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ORIGINAL RESEARCH

Analysis of two reperfusion techniques in uterine transplantation in an experimental model

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Abstract

Introduction: Uterine transplantation was developed for the treatment of absolute uterine factor infertility. As it is a new modality of transplantation, there is still room for technical improvement. A factor that impacts graft survival in organ transplantation is the warm ischemia time. In uterine transplantation specifically, at least two vascular anastomoses are performed on each side of the uterus, and the graft revascularization takes place when the vascular clamps of the arteries and veins are released on both sides simultaneously. For this reason, the warm ischemia time in uterine transplant is expected to be considerably long. The purpose of this study was to compare the sequential technique of uterine graft revascularization, which aims to reduce the warm ischemia time of the procedure, with the simultaneous revascularization technique.

Material and Methods: For the procedure, the uterine auto-transplantation technique was performed using 10 non-pregnant adult ewes weighing about 45 kg, divided into two groups: simultaneous revascularization group (5 animals) and sequential revascularization group (5 animals). To evaluate the groups, we analyzed the procedure and warm ischemia times, graft macroscopy, hemodynamic, laboratory, and histological parameters of the uterus.

Results: The sequential revascularization technique group had similar surgical procedure times, and the warm ischemia time was significantly shorter with medians of 32 min in the sequential group vs 72 min in the simultaneous group ($p < 0.008$). The

Abbreviations: AST, Aspartate aminotransferase; CPK, Creatine phosphokinase; LV, Left vein; MAP, Mean arterial pressure; RV, Right vein; SE, Sequential graft revascularization; SI, Simultaneous graft revascularization; T0, Time zero; T1, Time one; T2, Time two; WIT, Warm ischemia time.

graft macroscopy and hemodynamic, laboratory, and histological parameters evaluated were similar between the groups.

Conclusions: The sequential revascularization technique proved to reduce the warm ischemia time in the sheep uterine auto-transplantation model without compromising graft viability.

KEYWORDS
infertility, reperfusion, transplantation, uterus, warm ischemia

1 | INTRODUCTION

Uterine transplantation was developed to treat patients with absolute uterine factor infertility, for whom surgical repair is not an option.[1] Other options for these patients to achieve motherhood include pregnancy via surrogate uterus (SU) and adoption, but neither of these options allows the patient to conceive.[2]

Uterine factor infertility (IFU) is defined as an infertility condition exclusively related to the uterus, with a prevalence of approximately 3% to 5% of the general population.[3,4]

The first attempt at uterus transplantation in the modern era was a living donor uterus transplantation performed by Fageeh et al. in 2000 in Jeddah, Saudi Arabia.[5] The first clinical trial began in Sweden in 2012, with the first birth occurring in September 2014.[6,7]

A known factor impacting graft survival in organ transplantation is warm ischemia time (WIT).[8,9] Given the necessity of at least four small and delicate vascular anastomoses, combined with the revascularization of the graft after vascular anastomoses on both sides, a considerably long WIT is expected in uterine transplantation. Brannstrom et al. reported an average warm ischemia time in uterine transplantation of 1 h and 23 min (±9 min).[6] Although no clinical studies have evaluated the impact of WIT on uterine grafts, Garcia et al. demonstrated in an animal model that uterine grafts subjected to prolonged WIT exhibited more intense morphological changes, indicating harmful effects on the organ.[10]

In a previous study, our group described the sequential revascularization technique of the uterine graft in an animal model. This technique allows for a reduction in warm ischemia time in uterine transplantation.[11] The objective of this study was to compare the sequential revascularization technique with the simultaneous uterine graft revascularization technique.

Key message

The purpose of this study was to compare the sequential technique of uterine graft revascularization with the simultaneous revascularization technique. The sequential revascularization technique proved to reduce warm ischemia time in the sheep uterine auto-transplantation model without compromising graft viability.

2 | MATERIAL AND METHODS

All animals were handled at the University of São Paulo Medical School. Adult sheep weighing approximately 45 kg, not pregnant, were utilized. The uterine autotransplantation technique was performed, involving the removal of the uterus while preserving its anatomical structures and subsequent implantation in the same animal, albeit in a different location from the original. The experiment took place at the Medical Research Laboratory 37 (LIM 37) of Experimental Surgery at the University of São Paulo Medical School.

A total of 10 animals were utilized and divided into two groups:

- Simultaneous graft revascularization (SI) group, where vascular anastomoses (uterine arteries and veins) were performed on both sides of the uterus, starting on the right side, with graft revascularization conducted simultaneously on both sides.
- Sequential graft revascularization (SE) group, where the anastomoses of the right uterine artery and vein were initially performed, followed by graft revascularization, after which the anastomoses of the left uterine artery and vein were performed.

2.1 | Variables studied

To evaluate the groups, the following analyses were conducted: warm ischemia time, hemodynamic parameters (mean arterial pressure [MAP]), macroscopic evaluation of the graft, laboratory assessments (aspartate aminotransferase [AST], creatine phosphokinase [CPK], and lactate), and graft histology.

2.2 | Macroscopic analysis

The macroscopic evaluation of the graft was performed by the surgical team, considering the quality of the organ's perfusion based on the following parameters: homogeneous or heterogeneous, perfusion time ≤30 or >30 s, and unilateral or bilateral perfusion.

2.3 | Laboratory analysis

The laboratory analyses were conducted at the Special Analysis Laboratory of the Medical Research Laboratory 03 (LAE-LIM 03), Department of Pathology, using the Cobas C111 device (ROCHE, Indianapolis, IN, USA). All kits used in the exams were from ROCHE, employing UV kinetic methodology. The lactate test was performed using the ABL 800 FLEX device (Radiometer, Copenhagen, Denmark) with selective ion electrodes. Whole blood samples for laboratory analysis were collected at three-time points during the procedure: immediately after abdominal incision (T0), immediately after graft revascularization (T1), and 1 h after graft revascularization (T2). The first sample (T0) was collected via the central venous catheter, while the subsequent blood samples (T1 and T2) were collected from the right and left uterine veins and analyzed separately.

2.4 | Histological analysis

The histological evaluation of the graft was conducted by a specialized transplant pathologist from the Pathology Department of the Clinics Hospital of the University of São Paulo Medical School. Uterine biopsies were taken from both sides of the graft at three-time points: at the beginning of the procedure (T0), immediately after graft revascularization (T1), and 1 h after graft revascularization (T2). The uterine tissue fragments were fixed in a 2% formaldehyde solution and stained with hematoxylin and eosin. Subsequently, they were subjected to histological analysis via optical microscopy by a single pathologist, who was blinded to the group allocation of each studied fragment. The histological parameters used to assess tissue damage included edema, stasis, necrosis, apoptosis, and neutrophils in the tissue.[12,13]

2.5 | Anesthetic procedure

The anesthetic procedure began with intravenous induction using propofol at a dose of 5 mg/kg, followed by intubation with an orotracheal tube (size 7.5 or 8 mm), determined based on assessment by the veterinary anesthetist. Anesthesia maintenance was achieved with 1.5% isoflurane delivered in 100% oxygen, supplemented with a continuous infusion of fentanyl at a rate of 10 μg/kg/min and neuromuscular blockade using pancuronium at a dose of 0.05 mg/kg. Mechanical ventilation was employed with a controlled volume set at 10 mL/kg tidal volume, with adjustments made to the respiratory rate to maintain the end-tidal CO_2 concentration ($ETCO_2$) within the range of 35–45 mmHg. Hemodynamic monitoring was conducted using a Swan–Ganz catheter placed in the jugular vein. The entire anesthetic procedure was overseen and managed by a veterinary anesthetist experienced in experimental surgery.

2.6 | Surgical procedure

Abdominal access was gained through a median laparotomy, following which the visceral and vascular structures were assessed, and the animal's digestive tract was mobilized to enhance exposure of the pelvic cavity. At this juncture, the initial blood samples for laboratory analysis and biopsies (T0) were obtained. The procedure commenced with the sectioning of the broad ligament, followed by the bilateral dissection of the uterine vessels and external iliac vessels (later used for graft implantation). The uterine vessels were dissected bilaterally up to the iliac vessels. The internal iliac artery was dissected approximately 3 mm proximally and distally from the insertion of the uterine artery, place where the transection was performed, creating an arterial path to facilitate the arterial anastomosis at the time of implantation. The utero-ovarian vein was dissected approximately 10 mm before the insertion into the internal iliac vein, place where the transection was performed. The ovaries and oviducts were included in the uterine graft.

Subsequently, the bladder and ureters were dissected, and the vaginal vault was identified and sectioned. The uterine vessels were then severed, and the uterus was removed.

On the back table, the graft was cooled and perfused with approximately 250 mL of ice-cold Lactated Ringer's solution (5000 U of unfractionated heparin was added to the solution). The decision to use Ringer's lactate rather than preservation solution for organ perfusion was based on the fact that the graft was not kept in static storage. After the end of the back table procedure, the organ was immediately reimplanted. The back table procedure took an average of 35 min in both groups studied.

Perfusion was carried out through the uterine arteries until the solution returned completely clear through the uterine veins. At this stage, the uterine arteries and the distal portion of the uterino-ovarian veins were prepared for implantation.

The uterine graft was subsequently placed in the pelvic cavity, and vascular anastomoses were performed. The sutures for both the uterine artery and vein were performed with 7–0 Prolene in a continuous suture technique. In the SI group, anastomoses were executed on both sides of the uterus, followed by graft revascularization. In the SE group, after vascular anastomosis on the right side, reperfusion of the graft was conducted, followed by vascular anastomoses on the left side of the uterus. Immediately after revascularization, blood samples for laboratory analysis and biopsies (T1) were obtained. Following this, the graft was affixed to the peritoneum, and the vagina was sutured. One-hour post-graft revascularization, blood samples for laboratory analysis and biopsies (T2) were collected.

Upon completion of the procedure, the animal was euthanized by increasing the isoflurane vaporizer from 1.5% to 5% and administering intravenous potassium chloride. The animal was then placed in a designated plastic bag for infectious material and sent for incineration at the University of São Paulo School of Medicine.

2.7 | Statistical analyses

All analyses were conducted with a two-tailed alpha (p-value) of 0.05 and a confidence interval (CI) of 95%, utilizing computational support from R or IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, NY, USA).

Data were described using frequency and confidence intervals for qualitative variables, while measures of central tendency (mean and median) and measures of dispersion (standard deviation, interquartile range, minimum, and maximum) were employed for quantitative data. Non-parametric tests were chosen due to the number of animals utilized in the experiment.

The association between qualitative variables and the type of revascularization was assessed using the chi-square test, while the association between quantitative variables and the type of revascularization was evaluated using the Wilcoxon test.

3 | RESULTS

Warm ischemia time was significantly shorter in the sequential revascularization group ($p=0.008$). Table 1 presents the descriptive statistics for each surgical group, indicating that the warm ischemia time in the sequential technique group is less than half the time compared to the simultaneous technique. Additionally, Figure 1 displays a box plot illustrating that there is no overlap between the warm ischemia times of the groups, further supporting the result observed in the statistical analysis.

Regarding hemodynamic assessment, Supporting Information Table S1 presents the MAP data at each time point, separated by group. It was not possible to observe a significant difference between the groups. Additionally, in both groups, the MAP appears to decrease similarly over time.

TABLE 1 Descriptive statistics of surgery and ischemia times according to the type of revascularization.

	Revascularization type		p-value
	Sequential	Simultaneous	Wilcoxon
Surgery time (min)			
Average (±SD)	242 (±22.53)	242 (±18.28)	1
Median (IQR)	242 (220–263)	247 (234–248)	
Min–max	219–266	215–264	
Warm ischemia time (min)			
Average (±SD)	32 (±3.27)	70 (±4.15)	0.008
Median (IQR)	32 (31–33)	72 (68–73)	
Min–max	27–36	64–74	
Total ischemia time (min)			
Average (±SD)	76 (±16.69)	97 (±13.22)	0.151
Median (IQR)	68 (67–80)	100 (90–102)	
Min–max	61–103	78–113	

Regarding the macroscopic evaluation of the graft, in all 5 cases of the SI group, uterine revascularization occurred quickly and homogeneously on both sides of the graft simultaneously (Figure 2). In the SE group (Figure 3), following revascularization via the right uterine artery, perfusion occurred rapidly and uniformly on the right side of the graft in all cases. On the left side of the graft, perfusion was rapid and homogeneous in 4 cases, while in only 1 case (Animal 10), perfusion was slow, with complete perfusion achieved 55 s after reperfusion.

In the laboratory analysis (Table S2), it was not possible to observe a significant difference in the levels of AST enzyme, CPK, and lactate when comparing the surgical techniques between the groups. Although there were differences in AST values between different time points and among the groups, all differences fell within the statistical variation. At time zero, a considerable difference in AST parameters was observed between groups, but the high variability within each group prevented the establishment of a statistical difference. As for CPK, the values appeared to increase over time, but the difference between T2 and T0 was similar across all groups.

The results of the histopathological comparisons of the groups at each time and side evaluated are presented in Table 2, where none of the histological variables at each time found a significant association with the experimental groups. At T2, edema was present in 60% of cases in the right vein (RV) and 60% in the left vein (LV) in the sequential revascularization group, and in 75% of cases in RV and 80% in LV in the simultaneous group. Stasis was present in 100% of cases in RV and 80% in LV in the sequential revascularization group, and in 50% of cases in RV and 80% in LV in the simultaneous group. The presence of neutrophils was identified in 20% of cases in RV and 20% in LV in the sequential revascularization group, and in 20% in LV in the simultaneous group.

4 | DISCUSSION

The term WIT is used to describe the ischemia of cells and tissues under normothermic conditions. In the scenario of organ transplantation, this term refers to ischemia during implantation, which occurs from the removal of the organ from the ice until its reperfusion.[14] As at least four small and delicate vascular anastomoses are necessary, and the graft is revascularized only after anastomoses have been performed on both sides of the uterus, the WIT becomes considerably long. Brannstrom et al., in the first clinical trial on uterine transplantation, reported an average WIT of 1 h and 23 min (±9 min).[6]

The impact of WIT on the uterine graft was studied by Garcia et al., who evaluated, in an animal model using rats, the viability of the transplanted uterus after exposure to a long WIT. Evident signs of necrosis were observed in the group of animals subjected to extended warm ischemia.[10]

There are no clinical studies evaluating WIT in uterine transplantation; however, clinical studies carried out on other transplanted organs clearly demonstrate its impact. Marasco et al., in a

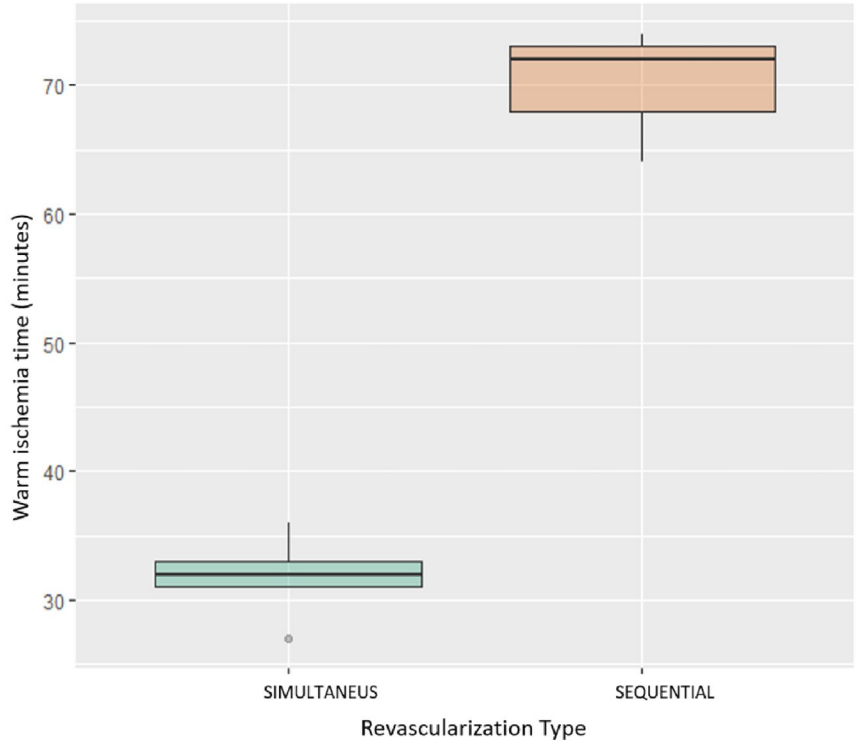

FIGURE 1 Warm ischemia time (in min), by type of revascularization.

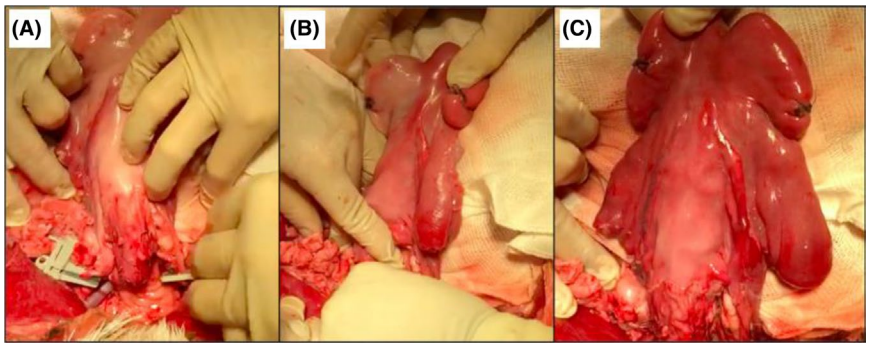

FIGURE 2 Example of the appearance of uterine perfusion (animal 5) in simultaneous revascularization at the initial (A), 30 s (B) and 60 s (C) moments after perfusion. Demonstrating the completely perfused uterus at 30 s post perfusion.

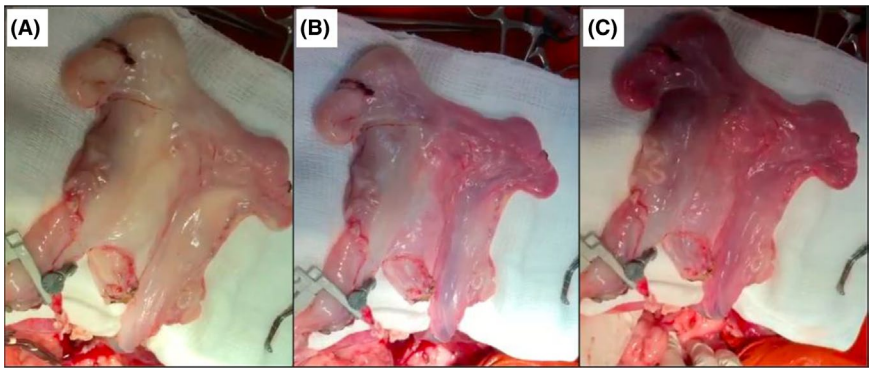

FIGURE 3 Example of the appearance of uterus perfusion (animal 02) in sequential revascularization at the initial (A), 30 s (B) and 60 s (C) moments after perfusion. Demonstrating the completely perfused uterus at 30 s post-perfusion.

retrospective study with 206 patients undergoing heart transplantation, demonstrated that a WIT of 80 min was associated with significantly reduced graft survival compared to a WIT of 60 min.[8] In a study on patients undergoing kidney transplantation, which evaluated the association between WIT and mortality and graft dysfunction, it was observed that the group that underwent a longer WIT (>60 min) presented a 23% increase in the adjusted relative risk of death or graft dysfunction.[9] Another study conducted in liver transplantation, which evaluated pre and perioperative factors associated with graft dysfunction, demonstrated that in an incidence of primary graft dysfunction of 31.9%, prolonged WIT (>45 min) was the only significant risk factor among other parameters evaluated.[15]

Therefore, based on experimental studies in uterine transplantation and clinical studies carried out on other transplanted organs, it

TABLE 2 Frequency of histological analysis variables by type of revascularization, with statistical association test (χ^2).

		Revascularization type				
		Sequential		Simultaneous		
		N	% (CI 95%)	*N*	% (CI 95%)	*p*-value (χ^2)
Edema						
RV						
(T1)	Absent	2	40 (9.44 to 79.06)	3	75 (28.38 to 97.15)	0.708
	Present	3	60 (20.94 to 90.56)	1	25 (2.85 to 71.62)	
(T2)	Absent	2	40 (9.44 to 79.06)	1	25 (2.85 to 71.62)	1
	Present	3	60 (20.94 to 90.56)	3	75 (28.38 to 97.15)	
LV						
(T1)	Absent	1	20 (2.25 to 62.86)	2	50 (12.28 to 87.72)	0.812
	Present	4	80 (37.14 to 97.75)	2	50 (12.28 to 87.72)	
(T2)	Absent	2	40 (9.44 to 79.06)	1	20 (2.25 to 62.86)	1
	Present	3	60 (20.94 to 90.56)	4	80 (37.14 to 97.75)	
Stasis						
RV						
(T0)	Absent	1	20 (2.25 to 62.86)			0.357
	Diffuse			1	25 (2.85 to 71.62)	
	Focal	4	80 (37.14 to 97.75)	3	75 (28.38 to 97.15)	
(T1)	Absent	1	20 (2.25 to 62.86)			0.165
	Diffuse			2	50 (12.28 to 87.72)	
	Focal	4	80 (37.14 to 97.75)	2	50 (12.28 to 87.72)	
(T2)	Absent			2	50 (12.28 to 87.72)	0.154
	Diffuse	4	80 (37.14 to 97.75)	1	25 (2.85 to 71.62)	
	Focal	1	20 (2.25 to 62.86)	1	25 (2.85 to 71.62)	
LV						
(T0)	Absent	1	25 (2.85 to 71.62)	1	25 (2.85 to 71.62)	0.549
	Diffuse			1	25 (2.85 to 71.62)	
	Focal	3	75 (28.38 to 97.15)	2	50 (12.28 to 87.72)	
(T1)	Absent			1	25 (2.85 to 71.62)	0.384
	Diffuse	3	60 (20.94 to 90.56)	1	25 (2.85 to 71.62)	
	Focal	2	40 (9.44 to 79.06)	2	50 (12.28 to 87.72)	
(T2)	Absent			1	20 (2.25 to 62.86)	0.368
	Diffuse	4	80 (37.14 to 97.75)	2	40 (9.44 to 79.06)	
	Focal	1	20 (2.25 to 62.86)	2	40 (9.44 to 79.06)	
Neutrophils in tissues						
RV						
(T1)	Absent	1	20 (2.25 to 62.86)			1
	Present	4	80 (37.14 to 97.75)	4	100 (100 to 100)	
(T2)	Absent	1	20 (2.25 to 62.86)			1
	Present	4	80 (37.14 to 97.75)	4	100 (100 to 100)	
LV						
(T1)	Absent	1	20 (2.25 to 62.86)			1
	Present	4	80 (37.14 to 97.75)	4	100 (100 to 100)	
(T2)	Absent	1	20 (2.25 to 62.86)	1	20 (2.25 to 62.86)	1
	Present	4	80 (37.14 to 97.75)	4	80 (37.14 to 97.75)	

seems very relevant to seek alternatives to reduce WIT in the context of uterine transplantation.

In a previous study, our group described the technique of sequential revascularization of the uterine graft, aiming to reduce WIT associated with the procedure.[11]

In the present study, comparing sequential revascularization with simultaneous revascularization tecniques, both groups had a similar surgical procedure time, with a median of 242 min (range: 220–263 min) in the SE group vs 247 min (range: 234–248 min) in the SI group ($p = 1.00$), demonstrating that the described technique does not increase the surgical time of the procedure. Additionally, in the group in which the sequential uterine graft revascularization technique was used, there was a significant reduction in the median WIT from 72.00 min in the SI group to 32.00 min in the SE group ($p = 0.008$).

There was also no significant difference in hemodynamic changes between the groups. Although a reduction MAP was observed mainly at moments T1 and T2, this reduction was not statistically significant and occurred similarly between the groups. Furthermore, in none of the procedures performed was there a need to use a vasoactive drug, and hemodynamic control was maintained solely with adequate volume replacement.

However, it is also important to demonstrate that there is no harm to the organ with the sequential revascularization technique. As previously described, direct vascular revascularization initially occurs on only one side of the graft. The contralateral side is revascularized through communicating vessels, making it necessary to assess whether, despite being indirect, it occurs adequately.

The native uterus has an extensive interconnected vascular network, and its perfusion is carried out by three pairs of arteries: the ovarian, uterine and vaginal arteries.[16] In this context, Shockley et al. have shown that, in baboons, uterine perfusion and drainage can be sustained even after bilateral ligation of the uterine vessels, relying solely on the utero-ovarian vessels.[17]

When evaluating the macroscopic appearance of the grafts, it was observed that in all cases in the SI group, revascularization occurred quickly and homogeneously on both sides of the graft simultaneously. In the SE group, on the left side of the graft, slow perfusion was observed in only 1 out of 5 cases. However, at the end of a period of 55 s, this graft was completely perfused and homogeneous. Therefore, although slower revascularization was observed in one case, this graft was completely perfused even before contralateral direct reperfusion. This demonstrates, in a macroscopic evaluation, adequate perfusion of the organ on both sides of the graft in both groups analyzed.

Unlike what happens, for example, in liver transplantation, where a laboratory test such as alanine aminotransferase can be used as a marker of liver injury, there are still no specific markers for evaluating the graft in the context of uterine transplantation.[18] The uterus is almost entirely composed of muscular tissue (myometrium). Therefore, the markers analyzed were AST, CK, and lactate as they are associated with muscle damage and tissue hypoperfusion.

Evaluating the AST enzyme, it was not possible to observe a significant difference comparing the surgical techniques between the groups. We observed differences in values between times and between groups, but all within the statistical variation.

Analyzing the pattern of CPK, which is a marker mainly related to muscle injury, it is observed that the values seem to increase between times, especially at moments T1 and T2. However, the difference between T2 and T0 was similar between groups. This pattern of CPK evolution demonstrates that although the left side of the graft in the sequential group was revascularized through communicating vessels, no greater impairment of the uterine muscular tissue was observed on this side.

Regarding the lactate variation, an important marker associated with tissue hypoperfusion, a temporal trend of increase is clearly observed. This trend affects groups and sides in a similar way, and despite the large overlap and lack of statistical support, the simultaneous revascularization group seems to present slightly increased values in relation to the other group. This could be explained due to the longer period of warm ischemia observed in this group.

In the present study, none of the histological variables at each time point showed a significant association between the groups studied. Analyzing the T2 moment, we observed that edema and stasis were quite prevalent changes. In the sequential revascularization group, edema was present in 60% of cases in RV and 60% of cases in VE, while in the simultaneous revascularization group, it was present in 75% of cases in RV and 80% of cases in VE. As for stasis, in the sequential revascularization group, it was present in 100% of cases in RV and 80% of cases in VE, and in the simultaneous revascularization group, it was present in 50% of cases in RV and 80% of cases in VE. On the other hand, necrosis and apoptosis were not found in any of the samples analyzed.

Corroborating these findings, Dahm-Kähler et al., when developing a model of uterine autotransplantation in sheep, subjected the grafts to cold ischemia of approximately 1 h followed by warm ischemia of approximately 1.5 h. They did not observe the presence of necrosis, edema, or fragments of apoptotic cells in any of the samples studied. Only mild blood vessel stasis was observed in one of the transplants, demonstrating a very subtle inflammatory reaction.[13]

On the other hand, Garcia et al., evaluating the impact of prolonged WIT in uterine grafts in an experimental model in rats, identified quite exuberant histological changes in grafts subjected to 4 h of warm ischemia. On the third day after the procedure, 3 out of 10 uteri showed severe stromal hemorrhage, thrombosis, edema, loss of endometrial cells, leukocyte infiltration, and necrosis; 4 out of 10 uteri had leukocyte infiltration and edema; 2 out of 10 uteri showed significant edema, with a total absence of lesions being evident in only one uterus. This demonstrates that prolonged WIT is associated with significant histological changes in the uterine graft.[10]

Among the main limitations of this study, we can mention the small number of animals studied in each group, which is associated with the fact that we chose to use the smallest possible number of

animals to carry out laboratory experiments, as well as with the high cost of these animals. Furthermore, the final evaluation of the graft was carried out in a short period of time (just 1 h after reperfusion), mainly due to the impossibility of keeping the animal alive in the available structure of the laboratory used. Finally, another limitation was the fact that the animals were not kept alive for a longer period after the procedure, and thus we were unable to compare the long-term uterine viability and the gestation capacity of the transplanted uterus in the groups analyzed.

5 | CONCLUSION

The technique of sequential revascularization of the uterine graft is associated with a significant reduction in WIT, and no important changes were identified in macroscopic, laboratory, and histological evaluations that would suggest a greater degree of tissue damage associated with this technique. A concern with the use of this technique was the possibility that the side of the graft that initially does not receive blood directly through the uterine vessels remains inadequately perfused for a long period, a fact that was not confirmed in the analyses carried out. This suggests that sequential revascularization is a viable technique for uterine transplantation, potentially reducing ischemic injury and contributing to the success of the procedure. However, further studies with larger sample sizes and longer follow-up periods are needed to fully assess the efficacy and safety of this technique in clinical practice.

AUTHOR CONTRIBUTIONS

All authors conception and design, collection and assembly of data, manuscript writing and final approval of manuscript. Additionally Rubens Macedo Arantes, Dani Ejzenberg and Wellington Andraus: surgical procedure. Ryan Yukimatsu Tanigawa: histological evaluation. Amadeu Batista da Silva Neto: anesthetic procedure.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ETHICS STATEMENT

All animals were handled at the University of São Paulo Medical School in accordance with the principles of Law no. 11794, dated October 8, 2008, Decree no. 6899, dated July 15, 2009, along with the regulations published by the National Council for Animal Experimentation Control, and resolution no. 714 of July 20, 2002, of the Federal Council of Veterinary Medicine, which governs euthanasia procedures. The study received approval from the Ethics Committee on the Use of Animals at the University of São Paulo School of Medicine under number 063/16 on June 22, 2016, following approval from the Ethics and Research Committee of the Gastroenterology Department at the Clinics Hospital of the University of São Paulo Medical School.

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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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