Focus on Fertility Preservation

Ovary and uterus transplantation

Roger G Gosden

Center for Reproductive Medicine and Infertility, Weill Medical College of Cornell University, 1305 York Avenue, New York, New York 10021, USA

Correspondence should be addressed to R G Gosden; Email: rgg2004@med.cornell.edu

Abstract

Ovarian and uterine transplantation are procedures gaining more attention again because of potential applications in respectively fertility preservation for cancer and other patients and, more tentatively, women with uterine agenesis or hysterectomy. Cryopreservation of tissue slices, and possibly whole organs, is providing opportunities for banking ovaries for indefinite periods before transplanting them back to restore fertility. The natural plasticity of this organ facilitates grafting to different sites where they can be revascularized and rapidly restore the normal physiology of secretion and ovulation. Ischemic damage is a chief limitation because many follicles are lost, at least in avascular grafts, and functional longevity is reduced. Nevertheless, grafts of young ovarian tissue, even after cryopreservation, can be highly fertile in laboratory rodents and, in humans, autografts have functioned for up to 3 years before needing replacement. Transplantation by vascular anastomosis provides potentially longer function but it is technically much more demanding and riskier for the recipient. It is the only practicable method with the uterus, and has enabled successful pregnancies in several species, but not yet in humans. Contrary to claims made many years ago, neither organ is privileged immunologically, and allografts become rapidly rejected except in hosts whose immune system is deficient or suppressed pharmacologically. All in all, transplantation of these organs, especially the ovary, provides a broad platform of opportunities for research and new applications in reproductive medicine and conservation biology.

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Introduction

Transplantation of the ovaries and uterus are old ideas gaining fresh attention. Following pioneering attempts to transplant ovaries for infertile women over a century ago, the focus turned to experimental models and, more recently, back to the clinic again. This circularity reflects research progress, more professional acceptance and evolving clinical priorities as cancer treatment becomes more successful at the price of sterilizing some patients at reproductive and even pre-reproductive ages.

The ovaries always garnered more attention than the uterus as candidates for transplantation because the surgery is less demanding or risky and applications more attainable. Moreover, ovarian transplants never suffered from association with phoney 'rejuvenation science' that brought testicular transplants into disrepute during the early 20th century (Hamilton 1986), and they have found valuable roles in experimental science ever since. Transplants of frozen-thawed ovarian tissue are now being tested as alternatives to oocyte banking for fertility preservation, providing that there is no risk of transmitting disease (Meirow *et al.* 1998) and confirming the cautious hope expressed in the

mid-1990s: 'Ovarian tissue cryopreservation prior to chemotherapy and abdominal/whole-body radiation is a distinctly promising technique for younger cancer patients' (Gosden & Aubard 1996). In recent years, there have been over 20 reports of transplanted fresh and frozen ovarian tissue representing nearly 50 cases, mainly for premature ovarian failure. Uterine transplants have not found any utility so far, but they are being investigated experimentally for potential application to women after hysterectomy or with Müllerian system anomalies. The focus of this review will mainly be the ovary, reflecting the preponderance of studies and because Brannström *et al.* (2003) have critically appraised literature on uterine transplantation.

Historical background

Paul Bert in Paris was probably the first to transplant ovaries. According to his medical thesis in 1863, he grafted ovaries from one rabbit to the abdominal cavity of a recipient animal, but they did not survive. Over 30 years later, Chrobach in Austria instructed his assistant, Emil Knauer, to repeat the experiment: only one allograft

survived, but this positive result encouraged Knauer to try autografts and they survived much better and restored fertility. In those days, and this was the dawn of endocrinology, there were none of the advantages taken for granted today, such as hormone immunoassays or appreciation of the allograft reaction and follicular biology, but the curiosity and skill of some pioneers led to impressive advances and encouraged others to enter the field.

Credit for the first success is not, however, due to Knauer but to Robert Morris (Fig. 1a) in New York, who had reported results with human ovarian transplants a year earlier in 1895 (Morris 1895). A medical graduate of the Columbia University College of Physicians and Surgeons, he was 38 years old at the time and would go on to establish a national reputation in abdominal surgery and become a Professor at the New York Postgraduate Medical School. His aim was to conserve ovarian function after hysterectomy, reflecting his vigorous opposition to the common and indiscriminate practice of removing normal ovaries, then called Battey's operation. He was aware of the emerging field of 'internal secretion' (endocrinology) and was encouraged by progress with thyroid gland transplants, hoping his ovarian transplants would restore secretion to counter menopausal symptoms and stimulate ovulation to reverse infertility.

At first, he transplanted ovarian tissues into the uterine cavity, later preferring to suture them to the broad ligaments. His first case was an allograft for a 20-year-old woman with primary amenorrhea; she had menses 2 months later but was lost to follow-up. The second was a woman with pelvic inflammatory disease in whom he transposed ovarian tissue to the stump of the ablated fallopian tube, and this enabled the patient to get pregnant, although she later aborted. In all, he performed about 26 such operations.

His most famous case, published in 1906, involved a woman with secondary amenorrhea, apparently from polycystic ovarian disease, in whom he grafted ovarian tissue biopsies to the broad ligament from a 33-year-old woman undergoing surgery for a uterine prolapse. Several years later, the recipient's general practitioner wrote to Morris announcing that she had given birth to a 7.5 lb daughter, and she later delivered two more children. It is impossible so long afterwards to verify the genetic identity of her children, whether matching the donor or the birth mother, but Morris was cautious and well aware that traces of recipient tissue can remain behind and even ovulate. Indeed, he had already encountered this problem as a Cornell biology student, and it continued to cloud interpretation of other reports and has sometimes been called 'ovarian remnant syndrome'. He believed that it could be ruled out because he had used a special instrument for excising tissue (Tuffier's angiotribe), and he had a senior medical observer present as a witness. Even more worrisome, the chances of allograft survival were slim, as Morris knew full well and stated, 'We are usually disappointed in heteroplastic grafting'. Most modern commentators have discounted his 1906 claim, although we cannot rule out the possibility that the case had a lucky genetic combination.

After his results became widely publicized, his methods were tried in other centers, but by the beginning of World War II, there were strong doubts whether even autografts can be very successful. The only exception was Estes' operation, named eponymously for its originator and his son (Estes Jr) who continued the practice for many years. The operation involved opening the uterus at its junction with a Fallopian tube, bisecting the proximal ovary and attaching it to the cut surface, somewhat like Morris's second transplant except the blood vessels and nerves were not severed. Estes'

Figure 1 (a) Robert Tuttle Morris (1857–1945; source: Morris RT 1935 *Fifty Years a Surgeon*, New York: E P Dutton & Co.) (b) Alexis Carrel (1873–1944; courtesy of the Rockefeller Archive Center).

operation directed ovulated oocytes into the uterine lumen, an environment that is not as hostile to fertilization in primates as in small laboratory animals (as later confirmed by clinical pregnancies from intrauterine transfer of zygotes conceived by *in vitro* fertilization (IVF)). When IVF was established clinically after 1979, Estes' operation fell into disuse; it had never been very successful and Adams (1979) deduced that many old claims had been exaggerated and the odds of becoming pregnant per cycle were <1%.

Overall, hundreds of autografts and allografts in approximately equal numbers were carried out during the early decades of the 20th century, and both orthotopic and heterotopic sites were used, including the rectus muscle, omentum, fallopian tube, uterus, breast, and under the skin. The aim of heterotopic transplants was purely to restore hormone production since technology was not available then for IVF. There were false hopes that they could stimulate the *in situ* ovaries to function again, but supportive evidence was never forthcoming.

Although clinical transplantation paused following widespread skepticism and emergence of IVF technology, ovarian transplants continued to serve as an important technique in experimental endocrinology and pathology. Among other applications, they were used to investigate ovarian ageing, angiogenesis, cryopreservation, luteolysis, innervation, sexual differentiation, steroidogenesis, tumorigenesis, and for rescuing lethal genotypes (Table 1). For the most part, studies were carried out using inbred strains of mice and rats to avoid rejection, and tissue implants succeeded without the demands of microsurgery. According to a leading investigator, the same ischemic grafts were unlikely to work with larger ovaries because they 'cannot be expected to survive adequately unless proper vascular anastomoses are prepared' (Krohn 1977). Krohn seems to have overlooked or discounted the claims of Morris and other pioneers but his pessimism has fortunately turned out to be unjustified. Starting in the 1990s, ovarian tissue implants from at least ten species, including rodents, companion animals, farm animals, and primates have all proved successful to varying extents, success rates varying according to a number of factors (Table 2). It is undoubtedly true, however, that larger organs benefit from vascular surgery, and uterine transplants have almost always involved vascular anastomoses.

Tissue implants

The small ovaries of laboratory rodents and fetuses can either be grafted intact or after bisection, whereas the cortical tissue of larger organs is peeled with a scalpel from its underlying medulla to create a 1–2 mm slice or if possible even thinner to minimize the duration of ischemia and accelerate revascularization. In either

Table 1 Ovarian transplants in animals: selected literature (adapted from Gosden & Aubard 1996).

Investigation	Selected references
Role of ovarian ageing	Aschheim (1965), Peng & Huang (1972) and Felicio <i>et al.</i> (1983)
Angiogenesis	Dissen <i>et al.</i> (1994)
Compensatory ovarian hypertrophy	Weems Chihil <i>et al.</i> (1976)
Conservation biology	Wolvekamp <i>et al.</i> (2001)
Cryopreservation	Parrott (1960), Gosden <i>et al.</i> (1994a), Harp <i>et al.</i> (1994), Salle <i>et al.</i> (1999, 2002) and Wang <i>et al.</i> (2002)
Hepatic inactivation of steroids	Leavitt & Carlson (1969) and Uilenbroek <i>et al.</i> (1978)
Immunological tolerance and allografting	Harris & Eakin (1949), Krohn (1958), Cornier <i>et al.</i> (1985) and Scott <i>et al.</i> (1987)
Luteolytic mechanisms	Goding <i>et al.</i> (1967) and Bland & Donovan (1968)
Innervation	Jacobowitz & Laties (1970)
Mesonephros differentiation	Taketo-Hosotani <i>et al.</i> (1985)
Mutant genotype rescue	Russell & Hurst (1945) and Sztejn <i>et al.</i> (1999)
Pregnancy physiology in marsupials	Tyndale-Biscoe & Hearn (1981)
Restoration of fertility by autografting	Krohn (1965) and Winston & McClure Browne (1974)
Reversing sterility in mutant animals	Wordinger <i>et al.</i> (1986)
Sex differences in the pituitary gland	van der Schoot & Zeilmaker (1970) and Norman & Spies (1986)
Steroidogenesis	Falck (1959), Farookhi <i>et al.</i> (1982) and Schnorr <i>et al.</i> (2002)
Tumor formation	Kim <i>et al.</i> (2001)
Xenografts	Gosden <i>et al.</i> (1994b), Gunasena <i>et al.</i> (1997), Weissman <i>et al.</i> (1999) and Snow <i>et al.</i> (2002)

case, it is important to anchor the tissue firmly with sutures or tissue glue, or secure it in a tight cavity such as the ovarian bursa of rodents, a subcutaneous pocket or under the renal capsule.

The surgical procedure is rapid, but warm ischemia may extend to 3–7 days during revascularization and it is during this time that 50% or more of the primordial follicles and virtually all growing follicles disappear (Jones & Krohn 1960, Newton *et al.* 1996, Baird *et al.* 1999). Antioxidants may provide some protection by combating reactive oxygen species from reperfusion injury (Nugent *et al.* 1998), but angiogenic factors and granulation tissue appear to be promising (Israely *et al.*

Table 2 Factors potentially affecting the performance of ovarian transplants.

1. Age of donor
2. Number of primordial follicles
3. Tissue mass/cortical thickness
4. Duration of ischemia/proximity to host vasculature
5. Immunological compatibility
6. Site of transplantation
7. Cryopreservation/vitrification
8. Reactive oxygen species

2006). Despite follicle losses, the transplant procedure has an excellent record, especially in mice where up to 17 litters and 79 pups were delivered after fresh, young ovaries were grafted in one study (Krohn 1965), and a normal reproductive lifespan was obtained in another after cryopreservation (Candy *et al.* 2000). Much of this success is attributable to favorable anatomy and physiology because primordial follicles in rodents are minute (16 μm in diameter), non-growing and lie close to the ovarian surface although, somewhat unexpectedly, their metabolic rate per unit mass is no lower than for growing follicles (Harris 2002).

After the demise of large follicles, estrogen production plummets to undetectable levels for at least a week until they are replaced by recruits from primordial stages. The recovery is faster in ovariectomized hosts, presumably because elevated serum follicle-stimulating hormone (FSH) stimulates follicle growth and secretion can be monitored externally by vaginal patency and epithelial cornification. After follicle dynamics are restored, the pyramid of follicle stages normalizes, estrous cycles return and fertile potential is restored. Scar tissue disappears quickly and the ovary can appear completely normal histologically, apart from a reduced follicle reserve corresponding to a more advanced chronological age. Cycle frequency and fertility can be indistinguishable from age-matched, unoperated animals, but they start to decline earlier (Aschheim 1965, Felicio *et al.* 1983). Young donor ovaries provide the longest functional service, and when prepubertal organs are grafted into adult hosts they start functioning prematurely as they become stimulated by adult levels of gonadotropins.

To conclusively prove offspring from mated hosts are derived from the donor and not residual host tissue, it is desirable to use genetically distinguishable combinations, such as transgenic animals expressing green fluorescent protein. But where autografts are used, some of the ambiguity can be reduced by grafting donor tissue into a resected host ovary after X-irradiation, which only requires a low dose to eliminate small follicles in mice ($\text{LD}_{50}=0.15\text{ Gy}$). However, the growing follicles are much less radiosensitive, taking at least a month to clear by natural processes, so it is necessary to delay the operation. Sterilized ovaries can also become fertile again after transferring enzymatically isolated primordial follicles, even after frozen storage (Carroll & Gosden 1993), but progress with isolated human follicles has been more difficult because of low recovery rates from the fibrous cortex. Notably, there is no evidence of any increase in congenital abnormalities from ovarian transplants (Candy *et al.* 2000), nor are there any reports of excess risk of ovarian tumors, with the notable exception of intrasplenic implants (Uilenbroek *et al.* 1978).

This is a reassuring background to clinical efforts for restoring fertility with cortical ovarian grafts (Fig. 2). Some

studies with fresh tissue have been particularly encouraging (Sanchez *et al.* 2007), and an opportunity to transplant ovaries in a series of monozygotic twins, one of whom was fertile and the other was sterile, has confirmed that the technique can be highly effective in our species. Tissue was transplanted bilaterally to recipient organs in seven women who had long been sterile and to another with gonad dysgenesis; six of them so far have conceived naturally and there are several ongoing pregnancies or deliveries (Silber *et al.* 2005, 2008, Silber & Gosden 2007). The surgery was uncomplicated, involving laparoscopic unilateral oophorectomy for the donors and mini-laparotomy of the recipients, with no adhesions encountered after the transplantation.

The first case illustrates an emerging pattern. During the initial 3–4 months post-transplant until the first menses, serum gonadotropins were falling rapidly and estradiol rising from postmenopausal levels (Fig. 3). The patient conceived in the third cycle and the pregnancy was uneventful leading to the birth of a healthy full-term baby. After breastfeeding, the patient had another nine menses before she conceived again, but this pregnancy ended in the miscarriage of a karyotypically normal fetus. Three years after the fresh transplant, her cycles ceased and hormones returned to pre-transplant levels. After she received a second transplant, this time using slices of frozen-thawed tissue also from her sister, the hormonal response was the same as before but this time she conceived an ongoing pregnancy at the first ovulation.

The implant technique is surprisingly successful with ovaries from many different species but it is unsuitable for large organs, like the human uterus. However, when the mid-segment of a mouse uterine horn was sutured orthotopically into a resected host uterus the tissue survived, although never supporting pregnancy even though the lumen remained patent and embryos implanted and developed normally on either side (the late Esther Jones, personal communication).

Vascular transplants

The chief disadvantage of avascular implants, namely a longevity limited to 3 years, should be overcome by vascular anastomosis because a minimal ischemia time drastically reduces follicle losses (Yin *et al.* 2003). Vascular surgery was pioneered at the Rockefeller Institute for Medical Research in New York City by Alexis Carrel (Fig. 1b) who was a contemporary of Robert Morris a few blocks away, though they never collaborated. In 1906, Carrel reported the transplantation of intact ovaries from cats with their pedicle and aorto-caval segment to a host animal, but he did not record their fate, turning his attention to transplanting other organs and garnering himself a Nobel Prize by 1912. Some gynecological surgeons took up the challenge with patients, but evidently with limited success, probably because of the tiny ovarian artery. Nevertheless, considering Carrel's

Figure 2 Schematic of human ovarian cortex transplantation (reprinted, with permission, from Silber *et al.* (2005); copyright 2005 Massachusetts Medical Society. All rights reserved).

technical feat and Morris's claim, 1906 was an *annus mirabilis* for ovarian transplantation.

Little progress could be made until advances in microsurgery and the realization of the advantages of *en bloc* preparations encouraged renewed efforts in animal models. Inclusion of the uterus and tubes with the ovaries was primarily a strategy to use larger vessels for anastomosis. Successful results were achieved in a number of species, including dogs, pigs, sheep, rabbits,

rats, and monkeys using either vascular anastomosis or omentopexy in which the omentum is wrapped around the organ to encourage revascularization (Gosden & Aubard 1996). Paldi *et al.* (1975) used both techniques in autotransplants for 40 dogs and obtained much better results with anastomosis, but only one animal had a successful pregnancy. At one time, ovo-tubo-transplantation seemed to be a remedy for tubal obstruction in patients if the allograft reaction could be avoided, but

Figure 3 Restoration of ovulatory cycles and fertility in a young woman with idiopathic, premature ovarian failure after transplanting fresh ovarian tissue (arrow) from a genetically identical sister. Three years later, after the first transplant failed, cryopreserved tissue from the same donor was transplanted (second arrow). Serum FSH and LH are expressed as mIU/ml and estradiol-17 β as pg/ml (from Silber *et al.* 2005, 2008).

it became redundant when clinical IVF became established (Winston & McClure Brown 1974). *En bloc* operations in all species were carried out using fresh tissue with the single exception of a study of cryopreserved ovaries in rats in which smaller organs favored success and allowed limited restoration of fertility (Wang *et al.* 2002).

In another remarkable technical feat, the sheep ovary was autografted heterotopically in a two-stage microsurgical operation to the neck, providing excellent access to ovarian venous blood for hormone analysis (Goding *et al.* 1967). A heterotopic transplant was also reported for an 18-year-old patient with subdiaphragmatic Hodgkin's disease in which the left ovary was moved to the subcutaneous tissue of the arm to avoid an irradiation field and the right ovary transposed intraperitoneally without dividing its vessels (Leporrier *et al.* 1987). Menstrual cycles were regular and cyclical changes in the circumference of the arm occurred in synchrony with the basal body temperature rhythm and presumptive growth of a corpus luteum. A secondary oocyte was aspirated from the graft but no fertilization attempt was made. This form of oophoropexy is beyond the scope of this review.

There is exciting new progress with orthotopic transplantation of whole ovaries of sheep and rabbits, which is all the more notable after low-temperature storage following perfusion of the vasculature with a cocktail of cryoprotective agents and inhibitors of reperfusion injury (Bedaiwy *et al.* 2003, Arav *et al.* 2005, Chen *et al.* 2006, Imhof *et al.* 2006). After thawing and reanastomosing the vessels end-to-end or end-to-side, some organs survived sufficiently to have endocrine function and generate oocytes for assisted reproduction for 2–3 years (Arav *et al.* 2005). But, even with a high degree of surgical skill, the procedure has a higher complication rate than cortical grafts. Moreover, the outcomes must be interpreted cautiously. It is possible that in an apparently successful operation there is thrombotic obstruction of the vasculature with some tissue surviving as an avascular graft, giving the appearance of a fully successful outcome. Only if the follicle reserve is subsequently shown to correspond closely to a control ovary, can we be sure the vascular transplant was fully successful (Yin *et al.* 2003). While this standard of evidence is too high in a clinical setting, the recent case reported by Silber *et al.* (2008) of a microvascular transplant between 37-year-old monozygotic twins appears to have been wholly successful, since serum FSH fell to very low levels in the recipient who had chronically elevated gonadotropins and was postmenopausal since she was a teenager.

Allografts, xenografts, and tolerance

In 1908, Marshall and Jolly reached a prescient conclusion based on studies of rat and monkey tissues

that can hardly be improved on today: 'Homoplastic transplantation (autotransplantation) of ovaries is very considerably easier to perform successfully than heteroplastic transplantation'. Notwithstanding Morris's own cautious view, their warning was either ignored or overlooked and even years later a comprehensive review of transplantation surgery stated that, 'It is almost impossible... after reading critically the early literature on the subject to escape the conclusion that ovarian homografts (allografts) may survive for weeks or even months in various different species' (Woodruff 1960). Billingham & Parkes (1955) drew similar conclusions by observing the comparatively slow rejection time of ovarian allografts in rats compared with skin grafts, stating, 'The majority of ovarian allografts are themselves incapable of invoking reactions'. There was a belief that in the hierarchy of antigenicity the ovaries are among the more anergic tissues, having fewer antigen presenting cells than, for example, skin and liver and therefore being more readily tolerated with mild or no immunosuppression.

This confidence was undermined from the 1980s by Cornier *et al.* (1985), Scott *et al.* (1987), and others who showed that immunosuppressive therapy, such as cyclosporine A plus steroids, is needed for ovarian allografts to survive. Even when circulating CD4+ cells were rarefied by immunodepletion, tolerance was not induced between mouse strains differing at the major histocompatibility complex (MHC) class I locus, though survival was extended in combinations of more closely related strains (Gosden 2007). If the donor and host were genetically unrelated and the host's immune system was competent, there was a heavy infiltration of leucocytes within a week that quickly rejected the graft. There is therefore experimental justification for vigorous immunosuppressive therapy for both vascular and avascular allotransplants, even where the donor and recipient are well matched (Mhatre *et al.* 2005). In the first fully authenticated case of its kind in humans, Donnez *et al.* (2007) have reported survival of an ovarian tissue implant in a woman from her genetically non-identical sister, but circumstances where a woman had already acquired specific tolerance to a donor (in this case her bone marrow transplant donor) will be rare.

Allografts and xenografts can, however, survive indefinitely in immunodeficient host animals, such as severe combined immunodeficiency (SCID) and nude mice, which have been used to test viability after tissue transport or cryopreservation or in disease (Newton *et al.* 1996, Kim *et al.* 2001). In the original report, ovarian tissue from cats and sheep were grafted under the kidney capsule of ovariectomized SCID mice (Gosden *et al.* 1994b). The follicles were evidently stimulated by host gonadotropins because the vaginal epithelium was cornified by superphysiological levels of estradiol. Follicle growth appeared normal but sheep or human follicles rarely grew larger than 5–6 mm in diameter,

much less than mature sizes for these species. Nevertheless, this is large enough to recover oocytes for *in vitro* maturation and IVF (Oktay *et al.* 1998, Gook *et al.* 2001, Kagawa *et al.* 2007). The feasibility of generating fertile gametes in xenografts has been demonstrated in a rodent model (Snow *et al.* 2002), and this technique may be useful in conservation biology (Gunasena *et al.* 1997, Wolvekamp *et al.* 2001).

False historical assumptions about immunological privilege for the ovary were mirrored for the uterus. By the 1960s, it was already apparent that uterine allografts required aggressive immunosuppression (Brannström *et al.* 2003, El-Akouri *et al.* 2003), but that is not the only reason why they are unlikely to be applied clinically in the foreseeable future. The first uterine transplant had to be abruptly removed after 99 days because of thrombosis and necrosis from torsion of blood vessels (Fageeh *et al.* 2002). Although procurement and short-term storage/shipment of organs is not a problem (Del Priore *et al.* 2007), major ethical issues need to be addressed before further attempts to undertake such a risky procedure that is not lifesaving and for which an effective alternative (IVF surrogacy) is legitimate at least in some countries. Nor is the danger to the transplant recipient the only consideration. The healthy babies of patients receiving immunosuppressive treatment for non-reproductive organ transplants (e.g. kidney) are reassuring although close monitoring is continuing for teratogenic risks (Armenti *et al.* 2005).

Fertility preservation

Transplantation research has been boosted by the ability to bank ovarian tissue in liquid nitrogen for patients needing to preserve a stock of follicles and postpone iatrogenic menopause. The original breakthrough with ovarian cryopreservation was made in the 1950s by the Parkes Group in London using 15% glycerol as the cryoprotectant. The climax of their work was achieved when Parrott (1960) obtained live pups from frozen-thawed isogenic grafts. Since there were no obvious clinical applications at the time, the subject attracted little interest until cancer survivorship for young people improved dramatically, which it had done by the 1990s. By that time, automated freezers and superior cryoprotective agents, such as dimethyl sulfoxide, propanediol, and ethylene glycol, were available (Hovatta *et al.* 1996). What is more, autografts of cryopreserved ovarian cortical tissue from large animals were being shown to restore fertility (Gosden *et al.* 1994a, Aubard *et al.* 1999, Baird *et al.* 1999, Salle *et al.* 1999, 2002), and human ovarian tissue was found to tolerate the same freezing and thawing protocols (Newton *et al.* 1996, Gook *et al.* 2001).

These studies laid a foundation for ovarian tissue banking in patients, which was much needed because oocyte freezing programs in those days were not very

effective. Increasingly, hematology and oncology services began to bank ovarian tissue for patients who were young (<35 years), had a good prognosis, no children yet and were at low risk of ovarian metastases. The first ovarian transplant after frozen storage was reported a few years later (Oktay & Karlikaya 2000), though it was unsuccessful in restoring spontaneous cycles and gonadotropin levels quickly returned to postmenopausal levels, probably because of a paucity of follicles. When the first baby was born in 2004, just as in Robert Morris's day, there was controversy about the origin of the ovulation because the thawed tissue was adjacent to residual ovarian tissue (Donnez *et al.* 2004). While some doubts about the origin of the fertilized oocyte remain, there are now five children born after similar procedures, all but one for former cancer patients and all from orthotopic grafts (Table 3).

The lower surgical burden of heterotopic transplants is attractive, because they involve a more superficial procedure where they can be monitored readily and oocytes can be aspirated for IVF (Kim *et al.* 2004, Oktay *et al.* 2004). To date, only early cleavage stages and a biochemical pregnancy have been attained using this strategy (Oktay *et al.* 2004, Rosendahl *et al.* 2006), although the generation of a baby monkey from an oocyte matured in a subcutaneous graft of fresh tissue bodes well for eventual success in humans (Lee *et al.* 2004). Nevertheless, the technique may remain inefficient even with gonadotropin stimulation because of the small harvest of oocytes for IVF, reflecting the size of tissue, and perhaps a poor vascular supply. Furthermore, the robustness of results in a series of patients undergoing fresh orthotopic transplantation through minimally invasive incisions argues against the heterotopic approach (Silber *et al.* 2008).

It remains to be seen whether outcomes with orthotopic or heterotopic transplantation will be improved by vitrification instead of the standard slow

Table 3 Clinical pregnancy successes after transplanting cryopreserved ovarian tissue.

Patient type	Transplant	Country	Reference
Hodgkin's lymphoma	Autograft Orthotopic cortical strip	Belgium	Donnez <i>et al.</i> (2004)
Non-Hodgkin's lymphoma	Autograft Orthotopic cortical strip	Israel	Meirow <i>et al.</i> (2005)
Ewing's sarcoma	Autograft Orthotopic cortical strip	Denmark	Claus Anderson (personal communication, 2007)
Hodgkin's lymphoma	Autograft Orthotopic cortical strip	Belgium	Demeestere <i>et al.</i> (2006)
Idiopathic, premature ovarian failure	Donated by MZ twin Orthotopic cortical strip	USA	Silber <i>et al.</i> (2008)

cooling method for cryopreservation. Vitrification avoids the formation of ice crystals inside cells and extracellularly, but requires a higher concentration of protective substances with a corresponding risk of toxicity. So far, it has been used in five species, and successful pregnancies have been obtained in mice and sheep (Migishima *et al.* 2003, Bordes *et al.* 2005, Kagawa *et al.* 2007).

This increasing diversity of techniques reflects a vibrant field and some pressing medical applications. The prospects of further improvements in tissue preservation and transplantation are excellent and better results are expected with younger subjects because they have a larger follicle reserve, and this is especially good news for child patients for whom oocyte and embryo banking are not options. All technologies are interim, however, and in the long-term transplantation could be superseded by culturing follicles after cryopreservation (Smitz & Cortvrindt 2002), theoretically avoiding the risk of disease transmission for cancer patients and making more efficient use of limited follicle numbers. Likewise, prenatal development may eventually be achieved *ex vivo* but for the foreseeable future transplantation of the ovary, and perhaps even the uterus, can provide some service in medicine as well as in research.

Declaration of interest

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