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ORIGINAL RESEARCH

Hypothermic machine perfusion in uterus transplantation in a porcine model: A proof of concept and the first results in graft preservation

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Abstract

Introduction: Graft optimization is a necessity in order to develop uterus transplantation from brain-dead donors, as a complement to living donors, as these grafts are rare and the last organs retrieved in multiple organ donation. The aim of this study was to assess the feasibility and interest of hypothermic machine perfusion (HMP) in uterus transplantation using a porcine model; secondary outcomes were the evaluation of the graft's tolerance to a prolonged cold ischaemia time and to find new biomarkers of uterus viability.

Material and Methods: Fifteen uterus allotransplantations were performed in a porcine model, after 18 h of cold ischaemia, divided in three groups: Static cold storage in

Abbreviations: CIT, cold ischemia time; HMP, hypothermic machine perfusion; SCS, static cold storage; UTx, uterus transplantation.

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a HTK solution, HMP (with the VitaSmart™ machine Bridge to Life Ltd.) with a UW-MP solution, and static cold storage in a UW solution. The main outcome was macroscopic: uterine arteries pulsatility, recoloration, and bleeding at the cut. Secondary outcomes were histological analyses (Zitkute and inflammation scores), caspase3 immunohistochemistry and plasmatic dosage of biomarkers.

Results: 14/15 allotransplantations were performed according to the protocol and met the criteria of macroscopic vitality. Grafts treated with HMP (MP did not show significantly more tissue) damage than the recipient's uterus, contrary to grafts in static cold storage, independently of the solution used. This difference disappeared one and 3h after uterus transplantation. Plasma dosages before and after uterus transplantation did not allow to identify a new biomarker of uterus viability.

Conclusions: HMP is feasible in a porcine model, without inflicting damage on the grafts during cold ischaemia time. Grafts exposed to HMP seemed to better endure reperfusion phenomena, but this advantage did not last over time.

KEYWORDS

animal model, assisted reproductive technologies, hypothermic machine perfusion, infertility, uterus transplantation

1 | INTRODUCTION

Since the first livebirth after a uterus transplantation (UTx) in Sweden in,¹ more than 90 UTxs were performed worldwide, leading to the birth of more than 45 healthy children,^{2,3} opening a new avenue in assisted reproductive technologies. This new procedure is mainly intended for patients with absolute uterine factor infertility, whether congenital (Mayer-Rokitansky-Küster-Hauser syndrome [MRKH]), or surgically induced (post-partum hemorrhage hysterectomy, or for oncologic purposes), and is to date their only option to become the genetic, legal and bearing mother of the child-to-be. Amongst them, the main indication reported in the registry of the International Society of Uterus Transplantation (ISUTx) is in almost 90% of cases³ the MRKH syndrome, which affects 1/4500 women⁴ of bearing age. If most of the reported cases were performed with living donors (approximately 80%³), a growing number of research teams are now working with brain-dead donors as their main source of grafts, or as an additional source to living donors.

The non-vital nature of the uterus implies that during a multiple organ procurement surgery, the hysterectomy is performed last in most institutions (the Baylor University Medical Center team in Dallas being an exception), once the thoracic and abdominal organs are retrieved. As a consequence, the cold ischaemia time will necessarily be longer for the uterus⁵ than vital organs. To this inevitable delay of organ procurement, the time required to transport and prepare the graft has to be added. To this day, the human uterus' tolerance to cold ischaemia remains unknown and can only be estimated empirically, as Fronek et al.⁶ reported a livebirth after 9h of cold ischaemia. Although it seems to tolerate cold ischaemia times of up to 24h in vitro from a histological point of view, in a human

Key message

The first animal model of hypothermic machine perfusion in uterus transplantation showed that this new technique was not only feasible, but it also seemed to provide a better preparation for ischaemia-reperfusion phenomena despite a significantly longer warm ischaemia time.

model⁷ as in animal models,^{8,9} and allow for live births in mice after 24h of cold ischaemia,¹⁰ the impact of such a lengthy time on the chances of embryo implantation after UTx in women is not known. While at least 5 live births were reported^{11,12} after a UTx using brain-dead donors since 2018, the rates of live births after UTx depending on the type of donors differ: 63% for brain-dead donors, 71% for living donors.¹¹ Although caution is advised as fewer cases were performed using brain-dead donors, various hypotheses were proposed to explain this difference, which persists even after adjustment for the cold ischaemia time, and which has already been described in other solid organ transplantations: hemodynamic instability stemming from the adrenergic storm in brain-dead patients, repeated phases of hypoperfusion resulting from it, and systemic inflammation.^{5,13} In order to optimize the preservation of uterine grafts, various teams started working on this issue using different non-human primate and non-primate animal models,^{9,14} experimenting with some preservation solutions currently used in other solid organ transplantations, sometimes paired with new pharmacological agents.^{9,15} Padma et al. published the results of their work involving normothermic machine perfusion, but as a way of studying

the impact of ischaemia-reperfusion phenomena in an ex-vivo ovine model.¹⁶ To this day, hypothermic machine perfusion (HMP) has not been studied yet, despite its proven positive impact in kidney¹⁷ and liver¹⁸ transplantation.

The aims of this study were to study the feasibility and impact of HMP in UTx in a porcine model. Secondary endpoints included the study of the graft's tolerance to a prolonged cold ischaemia time, and assessment of potential biomarkers that could reflect the damages inflicted upon the graft.

2 | MATERIAL AND METHODS

2.1 | Animals

The surgeries were performed on prepubescent crossbred Large White Landrace sows, born and bred in the INRAE experimental facilities (UE3P, Saint-Gilles, France), where they were housed in individual cages (2,3 by 2 m) with ropes and rubber duckboards aimed at enriching their environment. Free access to water and food rations adapted to their weight was given to the sows. Feeding was interrupted 24 h prior to surgery.

2.2 | Design

Fifteen uterus allotransplantations were performed after 18 h of cold ischaemia, according to three preservation groups: 18 h of static cold storage (SCS) in a HTK-Custodiol solution (PERISOC®, Laboratoires SERB, France) (SCS-HTK, $n=5$), HMP using the VitaSmart™ device (Bridge to Life Ltd., Etats-Unis) using the UWMP solution recommended by the manufacturer (Belzer MPS® UW Solution, Bridge To Life Ltd) (HMP-UWMP, $n=5$), and SCS in a UW solution (Belzer UW® Cold Storage Solution, Bridge To Life Ltd) (SCS-UW, $n=5$).

2.2.1 | Anesthesia

Induction started with an injection of Zoletil 100 (15–20 mg/kg, Rhône VIRBAC) and oral intubation was achieved after isoflurane inhalation (2%–2.5% v/v, Baxter, France). Anesthesia was sustained with a continued intravenous injection of Propofol (10–30 mL/kg/h). Hemodynamic parameters (heart rate, respiratory rate, pulse oxymetry and capnia) were monitored throughout the procedure.

2.2.2 | Surgery

This study was designed to mimic the conditions of a uterus retrieval in multiple organs procurement surgery. The surgery design was described in a video recording detailing the procedure, previously published.¹⁹ Cooling was performed in situ, through cannulas inserted in

the sows external iliac arteries; the outflow was assured by a cannula inserted in one of the external iliac veins (Figure 1). The retrieval was performed once the organ was deemed sufficiently washed, judging by its discoloration (Figure 1). Retrieval of the graft was performed *en bloc*, with the ovarian vessels, abdominal aorta and vena cava, external iliac vessels up to the cannulation sites, internal iliac vessels and uterine arteries. Ureters were sectioned near the bifurcation of the common iliac vessels, and colpotomy completed the procedure. The sow was then euthanized, and the graft was prepared. After the 18 h of either static cold storage or HMP, the recipient sow was prepared for the transplantation. The native uterus of the recipient was not removed, to allow for control samples. After a careful preparation of the anastomotic sites, that is, the external iliac vessels (either left or right, depending on the side providing the easiest access to this deep part of the sow's pelvis), end-to-side anastomoses were performed between the donor's aorta and vena cava and the recipient's external iliac vessels, with 3.5× magnifying glasses, using continuous sutures (5–0 polypropylene sutures, Corolene®, Peters Surgical, France). 3 h after the transplantation, the recipient sow was euthanized.

In the static cold storage groups (either with UW or HTK solution), the organ was immersed in the preservation solution and stored in a cooled hermetic container (with a measured temperature of 1°C) for 18 h.

2.2.3 | HMP settings

As there was no previously published study about HMP in UTx, and no data about uterine arteries physiological pressure in women or sows, the ideal perfusion flow and pressure were not known beforehand. Therefore, the parameters applied in liver preservation with this device were applied in our procedure. The preset pressure was 5 mmHg, and the preset flow was 250 mL/min; this pressure was never reached in the first two cases and peaked at 3 mmHg, and was therefore applied as the preset perfusion pressure for the next cases.

HMP started as soon as the organ was retrieved from the sow, and lasted 18 h.

2.3 | Measures

The main outcome was the macroscopic assessment of the graft's vitality using a composite criterion: uterine arteries pulsatility, bleeding at the incision, and coloration of the graft 5 min after opening clamps of graft vessels. Each criterion was assessed in a binary manner and scored one point when met, with a maximum score of 3 points and a minimum of 0 point.

Secondary outcomes included a blinded histological assessment of biopsies of the uterine horns retrieved from each animal, before and during the cold ischaemia time (H3, H6, H9, H12, H18) as well as after the transplantation, both on the native uterus and the graft,

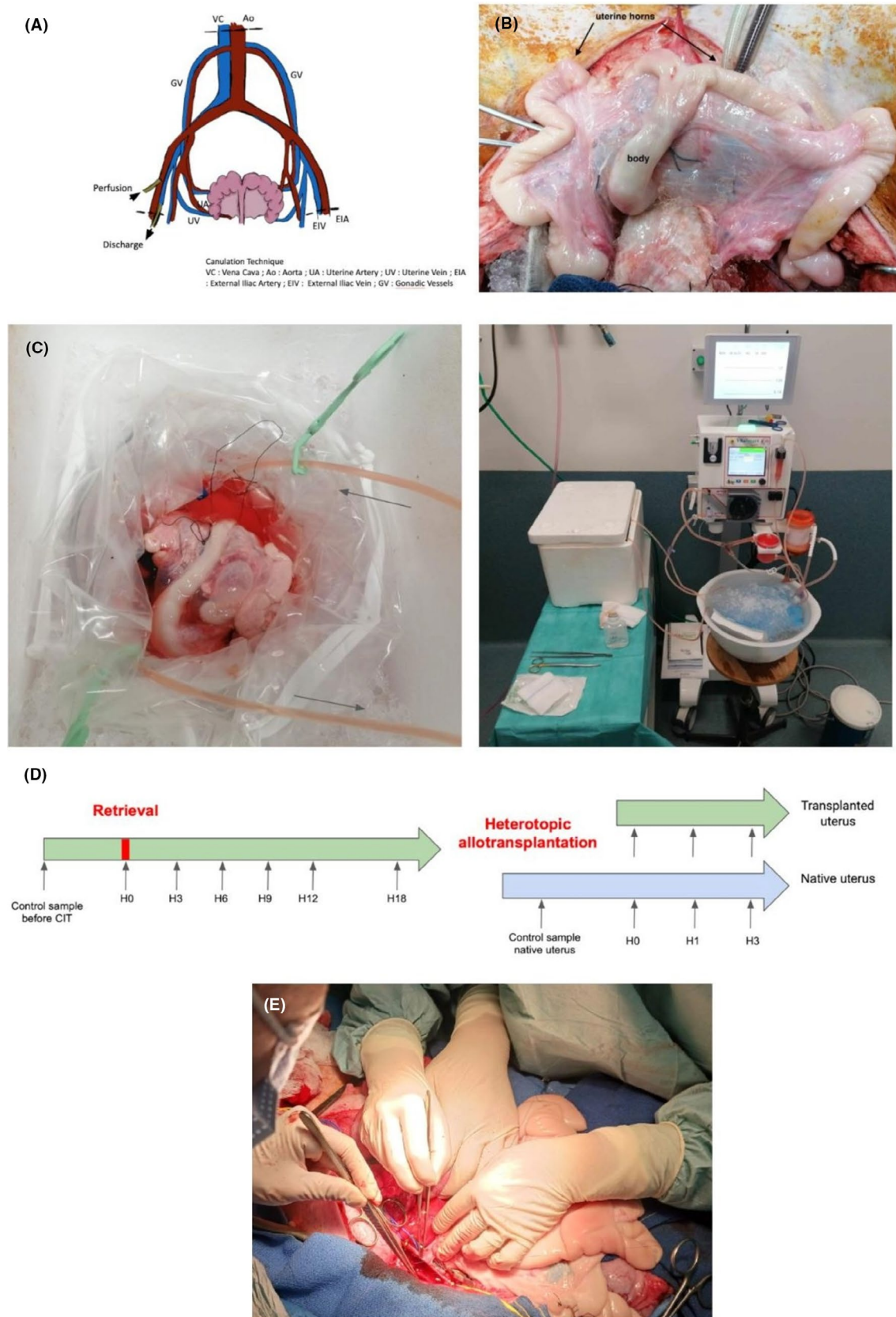


FIGURE 1 Surgical technique. (A) Uterus vascularization and cannulation site. (B) Discolored uterus after in situ cooling. (C) Hypothermic machine perfusion using the VitaSmart device, left: The graft is connected to the system via its aorta, and outflow is drained by a discharge cannula immersed in the solution; right: The VitaSmart machine during perfusion, with the graft kept at a 4°C temperature in its hermetic container, frequently controlled and refilled with ice, and the connecting tubes dispensing the solution immersed in ice to prevent the solution heating up as it passes through the machine at room temperature. (D) Samples timeline. (E) Arterial anastomosis, during allotransplantation n°14 (SCS-UW group). The venous anastomosis was already completed and tested. The discolored graft is here held by the assistant, in order to fully expose the vessels and to bring the graft's aorta closer to the external iliac artery of the recipient.

based on the score described by Zitkute et al.,¹⁵ and an evaluation of inflammation and apoptosis rate.

Uterine horn biopsies were immediately immersed in 4% buffered formaldehyde. After 24–48 h, samples were dehydrated with the Excelsior ES device (Thermo Scientific) then embedded in paraffin; 4 μ m sections were prepared with a microtome within the H2P2 laboratory (Histo Pathology High Precision, Rennes University, France). H&E staining (hematoxylin & eosin) by the ST5020 workstation (Leica Biosystems) completed the preparation of the biopsies, then examined under a light microscope by an experienced pathologist following the Zitkute score, a semi-quantitative morphological score assessing tissue damage during static cold storage. Inflammation was evaluated according to a semi-quantitative scoring system: 0: absence or mild infiltration of inflammatory cells under the epithelium (<25% of subepithelial cells are neutrophils); (1) moderate inflammation (25%–75%); (2) severe infiltration (>75%). A second analysis was conducted in immunohistochemistry using a cleaved Caspase 3 staining (Cell Signaling #9661; 1:1000 dilution of rabbit polyclonal Caspase 3 cleaved) prepared with the Discovery Ultra Research Staining System (Roche, Illkirsh, France) to detect apoptosis amongst epithelial cells (endometrium and glands). For positive control, porcine lymph node tissue was used. Each biopsy was entirely analyzed in a blinded fashion and the amount of apoptotic cells detected in the endometrial lining and glands was presented as a percentage of all epithelial cells of the biopsy.

Blood samples of the recipient were also taken before, immediately after, 1 h and 3 h after the transplantation to measure the concentration of various markers: kalemia, lactates, myoglobinemia, lactate dehydrogenase (LDH) and creatine kinase (CK) levels; as well as quantitative protein assays to find a new biomarkers amongst cytokines, chemokines and growth factors involved in inflammation and immune responses.

These blood samples were collected in heparin-coated tubes (BD Vacutainer® PST 4,0 mL, Becton-Dickinson), immediately followed by a 4°C centrifugation, 3500 rotations per minute, for 10 min. Plasma was then extracted and stored at a –80°C temperature before analysis. Plasma lactates, kalemia, myoglobinemia, LDH activity and CK activity were measured. Concentrations of interferon-gamma (IFN- γ), Tumor Necrosis Factor alpha (TNF- α), interleukin-1 (IL-1 α), interleukin-1 beta (IL-1 β), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), interleukin-12 (IL-12), interleukin-18 (IL-18), Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF), Metalloproteinase-1 (MMP-1), Serpin E1, Platelet Endothelial Cell Adhesion Molecule-1 (CD31/PECAM1), Platelet Derived Growth Factor BB (PDGF-BB), interleukin-1 receptor antagonist (IL-1RA) (Porcine Luminex® Discovery Assay, R&D Systems, Bio-technie, Minneapolis, USA), Tumor Growth Factors beta-1, beta-2 and beta-3 (TGF β 1, TGF β 2 and TGF β 3 respectively) (TGF-beta Premixed Magnetic Luminex® Performance Assay, R&D Systems, Bio-technie, Minneapolis, USA) were measured using the Luminex multiplex assay technology.

2.4 | Statistical analyses

Data were analyzed with the GraphPad Prism 10.0.0 software (GraphPad Software, Boston, Massachusetts, USA). A non-parametric Kruskal-Wallis test, followed by a multiple comparisons Dunn's test was applied when data were not matched or when data were missing; otherwise, a Friedman test followed by Dunn's test were used. Data are expressed as means (\pm standard deviation) and a *p*-value less than 0.05 was considered statistically significant.

3 | RESULTS

Characteristics of the sows population were similar in each group; mean age was 168 days (\pm 20 days) and mean weight was 106 kg (\pm 10 kg).

3.1 | Surgery results

All surgeries were fully completed. Mean incision to cannulation time was 119 min (\pm 32 min) in the SCS-HTK group, 122 min (\pm 18 min) in the HMP-UWMP group and 86 min (\pm 8 min) in the SCS-UW group, *p*=0.01. Mean hysterectomy time was 16 min (\pm 6 min) in the SCS-HTK group, 24 min (\pm 9 min) in the HMP-UWMP group and 19 min (\pm 3 min) in the SCS-UW group, *p*=0.33. Warm ischaemia times, i.e. vascular anastomoses times, were 58 min (\pm 15 min) in the SCS-HTK group, 88 min (\pm 15 min) in the HMP-UWMP group and 78 min (\pm 9 min) in the SCS-UW group, *p*=0.02. Mean venous anastomosis time was 32 min (\pm 8 min) in the SCS-HTK group, 47 min (\pm 14 min) in the HMP-UWMP group and 36 min (\pm 6 min) in the SCS-UW group, *p*=0.1. Mean arterial anastomosis time was 26 min (\pm 7 min) in the SCS-HTK group, 41 min (\pm 6 min) in the HMP-UWMP group and 42 min (\pm 3 min) in the SCS-UW group, *p*=0.01.

Of note, the recipient sow n°6 (HMP-UWMP group) showed signs of hemodynamic failure 2 h after the transplantation, and died shortly before the 3 h cut-off despite corrective measures (fluid resuscitation, sedation monitoring); therefore the last samples were taken before cardiac arrest, between 2 and 3 h after transplantation.

3.2 | Main outcome: macroscopic assessment

Immediately after the unclamping of the graft's vessels, all of the grafts, except the last one (n°15, SCS-UW group), met all the macroscopic criteria, amounting to a score of 3 points out of 3. The last allotransplantation (n°15, SCS-UW group) was given a score of 1: despite a good pulsatility of the uterine arteries, recoloration was segmental, incomplete, and bleeding at the incision was minor or venous (Figure 2). Discoloring of this graft could not be achieved, even though cannulas were correctly positioned and the graft had been washed again with the preservation solution on the backtable. Multiple thromboses were observed during cold ischaemia time within the vasculature of the



FIGURE 2 Post-transplantation aspect, recipient sow n°11 (left) and n°15 (right) (SCS-UW group). On the left, the transplanted uterus adequately colored; the native uterus of the recipient is larger, as oestrus occurred shortly before the surgery. On the right, the graft in the foreground is only partially recolored, with signs of edema, and many thromboses can be seen in its vasculature.

graft. Only half the uterine body and horns had a good coloration after the transplantation; an in situ thrombolysis was attempted by injecting 10000IU of heparin in the vena cava of the graft, allowing for a partial recoloration of the uterus.

3.3 | Secondary outcomes

3.3.1 | Histology

The analysis of the uterine horns biopsies after H&E staining did not reveal any difference between the three groups, whether in terms of inflammation (Figure 3B,D) or the Zitkute score of tissue damage during static cold storage. Zitkute scores were systematically inferior to 3, for a maximum score of 9, revealing a good preservation of the grafts during cold ischaemia time, and a low impact of ischaemia-reperfusion phenomena up to 3h after the transplantation (Figure 3A).

No significant difference was found between the study groups at each endpoint, even when the damages were expected to be the most important (after 18h of cold ischaemia or 3h after the transplantation). However, there was significantly more tissue damage in the transplanted uterus when compared to the native uterus of the recipient after reperfusion in the static cold storage groups (SCS-UW and SCS-HTK). This difference was not observed in grafts exposed to HMP (Figure 3C). This difference disappeared an hour after the transplantation, and was only found again 3 after the UTx in the SCS-UW group (Figure 3C).

Amongst the lesions described in the uterine tissue damage score published by Zitkute et al., each was observed, except for the smooth muscle contraction. Examples of the lesions in each group can be found in Figure 4. In the HMP-UWMP group, blood vessels appeared dilated, as soon as 3h after the start of the machine perfusion; this

aspect disappeared after the transplantation. They also seemed better washed, whereas in the static cold storage groups a few red cells were always found in the vessels. The uterine horn samples from the last allotransplantation (n°15, SCS-UW group) showed partially obstructive intravascular thromboses, and an intimal cell proliferation (Figure 4).

Neutrophilic infiltration was observed in glands and along the basement membrane, especially immediately after the transplantation. However, similar infiltrates were noted in the samples taken before graft retrieval in donor sows, and there was no significant difference between the 3 groups, or between the graft and the native uterus (Figure 4).

3.3.2 | Immunohistochemistry

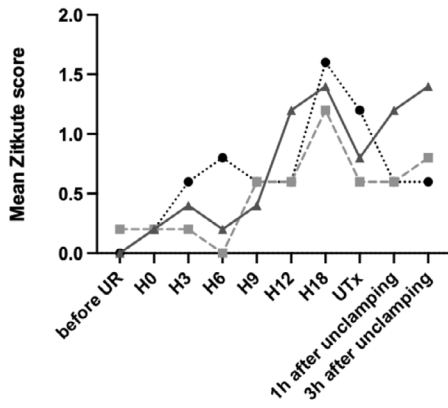
Apoptotic cells quantification in immunohistochemistry found very low rates of apoptosis, never higher than 2% of all epithelial cells; therefore, no significant difference was found between each group or between the graft and the native uterus (Figure 3G,H). Examples of cleaved caspase-3-stained samples from each group can be found in Figure 4B.

3.3.3 | Biochemical analysis on blood plasma

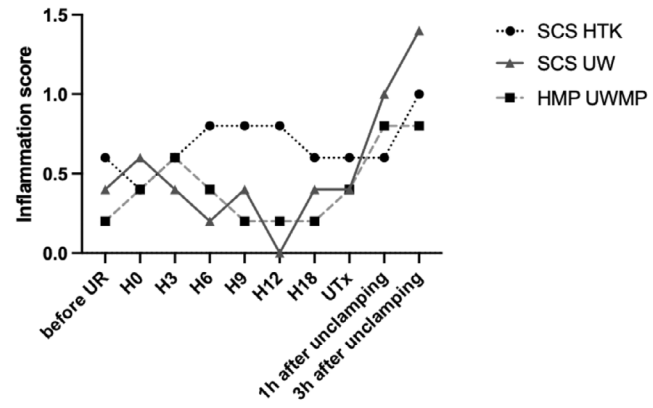
Peripheral blood samples of the recipient sow taken before the transplantation, immediately afterwards, and 1h and 3h after the transplantation, were analyzed in order to find a marker of the graft's vitality. Markers used in clinical practice on a daily basis were studied; their plasmatic dosage before transplantation were similar to values previously published in the adult sow of such lineage.²⁰⁻²³ No values could be found as references for myoglobinemia and LDH

FIGURE 3 (A) Evolution of the histological scores of uterine tissue damage during static cold storage, as described by Zitkute et al. (B) Evolution of the inflammation scores. (C) Comparison of the uterine tissue damage scores as described by Zitkute et al., (D) of the inflammation scores and (G) of the apoptotic cells rates following the transplantation, between the native uterus of the recipient sow, and the graft. (E) Comparison of Zitkute scores of each groups, immediately after the retrieval of the graft (H0, start of cold ischaemia time), after 18h of cold ischaemia, and 3h after the transplantation; (F) comparison of the inflammation scores and (H) of the apoptotic cells rates in epithelial cells. UR, Uterus retrieval; UTx, Uterus transplantation; SCS HTK, Static cold storage with a HTK solution; HMP UW, Hypothermic machine perfusion with UWMP solution; SCS UW, Static cold storage with a UW solution; CI, Cold ischaemia.

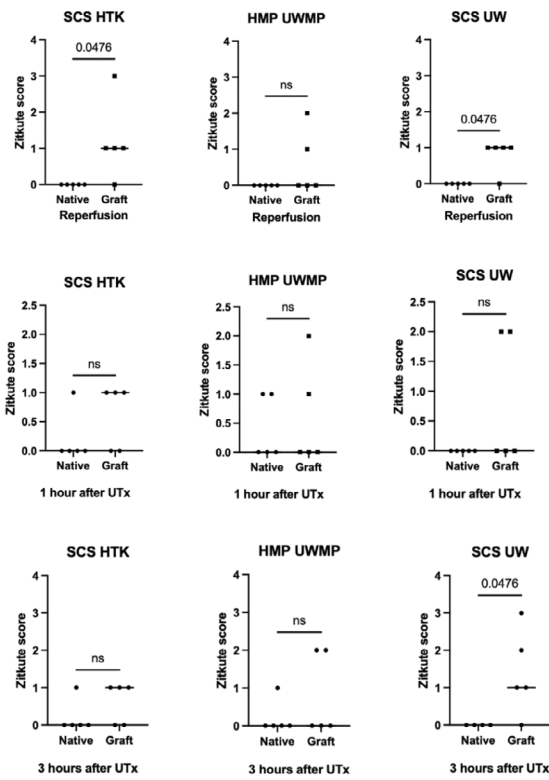
(A) Mean Zitkute score by study group



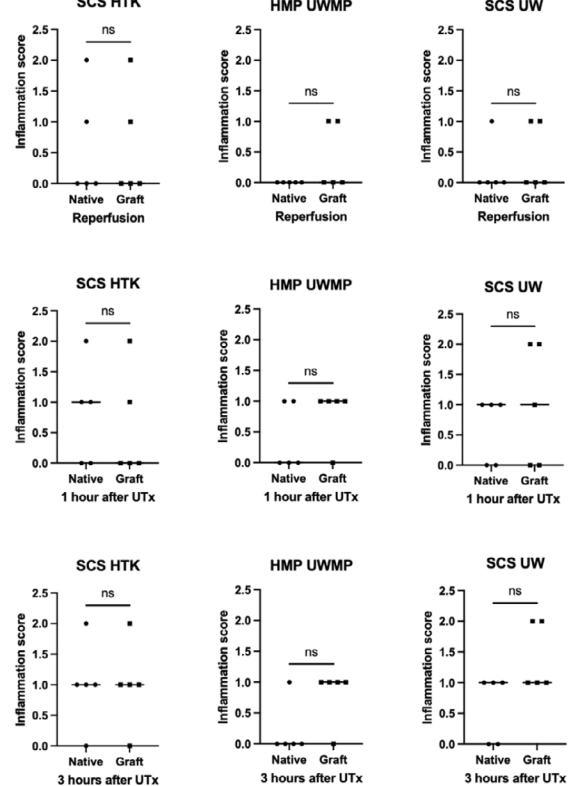
(B) Mean inflammation score by study group



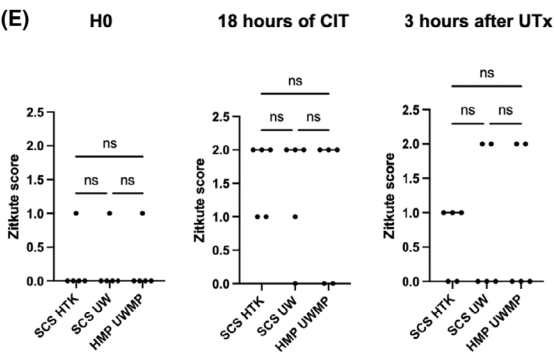
(C)



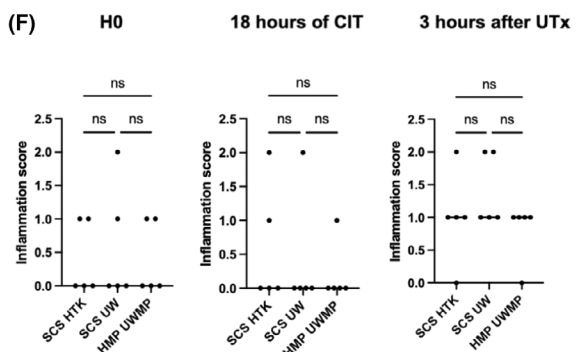
(D)



(E)



(F)



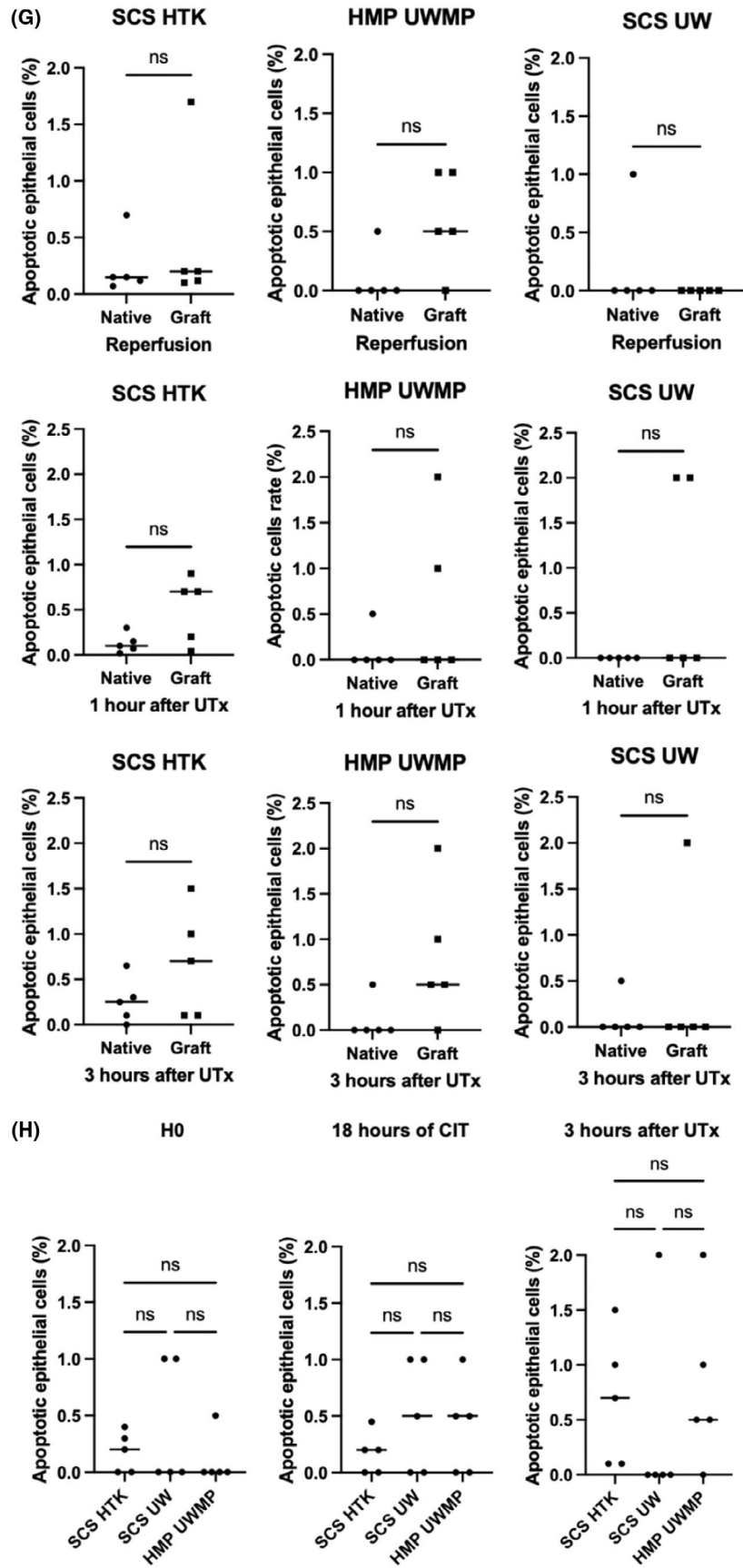


FIGURE 3 (Continued)

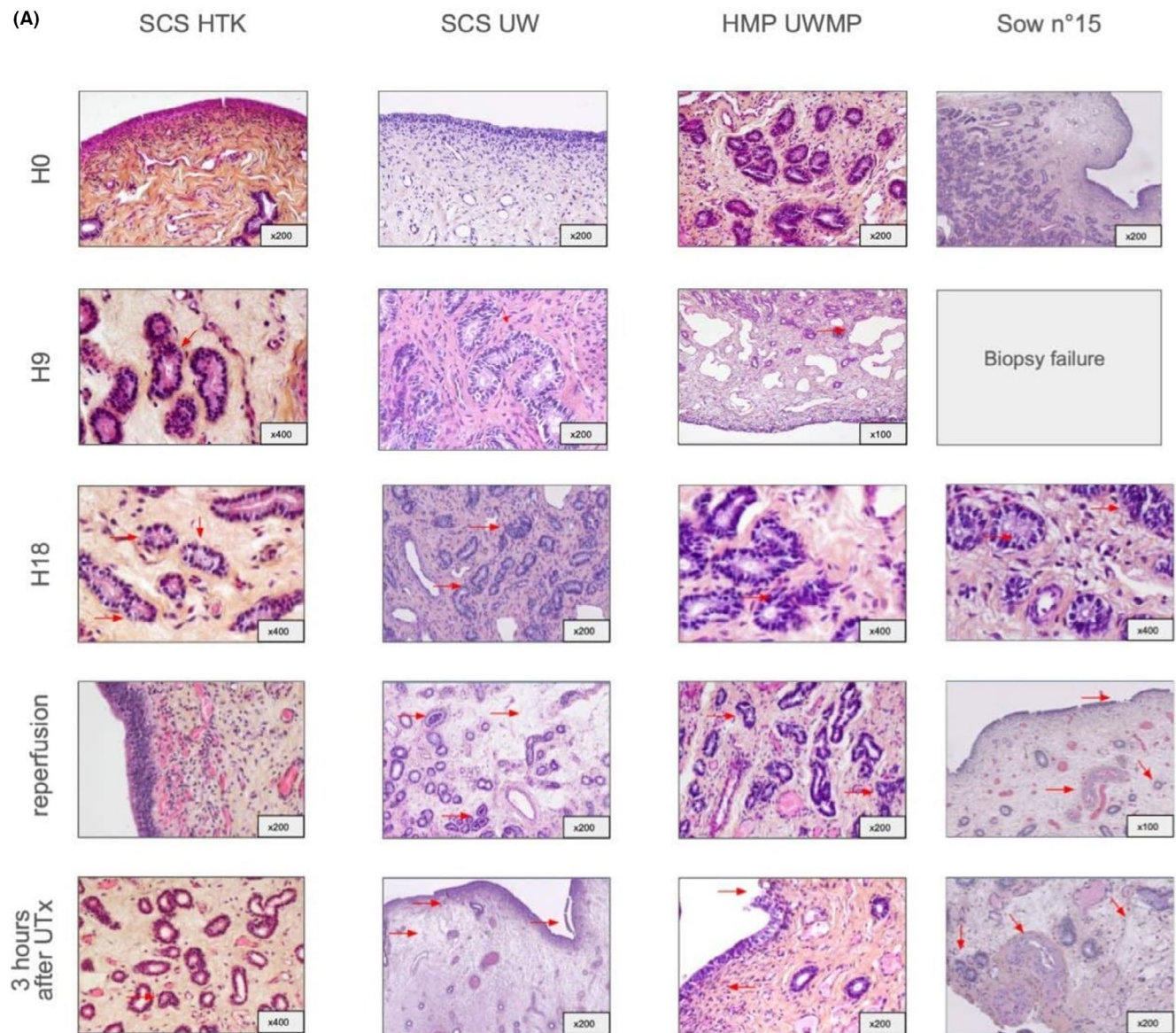


FIGURE 4 (A) Biopsies of uterine horns after H&E staining. Lesions of interest and/or described in the Zitkute score are indicated with red arrows: Apoptotic cells, impaired basement membrane integrity, endometrial cell loss, edema, and blood vessel dilation in the HMP-UWMP group after 9 and 18h of cold ischaemia. The three first columns showed one figure representative of all experiments. In the right column, uterine horn biopsies of the recipient sow n°15 (SCS-UW group) after H&E staining: Red arrows indicate lesions of impaired basement membrane integrity within the glands, edema, and abnormal vessels with partial thrombosis and intimal cell proliferation. (B) Biopsies of uterine horns under light microscopy after cleaved Caspase 3 staining. Apoptotic bodies are indicated with red arrows. The three first columns showed one figure representative of all experiments. In the right column, uterine horn biopsies of the recipient sow n°15 (SCS-UW group). SCS HTK: Static cold storage with a HTK solution; HMP UW: Hypothermic machine perfusion with UWMP solution; SCS UW: Static cold storage with a UW solution.

activity. Plasma lactates, kalemia, myoglobinemia, LDH activity and CK activity are represented in [Figure S2](#).

There was a significant increase in plasma lactates, glucose, potassium, and myoglobinemia after the transplantation ([Figure S2](#)). Three h after the transplantation, myoglobinemia was found to be significantly higher in the HMP-UWMP group than in the SCS-UW group; no difference was found between the HMP-UWMP group and the SCS-HTK group.

The plasma concentration of these six biochemical markers were higher in sow n°6, which died prematurely and experienced hemodynamic instability during the transplantation, but not in sow n°15,

where the graft was not reperfused appropriately and did not meet the macroscopic criteria established for this study.

3.3.4 | Plasmatic proteins dosage

Proteins detected in the blood samples taken from the sows are presented in [Figure S1](#).

Serpin E1,²⁴ a protein inhibiting fibrinolysis, PDGF-1,²⁵ chemokine involved in cellular growth and mobility, and recruitment of fibroblasts, and TGF-beta²⁶ involved in healing mechanisms and if

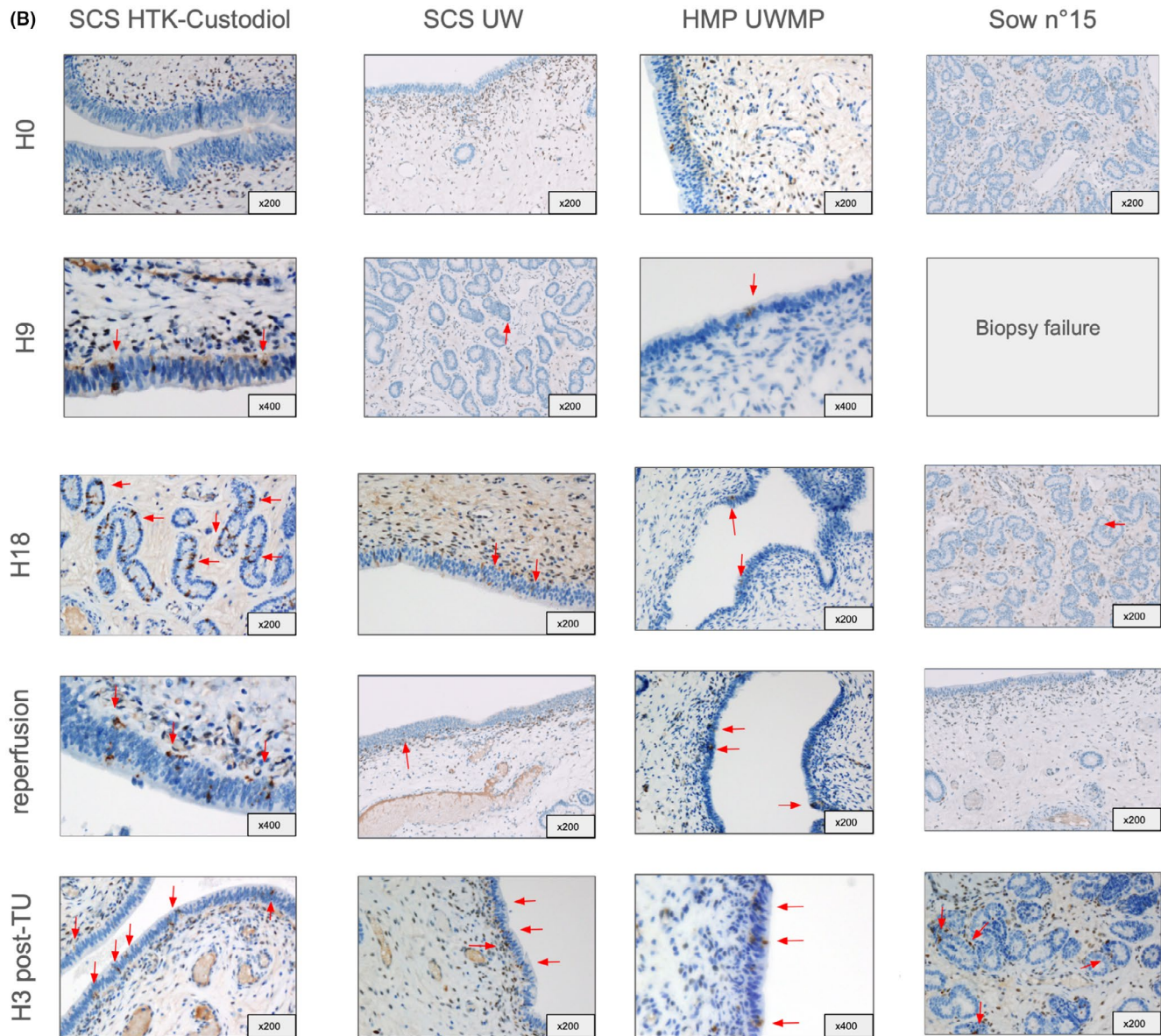


FIGURE 4 (Continued)

dysregulated, fibrosis, did not significantly increase during the 3h post-transplantation. Interleukins (IL-4, IL-12, IL-6, IL-18, IL1-RA), cytokines involved in inflammation pathways,²⁷⁻³⁰ MMP-1 involved in the regulation of the extracellular of the extracellular matrix, angiogenesis, and cell growth and apoptosis³¹; and CD31/PECAM1, involved in the maintaining and restoring of the vascular permeability barrier after a stress,³² significantly increased after the transplantation. The evolution of their plasmatic concentration in sow n°15 and n°6 are shown in Figure S1. The plasmatic concentration of MMP-1 seems higher in sow n°15, which did not meet macroscopic criteria of graft vitality; nevertheless, the mean plasmatic concentrations of all the transplanted sows were only significantly higher than before the transplantation 3h after the surgery, which is the only moment when the plasmatic concentration of MMP-1 in sow n°15 is finally inferior to the mean concentration (Figure S3).

The plasma concentration of IL-4 was significantly higher in the SCS-HTK group than in the HMP-UWMP group 3h after the transplantation, with no difference observed between the SCS-UW and HMP-UWMP groups (Figure S3).

4 | DISCUSSION

In the present study, a slightly better preservation of the uterine graft was observed with HMP despite longer warm ischaemia times in the HMP-UWMP group. Zitekute scores of the grafts exposed to static cold storage were significantly higher than the scores of native uteri; the clinical implication of this difference is yet to be defined. This impact disappears in the h following the transplantation, which could mean a progressive graft recovery after the acute reperfusion phase. It is worth noting that the grafts exposed to static cold

storage with the UW solution (SCS-UW group) had a significantly higher Zitekute score again 3h after the surgery, which is another argument in favor of the machine's own impact on the preservation of grafts. Thus, it seems that not only is machine perfusion not detrimental to grafts, but it could also prepare them better for the lesions induced by the ischaemia-reperfusion phenomena. This advantage, already exploited in kidney and liver transplantation, could allow for a longer cold ischaemia time, which could alleviate the time limitations between graft retrieval and transplantation, thus increasing the distance acceptable between the donor's center, and the transplantation center.

This brief difference, only observed during reperfusion, matches the data previously published, either on animal models and samples of human uteri. The uterus seems to be a very resilient organ, allowing for full-term pregnancies after 9h of cold ischaemia time,⁶ or after emergency embolization of the uterine arteries in cases of post-partum hemorrhage.³³

The preservation solution used in this study were chosen based on the previously published works in UTx: to this day, most teams involved in UTx worldwide work either with the HTK solution (Histidine-Tryptophan-Ketoglutarate), or either with the UW solution³⁴; the superiority of either solution in this context has not yet been studied. As for the brain-dead donor situation, HTK solution is preferred for multiple reasons: its cost, its acceptability by all the teams potentially involved in multiple organ harvesting procedures. Indeed, higher concentrations in potassium and calcium of the UW solution could be responsible for the lesser results observed in heart and lung transplantation³⁵ and its high viscosity impairs the vascular washout. The composition of the UW solution was slightly modified for machine perfusion by replacing potassium lactobionate by sodium gluconate, and increasing its concentration in sodium. This solution, UWMP, still uses HES (hydroxyethyl starch) of the original solution, therefore keeping a high viscosity, but in this pressurized perfusion situation, this could be an asset as it is expected to protect the vessels of the graft from the barotraumas inflicted by the waves of perfusion.³⁵

Some limitations should be considered in this work. First, the animals were euthanized 3h after the transplantation. There was no evaluation of the graft days of weeks or months after, nor any attempt for a pregnancy in the recipient sows. Therefore, the impact of this prolonged cold ischaemia time and of the HMP on the rates of live births after transplantation was not studied here and will need further evaluation. Second, the histological score of uterus tissue damage in static cold storage published by Zitekute et al. was initially described in rats; the absence of smooth muscle contraction in this study could be explained by the use of a score that does not suit the pig model. Nevertheless, to this day, there is no histological score for porcine uteri, as few studies focused on this organ's tolerance to cold ischaemia, and animal experimentation in UTx primarily focused on murine, ovine and non-human primate models. Another limitation was the uncomplete reproduction of the circumstances of a brain-dead donor setting, as there was no simulation of the hemodynamic instability usually encountered with this kind of donors, due to the

cytokine storm; therefore, the grafts were not exposed to the same level of hypoperfusion before the organ retrieval, and the levels of inflammation may have been underestimated.

The pioneering team led by Pr Brännström worked on a porcine model in 2006 but faced a low success rate of the transplantation: only seven grafts out of 19 autotransplantations performed were correctly discolored and only four of them seemed well-perfused at the end of the procedure.³⁶ The team therefore abandoned the pig model for the ovine and non-human primate model. However, in their study, the sows were older (6–8 months old) and oestrus already happened (usually between 5 and 9 months of age³⁷) and therefore had long uterine horns, approximately 1m long from one end to the other, which could have prevented an adequate exposure during the surgery. Vessels were sectioned at their origin, and anastomoses were performed on the same site as the section, and ovarian vessels were cut; and even though the vessels were continuously flushed with heparin during 2h of cold ischaemia time and during the vascular anastomoses, no systemic thromboprophylaxis was performed, by fear of uncontrolled bleeding; these elements may have contributed to the low rate of correctly discolored grafts, the presence of constriction and thrombosis on the anastomosis sites and the difficult access to the vessels chosen for the anastomoses. Another limitation of this model is the importance of the gonadic vessels in the vascularization of the uterus: their preservation required a large *en bloc* retrieval of the graft, larger than in the procedure performed in women. Here, gonadic vessels were preserved by retrieving the whole vasculature starting from the aorta and the vena cava, under the renal vessels; the graft was then connected to the machine following the same “one vein, one artery” mode already used for kidneys, using the vena cava and the aorta of the graft. Vascular anastomoses between the external iliac vessels of the recipient and the vena cava and aorta of the graft allowed for the correct reperfusion of the graft via its gonadic and uterine vessels, which were often of a very small caliber and hard to preserve. This technique made the anastomoses quicker and easier to perform in this model, but remains distinct from the procedure performed in women.

Secondary outcomes of this study involved the search for a specific tolerance and vitality marker for the uterine graft, which could be used in the early post-operative stages, and ideally, until the menses or until successful embryo transfer. Plasmatic markers already used in clinical practice and in solid organ transplantation, and proteins involved in inflammation, tissue homeostasis and healing studied in this work did not meet these criteria. The increase observed in biochemical markers (plasmatic lactates, kalemia, glucose, and myoglobinemia) was not correlated to the macroscopic aspect of the graft, which was the only clinically significant parameter available in this study. Immunoassays of plasmatic proteins met the same limitations, as interleukins and other chemokines are involved in inflammation and tissue homeostasis mechanisms, probably largely activated in sow n°6, as in the other animals.

The pro-inflammatory response seen in the first hour after the transplantation has been a subject of research in other solid

organ transplantations: the recruitment of chemokines, other pro-inflammatory factors (IFN- γ , TNF- α) potentiating the inflammatory response, the activation of pathways leading to apoptosis, increased endothelial permeability and tissue fibrosis, have been linked to chronic allograft rejection when the inflammation persists, for example due to ischemia–reperfusion injuries, potentially boosted by the systemic effects of the cytokinetic storm found in brain-dead donors.³⁸ Some of these pro-inflammatory factors, receptors and pathways could be potential targets for new or already existing drugs, to hamper their effect and reduce ischemia–reperfusion injuries.³⁹

The absence of identification of a specific and sensitive biomarker in this study could also be explained by the apparent very good tolerance of the graft to a prolonged cold ischaemia time, as shown in this histological analysis of the uterine horns biopsies. Further research is needed to evaluate its tolerance to a cold ischaemia time of more than 18 h.

5 | CONCLUSION

This study has proven the feasibility of HMP in UTx in a porcine model. Furthermore, the technique was not detrimental for the grafts, but it also seemed to preserve the grafts better during the early phases of reperfusion. The impact of HMP on the success rates of UTx through its potential effect on microvascularization will need further research, particularly on its impact on live births rates. The research for a sensitive and specific biomarker of vitality of the uterus graft amongst biochemical and protein plasma markers already exploited in clinical practice has to be continued. Further analysis using proteomics and transcriptomics technologies on endometrium samples taken during the cold ischaemia time and post transplantation period could provide new markers.

AUTHOR CONTRIBUTIONS

Carla Hélène Sousa: Writing—Original Draft, Formal Analysis, Funding Acquisition, Data Curation, Investigation; **Marion Mercier:** Data Curation, Investigation; **Nathalie Rioux-Leclercq:** Formal Analysis, Investigation, Methodology; **Erwan Flecher:** Investigation, Methodology; **Claude Bendavid:** Formal Analysis, Investigation, Methodology; **David Val-Laillet:** Methodology, Project Administration, Resources, Writing—Review & Editing; **Juliette Ferrant:** Methodology, Resources; **Sylvie Jaillard:** Methodology; **Emma Loiseau:** Writing—Review & Editing; **Julien Branchereau:** Writing: Review & Editing; **Yanis Berkane:** Writing: Review & Editing; **Krystel Nyangoh Timoh:** Resources; **Isis Carton:** Project Administration; **Maëla Le Lous:** Writing: Review & Editing; **Vincent Lavoue:** Conceptualization, Methodology, Supervision, Validation, Writing: Review & Editing; **Ludivine Dion:** Conceptualization, Funding Acquisition, Investigation, Methodology, Supervision, Writing: Review & Editing.

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CONFLICT OF INTEREST STATEMENT

The authors have nothing to report.

ETHICS STATEMENT

This study was conducted in strict application of the 2010/63/UE directive of the European parliament and the Council of 22 September 2010 on the protection of animals used for scientific purposes (Facility Agreement No C35-275-32). This work was authorized by the French Superior Education, Research and Innovation Ministry, registered under the APAFIS number #32127–2021.092.915.368.626.v2 on September 17, 2021.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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