Preclinical report on allogeneic uterus transplantation in non-human primates

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STUDY QUESTION: Is it possible to perform allogeneic uterus transplantation (UTx) with a donation from a live donor in a non-human primate species and what immunosuppression is needed to prevent rejection?

SUMMARY ANSWER: Allogeneic UTx in the baboon is a donor- and recipient-safe surgical procedure; immunosuppression with induction therapy and a triple protocol should be used.

WHAT IS KNOWN ALREADY: UTx may become a treatment for absolute uterine factor infertility. Autologous UTx models have been developed in non-human primates with reports on long-term survival of the uterine grafts.

STUDY DESIGN, SIZE AND DURATION: This experimental study included 18 female baboons as uterus donors and 18 female baboons as uterus recipients. The follow-up time was 5–8 weeks.

PARTICIPANTS/MATERIALS, SETTING AND METHODS: Uterus retrieval was performed with extended hysterectomy including bilateral uterine and internal iliac arteries and ovarian veins. After UTx, with vascular anastomoses unilateral to the internal iliac artery and the external iliac vein, the uterus recipients received one of the following: no immunosuppression (n = 4); monotherapy (oral slow release tacrolimus) (n = 4) or induction therapy (antithymocyte globulin) followed by triple therapy (tacrolimus, mycophenolate, corticosteroids; n = 10). Surgical parameters, survival, immunosuppression and rejection patterns were evaluated.

MAIN RESULTS AND THE ROLE OF CHANCE: The durations of uterus retrieval and recipient surgery were around 3 and 3.5 h, respectively. The total ischemic time was around 3 h. All the recipients and the donors survived the surgery. All the recipients presented rejection to some extent within the first weeks following UTx. In one recipient, the uterus was of normal appearance at the end of the study period. In spite of occasional high (>60 ng/ml) blood levels of tacrolimus, there was no evidence of nephrotoxicity.

LIMITATIONS AND REASONS FOR CAUTION: This initial non-human primate allogeneic UTx study indicates that further research is needed to optimize immunosuppression protocols in order to avoid uterine rejection.

WIDER IMPLICATIONS OF THE FINDINGS: The findings suggest that allogeneic UTx in primate species is feasible but continued work on this issue is needed.

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Key words: allogeneic / immunosuppression / rejection / transplantation / uterus
Introduction

Absolute uterine factor infertility (AUFI) is at present clinically untreatable. For women suffering from AUFI and wishing for motherhood, surrogacy and adoption have traditionally been the only feasible options, although uterus transplantation (UTx), still at an experimental stage, may become an alternative future treatment for AUFI (Diaz-Garcia et al., 2012). So far only one human UTx case has been published, in which the uterus with oviducts from a live donor was transplanted to a 26-year-old woman. Three months after the surgery, the graft was removed because of uterine prolapse with signs of necrosis and vascular thrombosis (Fageeh et al., 2002).

Subsequently, during the last decade, a large number of animal UTx models have been developed, demonstrating fertility in rodents following syngeneic (Racho El-Akouri et al., 2003; Wranning et al., 2011) and allogeneic (Diaz-Garcia et al., 2010) UTx. Since the experimental situation in rodents is far from human conditions, studies have also been conducted in large animals, showing long-term uterine viability after allogeneic UTx in miniature swine (Avison et al., 2009) and fertility after autologous (Wranning et al., 2010) and allogeneic (Ramirez et al., 2011) UTx in sheep. Lately, more focus in UTx research has been in experiments concerning non-human primates. Thus, methodologies for vascular UTx have been developed in three non-human primate species (Del Priore et al., 2008; Enskog et al., 2010; Kisu et al., 2012), to further explore the prospects of human UTx. All the previous studies of UTx in non-human primates have been on autologous UTx and a transition to allogeneic UTx, also involving immunosuppression, should precede before future human UTx attempts are made.

Transplanted organs of different types may vary when it comes to immune response and organ-specific immunosuppression protocols exist. In humans, immunosuppression therapy usually includes both induction therapy, to avoid cellular acute rejection, and maintenance therapy, to avoid acute and chronic rejection. There exist several studies of UTx and immunosuppression, one in human (Fageeh et al., 2002) and several in animal models (Wranning et al., 2007; Avison et al., 2009; Diaz-Garcia et al., 2010; Ramirez et al., 2011). Monotherapy with tacrolimus in allogeneic UTx in rats was shown to be sufficient during pregnancy to avoid graft rejection (Diaz-Garcia et al., 2010). In large animals, allogeneic UTx in sheep was performed with cyclosporine for immunosuppression, and pregnancy and live birth were reported (Ramirez et al., 2011). A miniature swine model of UTx initially used i.v. tacrolimus (Day 0–12) followed by oral cyclosporine plus methylprednisolone (1 month), and long-term survival and graft function in 50% of the animals were shown (Avison et al., 2009). In the published human case, immunosuppression was maintained by oral cyclosporine, azathioprine and prednisolone (Fageeh et al., 2002).

In this study, we present the first comprehensive non-human primate data in the literature of surgical techniques for UTx, and report our experiences of immunosuppressive treatment and rejection in allogeneic UTx transplantations in baboons, using grafts from live donors.

Materials and Methods

Animals

A total of 36 healthy female olive baboons (10–16 kg) were included in the experiments, which took place at Institute of Primate Research (IPR), Karen, Kenya. The animals had regular menses for ≥4 months before inclusion. The age and parity of the animals were not known, since they were not born in captivity. Following uterus recovery/ transplantation, the animals were held in single cages and fed a commercial monkey diet with additional fruits and vegetables. The study protocol was reviewed and approved by the Institutional Scientific Evaluation and Review Committee of IPR.

Experimental groups

The animals (n = 36) were selected to be either donors or recipients at UTx, depending on blood type and body weight (bw), the heavier animal usually being selected for donation. Subsequently, the animals were divided into three groups. All the groups were transplanted using a live donor concept with a previously described surgical technique (Johannesson et al., 2012a,b). The 18 recipients included four untreated animals (control group), 4 animals treated with slow-release tacrolimus as monotherapy (TAC group) and 10 animals treated with induction therapy and standard triple immunosuppressive (Table I) treatment (triple group).

Blood type

In baboons, expression of blood group antigens can be found only on epithelial and endothelial cells (Onol et al., 1984) in contrast to humans,

<p>| Table 1 Immunosuppressive therapy used in recipients of uterus grafts in the triple-group (n = 10). |
|---------------------------------|---------------------------------|------------|------------|------------|------------|------------|------------|</p>
<table>
<thead>
<tr>
<th>Medication</th>
<th>Route of administration</th>
<th>Day</th>
<th>10 and onwards</th>
</tr>
</thead>
<tbody>
<tr>
<td>Induction therapy</td>
<td></td>
<td>Day –2</td>
<td>–1</td>
</tr>
<tr>
<td>ATG i.v.</td>
<td></td>
<td>10 mg/kg</td>
<td>10 mg/kg</td>
</tr>
<tr>
<td>Corticosteroids i.m.</td>
<td></td>
<td>400 mg</td>
<td>400 mg</td>
</tr>
<tr>
<td>Maintenance therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMF Oral</td>
<td></td>
<td>0</td>
<td>50 mg/kg</td>
</tr>
<tr>
<td>Tacrolimus i.m.</td>
<td></td>
<td>0</td>
<td>0.1 mg/kg</td>
</tr>
<tr>
<td>Corticosteroids i.m.</td>
<td></td>
<td>0</td>
<td>60 mg/kg</td>
</tr>
</tbody>
</table>

Day 0 is defined as the day of transplantation. ATG, antithymocyte globulin; MMF, mycophenolate mofetil; corticosteroids. Day –2 to 6 methylprednisolone; Day 7 and onwards, prednisolone.

* Dose adjusted according to blood levels of tacrolimus.
where antigens are expressed on erythrocytes, epithelial and endothelial cells (Wiener and Moor-Jankowski, 1969). Identification of the ABO phenotypes can be performed on buccal mucosa epithelium (Neilsen-Cannarella and Bohn, 1987). In the study in question, buccal cell samples were taken with cotton swabs and then transferred to two glass slides per animal. The smears were air-dried and fixed in ice-cold acetone. Two cell areas on each slide were circled with a hydrophobic marker pen. The slides were subsequently rinsed in TBST (Tris-Buffered NaCl and Tween-20). Non-specific binding was blocked with Background Sniper® (Biocare Medical, Concord, CA, USA) for 20 min. Mouse monoclonal anti-human antibodies towards blood group A and B were used in 10-fold dilution and anti-A (021 anti-A type 2, Clone A005, Isotype: IgG3, BioCarb AB, Lund, Sweden) and anti-B (035 anti-B, Clone No. 15, Isotype: IgG3, National Institute of Hematology, Budapest, Hungary) were applied 10-fold dilution and anti-A (021 anti-A type 2, Clone A005, Isotype: IgG3, BioCarb AB, Lund, Sweden) and anti-B (035 anti-B, Clone No. 15, Isotype: IgG3, National Institute of Hematology, Budapest, Hungary) were applied in one encircled area each of the slides. The other area was left with only TBST as a negative control. The slides were incubated for 1 h at room temperature. Detection was performed with a MACH3 polymer detection kit with Vulcan Fast Red (both Biocare Medical). The slides were subsequently counterstained with hematoxylin (Histolab, Gothenburg, Sweden) and covered with Aqua Pertex (Histolab). Human buccal smears from blood-typed individuals were used as positive control cells for blood group A, B and O. The majority of the animals included in the study were B (39%) or AB (42%) with only a few type A (19%). Out of all the donor/recipient combinations, 55% were ABO identical and additionally 45% were ABO compatible.

**Anesthesia and peroperative pharmacological treatment**

After overnight fasting (water not withheld), anesthesia was induced with i.m. ketamine and xylazine and the animals were intubated with subsequent anesthesia maintained by spontaneously breathing of halothane in oxygen/air mixture. Preoperatively, antibiotics (trimethoprimsulphamethoxazole, 15 mg/kg bw; metronidazole, 5 mg/kg bw) were administered i.v. Anticoagulant (heparin, 3000 IU) was administered i.v. 15 min before clamping the uterine blood flow. Normothermia and adequate hydration were maintained throughout the surgery, using heating pads/blankets and continuous i.v. infusion (3 ml/kg bw/preoperative fasting hour and surgical hour) of warm (37°C) physiological saline. Anesthesia during sampling of biopsy was performed with i.m. ketamine and xylazine without intubation. Euthanization during anesthesia was done by i.v. administration of phenobarbital (Fenemal Recip, Recip AB, Solna, Sweden).

**Immunosuppressive therapy**

The target trough level was based on a local tradition of solid organ transplantation in humans (Transplantation Institute, Sahlgrenska University Hospital, Gothenburg, Sweden). The target trough levels (8–12 ng/ml) remained the same during the experimental period.

**Control group (n = 4)**

No immunosuppressive treatment was given.

**TAC group (n = 4)**

The tacrolimus dose was based on an initial dose-study, where oral administration of slow-release tacrolimus (Advagraf®; Astellas Pharma, Malmo, Sweden) of 30 and then 60 mg/kg bw once daily to two non-operated baboons demonstrated trough levels in the ranges 4–6 and 7–10 ng/ml, respectively (Fig. 1a). The starting dose was then decided to be 40 mg/kg bw and thereafter either 40 or 80 mg/kg bw, adjusted accordingly to blood levels of tacrolimus measured once weekly. The animals were closely observed to make sure that complete intake of all medications occurred.

**Triple group (n = 10)**

Immunosuppressive therapy for the triple group is shown in Table I.

On the basis of fluctuating blood levels of tacrolimus following oral administration (TAC group; Fig. 2), it was decided to use i.m. administration of tacrolimus as an alternative route for immunosuppressive therapy. A dose-study showed that administration of i.m. tacrolimus of 0.03 and then 0.06 and 0.09 mg/kg bw once daily resulted in trough levels of 2–5, 4–7 and 4–12 ng/ml, respectively (Fig. 1b); this determined the starting dose (0.1 mg/kg bw i.v.; ATG®; Fresenius Medical Care, Sollentuna, Sweden) together with i.m. corticoids (400 mg; Solu-Medrol®; Pfizer AB, Sollentuna, Sweden) were given on Day -2 and -1 (Day 0 defined as day of surgery). Maintenance therapy was performed by daily administration of tacrolimus (Prograf®; Astellas Pharma, Malmo, Sweden) from Day 1. Additionally, 50 mg/kg bw mycophenolate mofetil (Cellcept®; Roche, Stockholm, Sweden) was mixed with orange juice and given orally once daily from Day -1 with the animal under close observation. Corticosteroids were given i.m. daily, Day 0–2 60 mg/kg bw; Day 3–6 30 mg/kg bw; Day 7–9 16.25 mg/kg bw and Day 10 and thereafter 5 mg/kg bw (Day 0–6, methylprednisolone; Solu-Medrol®, Day 7 onwards, prednisolone; Precortalon®, Merck Sharp & Dohme AB, Sollentuna, Sweden).
Non-immunosuppressive medication

All other (non-immunosuppression) medications given are shown in Table II. Antiocoagulants were administered daily during the post-operative period to prevent thromboembolic complications. Pain relievers were given daily as long as needed and the minimum time was 4 days for both donors and recipients. As prophylaxis of cytomegalovirus, valganciclovir was given every other day regardless of whether the donor and the recipient were positive or negative for the virus.

Donor surgery and flushing

A midline incision was performed from the pubic bone up to around 5 cm above the umbilicus. All subsequent dissections were performed using diathermia (monopolar (20–40 W) and bipolar (8–16 W)) or scissors. The round ligaments were severed at a distance of around 2 cm from the uterine fundus and the ureters were mobilized from the bladder up to the kidneys to enable full access to the uterine and ovarian veins. The bladder was separated from its attachments to the cervix and upper part of the vagina. The uterine arteries were then dissected free from around 1 cm cranial to the crossover of the ureter and towards the intern- al iliac arteries. That included ligation and severance of several small branches off the uterine arteries. The two main posterior branches of the internal iliac artery were divided and the main trunk of the internal iliac artery with the largest branch on each side was dissected free for inclusion in the graft. On the venous side, the ovarian veins were dissected all the way to their inlets into the vena cava and the left kidney vein. Subsequently, the lateral vaginal vessels were ligated and severed and the vagina was transected. The graft was removed from the abdomen after clamping and ligating the proximal vessels. The abdomen was closed, by separate running sutures of fascia and skin. The graft was, after removal from the abdominal cavity, placed on sterile ice slush (physiological saline) and Teflon catheters (inner diameter 0.64 mm) were inserted into the internal iliac arteries and secured with hemostatic clamps. Initial flushing with a mixture of 10 ml of cold heparinized (3500 IU) physiological saline and xylocaine (190 mg) was followed by flushing with (~30 ml) 4 °C histidine-tryptophan-ketoglutarate (HTK®; Custodiol®, Nordmedica, Gentofte, Denmark).

Assessment

The total durations of anesthesia (from induction to skin closure) and surgery were noted as were durations of ischemia, which was subdivided into warm ischemia part 1 (time from vascular clamping to start of flushing), cold ischemia (time between the start of cold flushing and the start of anastomosis surgery) and warm ischemia part 2 (time of anastomosis surgery until graft perfusion) according to established definitions of ischemia (Halazun et al., 2007). The uterine grafts were weighed at the end of the cold ischemic period.

Recipient surgery

A midline incision was performed from the pubic bone to 2 cm above the umbilicus. A standard hysterectomy, with the uterine vessels and the super- ior vaginal vessels carefully dissected and ligated close to the body of the uterus, was then done. The vaginal vault was left open with marking sutures of its corners. Subsequently, the left external iliac vessels were dis- sected free for a distance of around 3 cm to enable end-to-side anasto- moses of the graft vessels. The iliac vein branches (2–3) towards the pelvic sidewall were severed and the segment for vascular anastomosis was clamped. Since the external iliac vein runs deeper in the pelvis than the corresponding artery, the venous anastomosis to the left external iliac vein (9–0 prolene) was done prior to the arterial anastomosis to

![Figure 2](image_url) Blood levels of tacrolimus (ng/ml) in the four recipients of uterus grafts in the TAC group. Immunosuppressive treatment was oral slow-release tacrolimus as a monotherapy. Day 0 is defined as the day of surgery.

Table II Pharmacological therapy (non-immunosuppressive) used in all recipients of uterus grafts (n = 20).

<table>
<thead>
<tr>
<th>Medication</th>
<th>Route of administration</th>
<th>Day</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4 and onwards</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>Oral</td>
<td>75 mg</td>
<td>75 mg</td>
<td>75 mg</td>
<td>75 mg</td>
<td>Continues</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>i.m.</td>
<td>0.01–0.03 mg/kg</td>
<td>0.01–0.03 mg/kg</td>
<td>0.01–0.03 mg/kg</td>
<td>0.01–0.03 mg/kg</td>
<td>As long as in pain</td>
</tr>
<tr>
<td>Dalteparin</td>
<td>s.c.</td>
<td>0</td>
<td>0</td>
<td>1250IE</td>
<td>1250IE</td>
<td>1250IE</td>
</tr>
<tr>
<td>Famotidine</td>
<td>Oral</td>
<td>10 mg</td>
<td>10 mg</td>
<td>10 mg</td>
<td>10 mg</td>
<td>Continues</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>i.m.</td>
<td>0.3 mg/kg</td>
<td>0.3 mg/kg</td>
<td>0.3 mg/kg</td>
<td>0.3 mg/kg</td>
<td>As long as in pain</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>i.v.</td>
<td>20 mg/kg</td>
<td>20 mg/kg</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>Oral</td>
<td>0</td>
<td>0</td>
<td>20 mg/kg</td>
<td>20 mg/kg</td>
<td>20 mg/kg</td>
</tr>
<tr>
<td>Trimethoprim + sulfadoxine</td>
<td>i.m.</td>
<td>15 mg/kg</td>
<td>15 mg/kg</td>
<td>15 mg/kg</td>
<td>15 mg/kg</td>
<td>15 mg/kg</td>
</tr>
<tr>
<td>Valganciclovir</td>
<td>Oral</td>
<td>0</td>
<td>0</td>
<td>450 mg</td>
<td>0</td>
<td>450 mg</td>
</tr>
</tbody>
</table>

Day 0 is defined as the day of transplantation.
the external iliac artery (8–0 prolene). Single sutures closed visible leakage if present. The round ligaments of the uterine graft were attached to the corresponding ligaments of the recipient for graft fixation and the vagina was reanastomosed by continuous suture (2–0 polydioxanone).

**Blood sampling**

Samples for blood levels of tacrolimus were routinely collected in the mornings twice weekly, before drug administration and measured by Tandem high-performance liquid chromatography/mass spectrometry (LC/MS), a high-performance mass spectrometer designer for LC/MS/MS operation. Quantification was achieved by use of appropriate internal standards and comparing responses with those of a standard curve for each compound of interest. The individual dose of tacrolimus was adjusted according to blood level. Creatinine levels were determined twice, prior to UTx and before euthanization. The creatinine level was measured on Vitros 5, 1 FS analyzer using isotop dilution MS (iDMS). The resulting change in reflection density was measured at two time points.

**Cyclicity**

The perineal skin in the baboon changes during the menstrual cycle and the appearances correlate well with the hormonal variations (Hendrickx, 1971). By ocular observations of the inflation/deflation pattern, the ovarian activity can be monitored easily in a non-invasive manner. The surveillance of the hormonal activity was performed by trained animal technicians/veterinarians.

**Biopsy samples and histologic analysis**

Biopsy samples were taken transvaginally from the uterine cervix and the endometrium once weekly. The biopsy samples were fixed in formaldehyde, embedded in paraffin, sectioned and stained with hematoxylin and eosin. Histologic severity of acute cellular graft rejection was scored, from none to severe, according to a subjective scale based on experience of rejection characteristics of other organs and uterine rejection of several animal species (Table III).

**Results**

**Surgical parameters**

**Donors**

The time of surgery (i.e. retrieval of the uterus) was around 3 h (Table IV). The surgical survival (>24 h) of the donors was 100%. After the immediate post-operative period of 4 days, the animals behaved normally and were not in need of pain relievers. The weight of the uterus, including vascular pedicle, was 31.4 ± 2.7 g (mean ± SEM).

**Recipients**

All the 18 animals survived the surgery as evaluated after the first 24 h. During the immediate post-operative period (3–6 days), inactivity and lack of interest in food were seen in some. The duration of surgery was ~3.5 h. The total ischemic time was around 3 h. The surgical and anesthesiological parameters are summarized in Table IV.

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### Table III Suggested classification of acute uterine rejection in endocervical biopsy samples after uterine transplantation.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Rejection</th>
<th>Biopsy findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No</td>
<td>Normal morphology</td>
</tr>
<tr>
<td>1</td>
<td>Mild</td>
<td>Mild diffuse mixed inflammatory cell infiltrate (mainly lymphocytes) Occasional epithelial apoptotic bodies, focal distribution Surface epithelium intact No necrosis</td>
</tr>
<tr>
<td>2</td>
<td>Moderate</td>
<td>Moderate, diffuse mixed inflammatory cell infiltrate (mainly lymphocytes) Increased amount of epithelial apoptotic bodies: Reduced thickness surface epithelium, possible focal erosion No necrosis</td>
</tr>
<tr>
<td>3</td>
<td>Severe</td>
<td>Significant, diffuse and aggregate, mixed inflammatory cell infiltrate (mainly lymphocytes; neutrophils and eosinophils may be present) Frequent apoptotic bodies Epithelial erosions, focal to total Focal necrosis</td>
</tr>
<tr>
<td>4*</td>
<td>Total necrosis</td>
<td>Necrotic tissue only</td>
</tr>
</tbody>
</table>

*Assumed to represent end stage rejection.

---

### Table IV Summary of surgical and ischemic durations (min) of animals undergoing UTx.

<table>
<thead>
<tr>
<th></th>
<th>Anesthesia time</th>
<th>Surgery time</th>
<th>Warm ischemia 1</th>
<th>Cold ischemia</th>
<th>Warm ischemia 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Donors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>292 (253–396)</td>
<td>169 (126–259)</td>
<td>9 (5–10)</td>
<td>121 (101–157)</td>
<td>47 (21–71)</td>
</tr>
<tr>
<td>Triple group</td>
<td>390 (285–567)</td>
<td>195 (160–230)</td>
<td>7 (1–13)</td>
<td>131 (100–222)</td>
<td>32 (30–87)</td>
</tr>
<tr>
<td><strong>Recipients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>288 (264–330)</td>
<td>210 (145–254)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAC group</td>
<td>303 (211–381)</td>
<td>183 (135–206)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triple group</td>
<td>325 (208–545)</td>
<td>164 (148–252)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The three live-donor-UTx groups included untreated animals (control group, n = 4), animals with a slow-release tacrolimus as monotherapy (TAC group, n = 4) and animals with induction therapy and standard triple immunosuppressive treatment (triple group, n = 10). Values are given in medians (ranges). For definitions of ischemia see the ‘Materials and Methods section’.
Immunosuppression and rejection

**TAC group**
Blood levels of tacrolimus are shown in Fig. 2. Severe rejection/necrosis was shown in the cervical biopsy samples of all the four animals within the first week following UTx (Table V).

**Triple group**
Blood levels of tacrolimus are shown in Fig. 3. Owing to misunderstandings and errata in administration of tacrolimus, four animals received tacrolimus daily from Day –1 to Day 10, and then only weekly (Day 10–25), with resumed daily administration after correction from Day 26 onwards. The remaining six animals received daily tacrolimus during the entire study period (Fig. 3). All the animals presented some degree of rejection in the cervical biopsy samples within 2 weeks, and after 3–4 weeks, three animals additionally showed necrosis (Table V). One animal rejected the uterus through the vagina post-operatively (Day 5).

Representative light micrographs of cervical biopsy samples of different levels of rejection are shown in Fig. 4.

Long-term outcome

**Control group**
Out of the four animals, hormonal cyclicity reappeared within 1 month in two animals but no menstruation was seen. Two animals were euthanized when the study period was terminated (Day 33 and Day 35) and the uterus, although of dark color and necrotic appearance, could be found in one animal (weight 31 g). In the other animal, no uterus was visible.

**TAC group**
None of the animals resumed either hormonal cyclicity or menstruation. All of the animals were euthanized when the experiment was terminated (Day 32–36) and the uterus could be visually found in all the animals although of necrotic appearance. The weight of the uteri recovered at termination of the study was $22.8 \pm 3.6$ g (mean ± SEM).

**Triple group**
Out of the 10 animals, hormonal cyclicity resumed in 5 but menstruation failed to reappear. Two animals died post-operatively (Day 5 and Day 37) and the necropsy procedure reported pulmonary edema in both cases. At the termination of the experiment, the uterus could

### Table V Pathologic interpretation of the cervical biopsy samples in the recipients of uterus grafts.

<table>
<thead>
<tr>
<th>Day</th>
<th>Control group</th>
<th>TAC group</th>
<th>Triple group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>14</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
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Histologic severity of graft rejection is defined as: 0 (normal morphology), 1 (mild), 2 (moderate), 3 (severe) and 4 (necrosis) according to Table III. Day 0 is defined as the day of transplantation. For definitions of groups see the ‘Materials and Methods’ section. [control group: no immunosuppression, TAC group: oral slow-release as monotherapy, triple group: induction with antithymocyte globulin followed by triple therapy (tacrolimus, mycophenolate, corticosteroids)].
be found in the orthotopic position in 7 of the 10 animals. However in only one of these animals, the uterus was of macroscopically normal appearance (weight 25 g compared with 30 g at UTx). In one animal, the uterus had prolapsed into the vagina.

**Discussion**

To our knowledge, this is the first major report in a non-human primate model of UTx showing an excellent surgical survival of 100% in both donors and recipients, indicating that allogeneic UTx in the baboon model is associated with minor surgical risk. Moreover, the durations of uterus recovery and transplantation, in this model with an anatomy similar to that of the human female, were reasonably (≈3 and 2 h, respectively) short. Rejection was seen in recipients without immunosuppression and with suboptimal treatment protocols.

AUFI, with a prevalence of 3–5% in the general population (Milliez, 2009), is one of the largest still untreatable causes of female infertility. Animal research in several species, including non-human primates (Johannesson et al., 2012a,b), has shown that UTx may be a possible future treatment of uterine factor infertility. Although only one case of human UTx has been published so far (Fageeh et al., 2002), preparations for further attempts are most likely being made in several centers around the world. Experience from and knowledge of non-human primate studies are important steps for every group considering starting this new and sometimes controversial treatment.

Survival and safety of the donor are the most vital issues in live donor transplantation. In the present study, the survival of the altogether 18 live donors was 100%. A contributing factor to the 0% mortality is most likely the short duration of retrieval surgery, given that long surgical interventions may increase the risk of donor complications. The median time for live uterus retrieval was around 3 h in the present study. This can be compared with previous UTx studies in non-human primates involving autologous UTx, where retrieval procedures have been reported to be around 2.5–3 h in the baboon (Enskog et al., 2010; Johannesson et al., 2012a,b) and 6.5 h in the cynomolgus macaque (Kisu et al., 2012). In our study in humans, where the uterine vessels were selectively dissected and recovered at radical hysterectomy in cancer patients, the time of surgery was almost 5 h (Johannesson et al., 2012a,b). However, that surgery also included total pelvic lymph node dissection and dissection of the ureters all the way to the bladder, a procedure that would not be

![Figure 4](representative_light_micrographs_of_cervical_biopsy_samples_showing_a_grade_0_normal_morphology_b_grade_1_mild_rejection_c_grade_2_moderate_rejection_and_d_grade_3_severe_rejection_for_definitions_of_the_classification_of_uterine_rejection_see_table_iii_epithelium_e_stroma_s_apoptotic_bodies_arrow_and_necrosis_and_loss_of_endothelium_star_are_marked_magnification_x20)

- **Figure 4** Representative light micrographs of cervical biopsy samples showing: (a) Grade 0: Normal morphology, (b) Grade 1: Mild rejection, (c) Grade 2: Moderate rejection and (d) Grade 3: Severe rejection. For definitions of the classification of uterine rejection, see Table III. Epithelium (E); stroma (S); apoptotic bodies (arrow) and necrosis and loss of endothelium (*) are marked. Magnification ×20.
included in a possible future human uterus recovery from live donors. Importantly, no complications were reported after the immediate post-operative period in the present study.

It is generally known that there exists a positive correlation between durations of anesthesia/surgery and the frequency of post-operative morbidity after transplantation. The present study showed a duration of transplantation surgery of around 3 h. However, surgical durations in the range of 2–4 h alone would not compromise the safety of an otherwise healthy individual as indicated in the present study by the low morbidity and mortality.

By minimizing the ischemic time and damage to the graft, both acute (Totsuka et al., 2004) and, to some extent, chronic (Schwarz et al., 2005) rejection can be prevented in solid organ transplantation. The total ischemic time of the present study was around 3 h and the warm ischemic time, corresponding to the rewarming/anastomosis (warm ischemia part 2), was <1 h in all the groups. A previous study of autologous UTx in baboons with approximately the same warm ischemic time as in the present study showed long-term graft function (resumed menstruation) in 60% of the animals (Johannesson et al., 2012). Other studies in non-human primates have shown longer episodes of warm ischemia (Enskog et al., 2010) with up to 4.5 h in the cynomolgus macaque (Kisu et al., 2012). Studies of autologous UTx in sheep demonstrated that pregnancy could occur in a uterus exposed to around 1 h of cold and 3 h of warm ischemia at transplantation, indicating that the uterus in sheep has great tolerance to ischemia (Wranning et al., 2010). It should be mentioned that the sheep uterus has a size equivalent to that of human females. In a human single case study, the retrieved uterus was subjected to cold ischemia for durations up to 48 h and light microscopy examination showed normal uterine tissue (Sieunarine et al., 2008), although it is questionable whether light microscopy is sensitive enough to detect early ischemic damage. Another study in humans showed that human myometrial tissue, if stored in a protective preservation solution, can tolerate cold ischemia for at least 24 h (Wranning et al., 2005). In that study, electron microscopy showed subcellular damage in tissue stored without appropriate preservation solution, showing the importance of a buffered environment as in the present study where HTK solution were used. The ischemic time presented in the present study is likely not the sole reason for the episodes of rejection.

Studies of UTx in rodents (Groth et al., 2009) and domestic animals (Avison et al., 2009; Gauthier et al., 2011) have shown that a transplanted uterus triggers the same rejection process as any solid organ and that rejection can be avoided only if the animals are treated with adequate immunosuppression (Wranning et al., 2007; Avison et al., 2009; Diaz-Garcia et al., 2010). In this article, we present two different immunosuppressive treatments, monotherapy with tacrolimus and standard triple therapy. There is, to our knowledge, no previous study of immunosuppression in non-human primates following UTx. However, in renal transplanted baboons, tacrolimus monotherapy decreased signs of graft rejection and prolonged animal survival with high (12–18 mg/kg bw) doses of oral administration resulting in mean trough plasma levels between 3.26 and 5.24 ng/ml (Todo et al., 1989). Importantly, the high doses of tacrolimus appeared to be non-toxic (Todo et al., 1989). The oral administration of medication in the TAC group of the present study gave variable trough levels of tacrolimus. Since all the animals showed fluctuating tacrolimus blood levels, we could not conclude whether the instable levels were due to non-adherence of drug delivery or variable metabolism of the drug. In humans, oral immunosuppressive medications are commonly used and with resulting stable blood levels. One main issue in human UTx regarding immunosuppression will instead likely be intake of immunosuppressive drugs during pregnancy. In a pregnant female, the immunosuppression must be monitored thoroughly to ensure stable immunosuppressive levels and all teratogenic substances must be excluded prior to pregnancy (Vento et al., 2008). Large sets of registry data on pregnancies of transplant patients do not show significant increases in congenital malformations, and although pregnancies following organ transplantation have an increased risk of complications, the increase was similar to pregnancies occurring before the transplantation (Kallen et al., 2005).

Although of different histological severity, signs of rejection were present in all animals during the study period. There is no organ-specific type of blood analysis that may reveal early dysfunction of a uterus graft. In the only published human UTx study, rejection was monitored by Doppler-ultrasound, magnetic resonance imaging and measurement of the CD4/CD8 ratio in peripheral blood (Fageeh et al., 2002). In that study, cervical biopsy samples were avoided in order to avoid an invasive procedure potentially harmful to the graft (Fageeh et al., 2002). In the present study, rejection was mainly monitored through biopsy samples of the cervix. The cervical biopsy samples were generally consistent, irrespective of ovarian hormonal activity and menstruation presented by the individual animal. The additionally sampled endometrium correlated poorly with the cervical samples and clinical picture in displaying rejection (data not shown). Unfortunately, there was a long delay in the histopathological interpretation of the cervical biopsy samples because of logistic problems. This limitation in rejection diagnosis of the study, due to the fact that the results of blood levels of tacrolimus were obtained several days after sampling, made it impossible to treat episodes of rejection adequately. We were not able to conclude whether the uterine damage was caused by rejection or impaired blood flow due to surgical complications. However, in our ongoing experiments in allogeneic UTx in the baboon using a deceased donor concept performed in Miami, we have received all the necessary test results the same day as sampling and we have been able to treat and resolve episodes of rejection with temporarily increased immunosuppression when detected early (our unpublished data).

Hormonal cyclicity, as a sign of health and well-being, was resumed in 40% of the recipients in the present study. This can be compared with previous autologous UTx in baboons where 60% (Enskog et al., 2010) and 75% (Johannesson et al., 2012) of the animals resumed hormonal cyclicity (note that in both these studies the ovaries were included in the transplant). In the present study, the ovaries had to be removed from the donor because of the fact that the ovarian veins were used for vascular anastomosis. In a situation of live human uterus donation, the uterine veins would be used. However, in the case of a post-menopausal donor, there would also be a possibility of using the ovarian veins if a concomitant oophorectomy is to be performed.

The present study was not designed to test fertility. Pregnancy and live offspring have been demonstrated following allogeneic UTx in rodents (Diaz-Garcia et al., 2010) and large animals (Ramirez et al., 2011), but it still remains to be proved in a primate species. The
high survival rate of the recipients (100%) in the present study indicates that UTx is associated with little recipient risk. Other previous studies of UTx in large non-human primates have shown similar (Enskog et al., 2010; Johannesson et al., 2012a,b) or lower (Kisu et al., 2012) animal survival. However, the latter have been studies of autologous UTx with long surgical durations including both recovery and transplantation of the same animal. In allogeneic sheep UTx, utilizing a simplified procedure with uterine switch between animals and anastomosis on the distal uterine vessels, animal survival of 100% (Ramirez et al., 2008) and 67% (Ramirez et al., 2011) after 6 and 4 months, respectively were reported.

Shortage of transplantation organs from deceased donors has led to an increasing interest in live donation. Transplants from live donors are common in kidney, partial liver and lung transplantation. In the human setting, UTx studies of the aspects of both deceased donors (Del Priore et al., 2007) and live donors (Fageeh et al., 2002; Johannesson et al., 2012a,b) have been published. An advantage of deceased donor UTx would be that large vessels such as the aorta and the vena cava could be used in the anastomoses. Possible advantages of live donation in human UTx would be that the surgical procedure can be well planned, avoiding a long waiting time and with both the donor and the recipient in optimal physical condition. Additionally, the ischemic damage to the graft due to long ischemic durations is minimal (Perico et al., 2004). The obvious drawback of live donation is the surgical risk of the donor, which will have no direct benefit of the surgical procedure.

In conclusion, this study shows that live donor UTx in the baboon is a donor- and recipient-safe surgical procedure. Furthermore, immunosuppression in baboon UTx should include both induction and maintenance therapy.

Authors’ roles
L.J. was involved in the design, execution and analysis of the study and drafting of the manuscript. M.B. was involved in the study design and execution and took part in drafting the manuscript and supervision. A.E., P.D.K., C.D.G., A.H. and M.O. were involved in execution of the study and contributed to the intellectual input in gynecology, anesthesiology and transplantation surgery. J.M., A.T., P.R. and A.T. contributed to the intellectual input in pathology and transplantation surgery. P.M., K.R and P.T. contributed with technical input.

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Conflict of interest
None declared.

References


