ELSEVIER

THE JOURNAL OF
MINIMALLY INVASIVE
GYNECOLOGY

Pilot Study

Pregnancy and Outcome of Uterine Allotransplantation and Assisted Reproduction in Sheep

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ABSTRACT This pilot study was performed from March 2008 through February 2010 to demonstrate that pregnancy can be achieved in a uterine allograft in the sheep model with the guidance of assisted reproductive technology. Uterine allotransplantation was performed in 12 sexually mature African sheep (Sudanese and Ethiopian). All animals underwent uterine transplantation via a minilaparotomy incision using a Mobius retractor device. A control group of pregnant Romney Marsh sheep with nontransplanted uteri were used to compare fetal development, uterine and placental histologic findings, and blood samples of progeny of the uterine transplant recipient sheep. Fetal size was obtained from ultrasound measurements during the early (crown-rump length) and late (biparietal diameter and abdominal circumference) gestational periods. The primary end point variables included preoperative and postoperative management, embryo transfer protocol, intraoperative assessments, and physiologic cardiopulmonary changes in the lamb during the first 5 hours of life. Four months after the initial uterine transplantation, 5 of 12 uterine allografts were considered candidates for the embryo transfer procedure. Fresh and frozen blastocyst donors were transferred accordingly to the remaining 5 uterine allografts via a minilaparotomy incision. Three of these resulted in pregnancies. One was an ectopic gestation, 1 sheep carried the pregnancy to 105 days, and 1 delivered a fully developed lamb from the transplanted uterus that was delivered via cesarean section. Neonatal lamb blood gas values and chemistry, gross organ examination, and ventilation and respiratory compliance studies yielded results normal for gestational age. This first reported case demonstrates that pregnancy can be carried in an allotransplanted uterus, with the end result a successful delivery. Journal of Minimally Invasive Gynecology (2011) 18, 238–245 © 2011 AAGL. All rights reserved.

Keywords: Sheep; Uterine-allotransplant; Immunosuppression; Embryo transfer; Pregnancy; Uterine factor infertility

Approximately 1 in 4000 to 10 000 female births results in an absent or nonfunctioning uterus [1]. The exact etiology of müllerian agenesis (Mayer-Rokitansky-Kuster-Hauser syndrome) is unknown. Several operative and nonoperative techniques have been designed to create a vaginal canal in patients who are emotionally mature and motivated [2]. Despite these treatments, most patients have feelings of inadequacy, low self-esteem, and lack of femininity when they realize they are unable to have children [3]. Surrogacy and adoption have been offered as a temporary solution for uterine factor infertility. However, these services are at the heart of a debate about whether they are considered valid options for reproduction or simply brokered commodities. Many argue that surrogacy and adoption do not adequately meet the needs of infertile couples who wish to carry their own biological child to term [4].

Advances in reproductive medicine have led to the discovery of treatments for patients who are unable to bear their own children because of uterine congenital malformations or acquired disease. The concept of uterine transplantation arose in the early 1900s; however, because of limited recognition of immunosuppression therapy, studies were deferred [5]. With the availability of newer immunosuppression therapies [6],

The authors have no commercial, proprietary, or financial interest in the products or companies described in this article.
This study was supported by a family corporation (RAFAM, Inc.).
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Submitted September 14, 2010. Accepted for publication November 18, 2010.
Available at www.sciencedirect.com and www.jmig.org

doi:10.1016/j.jmig.2010.11.006

transplantation of composite tissue gained popularity and motivated many scientists to search for different types of infertility treatments including transplantation of reproductive organs. The first human uterine transplantation was performed in 2000 in Saudi Arabia; however, this attempt resulted in removal of the allograft because of poor vascular reperfusion and anatomical support [7]. Since then, recent publications have documented successful pregnancies after syngeneic uterine transplantation in the mouse, and others have developed a surgical protocol for performing successful uterine allotransplantation in the ewe, rabbit, and swine models [8–11].

The primary objective of the present study was to demonstrate that pregnancy could be achieved in a uterine allograft in the sheep model with the guidance of assisted reproductive technology. The goal was to provide the medical community with valuable information while simultaneously introducing the concept of uterine transplantation as a central intervention for uterine factor infertility.

Methods

Animals

The study was approved by the Universidad de La Salle Animal Care and Use Committee, and ensured that all animals were bred, used appropriately, and treated in accordance with the highest standards of humane care. In addition, the animals were managed according to National Institutes of Health regulations and the *Guiding Principles in the Care and Use of Animals* of the American Physiological Society. Twelve mature female African sheep were purchased from an accredited sheep reproductive biotechnology center, where each animal was identified as being nonrelated and fertile. Selection criteria were based on the history of previous pregnancies, mean age of 3 to 4 years, history of good ovarian response to gonadotropin stimulation as determined at ultrasonography, follicular development (>5-mm diameter), and ovulation response. All animals weighed between 35 and 50 kg and were free of parasitic infection as determined using a modified McMaster technique that measures the number of nematode eggs per gram of feces [12]. During the trial, ewes were housed in straw-bedded pens. The consumption of green grass was monitored, and intake was limited to approximately 7 to 10 kg with daily supplements of 400-g concentrates (18% protein, 2.5% fat, 12% fiber, 10% ash, and 13% humidity).

The ewes were then subdivided into 2 experimental groups, Sudanese (n = 6) and Ethiopian (n = 6) sheep, in which each animal served as a donor and recipient. The polymorphic gene in the ovine model is composed of a large number of alleles, which makes genotyping difficult in the ewe [13]. Therefore, allogenicity was strictly based on phenotypic features, historical breed (Sudanese and Ethiopian, as the reproductive center had experience in handling these types of breeds), and characteristics of wool distribution. These two experimental groups were necessary in order to differentiate allogenicity since genotyping could not be obtained.

A control group of pregnant Romney Marsh sheep with nontransplanted uteruses were used to compare uterine histology, progression of pregnancy, and blood samples of progeny with the uterine transplant recipient sheep. The mean (SD) duration of pregnancy in small ruminants is 147 (3) days, and the estimated weight of the lamb is 2.5 to 5 kg.

Procedure Performed in Donor and Recipient

As reported by Ramirez et al [9], food was withdrawn 24 hours before the procedure, and the wool was clipped from the abdomen. Using a minimally invasive approach, 2 surgeries were performed simultaneously. A 5-cm vertical midline incision was made, and a 900 to 500 Mobius retractor (Apple Medical Corp., Marlborough, MA) was used to expose the underlying pelvic viscera and retroperitoneal structures. After documenting normal reproductive anatomy, both uteroovarian ligaments were identified and transected using bipolar thermal energy. The right and left fallopian tubes were separated away from the ovarian adnexa, and the mesosalpinx was transected medially without traumatizing the uterine vascular pedicles. Using a microsurgical approach and 2.5× magnifying loops (SA 451; Surgical Acuity Inc., Middleton, WI), both the right and left uterine artery and vein were identified laterally from the corpus of the uterus and dissected down to the anterior branch of the internal iliac artery. The uterine artery and vein were freely dissected from the peritoneal surface of the broad ligament and displaced laterally using microsurgical clamps. Eight vascular clamps were applied to each uterine artery and vein, securing the proximal and distal ends of the uterine vasculature. Both the right and left uterine artery and vein were transected using microsurgical scissors, and total abdominal hysterectomy without oophorectomy was performed.

All recipient sheep initially served as donors. Retrieval of uterine tissue was achieved by performing a hysterectomy without oophorectomy, as previously described in detail. Both uterine tissues were transferred to a sterile basin, and a 23-gauge intravenous catheter was secured to the uterine arteries. All 12 uterine allografts were preserved and flushed at 4°C using a histidine-tryptophan-ketoglutarate buffering solution (Custodiol HTK; Köhler Chemie GmbH, Germany). Continuous perfusion pressure was maintained at a flow of 75 mm Hg. When total cold ischemic time of 60 minutes was achieved, each donor uterus was transferred to each recipient. Donor and recipient vaginal tissues were reapproximated using 2-0 polyglactin 910 suture (Vicryl; Ethicon, Inc., Somerville, NJ) in a continuous noninterlocking fashion, and the vascular pedicles were isolated (2 arteries and 2 veins). The vascular anastomosis was initiated first by reapproximating the uterine veins with 6-0 polypropylene suture (Prolene 318-13 mm; Ethicon, Inc.) (Fig. 1). This enabled evaluation of venous vascular patency by infusing diluted sodium heparin, 10 mL, mixed in 25 L of physiologic solution (Viaflex 0.9% sodium chloride; Baxter, Cali, Colombia), into each donor uterine artery. After documenting venous vascular patency, the donor and recipient uterine arteries were reapproximated with 5-0 polypropylene