Uterus transplantation in a non-human primate: long-term follow-up after autologous transplantation

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BACKGROUND: Uterus transplantation (UTx) may provide the first available treatment for women affected by uterine infertility. The present study aimed to further develop a surgical technique for autologous UTx in a non-human primate species and to assess long-term function.

METHODS: Female baboons (n = 16) underwent autologous transplantation of the uterus with the Fallopian tubes and ovaries, performed with a previously published surgical technique (n = 6, Group 1) or using a modified technique (n = 10; Group 2). The uterine arteries were dissected to the proximal end of the anterior branch (Group 1) or the entire (Group 2) internal iliac artery, and the ovarian veins were dissected to the crossing over the ureter (Group 1) or further cranially to include greater lengths and patches of the cava/renal vein (Group 2). Back-table preparation created common venous and arterial ends with arterial anastomosis either end-to-side to the left external iliac artery (Group 1) or end-to-end to the left internal iliac artery (Group 2).

RESULTS: Overall short-time survival of the animals was 88% (66% in Group 1 and 100% in Group 2). Of all the operated animals, 75% (66% in Group 1 and 80% in Group 2) resumed ovarian cyclicity. Regular menstruation after UTx was demonstrated only in Group 2 (60%). Menstruating animals (n = 6) were each exposed to timed mating for ≥5 menstrual cycles, but pregnancy did not occur. Adhesions and tubal blockage were seen in post-mortem analysis.

CONCLUSIONS: The modified UTx model of Group 2 is a safe procedure and shows resumed long-term uterine function in a majority of the animals, although pregnancy could not be demonstrated.

Key words: autologous / baboon / infertility / transplantation / uterus

Introduction

Uterine infertility, due to either the absence of the uterus or to the presence of a non-functional uterus, remains a clinical field to conquer. A possible future treatment of uterine infertility is uterus transplantation (UTx) (Altchek, 2003; Brannstrom et al., 2003; Brannstrom, 2007), providing an alternative to adoption or gestational surrogacy to acquire motherhood.

A decade ago a Saudi Arabian team, with minimal preparatory animal studies, performed the first human UTx attempt (Fageeh et al., 2002). The graft was removed after 99 days due to prolapse and necrosis. Extensive research has been done since then and UTx models in the mouse (Racho El-Akouri et al., 2002, 2003a,b), rat (Wranning et al., 2008a,b, 2010a,b), rabbit (Sieunarine et al., 2008), pig (Avison et al., 2009) and sheep (Dahm-Kahler et al., 2008; Wranning et al., 2010a,b) now exist. Experimental UTx research has aimed towards development of surgical techniques and characterization of ischaemic tolerance (Racho El-Akouri et al., 2003a,b; Sieunarine et al., 2005a,b; Del Priore et al., 2007; Wranning et al., 2008a,b) and rejection after allogeneic UTx (El-Akouri et al., 2006; Sieunarine et al., 2008; Groth et al., 2009). Importantly, pregnancies have been achieved both after syngeneic (Racho El-Akouri et al., 2003a,b; Wranning et al., 2010a,b) and allogeneic (Diaz-Garcia et al., 2010; Ramirez et al., 2011) UTx.
The introduction of a new type of organ transplantation in humans should be preceded by extensive studies in non-human primate species to include experimental conditions similar to the human situation. In particular, since UTx would be a non-vital type of organ transplantation, the scientific background, ethical aspects and the potential benefits and/or risks of the procedure must be considered. UTx is comparable with hand and facial transplantation as quality-of-life enhancing and non-vital, but there is added complexity in that UTx also includes the concern of the future child. Only a few previous studies utilizing vascular UTx in non-human primates have been performed. In our first report on autologous transplantation of the uterus with adnexae in the baboon (Enskog et al., 2010), we demonstrated that 5 out of 10 animals resumed ovarian cyclicity, but only 2 out of the 10 animals had preserved uteri with menstruation. Thus, these poor results indicate that further surgical development is needed in a non-human primate auto UTx setting, and that allogeneic non-human primate UTx, with the added complexity of rejection and immunosuppression, should await further surgical modifications. In the initial study of autologous UTx in the cynomolgus macaque (Kisu et al., 2011), only one out of the two animals survived surgery and, in a follow-up study on uterine blood flow after autologous UTx (Mihara et al., 2011), three out of four animals died within 3 months post-operatively, with neither cyclicity nor menstruation being observed prior to death in these animals. The fourth surviving animal resumed cyclicity and menstruation within 6 months post-operatively.

The aim of the present study was to develop the surgery of the baboon UTx model further and to test long-term function, including tests of fertility after natural mating.

Materials and Methods

Animals
Sixteen female olive baboons (Papio anubis; 10–16 kg) with regular menses for 4 months were included. The animals were a fed commercial monkey diet with additional fruits and vegetables three times weekly. The Institutional Scientific Evaluation and Review Committee of Institute of Primate Research, Karen, Kenya approved the study protocol. In Group 1 (n = 6), UTx was performed using a previously described surgical technique (Enskog et al., 2010) and in Group 2 (n = 10) a modified surgical technique was used (see below).

Anaesthesia and post-operative care
Anaesthesia and post-operative treatments were identical in Groups 1 and 2, using essentially the same regimen as previously described in detail in our initial baboon UTx study (Enskog et al., 2010). Briefly, anaesthesia was induced by ketamine plus xylazine and maintained by halothane in oxygen/air. Antibiotics were administered preoperatively [15 mg/kg body weight (bw) intravenously (i.v.) of trimethoprim/sulphamethoxazole (17/83%) and 5 mg/kg bw i.v. of metronidazole] and post-operatively (15 mg/kg bw/day intramuscular (i.m.) of trimethoprim/sulphamethoxazole and 20 mg/kg bw/day orally of metronidazole) for 7 days. Anticoagulants were administered 15 min before clamping of the uterine blood flow (heparin, i.v. 3000 IU) and once daily for 3 weeks (low-molecular weight heparin, s.c. 1250 IU). As pain relief, meloxicam (0.3 mg/kg bw) and betamethasone (1 mg) were given i.m. for 4 days.

At second-look surgery (laparotomy), 6–18 months after UTx, anaesthesia was identical to that of UTx. Euthanization during anaesthesia was induced by ketamine plus xylazine and maintained by halothane in oxygen/air. Antibiotics were administered preoperatively and post-operatively as described above. For euthanization, 25 mg/kg bw of sodium pentobarbital (metason) were given i.m. for 4 days.

The surgery started by vaginal/abdominal disinfection by 0.1/1% chlorhexidine. Standard sterile techniques were used during surgery. Through a low midline incision, up to around 5 cm supraumblically, intra-abdominal dissection was performed using monopolar diathermia (20–40 W), scissors or bipolar diathermia (8–16 W). During dissection of the blood vessels, all larger (>1 mm) venous and all arterial branches were ligated towards the specimen and on the pelvic side either clamped by titanium clips (Premium Surgiclip; generously provided by Coviden, Solna, Sweden) or by bipolar diathermy. The first procedure was bilateral division of the round ligaments between double sutures at a distance of around 2 cm from the uterine body. The vascular dissection started on one side by separating the infundibulopelvic ligament (IPL) from the pelvic sidewall. The ureter was then mobilized to enable the dissection of the internal iliac artery. Meticulous dissection with separation of the internal iliac artery and its branches from attachments to the veins and the pelvic sidewall was performed. The bladder peritoneum was then detached from the cervix and the cranial portion (≈1.5 cm) of the vagina. All small arterial branches of the uterine artery, up to a level of 1 cm above the point where the uterine artery overrides the ureter, were severed. The ureter was dissected free from its attachments to the uterine artery and the cervix/vagina so that it was mobilized all the way to the entrance to the bladder. The procedure was then repeated on the contralateral side.

The bilateral vaginal arteries/veins on the lateral aspects of the vagina were divided between ligatures and then the vagina was transected by the use of monopolar diathermy. The dissected arterial inflow and the venous drainage were then clamped (titanium clips) at the most cranial position of dissection and severed to enable the removal of the graft followed by placement on sterile ice slush. Teflon catheters (inner diameter 0.64 mm) were inserted into the two main arteries of the specimen and
secured with haemostatic clamps. Three ischaemic periods were recorded according to the established criteria (Halazun et al., 2007). Warm ischaemia part 1 (WI-1) was the time between vascular clamping and flushing. Cold ischaemia (CI) was the time between cold flushing and start of anastomosis surgery and warm ischaemia part 2 (WI-2) was the time of anastomosis surgery until graft perfusion. The graft (uterus including vessels, ovaries and oviducts) was weighed prior to transplantation (graft weight = gw). After completion of transplantation, the abdomen was closed by sutures of fascia and skin.

Specific surgery (recovery and back-table) of Group 1

The uterine arteries/anterior portions of internal iliac arteries were dissected cranially only 1.5 cm above the umbilical artery branching, with the two posterior branches of the internal iliac artery left in situ (Fig. 2). The ovarian veins were dissected to the crossing of the IPL and the ureter. Back-table preparation included: (i) flushing for 10 min with histidine-tryptophan-ketoglutarate (HTK; generously provided by Nordmedica, Gentofte, Denmark) at 4°C; (ii) cleavage of the distal ends of the largest ovarian veins/arterial ends and (iii) suturing the ends side-to-side by 9-0 nylon/8-0 prolene, to create one venous and one arterial end.

Specific surgery (recovery and back-table) of Group 2

Owing to poor long-term outcome within Group 1 (see ‘Results’ section), some modifications were agreed upon. A transplant surgeon (M.O.) joined the team and, accordingly, the surgery was altered at several critical steps: (i) the main trunk of the internal iliac artery was included bilaterally after divisions of the two main posterior branches of the internal iliac artery (Fig. 2) but with the largest branch on each side included (length of ≥5 mm) to allow end-to-end anastomosis (interrupted 8-0 prolene) of one of the internal iliac artery ends to the largest posterior branch on the contra lateral side; (ii) the dissection of the IPL was extended up to the vena cava on the right hand side and the left kidney vein, to acquire venous ends with reasonable thick vessel walls, which would simplify back-table preparation (interrupted 9-0 nylon on the back and front sides) and anastomosis surgery and (iii) the flushing procedure was modified by pre-flushing the graft with 5–10 ml of cold heparinized saline supplemented with xylocaine and this was followed by flushing (80 mmHg) for 30 min with a large volume (≈300 ml) of 4°C HTK.

Surgery (Groups 1 and 2) at transplantation

Simultaneously to back-table preparation, the left external iliac vessels were dissected free for a distance of 3 cm, including severances of 2–3 veins that branch towards the pelvic sidewall. In both groups, venous anastomosis was done prior to arterial anastomosis, since the external iliac vein is deeper in the pelvis compared with the external/internal iliac artery. Anastomosis of the vein was end-to-side (9-0 nylon) to the left external iliac vein in both Groups 1 and 2. Anastomosis of the artery was performed end-to-side (8-0 prolene) to the external iliac artery by two gynaecology surgeons (Group 1) or end-to-end to the internal iliac artery by a transplant-surgeon with the assistance of one gynaecology surgeon (Group 2). Any major leakage points were sutured by single
sutures. The vagina was reanastomosed by continuous suture (2-0 poly-
dioxanone) and the round ligaments of the uterine body were re-attached
by a single suture (2-0 polydioxanone) for graft fixation. These were the
only points for graft fixation, apart from the blood vessel anastomosis sites.

Assessment of cyclicity and timed mating
The baboon pregnancy is 6 months long and the menstrual cycle is of 33
± 2 days (Hendrickx, 1971). Perineal skin changes correlate to hormonal
variations of the baboon menstrual cycle, and the observation of perineal
skin inflation/deflation is a well-established method to monitor ovarian ac-
tivity (Hendrickx, 1971). Every cycle is divided into nine stages, where five
stages cover the follicular phase. Animals showing resumed menstruation
were introduced (1:1) to males that had demonstrated fertility through
previous fatherhood and semen analysis. Mating was allowed in each
cycle for 7–10 days, between 08.30 and 15.00, starting in the late follicular
phase. The males were replaced every third cycle.

Second-look surgery
A second-look laparotomy was performed 6–7 months after transplant-
ation in animals failing to resume menstruation, and after 12–18 months
in menstruating animals. In cases of unplanned deaths (n = 4), autopsies
were performed. All procedures included registration of bw, visual inspec-
tion of the abdominal cavity, photographic documentation of surgical find-
ings, weight of the uterus (utw), chromopertubation to assess tubal patency
and biopsies from the ovary, cervix, uterine corpus and Fallopian tubes,
if present.

Histology
The biopsies were fixed in 4% buffered formaldehyde, dehydrated,
embedded in paraffin, sectioned and stained with haematoxylin–eosin
followed by examination by light microscopy.

<table>
<thead>
<tr>
<th>Table I</th>
<th>Weights and surgical durations at auto-UTx in the baboon.</th>
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<tr>
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<td>Group 1 (n = 6)</td>
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<tr>
<td>Weights</td>
<td></td>
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<tr>
<td>bw (kg)</td>
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<tr>
<td>Pre-UTx</td>
<td>13 (10–16)</td>
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<tr>
<td>Post-UTx</td>
<td>nra</td>
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<tr>
<td>gw (g)</td>
<td>38 (28–48)</td>
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<tr>
<td>utw (g)</td>
<td>nra</td>
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<tr>
<td>Surgical durations (min)</td>
<td></td>
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<tr>
<td>Anaesthesia</td>
<td>401 (437–550)</td>
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<tr>
<td>Organ recovery</td>
<td>214 (165–223)*</td>
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<tr>
<td>Anastomosis</td>
<td>58 (36–81)</td>
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<tr>
<td>Ischaemia</td>
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<td>WI-1</td>
<td>3 (2–5)</td>
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<tr>
<td>WI-2</td>
<td>58 (36–76)</td>
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<td>Cl</td>
<td>130 (118–170)</td>
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Values are given in medians (ranges); bw, body weight; Cl, cold ischaemia; gw, graft weight; utw, uterus weight; WI-1, warm ischaemia part 1; WI-2, warm ischaemia part 2.

Statistical analysis
Results are expressed as medians and ranges. Statistical analyses were
performed by the non-parametric Mann–Whitney U-test since normal dis-
tribution could not be assumed. A P < 0.05 was considered significant.

Results

Animals
All animals had similar baseline bw and gw values at uterus recovery
(Table I). At euthanization, the bw of Group 2 was unchanged. The
utw, only recorded in Group 2, was then somewhat lower than
previous gw (including also adnexae and blood vessels).

Surgical parameters
The duration of anaesthesia was comparable (∼8 h) in the two groups
(Table I). The time of organ recovery was shorter in Group 2, but no
significant difference was seen in total surgery time. Ischaemic times
were similar between Groups 1 and 2 and also between the subgroups
(only ovarian cyclicity versus ovarian cyclicity plus menses) of Group
2. Duration of anastomosis surgery (∼1 h) was similar between the
groups and uteri of all animals became reddish and showed venous
blood flow. In Group 2, also clear pulsations of the uterine arteries
were seen in all animals.

Long-term outcome

Group 1
Out of the six animals, two died shortly after surgery (peritonitis day
4, internal bleeding day 9). Cyclicity appeared within 4 months in the
surviving animals but none demonstrated menstruation. At second-
look laparotomy, the ovaries were identified and showed bleeding
at the ovarian incision in all animals. Fibrotic tissue covered the
vaginal vault but there was no visible uterus in any of the animals. Three animals showed macroscopically visible thrombi in the venous anastomosis site on the external iliacs and in one animal the anastomosis site could not be identified.

**Group 2**

The post-operative (>6 months) survival was 100%. An overview is presented in Fig. 3. Out of 10 animals, 6 resumed cyclicity and menstruation within 1–2 months and 2 resumed cyclicity within 4 months. In the two animals that exhibited neither cyclicity nor menstruation, uterine and ovarian tissue could not be found at second-look surgery. At second-look surgery of animals with only cyclicity (n = 2), a small normal-shaped uterus (utw = 4.4 g compared with gw = 29 g at UTx) was present in one animal and an even smaller uterine remnant was seen in the second animal. Uterine and ovarian blood flow was evident in these animals at incisions. The menstruating animals (n = 6) were allocated for timed mating, starting either 2 (n = 3), 10 (n = 2) or 12 (n = 1) months after UTx. Mating occurred in ≥5 cycles in each animal, but pregnancy failed to appear. Two of these animals (mating started 2 months post UTx) died (Months 10 and 12) due to pneumonia and sepsis. Their uteri were found to be macroscopically normal.

The remaining four menstruating animals revealed severe adhesions around the ovaries and oviducts at second-look laparotomy (Fig. 4) and tubal passage could not be demonstrated in any of these animals. Their uteri were of normal gross appearance (Fig. 5), with uterine and ovarian incisions demonstrating normal bleeding. The anastomosis sites showed patent lumina (Fig. 6).
Light microscopy showed normal histology of the endometrium, myometrium, cervix tissue and oviduct of all animals that showed macroscopically normal uterine appearance and resumed menstruation (Fig. 7). The ovarian tissue had a normal histological appearance with the presence of follicles of different sizes (Fig. 7).

**Discussion**

Uterine infertility remains a cause of infertility that is largely untreatable. The size of the group of uterine infertile women is not clear but in the UK it is suggested to include >12 000 women (Sieunarine et al., 2005a, b). This estimation would, on a total population basis, correspond to >180 000 uterine infertile women in Europe and 75 000 in the USA. UTx would, if developed into a safe method with a reasonable chance of success in terms of live birth, be an alternative for these women (Brannstrom et al., 2010), with their only genetic motherhood option today being gestational surrogacy (Brinsden, 2003). In this context, it should be mentioned that the ethics around UTx may be complex, with societal, religious and cultural issues influencing the outcome of an ethical analysis.

Since UTx is a novel type of quality-of-life enhancing transplantation, thorough animal research is imperative. Although we have previously performed UTx in large animal models such as the sheep and pig, we consider research on a non-human primate model, with the greatest anatomical and physiological resemblance to the human, mandatory before introduction of UTx in humans. We have recently published the results of a study on autologous UTx in a baboon model, showing a relatively low success rate of resumed menses in 20% of 10 animals. Also Rhesus macaques (Del Priore et al., 2008) and Cynomolgus macaques (Kisu et al., 2011) have recently been used in UTx research.

The main finding of the present study is that this modified UTx procedure in the baboon is associated with safe recovery and transplantation, despite a total surgical time of almost 6 h and anaesthesia for almost 8 h. The results improved considerably with a much higher rate of menstruation (60%) in Group 2, when compared after the use of the original technique as in our first study (Enskog et al., 2010) and in Group 1. Pregnancies were not achieved despite mating during a total of 61 cycles, and the cause of this post-transplantation infertility is likely to be pelvic adhesions and non-patent oviducts.

The animal survival of the present study (100% in Group 2) is higher than that of previous studies of UTx in large animals such as the baboon (90%; Enskog et al., 2010), cynomolgus macaque (50%; Kisu et al., 2011) and sheep (50%; Wranning et al., 2010a, b). It should be noted that all these studies involved autologous UTx, including both recovery and transplantation surgery. Naturally, the fact that two surgeries performed in one animal and with a delay during backtable preparation and organ flushing will incur a longer surgical time when compared with the allogeneic UTx live-donor concept. Two studies of allogeneic UTx in the sheep have shown animal survival rates of 100% (Ramirez et al., 2008) and 67% (Ramirez et al., 2011). Nevertheless, the high animal survival in the present study indicates that a live-donor concept is feasible for further studies on allogeneic UTx in the baboon model.

The first human UTx (Fageeh et al., 2002) used a graft from a live donor. However, uterine grafts from deceased donors may be feasible.
in a future human setting as suggested by results of a restricted study on human brain-dead, heart-beating multorgan donors. In that study, a satisfactory uterine graft with a vascular pedicle containing the complete internal iliac vessels was accomplished in only two out of seven donors and notably there was a total unilateral loss of uterine vessels in two donors. A live-donor concept in human UTx may provide a uterus of superior quality than would a deceased donor concept, due to the avoidance of influences from systemic inflammation at brain death (Schuurs et al., 2004; Barklin, 2009) and a shorter ischaemic time. Moreover, a uterus from a close relative, such as the mother/sister, would be of immunologic advantage with a possible haplo-identity and there would also be ample time for detailed pre-operative testing of uterine-specific pathology such as leiomyoma, dysplasia, endometrial hyperplasia or persistent human papilloma virus infection. Furthermore, sufficient time would exist for non-invasive diagnostics of pelvic vascular anatomy of the donor prior to UTx. A drawback of live uterine donation is of course the associated surgical risks imposed on person with no direct benefit of the procedure.

The duration of recovery surgery is of great importance in live donation, since less lengthy surgery will decrease the days of post-operative hospital care and complications. Despite more extensive surgery performed in Group 2, the time of organ recovery was decreased compared with that of Group 1. Previous primate UTx studies report recovery durations of around 2.5 h in the original baboon study (Enskog et al., 2010) and 6.5 h in the smaller cynomolgus macaque study (Kisu et al., 2011). The fact that the duration of the more extensive recovery surgery of Group 2 was similar to that of Group 1 may be related to both the team having improved further on the learning curve of UTx surgery (Wranning et al., 2008a,b) and also that an experienced transplant surgeon took part in the surgery of Group 2.

The improved long-term uterine function (menstruation), as demonstrated in Group 2, may be due to several modifications. The arterial anastomosis was altered between Groups 1 and 2. The artery was anastomosed bilaterally end-to-side to the external iliac artery by two gynaeoncology surgeons in Group 1, but in Group 2 a transplant surgeon modified this to end-to-end anastomosis to the internal iliac artery. The superiority of this anastomosis site was indicated by immediate and strong arterial pulsations in uterine arteries of Group 2. The uterus of Group 1 became reddish but lacked clear arterial pulsations. The presence of uterine artery pulsations of Group 2 would indicate a high blood flow over the anastomosis line. A suboptimal blood flow of the uterine graft could correlate to an early inflammatory response, in line with observations in human kidney grafts (Kruger et al., 2009). In the UTx study in two cynomolgus monkeys, the arterial anastomosis was either unilaterally end-to-end to the internal iliac artery or bilaterally end-to-side to the external iliac artery (Kisu et al., 2011). Only the animal with bilateral anastomosis survived and also resumed menstruation 4.5 months later (Kisu et al., 2011). In the follow-up study in cynomolgus monkeys, the uterine arteries were bilaterally end-to-side anastomosed to the external iliac arteries and only one out of four animals survived >3 months and resumed menstruation within 6 months (Mihara et al., 2011). In our previous baboon UTx study, a joined trunk of the anterior branches of the internal iliac arteries was anastomosed unilaterally end-to-side to the external iliac artery (Enskog et al., 2010). Despite the larger vessel size of the baboon, in comparison with the cynomolgus monkey, only 2 out of 10 animals, with end-to-side arterial anastomosis-resumed menstrual bleedings post-operatively (Enskog et al., 2010).

Another surgical modification of Group 2 was on the venous side. We speculated, on the basis of the previous 20% uterine graft success rate (Enskog et al., 2010) and the 0% success rate of Group 1, that anastomosis with the thin-walled ovarian veins lead to partial venous constriction, which in turn would decrease graft perfusion with secondary venous thrombosis and uterine necrosis. The venous modification of Group 2 included extended venous dissection and inclusion of patches of large veins with well-defined vessel walls. The use of patches, with solid walls (>5 times thicker than of ovarian veins), simplified the back-table preparation and also the anastomosis surgery. The inlet diameter of the vein and total area of the anastomosis site on the external iliac vein would thereby increase considerably, possibly resulting in alleviation of any blood flow turbulence at this site. In the cynomolgus UTx study, unilateral end-to-end anastomosis of the superficial uterine vein to the superficial vesical vein was performed in one case and bilateral end-to-side anastomosis of the deep uterine vein to the external iliac vein was used in the other case (Kisu et al., 2011). It is not further discussed whether the outcome was related to the different anastomosis sites. In our previous baboon UTx study (Enskog et al., 2010) and in Group 1, a venous pipe of two joined ovarian veins was anastomosed unilaterally end-to-side to the external iliac vein and we proposed that extended venous pedicles, with vessels having thicker walls, would facilitate the vascular anastomosis surgery. The other comparable studies in primates are either too small to be conclusive concerning the outcome of venous anastomosis (Kisu et al., 2011) or not reporting post-operative findings (Del Priore et al., 2008). The venous procurement and anastomosis will certainly differ in a future human UTx situation of a deceased when compared with a live uterus donor. The relatively large and easily dissected ovarian veins may be used, in addition to the uterine-internal iliac veins, at uterine recovery from a deceased donor. In a human live uterus donor, the uterine veins could be recovered up to their inlets into the internal iliac vein, and simultaneous use of at least one ovarian vein could be considered in a post-menopausal donor.

The anastomosis surgery took ~1 h in both Groups 1 and 2. Previous primate UTx studies exhibit longer durations of anastomosis surgery with over 1 h in our initial baboon study (Enskog et al., 2010) and 4.5 h in the cynomolgus macaque study (Kisu et al., 2011). In the present study, the menstruating animals exhibited slightly shorter, but not significantly different, ischaemic times when compared with the animals resuming only cyclicity indicating the importance to minimize both warm and CI. The markedly longer duration of anastomosis surgery in the cynomolgus macaque UTx study (Kisu et al., 2011) is most likely explained by the smaller vessel and body size of the macaque but also that bilateral anastomosis was performed. The size difference of the vessels between baboons and cynomolgus monkeys is exemplified by the sutures used for vein anastomosis, which was 8-0 and 11-0, respectively (Kisu et al., 2011).

Maintenance of organ viability during preservation is critical for successful long-term outcome. In an attempt to limit the ischaemia-reperfusion injury of the graft, preflushing with a xylocaine-heparin-saline solution was added in Group 2. The HTK flushing was also prolonged around 2-fold in Group 2, when compared with Group 1. The flushing time of around 30 min would be ample time for...
general distribution of the preservation solution throughout the organ. These changes in flushing may be a contributing factor to the improved transplant survival of Group 2.

To enable assessment of transplant viability and functionality in the present study, the ovaries were included in the graft. This allowed not only observance of menstruation (uterine viability) but also indirectly of ovarian function, by inspection of the perineal skin. Previous primate UTx studies have chosen to include (Enskog et al., 2010) or not include ovaries and oviducts (Del Priore et al., 2008; Kisu et al., 2011). Ovaries could be found in all 12 animals with ovarian cyclicity at second-look surgery. The fact that some animals showed preserved ovarian function without presence of uterus, despite that the uterine arteries supply both organs, indicates that the ovary is an organ of higher ischaemic tolerance than the uterus. This is in line with the clinical observations of resumed cyclicity and live births after a vascular auto-transplantation of ovarian cortex in primates (Lee et al., 2004; Donnez et al., 2011). All the menstruating animals regained their cyclicity within 1–2 months, whereas the non-menstruating with preserved ovaries resumed cyclicity later. This observation of early resumption of ovarian cyclicity in grafts with uterine survival correlates well with the results our preliminary study of UTx in the baboon.

The end-point of any UTx and definition of a successful transplantation is naturally the birth of a healthy offspring. Fertility and live births have been proved in our sheep auto-UTx model (Wranning et al., 2010a,b), with mating occurring from 2 months after transplantation. Despite 61 attempts of mating in the present study, no pregnancies occurred. Adhesions around the ovaries and oviducts were seen at second-look surgery and tubal blockage was seen in all animals. The tubal blockage seems to be secondary to the adhesions, in the light of the observation that histology of the oviducts of the grafts showed preserved and normal tubal epithelium. The findings of this study can be extrapolated to the human to suggest that oviducts should not be transplanted and that UTx should be combined with IVF. There have been several attempts to develop IVF in baboons but although fertilization of baboon oocytes in vitro has been demonstrated (Nyachieo et al., 2011), no live births have been reported. In the rhesus macaque, efficient IVF protocols have been used for several years.

In summary, using an autologous UTx baboon model, we could demonstrate that the procedure is possible with no mortality and that the majority of the animals resume uterine function. The baboon UTx model is now developed for further studies including also allogeneic transplantation, where only the uterus should be transplanted and cyclicity should rely on the native ovaries.

**Conflict of interest**

None declared.

**References**


**Authors’ roles**

L.J. and M.B. are responsible for the design of the experiment, evaluation of the results and elaboration of the manuscript. L.J., P.D.-K., A.H., C.D.-G., M.O. and M.B. are responsible for the surgical procedures. A.E. is responsible for the anaesthesiology procedure. D.C. and J.M. are responsible for the animal care during the procedures.

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