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Uterus tolerance to extended cold ischemic storage after auto-transplantation in ewes

Jérémy Tricard[a,b,c], Sébastien Ponsonnard[d], Yannick Tholance[e], Laura Mesturoux[f], Denis Lachatre[g], Claude Couquet[h], Faraj Terro[c], Catherine Yardin[c], Pierre Marquet[b], Alessandro Piccardo[a], Tristan Gauthier[b,i,*]

[a] Department of Thoracic and Cardiovascular Surgery and Angiology, Limoges University Hospital Center, 87000 Limoges, France
[b] Department of Pharmacology, Toxicology and Pharmacovigilance, INSERM UMR S-850, Limoges, France
[c] Equipe de Recherche Médicale Appliquée (ERMA), University of Limoges, 87000, France
[d] Department of Anesthesia and Intensive Care, Limoges University Hospital Center, 87000 Limoges, France
[e] Department of Biochemistry and Molecular Genetics, Limoges University Hospital Center, 87000 Limoges, France
[f] Department of Pathological Anatomy, Limoges University Hospital Center, 87000 Limoges, France
[g] Department of Radiology and Medical Imaging, Limoges University Hospital Center, 87000 Limoges, France
[h] Research and Analysis Laboratory of Haute-Vienne, 87000 Limoges, France
[i] Department of Obstetric and Gynecology, Limoges University Hospital Center, 87000 Limoges, France

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ABSTRACT

Objective: To assess how the uterus tolerates extended cold ischemic storage before auto-transplantation in ewes.
Study design: Fourteen uterine auto-transplantations were performed in ewes from November 2014 to June 2015 at the Analysis and Research Laboratory of Limoges, France. The animals were divided into 2 groups: 7 after 3 h of cold ischemia timeand 7 after 24 h. Transplant was assessed ≥8 days after transplantation. Histology and apoptosis analyses (TUNEL method and indirect immunohistochemistry of cleaved Caspase 3) were performed before uterus retrieval (control), after 90 min following reperfusion and ≥8 days after transplantation.
Results: Twelve uterine auto-transplantations were successfully performed. The histological analysis at 90 min following reperfusion revealed a moderate inflammation of the endometrium and serosa in the 3-h group and severe inflammation in the 24-h group, but no significant apoptotic signal was found in either group. Seven ewes were alive at ≥8 days after transplantation: the macroscopic and histological analyses revealed two viable uteri in the 3-h group and three in the 24-h group. In each group one uterus was necrotic.
Conclusion: These first results in ewes suggest that the uterus is an organ with a good tolerance to extended cold ischemic storage before transplantation.

Introduction

The first live birth after uterus transplantation (UTx) in Sweden reported by Brännström's team was a major advance for the treatment of uterine infertility [1,2]. In Europe, 1/500 woman of childbearing age suffers from uterine infertility and might benefit from UTx. Two types of donors may be considered: living donors and brain dead donors. The use of brain dead donors has the advantages of being anonymous and avoiding surgical risks for the donor [3,4]. One major drawback is the duration of cold ischemia when the procurement site is remote from the transplantation site. This requires some degree of uterus tolerance to extended cold ischemia. However, there are very few data on this subject, whether in women or in large animal models.

The aim of this study was to assess how the uterus of the ewe tolerates ischemia/reperfusion after extended cold ischemic storage followed by auto-transplantation.

Material and methods

Fourteen uterus auto-transplantations were performed in ewes, half after 3 h of cold ischemia time (CIT) and half after 24 h of CIT.

Limousin breeding ewes were 3–5 years of age and weighed between 45 and 65 kg. All ewes were in anoestrus during the

* Corresponding author at: 2 avenue Martin Luther King, 87042 Limoges, France.
E-mail address: tristan.gauthier@chu-limoges.fr (T. Gauthier).

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operation and shared the same nycthemeral cycle. Food was withdrawn 24 h before surgery. The study was approved by the "Limousin's Regional Animal Ethics Committee "(CREEAL) » *(n° 06-2014-06)*.Transplantations were performed at Haute-Vienne's Analysis and Research Laboratory of, France *(Approval number: 87.797)*.

• Anesthesia and analgesia

Premedication consisted of an intramuscular injection of ketamine 20 mg/kg and xylazine 1.5 mg/kg. General anaesthesia was ensured with an IV injection of 0.3 μg/kg of sufentanil and 2 mg/kg of propofol before endotracheal intubation. Anesthesia was maintained with isoflurane and regular injections of sufentanil. Post-operative analgesia was ensured with an intramuscular injection of flunixine (100 mg) at the end of the surgery and with an intramuscular injection of 5 mg/kg ketamine twice daily for 5 days.

• Surgery and postoperative follow-up

The surgical technique was inspired by a technique described by Brännström's team [5,6].

The ewe was positioned in the dorsoventral position and draped in sterile conditions. After midline laparotomy, the uterine horn intended for auto-transplantation was chosen depending on the size of the uterine artery. The control uterine horn was ligated and removed with an automatic stapler. The remaining uterine horn, the uterine body, the cervix and the upper part of the vagina were then dissected and isolated. The uterine artery and utero-ovarian vein were dissected to their root. The ipsilateral posterior internal iliac branch was dissected. The vagina was severed 2 cm below the cervix and closed by interrupted 2-0 sutures. A bolus of heparin (12 500 IU) was injected IV. The hypogastric trunk and the root of the internal iliac artery on the opposite side of the horn were clamped and the collateral small blood vessels ligated (defining the start of the initial warm ischemic period). The posterior internal iliac branch was catheterized with a 20-gauge catheter in a retrograde direction. The uterus was flushed with preservation solution Celsior® (Genzyme, Cambridge, Mass.) at 4 °C (100 mmHg pressure) [7]. The utero-ovarian vein was clamped and then cut distally 1 cm from the vena cava. The uterus was discolored and cooled and the preservation solution was collected in a cup (start of cold ischemia). When the solution from the venous side was clear, the infusion was stopped. The posterior internal iliac branch was then ligated and cut at its root and the hypogastric trunk was obliquely severed to get the widest possible arterial patch as the anastomotic site. The utero-ovarian stub was ligated and the hypogastric trunk closed. The uterus was placed in a sterile container with cold Celsior® and the container placed in an isothermal box filled with crushed ice.

In the 3-h CIT group, the ewes were maintained under general anesthesia during cold ischemia. In the 24-h CIT group, the ewes were woken up after uterus retrieval and operated again on the next day.

After reopening the laparotomy and surgical exposure as before, the external iliac vein and artery on the same side of the preserved horn were dissected. The uterus was taken out of the isothermal box (end of cold ischemia, start of the second warm ischemic period). A bolus of heparin (12 500 IU) was injected IV. Anastomoses were performed under microsurgical loupes (magnification, ×2.5). The utero-ovarian vein was tied end-to-side to the external iliac vein with continuous 6-0 polypropylene sutures. The utero-ovarian vein was clamped with an atraumatic clamp in order to avoid warming the uterus. End-to-side arterial anastomosis was performed with continuous 6-0 polypropylene sutures. After clamp release (end of warm ischemia), the presence of pulsations in the uterine artery, coloration of the graft and reperfusion edema were monitored over the following 90 min of reperfusion before a uterine horn biopsy was performed (Fig. 1). The vaginal cuff was sutured back to the vagina with interrupted 2-0 polyglactin sutures and the uterus was tied to the round ligaments and peritoneum.

Initial post-operative recovery was assessed by pulse, diuresis, food and water intake and ability to stand up. The animals were returned to the stables with a daily veterinarian follow-up. Low-molecular-weight heparin (Enoxaparin, 40 mg) was given once daily, up to reoperation. Prophylactic antibiotics (sustained-release amoxicillin, 15 mg/kg) were given for 7 days. The ewes were reoperated by laparotomy 8 days or more after transplantation (≥D8). The uterus characteristics (necrosis, contractions of the myometrium) were noted and Doppler ultrasound of the uterine artery performed. The animals were sacrificed while still under anesthesia with an injection of Embutramid (200 mg/ml, 6 ml/50 kg IV).

• Sampling and analysis

Three uterine biopsies were performed; of the discarded uterine horn (control) and of the auto-transplanted uterine horn at 90 min of reperfusion and at ≥D8. The biopsies included the entire uterine wall, and were fixed in formaldehyde (4%), paraffin embedded, sectioned and stained with eosin-hematoxylin-safran. The sections were analyzed by light microscopy (Leica®, Paris, France) by the same medical pathologist blinded to the group. Edema and necrosis were evaluated semi-quantitatively (absent or mild/moderate/severe). Mild was defined as less than 1/3 of parietal tissue, moderate between 1/3 and 2/3 and severe more than 2/3. Besides, the density of neutrophilic granulocytes in the endometrium, the myometrium and the serosa were quantified by counting tissue-bound neutrophils in 10 consecutive areas (magnification, ×40).

The apoptotic signal was assessed using TUNEL, and indirect immunohistochemistry of cleaved Caspase-3. The « In Situ Cell Death Detection Kit, POD » (Roche Diagnostic®, Indianapolis, IN, USA) and the cleaved caspase-3 kit (Cell Signaling Technology®, Danvers, MA, USA) were used on paraffin sections in accordance with the manufacturer's instructions. In the negative control, distilled water replaced TdT enzyme or primary antibodies. A

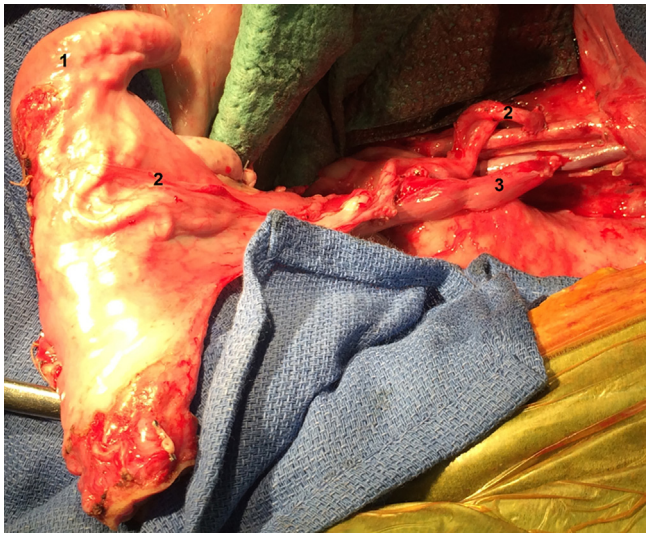

Fig. 1. Intra-operative picture of vascular anastomosis on external iliac vessels (ewe n°2–3 h of CIT group). 1: uterine horn: site of biopsy; 2: uterine artery; 3: uteroovarian vein.