorman, M. Friez, E. Crawford and R Gosden. Ovarian transplantation between monozygotic twins discordant for premature ovarian failure. *N Engl J Med* 353, 58-63 (2005)

14. S. Silber, N. Kagawa, M. Kuwayama and R. Gosden. Duration of fertility after fresh and frozen ovary transplantation. *Fertil Steril* [Epub ahead of print] (2010)

15. J. Donnez, J. Squifflet, MM Dolmans, B. Martinez-Madrid, P. Jadoul and A. Van Langendonckt. Orthotopic transplantation of fresh ovarian cortex: a report of two cases. *Fertil Steril* 84, 1018e1-1018e3 (2005)

16. J. Donnez, MM. Dolmans, D. Demylle, P. Jadoul, C. Pirard, J. Squifflet, B. Martinez-Madrid and A. Van Langendonckt. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. *Lancet* 364, 1405-1410 (2004)

17. J. Donnez, B. Martinez-Madrid, P. Jadoul, A. Van Langendonckt, D. Demylle and MM. Dolmans. Ovarian tissue cryopreservation and transplantation: a review. *Hum Reprod Update* 12, 519-535 (2006)

18. I. Demeestere, P. Simon, S. Emiliani, A. Delbaere and Y. Englert. Orthotopic and heterotopic ovarian tissue transplantation. *Hum Reprod Update* 15, 649-665 (2009)

19. JS. Jeruss and TL Woodruff. Preservation of fertility in patients with cancer. *N Engl J Med* 360, 902-911 (2009)

20. SJ. Lee, LR. Schover, AH. Partridge, P. Patrizio, WH. Wallace, K. Hagerty, LN. Beck, LV. Brennan and K. Oktay. American Society of Clinical Oncology. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. *J Clin Oncol* 24, 2917-31 (2006)

21. Ethics Committee of the American Society for Reproductive Medicine. Fertility preservation and reproduction in cancer patients. *Fertil Steril* 83, 1622-1628 (2005)

22. A. Cobo, G. Vajta and J. Remohí. Vitrification of human mature oocytes in clinical practice. *Reprod Biomed Online* 19, 4385 (2009)

23. A. Cobo, J. Domingo, S. Perez, J. Crespo, J. Remohí and A. Pellicer. Vitrification: an effective new approach to oocyte banking and preserving fertility in cancer patients. *Clin Transl Oncol* 10, 268-273 (2008)

24. E. Demirtas, SE. Elizur, H. Holzer, Y. Gidoni, WY Son, RC Chian and SL. Tan. Immature oocyte retrieval in the luteal phase to preserve fertility in cancer patients. *Reprod Biomed Online* 17, 520-523 (2008)

25. A. Borini, PE. Levi Setti, P. Anserini, R. De Luca, L. De Santis, E. Porcu, GB. La Sala, A. Ferraretti, T. Bartolotti, G. Coticchio and G. Scaravelli. Multicenter observational study on slow-cooling oocyte cryopreservation: clinical outcome. *Fertil Steril* 94, 1662-1668 (2010)

26. HM Picton, SE Harris, W Muruvi and EL Chambers. The invitro growth and maturation of follicles. *Reproduction* 136, 703-715 (2008)

27. CA. Amorim, A. Van Langendonckt, A. David, MM. Dolmans and J. Donnez. Survival of human pre-antral follicles after cryopreservation of ovarian tissue, follicular isolation and in vitro culture in a calcium alginate matrix. *Hum Reprod* 24, 92-99 (2009)

28. J. Donnez , S. Silber, CY. Andersen, I. Demeestere, P. Piver, D. Meirow, A. Pellicer and MM. Dolmans. Children born after autotransplantation of cryopreserved ovarian tissue. A review of 13 live births. Ann Med. 2011 Jan 13. [Epub ahead of print]

29. D. Meirow, J. Levron, T. Eldar-Geva, I. Hardan, E. Fridman, Y. Zalel, E. Schiff and J. Dor. Pregnancy alter transplantation of cryopreserved ovarian tissue in a patient with ovarian failure alter chemotherapy. *N Engl J Med* 353, 318-321 (2005)

30. I. Demeestere, P. Simon, S. Emiliani, A. Delbaere and Y. Englert. Fertility preservation: successful transplantation of cryopreserved ovarian tissue in a young patient previously treated for Hodgkin's disease. *Oncologist* 12, 1437-1442 (2007)

31. C. Andersen, M. Rosendahl, A. Byskov, A. Loft, C. Ottosen, M. Dueholm, K. Schmidt, A. Andersen and E. Ernst. Two successful pregnancies following autotransplantation of frozen/thawed ovarian tissue. *Hum Reprod* 23, 2266-2272 (2008)

32. P. Piver, C. Amiot, G. Agnani, J. Pech, PS. Rohrlich, E. Vidal, 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* i15 (2009)

33. C. Roux, C. Amiot, G. Agnani, Y. Aubard, PS. Rohrlich and Piver P. Live birth after ovarian tissue autograft in a patient with sickle cell disease treated by allogeneic bone marrow transplantation. *Fertil Steril* 93, 2413.e15-9 (2010)

34. E. Ernst, S. Bergholdt, JS. Jorgensen and CA. Andersen. The first woman to give birth to two children following transplantation of frozen/thawed ovarian tissue. *Hum Reprod* 25, 1280-1281 (2010)

35. I. Demeestere, P. Simon, F. Moffa, A. Delbaere and Y. Englert. Birth of a secondhealthy girl more than 3 years after cryopreserved ovarian graft. *Hum Reprod* 25, 1590-1591 (2010)

36. M. Sánchez-Serrano, J. Crespo, V. Mirabet, A.C. Cobo, MJ. Escribá, C. Simón and A. Pellicer. Twins born after transplantation of ovarian cortical tissue and oocyte vitrification. *Fertil Steril* 93, 268.e11-3 (2010)

37. J. Donnez, J. Squifflet, P. Jadoul, D. Demylle, AC. Cheron, A. Van Langendonckt and MM. Dolmans. Pregnancy and live birth after autotransplantation of frozen-thawed ovarian tissue in a patient with metastatic disease undergoing chemotherapy and hematopoietic stem cell transplantation. *Fertil Steril* 2010 [Epub aheadof print]

38. MM. Dolmans, W. Yuan, A. Camboni, A. Torre, A. Van Langendonckt, B. Martinez-Madrid and J. Donnez. Development of antral follicles after xenografting of isolated small human preantral follicles. *Reprod Biomed Online* 16, 705-711 (2008)

39. R. Gosden, D. Baird, J. Wade and R. Webb. Restoration of fertility to oophorectomized sheep by ovarian autografts stored at -196 degrees C. *Hum Reprod* 9, 597-603 (1994)

40. C. Amorim, A. David, A, Van Langendonckt and J. Donnez. Vitrification of human ovarian tissue: effect of different solutions and procedures. *Fertil Steril* 95, 1094-1097 (2011).

41. AS. Van Eyck, B. Jordan, B. Gallez, JF. Heilier, A. Van Langendonckt and J. Donnez. Electron paramagnetic resonance as a tool to evaluate human ovarian tissue reoxygenation after xenografting. *Fertil Steril* 92, 374-381 (2009)

42. AS Van Eyck, C. Bouzin, O. Feron, L. Romeu, A. Van Langendonckt, J. Donnez and MM. Dolmans. Both host and graft vessels contribute to revascularization of xenografted human ovarian tissue in a murine model. *Fertil Steril* 93, 1676-1685 (2010)

43. MM. Dolmans, C. Marinescu, P. Saussoy, A. Van Langendonckt, C. Amorim and J. Donnez. Reimplantation of cryopreserved ovarian tissue from patients with acute lymphoblastic leukemia is not safe. *Blood* 116, 2908-2914 (2010)

44. O. Hovatta, C. Wright, T. Krausz, K. Hardy and R. Winston. Human primordial, primary and secondary ovarian follicles in long-term culture: effect of partial isolation. *Hum Reprod* 14, 2519-2524 (1999)

45. M. Nisolle and J. Donnez. Peritoneal endometriosis, ovarian endometriosis, and adenomyotic nodules of the rectovaginal septum are three different entities. *Fertil Steril* 68, 585-596 (1997)

Key Words: Ovary, Ovarian Endometriosis, Endometrioma, Fertility Preservation, Cryopreservation, Ovarian Cortex, Review

Send correspondence to: Jacques Donnez, Universite Catholique de Louvain, Cliniques Universitaires St. Luc, Department of Gynecology, Avenue Hippocrate 10, 1200 Brussels, Belgium, Tel: 003227649501, Fax: 003227649507, E-mail: jacques.donnez@uclouvain.be

http://www.bioscience.org/current/vol4E.htm