OVARIAN TRANSPLANTATION IN THE PELVIC ORGANS – IS IT IN THE CLINICAL OR RESEARCH STAGE?

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Reported cases of autotransplantation of cryopreserved ovarian tissue, either to an orthotopic or heterotopic site, are summarized in Table 1, detailing in each case the age of the patient before freezing, whether the patient received chemotherapy before freezing, the indications for cryopreservation, the graft site and size, the interval before recovery of ovarian function after grafting and the outcome of transplantation (Oktay and Karlikaya, 2000; Radford et al., 2001; Callejo et al., 2001; Kim et al., 2004; Oktay et al., 2004, 2006; ; Donnez et al., 2004, 2006, unpublished data; Meirow et al., 2005; Schmidt et al., 2005; Wolner-Hanssen et al., 2005; Demeestere et al., 2006) Orthotopic autotransplantation of cryopreserved human ovarian tissue. In theory, natural pregnancy may be achieved via orthotopic tissue transplantation if the fallopian tubes remain intact.

In 2000, Oktay and Karlikaya reported laparoscopic transplantation of frozen-thawed ovarian tissue to the pelvic side wall in a 29-year-old patient, who had undergone bilateral oophorectomy for a non-malignant disease (Oktay and Karlikaya, 2000). Pieces of cryopreserved ovarian tissue were thawed and transplanted. The patient was stimulated by gonadotrophins once after 15 weeks and then again after ten months, follicular development was demonstrated by ultrasonography and ovulation occurred in response to hCG administration.

Radford et al. (2001) reported a patient with a history of Hodgkin's disease treated by chemotherapy, whose ovarian tissue had been biopsied and cryopreserved 4 years after chemotherapy and later reimplanted. In this case, histological section of the ovarian cortical tissue revealed only a few primordial follicles due to the previous chemotherapy. Estradiol was detected and the FSH and LH levels decreased 8 months after reimplantation. The patient had one menstrual period but, 9 months after reimplantation, her LH and FSH concentrations returned definitively to menopausal levels.

We reported the first successful transplantation of cryopreserved ovarian tissue (Donnez et al., 2004) resulting in a pregnancy and live birth. In 1997, a 25-year-old woman presented with clinical stage IV Hodgkin's lymphoma. Ovarian tissue cryopreservation was undertaken before chemotherapy. By laparoscopy, we took five cortical biopsies, about 12-15 mm long and 5 mm wide, from the left ovary. Removal of the whole ovary was not an option because one can never completely exclude recovery of ovarian function after chemotherapy. After laparoscopy, the patient received hybrid chemotherapy from August 1997 to February 1998, followed by supradiaphragmatic radiotherapy (38 Gy). According to Schilsky et al. (1981), the risk of POF after such a regimen in a woman of 26 years of age is more than 90%, while according to Wallace et al. (2005a), and Lobo et al. (2005), the risk of subfertility after Hodgkin's treatment with alkylating agents is more than 80%. Indeed, not only the type of drug and dose, but also the age, are important factors when evaluating the risk of POF after chemotherapy.

In 2003, once the patient had been declared completely disease-free, transplantation went ahead. A large strip and 35 small cubes of frozen-thawed ovarian tissue were implanted into a furrow created by the peritoneal window very close to the ovarian vessels and fimbria on the right side. Four months after transplantation, a laparoscopy was carried out to check the viability of the orthotopic graft and to reimplant the remaining 32 ovarian cortical cubes. A follicular structure was visible in the area where the tissue had been reimplanted, clearly outside the native ovary. Biopsy and analysis by vital fluorescent probe staining and histology revealed the presence of viable primordial follicles and a follicular structure with inhibin A-marked cells (Donnez et al., 2004). From 5 to 9 months after reimplantation, concentrations of FSH, 17$\beta$-estradiol and progesterone showed the occurrence of ovulatory cycles. At 11 months, the patient became pregnant and subsequently delivered a healthy baby. This birth was announced in the Lancet in September 2004.

Several lines of evidence lend support to our assertion that the origin of the pregnancy was indeed the autotransplanted cryopreserved tissue. The possibility that the egg was derived from the native ovary is highly unlikely, since vaginal echography demonstrated the development of a follicle of 18 x 22mm in size outside the native ovary during the cycle which led to the pregnancy. The same day, the estradiol level was 156 pg/ml and progesterone 0 ng/ml. This was extensively explained in the publication itself (Donnez et al., 2004), as well as in a letter later published by Donnez and Dolmans (2004) in response to comments by
Oktay and Tilly (2004). Another of our main arguments was that laparoscopy performed 4 ½ months after reimplantation proved, by direct visualization, the development of a follicle from the grafted tissue and, on histological examination, the biopsy samples indicated not only the survival of primordial follicles in the grafted tissue, but also maturation of a follicle (granulosa cells marked by inhibin B). It was the first histological proof of follicular maturation from reimplanted cryopreserved ovarian tissue. After delivery, the patient experienced ovulatory cycles every five to six weeks. Each time, an increase in FSH levels was observed.

In 2006, restoration of ovarian function after orthotopic (intraovarian and paraovarian) transplantation of cryopreserved ovarian tissue was reported in a woman treated by bone marrow transplantation (BMT) for a non-cancerous disease (sickle cell anemia) (Donnez et al., 2006). Thirty-nine small cryopreserved cubes were thawed and grafted into the ovary itself (24 cubes) and a peritoneal window (15 cubes). Vaginal echography and sequential measurement of FSH, LH, 17β-estradiol and progesterone concentrations revealed the onset of an ovulatory cycle 4 ½ months after reimplantation of ovarian tissue, demonstrating the efficacy of orthotopic transplantation and confirming, once again, the time interval between reimplantation and the onset of ovulation. The patient experienced 3 cycles in total, evidenced by the development of a follicle and raised estradiol levels. It should be noted that the estradiol peaks never exceeded 55 pg/ml and that FSH rose to 40 mIU/ml between the cycles. However, after these 3 cycles, LH and FSH concentrations returned to castrated levels. We then decided to reimplant the 30 remaining cubes into the ovary, the patient being under GnRH agonist in order to decrease LH and FSH levels. She experienced a first ovulatory cycle 4 months after reimplantation. The preovulatory estradiol level was 120 pg/ml. The follicle measured 20 mm before the LH peak and progesterone was at 14.7 ng/ml in the mid-luteal phase.

Very recently, we applied a technique similar to that used by Silber et al. (2005) for the transplantation of fresh ovarian cortex between monozygotic twins in a woman who had also undergone BMT and two regimens of alkylating agents in 2000 for non-Hodgkin’s lymphoma (Table 1). Cryopreservation of ovarian tissue was carried out 1 year after first-line chemotherapy. One ovary was removed and biopsies of cortical ovarian tissue revealed the presence of histologically normal primordial follicles. Six ovarian cortical pieces measuring 10 x 4-5mm were then grafted onto the remaining ovary after the cortex of this ovary had been removed. It was five months before a mature follicle (21 mm) developed and an increase in estradiol levels (194 pg/ml) was noted. The patient experienced an ovulatory cycle every 5 weeks, the preovulatory estradiol level reaching values between 210 and 356 pg/ml. Analysis of these cases raises some important points for discussion. First of all, in all three cases, it took between 4 ½ and 5 months after reimplantation before a follicle could be seen. The process of folliculogenesis takes ~ 4-6 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 (Gougeon, 1996). Thus, the appearance of the first follicle originating from the grafted tissue 5 months after reimplantation, proved by laparoscopy in one 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 (Baird et al., 1999, 2004) and human beings (see Table 1), although some variations may be observed. Indeed, as seen in Table 1, the delay between transplantation and follicular development was found varying from 6 weeks to eight months. Such a variation could be explained by a difference in follicular reserve at the time of cryopreservation.

Another very interesting finding is the persistence of relatively high FSH levels during the follicular phase. FSH levels remained as high as 25 mIU/ml during the 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 gonadotrophin 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. These results are in agreement with those obtained in sheep by Campbell et al. (2000).

A further significant observation is the return to an FSH level of > 35 mIU/ml immediately after each menstrual bleed, which supports the theory suggested by Baird et al. (2004) that some inhibitory mechanisms, such as inhibin B or anti-Müllerian hormones (AMH) normally produced by developing follicles in intact human ovaries, are probably almost nonexistent in transplanted tissue. After transplantation, the patient would have been regarded a poor responder because, of the 500-1000 primordial follicles that would have been transplanted,
more than 50% would have been lost owing to hypoxia (Donnez et al., 2004). This raises the question of the evaluation of the ovarian reserve. There is a lack of data on the ovarian reserve in cancer. Qu et al. (2000), Gook et al. (2005) and Schmidt et al. (2003) have all demonstrated an unequal distribution of primordial follicles in ovarian cortex.

In 2005, Meirow et al. 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. The rise in anti-Müllerian hormones and increased inhibin B levels were consistent with the presence of early growing follicles and ovulation respectively. Nine months after transplantation, the patient experienced a second spontaneous menstrual period. After a modified natural cycle, a single mature oocyte was retrieved and fertilized. Two days later, a four-cell embryo was transferred. The patient became pregnant from this embryo transfer and delivered a healthy infant weighing 3000g. The possibility that the oocyte was derived from the native ovary is highly unlikely given the consistent evidence of POF after high-dose chemotherapy in this patient, from whom ovarian tissue was harvested after administration of a first-line conventional chemotherapy regimen, prior to second-line high-dose chemotherapy.

Schmidt et al. (2005) recently reported the results of 3 cases of ovarian tissue transplantation. All three patients with autotransplanted ovarian tissue regained ovarian function, as confirmed by recovery of menses, follicles visible on ultrasonography and normal hormone levels. Two embryos were obtained from a total of three metaphase-II oocytes and one GV oocyte, but no pregnancy resulted from embryo transfer.

Demeestere et al. (2006) very recently reported a pregnancy after natural conception in a woman who had undergone orthotopic and heterotopic transplantation of cryopreserved ovarian tissue. They observed follicular development in all three transplantations sites: large follicles in the ovarian site, only one dominant follicle in the peritoneal site and follicles < 13mm in size in the heterotopic site. Detectable hCG levels and ultrasonography confirmed the presence of a viable intrauterine pregnancy. Unfortunately, this pregnancy obtained by natural conception ended in miscarriage at 7 weeks, due to aneuploidy. Interestingly, Demeestere et al. (2006) observed normal FSH values of after orthotopic and hysterotopic transplantation of cryopreserved ovarian tissue. As stressed by the authors, this may have been due to the young age of the patient and the large number of tissue fragments transplanted (Demeestere et al., 2006), which could have yielded a rich follicular reserve in the graft.

Heterotopic autotransplantation of cryopreserved human ovarian tissue: a better option than orthotopic transplantation? There are only six existing reports on this subject (Table 1). Callejo et al. (2001) evaluated the long-term function of cryopreserved heterotopic grafts but no conclusions could be drawn since the patient was perimenopausal at the time of ovarian biopsy for cryopreservation. In 2004, Kim et al. reported a case of a 37-year-old woman who underwent heterotopic (rectus and pectoralis muscle) transplantation of cryopreserved ovarian tissue (Kim et al., 2004). By 14 weeks of transplantation, restoration of endocrine function was demonstrated but, approximately 28 weeks after transplantation, cessation of ovarian function was evidenced by very high FSH levels (62-99 IU/L) and very low estradiol levels.

The same year, Oktay et al. (2004) reported transplantation of frozen-thawed ovarian tissue beneath the skin of the abdomen. A four-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.

In 2005, Schmidt et al. reported 2 cases of mixed (heterotopic and orthotopic) transplantation, as did Demeestere et al. in 2006. These cases are discussed in the section on orthotopic transplantation. Wolner-Hanssen et al. reported subcutaneous transplantation of frozen-thawed tissue to the forearm in 2005. Two follicles developed, but only to a maximum diameter of 12.6 and 6.7 mm respectively, and the tissue survived 7 months. The authors suggested that the risk of graft exposure to suboptimal temperatures or mechanical stress may depend on transplantation site, and thus tissue transplanted under the skin of the forearm will probably be exposed to both higher and lower temperatures than ovaries in their normal location. Very recently, Oktay et al. (2006) reported a pregnancy after heterotopic transplantation of cryopreserved ovarian tissue, but ovulation occurred from the native ovary. Papers describing heterotopic transplantation have all reported follicular development, but with follicles always less than 15mm in size. As stressed by Wolner-Hanssen (2005) and
Oktay et al. (2004), differences in temperature and pressure could interfere with follicular development in heterotopic sites. Whole ovary - As previously discussed, the main drawback of ovarian tissue cryopreservation followed by avascular transplantation is that the graft is completely dependent on the establishment of neovascularization and, as a result, a large proportion of follicles are lost during the initial ischemia occurring after transplantation (Baird et al., 1999; Aubard et al., 1999; Nisolle et al., 2000; Liu et al., 2002; Newton et al., 1996; Candy et al., 1997; Gunasena et al., 1997). Reducing the ischemic interval between transplantation and revascularization is therefore essential to maintaining the follicular reserve and extending the life span and function of the graft. In theory, the best way to achieve this is by transplantation of intact ovary with vascular anastomosis, allowing immediate revascularization of the transplant.

Ovarian vascular transplantation has already been successfully performed using intact fresh ovaries in rats (Wang et al., 2002; Yin et al., 2003), rabbits (Winston and Browne, 1974), sheep (Jeremias et al., 2002; Goding et al., 1967), dogs (Paldi et al., 1975), monkeys (Scott et al., 1981) and humans (Leporrier et al., 1987, Hilders et al., 2004, Mhatre et al., 2005). In the last few years, attempts at freezing and grafting whole ovaries in rats (Wang et al., 2002; Yin et al., 2003), rabbits (Chen et al., 2005) and sheep (Bedaiwy et al., 2003; Arav et al., 2005; Courbiere et al., 2005; Imhof et al., 2006) have also yielded encouraging results. The first case of restoration of fertility after whole frozen ovary transplantation was described by Wang et al. in 2002. They described successful vascular transplantation of frozen-thawed rat ovaries and reproductive tract in 4 out of 7 (57%) transplants, which survived for ≥ 60 days, were ovulatory and resulted in one pregnancy. Chen et al. (2005) showed that frozen-thawed rabbit ovaries remained functional for at least 7 months after microvascular transplantation in 13 out of 15 (86.7%) animals. It appears that, in large mammals and humans, cryopreserving such a large-sized intact ovary may prove more problematic than in small animals due to the difficulty of adequate diffusion of cryoprotective agents into large tissue masses and vascular injury caused by intravascular ice-formation. Nevertheless, Arav et al. (2005) reported progesterone activity 36 months after vascular transplantation of frozen-thawed sheep ovaries in 3 out of 8 transplants, and retrieval of 6 oocytes, resulting in embryonic development up to the 8-cell stage after parthenogenic activation. Bedaiwy et al. (2004) reported restoration of ovarian function after autotransplantation of intact frozen-thawed sheep ovaries with microvascular anastomosis, but it should be noted that 8 out of 11 ovaries were lost due to thrombotic events in the reanastomosed vascular pedicle. Imhof et al. (2006) recently demonstrated that autotransplantation of whole cryopreserved sheep ovaries with microanastomosis of the ovarian vascular pedicle could lead to pregnancy and delivery. Moreover, in this study, 6 out of 8 ovaries showed major ovarian vessels to be free of thrombosis, and with the structural integrity of the ovarian stroma largely retained 18-19 months after transplantation. Recently, Martinez-Madrid et al. described a cryopreservation protocol for intact human ovary with its vascular pedicle and proved high survival rates of follicles (75.1%) small vessels and stroma, and a normal histological structure in all the ovarian components after thawing (Martinez-Madrid et al., 2004). After freeze-thawing whole human ovaries using this protocol, no induction of apoptosis was observed in any cell types, assessed by both the TUNEL method and immunohistochemistry for active caspase-3 (Martinez-Madrid et al., 2005). Transmission electron microscopy (TEM) confirmed that the majority (96.7%) of primordial follicles were intact after cryopreservation (Camboni et al., 2005). Particular attention was paid to the evaluation of the endothelial cells: TEM revealed that 96.3% of these cells had a completely normal ultrastructure , and the percentage of active caspase-3-positive endothelial cells was less than 1%.

Our results in humans have led us to seriously consider proposing this option to women in the future, when there is no risk of transmitting malignant cells via the graft after transplantation (Jadoul et al., 2007). So far, in our department, five three whole ovaries have been cryopreserved with a view to future reimplantation (grafting) and vascular anastomosis. Developing new cryochambers and improving protocols for whole ovary cryopreservation must therefore be considered as vital directions in ongoing research to make transplantation of an entire ovary a feasible objective (Martinez-Madrid and Donnez, 2005; Donnez et al., 2005). Research and development of technology to cryopreserve whole organs, as well as surgical techniques for the autotransplantation of an entire ovary with its vascular pedicle, should be encouraged. This could lead to the transplantation of intact ovaries with
microvascular anastomosis carried out to restore immediate vascularization and minimize post-transplantation ischemia, responsible for the reduction in follicular density.

Safety and ethical issues: The transmission of lymphoma via grafts of ovarian tissue from diseased donor mice to healthy recipients was reported by Shaw et al. (1996). This study highlighted the risks of clinical transplantation of ovarian biopsy samples to women recovering from cancer, especially a blood-borne cancer (Shaw et al., 1996; Shaw and Trounson, 1997). However, there are certain circumstances where the risk of cancerous involvement of the ovary is absent or minimal (Meirov et al., 1998), and where autografting would present little or no danger (Gosden et al., 1997; Moomjy and Rosenwaks, 1998; Kim et al., 2001). Future experiments should help us address questions about the relevance of replacing residual malignant cells with grafted tissue in such cases. Screening methods must be developed to eliminate the risk of cancer cell transmission with reimplantation. In some diseases, other options must be considered, such as the transplantation of isolated follicles. Meanwhile, the debate rages on.

The Practice Committee of the ASRM (2004) has summarized some important points to be taken into consideration and Dudzinski (2004) recently underlined the need to develop policies to protect the patient’s right to self-determination with respect to her gametes. She conducted a normative analysis of ethical issues in the context of oocyte and ovarian tissue cryopreservation for adolescent cancer survivors and concluded that more research is required before adolescents can ethically be enrolled in clinical trials.

We do not fully agree with this conclusion. Indeed, approximately one third of pubertal women exposed to chemotherapy develop ovarian failure. In 2006, we believe it is our ethical responsibility to propose cryopreservation of ovarian tissue to all adolescents and young women under IRB protocols having to undergo chemotherapy with alkylating agents. Indeed, is it ethical to simply accept the existing discrepancy between males and females with regard to their chances of preserving their fertility following cancer treatments. What do we then say to young women facing POF following chemotherapy, knowing that ovarian cryopreservation has been an option for more than 10 years? It will be too late to say “we should have done something - we should at least have tried”.

This is why, since 1996, we have systematically proposed cryopreservation to all women under 35 years of age prior to chemotherapy, when there is a risk of premature ovarian failure. 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. But we wholeheartedly agree with Revel and Schenker (2004), who contributed to a debate published in Human Reproduction, arguing that ovarian cortex banking should be offered before chemotherapy in all cases where emergency IVF is not possible.

One of the most important ethical issues is to ensure that the intervention does not harm the patient by dangerously delaying cancer treatment and that no remnant cells are reintroduced by subsequent transplantation. Taking these points into account, we agree with Dudzinski (2004) that policies to protect the patient’s future rights to her gametes should be developed, as well as policies addressing the disposition of the gametes if the patient dies. Although an adolescent is more vulnerable when consent is sought in the rush to begin chemotherapy, she must be mature enough to understand the risks and benefits of the procedure. Consent must then be discussed extensively, the discussion including both the adolescent patient and her parents, in order to minimize the risk of conflict of interest or inadvertent caution (Bukovsky, 2005). Respecting the code of good practice, all patients who may become infertile have the right to receive proper consideration of their interests for future possibilities in the field of ovarian function preservation. The selection of cases should be carried out on the basis of a multidisciplinary staff discussion including oncologists, gynecologists, biologists, psychologists and pediatricians. Counselling should be given and informed consent obtained from the patient. Cancer treatment takes priority over potential restoration of fertility, but offering the chance to preserve fertility may greatly enhance quality of life for cancer survivors.

Conclusion: Advances in reproductive technology have made fertility preservation techniques a real possibility for patients whose gonadal function is threatened by premature menopause, or by treatments such as radiotherapy, chemotherapy or surgical castration. Decision making in this area is particularly difficult because of the experimental nature of some of these techniques. With their continued development and optimization, however, it may one day be possible to offer an individualized approach to management, be it through embryo
cryopreservation, oocyte cryopreservation or cryopreservation of ovarian tissue (isolated follicles, cortical fragments or whole ovary).

Cryopreservation of ovarian tissue should be seriously considered for any patient undergoing treatment likely to impair future fertility, the indications being pelvic, extrapelvic and/or systemic malignant diseases, as well as non-malignant diseases. 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 38 years, the procedure should probably be restricted to patients below this limit. In any case, irradiation and chemotherapy appear to be less harmful to the gonads of prepubertal than postpubertal women (Häie-Meder et al., 1993; Sanders et al., 1996; Meirow and Nugent, 2001).

It has been demonstrated that cryopreserved primordial follicles can survive the thawing process. Research must now focus on investigating current options and new alternatives in the field, to identify the best way of using tissue after thawing. It is probable that the answer lies in the use of culture environments adapted to each stage of follicular development. If autografting is the aim of cryopreservation of ovarian tissue, testing for malignant cells in the tissue must be carried out using adequate techniques, especially in case of hematological malignancies.

In conclusion, live births obtained after transplantation of frozen-thawed ovarian tissue in humans give hope to young cancer patients, but there is still much work to be done. Research programs need to determine whether active angiogenesis can be induced to accelerate the process of neovascularization in grafted tissue, if isolated human follicles can be grafted, or indeed if microvascular reanastomosis of a entire cryopreserved ovary is a valuable option.

References


