Uterine allotransplantation in ewes using an aortocava patch

T. Gauthier1,*, F. Bertin2, L. Fourcade3, A. Maubon4, F. Saint Marcoux5, P. Piver1, P. Marquet5, I. Pommepuy6, X. Plainard7, C. Couquet8, M.J. Cornuejols8, M. Essig9, and Y. Aubard1

1Obstetrics and Gynecology Department, Hôpital Mère-Enfant, CHU Dupuytren, av Larrey, Limoges 87000, France 2Vascular Surgery Department, CHU Dupuytren, 87000 Limoges, France 3Pediatric Surgery Department, Hôpital Mère-Enfant, CHU Dupuytren, av Larrey, 87000 Limoges, France 4Medical Imaging Department, CHU Dupuytren, 87000 Limoges, France 5Pharmacology, Toxicology and Pharmacosurveillance Department (UMR-S850 INSERM), CHU Dupuytren, 87000 Limoges, France 6Anatomy and Pathology Department, CHU Dupuytren, 87000 Limoges, France 7Urology Surgery Department, CHU Dupuytren, 87000 Limoges, France 8Research and Analysis Laboratory, Haute-Vienne, France 9Nephrology Department, CHU Dupuytren, 87000 Limoges, France

*Correspondence address. Tel: +33-05-55-05-61-07; E-mail: gauthiertristan@yahoo.fr

Submitted on December 4, 2010; resubmitted on July 19, 2011; accepted on August 4, 2011

BACKGROUND: We investigated a novel allotransplantation model using an aortocava patch in ewes.

METHODS AND RESULTS: We carried out 10 uterine orthotopic allotransplantations in ewes with end-to-side anastomosis of the aortocava donor patch on the left external iliac vessel recipient. The immunosuppressive protocol was a combination of cyclosporine (10 mg/kg/day) and mycophenolic acid (3 g/day). An estimation of the immunosuppressive therapy exposure was performed by measuring the area under the curve (AUC) of immunosuppressive plasma concentrations. The graft was assessed by vaginoscopy, magnetic resonance imaging (MRI) and second look laparotomy at 6, 8 and 10 weeks, respectively. The median (range) times for cold and warm ischemia were 95 min (75–130) and 91 min (55–165), respectively. All the vascular anastomoses were patent at the end of the surgery. The median AUC of cyclosporine and mycophenolic acid were 1.24 mg h/l (0.34–3.85) and 18.40 mg h/l (3.76–42.35), respectively. Of the 10 ewes receiving a transplant, 6 could be assessed. Cervical biopsies showed signs of necrosis in all six ewes. The MRI results correlated with the macroscopic observations of the second look laparotomy. The aortocava vascular pedicles were thrombosed, adding to the peripheral neovascularization. Graft histology showed endometrial tissue in two out of six ewes.

CONCLUSIONS: Mobility of the transplant within the pelvis, the length of the vascular pedicle and rejection can explain the high rate of transplant necrosis. The particular digestive anatomy and physiology of ruminants makes it difficult to administer an optimal immunosuppressive treatment. MRI appears to be a good non-invasive examination for graft estimation.

Key words: uterine allotransplantation / ewe / magnetic resonance imaging / cyclosporine / mycophenolic acid

Introduction

Patients with uterine infertility can only become parents through adoption or IVF gestational surrogacy, and this second option is only available in certain countries. For many women, pregnancy and giving birth are very significant milestones. Uterine transplantation (UTx), which could potentially fulfill this need, is an interesting alternative. Results of experimental UTx in mouse and ewe indicate that the uterus is resistant to ischemia-reperfusion injury (El-Akouri et al., 2003; Dahm-Kahler et al., 2008; Wranning et al., 2008). After transplantation, the uterus exhibits normal function and the capacity to carry pregnancies without risk of rejection (Brännström et al., 2010). Pregnancies have been described after heterotopic transplantation in mice (El-Akouri et al., 2003) and after orthotopic uterine autotransplantation in dogs (Eraslan et al., 1966; Paldi et al., 1975), and more recently in large animals (El-Akouri et al., 2003; Wranning et al., 2010). The first pregnancy after an allogeneic UTx was reported in a rat model (Diaz-Garcia et al., 2010). However, an autotransplantation surgical model in large animal species is more common in literature (Wranning et al., 2006, 2008, 2010; Dahm-Kahler et al., 2008; Enskog et al., 2010; Kisu et al., 2011) and allogeneic UTx has rarely been described (Sieunarine et al., 2005; Del Priore et al., 2008; Ramirez et al., 2008; Avison et al., 2009). The obvious next step in UTx research is to develop an allotransplantation model using immunosuppressive agents in larger animals before the next human trials are conducted.

Concerning allogeneic UTx in women, in our opinion, it would be necessary to retrieve the human uterus from a multi-organ donor to avoid injury in a live donor. In this study, we provide the first
description of a surgical model in sheep that aims to be as close as possible to human conditions for allogeneic UTx. We conducted orthotopic allogeneic UTx on ewes with an aortocava patch to preserve uterine and ovary vascularization. The advantage of using sheep is that the body size and pelvic vascular anatomy are similar to those in young women.

Our secondary aims were to assess the feasibility of prolonged immune suppression in ewes in conditions similar to those that would be required for humans, and to assess graft integrity by vaginoscopy and magnetic resonance imaging (MRI).

Materials and Methods

This study was conducted at the Haute-Vienne research and analysis laboratory in Limoges (France), where experiments on animals are authorized. The study was approved by the Limousin Regional Committee of Ethics and Animal Experiments (CRREAL) and received financial support from the French Biomedical Agency. We planned to carry out 10 uterine allotransplantations. Twenty-three-year-old Limousine ewes with prior history of gestation were purchased from a local breeder. All animals weighed between 40 and 70 kg. Major histocompatibility complex (MHC) tissue matching was not obtained. As the animals belonged to the same group of livestock, the degree of MHC uniformity might be high between the ewes.

Anaesthesia protocol

The wool on the lower abdominal region was clipped and food was withdrawn 48 h before the day of surgery. Premedication consisted of i.m. injection of 20 mg/kg ketamine (Imalgene®, Merial, Lyon, France) combined with 0.4 mg/kg 2% xylazine (Rompun®, Bayer, Puteaux, France). General anaesthesia was maintained using a continuous i.v. propofol (10 mg/ml) drip at a dosage rate of 25 ml/h. To compensate for fluid loss during surgery, the animal received low i.v. infusion of Ringer's Lactate solution.

Donor procedure

The aim of the surgery was to isolate a specimen that included the common uterine cavity and cervix, both uterine horns, both ovaries and oviducts, the upper vagina and a vascular pedicle, including the abdominal aortocava vessels, the internal iliac artery and vein, both uterine and ovarian arteries and both utero-ovarian veins (Fig. 1). All surgical procedures were performed using sterile techniques. Using a midline incision, the rumen, small intestine and colon were retained in the cranial portion of the abdomen with large gauzes and one abdominal retracting pad. A self-retaining hook was used during the subsequent surgery. The sigmoid colon was transected by an automatic stapling-dividing device to expose and remove the entire intact vascular bifurcation. The iliac vessels and the infrarenal aorta and infrahepatic vena cava were dissected gently and exposed. During the dissection procedures, unipolar diathermy was used to minimize bleeding. A 25 000 IU heparine bolus was injected by the anaesthetist before clamping and cutting the aortocava pedicle. The ewe was then euthanized with 20 ml of embutramide (T61®, Intervet, Beaucouze, France). The transplant procurement proceeded with the complete retrieval of the uterus, adnexa and upper vagina, along with the aortocava pedicle, including the preserved uterine and ovarian arteries as well as the utero-ovarian veins.

Graft preparation

Once collected, the uterus graft was kept and prepared ex vivo on a ‘back table’ in a sterile basin and chilled in ice. The graft was flushed through, by way of cannulated aorta, with 1000 ml of chilled (4 °C) protective Celsior® solution (Genzyme, Saint Germain en Laye, France) at 100 mmHg pressure. Flushing was continued until clear fluid drained from the vena cava.

Figure 1 Procurement of the uterus bicornis, adnexas and an aortocava patch to maintain utero-ovarian vascularization for allotransplantation in the ewe procurement limits.
Transplantation of uterus
The recipient ewe was taken into the operating room after the donor was removed. Premedication and general anaesthesia were achieved as described already. Antibiotic preventive therapy using 2 g clavulanic acid-amoxicillin (Augmentin®, GlaxoSmithKline, Marly-le-Roi, France) was administered i.v. Through an abdominal midline incision, the small intestine and colon were retained in the cranial portion of the abdomen with large gauzes and one abdominal retracting pad. A self-retaining hook was used during the subsequent surgery. A total hysterectomy with a bilateral oophorectomy was performed. Unipolar diathermy was used to minimize bleeding. The recipient hysterectomy started with division of the round ligaments using cautery. The broad ligament was opened and the ureters identified. The ovarian veins and arteries on both sides were ligated and cut. The bladder and rectum were dissected from the uterus, and uterine and cervix vessels were clamped, and cut and tied with 2–0 Polysorb® sutures (Covidien, France) using an extra-fascial hysterectomy technique. The vagina was then divided to the cervix using scissors to avoid tissue necrosis. To choose the anastomosis site, the retroperitoneum was incised over the left external iliac vessels, and the latter were gently dissected. Before transplantation of the uterus graft, methylprednisolone (500 mg) was given i.v. The orthotopic allo-transplantation began with vaginal anastomosis using interrupted 2–0 Polysorb® sutures (Covidien). Proximal and distal control of the recipient’s left external iliac vessels were obtained with short atraumatic vascular clamps. About 5000 IU of heparin were administered i.v. before clamping to avoid blood clotting. An incision into the recipient external iliac vein was made using a size 11 blade. Donor vena cava was anastomosed end-to-side with the recipient’s left external iliac vein using a continuous suture pattern with 6–0 Prolene® (Ethicon, France). The donor lower aorta anastomosis with left iliac external artery was carried out in a similar fashion (Fig. 2). After the vascular clamps were removed, the graft was observed for macroscopic signs of reperfusion, such as the change of tissue colour and pulsation of the graft aorta. We checked the graft staining (i.e. recolouring of the graft) and the aortic graft beats below the anastomosis. Interrupted 2–0 Polysorb® sutures (Covidien) were used to attach the graft to the peritoneum on the pelvic side and to the native round ligaments. The abdominal incision was closed with Polysorb® I (Covidien) in a continuous suture pattern. The skin was closed with surgical staples. The ewes were kept for 24 h in a closed room adjoining the operating room and then transferred to their regular holding space.

We collected the following data during transplantation: transplant procurement time, time of cold ischemia (time the graft spent in the ice basin), the first phase of warm ischemia (time between aorta clamping and cooling), the second phase of warm ischemia (time between clamping before removal of the ice basin and removal of the external iliac arterial clamp), the total time in warm ischemia and the overall surgery time, including transplant retrieval and transplantation. The surgery times were expressed as median and range.

Immune suppression
We used an immune suppression protocol, including a combination of cyclosporine (Néoral®; Novartis, Rueil-Malmaison, France) with mycophenolate mofetil (MMF) (CellCept®, Roche, Welwyn Garden City, UK), and a corticosteroid-sparing strategy. The immune suppression protocol was initiated 2 days before transplantation and consisted of Neoral® (10 mg/kg/day, per os) and CellCept® (3 g/day, per os). Corticosteroid treatment consisted of methylprednisolone given as one i.v. injection of 500 mg during surgery and then a daily dose of 40 mg (i.v.) from post-operative Day 1–7. For each ewe, a complete pharmacokinetic profile for cyclosporine and MMF was established at post-transplantation Days 7, 14, 28, 42 and 56 (+/- 3 days).

This protocol included blood sampling at T zero (before the morning treatment) using the external jugular veins, and then at T + 20 min, T + 40 min, T + 1H, T + 2H, T + 3H, T + 4H, T + 6H, T + 8H and T + 12H, which enabled us to calculate the area under the curve (AUC) for immunosuppressant drug plasma concentration between two times of administration (AUC⁰₋₁₂). The AUC values were expressed as median and range. We did not make any dosage adjustments.

All the samples have been measured at the laboratory of the Department of Pharmacology and Toxicology in the University Hospital of Limoges, France.

Cyclosporine determination was performed using turbulent flow chromatography-tandem mass spectrometry, in an Aria TLX TurboFlow 2300 HTLC™ System (Cohesive Technologies, Milton Keynes, UK) equipped with a CTC HTC PAL autosampler (CTC Analytics AG, Zwingen, Switzerland). Detection was performed with a TSQ Quantum® tandem mass spectrometer (ThermoFischer Scientific, Les Ulis, France) equipped with an electrospray ionization source and controlled by the X-Calibur computer program. The lower limit of quantification was 20 μg/l, and calibration curves obtained using quadratic regression from the limit of quantification up to 2000 μg/l yielded r² ≥ 0.99. The method was found to be accurate and precise with bias of –4.4–0.6% over the linearity range. The intra- and inter-day assay variabilities evaluated from the results of quality controls at four different stages (MassCheck® Immunosuppressants Whole Blood Control; Chromsystems Instrument & Chemicals GmbH, München, Germany) were <15%.

The measurement of total mycophenolic acid was performed using a validated high-performance liquid chromatography (HPLC) method with
ultraviolet (UV) detection. Briefly, a liquid–liquid extraction procedure was applied to a mixture of 1 ml whole blood and 4 μg of internal standard (thiopental sodium) and chromatographic separation was performed using a HPLC system, including an X-Terra column and precolumn (Waters Corporation, Saint Quentin en Yvelines, France) and with a UV detection at 254 nm. This method exhibits a lower limit of quantification of 0.1 mg/l and a coefficient of variation of 6.0% for inter-day assay accuracy at the 1 mg/l concentration.

Post-operative care and follow-up
Food and water intake, vaginal discharge and rectal temperatures were recorded daily. An i.m. injection of 0.85 mg/ml metomidine hydrochloride (Sedator®, Eurovet, Bladel, Netherlands) was provided for post-surgery analgesia. Clavulanic acid with amoxicillin at a dose of 4 g per day i.m. (Augmentin®, GlaxoSmithKline) was administered for 10 days as preventive antibiotic therapy. The animals were given s.c. injections of 4000 IU/day of low molecular weight heparin (Lovenox, Sanofi-Aventis, France) for 7 days to prevent post-operative thromboembolism. Serum chemistry levels and blood counts were recorded every week for 2 months to detect infections or complications related to treatment because of the potential hematologic and renal toxicity of mycophenolate and cyclosporine.

Assessment of graft viability
Cervical biopsies were taken at vaginoscopy at 6 weeks (ewes N°2, N°4, N°6, N°8) and at 2 weeks (ewes N°9 and N°10).

A non-invasive graft assessment was made by pelvic MRI at three Tesla (Intera, Philips, Eindhoven, Netherlands) at 8 weeks (ewes N°2, N°4, N°6, N°8) and at 3 weeks (ewes N°9 and N°10). In a preparatory study, MRI was performed in two control ewes with a native uterus. Food was removed 48 h before the examination in order to reduce rumen volume. Simple sedation was given by ketamine i.m. (Imalgene®, Merial, Lyon, France) and xylazine 2% (Rompum®, Bayer). The ewe was placed on her left side and maintained by a simple restraint. Three-plane localizer images; axial, sagittal and coronal T2-weighted fast spin-echo images and T1-weighted spoiled gradient-echo images were obtained. Ewes then received i.v. injection of contrast product (gadolinium) and multiplanar T1-weighted images were obtained.

A ‘second look’ median laparotomy was performed 10 weeks after the transplantation procedure. The graft and the anastomosis site were inspected. During this second operation, the graft was removed if it did not appear viable, and examined for signs of rejection or necrosis.

Histology
The analyses were performed by one anatomopathologist doctor in the anatomopathology department of the Limoges University Hospital. Each cervical biopsy and transplant procurement was fixed in formaldehyde, embedded in paraffin, and sectioned and stained with haematoxylin and eosin. The sections included the cervix, the entire uterine wall (serosa, myometrium and endometrium) and the aortocava patch. Signs indicating normal tissue, acute rejection injury (density of lymphocytes, oedema, intima arteritis, necrosis) and ischemic injury (density of neutrophils, necrosis, vascular thrombosis) were noted.

Results

Outcome of surgery
We carried out 10 surgical procedures between February and June 2010. The median duration of transplant procurement was 102 min (82–130). The median duration of the entire surgery (retrieval and transplantation) was 305 min (265–385). The median time of cold ischemia was 95 min (75–130). The median time of the first warm ischemia phase and second warm ischemia phase were 15 min (5–30) and 77 min (50–150), respectively. The median time of the total warm ischemia phase was 91 min (55–165). In all 10 transplanted ewes, recirculation of the graft was satisfactory according to checked graft staining and pulsation through the anastomosed vessels after the vascular clamps were removed. Less than 6 h after the surgery, ewes number 1 and 5 died of pelvic haemorrhage related to graft bleeding. After these adverse events, we modified the surgical procedure slightly, carrying out both the vascular anastomosis prior to the vaginal anastomosis. Once this modification was made, we did not observe any further haemorrhaging. The median time of the second warm ischemia phase was reduced from 84.5 min (60–150) to 57.5 min (50–80) in the modified procedure. Ewe number 7 died on Day 7 because of an acute intestinal obstruction. The autopsy showed a viable graft with no signs of necrosis or infection, and there were no signs of thrombosis in the vascular anastomosis.

Immune suppression
The absorption profile of cyclosporine was of the ‘continued perfusion’ type with no gastric absorption peak (Fig. 3). The results for the plasma concentration of cyclosporine showed a large inter-individual variability. The median AUC of cyclosporine and mycophenolic acid were, respectively, 1.24 mg h/l (0.34–3.85) and 18.40 mg h/l (3.76–42.35). The intra-individual variability was also notable.

![Figure 3](https://academic.oup.com/humrep/article-abstract/26/11/3028/657663) Cyclosporine (A) and mycophenolate mofetil (B) plasma concentration and absorption profile in ewe N°10.
Ewe follow up
Among the seven remaining ewes, the surgery was well tolerated. Monitoring of the hemoglobin rate showed a median loss due to surgery of 2.87 g/dl (0.9–3.7). The median rectal temperature for 28 days post-transplantation was 38.8°C (37.1–40.7). Two ewes presented fever owing to a subcutaneous abscess, which was managed by antibiotic therapy. Ewe number 3 spontaneously expelled the transplant vaginally 2 months after the transplantation date, with no prior clinical signs (there was no fetid vaginal discharge, no fever or hyperleukocytosis). This event did not have any major consequences for the ewe.

Graft assessment
The data for graft evaluation of six ewes are summarized in Table 1. Owing to signs of necrosis in the first four cervical graft biopsies taken at 6 weeks (ewe numbers 2, 4, 6, 8), we decided to conduct the biopsies earlier (at 2 weeks) for the last ewes, numbers 9 and 10, and no signs of infection were noted. Cervical bleeding was observed in only two cases (ewe numbers 4 and 9).

At MRI examination of two control animals, the uterus with two horns and ovaries were identified, with myometrial and endometrial signal intensity being similar to that found in women. In the transplanted ewes, we conducted the first four MRIs at 8 weeks and advanced the MRI to 3 weeks for the last ewes, numbers 9 and 10. The results were equivalent. In all six ewes, the T2-weighted images showed a globular and heterogeneous graft with peripheral oedema (Fig. 4). The individual uterine horns and the ovaries could not be identified. T1-weighted images allowed us to explore the vascular anatomy and showed that the six-pedicle grafts were thrombosed (Fig. 5). The grafts showed only peripheral contrast enhancement.

The second look laparotomy performed at 10 weeks showed many adhesions. The omentum and sigmoid were adhered to the graft, and the bladder was stuck to the graft and hard to identify. Abdominal adhesions were managed with meticulous, sharp dissection. There was a perfect correlation between the MRI pictures and the laparotomy observations (Fig. 6). The grafts were globular and ovaries were not identified. There was a superficial neovascularization with few bleeds (Fig. 6). Thrombosis of the donor pedicle was proximal in most of the cases. In two cases, thrombosis was distal below the aortic graft beats. In one case (ewe 9), the graft was twisted above the bladder. All the grafts were removed following euthanasia of the ewes. The time of second look laparotomy was maintained at 10 weeks because of the necessary immunosuppressive pharmacokinetics profile evaluation at 2 months.

Histology
Histology of the expelled graft from ewe number 3 showed that massive necrosis had occurred by 2 weeks post-transplantation. Cervical biopsies taken at 6 weeks (ewes N2, N4, N6, N8) showed necrosis with infiltration of inflammatory cells, consisting mainly of polynuclear cells and macrophages. The biopsies taken at 2 weeks (ewes N9 and N10) showed lymphocyte infiltration with signs of necrosis and arteritis (Fig. 7).

Graft histology analysis after second look laparotomy showed the presence of myometrium and endometrial tissue in two cases. Necrosis was extensive, with a specific neutrophil granulocyte and macrophage infiltration. Thrombosis was present in all vascular pedicles. No signs of arteritis or lymphocyte infiltration were observed.

Discussion
The ultimate objective of this project is UTx in women. As with kidney transplants, uterine grafts could be obtained from a live donor with a potentially high rate of graft survival (Terasaki et al., 1995). However, because uterine procurement from a live donor risks vascular and ureteral injuries (Fageeh et al., 2002), we believe that a more appropriate ethical approach would be to retrieve the uterus from a heartbeat- ing, brain-dead multiorgan donor. For this approach, we were inspired by the Del Priore’s work on primates and a woman in a brain- dead state (Del Priore et al., 2007, 2008).

In our study on ewes, all of the aortocava pedicles were thrombosed. The two cases that had viable tissue can be explained by peripheral neovascularization. The pedicle length and the graft mobility in the pelvis, despite peritoneal fixation, could give rise to mechanical stress on the vessels, leading to thrombosis and subsequent necrosis. In one case, we described torsion of the graft above the bladder. In the single human example of a uterine graft, necrosis occurred at 3 months, and uterine mobility and vascular torsion were proposed as reasons for this (Fageeh et al., 2002). Moreover, the ewe’s early mobility following surgery and quadruiped stance can lead to traction on the pedicle and thrombosis. Autopsy of the dead ewe number 7 on Day 7 showed no pedicle thrombus, which indicates the possibility of a secondary thrombosis away from the surgery.

Acute rejection could also be the cause of massive necrosis. Lymphocyte infiltration at 2 weeks, MRI graft oedema and artery thrombosis can reflect acute rejection (Racusen et al., 1999; Taylor et al., 2010), and can be explained by insufficient immune suppression. Without immune suppression, necrosis occurs rapidly (El-Akouri et al., 2006; Diaz-Garcia et al., 2010). We decided to undertake a double immune suppression based on the combination of cyclosporine and MMF, similar to the human protocol. In humans, this combination decreased the impact of acute rejection on renal, hepatic and cardiac transplantation (Ciancio et al., 2005). In spite of the higher dosages than those usually used in humans, the AUCs 12 h of each ewe were clearly inferior to those recommended for humans i.e. about a 5 mg h/l zone for cyclosporine and between 30 and 60 mg h/l for the MMF in the first post-transplantation days (Le Meur et al., 2007). We did not adjust the treatment because there is a lack of knowledge regarding immune suppression in ewes, and to avoid confusion because immune suppression exposure was only a secondary aim of this preliminary study. Ramirez et al. used cyclosporine starting at 10 mg/kg/day and then quickly reduced to 2 and 5 mg/kg/day (Ramirez et al., 2008); compared with our findings in this study, this seems insufficient. This phenomenon could be explained by the anatomy of ruminant species in which, because of rumen volume, pH and rumination, the absorption differs from monogastric species (Ruckebusch, 1977). A different manner of administration (i.v. or abomasal administration) or a different animal model will be necessary for future studies. A preparatory study will be useful to determine the relationship between dose and plasma concentration for immunosuppressive drugs in ruminants with different routes of administration—the aim will be to obtain an immune suppression model in the ewe. Diaz-Garcia et al. described the first
Table I  Data for the six ewes together with evaluation of the grafted uterus.

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<th>Ewe 2</th>
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<th>Ewe 9</th>
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<td>95</td>
<td>113</td>
<td>102</td>
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<td>105</td>
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<tr>
<td>Time of total warm ischemia (min)</td>
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<td>107</td>
<td>97</td>
<td>70</td>
<td>68</td>
<td>55</td>
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<tr>
<td>Median and range AUC cyclosporine (mg h/l) over the 8 weeks period</td>
<td>2.77 (0.34–3.81)</td>
<td>1.21 (0.98–2.62)</td>
<td>1.75 (0.61–3.85)</td>
<td>1.14 (0.74–1.87)</td>
<td>0.52 (0.38–2.12)</td>
<td>1.24 (0.72–1.74)</td>
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<td>Median and range AUC MMF (mg h/l) over the 8 weeks period</td>
<td>18.01 (3.76–21.02)</td>
<td>18.79 (8.30–37.83)</td>
<td>32.68 (14.70–42.35)</td>
<td>20.35 (7.58–31.14)</td>
<td>16.29 (8.21–38.62)</td>
<td>17.22 (5.85–26.55)</td>
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<td>Time of cervix biopsies (weeks)</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
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<td>Results of cervix biopsies</td>
<td>Neutrophilic granulocyte infiltration Necrosis</td>
<td>Neutrophilic granulocyte infiltration Necrosis</td>
<td>Neutrophilic granulocyte infiltration Necrosis</td>
<td>Neutrophilic granulocyte infiltration Necrosis</td>
<td>Moderate lymphocyte infiltration Necrosis</td>
<td>Lymphocyte infiltration Necrosis</td>
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<td>Time of MRI (weeks)</td>
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<td>MRI results</td>
<td>Pedicle thrombosis Heterogeneous graft Peripheral vascularization Necrosis</td>
<td>Pedicle thrombosis Heterogeneous graft Peripheral vascularization Necrosis</td>
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<td>Graft histology at 10 weeks</td>
<td>Neutrophilic granulocyte, macrophage infiltration</td>
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MRI, magnetic resonance imaging; AUC, area under curve; MMF, mycophenolate mofetil.
pregnancy after allogenic uterus transplantation in rats with a continuous administration of tacrolimus via an s.c. pump (Diaz-Garcia et al., 2010). In Avison’s study of swine, the rejection episodes were managed by i.v. tacrolimus followed by cyclosporine (10 mg/kg/day) and methylprednisolone (Avison et al., 2009). Compared with studies with rats or swine (Avison et al., 2009; Diaz-Garcia et al., 2010), we did not assess the degree of inbreeding between donors and recipients, which could influence the level of rejection. The ovine MHC has not been fully described, unlike for bovines and swine but complete ovine MHC sequence information should be available soon for studies of inbreeding (Gao et al., 2010). Compared with studies with rats or swine (Avison et al., 2009; Diaz-Garcia et al., 2010), we did not assess the degree of inbreeding between donors and recipients, which could influence the level of rejection. The ovine MHC has not been fully described, unlike for bovines and swine but complete ovine MHC sequence information should be available soon for studies of inbreeding (Gao et al., 2010). However, inbreeding in farm sheep could reduce variability of MHC polymorphisms between animals. One-third of sheep from a single farm maintaining excellent breeding practices may be too closely related to mount an immune response against donor tissue (Lehr et al., 2006).

There may also be a rheological problem. The difference in calibre between the donor (aorta and vena cava) and recipient vessels (external iliac) can explain secondary thrombosis linked to flow turbulence. It would appear that the preventive anticoagulation used in our study was inadequate and it may be necessary to increase the time and dosage of anticoagulant (Ao et al., 1999; Connell et al., 2007).

Ischemia and reperfusion, well tolerated by the uterus (El-Akouri et al., 2003; Wranning et al., 2008), are likely not the reason behind our high necrosis rate. The best examples of ischemia tolerance are the successful pregnancies described following auto-transplantation of a uterine horn in an ewe (Wranning et al., 2010). The surgical model in sheep of the Brannström research team, published after the beginning of our study, seems to be efficient (Wranning et al., 2010), and in future, it will be interesting to know the results in allotransplantation conditions of this Swedish team.

Two non-invasive methods were used for graft evaluation. We can easily reproduce the vaginoscopy, but this is not sufficient as this test evaluates only the distal part of the graft. Moreover, the uterine body could be functional even if the cervix is necrotic. Hysteroscopy evaluation will be more useful (Avison et al., 2009; Bigolin et al.,

**Figure 4** T2-weighted MRI transverse section of ewe with native uterus (**A**) and 8 weeks post-transplantation with globular graft (**B**). 1: uterine horns 2: bladder 3: rectum 4: oedema.

**Figure 5** T1-weighted MRI (injected with gadolinium), 8 weeks after transplantation of the uterus in ewe. Distal thrombosis of the aortic pedicle (1).

**Figure 6** Uterus graft with peripheral neovascularization, 10 weeks post transplantation in the ewe, observed by laparotomy.
MRI is also the reference examination in the female pelvis (Maubon et al., 2009) but with a potential risk of infection in immune-suppressed conditions.

The most interesting finding of the present study is the correlation between the MRI images and the morphological observation. To our knowledge, this is the first report of pelvic MRI in ewes. We chose MRI examination because of its reliability and imaging quality, and MRI is also the reference examination in the female pelvis (Maubon et al., 2001). Pelvic MRI in the ewe was easily performed and the results correlated well with second look laparotomy observations. Estimation of graft viability was satisfactory with the morphological examination in T2-weighted images. MRI with gadolinium injection appears to be a functional reference examination to check vascularization patency. In future clinical trials of uterus transplantation, pelvic MRI could offer a means to avoid invasive evaluation methods and serve as a reference examination in post-transplantation follow-up. However, further studies would be useful to assess the validity of MRI for detecting graft rejection. An endorectal and endovaginal scan combined with a Doppler could also be an option. Ramirez’s team used the endorectal scan but no details on the conditions, no examination results and no imaging are provided in the article (Ramirez et al., 2008). Arteriography was not used because this examination does not allow morphological analysis of the graft. Also, the gadolinium used in MRI has the advantage of not being nephrotoxic for patients already on cyclosporine, unlike the radioopaque contrast product used in arteriography.

This preliminary and technically difficult study had several weaknesses that can explain the low graft survival rate. However, through this experience, we have identified several of these points so that they can be addressed more properly in a future study. The lack of early graft assessment to discern whether the cause of necrosis is mechanical or related to rejection is a major weakness. It will be essential to perform a very thorough follow-up in any future study. To detect signs of rejection, biopsy monitoring of the uterine cervix at short intervals, as suggested by certain cardiac transplantation teams, would provide more information than a single later biopsy (Tan et al., 2007). Immunochemistry for neutrophilic granulocytes, macrophages, cytotoxic CD8⁺ T cells and CD4⁺ T-helper cells could be useful (Groth et al., 2009). An early MRI scan, shortly after the surgery, will be helpful to detect early failure of the transplantation.

Figure 7 Representative light micrograph of cervical biopsy in a ewe, 2 weeks post transplantation, showing arteritis with moderate lymphocytic infiltration (Haematoxylin and eosin stain, magnification ×100).

The lack of a control group is another obvious weak point of the present study. The nature of an appropriate control group is not entirely obvious. For example, a group with allo-transplanted and non-immunosuppressed animals was advised against by the ethics committee and would also be of little value when trying to discern between surgery-related and immunological mechanisms effecting outcome. The ideal control group for our specific surgical model would be a syngenic animal (monozygotic twin donor/recipient pair or animals tested for absence of functional allogenicity), who underwent the same surgical procedure without any immunosuppressant treatment. Monozygotic twins are not readily available in sheep but can be produced by embryo division and embryo transfer (Gatica et al., 1984) as this would be the only way to assess the results of the surgical procedure.

Conclusion

The feasibility of uterine autotransplantation and tolerance of this process has already been demonstrated in mouse, ewe, pig and monkey in the literature. It is now important to establish whether a uterus graft can tolerate an allotransplantation in a large-animal model. The use of the aortocava patch is associated with a high risk of thrombosis although rejection cannot be excluded as a cause of necrosis. The digestive specificity of ruminants makes it difficult to obtain the optimal level of immune suppression for this procedure. MRI appears as an interesting non-invasive technique to evaluate graft morphology and vascular patency. Examination of the results of histology and imaging screening by MRI in parallel would appear essential in order to distinguish ischemic risk from the risk of rejection.

Authors’ roles

T.G., Y.A. and P.P. had the original idea for the study. T.G., Y.A. and P.P. supervised the entire study. The surgery was done by T.G., F.B., L.F. and X.P. The follow-up of the ewe was done by T.G., M.J.C. and C.Q. The MRIs were performed by A.M. The pharmacokinetic profile was done by F.S.M., P.M. and M.E. The anatomy and pathology review was done by I.P.

Funding

This research was supported by grants from the Agence de la Biomédecine.

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