THE JOURNAL OF
Obstetrics and Gynaecology Research

doi:10.1111/jog.12256 J. Obstet. Gynaecol. Res. Vol. 40, No. 3: 754–762, March 2014

Test of long-term uterine survival after allogeneic transplantation in rabbits

Srdjan Saso[1], Simon Hurst[1], Jayanta Chatterjee[1], Eugene Kuzmin[1], Yau Thum[3], Anna L. David[4], Nadey Hakim[1], David J. Corless[8], Michael Boyd[5], David E. Noakes[6], Iain Lindsay[7], Sadaf Ghaem-Maghami[1], Giuseppe Del Priore[9] and J. Richard Smith[2]

[1]*Department of Surgery and Cancer, Institute of Reproductive and Developmental Biology,* [2]*West London Gynaecological Cancer Centre, Queen Charlotte's Hospital, Imperial College London,* [3]*The Lister Hospital,* [4]*Institute for Women's Health, Faculty of Population Health Sciences, University College London,* [5]*Biological Services Unit,* [6]*Veterinary Reproduction and Obstetrics, Royal Veterinary College,* [7]*Department of Pathology, Imperial College Healthcare NHS Trust, Charing Cross Hospital, London,* [8]*Mid Cheshire NHS Trust, Leighton Hospital, Crewe, UK; and* [9]*Indiana University, Indianapolis, Indiana, USA*

Abstract

Aim: To see if: (i) a large vessel aortocaval vascular patch technique may bring about long-term graft survival after allogeneic uterine transplantation (UTn) in a rabbit model; and (ii) fertility can be achieved following natural mating post-allogeneic UTn.

Methods: Allogeneic uterine cross transplantations were performed in New Zealand white rabbits using an aortocaval macrovascular patch harvested as part of the uterine allograft. Five rabbit recipients received a uterine graft from five unrelated donor rabbits. All female rabbits were unrelated and were of proven fertility with at least one previous litter each. Tacrolimus was administrated for immunosuppression post-transplant. Natural mating was attempted if long-term survival had been achieved. The main outcome measures were: (i) long-term recipient survival; (ii) long-term adequate uterine perfusion; and (iii) successful pregnancy post-UTn.

Results: All five recipient animals survived the surgery with satisfactory immediate postoperative recovery. Recipients 1, 2 and 4 died within the first 4 postoperative days. Both long-term survivors failed to conceive following introduction of a proven male breeder despite evidence of mating. Necropsy at 9 and 11 months showed a lack of patency of uterine cornua at the point of anastomosis, albeit a small uterus in recipient 3 and a reddish brown amorphous material at the site of the transplanted uterus in recipient 5.

Conclusion: We have demonstrated the feasibility of uterine allotransplantation using a macrovascular patch technique, but could not demonstrate conception because of blocked cornua. To address this, we propose using embryo transfer techniques in order to achieve conception.

Key words: allogeneic uterine transplantation, fertility, graft survival, rabbit model.

Introduction

Uterine transplantation (UTn) has been proposed as a treatment option for women diagnosed with absolute uterine factor infertility (AUFI) and who are willing to bear their own child.[1] AUFI renders a woman 'unconditionally infertile'. Causes are congenital, such as Müllerian duct anomalies, or acquired (e.g. a

Received: May 17 2013.
Accepted: August 1 2013.
Reprint request to: Miss Sadaf Ghaem-Maghami, Division of Surgery and Cancer, IRDB, Imperial College London, Hammersmith Hospital Campus; Du Cane Road, London W12 0NN, UK. Email: s.ghaem-maghami@imperial.ac.uk

hysterectomy performed secondary to obstetric hemorrhage, myoma, endometrial or cervical malignancy). Since the first human UTn attempt in 2000, in which the donor graft failed after 99 days because of thrombosis of the uterine vasculature leading to subsequent necrosis of the transplanted uterus, important advances have been made into several fields which define UTn. These include donor graft retrieval, minimization of ischemic–reperfusion and allorejection-related injury, optimization of surgical techniques to allow for an adequate uterine blood supply, and finally achieving the 'Holy Grail' of UTn: pregnancy.[2] Pregnancy has indeed been achieved following both syngeneic[3,4] and allogeneic[5,6] UTn in three animal models: murine, rat and sheep. However, only the sheep model led to the birth of a healthy, term lamb.

Achieving an adequate blood supply for the transplanted uterus still presents the biggest challenge in UTn. A number of animal models have been tried, including non-human primate,[7,8] sheep,[9,10] rat[11–13] and mouse.[14] Our previous experience (UK Team) regarding this question assessed the feasibility of uterine autotransplantation (auto-UTn) in a porcine model using microvascular anastomoses.[15] A supracervical hysterectomy was performed on eight sows of proven fertility. Postoperatively, one survived up to 3 months with no signs of estrus, and three sows were killed on days 6, 33 and 54, respectively, for pelvic infection. Histopathology of the uterine grafts revealed gradual vessel thromboses suggesting that microvascular re-anastomosis was unsuccessful in auto-UTn (and most likely in allo-UTn) because of gradual vessel thromboses. In addition, the porcine model proved complex as it is highly susceptible to postoperative infection.

To overcome this problem and following consultation with the intestinal transplant surgeons at our institution,[16] we chose to test a large vessel technique whereby an aortocaval macrovascular patch is harvested as part of the uterine allograft. We previously proved the feasibility of this technique both in anatomical terms and surgical vascular dissection in a preserved human cadaver and freshly killed porcine and rabbit cadaveric models. The infrarenal aorta, inferior vena cava (IVC), common and internal iliac vessels, and the uterine arterial and venous tree together with the uterus en bloc were successfully harvested intact as the large vessel patch and graft. We concluded that the large vessel patch harvest may be used in uterine allograft transplants with an end-to-side anastomosis to the recipient's aorta/IVC.[17]

The macrovascular patch technique was used in our next set of experiments, a case series of six uterine allotransplantations on live rabbits.[18] The operation involved harvesting the uterine allograft with an aortocaval vascular patch en bloc in the donor. After 1 h of cold ischemic storage, the uterine allograft was transplanted to the recipient using an aortic–aortal cavo–caval end-to-side anastomosis. Tacrolimus was administrated for immunosuppressive therapy. All six rabbit recipients survived the immediate postoperative period but death occurred on days 1–4 post-transplant as a result of the following postoperative complications: limb paraplegia ($n = 3$), pulmonary emboli ($n = 2$) and intraperitoneal hemorrhage ($n = 1$). After post-mortem and histological analyses in the short term, all of the uteri appeared viable with no evidence of graft vessel thromboses suggesting that the macrovascular patch technique was technically successful.

This study enlarges on previous work with the aim to achieve long-term graft survival after applying the large vessel aortocaval vascular patch technique and to attempt fertility following natural mating. The animal model is the same: rabbit allo-UTn.

Methods

Rabbit model

Ten outbred New Zealand white rabbits were used as both donor and recipient animals (five donors, five recipients). They were all unrelated female rabbits, with the suppliers ensuring a complete major histocompatibility complex mismatch but a blood group match. Furthermore, they were of proven fertility with at least one previous litter each, weighing between 3.5 kg and 4.5 kg. Animals were acclimatized to their surroundings for at least 1 week before surgery to reduce stress.

Ethics approval

All work was conducted in accordance with the UK Animals (Scientific Procedures) Act (1986). It was also approved by The Royal Veterinary College Ethics Committee.

Surgical protocol

After fasting overnight, the donor rabbit was anesthetized using a combination of i.m. ketamine (35 mg/kg) and xylazine (7 mg/kg). After intubation, anesthesia was maintained with 1.5–2.0% isoflurane. The anterior abdominal wall was shaved and cleaned, and the abdomen was opened through a midline incision. The

UTn procedure was performed as previously described[18] but with a number of alterations. Namely, the rabbits' hind legs were supported with the use of mini-bean bags, heparin was administrated more frequently and the perfusion solution was run through under low flow pressure produced by gravitational effect.

The end result of the donor graft harvest was a simple hysterectomy transecting across the vagina and the most lateral aspects of the uterine horns together with an aortocaval macrovascular patch. The uterine allograft along with the aorta, inferior vena cava, common and internal iliacs, and uterine arterial and venous tree, all intact, were harvested en bloc. Following graft harvesting, the aorta was cannulated and the uterine specimen with its vascular patch was generously flushed *ex vivo* with heparinized normal saline (500 mL) and transplant storage solution, Celsior (500 mL). The total volume used was therefore 1 L.

The uterus was stored for a maximum of 2 h in the Celsior solution, between 2°C and 8°C, while the recipient female rabbit was being prepared. After fasting overnight, the recipient animal was anesthetized and prepared in a similar manner as the organ donor. A midline laparotomy was performed, and the loose connective tissue overlying the infrarenal aorta and vena cava were removed to expose this area of the major vessels. The vagina was anastomosed first to ensure proper orientation and positioning of the specimen followed by the aortocaval patch end-to-side vascular re-anastomosis to the recipient's aorta/inferior vena cava, ensuring vascular integrity, hemostasis and patency. The aortocaval patch anastomosis was carried out as follows. Soft tissue vascular clamps were first placed around the recipient's aorta. A longitudinal cut was made in the anterior wall of the aorta to create an opening. An end-to-side anastomosis was performed to the aorta of the graft using three to five interrupted 6–8 sutures (7-0 Prolene). A similar procedure was carried out for the cava–cava end-to-side anastomosis.

The uterine horns were finally rejoined. Successful reperfusion of the graft was judged to have occurred when the color of the uterus changed during reperfusion from its blanched appearance after flushing to a pink color. Blood flow past the venous and arterial anastomosis sites revealed no leakage.

Medication

Heparin (250 IU, s.c.) was administrated to the donor before organ procurement and to the recipient preoperatively to prevent thrombosis. Empirical immunosuppression was achieved using tacrolimus, initially with an i.v. intraoperative dose of 7 μg/h starting during the vascular patch anastomosis, followed by a p.o. dose of 500 μg twice daily postoperatively. These doses were based on work by Giessler *et al*.[19] Blood tacrolimus levels were not measured. Quinolone enrofloxacin (Baytril) was used as antibiotic prophylaxis intraoperatively at a dose of 5 mg/kg through a slow i.v. infusion.

Postoperative course

The rabbits were closely monitored by veterinary specialists for the first 24 h postoperatively within a specialist recovery enclosure at The Royal Veterinary College, London, with regular hemodynamic measurements taken. Heart rate and oxygen saturation (SaO_2) were then recorded daily in the first 2 weeks with twice weekly measurements taken subsequently. Rabbits were allowed to eat freely and were fed with autoclaved hay as well as with a proprietary pellet called GD99 that contains a coccidiostat.

The room temperature varied between 19°C and 21°C, with a relative humidity of 47–62% and a lighting schedule of 12 h on and 12 h off.

Pulse oximetry and perfusion index (PI) measurements

The use of pulse oximetry and PI measurements to assess uterine perfusion post-UTn was first developed during a series of trachelectomy operations to establish the exact number of vessels required to maintain uterine viability post-trachelectomy and has been described previously.[20,21] The oximeter (Datex-Ohmeda 3600P) measured the systemic arterial SaO_2 and PI through a modified finger probe[22,23] that allowed the jaws to fit around the uterine right and left cornua. As a result of the relatively short width of the rabbit cornua, the infrared light used in oximetry can easily pass through the structure. The finger probe was placed in a sterile endoscope bag which did not interfere with the measurements. To minimize interference, the theatre lights were also switched off. The probe was applied on four specific points: the proximal and distal aspects of the right and left cornua. Measurements (SaO_2 and PI) were taken just prior to harvesting of the graft and 5 min after commencement of uterine reperfusion post-UTn. The heart rate recorded on the uterine oximeter was compared to that of the anesthetic oximeter to ensure accuracy. Three attempts were made to obtain a reading from each site. An average

was taken of the measurements and that average was the final result reported in the table. If the oximeter failed to register a reading, this was recorded as 'zero'. All five recipients underwent the above procedure.

Mating process

Those rabbits that survived UTn surgery were introduced to males of proven fertility at 8 weeks post-transplant and, if there was no evidence of pregnancy, every 4 weeks subsequently until necropsy. Examinations were performed routinely for the first three mornings following the introduction of a male rabbit in order to visualize a vaginal semen plug and/or the presence of sperm in the vaginal lavage suggesting that mating had occurred. At 2–4-weekly intervals after mating, the recipient rabbits underwent an ultrasound examination to examine the uterus for pregnancy (Acuson 128 XP10 ultrasound scanner and a 10 MHz transducer).

Statistical analysis

Data on SaO_2 and PI was analyzed by Mann–Whitney *U*-test for non-parametric data.

Results

The mean weight of the donor rabbits was 3.9 kg (3.6–4.2 kg) and of the recipient rabbits 4.2 kg (3.5–4.5 kg). Operative data is shown in Table 1. The actual transplantations were completed with the donor graft, together with the macrovascular patch harvested and grafted en bloc. All anastomoses were completed successfully.

Pulse oximetry

Adequate uterine allograft reperfusion after UTn was confirmed both by visual clinical appearance and the assessment of SaO_2 and PI. The restoration of the graft color during reperfusion from white (as a result of *ex vivo* flushing with Celsior solution) to pink/red was confirmed for all five recipients. SaO_2 and PI were measured in the recipient, pre- and post-UTn. The averages and standard deviations for SaO_2 and PI are provided in Table 2. Comparison of SaO_2 values between pre-UTn recipient and post-UTn recipient revealed a statistically significant decrease in saturation levels post-UTn in the left and right medial cornua but not in the left or right distal cornua. Despite this, overall post-UTn SaO_2 measured between $83.6 \pm 6.3\%$ and $88.8 \pm 5.2\%$ (left and right cornua, respectively). Promisingly, a similar comparison of PI values demonstrated a statistically significant fall in PI level post-UTn in the right medial cornua only. This variation in SaO_2 and PI values is depicted in Figure 1.

Intra- and postoperative course

All five recipient models survived the surgery with satisfactory immediate postoperative recovery. Recipients 1, 2 and 4 died within the first 4 postoperative days. Necropsy of the first two recipients showed a substantial amount of fresh blood and clots in the peritoneal cavity, paracolic gutters and the pelvis. There was no evidence of any bleeding or leakage in the area of the macrovascular patch anastomosis and the findings therefore suggested death secondary to intra-abdominal hemorrhage most likely originating from the cornua. Recipient 4 was found to have a thrombus in the thoracic IVC, with cause of death therefore being a pulmonary embolus.

Necropsy revealed a healthy looking, well-perfused and clinically viable uterine graft in four recipients. No significant adhesions between the pelvic organs and

Table 1 Operative details from the five transplant procedures in the rabbit model

UTn	Graft retrieval (min)	1st warm ischemia (min)	Cold ischemia (min)	2nd warm ischemia (min)	Clamp on (IVC/AA) (min)	Reperfusion (min)	Recipient hysterectomy (min)	Grafting (min)	EBL (mL)
1	120	8	105	140	45/27	20	50	140	20
2	130	8	110	145	65/29	23	65	145	40
3	185	7	110	150	50/35	15	60	135	10
4	108	6	120	140	37/31	28	55	140	50
5	120	6	135	165	35/30	27	52	130	15
Mean	133	7	116	148	46/30	23	56	138	27

1st warm ischemia: a period of time during organ retrieval defined as the time between vascular clamping and the start of graft flushing with cold preservation solution. 2nd warm ischemia: a period of time occurring after cold ischemia, when the vascular anastomoses are established. It commences at the point at which the graft is removed from the preservation solution and ends when vascular anastomoses are established and perfusion is therefore recommenced. Cold ischemia: the time commencing at the start of graft flushing and ending at the point at which the graft is removed from the preservation solution to commence the grafting process. AA, abdominal aorta; EBL, estimated blood loss; IVC, inferior vena cava.

Table 2 Oxygen saturation and perfusion index values when measured with a pulse oximeter

Rabbit	Side	Cornua	SaO_2					Mean ± SD	Perfusion index					Mean ± SD
			UTn 1	UTn 2	UTn 3	UTn 4	UTn 5	All UTn	UTn 1	UTn 2	UTn 3	UTn 4	UTn 5	All UTn
Recipient (pre-UTn)	Right	Medial	98%	93%	90%	99%	99%	95.8 ± 4.1%	0.43	0.37	0.38	0.45	0.45	0.42 ± 0.04
		Lateral	90%	95%	97%	89%	92%	92.6 ± 3.4%	0.60	0.45	0.42	0.34	0.60	0.48 ± 0.11
	Left	Medial	100%	99%	95%	97%	98%	97.8 ± 1.9%	0.70	0.24	0.45	0.60	0.36	0.47 ± 0.18
		Lateral	100%	98%	98%	93%	78%	93.4 ± 9.0%	0.63	0.78	0.56	0.65	0.48	0.62 ± 0.11
Recipient (post-UTn)	Right	Medial	97%	89%	86%	83%	89%	88.8 ± 5.2%	0.36	0.14	0.12	0.30	0.22	0.23 ± 0.1
		Lateral	93%	81%	87%	79%	78%	83.6 ± 6.3%	0.49	0.25	0.27	0.56	0.30	0.37 ± 0.14
	Left	Medial	88%	93%	81%	88%	92%	88.4 ± 4.7%	0.39	0.34	0.33	0.44	0.20	0.34 ± 0.09
		Lateral	85%	85%	89%	82%	82%	84.6 ± 2.9%	0.65	0.43	0.32	0.48	0.40	0.46 ± 0.12

NA, not applicable; SD, standard deviation.

the bowel were detected. The aortocaval vascular patch anastomoses were intact. The two long-term survivors showed a lack of patency of the uterine cornua at the point of anastomosis, a reddish brown amorphous material at the site of the transplanted uterus in recipient 5 and a healthy, albeit small, uterus in recipient 3.

Two rabbits (3 and 5) survived long term (>1 month). They were eating and drinking normally and mobilizing 2 weeks after surgery. The laparotomy wound had healed by this time, and their weight had returned to preoperative level by 3 weeks after surgery. Normal patterns of behavior such as self-grooming had been established around the same time. There was no evidence of conception on ultrasound examination or in rabbit behavior despite numerous attempts at mating (as well as evidence) from the eighth postoperative week onwards. Recipient 3 underwent a planned necropsy in the ninth month, even though she was fit and healthy at the time, in order to ascertain the cause of failed conception. Recipient 5 developed diarrhea in the 11th postoperative month which worsened her condition and had to be euthanized as a result.

Histopathology

Histological analyses showed a viable transplanted uterine graft in recipients 1, 2 and 4, with no microscopic signs of necrosis or acute rejection. Graft vessels were patent throughout in all transplanted uteri as well as proximally and distally to the aortocaval vascular patch anastomosis. No macroscopic suggestion of any thrombosis of the anastomotic site of graft or other graft vessels in general was seen.

Discussion

Study findings

The macrovascular patch technique in this study as in our previous six allotransplants proved successful in enabling a feasible allo-UTn with respect to anatomy and vascular viability in the immediate postoperative period. In all five transplants, oxygen saturations and PI measurements taken after graft perfusion had been reestablished were not statistically different to the pre-transplant values. Prior to abdominal closure, the uterine graft appeared well perfused each time. Long-term rabbit survival was 40% ($n = 2$; recipients 3 and 5). Clearly, operating on a rabbit model continues to present us with a steep learning curve for the surgical vascular technique, which can only be surmounted with further attempts in the future. Furthermore, a reddish brown amorphous material was witnessed at

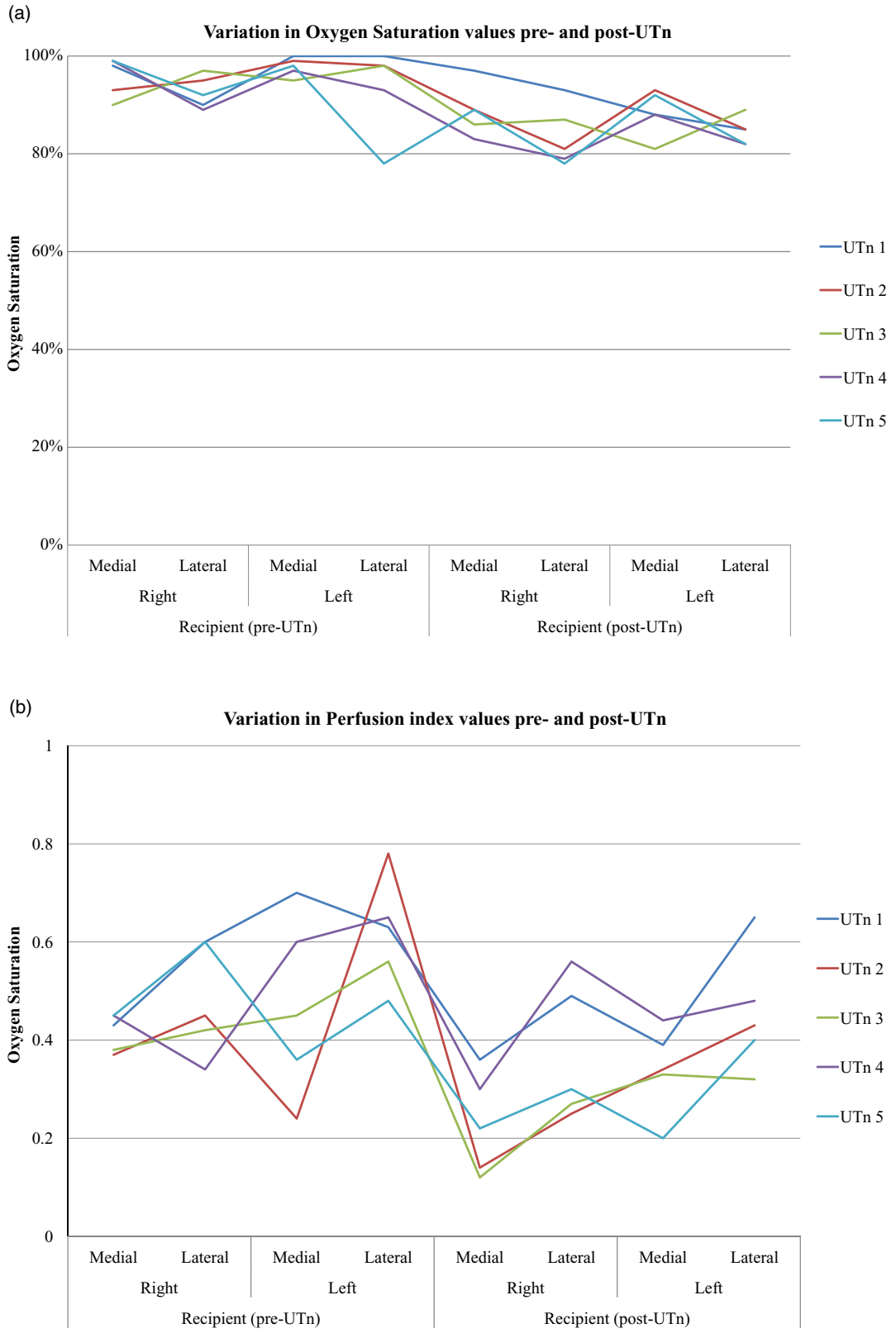

Figure 1 (a) Oxygen saturation values measured with a pulse oximeter. (b) Perfusion index values measured with a pulse oximeter.

the site of the transplanted uterus in recipient 3. Recipient 5 revealed a healthy, albeit small, uterus with a normal endometrium and myometrium. These appearances are most likely because of inadequate immunosuppression rather than hypoperfusion.

The two long-term surviving rabbits were mated every 4 weeks from the eighth postoperative week onwards. Pregnancy was not achieved despite evidence of mating, suggesting that the reason for infertility was secondary to an anatomical issue. This was most likely because of blocked cornua as a result of the native and donor cornua–cornua anastomoses. It is extremely unlikely that pregnancy would have been successful in recipient 3 even if the cornua were not blocked. Such an action would not allow the ova to reach the rabbit sperm in the cornua. *in vitro* fertilization techniques would help in solving this issue. By applying this method, one could be completely assured that the embryo has been formed and implanted in the correct place. There currently exists only one report of a pregnancy following a natural mating process in an animal model. The authors developed a rat model for orthotopic syngeneic UTn with the pregnancy rate, number of pups and growth trajectory of pups being similar to controls. Eleven rats out of 19 became pregnant, and from that group one rat delivered two healthy male pups.[24]

With respect to graft perfusion, the pulse oximeter was sensitive enough to detect a significant 8% reduction in SaO_2 when comparing the cornua pre- and post-UTn. Encouragingly, however, the absolute SaO_2 values were still 83–88%. This is a level perfectly adequate to maintain a viable uterus. Previous work by Moxey *et al*. has demonstrated that uterine perfusion when the ovarian, uterine and vaginal arteries are ligated together or individually is approximately 50–60%.[21] During an abdominal trachelectomy, only the ovarian arteries are spared. Therefore, a uterine perfusion of only 50% of the normal level is still capable of maintaining a healthy uterus, which can undergo menstrual cycles and subsequent pregnancies.

Furthermore, this also suggests that the surgical expertise necessary for adequate vessel anastomosis has improved in comparison to the previous cohort. The latter view is also supported by the fact that only the right medial cornua showed a significant change in PI level pre- and post-UTn. The difference in the pattern of SaO_2 and PI values pre- and post-UTn can be explained by two factors. First, the anastomosis appears to be patent in the immediate postoperative period which would explain the consistency in PI value. PI is an indicator of blood volume as explained previously. However, the delay in vessel vasodilatation secondary to both cold and warm ischemia as well as a minimal reperfusion time between re-anastomosis and pulse oximetry would have contributed to a reduction in SaO_2.

Published work review

This study focused on surgical techniques necessary to bring about long-term uterine graft survival. 'Long term' was defined as more than 1 month. Survival for this length of time can only be ensured with adequate vascular anastomotic technique and uterine structural support. Only by adhering to this can one bring about the correct uterine blood supply, in both pregnant and non-pregnant animals. Since the first human case in 2000,[8] a number of different animals have been used in attempts to characterize the optimal surgical method which would provide the uterine graft with a satisfactory blood supply. Auto- and/or syngeneic-UTn animal models are commonly used as they rather helpfully separate the damage caused by surgical trauma, ischemic–reperfusion injury and thrombosis, from immunological rejection by excluding the latter. We described our attempts to use a microvascular anastomotic method in a porcine model in the introduction. Wranning *et al*. used the same anastomotic technique (end-to-end anastomosis of the uterine artery and veins distal to their branching from the internal iliac vessels) to evaluate the early reperfusion events after short-term cold ischemia in an auto-UTn porcine model.[25] The authors demonstrated that acceptable reperfusion can be achieved with seven out of 19 autotransplanted pigs considered well flushed. Of these seven, four showed satisfactory reperfusion judged by change in gross appearance and presence of appropriate venous blood flow. Avison *et al*. performed 10 heterotopic and allogeneic UTn in genetically defined mini-pigs.[26] Tacrolimus was administrated i.v. for the first 12 days post-UTn followed by oral cyclosporin maintenance immunosuppression. At the end of the follow-up period, five animals were alive and healthy at 0.5–12 months post-transplantation. The anastomotic technique was the same macrovascular patch technique used by our team in both the previous[18] and present studies. The infrarenal donor aorta and IVC of the graft were anastomosed end-to-side with the recipient aorta and IVC.

A sheep model is another large animal model which can be used to 'mimic' a human pelvis. Ramirez *et al*. transplanted a uterus into 10 sexually matured sheep,

with bilateral end-to-end anastomosis of the uterine vessels. At 6 months post-UTn, hysterectomies were performed to reveal viable uterine tissue and uterine vessel re-anastomosis as well as vascular patency in six out of the 10 grafts.[9] An alternative method involved the anastomosis of the anterior division of the internal iliac artery and the utero-ovarian vein end-to-side to the external iliacs. In one study, reperfusion of blood was observed in five out of seven auto-transplanted uteri.[27] Wranning *et al*. studied the effects of cold ischemia and reperfusion after auto-UTn in a sheep model.[13] Uterine grafts were immediately re-perfused in 70% of all cases. The same team achieved pregnancy following auto-UTn in three of out of four mated animals.[4]

Study strengths and limitations

Although this case series of allo-UTn described only five cases, it highlighted a few challenges with the choice of animal model and the complexity of the surgical procedure involved. The macrovascular patch technique involves aorto–aorta and veno–vena anastomoses. This technique would not be attempted in a human because of its highly risky strategy involving the most important blood vessels. Damage to them could lead to catastrophic outcomes. Anastomosis at a lower level (common iliac, internal iliac or uterine arteries) would not be possible in a small animal model such as a rabbit as the diameter of these vessels is too small to allow for satisfactory anastomosis. Therefore, the macrovascular patch technique allows us to bypass this problem, and thus provide an adequate blood supply to the womb: the most important prerequisite to long-term graft survival and subsequent pregnancy. In a human, both live and brain-stem dead heart-beating donation, internal iliac or even uterine artery anastomosis would be possible, however, as the diameter of these vessels is large enough for precise microsurgery. In particular, anastomosis would be either internal iliac to internal iliac or internal iliac to external iliac vessels. Uterine artery anastomosis may indeed be feasible but our porcine data as well as the first human UTn attempt make this approach less desirable.

Tacrolimus was the only immunosuppressant used. It was administrated i.v. intraoperatively and as a daily p.o. dose postoperatively. This study did not endeavor to characterize the allorejection response of the recipient rabbit by assessing any of the uterine grafts for signs of tissue rejection or blood samples for markers of rejection. There was no gross or microscopic evidence of tissue rejection observed on analysis of allografts 1–4, which was a reassuring feature. However, allograft 5 was of an amorphic and disintegrated appearance, which could have been brought about by inadequate immunosuppression. It is difficult to make this judgment as there was only one other long-term survivor. In addition, using an i.v. infusion pump may be a more efficient way of administrating tacrolimus in preference to the p.o. route, especially for the first few postoperative weeks.

Our methodology uses a macrovascular patch technique to bring about adequate blood supply to the donor uterine graft. We have applied it with our previous six allotransplantations[18] and with a further five allotransplants described in this study. This technique has proved satisfactory in the immediate, early and late postoperative periods with respect to attaining adequate graft perfusion. However, as only two rabbits survived long term, it has highlighted a steep learning curve in both anatomical terms and surgical vascular anastomotic achievability. We propose that embryo transfer techniques are applied to the next set of rabbit allo-UTn in order to bring about pregnancy.

Disclosure

No authors have any financial, personal or professional competing interests to disclose.

References

1. Keith Louis G, Del Priore G. Uterine transplantation in humans: A new frontier. *Int J Gynecol Obstet* 2002; **76**: 243–244.
2. Brannstrom M, Wranning CA, Altchek A. Experimental uterus transplantation. *Human Reprod Update* 2010; **16**: 329–345.
3. Racho El-Akouri R, Wranning CA, Molne J, Kurlberg G, Brannstrom M. Pregnancy in transplanted mouse uterus after long-term cold ischaemic preservation. *Hum Reprod* 2003a; **18**: 2024–2030.
4. Wranning CA, Marcickiewicz J, Enskog A, Dahm-Kahler P, Brannstrom M. Fertility after autologous ovine-tubal-ovarian transplantation by vascular anastomosis to the external iliac vessels. *Hum Reprod* 2010; **25**: 1973–1979.
5. Diaz-Garcia C, Akhi SN, Wallin A, Pellicer A, Brannstrom M. First report on fertility after allogeneic uterus transplantation. *Acta Obstetricia et Gynecologia* 2010; **89**: 1491–1494.
6. Ramirez ER, Ramirez Nessetti DK, Nessetti MB *et al*. Pregnancy and outcome of uterine allotransplantation and assisted reproduction in sheep. *J Minim Invasive Gynecol* 2011; **18**: 238–245.
7. Scott JR, Pitkin RM, Yannone ME. Transplantation of the primate uterus. *Surg Gynecol Obstet* 1971; **133**: 414–418.

8. Fageeh W, Raffa H, Jabbad H, Marzouki A. Transplantation of the human uterus. *Int J Gynecol Obstet* 2002; **76**: 245–251.
9. Ramirez ER, Ramirez DK, Pillari VT, Vasquez H, Ramirez HA. Modified uterine transplant procedure in the sheep model. *J Minim Invasive Gynecol* 2008; **15**: 311–314.
10. Wranning CA, Dahm-Kahler P, Molne J, Nilsson UA, Enskog A, Brannstrom M. Transplantation of the uterus in the sheep: Oxidative stress and reperfusion injury after short-time cold storage. *Fertil Steril* 2008; **90**: 817–826.
11. Lee S, Mao L, Wang Y *et al*. Transplantation of reproductive organs. *Microsurgery* 1995; **16**: 191–198.
12. Jiga LP, Lupu CM, Zoica BS, Ionac M. Experimental model of heterotopic uterus transplantation in the laboratory rat. *Microsurgery* 2003; **23**: 246–250.
13. Wranning CA, Akhi SN, Kurlberg G, Brännström M. Uterus transplantation in the rat: Model development, surgical learning and morphological evaluation of healing. *Acta Obstet Gynecol Scand* 2008; **87**: 1239–1247.
14. Racho El-Akouri R, Kurlberg G, Dindelegan G, Molne J, Wallin A, Brannstrom M. Heterotopic uterine transplantation by vascular anastomosis in the mouse. *J Endocrinol* 2002; **74**: 157–166.
15. Sieunarine K, Zakaria FB, Boyle DC *et al*. Possibilities for fertility restoration: A new surgical technique. *Int Surg* 2005; **90**: 249–256.
16. Hakim NS, Papalois VE. Small bowel transplantation. *Int Surg* 1999; **84**: 313–317.
17. Sieunarine K, Hakim N, Corless DJ *et al*. Is it feasible to use a large vessel patch with a uterine allograft en bloc for uterine transplantation? *Int Surg* 2005b; **90**: 257–261.
18. Sieunarine K, Doumplis D, Kuzmin E *et al*. Uterine allotransplantation in the rabbit model using a macrovascular patch technique. *Int Surg* 2008; **93**: 288–294.
19. Giessler GA, Gades NM, Friedrich PF, Bishop AT. Severe tacrolimus toxicity in rabbits. *Exp Clin Transplant* 2007; **5**: 590–595.
20. Sieunarine K, Moxey P, Boyle DC *et al*. Selective vessel ligation in the pelvis: An invaluable tool in certain surgical procedures. *Int J Gynecol Cancer* 2005c; **15**: 967–973.
21. Moxey P, Sieunarine K, Cox J, Lawson AD, Ungar L, Smith JR. Pulse Oximetry and perfusion index measurement to assess uterine perfusion and viability. *Int Surg* 2006; **91**: 223–227.
22. Grace RF. Pulse oximetry. Gold standard or false sense of security? *Med J Aust* 1994; **160**: 638–644.
23. Middleton PM, Henry JA. Pulse oximetry: Evolution and directions. *Int J Clin Pract* 2000; **54**: 438–444.
24. Wranning CA, Akhi SN, Diaz-Garcia C, Brännström M. Pregnancy after syngeneic uterus transplantation and spontaneous mating in the rat. *Hum Reprod* 2011; **26**: 553–558.
25. Wranning CA, El-Akouri R, Lundmark C *et al*. Autotransplantation of the uterus in the domestic pig (sus scrofa): Surgical technique and early reperfusion events. *J Obstet Gynaecol Res* 2006; **32**: 358–367.
26. Avison DL, DeFaria W, Tryphonopoulos P *et al*. Heterotopic uterus transplantation in a swine model. *Transplantation* 2009; **88**: 465–469.
27. Dahm-Kahler P, Wranning C, Lundmark C *et al*. Transplantation of the uterus in the sheep: Methodology and early reperfusion events. *J Obstet Gynaecol Res* 2008; **34**: 784–793.