Uterine transplantation research: laboratory protocols for clinical application

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ABSTRACT: The aim of this review is to summarize the state-of-the-art methods that are used in clinical organ transplantation today, as well as the major findings of recent experimental uterine transplantation (UTx) research regarding organ donation/retrieval, ischemic preservation, surgical techniques for anastomosis, immunosuppression and pregnancy. Absolute uterine factor infertility lacks treatment despite the major developments in infertility treatment and assisted reproduction. Concerning uterine factor infertile patients, genetic motherhood is only possible through gestational surrogacy. The latter can pose medical, ethical and legal concerns such as lack of control of life habits during surrogate pregnancy, economic motives for women to become surrogate mothers, medical/psychological pregnancy-related risks of the surrogate mother and uncertainties regarding the mother definition. Thus, surrogacy is non-approved in large parts of the world. Recent advances in the field of solid organ transplantation and experimental UTx provide a favourable and safe background in a scenario in which a human clinical UTx trial can take place. Protocols based on animal research over the last decade are described with a view to providing a scientifically guided approach to human UTx as an experimental procedure in the future.

Key words: uterus / transplantation research / infertility / pregnancy / immunosuppression

Introduction

Absolute uterine factor infertility (Table I) still lacks treatment despite the major breakthroughs in infertility treatment and assisted reproduction during the last 25 years. The motherhood options for uterine factor infertile patients are today gestational surrogacy (genetic motherhood and after adoption legal motherhood) or adoption (legal motherhood). This patient group (several thousand in the UK alone) (Sieunarine et al., 2005) may be helped to attain genetic, gestational as well as legal motherhood with uterus transplantation (UTx).

The procedure of UTx may present some advantages compared with gestational surrogate. Thus, pregnancy-risks (thromboembolism, hypertension and eclampsia) are taken by the mother, the future mother can fully control lifestyle factors (medicine, alcohol and smoking) during pregnancy, natural bonding during pregnancy/childbirth is possible and the mother definition is clear from a legal point of view. On the other hand, UTx implies a potential surgical risk for both the donor and the recipient and the future pregnancy will take place under immunosuppression (IS).

In year 2000, the initial and so far sole human UTx attempt was performed, with transplantation of the uterus and oviducts to a young woman who previously had undergone emergency peripartum hysterectomy (Fageeh et al., 2002). This case, performed in Saudi Arabia, was—to a certain extent—successful, given that the surgery of the live donor and the recipient went without major complications and the uterus survived for almost 100 days.

The solitary human UTx case further stimulated research activities within the field of experimental UTx (Brannstrom et al., 2010) and in parallel, great progress was accomplished in the field of transplantation of highly immunogenic composite tissues, such as the hand, abdominal wall, face and larynx (Siemionow et al., 2010). These types of composite tissue transplantation (CTT) have, in line with UTx, the ultimate goal of restoring a missing body function in otherwise healthy patients and thereby to improve their quality-of-life. IS has been standardized (Szczech et al., 1998; McGuire et al., 2009; Costanzo et al., 2010) and can be maintained with low doses of medications (KDIGO Transplant Work Group, 2009).
The focus of this manuscript is to review the research accomplishments in the area of UTx (Table II). Indeed, several experimental animal models have been used to perfect the different aspects of UTx in order for it to be safely applied in humans. We present the current state of research of the various aspects of UTx, including IS and techniques, and our thoughts on the introduction of human UTx as an experimental procedure.

### Organ donation and retrieval

#### Organ donation and retrieval in solid organ transplantation

There are two main categories of donors, live and deceased. The majority of deceased donors (DDs) are brain-dead. Circulation is maintained because they are heart-beating. They are apneic and consequently ventilators support continued oxygenation of organs. In some terminal cases, brain-death cannot be declared due to residual neural activity but occurs soon after respiratory support is stopped (non-heart-beating donors) (Steen et al., 2003). The advantages with use of organs from DDs are the elimination of the risk to the donor and that an organ can be retrieved together with a large vascular pedicle containing central and vital vessels such as the aorta, vena cava and iliac vessels.

Live donation (LD) is practiced in renal transplantation, partial liver transplantation (Montgomery et al., 2005; Olsen and Brown, 2008) and less frequently as segmental pancreas or lung transplantation.
Advantages of LD transplantation include tissue matching. Indeed, if the donor is maternal or a sibling, they share at least one haplotype with the recipient. In addition, the timing and location of the retrieval are controlled, thereby allowing any necessary studies or treatments to be conveniently performed. The donor and graft can be electively studied in order to exclude viral infections, diminished organ function or vascular anomalies that may render an organ unsuitable for transplantation.

Cold ischemia of the graft (time from organ recovery to implantation) is kept at a minimum and the harmful systemic brain-death associated inflammation (Pratschke et al., 2005), that also affects the quality of the peripheral organs, is avoided.

Patient and graft survival in renal transplants are better with LD versus DD. (Segev et al., 2005). The major disadvantage of LD is the risks of the surgery to the donor. They include bleeding, damage of the remaining organs, infections, thromboses and pulmonary embolism (Olsen and Brown, 2008; Dols et al., 2010). Large retrospective kidney LD studies have shown mortality and morbidity up to 0.03 and 14%, respectively (Hadjianastassiou et al., 2007; Mjoen et al., 2009). However, a recent large cohort study comparing LD with paired-characteristics individuals did not demonstrate any difference in 6-years-mortality rates (Segev et al., 2010). Introduction of minimal invasive surgery at organ retrieval from LD may decrease morbidity. Thus, laparoscopic kidney retrieval from LD showed better quality-of-life of donors compared with classical kidney retrieval (Dols et al., 2010). The recent introduction of robotic-assisted laparoscopy (Oberholzer et al., 2010) in LD surgery should reduce the morbidity and convalescence of LD further.

Comparable general inclusion criteria of an organ donor should exist for LD and DD. Thus, systemic or organ-specific viral diseases such as human immunodeficiency virus and hepatitis should be ruled out and there should also be an upper age limit, specific for each organ and also adjusted to the recipient’s age.

New developments and research concerning organ donation and retrieval in UTx

Several animal models have examined the feasibility and techniques of uterus donation, comparable with an LD and DD situation, with DD being explored in most animal models (Table I). Initial descriptions of successful UTx in the mouse (Racho El-Akouri et al., 2002) and rat (Wranning et al., 2008a,b) used donors and recipient of the same inbred strain (syngeneic) to avoid rejection and the donors were sacrificed after harvesting the uterus and its vascular pedicle including parts of the aorta/inferior vena cava (IVC) (Racho El-Akouri et al., 2002; Wranning et al., 2008a,b) or the common iliacs (Fig. 1) (Diaz-Garcia et al., 2010; Wranning et al., 2011). In these rodent models, the large vessels had to be included to attain a graft with vessels large enough for microvascular anastomosis, using 10 to 11-0 sutures. In general, the duration of retrieval was around 60 min in these rodent models. In rabbit UTx, the uterus was harvested with vessels up to and including the aorta and IVC (Sieunarine et al., 2008a,b), but durations of retrieval surgery in these six animals were not given.

Another experimental pig UTx model, mimicking a DD situation, was used within our collaborative group (Avison et al., 2009). The bicornuate uterus of minipigs was harvested together with arteries and veins all the way up to the insertion of the renal vessels in the aorta/IVC (Fig. 2). The duration of retrieval surgery was around 3.5 h. Experimental designs mimicking LD include the models of auto-UTx, with the uterus removed from an animal and after some ex vivo time transplanted back. In auto-UTx, the vascular pedicle cannot include vital parts, and care must be taken during surgery to preserve function of other organs. The surgery time in auto-UTx is naturally extended since it involves uterus retrieval, back-table preparation and transplantation (Dahm-Kahler et al., 2008). Surgery in the sheep auto-UTx included separation of the uterine vessels from the ureter and dissection to also include the distal part of the internal iliac vessels in the graft. The durations of surgery were ~6 h when only the uterus was harvested (Wranning et al., 2008a,b) and 8 h when the ovary was also harvested (Wranning et al., 2010). A different type of uterus retrieval in the sheep (Ramirez et al., 2008) used minor dissection of the vessels and also excluded ureteric dissections. This method only has clinical relevance in the small number of cases, where a hysterectomy can be performed in the recipient as part of the transplantation procedure.

Lately, UTx research has come to include non-human primate species. In our report on auto-UTx in the baboon, we retrieved the uterus together with ovaries and oviducts (Enskog et al., 2010) in order to use resumption of ovarian cyclicity (visualized as perineal skin changes) as a non-invasive measure of successful transplantation. The vessels dissected for retrieval were the two uterine arteries up to a level including the anterior portions of the internal iliac arteries and the ovarian veins for a length up to the crossing over the iliacs. The uterine veins were found to be too short for subsequent anastomosis. The time for retrieval surgery in these animals was ~2.5 h, and all the...
10 animals survived the extended surgery over around 7 h, which included time of back-table preparation and transplantation. This model would not be suitable for a human case in which the ovaries will not be transplanted. A modified baboon model for allogeneic transplantation did not include the ovaries and oviducts and retrieval by this method had a similar duration (our unpublished data). In another study on UTx autotransplantation in two cynomolgus macaques (Kisu et al., 2011), the uterus together with uterine arteries/veins was retrieved. The long time for retrieval (6–8 h) was most likely due to the complicated microsurgery in these small (3–6 kg) primates. One animal died 2 days after surgery, probably because of acute renal failure (Kisu et al., 2011).

Implications concerning organ donation and graft retrieval in human UTx

The criteria for a suitable uterus donor should be discussed. Naturally, the general exclusion criteria in solid organ transplantation (SOT) such as malignancy, ongoing viral disease and major systemic disease should apply. The uterus has to be examined preoperatively to rule out pathological changes that may compromise the capability of the uterus to carry a pregnancy, such as large leiomyoma/polyp, or those increasing the risk of the immunosuppressed recipient, such as human papillomavirus infection or cervical/endometrial atypia.

The solitary human UTx case (Fageeh et al., 2002) used LD, but it was not further discussed in the article whether DD was ever considered. Our collaborative research group has used large animal models to investigate techniques that could be used with DD, as explored in the pig (Avison et al., 2009) and with LD, as explored in the sheep (Dahm-Kahler et al., 2008) and baboon (Enskog et al., 2010). We think that the LD concept has certain advantages over DD for UTx, at least during the initial phase of introduction. The drawback of LD is the surgical risk of the donor, but that is partly balanced by the fact that the organ recipient will get an organ that is thoroughly examined to exclude pathology. Moreover, since the donor would most likely be a close relative (older sister, mother and/or aunt) the probability of a good tissue-type matching would be greater. Regarding the age of the donor, data from oocyte donation programs suggest that the pregnancy rate is similar between ‘young’ recipients (<45-year-old) and ‘old’ recipients (>45-year-old) (Soares et al., 2005). The incidence of adverse perinatal outcome is increased in older patients, suggesting a potential role of the uterus (Soares et al., 2005). On the other hand, factors such as the cardiovascular, metabolic, renal and general health of the patients were not assessed in this study (Soares et al., 2005), making it difficult to draw any firm conclusions. Paradoxically, the perfect model to isolate the effect of the ‘aged’ uterus on perinatal outcome would be a UTx situation.

In humans, the arterial supply to a uterine graft provided by both uterine arteries should ensure satisfactory perfusion. In DD, the vascular pedicle can be extended to include the arterial tree up to the lower abdominal aorta, with severance of all other branching arteries, including the external iliacs and also the pudendals and gluteals. In LD, there is a compromise between achieving reasonable lengths and widths of the arteries to be anastomosed and to do as little harm with minimal risks of the donor. The standard site of uterine artery severance at radical hysterectomy is just distal to the branches of the umbilical artery and inferior vesical artery from the anterior portion of the internal iliac artery. This location of division will give around 6 cm (our unpublished observations) long free vascular pedicles on the arterial side and 5.5 cm of the veins (Fig. 3). It would also be possible to harvest longer pedicles, including at least the anterior portion of the iliac arteries, without side effects of the donor. This procedure would spare the gluteal and pudendal arteries. A further modification, with a combination of both uterine veins and one ovarian vein (Fig. 3) at human UTx should also be considered in cases of post-menopausal donors.

Ischemic preservation

Ischemic preservation in SOT

Ischemia–reperfusion injury has major impact on transplantation success increasing the frequency of acute rejection events (Howard et al., 1990; Tottsuka et al., 2004) and the rate of chronic rejection (Schwarz et al., 2005). To minimize ischemic damage and prolong ischemic time, cold storage with a buffered preservation solution is commonly used. The most widely used preservation solutions are University of Wisconsin (UW), Celsior, histidine-tryptophan-ketoglutarate (HTK) and Perfadex.

Kidney storage in cold ischemia preservation solutions up to 18 h does not affect functionality or survival, although each hour of cold

Figure 2 Schematic drawing of the allogeneic UTx model of the pig. End-to-side anastomoses were used between the graft and the recipient’s aorta and IVC. The vaginal rim of the graft was connected to a cutaneous stoma.
ischemia after 18 h increases risk of delay in the graft function by 10%, implies a greater incidence of acute rejection and decreases the time until the first rejection episode (Barba et al., 2011).

In liver transplantation, maximum patient and graft survival occurred with cold ischemic times ranging between 7.5–12.5 h (Stahl et al., 2008). In heart transplantation, cold ischemia beyond 8 h does not modify graft survival, number of rejection episodes and hospital readmissions during the first post-transplantation year when compared with cold ischemic times within 1 h 30 min (Scheule et al., 2002). Cold cardioplegic times up to 13 h have been reported (Wei et al., 2005).

New developments and research concerning ischemic preservation in UTx

Some studies have evaluated the tolerance of a uterine graft to ischemia. In our initial study of syngeneic UTx in the mouse, the uterus was subjected to cold ischemic preservation in UW for 24, 48 or 72 h before transplantation (Racho El-Akouri et al., 2003a,b). Uteri preserved for 24 h regained functionality and were capable of implanting embryos and later deliver normal offspring. It should be acknowledged that the uterus of the mouse has a tissue mass < 1/5000 of the human uterus and the time limit of 24 h may not apply to a human uterus. However, initial studies on cold ischemic tolerance of the human uterus demonstrated that small pieces of human myometrium remained contractile in response to prostaglandin F2a and were ultra-morphologically normal after 24 h cold ischemia in either UW or Perfadex (Wranning et al., 2005). In a study on whole human uteri retrieved from DD the histology, examined in one uterus, was normal 12 h after cold ischemia in UW (Del Priore et al., 2007) and in a follow-up study histology was reported to be normal after 48 h (Sieunarine et al., 2008a,b).

Concerning animals with a uterine size similar to the human, it seems as if the ewe uterus tolerates warm ischemia for at least 3 h, since pregnancy has been seen after this duration of ischemia at autotransplantation (Wranning et al., 2010). In our recent experiments in baboons the total ischemic time was around 3 h, with 1 h being the time for anastomosis and thereby with a gradual temperature increase of the uterus. The long-term success rate, with restored menstruation, was 60% (our unpublished data), indicating that the primate uterus is resistant to major ischemia-reperfusion injury after a period of at least 3 h. Further research in primate species is needed in order to improve results before moving to a clinical introduction.

Implications concerning ischemic preservation in human UTx

We think it is most advisable to use a similar ischemic preservation protocol as used in heart transplantation, since the heart and uterus are organs of similar tissue composition, with mainly muscle cells. The most widely used preservation solution in clinical heart transplantation today (Lee et al., 2010) is HTK preservation solution, which we also presently use in baboon UTx (our unpublished data). This preservation solution (at 4°C) should be administered in situ, preferentially via the femoral artery, in the DD situation and by cannulation of the arterial ends on back-table at the LD situation. Warm ischemia up to 3 h and cold ischemia up to 24 h (Wranning et al., 2005) seem to be reasonably well tolerated by a uterine graft although these times should be minimized as discussed above.

Organ recipients

Organ recipients in SOT

A universal rule in any transplantation situation is that the general health of the recipient should be as good as possible (KDIGO Transplant Work Group, 2009; Costanzo et al., 2010). Large series of transplantations have shown 5-year mortality rates for heart- and lung-transplant recipients of 28 and 37.9 respectively (Allen et al., 2010; Kreisel et al., 2011). Nevertheless, the patient groups that would be most comparable to recipients of a uterine graft would be young patients receiving CTT, such as the hand and the face. There is no reported transplantation-associated mortality in these fairly young patients and otherwise healthy patients (Petruzzo et al., 2010).

A blood group match between donor and recipient is advisable. However, blood group mismatch can be overcome by removing the recipient anti-ABO antibodies prior to transplantation (Oppenheimer et al., 2010). Today, with modern IS, less consideration to a major histocompatibility complex match is taken, since it appears to have less effect on organ function or survival (Kovarik et al., 2002).

New developments and research concerning recipients in UTx

The perioperative mortality and morbidity of uterine recipients in the experimental animal models were initially fairly high, probably due to a
steep learning curve. Thus, in our initial publication on the syngeneic mouse UTx model, the successful graft rate increased from 40 up to 90% after 20 surgeries (Racho El-Akouri et al., 2002). The procedure of UTx in the rat also included a learning curve with a mortality rate in the recipients decreasing from 30 to 0% (Wranning et al., 2008a,b). This learning curve-associated morbidity has also been shown in other animal models such as the sheep (Wranning et al., 2010), the rabbit (Sieunarine et al., 2008a, b), the pig (Avison et al., 2009) and the baboon (Enskog et al., 2010).

Implications regarding recipients in human UTx

In a human UTx situation, the recipient will be fairly young and in good health. The age limit is dependent on the extent of the recipient’s ovarian reserve, which could accurately be determined by measurement of anti-Mullerian hormone (Hendriks et al., 2007). An excellent general health status would be mandatory in the recipient. Presence of any chronic infectious disease or metabolic disease should be exclusion criteria. It would be important to rule out conditions such as activated protein C resistance or deficiencies of either protein C, S or antithrombin (Soare and Popa, 2010) that predispose for thromboembolism. In cases of previous malignancy such as cervical cancer, a time-period of at least 5 years should pass before UTx, since the risk for recurrent cancer after this time period is almost 0% in the absence of risk factors (Park et al., 2010).

Recipient surgery

Recipient surgery in SOT

Great variability exists regarding the sites and techniques used for graft re-implantation depending on the target organ. Generally, grafts of SOT are not fixed except for their vascular anastomoses and functional structures such as the hepatic duct (liver), ureter (kidney) or bronchus (lung). Vascular anastomoses are performed in specific ways depending on the vessels to be anastomosed. Two general categories of micro-vascular anastomosis exist: end-to-side and end-to-end. Although end-to-end anastomosis has proved to be equally effective, end-to-side anastomosis is preferred by many because of its ability to retain distal flow and usefulness in anastomosing donor and recipient vessels of different diameters (Adams et al., 2000).

New developments and research concerning recipient surgery in UTx

Large vessel anastomosis by aorto/caval end-to-side anastomoses have been used in UTx experiments in the mouse (Racho El-Akouri et al., 2002), rat (Wranning et al., 2008a,b), rabbit (Sieunarine et al., 2008a,b) and pig (Avison et al., 2009) (Fig. 2) with animal and graft survival rates ranging from 100 (Wranning et al., 2008a,b) to 50% (Sieunarine et al., 2008a,b). End-to-side anastomosis between external iliac vessels of the recipient and internal iliac vessels (Fig. 1) of the graft was used in our recent rat UTx model, with a 70% success rate (Diaz-Garcia et al., 2010; Wranning et al., 2011). End-to-end anastomosis between uterine vessels was tested in auto-UTx experiments in the pig but the success rate was low (Sieunarine et al., 2005; Wranning et al., 2006). The technical difficulty in anastomosing the small uterine vessels deep down in the pelvis may be a factor behind the poor outcome.

In our baboon auto-UTx model with end-to-side anastomosis to the external iliacs, preserved uteri were seen in 2 out of 10 animals of the first set of experiments (Enskog et al., 2010). In our follow-up study, we were able to increase the long-term success rate 3-fold by introducing some modifications, such as the inclusion of a transplant surgeon to perform vascular anastomosis and changing the arterial anastomosis site from end-to-side to the external iliac to end-to-end on the internal iliac (Fig. 4). This allowed us to decrease the thrombosis rate of the anastomosis site.

Implications regarding recipient surgery in human UTx

A general message from all the animal UTx studies is that the anastomosis of the thin walled veins is more challenging than that of the arteries, with a defined lumen and thicker walls. In most animal studies, the immediate blood flow and tissue perfusion of the uterus were satisfactory, but with vascular thrombosis occurring at high rate at a later stage (Fageeh et al., 2002; Sieunarine et al., 2005).

In the only human trial, authors claimed that twisting of the uterus and subsequent blood flow cessation could be the origin of uterine necrosis (Fageeh et al., 2002). In that study, the short ends of the uterine arteries/veins were elongated at back-table with grafts from the saphenous veins, and these may also provide a site for thrombosis formation. We think it is advisable to use direct anastomosis between the uterine vessels and the external iliac vessels and also to use one of the ovarian veins, which usually is larger in diameter that the uterine vein (Fig. 3). In order to prevent torsion or prolapse of the uterus, it should also be fixed to the recipient’s round ligaments and with some kind of fixation of the lower uterine body to the sacrum.

Immunosuppression

Immunosuppression in SOT

The immunogenicity of the various transplant organs differs and partly organ-specific IS protocols are used. Most of these protocols involve induction and maintenance IS. The induction therapy refers to depletion of circulating T-lymphocytes in order to avoid cellular acute rejection. This is generally achieved by administration of polyclonal antibodies (ATG, anti-thymocyte globulin) together with corticoids and/or calcineurin-inhibitors (CNI; mainly cyclosporine/tacrolimus) during the perioperative period. Monoclonal antibodies (basiliximab, daclizumab) against specific lymphocytic antigens have also been developed for this purpose (Kovarik et al., 2002; Aktas et al., 2011), with the possible advantage of a decreased rate of malignancies and infections (Liu et al., 2010). Maintenance IS refers to the combination of immunosuppressive drugs given during the complete lifespan of the graft to avoid episodes of acute rejection as well as chronic rejection. Maintenance IS in SOT/CTT usually includes a combination of CNI, corticosteroid and an antiproliferative agent. The combination of drugs with different mechanisms of action gives potent immunosuppressive effects with low dose-related toxicity. The combination of an induction and maintenance protocols results in a 2-year graft survival rate of 100% after highly immunogenic CTT (Petruzzo et al., 2010).
evaluated monotherapy with either cyclosporine (10 mg/kg/day) or tacrolimus (0.5 mg/kg/day) in a rat allogeneic UTx model and found a mild inflammation in the cyclosporine group after 7 days and fully normal histology 14 days after UTx in the tacrolimus group (our unpublished observations). These results indicate that CNI monotherapy may be immunosuppressive enough during pregnancy in a uterine allograft rat model (Diaz-Garcia et al., 2010).

**Implications concerning IS in human UTx**

Transplanting an organ such as the uterus implies some particularities compared with other types of SOT/CTT. The main problem regarding IS in UTx is that at least 9 months of the IS will take place during pregnancy. Any immunosuppressant causing malformations or miscarriage will be absolutely forbidden during pregnancy. Levels of immunosuppressive drugs must be closely monitored during pregnancy since pharmacokinetic changes occur then due to altered metabolism and volume distribution (Tendron et al., 2002). An induction protocol including ATG or anti-interleukin-2 receptor monoclonal antibodies, prednisolone, tacrolimus and micophenolate mofetil (MMF) would be suitable. Prednisolone, tacrolimus and MMF would be used as a maintenance protocol before pregnancy is intended. Tacrolimus and MMF doses would be adjusted according to their blood levels and the presence of rejection signs. Discontinuation of MMF should be mandatory before pregnancy, since its use has been related to fetal malformations (Vento et al., 2008) and the tacrolimus levels should be closely monitored in order to reach therapeutic levels.

In CTT, in which healthy patients are transplanted, no malignancies or life-threatening conditions have been reported after a 2-year follow-up (Petruzzo et al., 2010). A similar situation could be expected in UTx. Another particularity of UTx is its time-limited life: once the pregnancy is achieved and the baby is delivered the transplanted uterus can be removed, allowing for discontinuation of the IS therapy, therefore minimizing the risk of side effects in the recipient.

**Diagnosis of rejection**

**Diagnosis of rejection in SOT**

Early diagnosis of rejection is one of the main challenges in SOT (Sarwal et al., 2011). Imaging techniques such as sonography, magnetic resonance or computed sonography as well as the different ‘omics’ (genomics, proteomics, metabolomics and antibodyomics) could play central roles in early detection of acute rejection in the future (Sarwal et al., 2011).

Although conventional imaging technologies such as X-ray computed technology scan or 2D ultrasound unfortunately still have a limited value in diagnosing rejection (Cosgrove and Chan, 2008), new functional approaches, such as microbubble contrast agent dynamics (MCAD) or comprehensive magnetic resonance imaging (MRI), might supply clinicians with useful information for detection of early rejection signs (Browne and Tuite, 2006; Cosgrove and Chan, 2008) such as peripheral enhancement of MRI T2-weighted images corresponding to initial oedema or initial microvascular obstructions shown as Doppler lack of signal after MCAD. Despite all the progresses in the field of non-invasive diagnosis of rejection, biopsy remains the gold standard in SOT (Kasiske et al., 2010).

**Figure 4** Schematic drawing of the allogeneic UTx model of the baboon. The arterial anastomosis was done in end-to-end fashion using the internal iliac artery of the recipient and the internal iliac of the graft. The venous anastomosis was performed end-to-side to the external iliac vein, after back-table fusion of the two ovarian veins.

However, IS is not free of side-effects, especially metabolic (van Hooff et al., 2005) and nephrotoxic (European Multicenter trial group, 1983; Schwarz et al., 2005). New drugs aiming to reduce these side-effects are currently under phases II and III trials (Vincenti, 2002).

**New developments and research in IS at UTx**

The uterus has historically been considered as an immune-privileged organ due to its capacity to carry a semi-allogeneic pregnancy, but this has been refuted since a transplanted uterus triggers a normal rejection process that can only be suppressed by immunosuppressant drugs (Wranning et al., 2007; Avison et al., 2009; Diaz-Garcia et al., 2010). At least seven modern UTx studies in different allogeneic models under IS (Fageeh et al., 2002; Wranning et al., 2007; Ramirez et al., 2008, 2011; Steunarine et al., 2008a,b; Avison et al., 2009; Diaz-Garcia et al., 2010) have been put forward (Table II), but to our knowledge there is only one comparative study (Wranning et al., 2007). That study compared two doses of cyclosporine to placebo in a mouse model of allogeneic UTx (Wranning et al., 2007). Only the higher (20 mg/kg/day) dose of cyclosporine avoided rejection partially (Wranning et al., 2007).
New developments and research in diagnosis of rejection in UTx

Only two experimental studies have described the histological pattern of rejection at different time-points after allogeneic murine UTx without IS (El-Akouri et al., 2006; Groth et al., 2009). Inflammatory changes were seen from Day 2 post transplantation and at Day 28, massive necrosis was present (Groth et al., 2009). Rejection was characterized by an early (Day 2–5) myometrial invasion of neutrophils and macrophages, which was followed by cytotoxic T-cells (mainly CD8+) invasion of the endometrium and myometrium (Groth et al., 2009). These results must be extrapolated to the human with caution since different immune responses can be seen concerning the same type of organ transplantation depending on the species used as experimental rejection model (Wranning et al., 2007; Avison et al., 2009; Diaz-Garcia et al., 2010).

Implications regarding diagnosis of rejection in human UTx

For obvious reasons, we lack evidence on methods to monitor rejection in UTx. Duplex/Doppler ultrasound and the CD4/CD8 T-cell ratio in peripheral blood were used to monitor rejection in the only human UTx (Fageeh et al., 2002), but we doubt the sensitivity and specificity of these methods to diagnose early rejection. In a future clinical setting of UTx, we think that a combination of different tests must be used in order to minimize the probabilities of under-diagnosis of any acute rejection episode. These tests would include MRI and novel ultrasound approaches such as MCAD or power Doppler. The ultrasound techniques would have the advantage of being available for gynaecologists. Weekly cervical biopsies over a 2-month period were successfully used by our group to monitor acute rejection in an allogeneic non-human primate UTx model (our non-published data) and this or endometrial biopsies could of course be used for a non-graft transplanted human uterus.

Pregnancy

Pregnancy and SOT

The purpose of UTx is to treat the existing infertility, and pregnancy with a healthy offspring is thereby the natural end-point of UTx. During the last 50 years, more than 15 000 births have been reported in women under IS after transplantation from different organs (McKay and Josephson, 2006). Although some retrospective studies suggest the possibility that pregnancy after transplantation is associated with higher rate of obstetrical complications such as miscarriage, pre-eclampsia, preterm birth or low birthweight (Baird et al., 1976; Tendron et al., 2002; McKay and Josephson, 2006), there is one complete population-based study including more than 1000 newborns (Kallen et al., 2005), which suggests that these pregnancy complications are linked to the transplantation-causing disease and not to the immunosuppressants or the transplantation itself.

Transplanted patients are counselled not to get pregnant during the first year after transplantation to allow tapering of IS (McGuire et al., 2009; Zachariah et al., 2009) and also to avoid graft complications such as thrombosis, hematoma or infection that could compromise its viability (Costa et al., 2011). Such approach could also be used in UTx.

New developments and research in pregnancy and UTx

After UTx, multiple physiological connections such as vascularization, innervation and lymphatic drain are altered. In addition, the continuous IS could also modulate the implantation process and modulate the tolerance of the uterus towards the conception product. Normal live pups were described in a mouse model after syngeneic heterotopic UTx for the first time in 2003 (Racho El-Akouri et al., 2003a,b). These results prove that the anatomical modifications caused by the transplantation surgery do not affect the capability of the uterus to carry a full-term pregnancy. Nevertheless, they could affect the normal mechanisms of delivery (Wranning et al., 2011). Pregnancies have also recently been reported after allogeneic UTx and IS (Diaz-Garcia et al., 2010; Ramirez et al., 2011), with a long normal follow-up period in our rat model (our unpublished data). So far, pregnancy has never been achieved after UTx in any non-human primate model.

Implications regarding pregnancy in human UTx

It is difficult to estimate the potential risk for the mother and fetus after a human UTx. Preventive measures must be taken in order to minimize these risks. These measures could include the selection of the recipient following strict inclusion and exclusion criteria in order to avoid pathological conditions that could compromise the viability of the pregnancy; pregnancy should not be aimed before 1 year post-transplantation; frozen embryos/oocytes must be available before undergoing UTx, in order to ensure the couple’s fertility before UTx, as well as to minimize the risk of thrombosis due to ovarian hyperstimulation syndrome if an IVF procedure is needed.

If menstruation is not spontaneously resumed several months after UTx, the integrity of the uterine cavity must be checked by hysteroscopic direct examination and biopsy sampling. Once the possibility of necrosis or lack of endometrium has been discounted, sequential priming with estrogens and progestins could be used. If menstruation is not restored after a reasonable amount of time or functionality is not achieved after hormonal priming, a hysterectomy should be considered.

Strict monitoring of immunosuppressant blood levels must be done during the whole pregnancy and cervical biopsies should be taken when rejection is suspected. Growth and hemodynamic status of the fetus should be assessed fortnightly in the absence of any alarm signs. Since fibrotic tissue could be present in the vaginal anastomosis, we suggest the delivery be done by Caesarean section, in order to avoid potential dystocia as well as the torsion of the uterus. This could also allow for removal of the uterus after delivery without any additional intervention.

Summary

Experimental research efforts in UTx, performed mostly during the last 10 years, have provided essential information to design a clinical introduction of human UTx under a strict research protocol. Clinical evidence about different aspects of transplantation is available from other solid organs and CTT. This could be extrapolated to the UTx field to improve the donor and recipient management protocols.
Since a great deal of progress has been made during recent years, it is predicted that the second human UTx attempt will take place within the coming few years (Del Priore et al., 2011) and this attempt should have a good chance of successful outcome since it is based on solid animal research.

Authors’ roles
C.D.-G. was involved in elaboration of the manuscript and design. M.B. took part in elaboration of the manuscript, design and supervision. L.J. contributed to intellectual input in gynaecology. A.E. contributed to intellectual input in anaesthesia. M.O. and A.T. provided the intellectual input in transplantation surgery.

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