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Review

THE CONCEPT OF MACHINE PERFUSION IN UTERUS TRANSPLATATION

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Summary

Uterus transplantation represents the only opportunity for women affected by absolute uterine factor infertility to become pregnant. One of the major challenges in uterus transplantation is represented by the high rate of thrombotic complications, often resulting in graft loss and poor outcomes. Although the exact etiology remains unknown, it is possible that post-implantation thrombosis of the uterine vessels could be the expression of microcirculation failure resulting from ischemia and reperfusion injury (IRI). Uterus machine perfusion could offer a way to ameliorate IRI and protect the graft from vascular complications. Thanks to the experience gained in solid organ *ex-situ* perfusion, it can be speculated that the uterus graft could benefit from different perfusion techniques: hypothermic oxygenated perfusion would reduce the ischemia reperfusion injury, with an impact on graft, recipient and offspring outcomes that is currently unknown; normothermic machine perfusion would allow graft reconditioning, flow measurements and graft testing in a near-physiological environment, helping the physician to understand the relationship between flow, pressure and myometrial function. Lastly, machine perfusion could play a major role in improving the logistics of uterus transplantation, resulting in a safe expansion of the preservation time.

Key words: uterus transplant, machine perfusion, *ex-situ* organ perfusion, infertility

Abbreviations

ALT: alanine aminotransferase
AST; aspartate aminotransferase
ATP: adenosine triphosphate
AUFI: absolute uterine factor infertility
CIT: cold ischemia time
DD: deceased donor
HMP: hypothermic machine perfusion
HOPE: hypothermic oxygenated machine perfusion
IRI: ischemia reperfusion injury
LD: living donor
MP: machine perfusion
NMP: normothermic machine perfusion
ROS: reactive oxygen species
SCS: static cold storage
UT: uterus transplant
VCAs: vascularized composite allografts

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INTRODUCTION

Uterus Transplantation (UT) represents a valid alternative to surrogacy and adoption in women with absolute uterine factor infertility (AUFI). AUFI affects 3-5% of the female general population [1,2] and it can be caused by the complete absence of the uterus (congenital uterine agenesis, previous hysterectomy) or uterine malformations (uterine myomas, adhesions, congenital malformations), leading to implantation failure or placentation defects [3]. Although UT is not a life-saving procedure, it is the only possible treatment for AUFI [4] and remarkable improvements have been made over the last decade in the field of uterine transplantation [5].

In the past, numerous attempts of UT in animal models have been made [3]. In the modern age, the first UT performed in humans goes back to 2000 in Saudi Arabia, where a 26-year-old female was transplanted with a uterus from a 46-year-old living donor [6]. Three months later, unfortunately, the recipient underwent a hysterectomy due to a vascular thrombosis, which led to graft necrosis. The first birth following UT took place in Sweden in 2014 [7]. The recipient was a 35-year-old woman with congenital absence of the uterus who was transplanted with a uterus from a 61-year-old living donor [8]. In the US, the first birth from a living donor (LD) occurred in Texas in 2017 [9] and the first baby born after a deceased donor (DD) UT was in Cleveland - OH in 2019 [10].

Machine perfusion (MP) improves outcomes in solid organ transplantation such as liver and kidney, leading to an expansion of the donor pool. MP in UT is an innovative concept that aims to lower the recipient morbidity through the reconditioning and assessment of a relatively new graft and improve the outcomes of both recipient and offspring. Although significant efforts have been made to reduce recipient morbidity in a non-life-saving procedure such as UT, approximately 20% of the reported implanted grafts underwent early hysterectomy due to vascular thrombosis [11]. It is still unclear whether there is a common cause responsible for thrombi development in the graft after transplantation; therefore, the prediction and prevention of this major complication represents a real challenge for this emerging procedure.

In the uterus graft, inflow and outflow structures are represented by the uterine arteries and veins in the LD and by the internal iliac vessels in the DD. It could be argued that the diameter of the anastomosed vessels plays only a marginal or no role in thrombosis, which occurs in both LD and DD grafts at similar rates, whereas a major contribution to this vascular complication could be given by the microcirculation failure that follows ischemia and reperfusion injury (IRI) of the graft. Moreover, a co-factor that could trigger thrombi development can be found in the physiologic low flow of the uterus, which is mainly a resting muscle outside of a pregnancy. These graft characteristics along with microcirculation injury following IRI could trigger blood stasis and vascular thrombosis.

ISCHEMIA REPERFUSION INJURY IN VASCULARIZED COMPOSITE ALLOGRAFTS

Given its heterogeneous tissue composition, UT falls into the category of vascularized composite allografts (VCAs). VCAs donation and transplantation is an emerging area of transplant surgery that involves the transplantation of multiple different tissues, such as skin, bone, muscle, body vessels, connective tissue, and nerves either from a deceased or a living donor. For instance, VCAs include upper and lower limbs transplant, face, musculoskeletal segments, glands (such as parathyroids), and genitourinary organs (penis, uterus) [12-15].

As for solid organs, VCAs are affected by IRI [16,17]. The deprivation of oxygen occurring during the static cold storage (SCS) determines an ischemic injury of cells triggered by adenosine triphosphate (ATP) depletion, mitochondrial respiratory dysfunction and acidosis. Ischemia per se determines a depletion of ATP, causing the cells to switch to anaerobic glycolysis, leading to acidosis, succinate accumulation, reactive oxygen species (ROS) formation, and therefore, impairment of cell function. The damaging effects of ischemia on cells are subsequently aggravated once the blood flow is restored during reperfusion [18]. Additionally, prolonged cold ischemia time (CIT) and subsequent IRI trigger an inflammatory cascade that contributes to acute and chronic rejection in solid organ transplantation [18-21].

IRI in VCAs appears to be even more challenging to study, given these organs' heterogeneous histological composition and varying degrees of susceptibility to ischemia. For instance, muscle seems particularly vulnerable to cold ischemia compared to other tissues due to its elevated metabolism [22], and the degree of myocyte damage correlates with prolonged CIT [23]. The damage that occurs in the muscle manifests with edema, caused by an increase in endothelial permeability induced by inflammation in the tissue. This process eventually leads to cell dysfunction and necrosis. In addition, edema is responsible for raising the muscle pressure, leading to a compression of thin-walled capillaries and reduced blood flow, as previously demonstrated in the limbs [24-26]. Experimental models on the limb showed that the degree of myocyte damage correlates with CIT and becomes irreversible after 3-6 hours of SCS [27,28]. Van der Heiden et al. demonstrated that after 16 hours of SCS

at least 25% of muscle fibers were necrotic and unresponsive to electrical stimulation [23]. Another structure that happens to be very vulnerable to cold ischemia is vasculature. Upon ischemia, the integrity of the endothelial barrier is lost, leading to pro-inflammatory and pro-coagulatory pathways activation [29] and nitric oxide levels reduction, resulting in poor tissue perfusion and hypoxia [30].

UTERUS PHYSIOLOGIC PERFUSION

Uterus hemodynamics changes widely during pregnancy: in a non-pregnant woman, uterine artery physiologic blood flow is approximately 94, 5 mL/min, receiving 3% of the cardiac output, whereas during the second and third trimester the flow increases tremendously, reaching a bilateral flow of 605.6 ± 220.5 mL/min and a mean velocity of 58.9 ± 19.5 cm/s for each artery, which represents about 10% of the cardiac output [31,32]. Interestingly, up to date no hemodynamic complication or thrombi development have been reported after the first trimester in transplanted uteri, suggesting that the physiologic hemodynamic changes in pregnancy do not impact on graft vascular complications.

Another major difference between non-pregnant and pregnant uterine tissue is placentation and the consequent hemodynamic changes that occur in the uterus during pregnancy. At beginning of pregnancy, the remodeling of the spiral uterine arteries, that occurs to supply nutrients to the fetus, leads to a lowering in the velocity and rate of the blood flow from the uterus to the placenta [33]. One of the main causes of preeclampsia, for instance, is believed to be an altered remodeling of the spiral arteries, which determines an increased resistance to flow and consequently a faster flow, resulting in malperfusion of the uterine-placental tissue [34].

It can be speculated that one of the physiological changes that develop during pregnancy could be even protective: the increase of the left atrial volume index between both the first and second trimester and the second and third trimester correlates with parameters of doppler ultrasound of the fetal circulation and the uterine artery [35]. This maternal cardiovascular adaptation, which is a result of the uterus and fetal growth, might play a protective role in modulating the correct uterus perfusion according to the graft request. Moreover, further physiological changes that involve uterine arteries and veins such as matrix remodeling, circumferential artery enlargement due to shear stress and nitric oxide release could represent one of the reasons why most hemodynamic-related complications occurred during the first two weeks after transplantation.

As previously demonstrated in the kidney, MP plays a role in modulating the vascular resistances of the organ prior to transplantation. For instance, HMP tends to reduce vascular renal resistances during perfusion in kidneys [36] and as a result of HMP reconditioning effect, the grafts show better outcomes and a lower rate of delayed graft failure (DGF) [37], especially in ECDs [38,39] and DCDs [40] organs. It is not proved yet if MP reduce intratissue resistance in the human uterus graft, however this would represent an improvement that should not be underestimated for an organ with high resistance, lowering blood stasis and improving tissue perfusion.

From a surgical perspective, one of the main peculiarities of the uterus as a transplantable organ consists in the presence of two hila, each of them involving one inflow and two outflows. The inflow is represented by the uterine artery for each side, whereas the outflow is represented by the inferior uterine vein and that tract of vein that goes from the uterus fundus to the ovary defined as the superior uterus vein. Notably, the gonadal vein can be used if ovary sacrifice would not represent an issue in the donor, as it happens in DD.

MACHINE PERFUSION APPLICATION IN UTERUS TRANSPLANTATION

Modulation of IRI, viability assessment, immunological tolerance and logistic improvement are the pillars of dynamic preservation. The role of MP in improving graft and patient outcomes after transplantation has already been proven and different MP techniques are currently being introduced in clinical practice [41]. In solid organs, such as liver and kidney, the superiority of MP over SCS is clear and some benefits include lower rates of primary graft nonfunction and graft dysfunction, prolonged preservation time and reduced CIT [41]. Up to date, no human uterus has been transplanted after MP. However, according to the results achieved in kidney, liver and VCAs transplantation, MP could also play a key role in UT, given its potential in IRI modulation, flow measurements and graft viability assessment and feasibly preventing graft thrombosis, one of the major complications reported to date in UT.

As firstly described by Virchow in 1856, thrombosis occurs in the presence of endothelial damage, stasis and hypercoagulation state [42]. In UT, MP could find an application in modulating and studying all of these three aspects. The injury to the endothelial wall of a vessel alters blood flow dynamics, resulting in turbulence. This can depend on the quality of the graft (e.g. presence of irregular atheroma, bifurcations in the vessel, stenosis) or be a consequence of the procurement. Herein, MP would give an objective evaluation of the graft quality before implantation. Blood stasis can be caused by several factors, including microcirculation failure and

outflow obstruction: MP holds the potential to both protect microcirculation against IRI and allow outflow evaluation before transplant. Furthermore, IRI modulation would result in a milder immunological response in the recipient, lowering the risk of a hypercoagulative state.

MP to modulate IRI

The MP role in IRI modulation in VCAs has been investigated in both animal and human studies. Our colleagues from the Cleveland Clinic proved that a swine limb can be perfused with ex-situ normothermic machine perfusion (NMP) up until 12 hours, maintaining normal compartment pressure (16.4 ± 8.20 mmHg), minimal weight increase (0.54 ± 7.35%), and mean muscle temperature of 33.6 ± 1.678 °C [43]. Although an increase at the end of perfusion of myoglobin and creatine kinase concentrations of 875 ± 291.4 ng/mL and 53344 ± 14850.34 U/L, respectively, muscle contraction was present in all limbs until the end of NMP [43]. Animal studies [43-45] were confirmed by Werner et al. [46], which studied 5 human limbs after 24 hours of NMP. The authors found that neuromuscular electrical stimulation continually displayed contraction until the end of perfusion, with no changes in the maximum fiber isometric force or response to nerve stimulation. Moreover, vascular resistance was stable and histology showed no difference in fascicular architecture and shape, and no necrosis was reported at 0, 12 and 24 hours.

A previous attempt to extracorporeal perfusion of the human uterus was made in 2000 [47]. In this study, human uteri were subjected to 37°C extracorporeal perfusion with oxygenated Krebs-Ringer bicarbonate buffer, to simulate an *in vivo* situation. The results showed well-preserved myometrium and endometrium and no cellular edema, demonstrating that the viability and function of the organ could be maintained up to 24-hour perfusion time.

Up to date, viability assessment in UT remains an unexplored territory and yet a very crucial one. The uterus is mainly composed of muscle, the myometrium, which is particularly susceptible to the duration of CIT. There are multiple ways to test an organ for viability during transplantation: for the liver, we can assess bile production, lactates clearance, AST/ALT levels, bilirubin levels, coagulation factor synthesis and several parameters that can be measured during MP, such as cholangiocytes function or metabolism markers [48,49]. The success of a liver transplant and therefore the functionality of the organ can be assessed within days or weeks after surgery. UT success, on the other hand, is determined by not only the organ function but also the delivery of a healthy baby, which can take years [2]. In this scenario, MP could be a great tool to assess the organ's viability prior to transplantation, not only to recognize potentially damaged grafts but also for organ reconditioning to prevent major UT complications and improve global outcomes in recipient and offspring.

In a study conducted in 2005, the tolerability of the human uterus to cold ischemia was tested [50]. Grafts were preserved in cold storage (4°C) for 6 and 24 hours, in different preservation solutions. One parameter used to test the tissue viability is the ability of the myometrium fibers to generate spontaneous contractions, which resulted to be better preserved in the 6 h group.

We speculate that IRI modulation through MP might lead to a microcirculation enhancement, allowing physiologic perfusion parameters and, therefore, lowering the risk of graft thrombosis.

MP to evaluate uterus flows

Theoretically, in ex-situ NMP, a uterus graft with a significant turbulent flow, low flow and a high resistance would be less suitable for a transplant. Furthermore, MP would allow an evaluation of every inflow site of the uterus, which includes the right and the left uterine arteries, and the outflow sites, that is right and left inferior uterine veins and right and left superior uterine veins [51]. Knowing these data could be of utmost importance for the surgeon to decide on organ viability, but also the best vascular anastomotic site according to the hemodynamic parameters. For instance, poor venous drainage from the inferior uterine veins detected during NMP would lead to an optimization of the outflow through venous anastomoses of the superior uterine or ovarian veins, which could be monolateral or bilateral.

Furthermore, according to our transplant technique, we reperfuse the uterus graft after one side anastomosis to reduce the recipient's warm ischemia time. Although we begin with the vascular site that flushes better at the back table (i.e. has the lower resistance), a hemodynamic value registered during NMP would be an objective and better parameter to choose which side should be anastomosed first.

While in NMP we can compare *in-situ* physiologic hemodynamics with *ex-situ* parameters, in hypothermic machine perfusion (HMP) we expect to have lower flow and higher resistance than normothermia, and a comparison parameter is currently lacking.

Kristek et al. [52] reported the outcomes of the first 51 women who underwent UT, describing hysterectomy due to graft thrombosis in 19.6% (n = 10) of the recipients, which occurred in all the cases except for one within the first two postoperative weeks. Notably, 10 out of 12 graft failures (83.3%) occurred because of vascular thrombosis. A possible cause of thrombosis was found in focal atherosclerosis in 4 cases, vessel kinking due to graft displacement, artery dissection, and vein compression due to external hematoma in one case, whereas the cause was unknown in three recipients. In the Cleveland

Clinic series, three recipients experienced venous graft thrombosis resulting in one graft lossaz. In all cases no technical/anatomical issues were at the origin of the thrombosis.
Focal atherosclerosis in uterine arteries and/or internal iliac arteries could be difficult to predict considering the procurement technique that imposes the collection of a wide parametrium tissue to avoid injury to the anastomotic vascularization [53]. Furthermore, the amount of focal atherosclerosis could be difficult to interpret and its role to predict grafts failure may be very subjective. Ex-vivo uterus MP would allow an objective interpretation of the vasculature patency and resistance through flow measurements.
Future studies should focus on recipient selection considering uterine vessels' flows, velocity, and resistance and how the procurement and the IRI impact them, comparing flows and resistances between *in-situ* uteri and *ex-situ* normothermic perfused grafts.

POTENTIAL MACHINE PERFUSION DEMAND IN UTERUS TRANSPLANT AND FUTURE PERSPECTIVES

UT transplantation is a novel field with great potential, being the only method for women with AUFI to be pregnant. Assessing the real demand for UT is very challenging for many reasons. Firstly, not all women suffering from AUFI wish to have children. Secondly, there may be other medical, financial, psychosocial or logistical barriers that would preclude couples from accessing uterus transplantation. Data from adoption databases are also difficult to interpret; however, adoption and surrogacies can be very challenging or even not possible, depending on where people live and other societal variables.
Based on AUFI prevalence, it can be roughly estimated that between 3000-5000 women could be interested in uterus transplantation in the USA every year. However, in the near future, women with previous hysterectomy would access UT, rising the number of potential UT candidates to around 70000/year in the US only [54-56]. Therefore, lowering morbidity, optimization of donor-recipient selection, donor pool expansion and logistic improvement will be critical factors to improve in the future and MP holds the potential to improve all these four aspects. HOPE could potentially modulate IRI through reduction of ROS production, less inflammatory response and improve immune tolerance as it happens in the liver. The subsequent microcirculation enhancement might reduce graft failure and thrombosis development, which should be a focus of future studies.
Ex-situ NMP would represent a near-physiological environment for uterus viability assessment looking for myometrium contraction, response to flow modulating drugs, myorelaxants or vasodilators. Moreover, flow measurements of uterine arteries and veins flow can be studied deeper, in order to understand whether there is an association between a low-flow uterus type and complications development, such as graft failure due to thrombus formation, and a threshold of acceptable risk should be identified.
The ischemia-free technique would be a promising technique to apply to UT because the favorable anatomy of this organ. As mentioned, the uterus has two hila and a total of two inflows and four outflows. This anatomical peculiarity could make this organ suitable for ischemia-free perfusion technique, allowing *ex-vivo* perfusion to start before disconnecting the second hilum, and end the perfusion right after the graft *in-situ* reperfusion, through the first vascular side.
Normothermic regional perfusion would play a role in donor pool extension; however, it might find an application only if standard criteria DD and LD cannot supply the UT demand.
In conclusion, MP holds the potential to improve outcomes, facilitate donor-recipient matching in deceased donation, improve transplant logistics and extend the preservation time without compromising graft viability.

Conflict of interest statement

The Authors declare no conflict of interest.

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Authors' contributions

LDP, BC, CQ: conceptualized the ideas and concepts expressed in the manuscript; LDP, BC, CQ: wrote the article. All the Authors gave intellectual contribution to the manuscript and participated in the revision and approval of the final draft.

Ethical consideration

The research was conducted ethically, with all study procedures being performed in accordance with the requirements of the World Medical Association's Declaration of Helsinki.
Written informed consent was obtained from each participant/patient for study participation and data publication.

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