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A Case of Pregnancy following Allogeneic Uterine Transplantation in a Rabbit Model

Pregnancy following allogeneic rabbit UTn

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Abstract

Objective To assess whether fertility was possible following allogeneic uterine transplantation (UTn), whereby the recipient had demonstrated long-term survival and had been administered immunosuppression.

Design Case series.

Setting Royal Veterinary College, London, UK and Institute of Science and Animal Technology, Valencia, Spain.

Population 18 New Zealand white allogeneic rabbit does of proven fertility were used as donor and recipient animals (nine donors, nine recipients).

Methods Nine allogeneic UTn in the rabbit model were performed using a pre-determined protocol. Embryos were transferred into each cornua via a mini-midline laparotomy. The pregnancy was monitored with regular reproductive profiles and a colour Doppler ultrasound to measure fetal growth.

Main Outcome Measures Presence of conceptus, size and growth of fetus, markers of acute rejection, biochemical reproductive, renal and hepatic profiles.

Results Recipient #5 was the sole surviving doe out of the cohort of nine. Day 5 post-embryo transfer (D5) scan did not demonstrate a fetal sac. Fetal sac diameter on D9, D13, D16 and D18 was (mm): 17.3x13.1, 22.1x19.5, 25.2x22.7, and 10.2x6.3. Crown-rump length was only recordable on D9, D13 and D16. Subsequent scans on D22 and D25 did not demonstrate a fetal sac, which together with the fetal sac diameter was suggestive of resorption. The recipient was sacrificed on D27. Necropsy and histopathology confirmed evidence of a gravid uterus and presence of a gestational sac.

Conclusions Despite the end result of the experiment i.e. termination of pregnancy as a result of miscarriage, the study represents only the third example of conception and pregnancy following an allogeneic UTn.

Key Words Fertility, Uterine Transplantation, Rabbit, Pregnancy

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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 Mullerian duct anomalies, or acquired (for example, a hysterectomy performed secondary to obstetric haemorrhage, 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, minimisation of ischaemic-reperfusion and allorejection-related injury, optimisation of surgical techniques to allow for an adequate uterine blood supply, and finally achieving the 'Holy Grail' of UTn: pregnancy and a live birth of a healthy neonate following allogeneic UTn (**Figure 1**).[2]

The Brannstrom group has described five examples (three murine and two rat models) of successful pregnancy post-UTn. The first example in 2002 consisted of a syngeneic transplantation of the right uterine horn and cervix, with the native uterus remaining in situ and subsequent successful fertility following blastocyst transfer and implantation in a single mouse model. The mouse was 8 weeks old, with the embryo transfer occurring one week post-UTn. This proved, for the first time, the concept of pregnancy following a uterine transplant in a non-autotransplant model. The aim of the authors was to assess the macrovascular anastomotic model previously described; the pregnancy experiment was a secondary aim.[3]

The second example of pregnancy post-UTn was in fact a pregnancy-related study.[4] It was an extension of the above study with the focus on pregnancy-related factors. Transplantation of one uterine horn was performed in a syngeneic mouse model that used a macrovascular anastomosis concept to ensure uterine perfusion. Embryos were transferred 1-3 weeks post-UTn in both the transplanted and native uteri. The embryo preparation was as per standard mouse protocols.[5]19 mice

received a transplanted uterus. Out of those, 12 had viable grafts and thus received an embryo. Pregnancies were found in nine of 12 native uteri of transplanted mice and in those nine, eight also had fetuses within the transplanted uterus. With respect to the controls, eight out of 13 mice were pregnant. The lengths and weights of the fetuses, as well as weights of the placentae, were similar in pregnancies in native, grafted and the 'control' uteri. The placental aspect should be highlighted as the data may be extrapolated to the human model. In this experiment, UTn did not compromise placental development. Offspring from all three groups followed a similar post-natal growth pattern until eight weeks of age. They were then tested to see if they could continue the progeny. Both female and male offspring proved to be fertile and the pups reported birth weights within the normal range. Therefore, the experiments demonstrated similar implantation and pregnancy rates, as well as birthweight and growth curves (8 weeks) between the native and transplanted (syngeneic) uteri. The second-generation offspring from transplanted animals were all fertile with normal birth weights.[4]

However, the above murine experiments still differ somewhat from a human model. Three areas in particular are immediately obvious: gestational lengths, no immunological rejection mechanisms to consider and anatomical differences. The following experiments tried to counter some of these differences. In October of 2010, the Brannstrom group described the first recorded pregnancy in an allogeneic uterine transplant (rat model). It is therefore a central proof of a concept that a uterus can function following its transplantation from a donor to a recipient and that it can do so under immunosuppression.[6]

Their next experiment was also an advancement on the existing work - again a rat model, but with an attempt to bring about pregnancy following natural mating.[7] Furthermore, the actual transplant was orthotopic for the first time i.e. the native uterus was removed and the graft was placed *in lieu*. Female Lewis rats underwent hysterectomy and received syngeneic uterine transplants (with one horn removed). The uterine graft was placed in an orthotopic position i.e. in the pelvis with two tissue anastomoses: to the vagina and the upper part of the native uterine horn. The anastomosis was end-to-side between the common iliac vessels of the recipient and the graft. Control rats had only one uterine

horn removed. The overall pregnancy rates after introduction to male rats were comparable in UTn (11/19; 58%) and control (12/19; 63%) rats. From the UTn rats, only one rat delivered two healthy male pups. Two other UTn rats gave birth to at least two pups each but committed infanticide prior to the offspring count. The weight and development outcomes post-birth for the two surviving male rat pups from the one UTn rat were similar to the control rat offspring (32 live born pups). The main differences between the UTn and control groups were the increased number of resorptions and decreased number of successful deliveries in the former group. Thus, some unknown factor is compromising fetal wellbeing post-UTn, most likely blood flow or denervation. In the control group, no nerve manipulation occurred and there were no issues with labour. Also vaginal scarring at the anastomosis may cause a synthetic obstruction to the labour pathway. Obviously in future models, both animal and human, elective Caesarean section would remove these obstacles. In conclusion, this model may be reproduced to assess fertility outcomes following allogeneic UTn and ensuing natural mating. The study demonstrates that conception by natural mating, and subsequent pregnancy, and birth of live and healthy offspring is possible with orthotopic, syngeneic UTn.[7]

Transplant Immunology and Pregnancy

Today, immunosuppressants are routinely used after transplantation to minimize the risk of rejection. However, long-term use has its drawbacks, mainly the risk of recurrent infections and neoplastic disease, as well as necessary close and meticulous medical care.[8] UTn would be short term i.e. 2-4 years thus avoiding this risk. Once the uterus and immunosuppressants are removed, any associated neoplasm risk should theoretically return to baseline. Many transplant related lymphomas are known to regress after removal of the graft.

Nevertheless, care is taken with the type, dose and monitoring of immunosuppressants prescribed as they can cross the human placental barrier and enter the fetal circulation, possibly affecting the immune system of the fetus.[9] Medications used to suppress immunologic activity are summarised in **Table 1**. *McKay and Josephson* have summarised the outcomes of approximately 14000 post-

transplantation live births since the first birth to a transplant recipient occurred in 1963.[10] Therefore, the stratification of immunosuppressants is necessary as observations on post-transplant pregnancies indicate a higher incidence of preterm deliveries, low birth weights, and maternal hypertension but no increased rate of structural malformations.[11-14]

Nevertheless, we must acknowledge that long-term data on allograft loss, maternal survival after pregnancy (due to an increased risk of viral diseases and neoplasm secondary to immunosuppressants)[15] and offspring outcomes of transplant recipients is rather limited. Despite several reports indicating no increase of congenital anomalies among the offspring of recipients,[14,16] long-term outcomes focusing on physical and mental development, and immunological and oncological pathologies later in life in these offspring is necessary.[17] Unfortunately, currently, only one study has compared pregnancy outcomes in a single group of women before and after transplantation. It concluded that despite an initial increased risk for pre-eclampsia, growth restriction, pre-term birth, and the risk of miscarriage, the odds of these four outcomes were all statistically similar pre- and post-transplantation.[18]

Aims

The aim in this study was to assess whether fertility (conception, pregnancy and fetal well-being) was possible following orthotopic, allogeneic UTn, whereby the recipient had demonstrated long-term survival and had been administered immunosuppression.

Materials and Methods

The feasibility of the uterine allograft dissection together with its vascular supply that includes the internal and common iliacs, the abdominal aorta (AA) and the inferior vena cava (IVC) was first ensured by a series of uterine transplantations in the rabbit model performed by this team.[19] Here, we

performed two surgically explorative procedures and nine allogeneic uterine cross transplantations in the rabbit model using a pre-determined protocol.

18 New Zealand white rabbits were used as donor and recipient animals (nine donors, nine recipients). They were all allogeneic does of proven fertility with at least one previous litter each. The animals were acquired a few days before the operation to ensure appropriate acclimatization to their surroundings.

This particular surgical procedure has already been described in detail.[19,20] The uterine allograft along with the AA, IVC, common and internal iliacs, and uterine arterial and venous tree all intact were retrieved *en bloc*.

Rabbit embryo preparation

The protocol was prepared by the rabbit fertility team of the Polytechnic Institute, Valencia, Spain, led by Professor Jose Vicente. Sixty hours before insemination time, superovulation was induced in nine donor does following subcutaneous injection of 20IU/kg of eCG (Gonaser, Hipra, S.A.). Ejaculates from five proven males were collected using an artificial vagina. A 10µL aliquot samples from ejaculates were diluted 1:10 with Tris-citrate-glucose extender (250mM tris-hydroxymethylaminomethane, 83mM citric acid, 50mM glucose, pH 6.8-7.0, 300 mOsmkg-1) for a previous motility rate evaluation. Ejaculates with an estimated motility higher than 70% were pooled. From ejaculates, two aliquot sample of 10µL were taken, the first one was diluted 1:10 with Tris-citrate-glucose extender for motility rate evaluation in a computer assisted sperm analysis (CASA) system and the second one was diluted 1:10 with 1% of glutaraldehyde solution in phosphate buffered saline to calculate the concentration in a Thoma chamber and to evaluate both the percentages of normal intact acrosome and abnormal sperm by phase contrast at a magnification of x400. Only ejaculates with more than

70% of motility rate, 85% of normal intact acrosome, and less than 15% of abnormal sperm were pooled and used to inseminate donor does. Pooled semen was diluted to 40 million per milliliter adding Tris-citrate-glucose extender in order to elaborate the seminal doses. Donors were inseminated with a seminal dose of 0.5 ml..

Ovulation was induced in the donor does immediately after insemination by an intramuscular injection of 2µg of Buserelin Acetate (Hoechst, S.A.). The embryos were recovered *in vivo* by ventral midline laparoscopy 76-78 hours after the insemination, according to the method described by *Besenfelder et al.*[22] First, anaesthesia was induced by an intramuscular injection of 16mg xylacine (0.8ml of Rompun; Bayer AG, Leverkusen, Germany), followed by an intravenous reinjection of ketamine chlorohydrate (Imalgene; Merial, S.A., Lyon, France) to maintain does under anaesthesia during laparoscopy. Rabbit donor does were placed in a head-down position at a 45ºC angle. A Verres needle was inserted in the lower right abdominal quadrant and attached to CO_2 automatic insufflator. After CO_2 abdominal distension, a trocar-cannula unit of 5mm diameter was inserted into the abdominal cavity at 10cm caudal to the sternum. Another accessory (3mm diameter) trocar-cannula unit was inserted 4-5cm laterally to right of the former. After both were removed, they were respectively replaced by the laparoscope (Wolf paediatric 0º) and grasping forceps (length: 28.5cm). Subsequently, an epidural needle (inner diameter: 1mm, Vigor Epidural G17) was inserted near the ovary and a sterile polyethylene tube (inner diameter: 0.3 mm) attached to a 5ml sterile syringe was introduced for 2-3cm through the epidural needle into the oviduct. After fixation with the grasping forceps, ova and embryos were flushed from the oviducts to uterine horns with 5ml of Dulbecco´s phosphate buffered saline (DPBS, Sigma) containing 0.2 % (w/v) of Bovine Serum Albumin (BSA, Sigma) and supplemented with antibiotics (200 IU/mL penicillin G and 0.25mg/mL dihydrostreptomycin; Penivet 1). This was followed by flushing of the uterine horns, elevated with the grasping forceps placed near the oviduct-tube junction, with 50ml of the same flushing solution. It was achieved via insertion of a sterile epidural needle directly through abdominal wall into each uterine horn. Finally, the recovery medium was aspirated from the vagina by a Foley Catheter (Minitube, Tiefenbach, Germany)

connected with a vacuum pump of 20-40mmHg. After the recovery of does, the reproductive tract was washed with 0.1% ethylenediaminetetraacetic acid solution in PBS in order to prevent any abdominal adhesions post-laparoscopy.

In both groups, recovered embryos were scored by morphological criteria. Only embryos in the morula stage with homogeneous blastomeres as well as a visible regular mucin coat and zona pellucida were catalogued as normal embryos.

Vitrification and thawing process

All normal embryos (morulae) were vitrified by the methodology described by *Vicente et al.*[193] Briefly, the vitrification procedure was carried out in two steps at 20ºC. In the first step, embryos were placed for 2 minutes in a vitrification solution consisting of 12.5% (v/v) dimethyl sulphoxide (DMSO, Sigma) and 12.5% (v/v) ethylene glycol (EG, Sigma) in DPBS supplemented with 0.2% (w/v) of BSA. In the second step, embryos were suspended for 1 minute in a solution of 20% (v/v) DMSO and 20% (v/v) EG in DPBS supplemented with 0.2% (w/v) of BSA. Then, embryos suspended in vitrification medium were loaded into 0.125ml plastic ministraws (IMV, L´Aigle, France) between two drops of DPBS separated by air bubbles. Finally, the straws were sealed and plunged directly into liquid nitrogen. De-vitrification was performed in two steps, first ministraws were placed to 10 cm from vapour nitrogen until vitrified fraction begin to cristalize (20-30 sec) and, then, submerged into a water bath at 20 °C for 10 sec. To remove the vitrification, warmed embryos were introduced into a culture dish containing 0.33 M sucrose in DPBS supplemented with 0.2% BSA, after 5 min embryos were washed in DPBS for another 5 minutes. Thereafter, 30 de-vitrified embryos were cultured for 48 hours in Medium 199+20% FBS (Sigma) at 38.5ºC, 5% CO_2 and saturated humidity to asses post-vitrification viability. Twenty-four of them reached to state of hatching blastocyst (80%).

Embryo transfer

The recipient doe was injected intramuscularly with 25IU of eCG 60 hours prior to induce the ovulation with an intramuscular injection of 2μg of Buserelin Acetate (Hoechst, S.A.). The transfer was performed by mini-midline laparotomy, using previous scar to enter the abdomen. The uterus was externalised to allow for ease of access. Seventeen intact de-vitrified embryos were placed in cornua using an epidural catheter (G17, Baxter, Deerfield, Illinois, USA).

Pregnancy monitoring

Experiments were carried out in the first four weeks following the above ET. 3ml of blood was taken at appropriate intervals during the gestational period to obtain a reproductive profile (βhCG, FSH, LH, Testosterone, 17β-Oestradiol and Progesterone). Furthermore, a colour Doppler ultrasound with a two-dimensional 7.5MHz (range: 7-10MHz) probe was used to monitor fetal development and growth. The protocol has been described already by *Chavatte-Palmer et al*.[23] The ultrasound examination was performed from right to left with the probe in the sagittal orientation, following localisation of the urinary bladder. The initial scan was performed on Day 5 post-ET with the aim to judge whether any fetal sacks were present. Subsequent scans are done at 3-4 day intervals until the day of the elective Caesarean section. With respect to measurements, for the fetal sack, they are made when the largest surface area appears on the screen. Important for growth, crown-rump length (CRL) is determined as the maximum distance from crown to tail basis with the fetus on a sagittal plane.[23]

Results

Recipient #5 was the sole long-term (>30 days) surviving doe out of the cohort of nine. The doe showed no adverse effects post-operatively and throughout the recovery period. She was feeding and drinking well, with appropriate movement and affect. Following visualization of certain signs

suggesting a receptive doe, embryo transfer was performed on day 89 post-UTn. Vitrified embryos were prepared at the same time, with 17 transferred into the cornua of the sole surviving doe by open laparotomy and insertion of catheter transmurally (nine into the right and eight in the left cornua). The doe began to demonstrate signs of pregnancy, including nesting and an increase in abdominal size towards the end of the first gestational week.

D5 scan did not demonstrate a fetal sac. Fetal sac diameter on D9, D13, D16 and D18 was (mm): 17.3x13.1, 22.1x19.5, 25.2x22.7, and 10.2x6.3 (**Figure 2**). CRL was only recordable on D9, D13 and D16 (mm): 10.2x6.3, 14.3x9.5, and 16.4x15.3 respectively. Subsequent scans on D22 and D25 did not demonstrate a fetal sac, which together with the fetal sac diameter was suggestive of resorption. No bleeding was witnessed around the cage. The doe's behaviour was noticed to have returned to pre-embryo transfer period four days before she was culled. The recipient was sacrificed on D27. Necropsy and histopathology confirmed evidence of a gravid uterus and the presence of a gestational sac (**Figures 3 and 4**).

Unfortunately as a result of resistance from the doe, only one blood sample was possible during the gestational period. D102 was D13 of the gestational period (**Figure 5**). **Table 2** demonstrates renal, hepatic and reproductive profiles from D0 to D102.

Discussion

Main Findings

Our pregnancy did not result in the birth of a healthy term offspring. However the demonstration of pregnancy in this allogeneic model is a central proof of concept of UTn in a rabbit model and thereby an essential step in the research toward its clinical application in the human. The study represents only the third example of conception and pregnacy following an allogeneic UTn. To date, there has only

been one live birth following allogeneic UTn. The animal model was large (sheep), and thus, no live births have been achieved in a small animal model.

Interpretation

The macrovascular patch model functioned in that the uterine graft received an adequate blood supply, allowing for implantation and fetal growth. The renal, hepatic and reproductive profiles were normal throughout the post-operative period, during which time the recipient doe was taking daily tacrolimus. Its daily routine was also normal, with no adverse characteristics demonstrated with respect to behaviour, eating and drinking patterns, movement and fertility induction.

Embryo transfer techniques should be used to transport the already formed embryos into the uterine graft after transplantation in order to exclude any events which might compromise the graft. For example, it is likely that natural conception would not occur as the fallopian tubes would not be functional and if it did occur, it could lead to intra-/extra-uterine ectopic and tubal miscarriages. Some, including this team, propose that the Fallopian tubes should not be transplanted as part of an UTn.[2,6]

One of the key issues concerning UTn is finding a suitable immunosuppressive protocol which achieves graft protection from the body's rejection processes but also does not harm the embryo of the fetus. This resorbed pregnancy may therefore be explained by either the potential negative impact of tacrolimus on early fetal growth, or by a clinical rejected uterus because of inadequate immunosuppression. With regards to the former point, tacrolimus was selected as it is now widely used in clinical organ transplantation. It has lower nephrotoxicity, with reports from large studies stating fewer episodes of acute rejection compared with the traditional calcineurin inhibitor CsA.[24] Its mode of action suppresses T-lymphocyte activation via the inhibition of calcineurin-dependent interleukin IL-2 production.[25] Importantly it can be used after initial induction therapy as a single immunosuppressant in liver transplantation,[26] and tacrolimus has been used extensively during pregnancies in organ transplant recipients (a significant consideration for UTn).[27,28] The latest study

on the effects of tacrolimus on UTn, evaluated the effects of tacrolimus on the rejection of a transplanted rat uterus and on uterine expression of markers of inflammation and implantation.[28] Non-treated uterine grafts showed rejection with necrosis. Tacrolimus-treated transplanted group exhibited normal uterine morphology with low numbers of T-lymphocytes in all uteri except in two out of seven uteri of the tacrolimus-treated transplant group. Uteri of the non-treated transplanted group showed elevated mRNA expression of IL-1a and IP-10 and reduced galectin-1, compared with the tacrolimus-treated transplanted group. Therefore, tacrolimus monotherapy suppressed rejection of an allotransplanted uterus, normalized the expression of IL-1a and IP-10 and prevented T-lymphocyte infiltration. The tacrolimus in our experiment did overall decrease the rejection response (**Figure 5**). However, three rejection episodes did occur, with the ultimate episode occurring during pregnancy. Despite managing to control the first two (occurring on days 36 and 74), some uterine structural damage must have occurred, thus rendering the graft less suitable for carrying a pregnancy until term. The third episode on day 102 could have been the final insult which led to miscarriage, most probably occurring three days later (**Figure 2**). It is therefore unlikely that it was the effects of tacrolimus which led to intra-uterine death but rather that the rejection response itself was inadequate to protect the graft carrying the pregnancy. This could be either because the dose of tacrolimus was inadequate or that an additional immunosuppressant, in the form of a monoclonal antibody, should be added. Further such experiments are required but should be carried out in a larger-animal model, because of difficulties of using a rabbit model.

Advances in immunosuppression as well as original modalities to induce tolerance of a transplanted uterus may reduce these problems in the future. The induction of immunological tolerance to a transplanted graft offers a novel potential solution to inhibiting immune-mediated graft rejection. Tolerance is defined as the specific and permanent absence of an attack or response by the immune system to a foreign antigen, which in our case is represented by the uterine graft antigen, without immunosuppression.[29] It can be classified as either natural which occurs via either a central (in the thymus and bone marrow) or peripheral (circulating mature T and B cells) pathway, or acquired. The ultimate clinical goal would be for the patient to ‘acquire’ tolerance, both in the non-pregnant and

pregnant state, as natural tolerance cannot deal with foreign antigens. This would mean an almost complete allo-unresponsiveness to donor cells and therefore a diminished use of immunosuppressants and negligible damage to the uterine graft as a result of both the pre-clinical and clinical rejection process. Thus, it is important to differentiate between the role of immunosuppressants and tolerance modulation in the graft acceptance process. The level of an immunosuppressant administered to a patient can indeed portray the state of clinical tolerance, yet the drug does not provide tolerance induction in general. In certain cases, immunosuppressants can diminish the overall tolerance effect, for example, calcineurin inhibitors which downgrade the action of all T cells, including regulatory T cells (Tregs).[30]

The field of immunomodulation continues to develop, and could lead to the ultimate goal, a post-transplant world involving no regular immunosuppression medication. Aside from Tregs, immunosuppression may also be induced after a single treatment course. For example, T10B9, a monoclonal antibody directed against the alpha-beta heterodimer of the T-lymphocyte receptor complex, has been used successfully to treat acute cellular rejection in renal transplantation and as an immunosuppression induction agent in heart and simultaneous kidney-pancreas transplantation.[31] *Kawai et al* described combined bone marrow and kidney transplants from HLA single-haplotype mismatched living, related donors resulting in induced immunotolerance.[32]

Unfortunately, pregnant transplant recipients cannot wait for long term results to clearly demonstrate superiority of one regimen over the other. This is especially a problem regarding pregnancy outcomes among transplant patients. Regardless, if the pregnant transplant recipient has a kidney, heart, lung or uterus, it will be impossible to ensure safety to mother and fetus if the goal is a healthy child that matures to a healthy adult.

We cautiously predict that from the immunological point of view, the risks to mother and fetus will be no different to those faced by renal, hepatic or cardiac transplant patients undergoing pregnancy. The patient must be considered as high risk from the beginning of the pregnancy. She should be managed

under a multi-disciplinary team, involving in particular a physician specializing in high-risk obstetrics and a transplant immunologist. The care pathway would be very similar to any pregnant patient with a transplanted organ. Because of the effects of immunosuppressants on the maternal immune system, viral serology for cytomegalovirus as well as microbiological cultures of vaginal smears should be repeated monthly. Levels of immunosuppressive drugs in the blood should be monitored, thus permitting adjustments of drug dose relative to graft function and physiological changes of pregnancy. Doppler assessment of the vascular uterine supply as well as anastomosis may be helpful if not interesting. Visual inspection of the transplanted cervix would likely provide clinical clues of the graft's condition. Caesarean Section would be the preferred mode of delivery.[33]

Conclusion

Despite the end result of the experiment i.e. termination of pregnancy as a result of miscarriage, the study represents only the third example of conception and pregnancy following an allogeneic UTn. The surgical anatomical macrovascular model was successful. Post-operative recovery was uneventful, with no adverse effects recorded. The cause of fetal demise is most likely secondary to inadequate prevention of the immunological rejection response.

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Contribution to authorship *S Saso* was responsible for the original manuscript design, drafting and revision for important intellectual content. *S Ghaem-Maghami and G Del Priore (focus: pelvic surgery)* were responsible for providing important intellectual input into the work and preparation, drafting and final approval of the manuscript. *D Corless (focus: vessel anastomosis), M Boyd (focus: anaesthesia) and D Noakes (focus: general advice)*, together with *S Saso (focus: 1st assistant)* and *JR*

Smith (focus: chief surgeon) are all part of the surgical team that performed the actual uterine transplant surgeries in the rabbit model. *MY Thum* is a Fertility Specialist who has been a long-term advisor to the *Smith et al* research team on all fertility-related issues (natural/IVF/embryo transfers). *JS Vicente and F Marco-Jimenez* were responsible for preparing and advising on the embryo transfer process. *G Petts* and *I Lindsay* performed the histopathology on our samples. *G Del Priore* is the USA Uterine Transplantation lead and has been close collaborator of *JR Smith* on this topic for the past 15 years. *JR Smith* is the guarantor for this paper and accepts full responsibility for the work and/or the conduct of the study. His involvement was critical to every phase of this work and he controlled the decision to publish. In addition he is also the UK Uterine Transplantation lead.

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Table 1 Immunosuppressant risk categories according to US Food and Drug Administration[165]

Category	Drug	Animal / Human studies
A	**Paracetamol**	No risk in human studies
B	**Corticosteroids**	No risk in animal studies OR Risk in animal studies but that risk not demonstrated in human studies
C	**Tacrolimus** **Rapamycin** **Cyclosporine A** **Mycophenolate Mofetil**	Fetal risk demonstrated in animal studies BUT no adequate and well-controlled studies in humans. Drugs can be used if potential benefits outweigh risks.
D	**Azathioprine**	Fetal risk demonstrated in human studies. In exceptional circumstances, drugs can be used if potential benefits outweigh risks.

Table 2 Renal, hepatic and reproductive profiles from D0 to D102

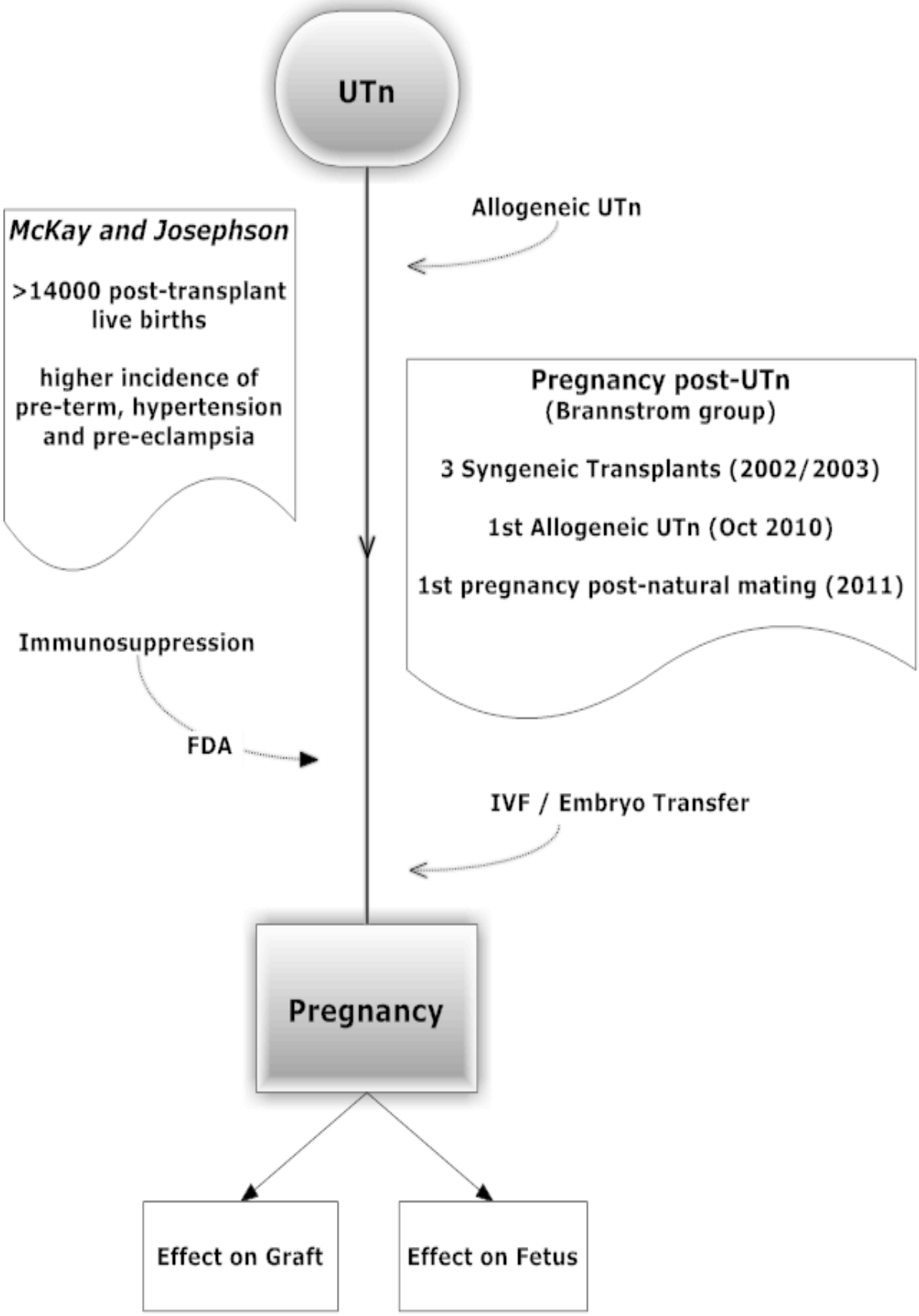
UTn 5											
Gestational Age	**D0**	**D2**	**D6**	**D9**	**D13**	**D20**	**D36**	**D46**	**D60**	**D74**	**D102**
Sodium	**151**	141	142	143	142	143	144	144	144	141	141
Potassium	3.9	4.7	NM	4.3	4.5	3.8	3.8	4.0	NM	4.2	4.4
Urea	8.9	**21.8**	**12.2**	7.6	6.2	6.8	5.8	6.7	7.6	7.2	7.6
Creatinine	131	115	91	89	82	79	82	91	106	95	95
AST	40	63	21	14	23	30	22	29	NM	25	36
ALT	28	71	44	26	17	22	21	27	31	23	19
ALP	41	**21**	NM	37	34	34	38	36	42	50	18
Albumin	**57**	42	**50**	**49**	45	**50**	**51**	**56**	**55**	**56**	48
CRP	NA	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	-
Reproductive profile											
17β-oestradiol	70	47	57	83	90	112	60	102	101	92	45
LH	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

FSH	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Testosterone	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Progesterone	0.5	0.5	0.5	0.5	0.5	0.6	0.5	0.7	0.5	0.5	15.5
Immunosuppressant											
Tacrolimus Levels	**NA**	**NM**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**-**

For ranges please, refer to *Hewitt et al*

Key **NA.** Non-applicable, **NM,** Not Measured (lab error, insufficient sample received, haemolysed sample)

Figure 1 Pregnancy and Uterine Transplantation [2]


UTn
Allogeneic UTn
McKay and Josephson
>14000 post-transplant live births
higher incidence of pre-term, hypertension and pre-eclampsia
Pregnancy post-UTn
(Brannstrom group)
3 Syngeneic Transplants (2002/2003)
1st Allogeneic UTn (Oct 2010)
1st pregnancy post-natural mating (2011)
Immunosuppression
FDA
IVF / Embryo Transfer
Pregnancy
Effect on Graft
Effect on Fetus

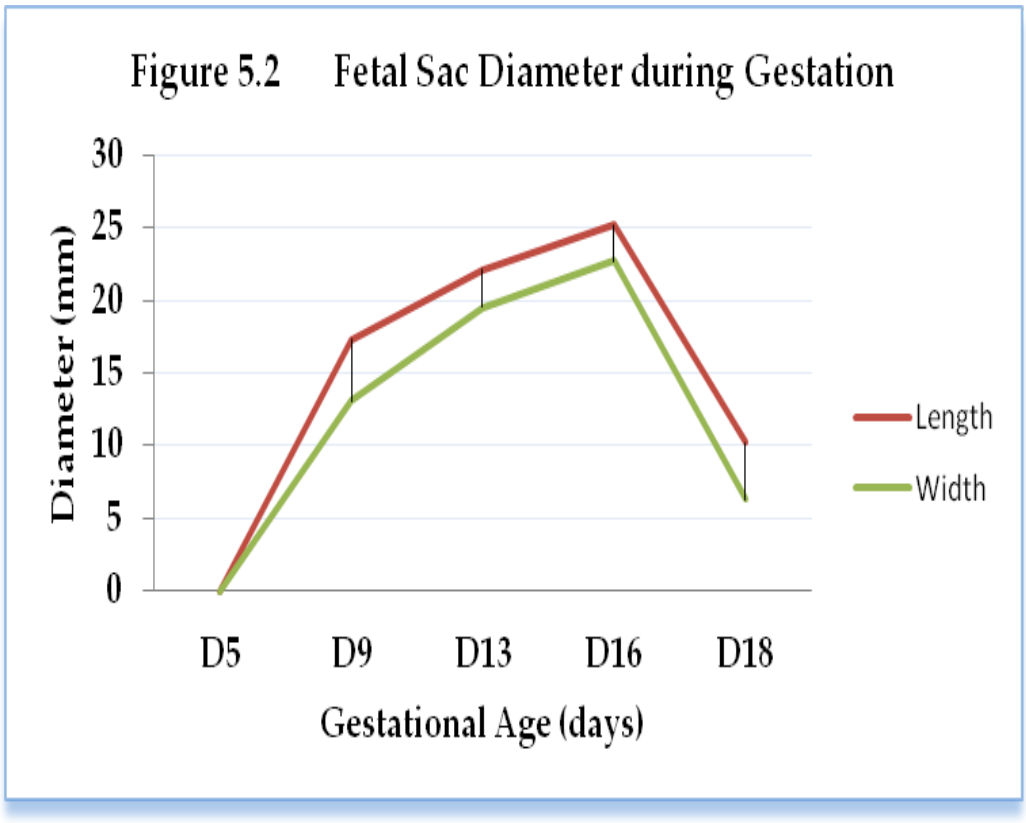


Figure 3 'Uterine Cavity' on D113 post-UTn (cull of doe)

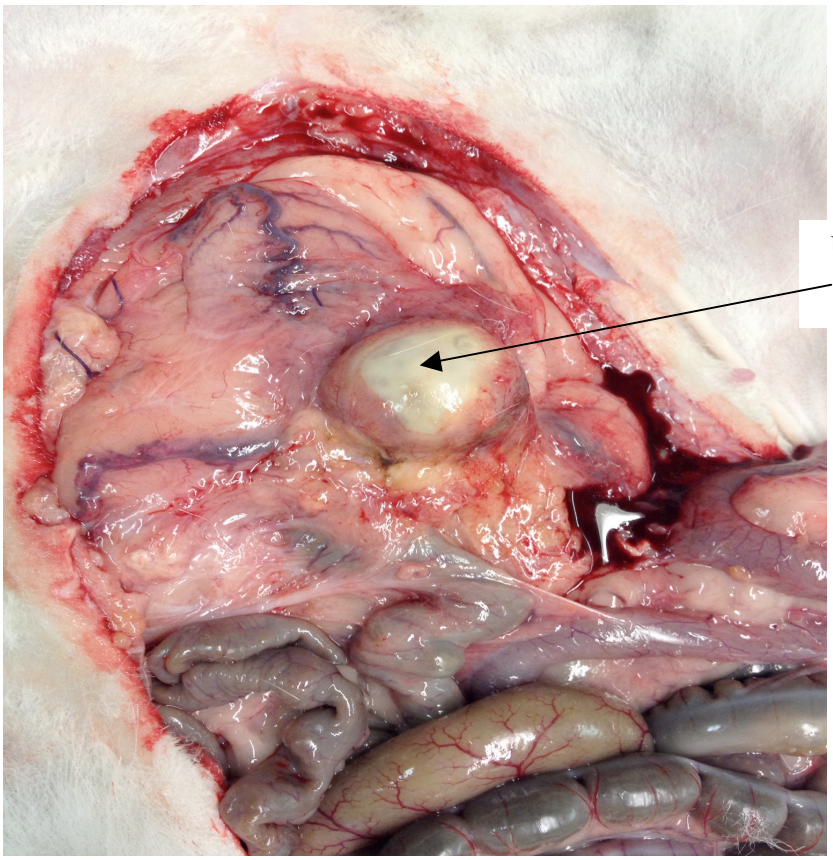


Figure 4 Products of conception: Chorionic Villi

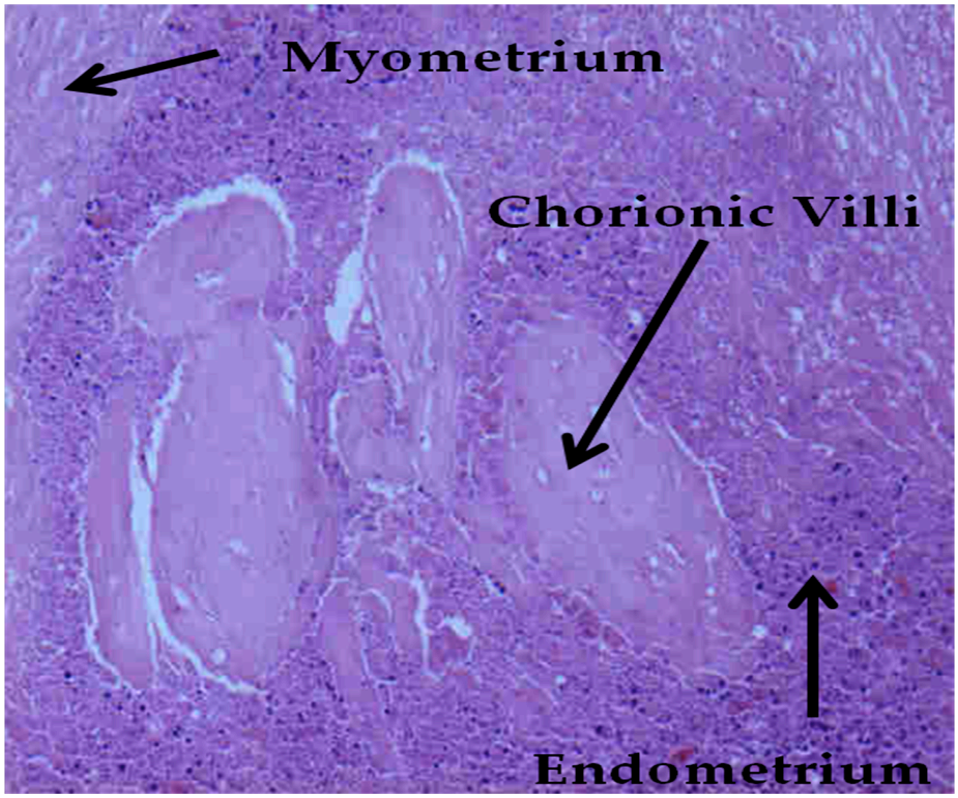


Figure 5 White cell subset levels including those taken during pregnancy