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Achieving an early pregnancy following allogeneic uterine transplantation in a rabbit model

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ABSTRACT

Objective: Uterine transplantation (UTx) has been proposed as a treatment option for women diagnosed with absolute uterine factor infertility (AUFI). The goal of UTx remains achieving pregnancy and live birth of a healthy neonate following allogeneic UTx. Our aim was to assess whether fertility was possible following allogeneic uterine transplantation (UTx), when the recipient had demonstrated long-term survival and had been administered immunosuppression.

Study design: Nine allogeneic UTx in New Zealand White rabbits were performed using a pre-determined protocol. Tacrolimus was the immunosuppressant selected. Embryos were transferred into both cornua of the sole living recipient via a mini-midline laparotomy. The pregnancy was monitored with regular reproductive profiles and serial trans-abdominal ultrasound to measure conceptus growth (gestation sac and crown rump length (CRL)).

Results: In the sole surviving doe a gestation sac was visualised on ultrasound from Day 9 (D9) after embryo transfer. Gestation sac diameter and CRL increased from D9 to D16 but by D18 the gestation sac had reduced in size. The fetus was no longer visible, suggesting fetal resorption had occurred. Subsequent scans on D22 and D25 did not demonstrate a gestation sac. Scheduled necropsy on D27 and histopathology confirmed evidence of a gravid uterus and presence of a gestational sac. A single episode of acute rejection occurred on D13.

Conclusion: Pregnancy was achieved after rabbit allogeneic UTx but serial ultrasound suggested that fetal demise occurred prior to scheduled necropsy. The study represents only the third example of conception and pregnancy following an animal allogeneic UTx.

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Condensation

This is only the third example of conception and pregnancy following animal allogeneic uterine transplantation, made possible by a successful surgical macrovascular model.

Introduction

Uterine transplantation (UTx) has been proposed as a treatment option for women diagnosed with absolute uterine factor infertility (AUFI) and who wish to bear their own child [1]. AUFI renders a woman 'unconditionally infertile'. Important advances have been made in several fields which define UTx. 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 ultimate goal of UTx: pregnancy and live birth of a healthy neonate following allogeneic UTx [2].

The Brannstrom group has described five examples (three murine and two rat models) of successful pregnancy post-UTx. The first three cases all 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 [3–5]. The lengths and weights of the fetuses, weights of the placentae, and implantation and pregnancy rates were similar in native, grafted and 'control' uteri [3–5]. Pregnancy was also achieved following a transplant operation after 24 h storage of the uterus in cold ischaemic conditions [5].

The Brannstrom group moved to a rat model with a longer gestation and known immunological transplant rejection mechanism. They described the first recorded pregnancy in a rat allo-UTx model with immunosuppression. This provided proof of the concept that a uterus can function following its transplantation from a donor to an allogeneic recipient with the use of immunosuppression [6]. In their next experiment involving natural (syngeneic) mating, the overall pregnancy rates after introduction to male rats were comparable in UTx (11/19; 58%) and control (12/19; 63%) rats. From the UTx rats, only one rat delivered two healthy male pups. Two other UTx 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 UTx rat were similar to the control rat offspring (32 live born pups). The main differences between the UTx and control groups were the increased number of resorptions and decreased number of successful deliveries in the former group. Some unknown factor is therefore compromising fetal wellbeing post-UTx, most likely blood flow or denervation. The study demonstrated that conception by natural mating, subsequent pregnancy and birth of live and healthy offspring are possible with orthotopic, syngeneic UTx [7].

The third animal model to demonstrate pregnancy following allo-UTx was the sheep model. This was the first case of pregnancy following large-animal allogeneic UTx in 12 sexually mature African sheep. Four months post-UTx, fresh and frozen blastocyst donors were transferred to the surviving five uterine allografts via a mini-laparotomy incision. Three of these resulted in pregnancies. One was an ectopic gestation, one sheep carried the pregnancy to 105 days, and one delivered a fully developed lamb from the transplanted uterus by caesarean section. Neonatal lamb blood gas values and chemistry, gross organ examination, and ventilation and respiratory compliance studies yielded results normal for gestational age [8].

It goes without saying that the ultimate goal of UTx is the achievement of pregnancy in a human model. This paper focuses on animal models but it is worth highlighting that in the last three years, three pregnancies have been demonstrated following human UTx: a chemical pregnancy and a pregnancy followed by an eight-week miscarriage in the same recipient operated on by a team in Turkey [9], and finally, the impressive news of a live birth at 32 weeks demonstrated by Professor Brannstrom's team in Sweden [10].

The aim of the present study was to assess whether fertility (conception, pregnancy and fetal well-being) was possible following orthotopic, allogeneic UTx with the use of immunosuppression.

Materials and methods

The feasibility of the uterine allograft dissection together with its vascular supply, which 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 [11]. Here, we performed two surgically explorative procedures and nine allogeneic uterine cross transplantations in the rabbit model using a pre-determined protocol.

Eighteen 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 acclimatisation to their surroundings. The focus here was the attempt to achieve fertility in a long-term surviving doe.

This particular surgical procedure has already been described in detail [11,12]. The uterine allograft along with the AA, IVC, common and internal iliacs, and uterine arterial and venous tree all intact were retrieved *en bloc*. The anastomosis was end-to-side aorto-aortic and cavo-caval. Fig. 1 demonstrates a close-up view of end-to-side vascular anastomoses between the common iliac artery and vein of the donor to the vena cava and the aorta of the recipient animal [13]. In our surgery, the anastomosis looked exactly the same except it was the aorta and vena cava that were used and not the common iliacs.

The protocol was prepared by the rabbit fertility team of the Polytechnic Institute, Valencia, Spain, led by Professor Jose Vicente and is described in Appendix A. All normal embryos (morulae) were vitrified by the methodology described by Vicente et al. [15]. For further details please refer to Appendix A. The recipient doe was injected intramuscularly with 25IU of eCG 60 h prior to

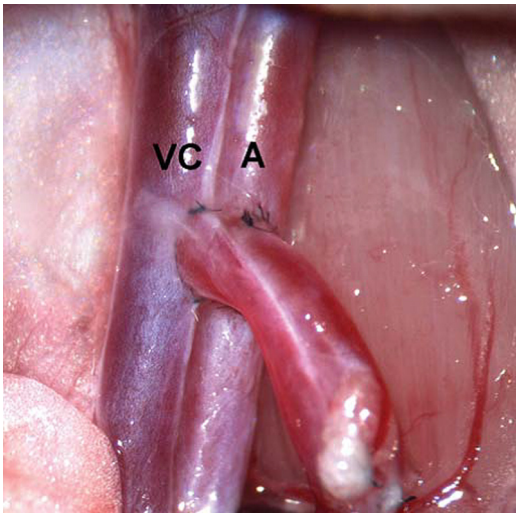

Fig. 1. Close-up view of end-to-side vascular anastomoses between the common iliac artery and vein of a rat to the vena cava (VC) and the aorta (A) of the recipient rat [13].