Cumulative Dissertation

REDUCTION OF ISCHEMIA AND REPERFUSION INJURY IN EXPERIMENTAL UTERUS TRANSPLANTATION

submitted by

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Declaration

I herewith formally declare that I have written the submitted dissertation independently. I did not use any outside support except for the quoted literature and other sources mentioned in the paper. I clearly marked and separately listed all of the literature and all of the other sources which I employed when producing this academic work, either literally or in content. Due acknowledgement has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the “Guidelines of the Medical University of Graz on Good Scientific Practice”.

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Disclosures

Part of this thesis has been published in the following original articles:

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Table of Contents

Abbreviations and Definitions

8-OHdG	8-hydroxy-2′-deoxyguanosine
ATP	Adenosine triphosphate
AUFI	Absolute uterine factor infertility
BMI	Body mass index
CAT	Catalase
FIGO	The International Federation of Obstetrics and Gynaecology
GSH	Reduced glutathione
GSH-Px	Glutathione peroxidase
IRI	Ischemia-reperfusion injury
MDA	Malondialdehyde
MPO	Myeloperoxidase
MRKH	Mayer-Rokitansky-Küster-Hauser
PER	Perfadex
ROS	Reactive oxygen species
SCS	Static cold storage
SOD	Superoxide dismutase
Tx	Transplantation
UTx	Uterus transplantation
UW	University of Wisconsin
WHO	World Health Organization
XO	Xanthine oxidase

List of Tables

Abstract in German

Hintergrund: Eine Gebärmuttertransplantation (UTx) ist die einzige verfügbare Behandlung für aufgrund von Uteruserkrankungen unfruchtbarer Frauen. Die klinische Anwendung ist jedoch durch den Mangel an Organen, ischämischer Reperfusionsschaden (IRI) sowie Immunsuppression nach der UTx eingeschränkt. Der IRI ist eines der Hauptproblem, das bei einer UTx gelöst werden muss. Der IRI umfasst warme und kalte Perioden während der Organentnahme und Organkonservierung. Zur Organkonservierung wurden mehrere unterschiedliche Lösungen bei experimentellen und klinischen UTx mit unterschiedlichen Erfolgen verwendet. Die neuartige Custodiol-N Lösung wurde kürzlich mit ausgezeichneten Ergebnissen bei der kalten Organkonservierung (static cold storage, (SCS)) entwickelt. Die neue Lösung wurde jedoch noch nicht bei einer UTx angewendet. Mit dem Wissen, dass Melatonin antioxidative und entzündungshemmende Eigenschaften hat, Glyzin den IRI durch direkte Zytoprotektion der Entzündungsreaktion in anderen Organen verhindert, haben wir beschlossen, die Schutzwirkungen beider Supplementierungen bei Ratten-Uteri mit warmer Ischämie, die zu Versuchszwecken IRI ausgesetzt waren, zu untersuchen. Wir haben zusätzlich beschlossen, die Wirkung von Custodiol-N auf die verlängerte kalte Konservierung der Gebärmutter im Vergleich zur Custodiol® Lösung auszutesten.

Materialien und Methoden: Für das Gebärmutter-IRI Ratten-Modell mit warmer Ischämie, wurden Sprague-Dawley Ratten (12 Wochen alt) zufällig in acht Gruppen randomisiert (n = 10/Gruppe). Die Hälfte der Tiere erhielt eine 5 %ige Glyzin-Ernährung, während der anderen Hälfte eine Kasein-beinhaltende Kontrollernährung verabreicht wurde. Nach fünf Tagen Ernährung wurden die Tiere in zwei Untergruppen aufgeteilt, die entweder 50 mg/kg Melatonin oder mikrokristalline Zellulose über eine Schlundsonde 2 h vor dem IRI-Experiment erhielten. Die Versuchsdurchführung umfasste einen Zeitraum von 1 h von Ischämie gefolgt von 1 h Reperfusion, die erhalten wurde, indem die Bauchaorta oberhalb der Bifurkation und der Eierstockarterien abgeklemmt wurde. Nach den Ischämie- und Reperfusionszeiträumen wurden die Gebärmutterproben für die biochemische und histopathologische Auswertung gesammelt. Für das Gebärmutterkonservierungsmodell wurden Gebärmuttergewebeproben (nach 8 und 24 h SCS) von erwachsenen Sprague-Dawley Ratten (n = 10/Guppe) genommen. Bei beiden Versuchsteilen wurde ein Expressionsniveau von Myeloperoxidase durch immunhistochemische Färbung bemessen. Zur histologischen Bewertung wurde ein semi-quantitatives morphologisches Bewertungssystem verwendet. Es wurden Superoxid-Dismutase-Aktivitäten in den Gewebeproben gemessen.

Ergebnisse: Melatonin alleine und zusammen mit Glyzin erhöhte die Konzentration der Superoxid-Dismutase-Aktivität signifikant (p = 0.015) in IRI-betroffenem Gebärmuttergewebe. Es wurden geringere Myeloperoxidase-Level in immunohistochemisch gefärbtem Gebärmuttergewebe während der Vorbehandlung mit Melatonin (p = 0.004), Glyzin (p < 0.001) oder ihrer Kombination (p < 0.001) gefunden. Die histopathologische Auswertung ergab, dass Melatonin- und Glyzin-Behandlungen bei IRI wesentlich geringere Neutrophil-Infiltration, Ödeme und Vasokongestion des endometrialen Stromas im Vergleich zum IRI + Kontrollbehandlung zur Folge hatte. Des Weiteren wurden nach 8 h SCS ein geringerer Anteil von Gewebeödemen (p = 0.004), der Myeloperoxidase-Expression (p = 0.002) sowie eine erhöhte Superoxid-Dismutase-Aktivität (p = 0.002) bei Custodiol-N im Vergleich zu Custodiol® festgestellt. Diese Unterschiede wurden nach 24 h kalter Konservierungszeit (p < 0.05) noch deutlicher.

Zusammenfassung: Die aktuellen Ergebnisse zeigen, dass eine Anwendung von Melatonin und Glyzin vielversprechende entzündungshemmende und antioxidative Wirkungen auf den Gebärmutter-IRI haben und bei einer UTx sicher eingesetzt werden können. Zusätzlich hat die Studie gezeigt, dass Custodiol-N die Gebärmuttertransplantate besser vor Verletzungen durch Ischämie schützt als die Standard Custodiol® Lösung, höchstwahrscheinlich durch Hemmung von oxidativem Stress und Gewebeödemen. Es scheint, dass Eisenchelatoren in der Zusammensetzung von Custodiol-N eine wichtige Schutzrolle gegen kalte Ischämie spielen.

Abstract in English

Background: Uterus transplantation (UTx) is a promising fertility-restoring treatment modality for women with absolute uterine factor infertility. However, clinical use of UTx is limited by the lack of organ donors, ethical issues, ischemia/reperfusion injury (IRI), and immunosuppressive therapy-related risks. IRI still remains the main problem to be solved in UTx. IRI includes warm and cold periods during organ retrieval and organ preservation. Custodiol® has been demonstrated to be a viable preservation solution for solid organs, including the uterus. More recently, an improved solution, Custodiol-N, was introduced with superior results in experimental models. However, this new solution was not studied in UTx. Knowing the fact that melatonin has antioxidant and anti-inflammatory effects, glycine prevents IRI by direct cytoprotection and inhibition of the inflammatory response in other organs, we chose to investigate the protective effects of both supplements, in experimental IRI exposed rat uteri warm ischemia. In addition, the protective effects of Custodiol-N solution in uterus prolonged static cold storage (SCS) were analysed and compared with Custodiol® solution.

Materials and Methods: For uterus IRI in a rat warm ischemia model, Sprague Dawley rats (12-weeks-old) were randomly assigned into eight groups (n = 10/group). Half of the animals received a glycine-enriched diet, while the others – control diet (without glycine). After five days of diet, animals were split into two subgroups, receiving either 50 mg/kg of melatonin or microcrystalline cellulose via gavage 2 h prior to IRI. The experimental procedure involved 1 h of ischemia followed by 1 h period of reperfusion, obtained by clamping the abdominal aorta above the bifurcation and ovarian arteries. After the ischemic and reperfusion periods, the uterus samples were collected for biochemical and histopathologic analysis. For the uterus preservation model, uterus tissue samples from rats (after 8 and 24 h of SCS; n = 10/group) were used. For both experiment parts expression levels of myeloperoxidase were assessed by immunohistochemical staining. Histological tissue damage was evaluated using the modified scoring system. Superoxide dismutase activities were measured in tissue samples.

Results: Melatonin alone and together with glycine significantly increased the concentration of superoxide dismutase activity ($p = 0.015$) in IRI affected uterus tissues. We found significantly lower myeloperoxidase levels in immunohistochemically stained uterus tissue while pretreatment with melatonin ($p = 0.004$), glycine ($p < 0.001$) or their combination ($p < 0.001$). Histopathological evaluation showed that melatonin and glycine treatment in IRI resulted in significantly less endometrial stroma neutrophil infiltration, oedema, and vasocongestion compared to the IRI + control treatment. Moreover, after 8 h of SCS, a lower percentage of tissue oedema ($p = 0.004$), myeloperoxidase expression ($p = 0.002$), as well as higher superoxide dismutase activity ($p = 0.002$) were found in Custodiol-N compared to Custodiol®. These differences were even more prominent after 24 h of SCS ($p < 0.05$).

Conclusion: The present results showed that application of melatonin and glycine has promising anti-inflammatory and antioxidant effects on the uterus IRI and can be safely used in UTx. Additionally, the novel Custodiol-N solution provides better protection of uterus grafts against cold ischemic injury than standard Custodiol®, most likely due to iron chelators in the composition of Custodiol-N.

1. Introduction

Reproductive medicine has made remarkable advances ever since the first in vitro fertilization baby, in 1978 was reported (1). After development of intracytoplasmic sperm injection and embryo cryopreservation, the most types of male and female infertility were treatable and led to successful pregnancy in many infertile couples worldwide (2, 3).

Up to date about 15 % of the reproductive population is infertile (4). Despite important advances in reproductive medicine, there remains one group of women with no available infertility treatment until now. These women suffer from absolute uterus factor infertility (AUFI). This condition can be described as the absence of a functional uterus and has a prevalence of 3 – 9.8 % in the general population (5). This abnormality generally leads women to consider adoption or surrogacy, however, the latter is not currently legal in the majority of nations and societies worldwide (1). Uterus transplantation (UTx), although still experimental, may be an option in these cases. This procedure can be performed with living or deceased donors (6). After successful childbirth after UTx, the transplanted uterus is surgically removed, eliminating the need for further immunosuppressive therapy.

Currently, more than 60 UTx procedures and at least 18 livebirths have been presented in the literature, with three centers in the United States and more than 15 teams worldwide actively performing UTx, although unpublished data suggest that the number of UTx and livebirths may be greater (7).

1.1. Indications for UTx

AUFI can be caused by congenital or acquired diseases. Congenital uterus malformations, as well as certain acquired diseases, can lead to reduced endometrial receptivity and an increased rate of repeated pregnancy loss or infertility due to embryo implantation failure (8). We could also divide patients into two groups: those with complete infertility and those with relative infertility. Causes of AUFI with estimated prevalence and infertility percent are shown in Table 1.

1.1.1. Congenital Absence or Anatomical Defect of the Uterus

Most uterus anomalies are formed during embryogenesis because of defect in the development or fusion of the paired Müllerian ducts (8). Müllerian malformations presents in one of 4,000 to 10,000 women (9, 10). Its most frequent clinical manifestation is utero-vaginal agenesis, or Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome. As a result, females do not have a uterus, cervix, or upper one-third of the vagina, but have a lower vagina and normal ovaries (11). Often, the initial symptom suggesting a diagnosis of MRKH is primary amenorrhea in an adolescent female with usually developed secondary sexual characteristics.

AUFI is also linked to other major congenital uterus malformations: arcuate, septate or bicornuate uterus, which are more common, and less frequent – unicornuate, didelphic or hypoplastic uterus (Table 1) (5, 12-14). These malformations are diagnosed by combining hysterosalpingography/hysteroscopy and magnetic resonance imaging or 3-dimensional ultrasound and have obviated the need to perform laparoscopy for the diagnosis (11, 12, 15). Based on literature review, arcuate, septate or bicornuate uterus can cause relative uterus factor infertility with an infertility prevalence of 17 – 37 % (Table 1). Typically, the surgical correction of a uterus malformation was indicated after two or more spontaneous abortions. As surgery has become less invasive, now the correction can be performed prophylactically when a disease is diagnosed and refers to infertility (14). Patients with relative infertility should be considered for UTx only after negative results of other treatment options.

Table 1. The main causes of absolute and relative uterus factor infertility.

Causes	**Prevalence (%)**	**Infertility (%)**
Congenital		
Arcuate-shaped uterus	0.7 – 6.8	17.3
Septate uterus	0.8 – 2.3	38
Bicornuate uterus	0.7 – 1.3	37.5
Uterus hypoplasia	0.038	100
Mayer-Rokitansky-Küster-Hauser Syndrome	0.0002 – 0.1	100
Acquired		
Leiomyoma	21 – 26	40
Intrauterine adhesions	1 – 2	50 – 70
Peripartum hysterectomy	0.04 – 1.25	100
Hysterectomy due to large myomas	1 – 3.5	100
Hysterectomy due to malignancies	0.00004 – 0.0001	100

Reproduced from reference (16).

1.1.2. Acquired Anatomical or Functional Absence of the Uterus

Acquired anatomical AUFI can be divided into two origin groups: gynaecological and obstetrics (Table 1). The most common gynaecological cause is leiomyomata, which is probably the main reason of hysterectomy in reproductive age (9). The incidence of uterus leiomyoma increases with age, the prevalence is about 21 – 50 %, some of them could be treated with hysteroscopy or laparoscopic/open myomectomy, but 1 – 3.5 % needs hysterectomy (8, 14). The mechanisms by which myomas may influence fertility are the following: shift of the cervix, deformation of the uterus cavity, obstruction of the proximal fallopian tubes, increased or disordered uterine contractility, distortion of the endometrium and therefore disturbance of implantation, impaired endometrial or whole uterus blood flow (8, 14).

The second frequent anatomical cause is intrauterine adhesions, known as Asherman's syndrome (14). Usually, it is caused by uterus curettage, intrauterine infections or hysteroscopic examination. Its prevalence is 3.7 – 23.4 % in women with one curettage, 5 – 39 % in women with recurrent miscarriages, and 40 % after repeated curettage (17).

Third and under a lot of debate, endometrial polyps, are causes of relative infertility and have been associated with decreased pregnancy rates and increased miscarriage rates. Endometrial polyps are identified by hysteroscopy in 16.5 – 26.5 % of women with unexplained infertility (8). It is related with sperm transport difficulties, embryo implantation or through intrauterine inflammation or altered production of endometrial receptivity factors (18). Nowadays there is evidence about endometrial polyps relation with endometriosis – the disease in which the endometrial cells are presented outside of the uterus (19). Endometriosis affects about 10 – 15 % of general female population (20). There is evidence that endometrial polyps are more common in women with endometriosis and might be cofactors in female infertility, as the prevalence of endometrial polyps can reach 47.8 – 68.3 % in women with endometriosis-associated infertility (19).

Other diseases, such as tuboperitoneal abnormalities, severe adenomyosis, cause relative infertility. In most of the cases all in this section mentioned diseases are treatable and can partially affect infertility.

It is important to mention patients who had hysterectomy at their reproductive age. Hysterectomies are the most frequent gynaecological surgeries with approximately 600,000 procedures per year performed in the US. More than 40 % of patients undergoing hysterectomy are younger than 44 years (21). Hysterectomy prevalence due to malignant uterus tumour is 0.00004 – 0.0001 % (3). The most common reason during the reproductive years is cervical carcinoma, with 30 – 50 % of affected women being under the age of 40 years (22). Other malignancies of the uterus, such as sarcoma or endometrial cancer, are less frequent at a young age (< 3 %) (21). Emergency hysterectomies due to obstetric causes are rare but significant, with a prevalence of approximately 5 in 10,000 deliveries including intractable post-partum haemorrhage, usually due to uterus atony, rupture or placenta acreta/increta/percreta (13).

1.2. Ethical, Legal, and Social Issues Regarding UTx

Nowadays, it is progressively feasible to transplant non-vital organs. Rather than being simple, these are particularly complicated operations, not only considering the clinical profile but also for the multiple issues and implications for the human race (23). Cozzoli wrote an article about ethical aspects of organ transplantation (Tx), where it is mentioned, that if Tx of non-vital organ is not necessary to save the life, it is nonetheless essential for quality of life and physical liberty (24). UTx is not lifesaving, but life maintaining. Wide clinical application of UTx is limited not only due to the complexity of the procedure but also ethical issues (25).

Since UTx poses a high risk compared to surrogate motherhood and adoption, as well as Tx of other organs, it needs to be more justified because its goals are quality of life rather than lifesaving (24, 26). Although surrogacy and adoption are the current reproductive options for women with AUFI, UTx offers several advantages over both currently available options. The main benefit of UTx is the potential for a genetic link between mother and child (possible in the case of surrogacy, but not in – adoption) (26). UTx may also be a legal alternative in countries where altruistic (France, Greece, Italy) or commercial surrogacy (Australia, Bulgaria, Croatia, Estonia, Finland, France, Germany, Greece, Hungary, Holland, Italy, Lithuania, New Zealand, Norway, Portugal, Slovakia, Spain, Sweden, South Africa, United Kingdom) is forbidden (9, 27).

Bioethical discussions and analyses played an important role in initiating the first studies of UTx. The cooperation between researchers, physicians, patients, and bioethicists has developed a scientific plan for human research. The initial work assessed whether there is an ethical justification for conducting this kind of studies. Ethical systems have focused on the value of UTx as a way of providing a pregnancy experience for women with AUFI (7). The safety of transplant donors, recipients and child is constantly being considered. It is therefore essential to have monitoring and regulatory mechanisms that address issues of supervision. In particular who will be responsible for monitoring outcomes and who will ensure that there is continued scientific strictness, transparency and reproducibility in the implementation of the uterus during Tx (28).

UTx is covered by the National Organ Transplant Act, which regulates the distribution of transplanted organs (7). When institutions plan an UTx program, one of the first remaining key questions is, how the organs will be distributed in an ethically justifiable manner. Current rules governing the distribution of organs to recipients, such as emergency medical care or priority, may not apply equally to uterus recipients. In 2016, around 70 clinical doctors and scientists, with special interest in UTx, formed the International Society of Uterus Transplantation. This society helps to protect patients' rights, educate the public and medical specialists, share current knowledge and new discoveries, promote multidisciplinary collaborative research, develop consensus and guidelines for UTx and maintain an international registry of uterus transplant cases, monitoring patients, children and donors (2).

To date, psychological research on uterus recipients has been limited to direct living donation, but future research may focus on undirected living and deceased donation (29, 30). The deceased donor model has significant advantages, including no physical harm to living donors, but has significant disadvantages, including organ shortage and the inability to undergo thorough medical examination, as in the case of a living donor (7, 31). If the organs of deceased donors were equally effective (or superior) to the organs of living donors, the question arises as to whether it is ethically justified to use living donors due to the shortage of organs from deceased donors.

1.3. Uterus Donation

Live or deceased (cadaver) organ donation can be used equally for UTx. In accordance with the basic principle of the World Health Organization (WHO), further development of organ donation from deceased donors is necessary avoiding any risk to living donors (25). However, despite the frequent use of material donated from deceased donors, donations from living donors are necessary for some types of transplants or to compensate the limited amount of material that can be obtained from deceased donors to meet patient needs. Both donor options have corresponding advantages and disadvantages.

Sweden is the leading country of successful UTx using living donors (1, 32, 33). Globally, there were some trials with deceased donors, however it has not been successful (34, 35), until 2018, when a Brazil Tx team announced the first livebirth after UTx of a deceased donor (36). In total five births following UTx using deceased donors' uteri have been reported in the literature: taking place in Brazil; Cleveland, USA; Dallas, USA; the Czech Republic; and Pennsylvania, USA (37-39).

1.3.1. Guidelines on Assessing Recipient and Donor Suitability

The International Federation of Obstetrics and Gynaecology (FIGO) published limited UTx guidelines in 2009, later in 2012 the Montreal Criteria were reported, which were the first criteria to consider the ethical feasibility of UTx (40).

Considering that UTx is an arising field that many active centers still consider to be experimental, donor and recipient selection is necessarily strict.

1.3.1.1. Recipient Suitability

Recipients are considered for UTx trial if suffering from uterus infertility that cannot be cured (see AUFI causes above). The main UK criteria, as with all UTx trials worldwide, require all recipients to produce their own ovum (38). The upper age limit is 38 years, apparently because the women involved must be able to produce their own eggs, and the number and quality of them are declining rapidly from the age of 35 (41). In the UK study, all recipients must undergo in vitro fertilization to produce at least ten embryos before transplantation. Other studies vary from two to ten embryos (42). The recipient should not have cancer (at least 5 years in remission), severe endometriosis, chronic infections such as HIV, tuberculosis or hepatitis, severe illnesses that are sensitive to, or worsened by, pregnancy or immunosuppressant medication (40). Additionally, the recipient should feel a strong desire to become a biological parent and had no history of significant psychiatric illness (38).

1.3.1.2. Donor Suitability

Donor selection criteria vary from institution to institution. There are still arising ethical and medical discussions whether there should be a preference for living or deceased donors. General and additional contraindications to uterus donation are provided in Table 2.

Table 2. Contraindications for uterus donation.

General contraindications to organ donation	**Additional contraindications to uterus donation**
Not matching blood/tissue type	Donor infertility or subfertility
Human immunodeficiency virus infection	Cervical or endometrial dysplasia
Acute hepatitis B or C	Human papillomavirus infection
Active or untreated tuberculosis	Gonorrhoea, chlamydia, herpes simplex type 1 or 2
Acute viral infections (e.g., rubella, rabies, adenoviruses, enteroviruses, parvoviruses)	Atherosclerosis of uterus vessels
The presence of systemic invasive fungal infections	Vascular anomalies of uterus vessels
Active metastatic or non-curable malignant disease	Uterus malformations
Past history of malignancy that poses risk for transmission	Intrauterine adhesions
Clinically uncontrolled sepsis	Myomas, adenomyosis, polyps
Systemic illness (diabetes, hypertension)	Age: < 18 or > 65 years old

Reproduced from reference (16).

1.3.1.2.1. Living Donor

Living donations are acceptable when the donor is informed, a voluntary consent is obtained; when professional supervision of donors is guaranteed, follow-up is well organized and when donor selection criteria are applied and monitored in good faith (43).

Main criteria for live uterus donor are: 40 – 65 years; negative for HPV, HIV, hepatitis B or C; negative history for sexually transmitted diseases including gonorrhoea, chlamydia, and syphilis; normal uterus ultrasound and computed tomography; at least one prior full-term live birth; BMI < 30; no active infections; no history of cancer in the last 5 years; at the discretion of the investigator, there are no clinical or health conditions that could pose a higher risk and should meet psychological donor criteria (30, 44, 45). It is noticed that donors should have at least one previous full-term delivery, no more than one caesarean delivery (46).

One of the undoubted advantages of living donors is screening for medical or gynaecological conditions, with a thorough morphological and microbiological evaluation, which is practically impossible with deceased donors due to urgent conditions. Second, convenient planning of the procedure which allows the donor and recipient to be nearby, even in adjacent operating rooms, which can reduce cold ischemia time and increases the likelihood of the transplant surviving (25, 47).

However, usage of a live uterus donor is at great risks of both physical and psychological harm (38). The main surgical risks are ureter damage, by devascularization during dissection of parameters, and the risk of ureterovaginal fistula (48-50). Based on literature, ≥ 12 % of live donors were reported to experience major complications, including bleeding, infection, ureter injury, and ovarian dysfunction (46, 51). In addition, to remove the uterus the laparotomy method is required, which is bearing specific complications itself. But with the development of new methods, the risk of surgical complications is likely to be reduced using minimally invasive techniques (laparoscopic or robotic surgery) (25, 52). The long duration of surgery in living donors (average 12 h) poses an aesthetic risk and increases the thromboembolic complications (49). Difficult surgical isolation of uterus blood vessels, especially veins, is reported to be a major time-consuming part of the procedure (15).

1.3.1.2.2. Deceased Donor

Challenges in the selection of deceased donors include the low mortality of women of reproductive age without significant comorbidities in developed countries (46).

The main advantage of the deceased donor model is that it eliminates surgical and medical risks to the donor. Usage of a deceased donor allows longer and larger diameter blood vessels to be prepared for Tx and to ensure adequate perfusion of the graft (34). Furthermore, usage of a bladder peritoneum allows better graft support and ability to prepare a longer vaginal cuff ensures better vaginal-vaginal anastomosis (47, 53). The psychological aspect is also very important due to anonymity of donation and recipient's less feeling of 'debt' to the donor, no emotional guilt or regret (38).

However, for a deceased donor, the time of uterus removal is significantly shorter, which can affect graft viability (35, 47). In the case of multiorgan donations, the uterus will be usually removed last, as it is both an experimental and a non-life-saving procedure, as well as to prevent any contamination of other organs or vascular transplants from the vaginal microbiome. Another limitation associated with the deceased donor model is failure to plan procedures, thus prolonging the period of cold ischemia, lack of time before Tx, and lack of donors (38).

1.4. Uterus IRI

1.4.1. Uterus IRI Mechanism

IRI is a pathological condition characterized by an initial restriction of blood supply to an organ followed by the later restoration of perfusion and attendant reoxygenation (7). IRI can affect different organs and tissues after difficult operations or conditions due to patient diseases (e.g., myocardial infarction, hypovolemic shock, or thromboembolism) (54). The ischemic-reperfusion mechanism is multifunctional and involves different biological mechanisms such as immune activation, ion accumulation, and the formation of toxic substances reactive oxygen species (ROS) (55).

Firstly, injury reveals itself on an intracellular level, because of ROS, which interact with lipids, proteins and nucleic acids, resulting in cell damage, apoptosis, and inflammatory events (56-58). Secondly, IRI effects the immune system and contributes to vasculopathy, graft dysfunction, and rejection (54). In experimental models of IRI, it was established that ischemia time is directly associated with severity of injury damage after reperfusion period. Therefore, reduction of organ ischemia time is one of the key points in order to improve solid organ Tx outcomes (59).

During elongated ischemia, the lack of oxygen supply induces anaerobic metabolisms, resulting in a decrease in cell pH due to low adenosine triphosphate (ATP) production (59, 60). The result of this damage promotes acidosis, oedema, perturbation of cellular Na^+ and Ca^{2+} homeostasis, which initiates inability to maintain cell membranes integrity and resulting in possible leakage of autolyzing proteolytic enzymes into the cell and surrounding tissues (59, 61, 62).

Restoration of blood supply to ischemic tissues can cause further damage known as reperfusion injury that can be more damaging than the primary ischemia (58). Reintroduction of blood flow brings oxygen back to the tissues, and then xanthine oxidase converts the excess hypoxanthine to toxic ROS, which results in cellular damage by lipid peroxidation (50, 57). Overproduction of ROS, such as superoxide anion, nitric oxide, hydrogen peroxide, hydroxyl radical and free radicals cause pathological states (63). It also brings more calcium ions to the tissues causing further calcium overloading and accelerates cellular self-destruction. The restored blood flow also overstates the inflammation response of damaged tissues, causing white blood cells to destroy damaged cells that may otherwise still be viable (59).

There are several different cell death programs that are activated following IRI:

- Apoptosis – involves a caspase signalling cascade resulting in cellular self-destruction. This physiological process is described by nuclear fragmentation, plasma membrane blebbing, normotonic shrinkage of cells, and loss of mitochondrial membrane potential and solidity (60).
- Autophagy-associated cell death – cytoplasmic vacuolization, loss of organelles and conglomeration of vacuoles with membrane whorls (59).
- Necrosis – characterized by progressive cell and organelle oedema with subsequent plasma membrane rupture and leakage of proteases and lysosomes into the extracellular space (60). When cells are necrotizing, they produce an immunostimulatory effect and lead to inflammatory cell infiltration and cytokine production around the necrosed area.

1.4.2. Uterus IRI Markers

Usually, tissues and cells are protected against ROS injury by various mechanisms. They are equipped with different antioxidant enzymes and compounds. Several ROS-forming enzymes that are usually situated within a cell can also be detected in the blood, as IRI markers, independently of the underlying mechanism of their release (59). Basically, IRI markers could be divided into two groups – antioxidants (protectors) and oxidative stress markers (indicating injury degree). However, there are no specific markers for the evaluation of uterus IRI. Markers that are the most important and frequently used in the research are given below.

- Reduced glutathione (GSH) – is an antioxidant found in every cell and is produced naturally within our body. GSH is able to prevent damage to cellular components caused by ROS, such as free radicals, peroxides, lipid peroxides, and heavy metals (64). Firstly, GSH needs to be in a reduced state to neutralize free radicals by binding its extra electron to the ROS molecules free radical electron. The intracellular ratio of reduced and oxidized GSH is commonly used as a marker of cellular oxidative stress (65). Manageably low levels result in the systematic breakage of the cell whereas too low GSH levels result in rapid cell death (59).

- Glutathione peroxidase (GSH-Px) – is an antioxidant enzyme that effectively reduces H_2O_2 and lipid peroxides to water and lipid alcohols and turns oxidized GSH to GSH disulphide. Although GSH-Px shares the substrate, H_2O_2, with catalase, it alone can react effectively with lipid and other organic hydroperoxides and it is thought to be a major defence in low-level oxidative stress (66).
- Catalase (CAT) – is an enzyme responsible for the degradation of hydrogen peroxide. This enzyme is present in nearly all animal cells. It converts H_2O_2 to water and molecular oxygen, reacts with H donors (methanol, ethanol, formic acid, or phenols) with peroxidase activity and prevents the formation of hydroxyl radicals (57, 66). Although CAT is not essential for some cell types under normal conditions, it plays a significant part in the acquisition of tolerance to oxidative stress in the adaptive response of cells (66).
- Superoxide dismutase (SOD) - is an antioxidant enzyme that catalyzes the dismutation of the highly reactive superoxide anion to O_2 and to the less reactive species – H_2O_2. Peroxide can be decomposed by CAT or GSH-Px reactions. In humans, there are three forms of SOD: cytosolic, mitochondrial, and extracellular (65).
- Xanthine oxidase (XO) – catalyzes the hydroxylation (oxidation) of hypoxanthine to xanthine. Moreover, it can further catalyze the hydroxylation of xanthine to uric acid. The activity and levels of XO in plasma are increased in response to inflammation due to the release of interferons (65).
- Myeloperoxidase (MPO) – is a haem peroxidase, mainly expressed in neutrophils, that catalyzes the reaction of H_2O_2 with chloride ions to yield hypochlorous acid as the primary oxidant. Therefore, MPO is suitable for usage as a prognostic marker of inflammation (a marker of neutrophil infiltration) (67).
- Malondialdehyde (MDA) – is a secondary product of oxidative stress formed during lipid peroxidation and is significantly increased by IRI (57, 64). It is released as a result of the toxic effects of ROS which destroy unsaturated fatty acids in the cell membrane (56).
- 8-hydroxy-2′-deoxyguanosine (8-OHdG) – is another well-defined oxidative stress marker representing deformation in DNA (67). It has been demonstrated that oxidative damage constantly occurs to proteins, membrane lipids, and DNA. In DNA (mitochondrial and nuclear), 8-OHdG is one of the prominent forms of free radical-induced oxidative lesions. Hence, it is used as a potential marker for oxidative stress and carcinogenesis (68).

- Serum ischemia modified albumin – is a form of human serum albumin in which the N-terminal amino acids have been modified by ischemia. This modification reduces the affinity of plasma albumin to bind to heavy metal ions such as cobalt (67).

1.4.3. Warm and Cold Ischemia

Ischemia is very costly for all cells with aerobic metabolism, and cells are injured through the complex and interconnected chain of many failed mechanisms necessary for homeostasis, which results in cell death (69).

Warm ischemia is a term used to characterize ischemia of cells and tissues under normothermic conditions. In the Tx context, this term is used to describe two physiologically different periods of ischemia: donor ischemic time – from the time of clamping the vessels until cold perfusion is started (59, 70). Recipient ischemic time – from the removal of the organ from ice until reperfusion (time during anastomosis surgery) (70, 71).

Cold ischemia – is the time when a tissue or organ after its blood supply has been reduced, starts washing with cold preservation solution until the time it is placed to the abdominal cavity and warmed by having its blood supply restored (71).

Cold and warm ischemia times are very relevant for the good surgical recovery of organs after Tx. These periods influence the long-term survivability and function of the organ in the recipient. Procedures for removal and transport of life-supporting organs are based on the proper ischemic time by organ, but this time is unknown for the uterus. There is a wide range in time of ischemic tolerance for other organs, for example, the clinically practiced time limit for cold storage ranges from six hours for heart up to 36 h for kidney (72). Several experimental studies indicate that uterus is resistant to both warm and cold ischemia; however, the time limit for cold ischemia has not yet been established (15, 73).

Cold storage with organ preservation solutions today is the most common method used to preserve an organ intended for Tx during the ischemic time between procurement and reperfusion within the recipient. Hypothermia with the different preservation solutions lowers tissue metabolism and slows down the development of ischemic injuries, but the tolerable time window is still limited (54).

In 2005, a preclinical human UTx study by Wranning et al. tested the tolerability of human uterus tissue to cold ischemic storage. They demonstrated that uterus tissue did not reveal any significant histological changes after 6 and 24 h of cold ischemic storage in University of Wisconsin (UW) or Perfadex (PER) solutions (72). Another study of the same group was performed in mice. They approved that uterus cold storage for 24 h displayed normal morphology. However, after 48 h preservation minimal degenerative changes were seen. And most important, transplanted uteri which had been preserved for 24 h developed normal pregnancies with healthy offspring (74).

In a clinical study published by Priore et al, eight uteri were taken during multi-organ donor procurement surgeries. After retrieval, histology sections during the period of cold ischemia (flushing with UW solution at 4 °C), taken every 15 – 30 min, showed no signs of changes over 12 h of cold ischemia (75). Later in 2014, Gauthier et al. established that after 24 h of cold ischemia there were no major morphologic changes after evaluation of histological and apoptosis analyses (76). More recently, researchers from France performed static cold storage (SCS) following UTx with ewes uteri, resulting normal morphology after 3 and 24 h of cold ischemia and good uteri viability after 8 days of UTx (77).

Usually, warm ischemic time in solid organ transplantation lasts less than one hour. However, in complicated cases it can increase to 2 – 3 h. In a study from 2013, the allowable warm ischemic time has been found to be up to 5 h in a rat model (71). Adachi and colleagues performed uterus auto-Tx in cynomolgus monkeys. Based on uterus function, like pregnancy and delivery, the maximum uterus warm ischemic time in non-human primates is estimated to be between 3 – 4 h (78). After four years, the same group repeated the study with monkeys. They confirmed that tolerable warm ischemia time is up to 4 h (70). In UTx in humans accomplished by Brännström et al., the warm ischemia time in the uterus was 1 h 13 min after Tx in the first delivery case in the world (33). In the following seven cases warm ischemia time was 1 h 23 ± 9 min, in which all recipients had restart of menstruation and four women delivered a child (49).

1.4.4. Organ Preservation and Uterus

Organ preservation has been very important since Tx became a global clinical activity. Historically, the development of effective organ preservation solutions has always focused on mechanisms and strategies for inhibiting IRI-induced cell damage (69). Although cooling initially slows the metabolic rate, prolonged cold ischemia results in depletion of cellular ATP and accelerated glycolysis and lactic acid production (59). Cellular acidosis disrupts pH and energy-dependent cellular processes, including transmembrane ion pumps (Na/K^+ and Ca^{2+}), which ultimately lead to the inflow of ions (Na^+ and Cl^-) and water, resulting in loss of membrane potential and progression of cell swelling. Damage of the cell membrane results from the release of lysosomal enzymes in response to intercellular acidosis and direct oxidation damage by ROS (63). This basic understanding of the cell injury mechanisms during SCS is based on the biochemical properties of preservative solutions that aim to target presumptive pathways that soften the processes that lead to cell death (59). Therefore, the main components of such solutions are: impermeable substances and colloids that neutralize the movement of water and electrolytes across the cell membrane and prevent cell swelling. It is also needed buffers to control pH changes, antioxidants in the form of free radical scavengers, nutrient precursors for ATP production and energy generation (69).

Preservation methods and choice of solution influence organs in a wide range of molecular responses, thus providing essential information on the timing of injury processes and their physical and chemical differences associated with the used solution. Currently, in experimental studies and clinical practice several solutions for uterus preservation has been used, including IGL-1® (79), Celsior® (61, 77, 80), University of Wisconsin (72, 81), and Custodiol® (9, 32, 36, 82-85).

According to the literature, ROS formation during uterus SCS could be prevented by modulation of the preservation solution, leading to reduced cellular damage, acidosis, and swelling (81). In our UTx research, we analysed a new trending preservation solution, Custodiol-N, which has never been used for UTx previously. This novel solution was developed by modifying the standard Custodiol® (HTK; histidine-tryptophan-ketoglutarate) solution. In order to inhibit the formation of hypoxia-induced plasma membrane pores and sodium influx, Custodiol-N was supplemented with glycine and alanine, with iron chelators (deferoxamine and LK-614) to inhibit SCS-induced cell injury, and with L-arginine to potentially decrease microcirculatory disturbances (54, 86). A higher concentration of aspartate complements the Krebs cycle and, as an energy substrate, appears to protect cells against cold injury (87). Moreover, since mannitol is not permeable to all types of cells, it was replaced by sucrose in Custodiol-N formulation (54). The complete composition of the preservation solutions is shown in Table 3. In several experiments, Custodiol-N has been demonstrated to provide better protection of organ grafts against cold ischemic injury than standard Custodiol® (86-89). Currently, there is an ongoing ulticentre, randomized, single blind comparison study (phase III), which aims to demonstrate the non-inferiority of Custodiol-N to Custodiol® in Tx of kidney, kidney-pancreas, and liver (Trial registration: Eudra-CT, 2017–002198-20) (9).

Table 3. Composition of organ preservation solutions: Custodiol® vs. Custodiol-N.

Components	Custodiol® (HTK)	Custodiol-N
Na^+ (mmol/L)	16	16
K^+ (mmol/L)	10	10
Mg^{2+} (mmol/L)	4	8
Ca^{2+} (mmol/L)	0.015	0.02
Cl^- (mmol/L)	50	30
Sucrose (mmol/L)	0	33

Mannitol (mmol/L)	30	0
α-Ketoglutaric acid (mmol/L)	2	2
L-Histidine (mmol/L)	198	124
N-α-Acetyl-L-Histidine (mmol/L)	0	57
Aspartate (mmol/L)	1	5
Tryptophan (mmol/L)	2	2
Arginine (mmol/L)	0	3
Alanine (mmol/L)	0	5
Glycine (mmol/L)	0	10
Deferoxamine * (µmol/L)	0	25
LK-615 * (µmol/L)	0	0.75

*Data modified from reference (90). * Iron chelators. HTK: histidine-tryptophan-ketoglutarate solution.*

1.5. Animal-Based UTx Research

Experiments with laboratory animals, including rodents (mice and rats), large domestic species (pigs and sheep), and non-human primates (monkeys and baboons), have paved the way for clinical UTx application. These studies are discussed below.

1.5.1. Small Animals (Mice, Rats)

To date, a limited number of studies have explored different approaches to reduce IRI, particularly in UTx. Wranning el al. performed semi-allogeneic mice UTx. In their research immunosuppressant cyclosporine A was applied in two doses (10 or 20 mg/kg/day). Histology analysis revealed less necrosis and suppressed apoptosis and inflammation in the immunosuppressant group (91). In 2014 an IRI experiment with tacrolimus was done in a rat model. Following aortic IRI, usage of tacrolimus improved uterus GSH levels and CAT activity, moreover, it prominently recovered MDA levels (92). In 2015 Atalay et al. showed that remifentanil could be used as an antioxidant to reduce IRI to result in significantly lower epithelial leucocytosis and cell degeneration, significantly decreased concentrations of MDA, increased CAT and SOD enzyme activities (57). Mycophenolate mofetil can also positively affect IRI due to significant reduction in serum IMA, uterine tissue 8-OHdG, MDA and MPO in a rat IRI model (67). Later in 2017 Aslan et al. demonstrated rat IRI experiment with oxytocin and kisspeptin-10, where the cellular damage of the uterus and MDA levels were decreased, while SOD activity and GSH levels were increased in treated groups (64). In a recent publication of Tsompos et al. an antioxidant lazaroid agent “U-74389G” was used. The results were non-significant, but U-74389G tendentially decreased endometrial oedema and uterus inflammation (93).

In 2013 the group of Akhi et al. performed an allogenic rat UTx with tacrolimus. Nontreated uterine grafts showed rejection with necrosis. Uteri of the nontreated transplanted group showed elevated mRNA expression of IL-1a and IP-10, and reduced galectin-1, compared to the tacrolimus-treated transplanted group (94).

1.5.2. Domestic Species (Pigs and Sheep)

In 2017, the group of Oliveira et al. performed six procedures of pig UTx, almost all were unsuccessful. Potentially, they used a low cyclosporine A dose since there was no control of the level of immunosuppressant in the serum; therefore, five out of six implanted uteri showed histologically confirmed acute rejection (95). Another heterogenic swine UTx model confirmed that it is really important to use the right amount of immunosuppressants. In their case tacrolimus and cyclosporine were applied, resulting in successfully treated acute graft rejections (96). A recent study in sheep showed successful results while UTx was accomplished in four sheep (97). During this study warm ischemia time was reduced from 42 to 22 min and whole surgical time was shortened from 240 to 185 min.

1.5.3. Non-Human Primates (Monkeys and Baboons)

In one study, uteri of two monkeys were removed, cooled at 4 °C and perfused with heparin saline. The uteri were interchanged with each other and then transplanted orthotopically. For immunosuppressive treatment they used three agents in case 1 (tacrolimus, mycophenolate mofetil and methylprednisolone) and two agents in case 2 (tacrolimus and methylprednisolone). After observation of blood flow in the uterus artery of the transplanted uterus B-Mode or Doppler ultrasonography, histology and menstrual cycle evaluation, results showed better recovery in case 1 with no uterus atrophy and menstrual cycle resumption (98). Adachi et al. examined the maximum warm ischemia time (during the periods of 0.5, 1, 2, 4 and 8 h), after subsequent reperfusion for 3 h biopsies were taken. The results showed that there were no significant changes after ischemia for up to 4 h but found some cell damage after 3 h reperfusion. Also, menstruation restarted in all animals with ischemia up to 4 h. However, animals with ischemia for 8 h had amenorrhea and uterus atrophy (70).

1.6. Melatonin Biological Activities and Effect on Uterus

Melatonin is a hormone secreted by the pineal gland and has long been associated with the management of the sleep and wake cycle, moreover, it has much more biological activities, including immunoregulatory and antioxidant effects (99). The discovery that melatonin is an antioxidant was made more than 25 years ago, in 1993 (100). Several approaches to melatonin include stimulation of antioxidant enzymes, regulation of glutathione synthesis, neutralization of nitrogen-based toxicants, and inhibition of prooxidant enzyme activity (99). For a long time, melatonin was considered a strong stimulant of immunity and did not receive the attention of the transplant society. Raising knowledge has shown that melatonin has a variety of effects on the immune system, including some immunosuppressive properties (101), also due to its lipophilic characteristic melatonin can access intracellular structures easily and act there (102). It is proved that melatonin has very low toxicity, as shown by animal (103) and human studies (104). Melatonin has been shown to be twice as active as vitamin E, which was considered to be the most effective lipophilic antioxidant (105).

Two membrane-associated melatonin receptors, MT1 and MT2, were identified and characterized (106). These melatonin receptors are expressed in many different types of cells, as well as in organs of human female reproductive system (ovary, uterus, breast and placenta) (107, 108). Functional MT1 and MT2 receptors were detected in the human uterus (109). Based on human myometrium biopsies and specific receptor antibodies, the MTNR1B protein of MT2 receptor was found to be strongly regulated in the myometrium of labouring women compared to the expression of MTNR1B in the myometrium of pregnant women (106). Melatonin acts via MTNR1B and synergistically enhances oxytocin-dependent signalling and contractions.

Melatonin has a direct free radical scavenger effect as well as indirect antioxidant influence by stimulating the cellular antioxidant defence system, i.e. by increasing the amount of mRNA and the activity of some valid antioxidant enzymes (110). Not only with above mentioned stimulation but also by transmitting a signal through melatonin receptors, it promotes the expression of various antioxidant enzymes, including SOD, GSH-Px, glutathione reductase, CAT and glutamyl cysteine ligase, which promotes the synthesis of another important intracellular antioxidant, glutathione (110, 111). At physiological and pharmacological concentrations, melatonin attenuates or neutralizes oxidative stress and regulates cellular metabolism, and some of these protective effects of melatonin are even shared by its metabolites (108).

1.7. Glycine Biological Activities and Effect on Uterus

Glycine is the simplest (and only achiral) proteinogenic amino acid and an important component and precursor of many cellular macromolecules (112, 113). Research with animals proved that glycine is synthesized through the three main pathways: from threonine (through threonine dehydrogenase pathway), choline (via formation of sarcosine), and serine (through serine hydroxy methyltransferase) (114). Glycine is taken up by cells through various glycine transporters, so due to its small size, glycine is very easily adapted and is the most suitable candidate for the internal position of proteins (112). It is an essential amino acid for human and other mammals and accounts for 11.5 % of all amino acids in the human body (114).

Without neurotransmitter function, glycine has a wide range of anti-inflammatory, cytoprotective and immunomodulatory properties in different cells (115, 116). Modulatory effects were mainly described in immune cells, endothelial cells and macroglial cells, where glycine regulates proliferation, differentiation and cytokine production (117). To protect cells from ischemic cell death, glycine acts by stabilizing defects in the plasma membrane porous that occur during the ischemia period, therefore protecting them from leakage of macromolecules and triggering later mechanisms of cell death (118). In many IRI models with different organs, including kidney, liver, and heart, glycine demonstrated the ability to diminish hypoxic cell injury (112, 113, 115, 119-121). There are clinical studies showing positive effects of glycine on human liver grafts after Tx (122). Moreover, glycine can be used as a therapeutic agent for inflammatory or angiogenic disorders without causing serious side effects on the female reproductive tract (123).

2. Discussion

Damage caused by ischemia and reperfusion persist as the most complicated pathophysiological process during organ Tx and the major challenge affecting clinical outcome. IRI could be described as an interrelated two-step process: the first phase of cell damage occurs during graft storage under ischemic conditions, leading to oxygen and nutrient deprivation, followed by the second phase when blood flow is restored, resulting in reperfusion injury, which includes mitochondrial dysfunction, production of ROS, calcium overload, pH alteration, and extensive inflammation (124). In ischemia metabolism, there is a big difference between supply and demand, which causes severe microvascular dysfunction, and later reperfusion stimulates the activation of immune systems, leading to acute or chronic organ rejection (60, 125).

Despite the fact that the area of organ preservation is continually expanding as new clinical trials establish a new concept of organ preservation, one of the most important parts remains to investigate the appropriate preservation method for the particular organ.

To date, only a few studies have looked into the ability of different treatments to minimize IRI, specifically in UTx (16). Moreover, we studied the impacts of Custodiol-N in a rat uterus extended preservation model for the first time worldwide.

2.1. Uterus Preservation Solutions

In the 1970s, as the number of organ transplants increased, many preservation solutions were created. All solutions were developed considering similar acting principles (124, 126):

- The use of substances with osmotic properties (e.g., glucose, mannitol, raffinose) to reduce the transplant swelling caused by the retention of sodium and water during SCS.
- antioxidant supplements (e.g., glutathione, tryptophan, histidine) can help to decrease ROS.
- metabolic acidosis neutralization using buffers (e.g., phosphate, bicarbonate, histidine).
- the use of energy precursors like adenosine or glutamate boosts ATP levels and enhances mitochondrial activity.

Up to date, there are described quite a few preservation solutions that were used in research with uterus. To begin with, Collins solution was the first preservation solution to enter the commercial market in 1969, with the purpose to preserve kidney, heart, liver, and lung grafts (127). Moreover, it was the first solution used in human UTx in Saudi Arabia, with the result of developed acute vascular thrombosis three months after UTx, and followed by hysterectomy

(50). Euro-Collins solution has a higher concentration of potassium and nowadays it is known to have a detrimental effect on the graft (81). Washing an organ with a low-potassium medium prior to preservation can protect micro vessels from the adverse effects of cold storage and improve post-transplant reperfusion in cases where vascular stenosis can be caused by high potassium levels in preservation solution (128). Another study provided data that rinsing with a low potassium solution after cold storage before organ implementation significantly reduced postoperative damage to sinusoidal endothelial cells (69). Wranning et al. tested tolerability of human uterus tissue to SCS using small uterus tissue samples and three preservation solutions: Ringer acetate, UW and PER (72). This study revealed that human uterus myometrial tissue is resistant towards SCS for at least 6 h if stored in UW and PER solutions, however usage of Ringer acetate solution showed significant degenerative uterus tissue changes. Research with animal auto-UTx, using Celsior solution resulted in increased oxidative stress markers and significant degradation of uterus samples (61, 77). All of the above-mentioned preservation solutions did not yield promising results, mainly due to their composition, due to quite high potassium and phosphate levels. Phosphate is known for its ability to stimulate mitochondrial membrane potential and lead to increased production of ROS (129).

Custodiol® (also known as Histidine-tryptophan-ketoglutarate solution) is probably the most popular solution in uterus and other organs Tx. It was developed as a cardioplegic solution, but found to be appropriate to use for both, abdominal and thoracic cavities organs. In the literature there are few studies where Custodiol® was used in experimental and clinical UTx practise with promising results until the new, Custodiol-N solution, was added to the market (130). In our study, for the first time, we used Custodiol-N solution in uterus SCS, which has never been described in literature before.

In our study, we chose to investigate different formulations of Custodiol-N, using it in full and incomplete composition and compare it to Custodiol® (90). When producing the Custodiol-N solution, four important strategies to improve the protective capability of its precursor Custodiol® were considered (90, 130, 131): 1) Reduced chloride concentration to avoid chloride-induced damage; 2) Including cytoprotective amino acids like L-arginine, glycine, and alanine; 3) N-acetyl-L-histidine is used to partially replace histidine to prevent histidine-induced cytotoxic effects; 4) Iron-chelators deferoxamine and LK-614 were added to minimize iron-dependent damage.

In our studies, this innovative solution obtained much better results than the traditional Custodiol® in both partial (without iron chelators) and full composition (90).

2.2. Iron Chelators Role in Custodiol-N

Knowing the fact that every transplant is a re-perfused organ and, therefore, undergoes some degree of oxidative damage via iron-dependent ROS formation, which has been revealed to play a key role in cold ischemia damage (130) in several cell types, including hepatocytes, endothelial, lung and kidney cells (132-134). During SCS the organ faces ROS overproduction and inadequate antioxidant defence, this balance is disturbed favouring the ROS increase that end up in oxidative stress. According to recent research, adding iron chelators to preservation solutions reduces cold preservation harm (89, 132-135). Many radical reactions can be formed from ferrous iron (Fe^{2+}), hence, iron chelation may be an effective approach for IRI reduction in the Tx field (136). Iron chelating agents function is firm bind iron and prevent it acting as a catalyst for redox reactions (133). Nowadays attention is focused on weak and strong chelator combination in preservation solution due to their known improved effect (137). Custodiol-N solution is enriched with deferoxamine and LK 614. Deferoxamine is a hydrophilic iron chelator with high molecular weight, which has been shown to reduce the formation of hydroxyl radicals or iron-oxygen species, but due to its size and hydrophilicity, cell penetration is restricted (134). Thus, LK 614, a smaller and more lipophilic iron chelator, is used to guarantee intracellular availability of an iron chelator (133). Custodiol-N with iron chelators suppresses oxidative stress-induced cell damage, polymorphonuclear infiltration, and tissue activation, as seen by lower MPO levels in our study's Custodiol-N groups.

There is evidence that iron chelators suppress reduction of antioxidants during oxidative stress (138). When compared to regular Custodiol®, the complete composition Custodiol-N group had significantly greater SOD activity, resulting in lower oxidative stress. During ischemia, oxygen free radical scavengers like SOD have been found to preserve the subcellular architecture (139).

2.3. Histological Improvement Using Custodiol-N

Our histological findings supplemented information provided in the literature, that in various SCS affected tissue cells, complex processes are beginning: generation of inflammatory proteins, enhanced formation of ROS, disrupted epithelial integrity, microvascular injury with enhanced permeability, and cell infiltration leading to cell death (90, 133). Custodiol-N with and without iron chelators showed superior results while in these groups a significantly lower percentage of uterus tissue oedema and necrosis signs were found compared to Custodiol®. During ischemia and hypothermia, the lack of substrate supply is estimated to involve Na+/K+ protein pump failure, resulting in sodium and water retention in transplant tissue (126). The capacity of preservation solutions to minimize graft oedema has been identified as the most crucial characteristic (126, 140). Recent studies have shown that Custodiol-N, with or without iron chelators, can reduce tissue oedema (90). This is due to the replacement of mannitol with sucrose in Custodiol-N solution. Cold ischemia-induced tissue oedema can be prevented by using less permeable carbohydrates in the preservation solution, especially for prolonged SCS (140, 141).

When analysing uterus samples by light microscopy, the pathologist has also noticed less expression of vasoconstriction, smooth muscle contraction, loss of endometrial cells and thickening of the perimetrium layer in the Custodiol-N group, however, the differences did not reach statistical significant when comparing all three groups (Custodiol®, Custodiol-N in full and partial composition). Upon histologically evaluating other in Custodiol-N solution preserved organs, a protective effect was observed. Animal studies with heart revealed lower apoptosis index and interstitial oedema, less inflammatory infiltration, improved myocyte preservation with usage of Custodiol-N (130, 131). In research on lung preservation, this novel solution displayed a trend toward attenuated areas of necrosis or hyaline membranes (142). SCS with Custodiol-N also showed promising results in usage with abdominal organs (124). The benefits of the Custodiol-N solution have been confirmed in most in vivo experiments using animal models for liver (87, 135), kidney (143) and small bowel (144, 145) Tx.

2.4. Uterus Tolerance to Extended SCS

The uterus's tolerance to one hour SCS has been shown in an animal model by obtaining live births in ewes after UTx (146). Moreover, promising results due to uterus tolerance to a long cold ischemic time in women have been announced, but these results were limited by the absence of reperfusion (72, 75). The study involving human subjects published by Priore et al, showed no signs of changes over 12 h of cold ischemia (75). Our study with Custodiol-N solution reported good uterus tolerance to 24 h SCS. Additionally, earlier research describing prolonged SCS in experimental UTx corroborates these findings (72, 77, 147).

Preclinical human UTx study showed non-significant histological changes after 6 and 24 h of uterus SCS in UW or PER solutions, however, in general it was seen tendentious uterus tissue tolerance to extended SCS (72). The same group performed a murine study and approved that uterus cold storage for 24 h displayed normal morphology. Moreover, uteri which had been preserved for 24 h and transplanted, developed normal pregnancies with healthy offspring (74). Successful pregnancies following embryo transfer to murine uterus transplants that had SCS for 24 hours were verified by El-Akouri et al (147). In 2014, Gauthier et al. established that after 24 h of cold ischemia there were no major morphologic changes after evaluation of histological and apoptosis analyses (76). More recently, researchers from France improved normal morphology after 3 and 24 h of cold ischemia in ewes uteri and after 8 days of UTx in both groups uteri were viable (77).

2.5. Melatonin and IRI Reduction

2.5.1. Melatonin Acting Pathways

IRI continues to be an issue in solid organ Tx, impacting clinical outcome. The potential advantages of melatonin in the treatment of Tx have been documented for many years (99, 110). According to research, melatonin is a powerful free radical scavenger that protects organ transplants against apoptosis, immunological reactivity, and oxidative stress (110, 148). It operates as a high-capacity free radical scavenger within the mitochondria which also promotes the expression of antioxidants such as SOD, GSH-Px, glutathione reductase, and CAT via signal transduction through melatonin receptors (149). Melatonin appears at high concentrations within mitochondrial fluid which greatly overstep the plasma concentration of melatonin (150). Due to its indirect effects on the expression of antioxidant enzymes, and its significant concentrations within mitochondria, several authors have indicated that melatonin has an important physiological function as a mitochondrial antioxidant (100, 151, 152). Melatonin attenuates IRI primarily through its antioxidative qualities and ability to inhibit nuclear factor κB (NF-κB), IκB kinase (IKK) and c-Jun N-terminal kinase (JNK) in the mitogen-activated protein kinase (MAPK) pathway (153-156). Additionally, the capacity of melatonin to increase Akt activation in the context of IRI has been previously noted as a potential benefit (155-157). Inflammation and cell death are influenced by all of these factors.

2.5.2. Melatonin Administration

In the literature authors described several forms of melatonin that can be used, and it seems that oral administration reaches quite good effect and at the same time is a safer method than injection (158). Administration time and route can limit melatonin consumption in many clinical situations, but not in transplant surgery. There is no restrictions for melatonin administration before organ retrieval, during storage, at any stage of implantation, or during the postoperative course (99). We used a high-dose single melatonin application 2 h before start of ischemia time based on the half-life time which is up to 2 h and results of previous experiments with other organs (154).

2.5.3. Melatonin Effect on Other Solid Organs

In the last decades, its superb effects in heart (101, 159, 160), bone (161), lung (162), pancreas (163, 164), kidney (153, 165, 166), and liver (167-169) have been described in the Tx setting. The potential role of melatonin in liver Tx has been extensively investigated. The benefit of melatonin against IRI has been demonstrated in several experimental animal models, including studies simulating healthy and damaged liver (99, 170). Nevertheless, the role of melatonin in liver Tx has been better investigated than in other solid organ transplants.

The superb effect of melatonin was shown in lung Tx research, where melatonin treatment diminished lipid peroxidation and MPO activity in lung tissue and nitrite levels in bronchoalveolar lavage with amelioration of functional parameters (162). In the cardiac Tx field, melatonin reduced the proliferative capacity of lymphocytes, abolished acute allograft rejection, and prolonged graft survival in animals (101). Even ultrastructural analysis revealed that mitochondrial damage was blurred and glycogen granules were well preserved when MLT was applied during cardiac Tx (171). A recent systematic review evaluated the relationship between melatonin and organ Tx (mainly heart and lung), verifying the positive effect of melatonin on the short-term success of Tx due to reduced oxidative stress, inflammatory process and reduced apoptosis (148).

There are many studies with kidneys because it is the most frequently transplanted solid organ. The authors described melatonin as an additive that can significantly reduce the histological index of tubular damage, induce enzymatic SOD activity, and reduce lipid hydroperoxide activity in the kidney (156, 166). At the same time, it is known, this supplement can reduce the expression of NF-κB p65, iNOS, and caspase-3 during kidney IRI (153).

2.5.4. Melatonin Effect on Female Reproductive System Organs

The impact of melatonin on the uterus and ovary IRI was examined since melatonin receptors are present in the female reproductive system. After uterus IRI, we found that there was less histopathologic damage when analysing hematoxylin and eosin stained samples, unfortunately we did not perform electron microscopy for further cell and its organelles analysis, but it will be included in future studies. Furthermore, as compared to control groups, pre-treatment with melatonin significantly attenuated the observed reduction in SOD activity after 1 h of ischemia followed by 1 h of reperfusion, resulting in lower oxidative stress. As a result, melatonin use greatly diminished polymorphonuclear infiltration and tissue activation, as seen by lower MPO levels. The effects of melatonin on uterus tissue in pregnant rats with experimentally induced uterine torsion have recently been reported (102). This study shows that melatonin reduced uterine torsion-induced tissue damage due to decreased mRNA levels in the Rock1, Hox4, TLR4, NFκB1, Cav1, and Hsp90 genes. Another study in uterus torsion / detorsion in rats found that melatonin reduced tissue damage, in addition, the use of this supplement during torsion was more effective than at the end of detorsion (172). Research also showed that melatonin works through the TLR4/NF-κB pathway, especially in IRI (102, 172).

Shiroma et al. published a systematic review focusing on melatonin influence in ovary Tx (173). Friedman et al. published a study where donated human ovarian samples were transplanted into immunodeficient mice and melatonin was supplemented, resulting in a reduced number of apoptosis and atretic follicles (174). Hemadi et al. described that with low dosage of melatonin enhanced follicle quality, quantity, and graft size was noticed. While by using high dosage of melatonin diminished Th1/Th2 lymphocytes immunological reaction and longer graft lifespan was achieved (175). Sapmaz et al. conducted autologous intraperitoneal ovary Tx in rats where the effect of melatonin as an antioxidant was investigated. This experiment revealed diminished ovarian and plasmatic MDA and ovarian necrosis, enhanced GSH-Px and SOD (173).

2.6. Glycine and IRI Reduction

2.6.1. Glycine Acting Pathways

Laschke et al. was the first one that analyses in detail the effect of dietary glycine on the female reproductive tract (123). In this study, inhibition of apoptosis was associated with decreased NF-κB expression, moreover, glycine regulated the expression of early-response genes that mediate inflammation in endometrial and ovarian tissue. Glycine inhibits IRI in almost the same way as melatonin does: it increases Akt activity while decreasing the activation of extracellular signal-regulated kinase (ERK), JNK, and p38 in the MAPK signalling pathway (113, 176, 177). The mechanisms responsible for the cytoprotective effects of glycine are largely unknown and may include stimulation of glutathione synthesis, activation of chlorine channels with glycine, and inhibition of apoptosis. The method of cell death and the molecular mechanisms underlying the protective effect of glycine were investigated by flow cytometric analysis and evaluation of the effect of glycine on the abundance of MAPK (extracellular signal-regulated kinases (ERKs), c-Jun amino terminal kinases (JNK) and p38) (176). In our investigation, glycine caused effects that were similar to those described for melatonin. As shown by MPO expression, glycine was able to retain the function of antioxidant mechanisms during uterus IRI, leading to decreased oxidative stress and inflammatory cell activation. Although the combination of melatonin and glycine did not produce substantial additive effects as predicted when these two substances were combined, this finding raised new questions that may be resolved by additional research.

2.6.2. Glycine Administration and Safety

Glycine is already rich in the diet as a component excreted in the digestion of proteins and is considered to be non-toxic even at high doses. The usual daily diet contains about two grams of glycine, but up to 40 – 90 g per day have been used for up to 6 weeks in clinical trials without serious adverse effects (178). The normal blood glycine concentration is about 300 µM (119). After taking 40 – 90 g of glycine per day, blood levels increase steadily to more than 3 times of normal levels (178). As used by Schemmer et al. for liver transplant recipients, an infusion of 250 ml of 300 mM glycine over 1 h increased usual glycine levels more than six times (122).

The half-life of glycine after intravenous infusion or oral administration depends on the dose used and ranges from about 0.5 to 4 h (119).

However, some studies suggest that glycine may have toxic effects. In an animal study, intravenous infusion of high-dose glycine induced bradycardia and reduced survival. However, this dose was approximately 50 times higher than that used in most studies that have shown a protective effect of glycine on animals or humans after liver Tx (179).

2.6.3. Glycine Effect on Other Solid Organs

The anti-inflammatory, cytoprotective and immunomodulatory properties of glycine rely on glycine receptors, which were primarily discovered in the CNS, where glycine acts as an inhibitory neurotransmitter, later its receptors were found to be present on peripheral cells (180). Studies on the therapeutic role of glycine of various organs have since begun. After demonstrating its cytoprotective effect on hypoxic cultured renal tubular cells, further studies have established a mechanism of its anti-inflammatory action that depends on the stimulation of glycine-sensitive chloride channel receptors in the cell membrane (116).

A significant number of experimental and clinical investigations have revealed that glycine has a significant potential to protect against IRI. Perhaps most experimental studies using glycine have been performed with the liver. Habib et al. reported about the role of glycine in hepatic IRI (119). This research provided relevant information that glycine could significantly increase survival, and reduction of hepatocellular injury markers during liver Tx in animal experiments and human clinical trials. Glycine also reduces alcohol-induced liver damage and eliminates lipid peroxidation reperfusion injury and glutathione deficiency caused by several types of hepatotoxins (114). Glycine has shown promise in reducing hepatic IRI. Schemmer et al. performed liver studies with glycine usage during liver Tx, IRI or cancer treatment. They reported that glycine was infused into donors for 1 h before harvest to prevent Kupffer cell activation and proved that pre-treatment of donors with intravenous glycine protects against the harmful effects of graft manipulation during harvest (181). Later Schemmer et al. performed human liver Tx with administration of 250 ml of 300 mM glycine infusion over 1 h before Tx and a similar dose of glycine daily for one week post liver Tx, resulting in significant reduction of liver enzymes (122). In 2005 Luntz et al. published data from a randomized, placebo controlled, multicentre, double-blind clinical trial where hepatoprotective effects of glycine in the postoperative phase of liver Tx was investigated (182). Based on our research group's findings, hepatoprotective glycine has been also shown to have anti-tumorigenic properties (121, 170, 183) and cardioprotective effects against FOLFOX chemotherapy (115).

Petrat et al. checked the protective effects of glycine at low doses in IRI of the rat small intestine, and showed full protection as indicated by less intestinal haemorrhages and better preserved mean arterial blood pressure, among other signs (120). It should be noted that this protective ability does not apply to all organs or diseases. Several studies proving organ protection have been reported for the liver, heart, and small intestine, but it is questionable for the kidney (112).

2.7. Limitations of This Study

There are few limitations of our study. First, the IRI model (1 h of ischemia followed by 1 h of reperfusion) does not accurately reflect the clinical situation. It is, however, sufficient time to initiate tissue oxidative damage pathways and examine the possible positive effects of possibly helpful substances. Second, the uterus affected by IRI was not transplanted. Nonetheless, because this was the first time that the effects of melatonin and glycine were explored in a model of uterus IRI, this study was primarily concerned with providing preliminary answers to the problems that had been raised. Third, only after reperfusion and/or pregnancy in a transplanted uterus can the unquestionable tolerance of the uterus to extended SCS be proven.

2.8. Future perspectives

Further research in large animal models of UTx using non-toxic substances, such as glycine and melatonin, must be conducted, and these experiments should also include Tx phase and tests of pregnancy potential.

New treatment techniques are required, since limiting IRI during Tx may possibly decrease the immune response to the allograft, resulting in a reduced requirement for systemic immunosuppression and therefore lowering extra hazards, particularly during pregnancy following UTx. The main problem in UTx is the “serious” possibility of rejection and the obligatory use of immunosuppressants. In future, with increased understanding of transplant immunology, it could be possible to reduce the immunosuppressant dose to a minimum with usage of non-toxic supplements.

Knowing Custodiol-N’s superiority against other solutions, further research in large animal uterus preservation and usage in clinical trials is an aim. It is also worth trying to use melatonin and glycine in preservation solutions, as there are several studies reporting positive effects.

3. Conclusions

This research reveals that Custodiol-N solution protects uterus transplants against cold ischemia damage more effectively than regular Custodiol® solution, most likely via inhibiting oxidative stress and tissue oedema. Moreover, the results suggest that iron chelators in the composition of Custodiol-N has an essential function in the prevention of cold ischemia. This new preservation solution has beneficial effects and further investigations are needed to deepen the knowledge about underlying molecular mechanisms.

This study indicates that pre-treatment with melatonin and glycine provides protection against IRI in a rat warm ischemia model. There is sufficient evidence that these substances can be safely used in clinical practice, since both are harmless and natural compounds. This research is significant in terms of gaining a better knowledge of the effects of melatonin and glycine, which are often used as cytoprotective substances because of their antioxidative and anti-inflammatory effects, on IRI during Tx. Despite evidence that melatonin and glycine when taken independently, had IRI-reducing properties, the two supplements, when taken together, had no additive effects. Thus, additional studies are warranted to include these substances as promising therapeutic agents in UTx.

Bibliography

1. Brannstrom M. The Swedish uterus transplantation project: the story behind the Swedish uterus transplantation project. Acta Obstet Gynecol Scand. 2015;94(7):675-9.

2. Suganuma N, Hayashi A, Kisu I, Banno K, Hara H, Mihara M. Uterus transplantation: Toward clinical application in Japan. Reprod Med Biol. 2017;16(4):305-13.

3. Brannstrom M, Dahm-Kahler P. Uterus transplantation and fertility preservation. Best Pract Res Clin Obstet Gynaecol. 2019;55:109-16.

4. Ejzenberg D, Mendes LR, Haddad LB, Baracat ED, D'Albuquerque LA, Andraus W. Uterine transplantation: a systematic review. Clinics. 2016;71(11):679-83.

5. Jacquinet A, Millar D, Lehman A. Etiologies of uterine malformations. American Journal of Medical Genetics Part A. 2016;170(8):2141-72.

6. Mahmood S, Johannesson L, Testa G, de Prisco G. DUETS (Dallas UtErus Transplant Study): The role of imaging in uterus transplantation. SAGE Open Medicine. 2019;7:2050312119875607.

7. Farrell RM, Johannesson L, Flyckt R, Richards EG, Testa G, Tzakis A, et al. Evolving ethical issues with advances in uterus transplantation. Am J Obstet Gynecol. 2020;222(6):584 e1- e5.

8. Taylor E, Gomel V. The uterus and fertility. Fertil Steril. 2008;89(1):1-16.

9. Castellon LAR, Amador MIG, Gonzalez RED, Eduardo MSJ, Diaz-Garcia C, Kvarnstrom N, et al. The history behind successful uterine transplantation in humans. JBRA Assist Reprod. 2017;21(2):126-34.

10. Brannstrom M, Kvarnstrom N, Dahm-Kahler P. Novel approaches in uterus transplantation. Curr Opin Organ Transplant. 2020;25(6):584-93.

11. Bhagavath B, Ellie G, Griffiths KM, Winter T, Alur-Gupta S, Richardson C, et al. Uterine Malformations: An Update of Diagnosis, Management, and Outcomes. Obstetrical & gynecological survey. 2017;72(6):377-92.

12. Chan YY, Jayaprakasan K, Zamora J, Thornton JG, Raine-Fenning N, Coomarasamy A. The prevalence of congenital uterine anomalies in unselected and high-risk populations: a systematic review. Hum Reprod Update. 2011;17(6):761-71.

13. Brannstrom M, Diaz-Garcia C, Hanafy A, Olausson M, Tzakis A. Uterus transplantation: animal research and human possibilities. Fertil Steril. 2012;97(6):1269-76.

14. Abrao MS, Muzii L, Marana R. Anatomical causes of female infertility and their management. International Journal of Gynecology & Obstetrics. 2013;123:S18-S24.

15. Flyckt R, Davis A, Farrell R, Zimberg S, Tzakis A, Falcone T. Uterine Transplantation: Surgical Innovation in the Treatment of Uterine Factor Infertility. J Obstet Gynaecol Can. 2018;40(1):86-93.

16. Zitkute V, Kvietkauskas M, Leber B, Strupas K, Stiegler P, Schemmer P. Ischemia and reperfusion injury in uterus transplantation: A comprehensive review. Transplant Rev (Orlando). 2020;34(3):100550.

17. Fernandez H, Al-Najjar F, Chauveaud-Lambling A, Frydman R, Gervaise A. Fertility after treatment of Asherman's syndrome stage 3 and 4. J Minim Invasive Gynecol. 2006;13(5):398-402.

18. Al Chami A, Saridogan E. Endometrial Polyps and Subfertility. J Obstet Gynaecol India. 2017;67(1):9-14.

19. Kim MR, Ah Kim Y, Jo MY, Hwang KJ, Ryu HS. High Frequency of Endometrial Polyps in Endometriosis. The Journal of the American Association of Gynecologic Laparoscopists. 2003;10(1):46-8.

20. Parasar P, Ozcan P, Terry KL. Endometriosis: Epidemiology, Diagnosis and Clinical Management. Curr Obstet Gynecol Rep. 2017;6(1):34-41.

21. Brännström M, Dahm Kähler P, Greite R, Mölne J, Díaz-García C, Tullius SG. Uterus Transplantation: A Rapidly Expanding Field. Transplantation. 2018;102(4):569-77.

22. Quinn MA, Benedet JL, Odicino F, Maisonneuve P, Beller U, Creasman WT, et al. Carcinoma of the Cervix Uteri. International Journal of Gynecology & Obstetrics. 2006;95:S43-S103.

23. Taneja A, Das S, Hussain SA, Madadin M, Lobo SW, Fatima H, et al. Uterine Transplant: A Risk to Life or a Chance for Life? Sci Eng Ethics. 2018.

24. Cozzoli M. Ethical Issues of Organ Transplantation in Non-Life-Saving Situations. In: Lanzetta M, Dubernard J-M, Petruzzo P, editors. Hand Transplantation. Milano: Springer Milan; 2007. p. 111-4.

25. Johannesson L, Jarvholm S. Uterus transplantation: current progress and future prospects. Int J Womens Health. 2016;8:43-51.

26. Arora KS, Blake V. Uterus transplantation: ethical and regulatory challenges. J Med Ethics. 2014;40(6):396-400.

27. Sreenivas K, Campo-Engelstein L. Domestic and international surrogacy laws: implications for cancer survivors. Cancer Treat Res. 2010;156:135-52.

28. O'Donovan L, Williams NJ, Wilkinson S. Ethical and policy issues raised by uterus transplants. Br Med Bull. 2019;131(1):19-28.

29. Kvarnstrom N, Jarvholm S, Johannesson L, Dahm-Kahler P, Olausson M, Brannstrom M. Live Donors of the Initial Observational Study of Uterus Transplantation-Psychological and Medical Follow-Up Until 1 Year After Surgery in the 9 Cases. Transplantation. 2017;101(3):664-70.

30. Johannesson L, Wallis K, Koon EC, McKenna GJ, Anthony T, Leffingwell SG, et al. Living uterus donation and transplantation: experience of interest and screening in a single center in the United States. Am J Obstet Gynecol. 2018;218(3):331 e1- e7.

31. Richards EG, Flyckt R, Tzakis A, Falcone T. Uterus transplantation: organ procurement in a deceased donor model. Fertil Steril. 2018;110(1):183.

32. Brannstrom M, Bokstrom H, Dahm-Kahler P, Diaz-Garcia C, Ekberg J, Enskog A, et al. One uterus bridging three generations: first live birth after mother-to-daughter uterus transplantation. Fertil Steril. 2016;106(2):261-6.

33. Brännström M, Johannesson L, Bokström H, Kvarnström N, Mölne J, Dahm-Kähler P, et al. Livebirth after uterus transplantation. The Lancet. 2015;385(9968):607-16.

34. Ozkan O, Akar ME, Ozkan O, Erdogan O, Hadimioglu N, Yilmaz M, et al. Preliminary results of the first human uterus transplantation from a multiorgan donor. Fertil Steril. 2013;99(2):470-6.

35. Flyckt R, Kotlyar A, Arian S, Eghtesad B, Falcone T, Tzakis A. Deceased donor uterine transplantation. Fertil Steril. 2017;107(3):e13.

36. Ejzenberg D, Andraus W, Baratelli Carelli Mendes LR, Ducatti L, Song A, Tanigawa R, et al. Livebirth after uterus transplantation from a deceased donor in a recipient with uterine infertility. The Lancet. 2018;392(10165):2697-704.

37. Flyckt R, Falcone T, Quintini C, Perni U, Eghtesad B, Richards EG, et al. First birth from a deceased donor uterus in the United States: from severe graft rejection to successful cesarean delivery. Am J Obstet Gynecol. 2020;223(2):143-51.

38. Hammond-Browning N. UK criteria for uterus transplantation: a review. BJOG. 2019.

39. Ejzenberg D, Andraus W, Baratelli Carelli Mendes LR, Ducatti L, Song A, Tanigawa R, et al. Livebirth after uterus transplantation from a deceased donor in a recipient with uterine infertility. Lancet. 2019;392(10165):2697-704.

40. Lefkowitz A, Edwards M, Balayla J. The Montreal Criteria for the Ethical Feasibility of Uterine Transplantation. Transpl Int. 2012;25(4):439-47.

41. ASRM. Age and Fertility: A Guide for Patients Accessed 26 August 2016 [Available from: https://www.asrm.org/BOOKLET_Age_And_Fertility/.

42. Practice Committee of the American Society for Reproductive Medicine. Electronic address aao, Practice Committee of the American Society for Reproductive M. American

Society for Reproductive Medicine position statement on uterus transplantation: a committee opinion. Fertil Steril. 2018;110(4):605-10.

43. Kvarnstrom N, Enskog A, Dahm-Kahler P, Brannstrom M. Live versus deceased donor in uterus transplantation. Fertil Steril. 2019;112(1):24-7.

44. Richards EG, Farrell RM, Ricci S, Perni U, Quintini C, Tzakis A, et al. Uterus transplantation: state of the art in 2021. Journal of assisted reproduction and genetics. 2021.

45. Testa G, Koon EC, Johannesson L, McKenna GJ, Anthony T, Klintmalm GB, et al. Living Donor Uterus Transplantation: A Single Center's Observations and Lessons Learned From Early Setbacks to Technical Success. Am J Transplant. 2017;17(11):2901-10.

46. Ricci S, Bennett C, Falcone T. Uterine Transplantation: Evolving Data, Success, and Clinical Importance. J Minim Invasive Gynecol. 2021;28(3):502-12.

47. Flyckt RL, Farrell RM, Perni UC, Tzakis AG, Falcone T. Deceased Donor Uterine Transplantation: Innovation and Adaptation. Obstetrics and gynecology. 2016;128(4):837-42.

48. Lavoué V, Vigneau C, Duros S, Boudjema K, Levêque J, Piver P, et al. Which Donor for Uterus Transplants. Transplantation. 2017;101(2):267-73.

49. Brännström M, Johannesson L, Dahm-Kähler P, Enskog A, Mölne J, Kvarnström N, et al. First clinical uterus transplantation trial: a six-month report. Fertility and Sterility. 2014;101(5):1228-36.

50. Fageeh W, Raffa H, Jabbad H, Marzouki A. Transplantation of the human uterus. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 2002;76(3):245-51.

51. Kisu I, Mihara M, Banno K, Umene K, Araki J, Hara H, et al. Risks for donors in uterus transplantation. Reprod Sci. 2013;20(12):1406-15.

52. Brannstrom M, Dahm-Kahler P, Kvarnstrom N. Robotic-assisted surgery in live-donor uterus transplantation. Fertil Steril. 2018;109(2):256-7.

53. Chmel R, Novackova M, Janousek L, Matecha J, Pastor Z, Maluskova J, et al. Revaluation and lessons learned from the first 9 cases of a Czech uterus transplantation trial: Four deceased donor and 5 living donor uterus transplantations. Am J Transplant. 2019;19(3):855-64.

54. Kahn J, Schemmer P. Comprehensive Review on Custodiol-N (HTK-N) and its Molecular Side of Action for Organ Preservation. Current pharmaceutical biotechnology. 2017;18(15):1237-48.

55. Halladin NL. Oxidative and inflammatory biomarkers of ischemia and reperfusion injuries. Danish medical journal. 2015;62(4):B5054.

56. Turkoz Y, Celik O, Hascalik S, Cigremis Y, Hascalik M, Mizrak B, et al. Melatonin reduces torsion-detorsion injury in rat ovary: biochemical and histopathologic evaluation. J Pineal Res. 2004;37(2):137-41.

57. Atalay YO, Aktas S, Sahin S, Kucukodaci Z, Ozakpinar OB. Remifentanil protects uterus against ischemia-reperfusion injury in rats. Acta Cir Bras. 2015;30(11):756-61.

58. Ersoy Canillioglu Y, Erkanli Senturk G. Alterations of IL-1 and VEGF After Ischemia-Reperfusion Injured Uterus and Ovary in Rats. Medeni Med J. 2020;35(2):106-15.

59. Kalogeris T, Baines CP, Krenz M, Korthuis RJ. Cell Biology of Ischemia/Reperfusion Injury. International Review of Cell and Molecular Biology Volume 298. International Review of Cell and Molecular Biology2012. p. 229-317.

60. Eltzschig HK, Eckle T. Ischemia and reperfusion--from mechanism to translation. Nat Med. 2011;17(11):1391-401.

61. Tardieu A, Chazelas P, Faye PA, Favreau F, Nadal-Desbarats L, Sallee C, et al. Changes in the metabolic composition of storage solution with prolonged cold ischemia of the uterus. Journal of assisted reproduction and genetics. 2019.

62. Kisu I, Umene K, Adachi M, Emoto K, Nogami Y, Banno K, et al. Allowable warm ischemic time and morphological and biochemical changes in uterine ischemia/reperfusion injury in cynomolgus macaque: a basic study for uterus transplantation. Hum Reprod. 2017;32(10):2026-35.

63. Sies H, Jones DP. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. Nat Rev Mol Cell Biol. 2020;21(7):363-83.

64. Aslan M, Erkanli Senturk G, Akkaya H, Sahin S, Yilmaz B. The effect of oxytocin and Kisspeptin-10 in ovary and uterus of ischemia-reperfusion injured rats. Taiwan J Obstet Gynecol. 2017;56(4):456-62.

65. Frijhoff J, Winyard PG, Zarkovic N, Davies SS, Stocker R, Cheng D, et al. Clinical Relevance of Biomarkers of Oxidative Stress. Antioxidants & Redox Signaling. 2015;23(14):1144-70.

66. Mates JM, Perez-Gomez C, Nunez de Castro I. Antioxidant enzymes and human diseases. Clinical biochemistry. 1999;32(8):595-603.

67. Sahin Ersoy G, Kurek Eken M, Cevik O, Cilingir OT, Tal R. Mycophenolate mofetil attenuates uterine ischaemia/reperfusion injury in a rat model. Reprod Biomed Online. 2017;34(2):115-23.

68. Valavanidis A, Vlachogianni T, Fiotakis C. 8-hydroxy-2' -deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. Journal of environmental science and health Part C, Environmental carcinogenesis & ecotoxicology reviews. 2009;27(2):120-39.

69. Petrenko A, Carnevale M, Somov A, Osorio J, Rodriguez J, Guibert E, et al. Organ Preservation into the 2020s: The Era of Dynamic Intervention. Transfus Med Hemother. 2019;46(3):151-72.

70. Adachi M, Kisu I, Nagai T, Emoto K, Banno K, Umene K, et al. Evaluation of allowable time and histopathological changes in warm ischemia of the uterus in cynomolgus monkey as a model for uterus transplantation. Acta Obstet Gynecol Scand. 2016;95(9):991-8.

71. Diaz-Garcia C, Akhi SN, Martinez-Varea A, Brannstrom M. The effect of warm ischemia at uterus transplantation in a rat model. Acta Obstet Gynecol Scand. 2013;92(2):152-9.

72. Wranning CA, Molne J, El-Akouri RR, Kurlberg G, Brannstrom M. Short-term ischaemic storage of human uterine myometrium--basic studies towards uterine transplantation. Hum Reprod. 2005;20(10):2736-44.

73. Brännström M. Uterus transplantation. Curr Opin Organ Transplant. 2015;20(6):621-8.

74. El-Akouri RR. Pregnancy in transplanted mouse uterus after long-term cold ischaemic preservation. Human Reproduction. 2003;18(10):2024-30.

75. Del Priore G, Stega J, Sieunarine K, Ungar L, Smith JR. Human uterus retrieval from a multi-organ donor. Obstetrics and gynecology. 2007;109(1):101-4.

76. Gauthier T, Piver P, Pichon N, Bibes R, Guillaudeau A, Piccardo A, et al. Uterus retrieval process from brain dead donors. Fertil Steril. 2014;102(2):476-82.

77. Tricard J, Ponsonnard S, Tholance Y, Mesturoux L, Lachatre D, Couquet C, et al. Uterus tolerance to extended cold ischemic storage after auto-transplantation in ewes. Eur J Obstet Gynecol Reprod Biol. 2017;214:162-7.

78. Mihara M, Kisu I, Hara H, Iida T, Araki J, Shim T, et al. Uterine autotransplantation in cynomolgus macaques: the first case of pregnancy and delivery. Human Reproduction. 2012;27(8):2332-40.

79. Padma AM, Truong M, Jar-Allah T, Song MJ, Oltean M, Brännström M, et al. The development of an extended normothermic ex vivo reperfusion model of the sheep uterus to evaluate organ quality after cold ischemia in relation to uterus transplantation. Acta Obstetricia et Gynecologica Scandinavica. 2019.

80. Tholance Y, Tricard J, Chianea T, Marquet P, Ponsonnard S, Sturtz F, et al. Metabolic alterations of uterine grafts after extended cold ischemic storage: experimental study in ewes. Mol Hum Reprod. 2019;25(10):647-59.

81. Tardieu A, Dion L, Lavoué V, Chazelas P, Marquet P, Piver P, et al. The Key Role of Warm and Cold Ischemia in Uterus Transplantation: A Review. J Clin Med. 2019;8(6).

82. Obara H, Kisu I, Kato Y, Yamada Y, Matsubara K, Emoto K, et al. Surgical technique for allogeneic uterus transplantation in macaques. Sci Rep. 2016;6:35989.

83. Wei L, Xue T, Yang H, Zhao GY, Zhang G, Lu ZH, et al. Modified uterine allotransplantation and immunosuppression procedure in the sheep model. PLoS One. 2013;8(11):e81300.

84. Johannesson L, Enskog A, Mölne J, Diaz-Garcia C, Hanafy A, Dahm-Kähler P, et al. Preclinical report on allogeneic uterus transplantation in non-human primates. Hum Reprod. 2013;28(1):189-98.

85. Clancy NT, Saso S, Stoyanov D, Sauvage V, Corless DJ, Boyd M, et al. Multispectral imaging of organ viability during uterine transplantation surgery in rabbits and sheep. J Biomed Opt. 2016;21(10):106006.

86. Kniepeiss D, Houben P, Stiegler P, Berghold A, Riedl R, Kahn J, et al. A prospective, randomized, single-blind, multicentre, phase III study on organ preservation with Custodiol-N solution compared with Custodiol(R) solution in organ transplantation (kidney, liver and pancreas). Trials. 2020;21(1):62.

87. Liu Q, Bruns H, Schultze D, Xue Y, Zorn M, Flechtenmacher C, et al. HTK-N, a modified HTK solution, decreases preservation injury in a model of microsteatotic rat liver transplantation. Langenbecks Arch Surg. 2012;397(8):1323-31.

88. Pless G, Sauer IM, Rauen U. Improvement of the cold storage of isolated human hepatocytes. Cell Transplant. 2012;21(1):23-37.

89. Rauen U, Petrat F, Li T, De Groot H. Hypothermia injury/cold-induced apoptosis--evidence of an increase in chelatable iron causing oxidative injury in spite of low O2-/H2O2 formation. Faseb j. 2000;14(13):1953-64.

90. Zitkute V, Kvietkauskas M, Maskoliunaite V, Leber B, Ramasauskaite D, Strupas K, et al. Custodiol-N Is Superior to Custodiol((R)) Solution in Experimental Rat Uterus Preservation. Int J Mol Sci. 2020;21(21).

91. Wranning CA, El-Akouri RR, Groth K, Molne J, Parra AK, Brannstrom M. Rejection of the transplanted uterus is suppressed by cyclosporine A in a semi-allogeneic mouse model. Hum Reprod. 2007;22(2):372-9.

92. Sahin S, Ozakpinar OB, Ak K, Eroglu M, Acikel M, Tetik S, et al. The protective effects of tacrolimus on rat uteri exposed to ischemia-reperfusion injury: a biochemical and histopathologic evaluation. Fertil Steril. 2014;101(4):1176-82.

93. Tsompos C PC, Toutouzas K, Triantafyllou A, Zografos G, Papalois A. The Co-evaluation of Endometrial Edema and Uterus Inflammation after the “U-74389G” Effect on Uterine Ischemia Reperfusion Injury. Journal of Contraceptive Studies. 2018;03(01).

94. Akhi SN, Diaz-Garcia C, El-Akouri RR, Wranning CA, Molne J, Brannstrom M. Uterine rejection after allogeneic uterus transplantation in the rat is effectively suppressed by tacrolimus. Fertil Steril. 2013;99(3):862-70.

95. Oliveira E, Tavares K, Gomes MTV, Salzedas-Netto AA, Sartori MGF, Castro RA, et al. Description and evaluation of experimental models for uterine transplantation in pigs. Einstein (Sao Paulo). 2017;15(4):481-5.

96. Avison DL, DeFaria W, Tryphonopoulos P, Tekin A, Attia GR, Takahashi H, et al. Heterotopic uterus transplantation in a swine model. Transplantation. 2009;88(4):465-9.

97. Castro-Villegas F, Canaval-Erazo GA, Rico-Juri JM, Gutierrez-Montes JO, Vanin-Aguas A, Canaval-Erazo HO. [Orthotopic uterus transplantation. Sheep model experiment, Cali (Colombia)]. Rev Colomb Obstet Ginecol. 2020;71(3):265-74.

98. Kisu I, Mihara M, Banno K, Hara H, Masugi Y, Araki J, et al. Uterus allotransplantation in cynomolgus macaque: a preliminary experience with non-human primate models. J Obstet Gynaecol Res. 2014;40(4):907-18.

99. Stiegler P, Bausys A, Leber B, Strupas K, Schemmer P. Impact of Melatonin in Solid Organ Transplantation—Is It Time for Clinical Trials? A Comprehensive Review. International Journal of Molecular Sciences. 2018;19(11).

100. Poeggeler B RR, Tan D-X, Chen L-D, Manchester LC. Melatonin, hydroxyl radical-mediated oxidative damage, and aging: A hypothesis. J Pineal Res. 1993;14:151-68.

101. Jung FJ, Yang L, Harter L, Inci I, Schneiter D, Lardinois D, et al. Melatonin in vivo prolongs cardiac allograft survival in rats. J Pineal Res. 2004;37(1):36-41.

102. Saat N, Risvanli A, Dogan H, Onalan E, Akpolat N, Seker I, et al. Effect of melatonin on torsion and reperfusion induced pathogenesis of rat uterus. Biotech Histochem. 2019;94(7):533-9.

103. Guardiola-Lemaitre B. Toxicology of melatonin. J Biol Rhythms. 1997;12(6):697-706.

104. Nordlund JJ LA. The effects of oral melatonin on skin color and on the release of pituitary hormones. J Clin Endocrinol Metab. 1977;45(4):768-74.

105. Pieri CM, M; Moroni, F; Recchioni, R; Marcheselli, F. Melatonin: a peroxyl radical scavenger more effective than vitamin E. Life Science. 1994; 55:271-6.

106. Olcese J, Beesley S. Clinical significance of melatonin receptors in the human myometrium. Fertility and Sterility. 2014;102(2):329-35.

107. Olcese JM. Melatonin and Female Reproduction: An Expanding Universe. Front Endocrinol (Lausanne). 2020;11:85.

108. Slominski RM, Reiter RJ, Schlabritz-Loutsevitch N, Ostrom RS, Slominski AT. Melatonin membrane receptors in peripheral tissues: distribution and functions. Mol Cell Endocrinol. 2012;351(2):152-66.

109. Schlabritz-Loutsevitch N, Hellner N, Middendorf R, Muller D, Olcese J. The human myometrium as a target for melatonin. J Clin Endocrinol Metab. 2003;88(2):908-13.

110. Esteban-Zubero E, Garcia-Gil FA, Lopez-Pingarron L, Alatorre-Jimenez MA, Inigo-Gil P, Tan DX, et al. Potential benefits of melatonin in organ transplantation: a review. J Endocrinol. 2016;229(3):R129-46.

111. Jockers RD, P; Dubocovich, ML; Markus, RP; Renault, N; Tosini, G; Cecon, E; Zlotos, DP. Update on Melatonin Receptors. IUPHAR Review. British Journal of Pharmacology. 2016;173 (18):2702-25.

112. Petrat F, Boengler K, Schulz R, de Groot H. Glycine, a simple physiological compound protecting by yet puzzling mechanism(s) against ischaemia-reperfusion injury: current knowledge. British Journal of Pharmacology. 2012;165(7):2059-72.

113. Zhong X, Li X, Qian L, Xu Y, Lu Y, Zhang J, et al. Glycine attenuates myocardial ischemia-reperfusion injury by inhibiting myocardial apoptosis in rats. J Biomed Res. 2012;26(5):346-54.

114. Razak MA, Begum PS, Viswanath B, Rajagopal S. Multifarious Beneficial Effect of Nonessential Amino Acid, Glycine: A Review. Oxid Med Cell Longev. 2017;2017:1716701.

115. Maneikyte J, Bausys A, Leber B, Feldbacher N, Hoefler G, Kolb-Lenz D, et al. Dietary Glycine Prevents FOLFOX Chemotherapy-Induced Heart Injury: A Colorectal Cancer Liver Metastasis Treatment Model in Rats. Nutrients. 2020;12(9).

116. Bi W, Wang F, Bi Y, Wang T, Xue P, Zhang Y, et al. Renal ischemia/reperfusion injury in rats is attenuated by a synthetic glycine derivative. Eur J Pharmacol. 2009;616(1-3):256-64.

117. Van den Eynden J, Ali SS, Horwood N, Carmans S, Brone B, Hellings N, et al. Glycine and glycine receptor signalling in non-neuronal cells. Front Mol Neurosci. 2009;2:9.

118. Frank AR, U.; de Groot, H. Protection by glycine against hypoxic injury of rat hepatocytes: inhibition of ion fluxes through nonspecific leaks. Journal of Hepatology. 2000;32(1):58-66.

119. Habib MM, Hodgson HJ, Davidson BR. The role of glycine in hepatic ischemia-reperfusion injury. Curr Pharm Des. 2006;12(23):2953-67.

120. Petrat F, Drowatzky J, Boengler K, Finckh B, Schmitz KJ, Schulz R, et al. Protection from glycine at low doses in ischemia-reperfusion injury of the rat small intestine. Eur Surg Res. 2011;46(4):180-7.

121. Maneikyte J, Bausys A, Leber B, Horvath A, Feldbacher N, Hoefler G, et al. Dietary glycine decreases both tumor volume and vascularization in a combined colorectal liver metastasis and chemotherapy model. Int J Biol Sci. 2019;15(8):1582-90.

122. Schemmer PG, M; Kraus, T; Mayatepek, E; Herfarth, C; Klar, E. Glycine reduces reperfusion injury in human liver transplantation: our first patients. Transplantation Proceedings. 2001;33:3750–2.

123. Laschke MW, Schwender C, Scheuer C, Vollmar B, Menger MD. Dietary glycine does not affect physiological angiogenesis and reproductive function, but inhibits apoptosis in endometrial and ovarian tissue by down-regulation of nuclear factor-kappaB. Fertil Steril. 2008;90(4 Suppl):1460-9.

124. Mohr A, Brockmann JG, Becker F. HTK-N: Modified Histidine-Tryptophan-Ketoglutarate Solution-A Promising New Tool in Solid Organ Preservation. Int J Mol Sci. 2020;21(18).

125. Brannstrom M, Wranning CA, Altchek A. Experimental uterus transplantation. Hum Reprod Update. 2010;16(3):329-45.

126. Latchana N, Peck JR, Whitson BA, Henry ML, Elkhammas EA, Black SM. Preservation solutions used during abdominal transplantation: Current status and outcomes. World J Transplant. 2015;5(4):154-64.

127. Collins GMB-S, M.; Terasaki, P. I. Kidney preservation for transportation. Initial perfusion and 30 hours' ice storage. Lancet. 1969;2(7632):1219-22.

128. Olschewski P, Hunold G, Eipel C, Neumann U, Schoning W, Schmitz V, et al. Improved microcirculation by low-viscosity histidine- tryptophan-ketoglutarate graft flush and subsequent cold storage in University of Wisconsin solution: results of an orthotopic rat liver transplantation model. Transpl Int. 2008;21(12):1175-80.

129. Al-Aly Z. Phosphate, oxidative stress, and nuclear factor-kappaB activation in vascular calcification. Kidney Int. 2011;79(10):1044-7.

130. Loganathan S, Radovits T, Hirschberg K, Korkmaz S, Koch A, Karck M, et al. Effects of Custodiol-N, a novel organ preservation solution, on ischemia/reperfusion injury. J Thorac Cardiovasc Surg. 2010;139(4):1048-56.

131. Veres G, Radovits T, Merkely B, Karck M, Szabo G. Custodiol-N, the novel cardioplegic solution reduces ischemia/reperfusion injury after cardiopulmonary bypass. J Cardiothorac Surg. 2015;10:27.

132. Rauen U, Kerkweg U, de Groot H. Iron-dependent vs. iron-independent cold-induced injury to cultured rat hepatocytes: a comparative study in physiological media and organ preservation solutions. Cryobiology. 2007;54(1):77-86.

133. Pizanis N, Gillner S, Kamler M, de Groot H, Jakob H, Rauen U. Cold-induced injury to lung epithelial cells can be inhibited by iron chelators - implications for lung preservation. Eur J Cardiothorac Surg. 2011;40(4):948-55.

134. Radovits T, Lin LN, Zotkina J, Koch A, Rauen U, Kohler G, et al. Endothelial dysfunction after long-term cold storage in HTK organ preservation solutions: effects of iron chelators and N-alpha-acetyl-L-histidine. J Heart Lung Transplant. 2008;27(2):208-16.

135. Stegemann J, Hirner A, Rauen U, Minor T. Use of a new modified HTK solution for machine preservation of marginal liver grafts. J Surg Res. 2010;160(1):155-62.

136. Adjimani JP, Asare P. Antioxidant and free radical scavenging activity of iron chelators. Toxicol Rep. 2015;2:721-8.

137. Hershko C, Link G, Konijn AM, Cabantchik ZI. Objectives and mechanism of iron chelation therapy. Ann N Y Acad Sci. 2005;1054:124-35.

138. Basaran UN, Ayvaz S, Aksu B, Karaca T, Cemek M, Karaboga I, et al. Desferrioxamine reduces oxidative stress in the lung contusion. ScientificWorldJournal. 2013;2013:376959.

139. Gharagozloo F, Melendez FJ, Hein RA, Shemin RJ, DiSesa VJ, Cohn LH. The effect of superoxide dismutase and catalase on the extended preservation of the ex vivo heart for transplantation. The Journal of Thoracic and Cardiovascular Surgery. 1988;95(6):1008-13.

140. Ahmad N, Hostert L, Pratt JR, Billar KJ, Potts DJ, Lodge JP. A pathophysiologic study of the kidney tubule to optimize organ preservation solutions. Kidney Int. 2004;66(1):77-90.

141. Kobayashi T, Sumimoto R, Shimada H, Kamada N, Nakagawara G. Effect of sugars in the preservation solution on liver storage in rats. Cryobiology. 1991;28(5):428-35.

142. Pizanis N, Petrov A, Heckmann J, Wiswedel I, Wohlschlager J, de Groot H, et al. A new preservation solution for lung transplantation: evaluation in a porcine transplantation model. J Heart Lung Transplant. 2012;31(3):310-7.

143. Minor T, Paul A, Efferz P, Wohlschlaeger J, Rauen U, Gallinat A. Kidney transplantation after oxygenated machine perfusion preservation with Custodiol-N solution. Transpl Int. 2015;28(9):1102-8.

144. Chen Z, Kebschull L, Foll DA, Rauen U, Hansen U, Heitplatz B, et al. A novel histidine-tryptophan-ketoglutarate formulation ameliorates intestinal injury in a cold storage and ex vivo warm oxygenated reperfusion model in rats. Biosci Rep. 2020;40(5).

145. Lautenschlager I, Pless-Petig G, Middel P, de Groot H, Rauen U, Stojanovic T. Cold Storage Injury to Rat Small-bowel Transplants-Beneficial Effect of a Modified HTK Solution. Transplantation. 2018;102(10):1666-73.

146. Wranning CA, Marcickiewicz J, Enskog A, Dahm-Kahler P, Hanafy A, Brannstrom M. Fertility after autologous ovine uterine-tubal-ovarian transplantation by vascular anastomosis to the external iliac vessels. Hum Reprod. 2010;25(8):1973-9.

147. Racho El-Akouri R, Wranning CA, Molne J, Kurlberg G, Brannstrom M. Pregnancy in transplanted mouse uterus after long-term cold ischaemic preservation. Hum Reprod. 2003;18(10):2024-30.

148. Haddad CF, Haddad JM, Veiga ECA, Sorpreso ICE, Simoes RS, Baracat EC, et al. Melatonin and organ transplantation: what is the relationship? Rev Assoc Med Bras (1992). 2020;66(3):353-8.

149. Reiter RJ, Rosales-Corral S, Tan DX, Jou MJ, Galano A, Xu B. Melatonin as a mitochondria-targeted antioxidant: one of evolution's best ideas. Cell Mol Life Sci. 2017;74(21):3863-81.

150. Reiter RJ, Mayo JC, Tan DX, Sainz RM, Alatorre-Jimenez M, Qin L. Melatonin as an antioxidant: under promises but over delivers. J Pineal Res. 2016;61(3):253-78.

151. Mayo JC, Sainz RM, Gonzalez-Menendez P, Hevia D, Cernuda-Cernuda R. Melatonin transport into mitochondria. Cell Mol Life Sci. 2017;74(21):3927-40.

152. Manchester LC, Coto-Montes A, Boga JA, Andersen LP, Zhou Z, Galano A, et al. Melatonin: an ancient molecule that makes oxygen metabolically tolerable. J Pineal Res. 2015;59(4):403-19.

153. Li Z, Nickkholgh A, Yi X, Bruns H, Gross ML, Hoffmann K, et al. Melatonin protects kidney grafts from ischemia/reperfusion injury through inhibition of NF-kB and apoptosis after experimental kidney transplantation. J Pineal Res. 2009;46(4):365-72.

154. Liang R, Nickkholgh A, Hoffmann K, Kern M, Schneider H, Sobirey M, et al. Melatonin protects from hepatic reperfusion injury through inhibition of IKK and JNK pathways and modification of cell proliferation. J Pineal Res. 2009;46(1):8-14.

155. Koh PO. Melatonin prevents hepatic injury-induced decrease in Akt downstream targets phosphorylations. J Pineal Res. 2011;51(2):214-9.

156. Hadj Ayed Tka K, Mahfoudh Boussaid A, Zaouali MA, Kammoun R, Bejaoui M, Ghoul Mazgar S, et al. Melatonin modulates endoplasmic reticulum stress and Akt/GSK3-beta signaling pathway in a rat model of renal warm ischemia reperfusion. Anal Cell Pathol (Amst). 2015;2015:635172.

157. Song J, Kang SM, Lee WT, Park KA, Lee KM, Lee JE. The beneficial effect of melatonin in brain endothelial cells against oxygen-glucose deprivation followed by reperfusion-induced injury. Oxid Med Cell Longev. 2014;2014:639531.

158. Kireev R, Bitoun S, Cuesta S, Tejerina A, Ibarrola C, Moreno E, et al. Melatonin treatment protects liver of Zucker rats after ischemia/reperfusion by diminishing oxidative stress and apoptosis. Eur J Pharmacol. 2013;701(1-3):185-93.

159. Liu C, Hong T, Shao M, Chen Z, Wang C. Melatonin synergized with cyclosporine A improves cardiac allograft survival by suppressing inflammation and apoptosis. Mol Med Rep. 2014;10(3):1323-8.

160. Zhou H, Ma Q, Zhu P, Ren J, Reiter RJ, Chen Y. Protective role of melatonin in cardiac ischemia-reperfusion injury: From pathogenesis to targeted therapy. J Pineal Res. 2018;64(3).

161. Takechi M, Tatehara S, Satomura K, Fujisawa K, Nagayama M. Effect of FGF-2 and melatonin on implant bone healing: a histomorphometric study. J Mater Sci Mater Med. 2008;19(8):2949-52.

162. Inci I, Inci D, Dutly A, Boehler A, W. W. Melatonin Attenuates Posttransplant Lung Ischemia-Reperfusion Injury. Ann Thorac Surg. 2002(73):220–5.

163. Lin GJ, Huang SH, Chen YW, Hueng DY, Chien MW, Chia WT, et al. Melatonin prolongs islet graft survival in diabetic NOD mice. J Pineal Res. 2009;47(3):284-92.

164. Munoz-Casares FC, Padillo FJ, Briceno J, Collado JA, Munoz-Castaneda JR, Ortega R, et al. Melatonin reduces apoptosis and necrosis induced by ischemia/reperfusion injury of the pancreas. J Pineal Res. 2006;40(3):195-203.

165. Panah F, Ghorbanihaghjo A, Argani H, Haiaty S, Rashtchizadeh N, Hosseini L, et al. The effect of oral melatonin on renal ischemia-reperfusion injury in transplant patients: A double-blind, randomized controlled trial. Transpl Immunol. 2019;57:101241.

166. Shi S, Lei S, Tang C, Wang K, Xia Z. Melatonin attenuates acute kidney ischemia/reperfusion injury in diabetic rats by activation of the SIRT1/Nrf2/HO-1 signaling pathway. Biosci Rep. 2019;39(1).

167. Esteban-Zubero E, Garcia-Gil FA, Lopez-Pingarron L, Alatorre-Jimenez MA, Ramirez JM, Tan DX, et al. Melatonin role preventing steatohepatitis and improving liver transplantation results. Cell Mol Life Sci. 2016;73(15):2911-27.

168. Mortezaee K, Khanlarkhani N. Melatonin application in targeting oxidative-induced liver injuries: A review. J Cell Physiol. 2018;233(5):4015-32.

169. Kim SH, Lee SM. Cytoprotective effects of melatonin against necrosis and apoptosis induced by ischemia/reperfusion injury in rat liver. J Pineal Res. 2008;44(2):165-71.

170. Kvietkauskas M, Zitkute V, Leber B, Strupas K, Stiegler P, Schemmer P. Dietary Melatonin and Glycine Decrease Tumor Growth through Antiangiogenic Activity in Experimental Colorectal Liver Metastasis. Nutrients. 2021;13(6).

171. Dobsak P. Melatonin protects against ischemia-reperfusion injury and inhibits apoptosis in isolated working rat heart. Pathophysiology. 2003;9(3):179-87.

172. Rişvanlı A, Dogan H, Saat N, Gül F, İlhan N, Seker İ, et al. The effect of melatonin in rats with uterine torsion on uterus contractions, and the levels of ADMA, SDMA, arginine, Hsp90, TLR4, and NF-κB. Ankara Üniversitesi Veteriner Fakültesi Dergisi. 2019.

173. Shiroma ME, Botelho NM, Damous LL, Baracat EC, Soares JM, Jr. Melatonin influence in ovary transplantation: systematic review. J Ovarian Res. 2016;9(1):33.

174. Friedman O, Orvieto R, Fisch B, Felz C, Freud E, Ben-Haroush A, et al. Possible improvements in human ovarian grafting by various host and graft treatments. Hum Reprod. 2012;27(2):474-82.

175. Hemadi M, Shokri S, Pourmatroud E, Moramezi F, Khodadai A. Follicular dynamic and immunoreactions of the vitrified ovarian graft after host treatment with variable regimens of melatonin. Am J Reprod Immunol. 2012;67(5):401-12.

176. Wang W, Wu Z, Lin G, Hu S, Wang B, Dai Z, et al. Glycine stimulates protein synthesis and inhibits oxidative stress in pig small intestinal epithelial cells. J Nutr. 2014;144(10):1540-8.

177. Lu Y, Zhang J, Ma B, Li K, Li X, Bai H, et al. Glycine attenuates cerebral ischemia/reperfusion injury by inhibiting neuronal apoptosis in mice. Neurochem Int. 2012;61(5):649-58.

178. Heresco-Levy UJ, D.C.; Ermilov, M.; Mordel, C.; Silipo, G.; Lichtenstein, M. Efficacy of high-dose glycine in the treatment of enduring negative symptoms of schizophrenia. Arch Gen Psychiatry. 1999;56:29-36.

179. Zhong ZW, M.D.; Li, X.; Froh, M.; Schemmer, P.; Yin, M.; Bunzendaul, H.; Bradford, B.; Lemasters, J.J. L-Glycine: a novel antiinflammatory, immunomodulatory, and cytoprotective agent. Curr Opin Clin Nutr Metab Care. 2003;6:229–40.

180. Lynch JW. Molecular structure and function of the glycine receptor chloride channel. Physiol Rev. 2004;84:1051-95.

181. Schemmer P, ; Bunzendahl, H.; Thurman, R.G. Preparing the donor with glycine improves survival after liver transplantation in the animal model. Langenbecks Archiv Fur Chirurgie Supplement Kongressband Deutsche Gesellschaft Fur Chirurgie Kongress. 1998;115:589-93.

182. Luntz SP, Unnebrink K, Seibert-Grafe M, Bunzendahl H, Kraus TW, Buchler MW, et al. HEGPOL: randomized, placebo controlled, multicenter, double-blind clinical trial to investigate hepatoprotective effects of glycine in the postoperative phase of liver transplantation [ISRCTN69350312]. BMC Surg. 2005;5:18.

183. Mikalauskas S, Mikalauskiene L, Bruns H, Nickkholgh A, Hoffmann K, Longerich T, et al. Dietary glycine protects from chemotherapy-induced hepatotoxicity. Amino Acids. 2011;40(4):1139-50.

Appendix

I have met all criteria for a Cumulative Dissertation. I have published three first author articles in SCI-listed journals with the results of my dissertation project (PDFs are attached below):

1. Zitkute V, Kvietkauskas M, Leber B, Strupas K, Stiegler P, Schemmer P. Ischemia and Reperfusion Injury in Uterus Transplantation: A comprehensive Review. Transplant Rev (Orlando). 2020;34(3):100550; doi: 10.1016/j.trre.2020.100550
2. Zitkute V, Kvietkauskas M, Maskoliunaite V, Leber B, Ramasauskaite D, Strupas K, Stiegler P, Schemmer P. Custodiol-N Is Superior to Custodiol® Solution in Experimental Rat Uterus Preservation. Int. J. Mol. Sci. 2020;21:8015; doi:10.3390/ijms21218015
3. Zitkute V, Kvietkauskas M, Maskoliunaite V, Leber B, Ramasauskaite D, Strupas K, Stiegler P, Schemmer P. Melatonin and Glycine Reduce Uterus Ischemia/ Reperfusion Injury in a Rat Model of Warm Ischemia. Int. J. Mol. Sci. 2021;22:8373; doi:10.3390/ijms22168373

I have received confirmation from the Dean of Doctoral Studies, that based on my track record I am allowed to submit a Cumulative Dissertation (proof was sent to dissertation@medunigraz.at).

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Review article

Ischemia and reperfusion injury in uterus transplantation: A comprehensive review

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abstract

Uterus transplantation (UTx) is the only available treatment for human beings who cannot carry children out to term. However, despite several clinical studies with a very limited number of UTx many issues have to be addressed. Up to date, there is a limited number of successful UTx with livebirth and the majority was achieved with live donors. Wide clinical application is inherently limited by the lack of organs, ischemia/reperfusion injury (IRI) as well as immunosuppression after UTx. The objective of this comprehensive literature review is to discuss these arising limitations of UTx with main focus on strategies to reduce IRI. This review showed, that usage of immunosuppressants, opioids or supplements, like amino acids, protects uterus from IRI, improving rising level of antioxidants and decreasing level of oxidative stress markers. The available data of experimental and clinical studies was compiled and will be discussed.

Contents

Abbreviations: ATP, Adenosine triphosphate; AUFI, Absolute uterus factor infertility; CAT, Catalase; CIT, Cold ischemia time; CyA, Cyclosporine A; GSH, Glutathione; HTK, Histidine-tryptophan-ketoglutarate solution; IMA, Ischemia modified albumin; IRI, Ischemia and reperfusion injury; MDA, Malondialdehyde; MMF, Mycophenolate mofetil; PER, Perfadex solution; RIN, Ringer acetate solution; ROS, Reactive oxygen species; SCS, Static cold storage; SOD, Superoxide dismutase; UTx, Uterus transplantation; UW, University of Wisconsin solution; WIT, Warm ischemia time.

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1. Introduction

During the last few decades, transplantation of non-vital organs is of increasing interest in the transplantation community focusing on quality of life and physical liberty of the recipients [1,2]. Uterus transplantation (UTx) is known to be a challenging procedure related not only to medical and psychological aspects but also to ethical, moral, and cultural concerns and expectations [3].

Despite remarkable advances in reproductive medicine there remains a group of women suffering from absolute uterus factor infertility (AUFI) describing the absence of a functional uterus with a prevalence of 5–7% in the general population [4]. Congenital or acquired diseases can cause AUFI (Table 1). In general, patients with absolute or relative infertility have to be distinguished, whereas those suffering from relative infertility should be considered for UTx, only after negative results of other treatment options. However, UTx is the single treatment option for patients with complete infertility.

The prevalence of uterus malformations in the general population is closely reflecting the portion of infertile women (7.3%). There is no exact number of how many UTx are needed worldwide, but knowing that approximately 1 in 500 women are affected by AUFI, there are between 60,000 and 70,000 patients in Japan [5] and about 80,000 women in the United States that are at reproductive age (20–40 years of age) and with probable need of UTx [6]. While not all of those with anatomical absent or a dysfunctional uterus will be interested in UTx, the number of those seeking the procedure is expected to be substantial.

The first UTx was performed in 2000 by a Saudi Arabian team [3]. It was performed in a 26-year-old woman who lost her uterus due to peripartum haemorrhage 6 years earlier following a caesarean section. UTx resulted in graft loss and hysterectomy 3 months thereafter [7].

After 16 years the first successful UTx from a deceased donor was performed in Brasil. Recipient was 32-year-old woman with congenital uterine absence (Mayer-Rokitansky-Küster- Hauser syndrome) and the donor was 45 years old, had three previous vaginal deliveries and died of subarachnoid haemorrhage. Seven months after UTx single embryo transfer was performed, throughout pregnancy everything went fluently without complications. On 35 gestational week caesarean delivery occurred with the result of healthy baby.

The wide application of UTx is inherently limited by the lack of donor organs, ischemia/reperfusion injury (IRI) as well as immunosuppression after the UTx. The objective of this comprehensive literature review is to discuss these arising limitations of UTx with main focus on strategies to reduce IRI. The available data of experimental and clinical studies was compiled and will be discussed.

Table 1
The main causes of absolute and relative uterus factor infertility.

Causes	Prevalence (%)	Infertility (%)
Congenital		
Arcuate-shaped uterus	0.7–6.8	17.3
Septate uterus	0.8–2.3	38
Bicornuate uterus	0.7–1.3	37.5
Uterus hypoplasia	0.038	100
Mayer–Rokitansky–Küster–Hauser syndrome	0.0002–0.1	100
Acquired		
Leiomyoma	21–26	40
Intrauterine adhesions	1–2	50–70
Peripartum hysterectomy	0.04–1.25	100
Hysterectomy due to large myomas	1–3.5	100
Hysterectomy due to malignancies	0.00004–0.0001	100

Data summarized from references [9], [51–58].

2. Methodology

Multiple databases were searched, including Medline (PubMed) and the Cochrane Database. A detailed search including the key words "uterus transplantation", "ischemia", "infertility" revealed a total of 246 articles. A total of 45 articles involving humans and animals were retrieved. There were no restrictions on date, but the study was limited to English-language publications.

2.1. Uterus donation

A uterus can be donated from either a living or deceased donor. According to the World Health Organization Guiding Principle, organ donations from deceased donors should always be developed to their maximum potential, avoiding the risks to live donors [3]. However, because of the lack of eligible organs from deceased donors and inferior outcomes, donations from a live donor are more attractive [8]. Based on literature, more than 30 UTx have been performed worldwide with most cases using living donation [9], and only a limited number of UTx cases from a uterus of a deceased donor were published with a single reported birth [10]. Currently, a study comparing the efficacy of UTx from living donors versus deceased brain-dead donors is conducted at the Institute of Clinical and Experimental Medicine in Prague, Czech Republic. Estimated completion date is at the end of 2025 [11].

For a living donor the main surgical risk is the laparotomy with all its potential complications especially damaging the ureter, with devascularization during dissection of parametria and risk of ureterovaginal fistula [12,13]. However, the risk of surgical complications is likely to decrease with the use of new less invasive methods, such as laparoscopic or robotic-assisted surgery [3,14]. In contrast to the deceased donor organ donation, the indisputable advantage of living donation is the possibility of screening with complete morphological and microbiological evaluation of the uterus in order to exclude unsuitable donor candidates. Contraindications for uterus donation are compiled in Table 2. However, there are no general guidelines on assessing donor suitability for UTx.

2.2. IRI in UTx

IRI is characterized as a pathological condition by an initial sustained blood supply with subsequent restoration of perfusion and attendant reoxygenation [7]. Firstly, IRI acts on an intracellular level, because of

Table 2
Contraindications for uterus donation.

General contraindications to organ donation	Additional contraindications to uterus donation
Not matching blood/tissue type	Donor infertility or subfertility
Human immunodeficiency virus infection	Cervical or endometrial dysplasia
Acute hepatitis B or C	Human papillomavirus infection
Active or untreated tuberculosis	Gonorrhea, chlamydia, herpes simplex type 1 or 2
Acute viral infections (e.g., rubella, rabies, adenoviruses, enteroviruses, parvoviruses)	Atherosclerosis of uterus vessels
The presence of systemic invasive fungal infections	Vascular anomalies of uterus vessels
Active metastatic or non-curable malignant disease	Uterus malformations
Past history of malignancy that poses risk for transmission	Intrauterine adhesions
Clinically uncontrolled sepsis	Myomas, adenomyosis, polyps
Systemic illness (diabetes, hypertension)	Age: b18 or N65 years old

Data summarized from references [3, 6], [58,59].

reactive oxygen species (ROS), which interact with lipids, proteins and nucleic acids, resulting in cell damage, apoptosis, and inflammatory events [15,16]. Secondly, IRI effects the immune system and contributes to vasculopathy, graft dysfunction, and rejection [17]. During elongated ischemia, anaerobic metabolism prevails, which produces a decrease in cell pH. Moreover, ischemia depletes cellular adenosine triphosphate (ATP), and ATPase-dependent ion transport mechanisms become dysfunctional [18,19]. The result of this damage is the development of metabolic waste products, inability to maintain cell membranes, increased intracellular and mitochondrial calcium levels, and possible leakage of autolyzing proteolytic enzymes into the cell and surrounding tissues [18]. Restoration of blood supply to ischemic tissues can cause further damage known as reperfusion injury that can be more damaging than the primary ischemia [7,16].

There are no uterus specific markers for the evaluation of IRI. However, there is a special histopathological scoring system proposed by Diaz-Garcia et al. [20] and Sahin Ersoy et al. [30], which based on different cellular characteristics, such as interstitial oedema, vascular dilatation, haemorrhage, and polymorphonuclear leukocyte infiltrations allowing to evaluate IRI of the uterus. This scoring system is compiled in Table 3.

2.3. Warm and cold ischemia times

Warm (WIT) and cold ischemia times (CIT) are highly relevant for IRI and have detrimental effects on the long-term survival and function after UTx. According to the available data, it seems that the uterus has better tolerance to ischemia compared to other organs.

In the first systematic study on the effects of WIT published in 2013, the allowable WIT has been found to be up to 5 h in rat UTx experiments [20]. While in a uterus IRI model with cynomolgus monkeys allowable WIT for uterus was estimated to range from 3 to 4 h [21,22]. The results were based on morphological, biochemical changes, and most important uteri function, including resumption of menstruation. However, actual UTx was not exactly mimicked in this IRI model because uteri were not perfused, cooled, transplanted or reanastomosed with vessels, which were kept connected and clamped during the period of ischemia.

Static cold storage (SCS) remains the most prevalent method for organ preservation prior to transplantation. A study performed with mice demonstrated that uteri SCS for 24 h displayed normal morphology, and recipients developed normal pregnancies (in five out of six animals) with healthy offspring after UTx. Since grafts preserved for 48 h had minimal degenerative changes, after UTx decreased blood flow was observed and morphology showed total necrosis of the transplants [23]. More recently, researchers from France proved that the uterus is an organ with good tolerance to extended SCS. They performed 14 auto-UTx in ewes and 12 UTx were successfully completed. The histological analysis after 3 and 24 h of SCS and at 90 min following reperfusion revealed an inflammation of the endometrium and serosa (moderate and severe, respectively), but no significant apoptotic signal was found in either group. During a follow-up period of 8 days after UTx, seven ewes survived [24]. Lately another group performed 18 auto-UTx in ewes and found significant degradation of uteri during 24 h of SCS by analysing metabolic composition of the Celsior storage solution [25].

Wranning et al. preclinically analysed the susceptibility of human uterus to SCS revealing that myometrial tissue is resistant towards CIT for at least 6 h [26]. However, they used small tissue samples of human uteri instead of whole organs. Subsequently, studies with uteri retrieved during multi-organ donor procurement showed that SCS for 12 h in University of Wisconsin (UW) solution did not induce any histological changes over time [27]. Gauthier et al. supported these findings with uteri donated after brain death, and stated that even 24 h of SCS in Celsior solution are feasible [28].

The first successful human UTx accomplished by Brännström et al., reported WIT of 1 h 13 min and SCS of 1 h 6 min in the first delivery case in the world [13]. In the following seven cases WIT was 1 h 23 ± 9 min, SCS was 1 h 18 ± 23 min, and all recipients had restart of menstruation and 4 women delivered a child eventually [29].

Table 3
Scoring of uterus histopathological damage of IRI [20,30].

	Score		
	0	1	2
Inflammatory cells	Absent	Moderate number of cells	Severe infiltration of cells
Vasoconstriction	Absent	Moderate b20% small vessels	Severe N20% of small vessels
Haemorrhage	Absent	Subendometrial	Myometrial + endometrial
Necrosis	Absent	b20%*	N20%*
Oedema	Absent	b50%*	N50%*
Thrombosis	Absent	b50% of the vessels	N50% of the vessels
Endometrial loss of cells	Absent	b20%*	N20%*

Maximum score for uterus IRI − 14.

* Percentages are calculated as (surface of the affected area/surface of the whole section) x 100.

2.4. Uterus protection against IRI

It is well known that IRI provokes an inflammatory response with overproduction of ROS, resulting in cell necrosis and apoptosis [16,30]. Therefore, preventative strategies to reduce IRI are clinically important in UTx. To date, only a limited number of studies have investigated different approaches in order to minimize IRI particularly in UTx. Those experimental studies are briefly described below and additionally summarized in Table 4.

2.4.1. Studies with small animals

Atalay et al. proposed the possibility to use opioids, such as remifentanil, as an antioxidant to reduce IRI during UTx [16]. It helped to reduce epithelial leucocytosis and cell degeneration, significantly decreased concentrations of malondialdehyde (MDA), and increased catalase (CAT) and superoxide dismutase (SOD) activities in an experimental IRI rat model (2 h period of ischemia was followed by 1 h of reperfusion) [16]. Another study successfully investigated the potential protective effects of kisspeptin-10 and oxytocin as antioxidants on IRI injured rat uteri [31]. More recently, studies on erythropoietin and a lazaroid agent "U-74389G" in a rat IRI models (45 min ischemia followed by 60 and 120 min reperfusion) showed non-significant results [32,33].

Ugurlu et al. showed that acetyl L-carnitine, which known to be a potential antioxidative agent, protected rat uteri during SCS for up to 24 h [34]. Moreover, addition of iloprost, a prostacyclin analog, to Histidine-tryptophan-ketoglutarate (HTK) solution reversed the histological alterations after 24 h SCS in the same experimental setup [35].

2.4.2. Studies with large domestic animals

IRI after short-time SCS was studied by Wranning et al. in an auto-UTx model using sheep. They compared the preservation solution Perfadex (PER) with Ringer's acetate (RIN). According to the study protocol, 1 h of SCS was followed by 3 h of reperfusion. Results showed that short-time SCS of uteri does not induce any severe reperfusion damage, but the use of the protective buffer PER decreases oxidative stress and inflammation when compared with a more simple solution [36].

Dittrich et al. tested the possibility to successfully freeze and then thaw entire uteri with a slow freezing protocol by using of different cryoprotectant concentration, storage temperatures and duration periods [37]. They demonstrated the ability to contract frozen / thawed uteri from pigs using oxytocin. The key factor for successful contraction was found to be perfusion of uteri with a cryoprotectant (5% DMSO).

Table 4
Evidence for IRI protection in uterus.

Reference	Treatment	Experimental model	Outcome parameter
Atalay et al. [16]	Remifentanil (2 μg/kg/min)	Rat uterus IRI (2 h of ischemia, 1 h of reperfusion)	↓ Epithelial leucocytosis, cell degeneration, and MDA; ↑ CAT and SOD enzyme activities.
Aslan et al. [31]	Oxytocin (0.5 μg/kg) and Kisspeptin-10 (0.5 μg/kg)	Rat uterus and ovaries IRI (90 min of ischemia, 90 min of reperfusion)	Combined treatment ↓ cellular damage of uterus and ovaries. Both drugs alone ↓ MDA, ↑ SOD and GSH.
Tsompos et al. [32]	Erythropoietin (10 mg/kg)	Rat uterus IRI (45 min of ischemia, 60/120 min of reperfusion)	Non-sign. ↓ the uterus inflammation score.
Tsompos et al. [33]	Antioxidant lazaroid agent "U-74389G" (10 mg/kg)	Rat uterus IRI (45 min of ischemia, 1 and 2 h of reperfusion)	Non-sign. ↓ endometrial edema, while sign. ↓ the uterus inflammation.
Ugurlu et al. [34]	Acetyl L-carnitine (10^{-8} M)	Rat uterus SCS in HTK solution (4 and 24 h)	Prevented the formation of free radicals and protected the uterus stored in SCS for 4 and 24 h periods.
Barun et al. [35]	Iloprost (10^{-8} M)	Rat uterus SCS in HTK solution (4 and 24 h)	Non-sign. difference in MDA and nitric oxide levels between groups. SCS for 24 h with iloprost made less alterations in histological samples.
Wranning et al. [36]	PER vs. RIN	Sheep auto-UTx (1–2 h of CIT, 60 min of WIT)	SCS in PER: ↑ pH, ↓ lactate and pCO2-pO2, ↓ antioxidant capacity, lipid peroxidation, and intensity of ascorbyl radical electron spin resonance signal.
Dittrich et al. [37]	Dimethyl sulfoxide (DMSO) (1, 5 or 10%)	Pig uteri cryopreservation	All uteri frozen with 5 and 10% DMSO showed rhythmic contractions after thawing for at least 1 h, whereas only 40% - using 1% DMSO. No difference regarding the survival rates after cryopreservation from 4 to 16 weeks in 70 °C or 130 °C.
Padma et al. [10]	Normothermic machine perfusion	Sheep uteri perfusion (SCS for 4 or 48 h followed by 48 h normothermic ex vivo reperfusion)	No sign. histological changes in 4 h SCS group, while faster and severe IRI of all uterine layers were evident in 48 h of SCS group after ex vivo reperfusion.
Wranning et al. [26]	RIN vs. UW vs. PER	SCS of small tissue samples of human uteri (6 and 24 h)	Better contractility after 6 h in UW and PER. No major histological changes in UW and PER after both timepoints. UW ↑ GSH in myometrium. UW and PER preserved ATP concentrations sign. better than RIN.
Wranning et al. [45]	Cyclosporine A (10 or 20 mg/kg/day)	Semi-allogeneic mice UTx	Better histology in the CyA groups. Apoptosis and inflammation more suppressed in higher dose CyA group. Interestingly, CyA ↑ the number of CD8+ cells compared with control.
Groth et al. [46]	Cyclosporine A (10 mg/kg/day)	Allogenic rat UTx	CyA normalize mRNA levels of the proinflammatory cytokine IL-1a and the glycan-binding protein galectin-1.
Oliveira et al. [47]	Cyclosporine (5 mg/kg/day)	Pig UTx	Four of six animals had acute rejection with histologically notable presence of inflammatory infiltrate with glandular aggression and capillaritis. One animal had hiperacute rejection with extreme glandular damage and capillaritis.
Sahin et al. [48]	Tacrolimus (0.3 mg/kg/day)	Rat uterus IRI (30 min of ischemia, 60 min of reperfusion)	Sign. ↑ MDA, ↓ GSH levels, CAT and SOD activities.
Akhi et al. [49]	Tacrolimus (0.5 mg/kg/day)	Allogenic rat UTx	Suppressed rejection of uterus and normalized expression of IL-1a and IP-10, prevented T-lymphocyte infiltration.
Ersoy et al. [30]	Mycophenolate mofetil (20 mg/kg/day)	Rat uterus IRI (30 min of ischemia, 4 h of reperfusion)	Sign. ↓ serum IMA, uterus tissue 8-OHdG, MDA, and MPO. Sign. ↑ SOD. Less tissue, cellular damage and apoptosis.
Kisu et al. [50]	Tacrolimus (0.15 mg/kg) and methylprednisolone (4–10 mg/day) with or without MMF (20 mg/kg)	Allogeneic cynomolgus monkey UTx	Triple combination showed better recovery with no uterus atrophy and menstrual cycle resumption.

This approach could prolong the time period necessary to find a possible recipient and further researches are needed.

In a recent study from Sweden, 13 isolated sheep uteri were perfused with the preservation solution IGL-1, then exposed to SCS for either 4 h or 48 h, afterwards reperfused for 48 h under normothermic conditions with an oxygenated recirculating perfusate containing growth factors and synthetic oxygen carriers [10]. Results revealed that uteri exposed to 4 h SCS and 48 h normothermic ex vivo reperfusion had no significant changes in the myometrium or in the endometrium. However, much faster and severe reperfusion damage of all uterus layers were apparent during the reperfusion experiment following 48 h of SCS.

2.4.3. Preclinical UTx studies

In 2005 Wranning et al. evaluated the tolerability of human uterus tissue to SCS in different solutions. Small endometrial and myometrial tissue samples were taken from uteri of pre-menopausal patients. Specimens were kept at 4 °C for 6 or 24 h in RIN, UW or PER solution. The results showed, that contractility and response to prostaglandin $F_{2\alpha}$ were better preserved after 6 h SCS in UW and PER solutions. Also, histological examination did not reveal any significant changes after 6 and 24 h of SCS in both those solutions, while specimens stored in RIN for 24 h showed degenerative changes [26].

2.4.4. UTx and immunosuppression

The recipient starts immunosuppression few days before UTx. For induction therapy usually cyclosporine and prednisolone / methylprednisolone are used [7,38]. Nowadays anti-thymocyte globulin is also used for induction therapy [39,40]. Maintenance immunosuppression is composed of triple or double drugs, including calcineurin inhibitors (usually tacrolimus), mycophenolic mofetil (MMF), and additionally, in situations with frequent acute rejections, steroids are initiated [9,41,42]. In some cases, a monotherapy for immunosuppression maintenance after UTx was evaluated; however, in all cases rejection periods occurred and triple therapy had to be recontinued [40,43].

The main two differences of immunosuppression after UTx from other solid organ transplants are that it is short-term therapy and treatment must imperatively be modified before embryo transfer. Immunosuppressive therapy is continued until childbirth, when a caesarean section is performed and the allograft uterus is removed (currently considered to be approximately 5 years with up to 2 pregnancies) [39]. Knowing the potentially teratogenic side-effect of MMF to the child, this treatment must be ended at least 6 months before the planned initial embryo transfer (10 to 12 months after UTx) [13,43,44]. For MMF replacement azathioprine is used, which is considered to be safe during pregnancy [9].

Studies with animals showed positive effects of using immunosuppressants, including reduction of oxidative stress markers, increase of antioxidants and suppression of histological changes. Wranning el al. performed UTx in mice using two different doses of cyclosporine A (CyA) (10 or 20 mg/kg/day). Histology analysis revealed necrosis in controls, while in CyA groups it showed lesser extent. Apoptosis and inflammation were suppressed in higher dose of CyA group [45]. Moreover, CyA decrease uterus mRNA levels of proinflammatory cytokine IL-1α and glycan-binding protein galectin-1 [46]. However, in 2017 the group of Oliveira et al. performed UTx model with pigs using CyA which was unsuccessful. In their research, they used a minimal dose (5 mg/kg/day), and this probably led to a high frequency of rejection and they did not control the serum levels of the CyA. Four out of six implanted uteri showed acute rejection [47].

In 2014, rat a uteri IRI experiment with tacrolimus was performed where MDA levels were significantly decreased while glutathione (GSH), CAT, and SOD were found to be increased compared with control animals [48]. Another study with tacrolimus showed reduction in mRNA expression of IL-1α and IP-10, and reduced galectin-1 [49].

MMF is known to have antioxidant properties in addition to its immunosuppressive effects. Ersoy et al. proved that MMF can positively affect IRI by reducing ischemia modified albumin (IMA), uterus tissue 8-hydroxy-2′-deoxyguanosine (8-OHdG), MDA, and myeloperoxidase (MPO) in model with rats UTx [30].

In a preliminary experience with monkeys, two different immunosuppression strategies were applied. In first case tacrolimus, MMF and methylprednisolone were used, while in second case - only two agents, including tacrolimus and methylprednisolone. After ultrasound examination, histology and menstrual cycle evaluation, results showed better recovery in first case with no uteri atrophy and menstrual cycle resumption [50].

3. Conclusion

In this literature review, detailed information on the effects of IRI on UTx in different animal models could be summarized. It is known that IRI mechanism is complicated and has a lot of influence on UTx success. Not only CIT and WIT, storage solution or operation technique impact on results, but also antioxidants and immunosuppressants that are used to minimize IRI effects. Based on this review it seems that the uterus is an organ with good tolerance to SCS and WIT. However, a uterus with significant IRI will not start menstruation after UTx, resulting in no pregnancy and birth, even if UTx will be successful. Data collection from experimental studies has stimulated to clinical application of UTx with successful deliveries in humans; however, UTx is still in an early stage and there are many medical and technical issues to be resolved. Therefore, additional experimental studies in animals are needed for the accumulation of data that will provide valuable information for the establishment of UTx in humans.

Authors' role

Authors PS and PS were responsible for defining the research question. VZ, MK, BL did the literature research and wrote the first draft of the manuscript. PS, PS, KS corrected the article and approved the final version for publication, and all authors revised the manuscript critically for important intellectual content. All authors have approved the final version of the manuscript.

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Declaration of Competing Interest

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References

[1] Taneja A, Das S, Hussain SA, Madadin M, Lobo SW, Fatima H, et al. Uterine transplant: a risk to life or a chance for life? Sci Eng Ethics 2019;25:635–42.

[2] Cozzoli M. Ethical issues of organ transplantation in non-life-saving situations. In: Lanzetta M, Dubernard J-M, Petruzzo P, editors. Hand transplantation. Milano: Springer Milan; 2007. p. 111–4.

[3] Johannesson L, Jarvholm S. Uterus transplantation: current progress and future prospects. Int J Womens Health 2016;8:43–51.

[4] Campo H, Baptista PM, Lopez-Perez N, Faus A, Cervello I, Simon C. De- and recellularization of the pig uterus: a bioengineering pilot study. Biol Reprod 2017; 96:34–45.

[5] Suganuma N, Hayashi A, Kisu I, Banno K, Hara H, Mihara M. Uterus transplantation: toward clinical application in Japan. Reprod Med Biol 2017;16:305–13.

[6] Johannesson L, Wallis K, Koon EC, McKenna GJ, Anthony T, Leffingwell SG, et al. Living uterus donation and transplantation: experience of interest and screening in a single center in the United States. Am J Obstet Gynecol 2018;218 331 e1- e7.

[7] Fageeh W, Raffa H, Jabbad H, Marzouki A. Transplantation of the human uterus. Int J Gynaecol Obstet 2002;76:245–51.

[8] Hammond-Browning N. UK criteria for uterus transplantation: a review. BJOG 2019; 126:1320–6.

[9] Brännström M, Dahm Kähler P, Greite R, Mölne J, Díaz-García C, Tullius SG. Uterus transplantation. Transplantation 2018;102:569–77.

[10] Padma AM, Truong M, Jar-Allah T, Song MJ, Oltean M, Brännström M, et al. The development of an extended normothermic ex vivo reperfusion model of the sheep uterus to evaluate organ quality after cold ischemia in relation to uterus transplantation. Acta Obstet Gynecol Scand 2019;98:1127–38.

[11] ClinicalTrials.gov [Internet]. Uterus Transplantation From Live Donors and From Deceased Donors - Clinical Study. Available from: https://clinicaltrials.gov/ct2/show/NCT03277430; 2019 May 12.

[12] Lavoué V, Vigneau C, Duros S, Boudjema K, Levêque J, Piver P, et al. Which donor for uterus transplants. Transplantation 2017;101:267–73.

[13] Brännström M, Johannesson L, Bokström H, Kvarnström N, Mölne J, Dahm-Kähler P, et al. Livebirth after uterus transplantation. Lancet 2015;385:607–16.

[14] Brannstrom M, Dahm-Kahler P, Kvarnstrom N. Robotic-assisted surgery in live-donor uterus transplantation. Fertil Steril 2018;109:256–7.

[15] Turkoz Y, Celik O, Hascalik S, Cigremis Y, Hascalik M, Mizrak B, et al. Melatonin reduces torsion-detorsion injury in rat ovary: biochemical and histopathologic evaluation. J Pineal Res 2004;37:137–41.

[16] Atalay YO, Aktas S, Sahin S, Kucukodaci Z, Ozakpinar OB. Remifentanil protects uterus against ischemia-reperfusion injury in rats. Acta Cir Bras 2015;30:756–61.

[17] Kahn J, Schemmer P. Comprehensive review on Custodiol-N (HTK-N) and its molecular side of action for organ preservation. Curr Pharm Biotechnol 2017;18:1237–48.

[18] Kalogeris T, Baines CP, Krenz M, Korthuis RJ. Cell biology of ischemia/reperfusion injury. Int Rev Cell Mol Biol 2012;298:229–317.

[19] Eltzschig HK, Eckle T. Ischemia and reperfusion–from mechanism to translation. Nat Med 2011;17:1391–401.

[20] Diaz-Garcia C, Akhi SN, Martinez-Varea A, Brannstrom M. The effect of warm ischemia at uterus transplantation in a rat model. Acta Obstet Gynecol Scand 2013;92: 152–9.

[21] Kisu I, Umene K, Adachi M, Emoto K, Nogami Y, Banno K, et al. Allowable warm ischemic time and morphological and biochemical changes in uterine ischemia/reperfusion injury in cynomolgus macaque: a basic study for uterus transplantation. Hum Reprod 2017;32:2026–35.

[22] Adachi M, Kisu I, Nagai T, Emoto K, Banno K, Umene K, et al. Evaluation of allowable time and histopathological changes in warm ischemia of the uterus in cynomolgus monkey as a model for uterus transplantation. Acta Obstet Gynecol Scand 2016; 95:991–8.

[23] El-Akouri RR. Pregnancy in transplanted mouse uterus after long-term cold ischaemic preservation. Hum Reprod 2003;18:2024–30.

[24] Tricard J, Ponsonnard S, Tholance Y, Mesturoux L, Lachatre D, Couquet C, et al. Uterus tolerance to extended cold ischemic storage after auto-transplantation in ewes. Eur J Obstet Gynecol Reprod Biol 2017;214:162–7.

[25] Tardieu A, Chazelas P, Faye PA, Favreau F, Nadal-Desbarats L, Sallee C, et al. Changes in the metabolic composition of storage solution with prolonged cold ischemia of the uterus. J Assist Reprod Genet 2019;36:1169–78.

[26] Wranning CA, Molne J, El-Akouri RR, Kurlberg G, Brannstrom M. Short-term ischaemic storage of human uterine myometrium–basic studies towards uterine transplantation. Hum Reprod 2005;20:2736–44.

[27] Del Priore G, Stega J, Sieunarine K, Ungar L, Smith JR. Human uterus retrieval from a multi-organ donor. Obstet Gynecol 2007;109:101–4.

[28] Gauthier T, Piver P, Pichon N, Bibes R, Guillaudeau A, Piccardo A, et al. Uterus retrieval process from brain dead donors. Fertil Steril 2014;102:476–82.

[29] Brännström M, Johannesson L, Dahm-Kähler P, Enskog A, Mölne J, Kvarnström N, et al. First clinical uterus transplantation trial: a six-month report. Fertil Steril 2014;101:1228–36.

[30] Sahin Ersoy G, Kurek Eken M, Cevik O, Cilingir OT, Tal R. Mycophenolate mofetil attenuates uterine ischaemia/reperfusion injury in a rat model. Reprod Biomed Online 2017;34:115–23.

[31] Aslan M, Erkanli Senturk G, Akkaya H, Sahin S, Yilmaz B. The effect of oxytocin and Kisspeptin-10 in ovary and uterus of ischemia-reperfusion injured rats. Taiwan J Obstet Gynecol 2017;56:456–62.

[32] Tsompos C, Panoulis C, Toutouzas K, Triantafyllou A, Zografos G, Papalois A. The effect of erythropoietin on uterus inflammation during ischemia reperfusion injury in rats. Cesk Gynekol 2016;81:342–8.

[33] Tsompos CPC, Toutouzas K, Triantafyllou A, Zografos G, Papalois A. The co-evaluation of endometrial edema and uterus inflammation after the "U-74389G" effect on uterine ischemia reperfusion injury. J Contra Stud 2018;3.

[34] Ugurlu T, Ozogul C, Saribas GS, Gurgen SG, Akyol SN, Kartal B. The effect of antioxidants on angiogenesis in uterine transplantation. J Obstet Gynaecol 2018;38:382–7.

[35] Barun S, Özat M, Güngor T, Demirog˘Ullari B, Sökmensüer LK, Aksakal FN, et al. The use of a prostacyclin analog, iloprost, as an adjunct to uterus preservation with histidine-tryptophan-ketoglutarate solution. Transp Proceed 2011;43.

[36] Wranning C, Dahmkahler P, Molne J, Nilsson U, Enskog A, Brannstrom M. Transplantation of the uterus in the sheep: oxidative stress and reperfusion injury after short-time cold storage. Fertil Steril 2008;90:817–26.

[37] Dittrich R, Beckmann MW, Mueller A, Binder H, Hoffmann I, Maltaris T. Uterus cryopreservation: maintenance of uterine contractility by the use of different cryoprotocols. Reprod Domest Anim 2010;45:86–91.

[38] Ozkan O, Dogan NU, Ozkan O, Mendilcioglu I, Dogan S, Aydinuraz B, et al. Uterus transplantation: from animal models through the first heart beating pregnancy to the first human live birth. Womens Health 2016;12:442–9.

[39] Ejzenberg D, Mendes LR, Haddad LB, Baracat ED, D'Albuquerque LA, Andraus W. Uterine transplantation: a systematic review. Clinics 2016;71:679–83.

[40] Brannstrom M, Bokstrom H, Dahm-Kahler P, Diaz-Garcia C, Ekberg J, Enskog A, et al. One uterus bridging three generations: first live birth after mother-to-daughter uterus transplantation. Fertil Steril 2016;106:261–6.

[41] Sahin S, Selcuk S, Eroglu M, Karateke A. Uterus transplantation: experimental animal models and recent experience in humans. Turk J Obstet Gynecol 2015;12:38–42.

[42] Wei L, Xue T, Yang H, Zhao GY, Zhang G, Lu ZH, et al. Modified uterine allotransplantation and immunosuppression procedure in the sheep model. PLoS One 2013;8:e81300.

[43] Johannesson L, Kvarnstrom N, Molne J, Dahm-Kahler P, Enskog A, Diaz-Garcia C, et al. Uterus transplantation trial: 1-year outcome. Fertil Steril 2015;103:199–204.

[44] Leroy C, Rigot JM, Leroy M, Decanter C, Le Mapihan K, Parent AS, et al. Immunosuppressive drugs and fertility. Orphanet J Rare Dis 2015;10:136.

[45] Wranning CA, El-Akouri RR, Groth K, Molne J, Parra AK, Brannstrom M. Rejection of the transplanted uterus is suppressed by cyclosporine A in a semi-allogeneic mouse model. Hum Reprod 2007;22:372–9.

[46] Groth K, Akhi SN, Molne J, Wranning CA, Brannstrom M. Effects of immunosuppression by cyclosporine A on allogenic uterine transplant in the rat. Eur J Obstet Gynecol Reprod Biol 2012;163:97–103.

[47] Oliveira E, Tavares K, Gomes MTV, Salzedas-Netto AA, Sartori MGF, Castro RA, et al. Description and evaluation of experimental models for uterine transplantation in pigs. Einstein (Sao Paulo) 2017;15:481–5.

[48] Sahin S, Ozakpinar OB, Ak K, Eroglu M, Acikel M, Tetik S, et al. The protective effects of tacrolimus on rat uteri exposed to ischemia-reperfusion injury: a biochemical and histopathologic evaluation. Fertil Steril 2014;101:1176–82.

[49] Akhi SN, Diaz-Garcia C, El-Akouri RR, Wranning CA, Molne J, Brannstrom M. Uterine rejection after allogeneic uterus transplantation in the rat is effectively suppressed by tacrolimus. Fertil Steril 2013;99:862–70.

[50] Kisu I, Mihara M, Banno K, Hara H, Masugi Y, Araki J, et al. Uterus allotransplantation in cynomolgus macaque: a preliminary experience with non-human primate models. J Obstet Gynaecol Res 2014;40:907–18.

[51] Fernandez H, Al-Najjar F, Chauveaud-Lambling A, Frydman R, Gervaise A. Fertility after treatment of Asherman's syndrome stage 3 and 4. J Minim Invasive Gynecol 2006;13:398–402.

[52] Taylor E, Gomel V. The uterus and fertility. Fertil Steril 2008;89:1–16.

[53] Quinn MA, Benedet JL, Odicino F, Maisonneuve P, Beller U, Creasman WT, et al. Carcinoma of the Cervix Uteri. Int J Gynecol Obstet 2006;95:S43–S103.

[54] Jacquinet A, Millar D, Lehman A. Etiologies of uterine malformations. Am J Med Genet Part A 2016;170:2141–72.

[55] Chan YY, Jayaprakasan K, Zamora J, Thornton JG, Raine-Fenning N, Coomarasamy A. The prevalence of congenital uterine anomalies in unselected and high-risk populations: a systematic review. Hum Reprod Update 2011;17:761–71.

[56] Fatemi HM, Kasius JC, Timmermans A, van Disseldorp J, Fauser BC, Devroey P, et al. Prevalence of unsuspected uterine cavity abnormalities diagnosed by office hysteroscopy prior to in vitro fertilization. Hum Reprod 2010;25:1959–65.

[57] Abrao MS, Muzii L, Marana R. Anatomical causes of female infertility and their management. Int J Gynecol Obstet 2013;123:S18–24.

[58] Brannstrom M, Diaz-Garcia C, Hanafy A, Olausson M, Tzakis A. Uterus transplantation: animal research and human possibilities. Fertil Steril 2012;97:1269–76.

[59] Westphal GA, Garcia VD, Souza RL, Franke CA, Vieira KD, Birckholz VR, et al. Guidelines for the assessment and acceptance of potential brain-dead organ donors. Rev Bras Ter Intensiva 2016;28:220–55.

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Article

Custodiol-N Is Superior to Custodiol® Solution in Experimental Rat Uterus Preservation

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Abstract: Uterus transplantation (UTx) is the first and only available treatment for women with absolute uterine factor infertility. However, clinical application is limited by the lack of organs, ischemia/reperfusion injury, as well as immunosuppression after UTx. Several different preservation solutions are used in experimental and clinical UTx, including Custodiol® solution. Recently, the novel Custodiol-N solution was developed with superior results in organ preservation. However, the solution was not tested yet in UTx. Therefore, the aims of this study were to evaluate the effect of Custodiol-N in uterus prolonged cold preservation time (8 and 24 h), compared to Custodiol® solution. Uterus tissue samples were obtained from adult Sprague Dawley rats (n = 10/group). Cold ischemic injury was estimated by histology, including immunohistochemistry, and biochemical tissue analyses. After 8 h of cold ischemia, higher percentage of tissue edema, necrosis signs and myeloperoxidase expression, as well as lower superoxide dismutase activity were found in Custodiol® compared to Custodiol-N ($p < 0.05$). These differences were more pronounced after 24 h of cold preservation time ($p < 0.05$). This study demonstrated that Custodiol-N protects uterus grafts from cold ischemic injury better than standard Custodiol® most likely via inhibition of oxidative stress and tissue edema. It seems that iron chelators in the composition of Custodiol-N play an important protective role against cold ischemia.

Keywords: uterus transplantation; infertility; ischemia reperfusion injury; preservation; static cold storage

1. Introduction

Uterus transplantation (UTx) is the first and only available treatment for absolute uterine factor infertility (AUFI). Up to 7% of women suffer from AUFI [1,2], which is linked to either congenital uterine agenesis (Mayer–Rokitansky–Küster–Hauser syndrome), major congenital uterine malformation (hypoplastic uterus, fraction of bicornuate/unicornuate uterus), a surgically absent uterus, or an acquired condition (intrauterine adhesions, leiomyoma) related to uterine malfunction that causes implantation failure or defect placentation [3].

In 2014, the first birth of a healthy child to a woman who underwent UTx under the care of Brännström's team in Sweden finally removed the doubts and skepticism in the medical community [4,5].

Since then, several births have occurred in multiple centers worldwide utilizing uterus grafts from both living and deceased donors [6]. However, wider clinical application is inherently limited by an organ shortage, ischemia/reperfusion injury (IRI), since all of these factors limit success rates of UTx [2]. To date, the living donor is preferred in UTx, [7] due to the possibility of better donor evaluation and elective planning of the operation. On the other hand, the use of deceased donors is indisputable advantageous because of avoiding surgical risks for the donor. The main risk for a living donor is a thromboembolic event development due to the long surgical duration together with the possibility of anesthetic complications [8]. Long-term risks include ureter injury (ureteric-vaginal fistula [9] and ureteric laceration [10]). Whereas a cold ischemia time is short in living donation, longer ischemia times have to be taken into consideration when using deceased donors. Therefore, more precise knowledge about the tolerance of the uterus to prolonged cold ischemia is required in order to increase the organ donor pool and improve the general outcomes.

In separate experiments, the uterus graft was proven to be resistant to both warm and cold ischemia [11,12]. To date, several different preservation solutions, such as IGL-1® [13], Celsior® [14–16], University of Wisconsin [17,18], NaCl [17], Ringer's acetate [18], Perfadex® [18], and Custodiol® [19–25], were used in experimental and clinical practice of UTx. Based on literature, modulation of the solution used for uterus graft static cold storage (SCS) could prevent ROS formation with resulting in reduced cell damage [17]. On the basis of Custodiol® solution, the novel Custodiol-N solution was developed and supplemented with glycine and alanine to inhibit formation of hypoxia-induced plasma membrane pores and fortified with iron chelators, including deferoxamine and LK-614, to inhibit cold-induced cell injury as well as L-arginine to decrease microcirculatory disturbances [26,27]. Moreover, mannitol has been replaced by sucrose. The complete composition of the preservation solution is compiled in Table 1. In previous experiments, Custodiol-N proved to be superior to Custodiol® solution concerning inhibition of hypoxic cell injury and cold-induced cell injury [26,28–30]. An ongoing prospective, randomized, single blind, multicenter, phase III comparison study intends to demonstrate non-inferiority of Custodiol-N against Custodiol® in kidney, combined kidney-pancreas and liver transplantation (ClinicalTrials.gov Identifier: NCT03627013) [21]. Preliminary results are expected in early 2023. However, currently, no studies using the novel Custodiol-N for preserving the uterus were published.

Table 1. Composition of the clinically used Custodiol® and Custodiol-N solution.

Components (mmol/L)	Custodiol®	Custodiol-N Base Solution	Custodiol-N
Sodium	16	16	16
Potassium	10	10	10
Magnesium	4	8	8
Calcium	0.015	0.02	0.02
Chloride	50	30	30
L-Histidine	198	124	124
N-α-acetyl-L-Histidine	–	57	57
Aspartate	1	5	5
Tryptophan	2	2	2
α-Ketoglutarate	2	2	2
Arginine	–	3	3
Alanine	–	5	5
Sucrose	–	33	33
Mannitol	30	–	–
Glycine	–	10	10
Deferoxamine	–	–	0.025
LK-615	–	–	0.0075

Data modified from Kniepeiss et al. [26]. Custodiol-N base solution is Custodiol-N without iron chelators, deferoxamine, and LK-615.

The aim of the present study was to evaluate the effect of Custodiol-N in an experimental model of rat uterus prolonged cold preservation time compared to standard Custodiol® solution. By using Custodiol-N base solution, the role of iron chelators in the composition of Custodiol-N was evaluated. Cold ischemic injury was documented by histology, including immunohistochemistry (IHC), and biochemical tissue analysis.

2. Results

2.1. Histology

After 8 h SCS, H&E staining revealed no differences between all three solutions (Figure 1) when comparing the total score based on our adapted scoring system for cold injury (Table 2). The median score in the Custodiol® group was 2.5 (2; 3) out of 9, Custodiol-N base—2.5 (1; 3) and Custodiol-N—2 (1; 3) ($p = 0.728$). However, a significantly higher percentage of tissue edema was found in the Custodiol® group when compared to Custodiol-N base and Custodiol-N (10% (5; 15) vs. 4% (0; 5) vs. 3% (3; 3), respectively, $p = 0.004$). After 8 h of SCS, the median percentage of tissue necrosis was highest in the Custodiol® group when compared to the other groups, without statistical significance ($p = 0.138$). There were no significant differences observed between Custodiol-N base and Custodiol-N groups after 8 h of cold preservation.

After 24 h of SCS, the median score of the Custodiol® group was highest—6 (5; 7) out of 9, Custodiol-N base—3.5 (3; 5) and Custodiol-N—3 (3; 4) ($p = 0.008$). Moreover, a significantly higher percentage of tissue edema (15% (15; 25) vs. 5% (5; 10) vs. 5% (5; 10), respectively, $p = 0.003$) and the median percentage of tissue necrosis signs (20% (10; 30) vs. 10% (5; 10) vs. 7.5% (5; 10), respectively, $p = 0.009$) were found in the Custodiol® group compared to Custodiol-N base and Custodiol-N. There were no significant differences observed between Custodiol-N base and Custodiol-N groups after 24 h of SCS.

Other histological features, such as endometrial cell loss, perimetrium layer thickening, vasoconstriction, and smooth muscle contraction, were similar at all time in all three groups.

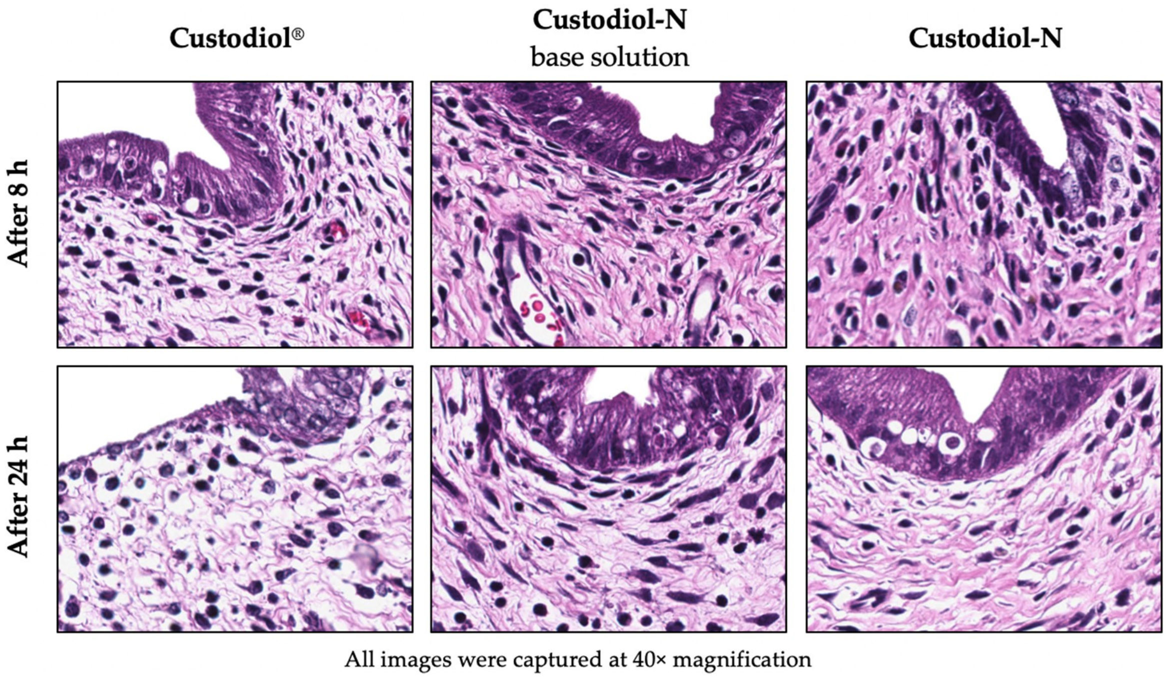

Figure 1. Effects of different preservation solutions on uterus SCS damage in H&E stained tissue samples.

2.2. Tissue MPO Expression

After 8 h of SCS, the median percentage of MPO expression in the Custodiol® group was highest—21.34% (12.43; 25.27), followed by Custodiol-N base—15.50% (12.50; 25.31) and Custodiol-N—8.03% (5.81; 10.26) (Figure 2). Significant differences were found when Custodiol® was compared to Custodiol-N ($p = 0.002$) as well as Custodiol-N base compared to Custodiol-N ($p = 000.8$). Furthermore, MPO expression increased in all three groups over time and was found to be 32.83% (27.66; 34.51) in Custodiol®, 17.48% (12.93; 21.11) in Custodiol-N base, and 11.07% (7.39; 12.86) in Custodiol-N group after 24 h of SCS; however, this increase was only statistically significant in the Custodiol® group ($p = 0.007$). MPO expression after 24 h of SCS was significantly lower in Custodiol-N compared to Custodiol® and Custodiol-N base ($p < 0.001$ and $p = 0.008$, respectively), and significantly lower in Custodiol-N base compared with Custodiol® group ($p = 0.003$).

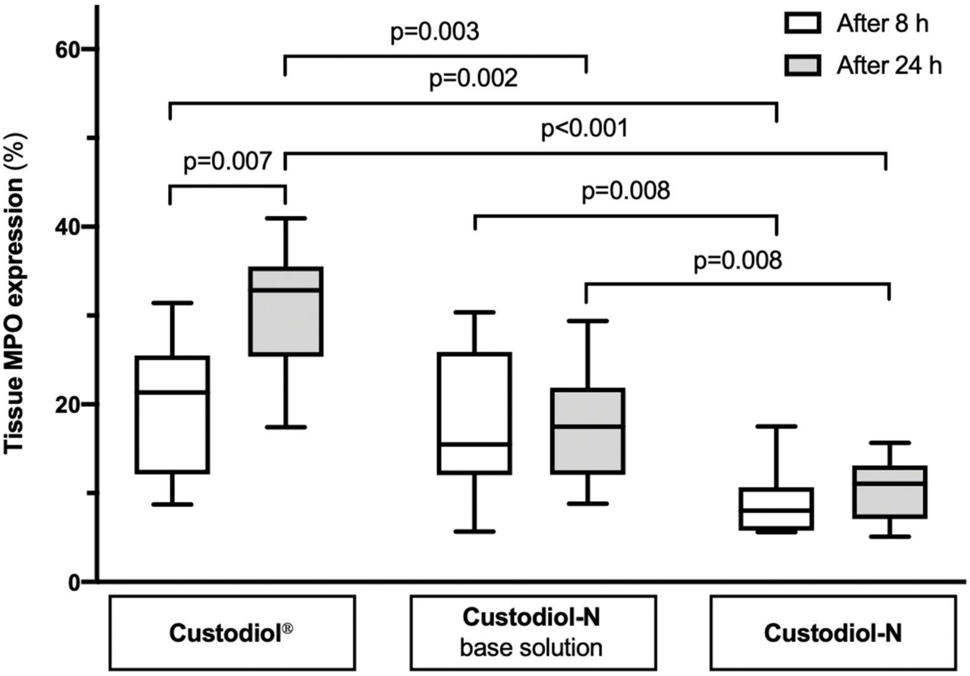

Figure 2. The percentage of myeloperoxidase (MPO) expression in uterus tissue cells preserved in different solutions for 8 and 24 h.

2.3. Tissue SOD Activity

After 8 h of SCS, the SOD activity in uterus tissue samples was significantly increased in both Custodiol-N base (1.85 (1.66; 2.62) U/mg protein; $p = 0.019$) and Custodiol-N (2.04 (1.82; 3.08); $p = 0.002$) groups compared to the Custodiol® group (1.49 (1.21; 2.10)). There was no significant difference between Custodiol-N base and Custodiol-N ($p = 0.436$) (Figure 3). Further, after 24 h of SCS, the SOD activity increased in all three groups and was found to be 1.93 (1.69; 2.42) U/mg protein in Custodiol®, 2.29 (2.13; 3.15) in Custodiol-N base, and 2.33 (1.86; 3.24) in Custodiol-N group. However, this increase was only statistically significant in the Custodiol® group ($p = 0.019$, $p = 0.089$, $p = 0.579$, respectively). On the other hand, SOD activity after 24 h was significantly higher in Custodiol-N base ($p = 0.007$) but similar in Custodiol-N ($p = 0.123$) compared to the Custodiol® group.

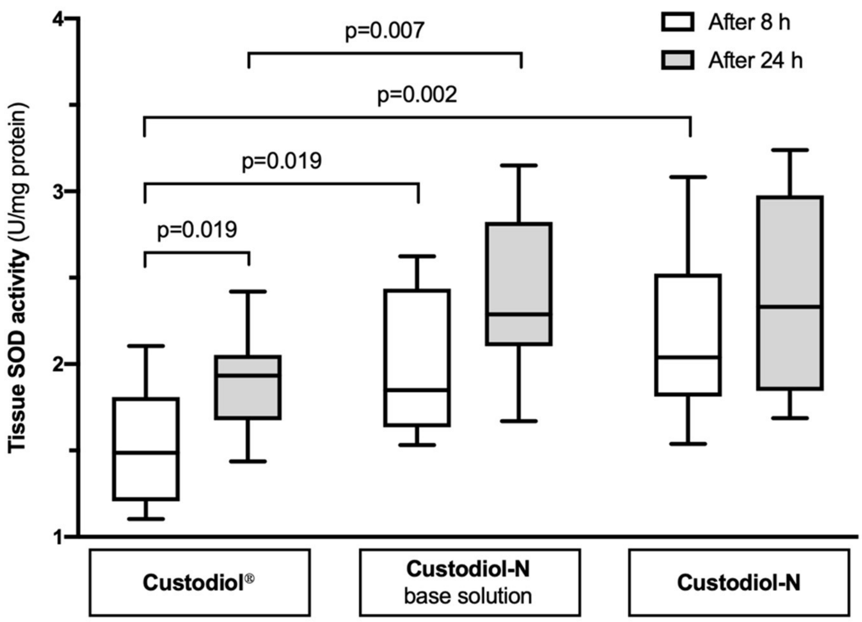

Figure 3. The SOD activity in uterus tissue samples preserved in different solutions for 8 and 24 h.

3. Discussion

In this study, the effects of Custodiol-N, a modified Custodiol® solution, were investigated in a rat uterus prolonged preservation model. This novel solution in both partial (without iron chelators) and full composition achieved significant superior results over the standard Custodiol® in our experiments.

In general, the formulation of Custodiol-N solution was modified in four important ways to improve the protective capacity of its predecessor Custodiol® [31,32]. (1) Reduction of chloride concentration to reduce chloride-induced injury. (2) Addition of cytoprotective amino acids, such as L-arginine, glycine, and alanine. (3) Partial substitution of histidine by N-α-acetyl-L-histidine to inhibit the histidine-induced cytotoxicity. (4) Addition of the iron-chelators deferoxamine and LK-614, to reduce iron-dependent injury.

Extensive research examining cell damage after tissue SCS has shown a complex interaction of different events including production of inflammatory cytokines, increased release of reactive oxygen species (ROS), loss of epithelial integrity, microvascular damage with subsequently increased permeability, and cellular infiltration leading to cell death [33]. ROS formed in an iron-dependent manner have been discovered to play a crucial role in cold ischemia injury [32] in different cell types, including hepatocytes, endothelial, lung, and kidney cells [33–35]. Current research showed that addition of iron chelators to preservation solutions decreased cold preservation injury [29,33–36].

Oxygen free radical scavengers, including SOD, have been shown to protect the subcellular architecture during ischemia [37]. Significantly higher SOD activity was found in both Custodiol-N groups (with and without iron chelators) compared to Custodiol® leading to reduced oxidative stress. Due to preserved antioxidative agents in ischemic cells, the generation of ROS could be reduced upon reperfusion, leading to less severe endothelial and DNA damage and local inflammatory responses [38]. Iron chelators added to Custodiol-N inhibit oxidative stress induced cell damage as well as further polymorphonuclear infiltration and tissue activation as documented by reduced MPO levels in the Custodiol-N groups.

The absence of substrate delivery during ischemia and hypothermia is known to induce Na+/K+ protein pump dysfunction leading to sodium and water retention in graft tissue [39]. The ability to counteract this effect is supposed to be one of the most important properties of preservation solutions [39,40]. In the current experiments, both Custodiol-N, with and without iron chelators, demonstrated the ability to prevent tissue edema mainly due to the fact that mannitol has been replaced with sucrose in Custodiol-N solution. Previous studies suggested that the inclusion of less permeable sugars in the preservation solution suppresses the cold ischemia-induced tissue edema more efficiently, especially during prolonged cold preservation [40,41].

Our findings are supported by previous studies that described extended SCS in experimental UTx [14,18,42]. El-Akouri et al. confirmed this tolerability by the successful pregnancies after embryo transfer to murine uterus grafts that had been preserved for 24 h [42].

The undeniable tolerance of the uterus to prolonged SCS can only be confirmed at least after reperfusion and/or pregnancy in a transplanted uterus which is the main limitation of this study. However, further research is mandatory to improve UTx results by optimization of organ preservation.

4. Materials and Methods

4.1. Animals

A total of 20 female Sprague Dawley rats (12-week-old; weight 270–360 g) were used for uterus procurement. The animals were obtained from Janvier Labs (Le Genest-Saint-Isle, France) and arrived at the research facility 7 days prior to surgery. Rats were housed four animals per cage in a controlled environment (22 ± 1 °C; 12 h/12 h light/dark cycle) and had access to fresh water and chow ad libitum. The study followed the guidelines for the handling and care of experimental animals issued by the Federation of European Laboratory Animal Science Associations (FELASA) and was approved by the Austrian Federal Ministry of Science, Research and Economy.

4.2. Uterus Procurement

During the procedure of uterus procurement, rats were in deep anesthesia in a supine position on a 37 °C heating pad. Anesthesia was infused by 2%, 2 L/min isoflurane applied in a rat anesthesia box. After induction therapy, rats were anesthetized with intramuscular injection of ketamine (50 mg/kg) and xylazine (9 mg/kg). A median abdominal laparotomy was performed with subsequent preparation and removal of the whole uterus. The animals were euthanized immediately afterwards by terminal blood withdrawal.

4.3. Experimental Groups, Static Cold Storage (SCS), and Sampling

The removed uterus horns were randomly assigned to the respective experimental groups: Custodiol® ($n = 10$), Custodiol-N base ($n = 10$), and Custodiol-N ($n = 10$). Custodiol-N base solution represents Custodiol-N solution without iron chelators (Table 1). The uterus cavity was, gently without force, flushed with 100 µL respective cold preservation solution, immediately packaged in small plastic bags filled with 30 mL of cold solution, and stored on ice at 4 °C. Tissue samples were obtained after 8 and 24 h of SCS. One part of the tissue specimen was fixed in 4% formalin and embedded into paraffin blocks for histological analyses and IHC while another part was frozen and stored in liquid nitrogen for later biochemical analyses.

4.4. Histology

Tissue sections (2 µm thick) were stained with hematoxylin and eosin (H&E) and subsequently examined under a light microscope by a blinded, experienced pathologist. For histological evaluation, a semi-quantitative morphological scoring system was modified based on previously published methods [43,44] (Table 2).

Table 2. The scoring system for uterus static cold storage (SCS) damage evaluation.

Histological Findings	**Score**			
	0	**1**	**2**	**3**
Edema	<5%	<5–15%	15–30%	>30%
Necrosis	Absent	<15%	15–30%	>30%
Smooth muscle contraction	Absent	Present		
Impaired basement membrane integrity	Absent	Present		
Endometrial cells loss	Absent	Present		

The maximum score for uterus SCS injury is 9. Percentages are calculated as (surface of the affected area/surface of the whole section) × 100.

4.5. Immunohistochemical (IHC) Staining

Expression levels of the oxidative stress marker myeloperoxidase (MPO) in uterus tissue was assessed by IHC. Anti-MPO (Dako, Via Real Carpinteria, CA, USA; dilution 1:800; polyclonal rabbit anti-human) antibodies were used in combination with the UltraVision LP Detection System HRP Polymer (Thermo Fisher Scientific, Waltham, MA, USA) and DAB chromogen (Dako, Via Real Carpinteria, CA, USA). For positive control, rat spleen tissue was used, while for negative control, primary antibodies were omitted. Stained slides were scanned and analyzed using the QuPath software version 0.2.0-m5 (Belfast, Northern Ireland) [45]. The number of positive cells was counted in a blinded fashion and expressed as percentage stained cells of total nuclei in the entire tissue comprising all uterus layers.

4.6. Biochemistry

For biochemical analyses, frozen tissue samples were homogenized in ice cold phosphate-buffered saline using a bead-beater, MagNA Lyser (at 6500 rpm/30 s × 3). To prevent excess sample peroxidation while processing, 5 mM butylated hydroxytoluene (antioxidant) was added in advance. The supernatant was collected, aliquoted, and stored at −80 °C for later analyses. Superoxide dismutase (SOD) activity was determined using the commercially available SOD Colorimetric Activity Kit produced by Thermo Fisher Scientific (Waltham, MA, USA) exactly as described by the manufacturer. Results were adjusted to total protein levels determined by the BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) and expressed as units per mg of protein.

4.7. Statistical Analyses

Statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) version 23.0 (IBM Corp., Armonk, NY, USA). Kruskal–Wallis and Mann–Whitney U tests were used to analyze statistical difference between groups according to their distribution. Non-parametric data is presented as median and quartiles (Q1; Q3). A p value less than 0.05 was considered statistically significant.

5. Conclusions

This study demonstrates the superiority of Custodiol-N solutions for uterus graft preservation when compared to standard Custodiol® most likely via inhibition of oxidative stress and tissue edema. It seems that iron chelators included in Custodiol-N play an important protective role against cold ischemic injury. The effects of this novel preservation solution are promising and further research is warranted.

Author Contributions: P.S. (Philipp Stiegler) and P.S. (Peter Schemmer) were responsible for the study concept, design, and critical revision of the drafted manuscript. V.Z., M.K., V.M., B.L., D.R., and K.S. were responsible for the data collection and analysis, literature review, interpretation of data, writing, review, and revision of the manuscript. All authors have read and agree to the published version of the manuscript.

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Abbreviations

AUFI	absolute uterine factor infertility
UTx	uterus transplantation
H&E	hematoxylin and eosin
IHC	immunohistochemistry
IRI	ischemia/reperfusion injury
MPO	myeloperoxidase
ROS	reactive oxygen species
SCS	static cold storage
SOD	superoxide dismutase

References

1. Groth, K.; Akhi, S.N.; Mölne, J.; Wranning, C.A.; Brännström, M. Effects of immunosuppression by cyclosporine A on allogenic uterine transplant in the rat. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **2012**, *163*, 97–103. [CrossRef] [PubMed]
2. Zitkute, V.; Kvietkauskas, M.; Leber, B.; Strupas, K.; Stiegler, P.; Schemmer, P. Ischemia and reperfusion injury in uterus transplantation: A comprehensive review. *Transplant. Rev.* **2020**, *34*, 100550. [CrossRef]
3. Brännström, M.; Dahm-Kähler, P.; Greite, R.; Mölne, J.; Diaz-Garcia, C.; Tullius, S.G. Uterus transplantation: A rapidly expanding field. *Transplantation* **2018**, *102*, 569–577. [CrossRef] [PubMed]
4. Brännström, M.; Johannesson, L.; Bokström, H.; Kvarnström, N.; Mölne, J.; Dahm-Kähler, P.; Enskog, A.; Milenkovic, M.; Ekberg, J.; Diaz-Garcia, C.; et al. Livebirth after uterus transplantation. *Lancet* **2015**, *385*, 607–616. [CrossRef]
5. Dion, L.; Tardieu, A.; Piver, P.; Aubard, Y.; Ayoubi, J.; Garbin, O.; Agostini, A.; Collinet, P.; Gauthier, T.; Lavoué, V.; et al. Uterus transplantation: Where do we stand in 2018? *J. Gynecol. Obstet. Hum. Reprod.* **2019**, *48*, 11–13. [CrossRef] [PubMed]
6. Johannesson, L.; Testa, G.; Flyckt, R.; Farrell, R.M.; Quintini, C.; Wall, A.E.; O'Neill, K.E.; Tzakis, A.G.; Richards, E.G.; Gordon, S.M.; et al. Guidelines for standardized nomenclature and reporting in uterus transplantation: An opinion from the United States Uterus Transplant Consortium. *Arab. Archaeol. Epigr.* **2020**. [CrossRef]
7. Lavoué, V.; Vigneau, C.; Duros, S.; Boudjema, K.; Levêque, J.; Piver, P.; Aubard, Y.; Gauthier, T. Which donor for uterus transplants: Brain-dead donor or living donor? A systematic review. *Transplantation* **2017**, *101*, 267–273. [CrossRef]
8. Kvarnström, N.; Enskog, A.; Dahm-Kähler, P.; Brännström, M. Live versus deceased donor in uterus transplantation. *Fertil. Steril.* **2019**, *112*, 24–27. [CrossRef]
9. Brännström, M.; Johannesson, L.; Dahm-Kähler, P.; Enskog, A.; Mölne, J.; Kvarnström, N.; Diaz-Garcia, C.; Hanafy, A.; Lundmark, C.; Marcickiewicz, J.; et al. First clinical uterus transplantation trial: A six-month report. *Fertil. Steril.* **2014**, *101*, 1228–1236. [CrossRef]
10. Fageeh, W.; Raffa, H.; Jabbad, H.; Marzouki, A. Transplantation of the human uterus. *Int. J. Gynecol. Obstet.* **2002**, *76*, 245–251. [CrossRef]
11. Brännström, M. Uterus transplantation. *Curr. Opin. Organ Transplant.* **2015**, *20*, 621–628. [CrossRef] [PubMed]
12. Flyckt, R.; Davis, A.; Farrell, R.; Zimberg, S.; Tzakis, A.; Falcone, T. Uterine transplantation: Surgical innovation in the treatment of uterine factor infertility. *J. Obstet. Gynaecol. Can.* **2018**, *40*, 86–93. [CrossRef] [PubMed]
13. Padma, A.M.; Truong, M.; Jar-Allah, T.; Song, M.J.; Oltean, M.; Brännström, M.; Hellström, M. The development of an extended normothermic ex vivo reperfusion model of the sheep uterus to evaluate organ quality after cold ischemia in relation to uterus transplantation. *Acta Obstet. Gynecol. Scand.* **2019**, *98*, 1127–1138. [CrossRef]

14. Tricard, J.; Ponsonnard, S.; Tholance, Y.; Mesturoux, L.; Lachatre, D.; Couquet, C.; Terro, F.; Yardin, C.; Marquet, P.; Piccardo, A.; et al. Uterus tolerance to extended cold ischemic storage after auto-transplantation in ewes. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **2017**, *214*, 162–167. [CrossRef] [PubMed]
15. Tardieu, A.; Chazelas, P.; Faye, P.-A.; Favreau, F.; Nadal-Desbarats, L.; Sallée, C.; Margueritte, F.; Couquet, C.-Y.; Marquet, P.; Guellec, C.B.-L.; et al. Changes in the metabolic composition of storage solution with prolonged cold ischemia of the uterus. *J. Assist. Reprod. Genet.* **2019**, *36*, 1169–1178. [CrossRef] [PubMed]
16. Tholance, Y.; Tricard, J.; Chianea, T.; Marquet, P.; Ponsonnard, S.; Sturtz, F.; Piccardo, A.; Gauthier, T. Metabolic alterations of uterine grafts after extended cold ischemic storage: Experimental study in ewes. *Mol. Hum. Reprod.* **2019**, *25*, 647–659. [CrossRef] [PubMed]
17. Tardieu, A.; Dion, L.; Lavoué, V.; Chazelas, P.; Marquet, P.; Piver, P.; Sallée, C.; Aubard, Y.; Le Guellec, C.; Favreau, F.; et al. The key role of warm and cold ischemia in uterus transplantation: A review. *J. Clin. Med.* **2019**, *8*, 760. [CrossRef]
18. Wranning, C.A.; Mölne, J.; El-Akouri, R.; Kurlberg, G.; Brännström, M. Short-term ischaemic storage of human uterine myometrium—Basic studies towards uterine transplantation. *Hum. Reprod.* **2005**, *20*, 2736–2744. [CrossRef]
19. Obara, H.; Kisu, I.; Kato, Y.; Yamada, Y.; Matsubara, K.; Emoto, K.; Adachi, M.; Matoba, Y.; Umene, K.; Nogami, Y.; et al. Surgical technique for allogeneic uterus transplantation in macaques. *Sci. Rep.* **2016**, *6*, 35989. [CrossRef]
20. Brännström, M.; Bokström, H.; Dahm-Kähler, P.; Diaz-Garcia, C.; Ekberg, J.; Enskog, A.; Hagberg, H.; Johannesson, L.; Kvarnström, N.; Mölne, J.; et al. One uterus bridging three generations: First live birth after mother-to-daughter uterus transplantation. *Fertil. Steril.* **2016**, *106*, 261–266. [CrossRef]
21. Castellón, L.A.R.; Amador, M.I.G.; González, R.E.D.; Eduardo, M.S.J.; Díaz-García, C.; Kvarnström, N.; Bränström, M. The history behind successful uterine transplantation in humans. *JBRA Assist. Reprod.* **2017**, *21*, 126–134. [CrossRef] [PubMed]
22. Wei, L.; Xue, T.; Yang, H.; Zhao, G.-Y.; Zhang, G.; Lu, Z.-H.; Huang, Y.-H.; Ma, X.-D.; Liu, H.-X.; Liang, S.-R.; et al. Modified uterine Allotransplantation and immunosuppression procedure in the sheep model. *PLoS ONE* **2013**, *8*, e81300. [CrossRef] [PubMed]
23. Ejzenberg, D.; Andraus, W.; Mendes, L.R.B.C.; Ducatti, L.; Song, A.; Tanigawa, R.; Rocha-Santos, V.; Arantes, R.M.; Soares, J.M.; Serafini, P.C.; et al. Livebirth after uterus transplantation from a deceased donor in a recipient with uterine infertility. *Lancet* **2018**, *392*, 2697–2704. [CrossRef]
24. Johannesson, L.; Enskog, A.; Molne, J.; Diaz-Garcia, C.; Hanafy, A.; Dahm-Kahler, P.; Tekin, A.; Tryphonopoulos, P.; Morales, P.; Rivas, K.; et al. Preclinical report on allogeneic uterus transplantation in non-human primates. *Hum. Reprod.* **2012**, *28*, 189–198. [CrossRef]
25. Clancy, N.T.; Saso, S.; Stoyanov, D.; Sauvage, V.; Corless, D.J.; Boyd, M.; Noakes, D.E.; Thum, M.-Y.; Ghaem-Maghami, S.; Smith, J.R.; et al. Multispectral imaging of organ viability during uterine transplantation surgery in rabbits and sheep. *J. Biomed. Opt.* **2016**, *21*, 106006. [CrossRef] [PubMed]
26. Kniepeiss, D.; Houben, P.; Stiegler, P.; Berghold, A.; Riedl, R.; Kahn, J.; Schemmer, P. A prospective, randomized, single-blind, multicentre, phase III study on organ preservation with Custodiol-N solution compared with Custodiol(R) solution in organ transplantation (kidney, liver and pancreas). *Trials* **2020**, *21*, 62. [CrossRef]
27. Kahn, J.; Schemmer, P. Comprehensive review on Custodiol-N (HTK-N) and its molecular side of action for organ preservation. *Curr. Pharm. Biotechnol.* **2018**, *18*, 1237–1248. [CrossRef]
28. Pless, G.; Sauer, I.M.; Rauen, U. Improvement of the cold storage of isolated human hepatocytes. *Cell Transplant.* **2012**, *21*, 23–37. [CrossRef]
29. Rauen, U.; Petrat, F.; Li, T.; De Groot, H. Hypothermia injury/cold-induced apoptosis–evidence of an increase in chelatable iron causing oxidative injury in spite of low O2-/H2O2 formation. *FASEB J.* **2000**, *14*, 1953–1964.
30. Liu, Q.; Bruns, H.; Schultze, D.; Xue, Y.; Zorn, M.; Flechtenmacher, C.; Straub, B.K.; Rauen, U.; Schemmer, P. HTK-N, a modified HTK solution, decreases preservation injury in a model of microsteatotic rat liver transplantation. *Langenbeck's Arch. Surg.* **2012**, *397*, 1323–1331. [CrossRef]
31. Veres, G.; Radovits, T.; Merkely, B.; Karck, M.; Szabo, G. Custodiol-N, the novel cardioplegic solution reduces ischemia/reperfusion injury after cardiopulmonary bypass. *J. Cardiothorac. Surg.* **2015**, *10*, 27. [CrossRef] [PubMed]

32. Loganathan, S.; Radovits, T.; Hirschberg, K.; Korkmaz, S.; Koch, A.; Karck, M.; Szabó, G. Effects of Custodiol-N, a novel organ preservation solution, on ischemia/reperfusion injury. *J. Thorac. Cardiovasc. Surg.* **2010**, *139*, 1048–1056. [CrossRef] [PubMed]
33. Pizanis, N.; Gillner, S.; Kamler, M.; De Groot, H.; Jakob, H.; Rauen, U. Cold-induced injury to lung epithelial cells can be inhibited by iron chelators—Implications for lung preservation. *Eur. J. Cardio-Thorac. Surg.* **2011**, *40*, 948–955. [CrossRef]
34. Rauen, U.; Kerkweg, U.; De Groot, H. Iron-dependent vs. iron-independent cold-induced injury to cultured rat hepatocytes: A comparative study in physiological media and organ preservation solutions. *Cryobiology* **2007**, *54*, 77–86. [CrossRef]
35. Radovits, T.; Lin, L.-N.; Zotkina, J.; Koch, A.; Rauen, U.; Köhler, G.; Karck, M.; Szabo, G. Endothelial dysfunction after long-term cold storage in HTK organ preservation solutions: Effects of iron Chelators and N-α-acetyl-l-histidine. *J. Heart Lung Transplant.* **2008**, *27*, 208–216. [CrossRef]
36. Stegemann, J.; Hirner, A.; Rauen, U.; Minor, T. Use of a new modified HTK solution for machine preservation of marginal liver grafts. *J. Surg. Res.* **2010**, *160*, 155–162. [CrossRef]
37. Gharagozloo, F.; Melendez, F.J.; Hein, R.A.; Shemin, R.J.; Disesa, V.J.; Cohn, L.H. The effect of superoxide dismutase and catalase on the extended preservation of the ex vivo heart for transplantation. *J. Thorac. Cardiovasc. Surg.* **1988**, *95*, 1008–1013. [CrossRef]
38. Wu, M.-Y.; Yiang, G.-T.; Liao, W.-T.; Tsai, A.P.-Y.; Cheng, Y.-L.; Cheng, P.-W.; Li, C.-Y. Current mechanistic concepts in ischemia and reperfusion injury. *Cell. Physiol. Biochem.* **2018**, *46*, 1650–1667. [CrossRef] [PubMed]
39. Latchana, N. Preservation solutions used during abdominal transplantation: Current status and outcomes. *World J. Transplant.* **2015**, *5*, 154–164. [CrossRef] [PubMed]
40. Ahmad, N.; Hostert, L.; Pratt, J.R.; Billar, K.J.; Potts, D.J.; Lodge, J.P.A.; Ahmad, L.H.N. A pathophysiologic study of the kidney tubule to optimize organ preservation solutions. *Kidney Int.* **2004**, *66*, 77–90. [CrossRef]
41. Kobayashi, T.; Sumimoto, R.; Shimada, H.; Kamada, N.; Nakagawara, G. Effect of sugars in the preservation solution on liver storage in rats. *Cryobiology* **1991**, *28*, 428–435. [CrossRef]
42. El-Akouri, R.R.; Wranning, C.A.; Mölne, J.; Kurlberg, G.; Brännström, M. Pregnancy in transplanted mouse uterus after long-term cold ischaemic preservation. *Hum. Reprod.* **2003**, *18*, 2024–2030. [CrossRef] [PubMed]
43. Ersoy, G.S.; Eken, M.; Cevik, O.; Çilingir, Ö.T.; Tal, R. Mycophenolate mofetil attenuates uterine ischaemia/reperfusion injury in a rat model. *Reprod. Biomed. Online* **2017**, *34*, 115–123. [CrossRef]
44. Diaz-Garcia, C.; Akhi, S.N.; Martínez-Varea, A.; Brännström, M. The effect of warm ischemia at uterus transplantation in a rat model. *Acta Obstet. Gynecol. Scand.* **2012**, *92*, 152–159. [CrossRef] [PubMed]
45. Bankhead, P.; Loughrey, M.B.; Fernández, J.A.; Dombrowski, Y.; McArt, D.G.; Dunne, P.D.; McQuaid, S.; Gray, R.T.; Murray, L.J.; Coleman, H.G.; et al. QuPath: Open source software for digital pathology image analysis. *Sci. Rep.* **2017**, *7*, 16878. [CrossRef]

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Article

Melatonin and Glycine Reduce Uterus Ischemia/Reperfusion Injury in a Rat Model of Warm Ischemia

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Abstract: Ischemia/reperfusion injury (IRI) remains a significant problem to be solved in uterus transplantation (UTx). Melatonin and glycine have been shown to possess direct cytoprotective activities, mainly due to their antioxidative and anti-inflammatory properties. The aim of this study was to investigate the protective effects of melatonin and glycine and their combination on IRI in a rat model of warm ischemia. In this study, *Sprague-Dawley* rats were assigned to eight groups, including sham and IRI ($n = 80$). Melatonin and glycine alone or their combination were administered prior to 1 h of uterus ischemia followed by 1 h of reperfusion. Melatonin (50 mg/kg) was administered via gavage 2 h before IRI and glycine in an enriched diet for 5 days prior to intervention. Uterus IRI was estimated by histology, including immunohistochemistry, and biochemical tissue analyses. Histology revealed that uterus IRI was significantly attenuated by pretreatment with melatonin ($p = 0.019$) and glycine ($p = 0.044$) alone as well as their combination ($p = 0.003$). Uterus IRI led to increased myeloperoxidase expression, which was significantly reduced by melatonin ($p = 0.004$), glycine ($p < 0.001$) or their combination ($p < 0.001$). The decline in superoxide dismutase activity was significantly reduced in the melatonin ($p = 0.027$), glycine ($p = 0.038$) and combined treatment groups ($p = 0.015$) when compared to the IRI control group. In conclusion, melatonin, glycine and their combination significantly reduced oxidative stress-induced cell damage after IRI in a small animal warm ischemia model, and, therefore, clinical studies are required to evaluate the protective effects of these well-characterized substances in uterus IRI.

Keywords: melatonin; glycine; ischemia and reperfusion injury; uterus transplantation

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1. Introduction

Successful uterus transplantation (UTx) has been shown to be the best treatment option for patients with absolute uterus factor infertility (AUFI) [1]. AUFI specifies women who are unable to become pregnant or maintain pregnancy because of the absence of a uterus (due to Mayer-Rokitansky-Küster-Hauser syndrome, or hysterectomy due to uterine benign/malignant tumors or postpartum bleeding), or the presence of a uterus that is anatomically dysfunctional (Asherman syndrome, septate, bicornuate, arcuate, hypoplastic or myomic uterus) [2–4].

In 2014, Brännström et al. reported the first human live birth following UTx, arousing interest in research on UTx as AUFI treatment and resulting in at least 20 clinical trials and even more animal experiments currently taking place across the world [5]. The need for UTx is growing in addition to the increasing number of successful clinical trials worldwide.

To date, about 100 human UTx have been performed with around 20 reported successful live births [2].

Unlike other solid organ transplantation (Tx), UTx is the only ephemeral type of Tx, where the transplant is not intended for life-long use but for a limited time in the recipient (until delivery), and this restricted duration greatly reduces the risk of the well-known long-term immunosuppressive-related side effects [6,7]. Although UTx is still at the stage of animal experiments and clinical trials, its necessity is undeniable, and it is only a matter of time before this procedure becomes routine worldwide.

Transplant success is limited by ischemia/reperfusion injury (IRI), and researchers are still struggling to answer key questions about uterus tolerance to IRI [4]. During ischemia, severe imbalance of metabolic supply and demand occurs, subsequently causing tissue hypoxia [8,9]. Moreover, restoration of blood flow and reoxygenation itself paradoxically further enhance the activation of innate and adaptive immune responses and cell death programs (reperfusion injury) [10]. These processes increase the likelihood of short- and long-term complications, such as delayed graft function and acute or chronic rejection [11]. Different strategies to reduce IRI, such as optimization of organ perfusion and storing conditions, or the development of drugs targeting IRI, are under investigation [12,13].

Melatonin is a hormone produced by various tissues in the body, although the major source is the pineal gland in the brain [14,15]. It is naturally produced from the amino acid tryptophan and comprises biological activities such as immunoregulatory, antioxidative and anti-inflammatory effects, as well as the ability to stabilize cell membranes; furthermore, its metabolites are able to reduce free radicals [14,16–18]. Melatonin receptors are expressed in a variety of cell types in rodents or primates as well as in the human female reproductive system, including the ovary, uterus, breast and placenta [14,19]. The potentially beneficial effects of melatonin have been shown in various animal models of IRI [12,20–22].

Glycine, the simplest amino acid, is involved in the synthesis of a variety of biomolecules and metabolisms, and is also an inhibitory neurotransmitter and an inhibitor of the activation of immune cells [23]. It is able to attenuate hypoxic cell injury by direct cytoprotection in isolated cells, organ perfusion and in vivo models targeting different organs, including the heart, liver, kidney and skeletal muscle [23–28].

This study was thus designed to evaluate the protective effects of melatonin and glycine, as separate supplements or in combination, on IRI in a rat model of warm ischemia.

2. Results

2.1. General Data

All animals were in good general health throughout the study period. One rat (1.25%) died after induction of anesthesia prior to surgery. The median body weight slightly increased from 303.9 (296.9; 322.1) g at the beginning of the study to 313.6 (304.9; 329.7) g at the end ($p = 0.001$). Five days of different diets (casein vs. glycine) did not affect body weight gain (3.04% (0.66; 6.84) vs. 2.7% (1.01; 4.42), $p = 0.494$), despite differences in median daily food intake (22.3 (21.4; 23.6) vs. 20.3 (19.3; 21.6) g, $p < 0.001$).

2.2. Glycine Concentration

The median glycine concentration in serum, before switching to special diets (baseline), was 342.6 (303.3; 412.5) µmol/L. After 5 days of a glycine-enriched diet, median serum glycine concentration was 4.2-fold higher compared to the casein diet group (745.1 (558.7; 991.2) vs. 176.9 (155.4; 220.6) µmol/L, $p < 0.001$). The casein diet resulted in decreased glycine concentration compared to baseline values ($p < 0.001$), while the glycine-enriched diet was associated with significantly higher serum glycine levels ($p < 0.001$). The gavage with melatonin, 2 h before IRI or sham procedure, did not affect glycine levels at the end of the experiment (Figure 1).

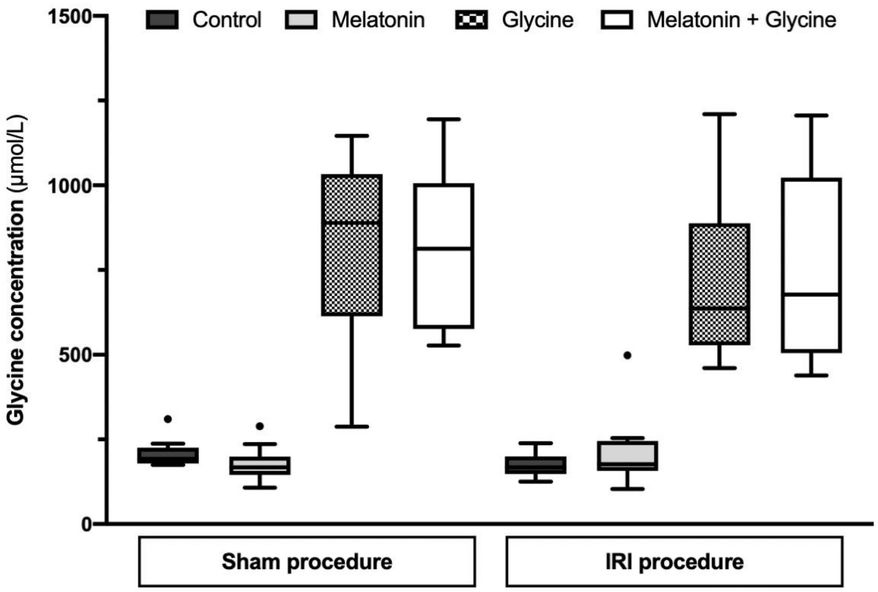

Figure 1. Glycine concentration in serum after 5 days of pretreatment in study groups. IRI: ischemia/reperfusion injury. Data presented as median and interquartile range (n = 10/group, except n = 9 in sham combined treatment group).

2.3. Histology

The total scores of uterus histological evaluation for IRI in all sham groups (control, melatonin, glycine and their combination) were similar (5 (4; 6) vs. 4 (3; 6) vs. 4.5 (4; 6) vs. 4 (3.5; 6), $p = 0.719$, respectively) (Figure 2). Uterus IRI procedure led to an increase in total scores, which were found to be 8.5 (7; 10.25) in control ($p < 0.001$), 6 (5; 8) in melatonin ($p = 0.005$), 7 (6; 8) in glycine ($p < 0.001$) and 6 (6; 7) in the combined treatment group ($p = 0.022$) compared to corresponding sham groups. The elevation in total score after IRI procedure was significantly attenuated by pretreatment with melatonin ($p = 0.019$) and glycine ($p = 0.044$) alone as well as with their combination ($p = 0.003$) compared to IRI control group.

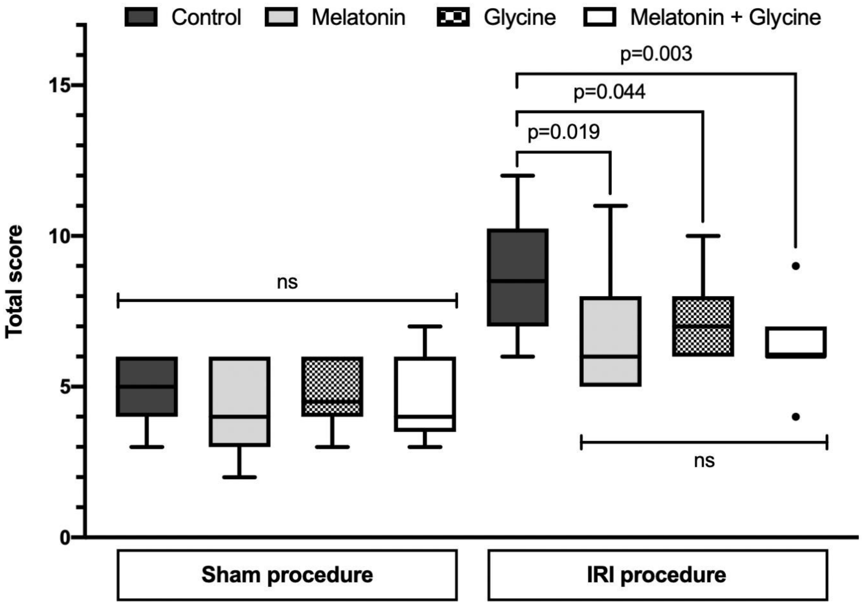

Figure 2. Total score of uterus histological evaluation for IRI. IRI: ischemia/reperfusion injury. Data presented as median and interquartile range (n = 10/group, except n = 9 in sham combined treatment group).

When comparing the individual histological features of the scoring system between IRI groups, there was a significant reduction in tissue edema ($p = 0.003$) after pretreatment using the combined treatment. In addition, there was a tendency toward reduction in inflammatory cells ($p = 0.074$) and tissue edema ($p = 0.068$) after pretreatment with melatonin, while the same occurred with tissue edema ($p = 0.074$) and perimeter thickening ($p = 0.068$) after pretreatment with glycine, and with inflammatory cells ($p = 0.068$) after pretreatment of their combination (Table 1). Other histological features were similar in all IRI groups.

Table 1. Results of the scoring system for uterus IRI evaluation.

	Sham Procedure				IRI Procedure			
	Control	**Melatonin**	**Glycine**	**Melatonin + Glycine**	**Control**	**Melatonin**	**Glycine**	**Melatonin + Glycine**
Inflammatory cells	1 (0.75; 1)	1 (0.75; 1)	1 (1; 1)	1 (0.5; 1)	2 (1; 2)	1 (1; 1.25)	1 (1; 2)	1 (1; 1.25)
Vasoconstriction	0 (0; 0.25)	0 (0; 0)	0 (0; 0.25)	0 (0; 0)	0 (0; 1)	0 (0; 1)	0 (0; 0.25)	0 (0; 0.25)
Hemorrhage	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)
Necrosis	1 (1; 1)	1 (0.75; 1)	1 (0.75; 1)	1 (1; 1)	1 (1; 2)	1 (1; 1.25)	1 (1; 2)	1 (1; 1.25)
Edema	1 (0.75; 1)	1 (0; 1)	1 (1; 1)	1 (0; 1)	2 (1; 2)	1 (1; 2)	1 (1; 2)	1 (1; 1)
Thrombosis	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 0.25)	0 (0; 0)	0 (0; 0)	0 (0; 0)
Endometrial loss of cells	1 (1; 1)	1 (0.75; 1)	1 (0.75; 1)	1 (0.5; 1)	1 (1; 2)	1 (1; 1.25)	1 (1; 2)	1 (1; 1)
Smooth muscle contraction	0 (0; 0)	0 (0; 0)	0 (0; 0)	0 (0; 1)	0 (0; 1)	0 (0; 1)	1 (0; 1)	0 (0; 0.25)
Impaired basement membrane integrity	1 (0; 1)	0 (0; 1)	0 (0; 0.25)	0 (0; 1)	1 (0; 1)	0.5 (0; 1)	1 (0; 1)	1 (0.75; 1)
Perimeter thickening	0 (0; 0.25)	0 (0; 1)	0.5 (0; 1)	1 (0; 1)	1 (0.75; 1)	0.5 (0; 1)	0 (0; 1)	0.5 (0; 1)
Total score	5 (4; 6)	4 (3; 6)	4.5 (4; 6)	4 (3.5; 6)	8.5 (7; 10.25)	6 (5; 8)	7 (6; 8)	6 (6; 7)

IRI: ischemia/reperfusion injury. Data presented as median and quartiles (Q1; Q3); $n = 10$/group, except $n = 9$ in sham combined treatment group.

2.4. Tissue MPO Expression

MPO levels in uterus tissue did not significantly vary between sham groups (Figure 3), with 2.92% (1.52; 3.89), 3.08% (1.64; 3.96), 3.61% (2.94; 4.25) and 3.52% (2.87; 4.06) in the sham control group, the sham melatonin group, the sham glycine group and the combined treatment group, respectively. Uterus IRI resulted in increased MPO levels (7.18% (6.57; 7.75) in the control group, 5.61% (3.58; 6.36) in the melatonin group, 5.31% (4.62; 6.16) in the glycine group and 4.04% (2.86; 5.2) in the combined treatment group). Pretreatment with melatonin ($p = 0.004$) and glycine ($p < 0.001$) alone or with their combination ($p < 0.001$) attenuated MPO increase following IRI compared to the IRI control group. While the combination treatment of glycine plus melatonin totally blocked the IRI effect of MPO expression ($p = 0.224$), all other IRI groups were different to their corresponding controls with $p < 0.001$, $p = 0.005$ and $p = 0.002$, for IRI, IRI plus melatonin and IRI plus glycine, respectively.

2.5. Tissue SOD Activity

The SOD activity in uterus tissue samples was similar in all sham groups with 3.66 (2.97; 3.78) in control vs. 2.93 (2.44; 3.89) in melatonin vs. 3.55 (2.81; 3.77) in glycine vs. 3.13 (2.47; 3.93) U/mg protein in combined treatment group, $p = 0.699$) (Figure 4). IRI significantly decreases SOD activity. This decrease was attenuated by pretreatment with melatonin (2.61 (2.12; 3.74) U/mg protein, $p = 0.027$), glycine (2.52 (1.93; 3.44) U/mg protein, $p = 0.038$) and the combination of both supplements (2.69 (2.23; 3.04) U/mg protein, $p = 0.015$) as compared to the corresponding IRI control group (2.04 (1.57; 2.35) U/mg protein).

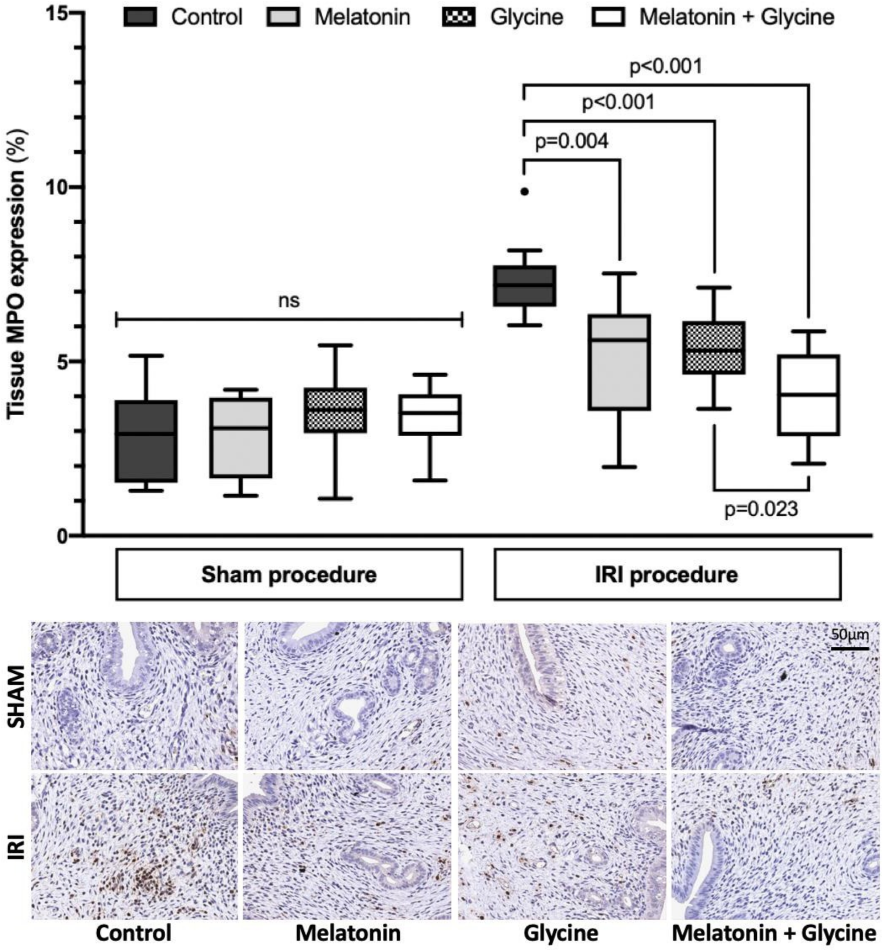

Figure 3. Myeloperoxidase expression in uterus tissue. IRI: ischemia/reperfusion injury. MPO: myeloperoxidase. Data presented as median and interquartile range (*n* = 10/group, except *n* = 9 in sham combined treatment group).

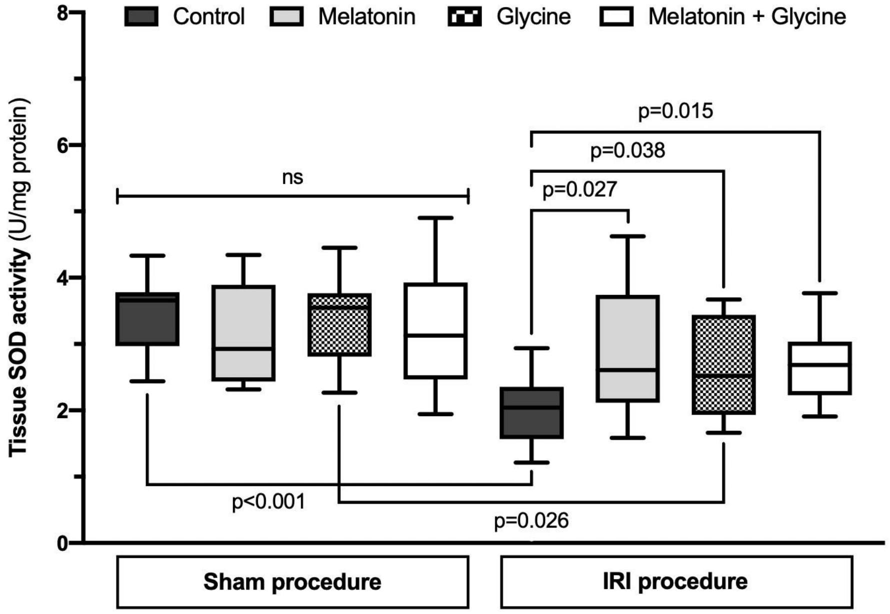

Figure 4. Superoxide dismutase activity in uterus tissue. IRI: ischemia/reperfusion injury. SOD: superoxide dismutase. Data presented as median and interquartile range (*n* = 10/group, except *n* = 9 in sham combined treatment group).

3. Discussion

In organ Tx IRI is a major challenge affecting clinical outcome. An imbalance in metabolic supply and demand within the ischemic organ results in profound tissue hypoxia and microvascular dysfunction; furthermore, subsequent reperfusion enhances the activation of innate and adaptive immune responses and cell death programs, culminating in acute or chronic organ rejections [10,29]. To date, only a limited number of studies investigating agents capable of minimizing IRI, particularly in UTx, are available [4]. The data of this study clearly demonstrate the protective effects of melatonin and glycine, both separately and combined, against experimental uterus IRI. This is the first implication of these non-toxic substances in the research of UTx.

The potential benefits of melatonin in other solid organ Tx have been described previously [12,16]. Melatonin is a potent free radical scavenger of reactive oxygen species (ROS) and, therefore, improves morphology, apoptosis, immunological reaction, and oxidative stress of grafts, among other processes. [16,30]. It has been proven to be a potentially useful therapeutic tool in reducing graft rejection [8]. In the last decades, its positive effects on the heart [31–33], bone [34], lung [35], pancreas [36,37], kidney [38–40] and liver [41–43] have been described in the Tx setting. Melatonin ameliorates IRI most likely through its antioxidative properties and inhibitory capacity of nuclear factor κB (NF-κB), IκB kinase (IKK) and c-Jun N-terminal kinase (JNK) in the mitogen-activated protein kinase (MAPK) pathway [40,44–46]. In addition, the capability of melatonin to enhance Akt activation in the setting of IRI has been documented previously [45–47]. All of these factors play an important role in inflammation and cell death. Our results demonstrated reduced histopathologic damage after uterus IRI. Moreover, the observed decline in SOD activity after 1 h of ischemia followed by 1 h of reperfusion was significantly attenuated by pretreatment with melatonin compared with control groups, leading to reduced oxidative stress. As a result, further polymorphonuclear infiltration and tissue activation, as documented by reduced MPO levels, significantly decreased with melatonin supplementation.

Numerous experimental and clinical studies have suggested the great potential of glycine in providing protection against IRI [24,25,48,49]. However, this protective ability does not apply to all organs and conditions. For the liver, heart and small intestine, several reports demonstrating organ protection have been published, while in the case of the kidney, it appears to be unlikely [23]. It seems that glycine attenuates IRI through similar pathways to melatonin: by enhancing Akt activation, while reducing the activation of extracellular signal-regulated kinase (ERK), JNK, and p38 in the MAPK signaling pathway [26,50,51]. Within this study, glycine mediated similar effects to those reported for melatonin. Glycine was able to preserve the activity of antioxidant pathways during uterus IRI, resulting in reduced oxidative stress and inflammatory cell activation as shown by MPO expression. Interestingly, the combination of melatonin and glycine did not result in significant additive effects as supposed, giving rise to new questions that may be answered by further investigations.

There were a number of limitations in our study. First, this model (1 h of ischemia and 1 h of reperfusion) did not reflect a real clinical situation. However, the time allowed for these processes was sufficient to trigger tissue-oxidative damage pathways and to investigate the effects of potentially beneficial agents. Second, the uteri exposed to IRI were not transplanted. However, as the effects of melatonin and glycine were being investigated in a model of uterus IRI for the first time, this study focused on answering preliminary questions. Within this study, melatonin and glycine revealed antioxidative and anti-inflammatory properties in uterus IRI, but further research is warranted. Novel therapeutic strategies are necessary, as minimizing IRI during Tx may potentially suppress the immune response against the allograft, leading to reduced need of systemic immunosuppression, thereby reducing additional risks [52], especially during pregnancy after UTx.

4. Materials and Methods

4.1. Animals

A total of 80 adult female (12-week-old; weight 270–360 g) *Sprague-Dawley* rats were obtained from Janvier Labs (Le Genest-Saint-Isle, France) and arrived at the research facility 7 days prior to intervention. All rats were kept under a controlled environment (22± 1 °C; 12 h/12 h light/dark cycle) and had access to fresh water and chow ad libitum. The study followed the guidelines for the handling and care of experimental animals issued by the Federation of European Laboratory Animal Science Associations (FELASA) and was approved by the Austrian Federal Ministry of Education, Science and Research (BMBWF-66.010/0197-V/3b/2018, 26 November 2018).

4.2. Animal Groups and Experimental Design

The rats (n = 80) were randomly assigned to either sham groups or experimental groups (n = 10/group) and subjected to a 7-day acclimatization period. Half of the animals in each group were then switched to a 5% glycine-enriched diet (containing 15% casein and 5% glycine for the glycine and combined treatment groups), while the other half received a control diet (containing 20% casein and 0% glycine for the control and melatonin groups), purchased from Altromin International (Lage, Germany), for 5 days. Two hours prior to IRI or sham procedure, the melatonin and combined treatment groups received 1.5 mL of milk (3.5% fat) containing 50 mg/kg melatonin (Sigma-Aldrich, St. Louis, MO, USA) via gavage, while the control and glycine groups received the same type of milk containing a corresponding amount of microcrystalline cellulose (placebo; from Sigma-Aldrich, St. Louis, MO, USA). The administration route and dose of both investigated substances (melatonin and glycine) were adopted based on previous experiments [27,28,40,44,53,54]. Water consumption, food intake and body weight were recorded regularly (for details, see Figure 5).

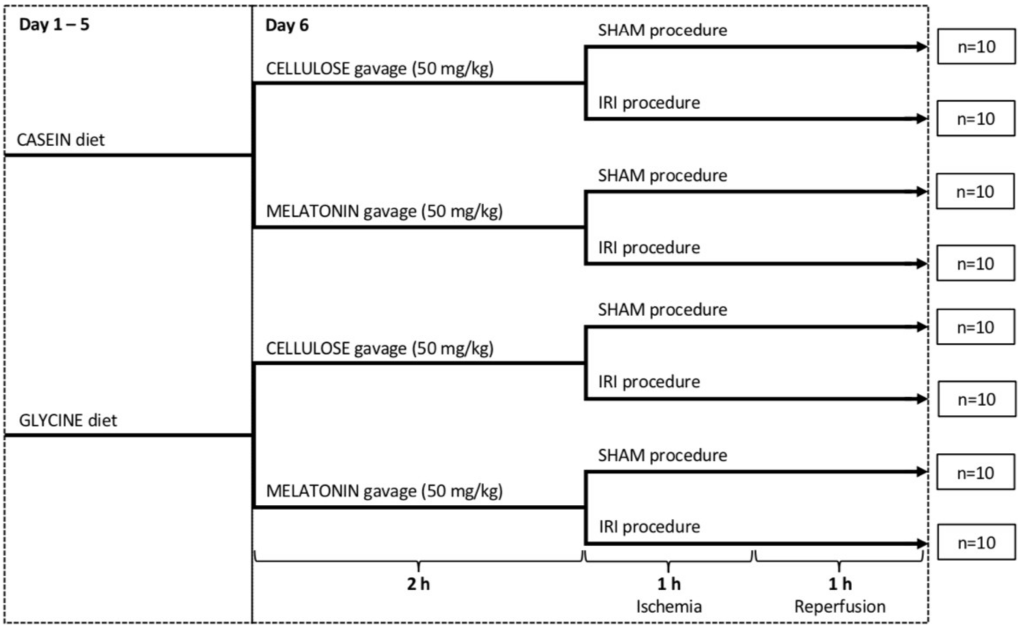

Figure 5. Study design. IRI: ischemia/reperfusion injury.

4.3. IRI and Sham Procedure

Anesthesia was performed using 2%, 2 L/min isoflurane inhalation and intramuscular injection of fentanyl (5 µg/kg), midazolam (2 mg/kg) and medetomidine (0.15 mg/kg). Animals were placed in a supine position on an automatically regulated heating pad to maintain normothermia during intervention. After shaving and disinfecting the surgical area, a horizontal laparotomy (measuring about 3 cm) was performed (Figure 6A). Ischemia was induced by clamping the distal abdominal aorta about 0.5 cm above the bifurcation with a micro-bulldog clamp (clamping pressure 20–25 g; GEISTER Medizintechnik, Tuttlingen, Germany; Figure 6B,C). Subsequently both ovarian arteries, including the surrounding fatty tissue, were temporarily ligated with Vicryl 3-0 sutures. The abdomen was closed and the animal was returned to a prone position. Warm ischemia was maintained for 1 h followed by relaparotomy restoration of the uterus blood flow by removing the micro-bulldog clamp and sutures from the ovarian arteries (see Figure S1 in Supplementary Materials). Reperfusion was maintained with closed abdomen in a prone position for 1 h. The duration of warm ischemia and reperfusion was based on previous preclinical studies and clinical case series [2,55–57]. At the end of the reperfusion period, animals were euthanized by terminal blood collection from the vena cava inferior. The right uterus horn was fixed in 4% formalin and prepared for histology and immunohistochemistry (IHC), while the left uterus horn was frozen in liquid nitrogen and stored at −80 °C for further biochemical analysis.

The sham procedure was performed in exactly the same manner, omitting vessel occlusion.

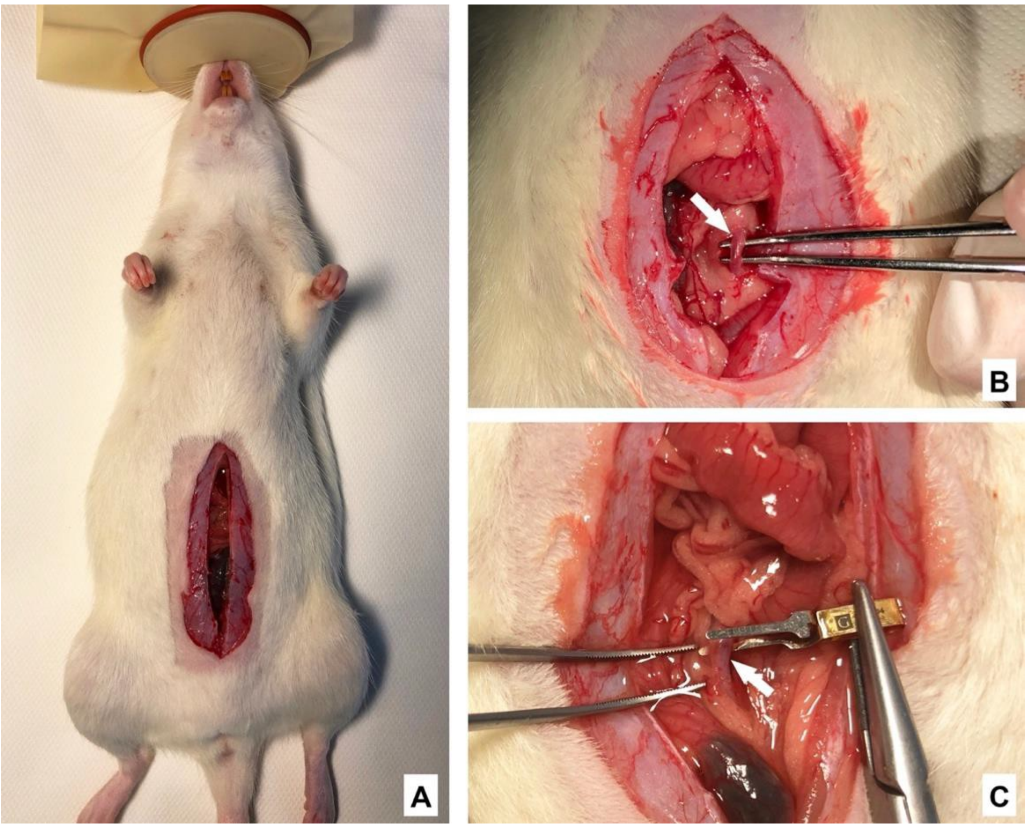

Figure 6. Surgical procedure. (**A**): laparotomy. (**B**): mobilization of the abdominal aorta (white arrow) about 0.5 cm above the bifurcation. (**C**): micro-bulldog clamp to be placed on the aorta (white arrow).

4.4. Histology

Paraffin-embedded samples were cut in 2 μm thick sections, stained with hematoxylin and eosin (H&E) [58], and subsequently examined by an experienced pathologist under a light microscope in a blinded manner. A modified semi-quantitative morphological scoring system was used for histological evaluation (Table 2; for details, see Supplementary Information) [59,60].

Table 2. Scoring system for the evaluation of uterus IRI.

	Score		
	0	**1**	**2**
Inflammatory cells	Absent	Moderate number of cells	Severe infiltration of cells
Vasoconstriction	Absent	<20% of small vessels	>20% of small vessels
Hemorrhage	Absent	Subendometrial	Myometrial and endometrial
Necrosis	Absent	<20%	>20%
Edema	Absent	<50%	>50%
Thrombosis	Absent	<50% of the vessels	>50% of the vessels
Endometrial loss of cells	Absent	<20%	>20%
Smooth muscle contraction	Absent	Present	
Impaired basement membrane integrity	Absent	Present	
Perimeter thickening	Absent	Present	

Maximum score for uterus IRI—17. IRI: ischemia/reperfusion injury.

4.5. IHC Staining

The expression of myeloperoxidase (MPO), the oxidative stress marker and the indicator of neutrophils accumulation [61] was assessed by IHC staining with a rabbit polyclonal antibody to human MPO (Dako, Via Real, Carpinteria, CA, USA; dilution 1:800) in combination with the UltraVision LP Detection System HRP Polymer (Thermo Fisher Scientific, Waltham, MA, USA) and DAB chromogen (Dako, Via Real, Carpinteria, CA, USA). Rat spleen tissue was used as positive control, while primary antibody was omitted as negative control. The slides were scanned using the QuPath software version 0.2.0-m5 (Belfast, Northern Ireland) [13,62] and analyzed by a blinded examiner. Results are given as the ratio of MPO-positive cells to the total number of cells (percent MPO-positive cells).

4.6. Biochemistry

Frozen tissue samples were homogenized in 2 mL MagNA Lyser Green Beads tubes (Roche Diagnostics, Mannheim, Germany) containing 1 mL ice cold phosphate-buffered saline and 5 mM butylated hydroxytoluene (antioxidant) by homogenizing 3 times at 600 rpm for 30 s in the MagNA Lyser Instrument (Roche Diagnostics, Mannheim, Germany). Supernatant was collected and stored at −80 °C for batch analysis. Superoxide dismutase (SOD) activity was determined using the commercially available SOD Colorimetric Activity Kit by Thermo Fisher Scientific (Waltham, MA, USA) exactly as described by the manufacturer. The BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) was used to adjust values to total protein levels. Results are expressed as units per mg of protein.

4.7. Blood Sample Analysis

Venous blood samples were collected from jugular veins before diet administration and at the end of the experiment at terminal blood collection from vena cava inferior under general anesthesia. Blood cells were separated from serum at 1970× *g* at 4 °C for 10 min and subsequently stored at −80 °C for further analyses. Determination of serum glycine levels was performed in the routine hospital laboratory.

4.8. Statistical Analysis

Statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) version 23.0 (IBM Corp., Armonk, NY, USA). The Kruskal-Wallis test was used to compare sham and IRI groups (comparison of more than two groups with Bonferroni correction). Experimental groups with representative control groups (comparison of two groups) were analyzed using the Mann-Whitney U test. Data are presented as median and quartiles (Q1; Q3). A p value less than 0.05 was considered as statistically significant.

5. Conclusions

Pretreatment with melatonin and glycine provided protection against IRI in a rat warm ischemia model. This study represents a step toward understanding the effects of melatonin and glycine, which are commonly used as cytoprotective agents due to their antioxidative and anti-inflammatory properties in IRI. Since both are natural and nontoxic molecules, their use in UTx is considered safe. Although dietary melatonin and glycine, given separately, exert beneficial effects, the combined supplementation did not yield additive properties. Further investigations replicating the clinical situation are warranted in order to pave the way for clinical studies focusing on new organ-protective strategies in UTx using glycine, melatonin or their combination.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/ijms22168373/s1.

Author Contributions: P.S. (Philipp Stiegler) and P.S. (Peter Schemmer) were responsible for the study concept, design and critical revision of the drafted manuscript. V.Z., M.K., V.M., B.L., D.R. and K.S. were responsible for the data collection and analysis, literature review, interpretation of data and drafting of the manuscript. All authors have read and agree to the published version of the manuscript.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available from the first author (V.Z.) upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Brannstrom, M.; Enskog, A.; Kvarnstrom, N.; Ayoubi, J.M.; Dahm-Kahler, P. Global results of human uterus transplantation and strategies for pre-transplantation screening of donors. *Fertil. Steril.* **2019**, *112*, 3–10. [CrossRef]
2. Jones, B.P.; Saso, S.; Bracewell-Milnes, T.; Thum, M.Y.; Nicopoullos, J.; Diaz-Garcia, C.; Friend, P.; Ghaem-Maghami, S.; Testa, G.; Johannesson, L.; et al. Human uterine transplantation: A review of outcomes from the first 45 cases. *BJOG* **2019**, *126*, 1310–1319. [CrossRef]
3. Ozkan, O.; Dogan, N.U.; Ozkan, O.; Mendilcioglu, I.; Dogan, S.; Aydinuraz, B.; Simsek, M. Uterus transplantation: From animal models through the first heart beating pregnancy to the first human live birth. *Women's Health* **2016**, *12*, 442–449. [CrossRef] [PubMed]
4. Zitkute, V.; Kvietkauskas, M.; Leber, B.; Strupas, K.; Stiegler, P.; Schemmer, P. Ischemia and reperfusion injury in uterus transplantation: A comprehensive review. *Transplant. Rev.* **2020**, *34*, 100550. [CrossRef] [PubMed]
5. O'Donovan, L.; Williams, N.J.; Wilkinson, S. Ethical and policy issues raised by uterus transplants. *Br. Med. Bull.* **2019**, *131*, 19–28. [CrossRef] [PubMed]
6. Brannstrom, M.; Dahm-Kahler, P. Uterus transplantation and fertility preservation. *Best Pract. Res. Clin. Obstet. Gynaecol.* **2019**, *55*, 109–116. [CrossRef] [PubMed]
7. Castellon, L.A.R.; Amador, M.I.G.; Gonzalez, R.E.D.; Eduardo, M.S.J.; Diaz-Garcia, C.; Kvarnstrom, N.; Branstrom, M. The history behind successful uterine transplantation in humans. *JBRA Assist. Reprod.* **2017**, *21*, 126–134. [CrossRef]
8. Halladin, N.L. Oxidative and inflammatory biomarkers of ischemia and reperfusion injuries. *Dan. Med. J.* **2015**, *62*, B5054.
9. Wu, M.Y.; Yiang, G.T.; Liao, W.T.; Tsai, A.P.; Cheng, Y.L.; Cheng, P.W.; Li, C.Y.; Li, C.J. Current Mechanistic Concepts in Ischemia and Reperfusion Injury. *Cell. Physiol. Biochem.* **2018**, *46*, 1650–1667. [CrossRef] [PubMed]
10. Eltzschig, H.K.; Eckle, T. Ischemia and reperfusion–from mechanism to translation. *Nat. Med.* **2011**, *17*, 1391–1401. [CrossRef]
11. Horvat, M.; Iltis, A. What Are Good Guidelines for Evaluating Uterus Transplantation? *AMA J. Ethics* **2019**, *21*, 988–995. [CrossRef]
12. Stiegler, P.; Bausys, A.; Leber, B.; Strupas, K.; Schemmer, P. Impact of Melatonin in Solid Organ Transplantation—Is It Time for Clinical Trials? A Comprehensive Review. *Int. J. Mol. Sci.* **2018**, *19*, 3509. [CrossRef]
13. Zitkute, V.; Kvietkauskas, M.; Maskoliunaite, V.; Leber, B.; Ramasauskaite, D.; Strupas, K.; Stiegler, P.; Schemmer, P. Custodiol-N Is Superior to Custodiol((R)) Solution in Experimental Rat Uterus Preservation. *Int. J. Mol. Sci.* **2020**, *21*, 8015. [CrossRef] [PubMed]

14. Slominski, R.M.; Reiter, R.J.; Schlabritz-Loutsevitch, N.; Ostrom, R.S.; Slominski, A.T. Melatonin membrane receptors in peripheral tissues: Distribution and functions. *Mol. Cell. Endocrinol.* **2012**, *351*, 152–166. [CrossRef]
15. Crupi, R.; Mazzon, E.; Marino, A.; La Spada, G.; Bramanti, P.; Spina, E.; Cuzzocrea, S. Melatonin's stimulatory effect on adult hippocampal neurogenesis in mice persists after ovariectomy. *J. Pineal Res.* **2011**, *51*, 353–360. [CrossRef] [PubMed]
16. Esteban-Zubero, E.; Garcia-Gil, F.A.; Lopez-Pingarron, L.; Alatorre-Jimenez, M.A.; Inigo-Gil, P.; Tan, D.X.; Garcia, J.J.; Reiter, R.J. Potential benefits of melatonin in organ transplantation: A review. *J. Endocrinol.* **2016**, *229*, R129–R146. [CrossRef] [PubMed]
17. Campolo, M.; Ahmad, A.; Crupi, R.; Impellizzeri, D.; Morabito, R.; Esposito, E.; Cuzzocrea, S. Combination therapy with melatonin and dexamethasone in a mouse model of traumatic brain injury. *J. Endocrinol.* **2013**, *217*, 291–301. [CrossRef] [PubMed]
18. Fusco, R.; Siracusa, R.; D'Amico, R.; Peritore, A.F.; Cordaro, M.; Gugliandolo, E.; Crupi, R.; Impellizzeri, D.; Cuzzocrea, S.; Di Paola, R. Melatonin Plus Folic Acid Treatment Ameliorates Reserpine-Induced Fibromyalgia: An Evaluation of Pain, Oxidative Stress, and Inflammation. *Antioxidants* **2019**, *8*, 628. [CrossRef]
19. Olcese, J.M. Melatonin and Female Reproduction: An Expanding Universe. *Front. Endocrinol.* **2020**, *11*, 85. [CrossRef]
20. Saat, N.; Risvanli, A.; Dogan, H.; Onalan, E.; Akpolat, N.; Seker, I.; Sahna, E. Effect of melatonin on torsion and reperfusion induced pathogenesis of rat uterus. *Biotech. Histochem.* **2019**, *94*, 533–539. [CrossRef]
21. Turkoz, Y.; Celik, O.; Hascalik, S.; Cigremis, Y.; Hascalik, M.; Mizrak, B.; Yologlu, S. Melatonin reduces torsion-detorsion injury in rat ovary: Biochemical and histopathologic evaluation. *J. Pineal Res.* **2004**, *37*, 137–141. [CrossRef]
22. Aslaner, A.; Gunal, O.; Turgut, H.T.; Celik, E.; Yildirim, U.; Demirci, R.K.; Gunduz, U.R.; Calis, H.; Dogan, S. Effect of melatonin on kidney cold ischemic preservation injury. *Int. J. Clin. Exp. Med.* **2013**, *6*, 794–798. [PubMed]
23. Petrat, F.; Boengler, K.; Schulz, R.; de Groot, H. Glycine, a simple physiological compound protecting by yet puzzling mechanism(s) against ischaemia-reperfusion injury: Current knowledge. *Br. J. Pharmacol.* **2012**, *165*, 2059–2072. [CrossRef]
24. Habib, M.M.; Hodgson, H.J.; Davidson, B.R. The role of glycine in hepatic ischemia-reperfusion injury. *Curr. Pharm. Des.* **2006**, *12*, 2953–2967. [CrossRef]
25. Petrat, F.; Drowatzky, J.; Boengler, K.; Finckh, B.; Schmitz, K.J.; Schulz, R.; de Groot, H. Protection from glycine at low doses in ischemia-reperfusion injury of the rat small intestine. *Eur. Surg. Res.* **2011**, *46*, 180–187. [CrossRef] [PubMed]
26. Zhong, X.; Li, X.; Qian, L.; Xu, Y.; Lu, Y.; Zhang, J.; Li, N.; Zhu, X.; Ben, J.; Yang, Q.; et al. Glycine attenuates myocardial ischemia-reperfusion injury by inhibiting myocardial apoptosis in rats. *J. Biomed. Res.* **2012**, *26*, 346–354. [CrossRef]
27. Maneikyte, J.; Bausys, A.; Leber, B.; Feldbacher, N.; Hoefler, G.; Kolb-Lenz, D.; Strupas, K.; Stiegler, P.; Schemmer, P. Dietary Glycine Prevents FOLFOX Chemotherapy-Induced Heart Injury: A Colorectal Cancer Liver Metastasis Treatment Model in Rats. *Nutrients* **2020**, *12*, 2634. [CrossRef] [PubMed]
28. Maneikyte, J.; Bausys, A.; Leber, B.; Horvath, A.; Feldbacher, N.; Hoefler, G.; Strupas, K.; Stiegler, P.; Schemmer, P. Dietary glycine decreases both tumor volume and vascularization in a combined colorectal liver metastasis and chemotherapy model. *Int. J. Biol. Sci.* **2019**, *15*, 1582–1590. [CrossRef]
29. Brannstrom, M.; Wranning, C.A.; Altchek, A. Experimental uterus transplantation. *Hum. Reprod. Update* **2010**, *16*, 329–345. [CrossRef] [PubMed]
30. Haddad, C.F.; Haddad, J.M.; Veiga, E.C.A.; Sorpreso, I.C.E.; Simoes, R.S.; Baracat, E.C.; Soares Junior, J.M. Melatonin and organ transplantation: What is the relationship? *Rev. Assoc. Med. Bras. (1992)* **2020**, *66*, 353–358. [CrossRef] [PubMed]
31. Jung, F.J.; Yang, L.; Harter, L.; Inci, I.; Schneiter, D.; Lardinois, D.; Keel, M.; Weder, W.; Korom, S. Melatonin in vivo prolongs cardiac allograft survival in rats. *J. Pineal Res.* **2004**, *37*, 36–41. [CrossRef]
32. Liu, C.; Hong, T.; Shao, M.; Chen, Z.; Wang, C. Melatonin synergized with cyclosporine A improves cardiac allograft survival by suppressing inflammation and apoptosis. *Mol. Med. Rep.* **2014**, *10*, 1323–1328. [CrossRef] [PubMed]
33. Zhou, H.; Ma, Q.; Zhu, P.; Ren, J.; Reiter, R.J.; Chen, Y. Protective role of melatonin in cardiac ischemia-reperfusion injury: From pathogenesis to targeted therapy. *J. Pineal Res.* **2018**, *64*, e12471. [CrossRef] [PubMed]
34. Takechi, M.; Tatehara, S.; Satomura, K.; Fujisawa, K.; Nagayama, M. Effect of FGF-2 and melatonin on implant bone healing: A histomorphometric study. *J. Mater. Sci. Mater. Med.* **2008**, *19*, 2949–2952. [CrossRef]
35. Inci, I.; Inci, D.; Dutly, A.; Boehler, A.; Weder, W. Melatonin Attenuates Posttransplant Lung Ischemia-Reperfusion Injury. *Ann. Thorac. Surg.* **2002**, *73*, 220–225. [CrossRef]
36. Lin, G.J.; Huang, S.H.; Chen, Y.W.; Hueng, D.Y.; Chien, M.W.; Chia, W.T.; Chang, D.M.; Sytwu, H.K. Melatonin prolongs islet graft survival in diabetic NOD mice. *J. Pineal Res.* **2009**, *47*, 284–292. [CrossRef]
37. Munoz-Casares, F.C.; Padillo, F.J.; Briceno, J.; Collado, J.A.; Munoz-Castaneda, J.R.; Ortega, R.; Cruz, A.; Tunez, I.; Montilla, P.; Pera, C.; et al. Melatonin reduces apoptosis and necrosis induced by ischemia/reperfusion injury of the pancreas. *J. Pineal Res.* **2006**, *40*, 195–203. [CrossRef]
38. Panah, F.; Ghorbanihaghjo, A.; Argani, H.; Haiaty, S.; Rashtchizadeh, N.; Hosseini, L.; Dastmalchi, S.; Rezaeian, R.; Alirezaei, A.; Jabarpour, M.; et al. The effect of oral melatonin on renal ischemia-reperfusion injury in transplant patients: A double-blind, randomized controlled trial. *Transpl. Immunol.* **2019**, *57*, 101241. [CrossRef]
39. Shi, S.; Lei, S.; Tang, C.; Wang, K.; Xia, Z. Melatonin attenuates acute kidney ischemia/reperfusion injury in diabetic rats by activation of the SIRT1/Nrf2/HO-1 signaling pathway. *Biosci. Rep.* **2019**, *39*, BSR20181614. [CrossRef]
40. Li, Z.; Nickkholgh, A.; Yi, X.; Bruns, H.; Gross, M.L.; Hoffmann, K.; Mohr, E.; Zorn, M.; Buchler, M.W.; Schemmer, P. Melatonin protects kidney grafts from ischemia/reperfusion injury through inhibition of NF-kB and apoptosis after experimental kidney transplantation. *J. Pineal Res.* **2009**, *46*, 365–372. [CrossRef]

41. Esteban-Zubero, E.; Garcia-Gil, F.A.; Lopez-Pingarron, L.; Alatorre-Jimenez, M.A.; Ramirez, J.M.; Tan, D.X.; Garcia, J.J.; Reiter, R.J. Melatonin role preventing steatohepatitis and improving liver transplantation results. *Cell. Mol. Life Sci.* **2016**, *73*, 2911–2927. [CrossRef]
42. Mortezaee, K.; Khanlarkhani, N. Melatonin application in targeting oxidative-induced liver injuries: A review. *J. Cell. Physiol.* **2018**, *233*, 4015–4032. [CrossRef]
43. Kim, S.H.; Lee, S.M. Cytoprotective effects of melatonin against necrosis and apoptosis induced by ischemia/reperfusion injury in rat liver. *J. Pineal Res.* **2008**, *44*, 165–171. [CrossRef]
44. Liang, R.; Nickkholgh, A.; Hoffmann, K.; Kern, M.; Schneider, H.; Sobirey, M.; Zorn, M.; Buchler, M.W.; Schemmer, P. Melatonin protects from hepatic reperfusion injury through inhibition of IKK and JNK pathways and modification of cell proliferation. *J. Pineal Res.* **2009**, *46*, 8–14. [CrossRef] [PubMed]
45. Koh, P.O. Melatonin prevents hepatic injury-induced decrease in Akt downstream targets phosphorylations. *J. Pineal Res.* **2011**, *51*, 214–219. [CrossRef]
46. Hadj Ayed Tka, K.; Mahfoudh Boussaid, A.; Zaouali, M.A.; Kammoun, R.; Bejaoui, M.; Ghoul Mazgar, S.; Rosello Catafau, J.; Ben Abdennebi, H. Melatonin modulates endoplasmic reticulum stress and Akt/GSK3-beta signaling pathway in a rat model of renal warm ischemia reperfusion. *Anal. Cell. Pathol.* **2015**, *2015*, 635172. [CrossRef] [PubMed]
47. Song, J.; Kang, S.M.; Lee, W.T.; Park, K.A.; Lee, K.M.; Lee, J.E. The beneficial effect of melatonin in brain endothelial cells against oxygen-glucose deprivation followed by reperfusion-induced injury. *Oxid. Med. Cell. Longev.* **2014**, *2014*, 639531. [CrossRef] [PubMed]
48. Schemmer, P.; Bradford, B.U.; Rose, M.L.; Bunzendahl, H.; Raleigh, J.A.; Lemasters, J.J.; Thurman, R.G. Intravenous glycine improves survival in rat liver transplantation. *Am. J. Physiol.* **1999**, *276*, G924–G932. [CrossRef]
49. Wheeler, M.D.; Ikejema, K.; Enomoto, N.; Stacklewitz, R.F.; Seabra, V.; Zhong, Z.; Yin, M.; Schemmer, P.; Rose, M.L.; Rusyn, I.; et al. Glycine: A new anti-inflammatory immunonutrient. *Cell. Mol. Life Sci. CMLS* **1999**, *56*, 843–856. [CrossRef]
50. Wang, W.; Wu, Z.; Lin, G.; Hu, S.; Wang, B.; Dai, Z.; Wu, G. Glycine stimulates protein synthesis and inhibits oxidative stress in pig small intestinal epithelial cells. *J. Nutr.* **2014**, *144*, 1540–1548. [CrossRef]
51. Lu, Y.; Zhang, J.; Ma, B.; Li, K.; Li, X.; Bai, H.; Yang, Q.; Zhu, X.; Ben, J.; Chen, Q. Glycine attenuates cerebral ischemia/reperfusion injury by inhibiting neuronal apoptosis in mice. *Neurochem. Int.* **2012**, *61*, 649–658. [CrossRef]
52. Kvietkauskas, M.; Leber, B.; Strupas, K.; Stiegler, P.; Schemmer, P. Machine Perfusion of Extended Criteria Donor Organs: Immunological Aspects. *Front. Immunol.* **2020**, *11*, 192. [CrossRef]
53. Kvietkauskas, M.; Zitkute, V.; Leber, B.; Strupas, K.; Stiegler, P.; Schemmer, P. Dietary Melatonin and Glycine Decrease Tumor Growth through Antiangiogenic Activity in Experimental Colorectal Liver Metastasis. *Nutrients* **2021**, *13*, 2035. [CrossRef] [PubMed]
54. Mikalauskas, S.; Mikalauskiene, L.; Bruns, H.; Nickkholgh, A.; Hoffmann, K.; Longerich, T.; Strupas, K.; Buchler, M.W.; Schemmer, P. Dietary glycine protects from chemotherapy-induced hepatotoxicity. *Amino Acids* **2011**, *40*, 1139–1150. [CrossRef]
55. Johannesson, L.; Enskog, A.; Dahm-Kahler, P.; Hanafy, A.; Chai, D.C.; Mwenda, J.M.; Diaz-Garcia, C.; Olausson, M.; Brannstrom, M. Uterus transplantation in a non-human primate: Long-term follow-up after autologous transplantation. *Hum. Reprod.* **2012**, *27*, 1640–1648. [CrossRef] [PubMed]
56. Sahin, S.; Ozakpinar, O.B.; Ak, K.; Eroglu, M.; Acikel, M.; Tetik, S.; Uras, F.; Cetinel, S. The protective effects of tacrolimus on rat uteri exposed to ischemia-reperfusion injury: A biochemical and histopathologic evaluation. *Fertil. Steril.* **2014**, *101*, 1176–1182. [CrossRef] [PubMed]
57. Atalay, Y.O.; Aktas, S.; Sahin, S.; Kucukodaci, Z.; Ozakpinar, O.B. Remifentanil protects uterus against ischemia-reperfusion injury in rats. *Acta Cir. Bras.* **2015**, *30*, 756–761. [CrossRef]
58. Siracusa, R.; Impellizzeri, D.; Cordaro, M.; Gugliandolo, E.; Peritore, A.F.; Di Paola, R.; Cuzzocrea, S. Topical Application of Adelmidrol + Trans-Traumatic Acid Enhances Skin Wound Healing in a Streptozotocin-Induced Diabetic Mouse Model. *Front. Pharmacol.* **2018**, *9*, 871. [CrossRef]
59. Diaz-Garcia, C.; Akhi, S.N.; Martinez-Varea, A.; Brannstrom, M. The effect of warm ischemia at uterus transplantation in a rat model. *Acta Obstet. Gynecol. Scand.* **2013**, *92*, 152–159. [CrossRef]
60. Sahin Ersoy, G.; Kurek Eken, M.; Cevik, O.; Cilingir, O.T.; Tal, R. Mycophenolate mofetil attenuates uterine ischaemia/reperfusion injury in a rat model. *Reprod. Biomed. Online* **2017**, *34*, 115–123. [CrossRef]
61. Fusco, R.; Gugliandolo, E.; Siracusa, R.; Scuto, M.; Cordaro, M.; D'Amico, R.; Evangelista, M.; Peli, A.; Peritore, A.F.; Impellizzeri, D.; et al. Formyl Peptide Receptor 1 Signaling in Acute Inflammation and Neural Differentiation Induced by Traumatic Brain Injury. *Biology* **2020**, *9*, 238. [CrossRef] [PubMed]
62. Bankhead, P.; Loughrey, M.B.; Fernandez, J.A.; Dombrowski, Y.; McArt, D.G.; Dunne, P.D.; McQuaid, S.; Gray, R.T.; Murray, L.J.; Coleman, H.G.; et al. QuPath: Open source software for digital pathology image analysis. *Sci. Rep.* **2017**, *7*, 16878. [CrossRef] [PubMed]