

Uterus transplantation

An experimental study in primates

by

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Cover illustration: Julie

“The way I see it, if you want the rainbow, you gotta put up with the rain!”

***Dolly Parton** American singer born in 1946. She underwent a partial hysterectomy after collapsing on stage at the age of 38. She never had biological children.*

Till min familj

Abstract

Most causes of infertility are nowadays treatable, but for women that are infertile due to lack of, or severe malfunction of the uterus, there is as of today no available cure. Uterus transplantation (UTx) may provide a future possibility for treatment of these females with uterine factor infertility (UFI). It may be the only alternative for women with absolute UFI and as the last option in patients with relative UFI, not amenable to conventional surgery. During recent years, UTx has been developed and extensively studied in different classical rodent and large animal models and the remaining necessary last step before a potential clinical introduction is to include experiments on nonhuman primates. The aims of this thesis were to develop a surgical technique for autologous and allogeneic UTx in a nonhuman primate model and to evaluate the surgical feasibility of both live and deceased donor uterus transplantation in humans.

A baboon model for autologous UTx was developed wherein the ovaries and Fallopian tubes were included in the graft. Despite long durations of surgery (6 h) and ischemia (3 h), the animal survival rate (90 %) was high. However, a poor graft survival (20%) advocated the UTx procedure to be refined. A second study of auto-transplanted baboons initially included an increased perfusion with HTK-solution as the only modification. Continued poor outcome (survival rate 66 % but no well functioning grafts) led to further alterations of the surgical procedure, such as inclusion of larger vessels, modified anastomosis and extensive graft perfusion. Subsequently, the graft function improved considerably (60 %). In a third study using an allogeneic UTx baboon model, the uteri were retrieved from either live or deceased donor and transplanted using the previously described technique (live donation) or with aortal/aortal and caval/caval anastomoses (deceased donation). The recipients were either left untreated or received monotherapy of tacrolimus or induction with antithymocyte globulin followed by triple therapy (tacrolimus, mycophenolate, corticosteroids). Good survival rate (100 %) of the

animals was seen. Long-term graft survival was proven with a triple immunosuppression therapy. In a human study, women undergoing modified radical hysterectomy, mimicking live donor organ retrieval, were subjected to meticulous surgical dissection of the uterine arteries and veins. Uterine vessels of 50-70 mm lengths were procured, without compromised post-operative recovery of the patients. In another study in deceased multi-organ donors, the procured vascular pedicles included either the lower aorta and vena cava or the bilateral common iliacs. Surgical feasibility of UTx with live donors, with anastomoses to the recipients' bilateral external iliacs, or deceased donors, with anastomoses to aorta and cava or external iliacs was demonstrated by the results of the two human studies.

In summary, autologous and allogeneic UTx have been demonstrated in a nonhuman primate model proving to be a donor- and recipient safe surgical procedure, regardless whether the graft is from a live or a deceased donor. Additionally, feasibility of human uterus retrieval was shown in both deceased donor and in a potential live donor setting without comprising donor safety.

It is concluded that UTx, based on solid experimental research, today stand a good chance of a successful outcome if performed in a facility with experienced expertise following a strict management protocol.

Key words: infertility, human, immunosuppression, nonhuman primates, transplantation, uterus

Sammanfattning på svenska

Ofrivillig barnlöshet drabbar i snitt 1 av 10 par. De flesta orsaker till barnlöshet är idag möjliga att behandla men för kvinnor som saknar, eller har en livmoder som ej kan bära en graviditet, finns ingen botande behandling. Livmoderstransplantation har föreslagits som en eventuell framtida behandling för denna stora grupp kvinnor som lider av en obotlig infertilitet till följd av avsaknad av, eller förekomst av en icke fungerande livmoder och när alla andra behandlingar till graviditet misslyckats. Transplantation av organ är idag inte längre förbehållet endast dem med livshotande sjukdom. De senaste åren har de så kallade livskvalitetshöjande transplantationerna, såsom transplantation av ansikte, hand eller ben, blivit en medicinsk verklighet. De övergripande målen med denna avhandling var dels att utveckla en kirurgisk metod för autolog transplantation av livmodern i en primatmodell för att senare vidareutveckla denna metod till att omfatta även allogen transplantation i en primatmodell, samt att utvärdera den kirurgiska möjligheten att uthämta livmodern i ett transplantationssyfte från både möjliga levande och avlidna mänskliga donatorer.

För babianer utformades en metod för autolog transplantation av livmodern med äggstockar och äggledare. Transplantatets kärl (ovarica vener och uterina artärer) syddes ihop med mottagarens bäckenkärl (externa iliaca venen och artären). Trots tidsmässigt lång kirurgi (6 timmar) och avsaknad av kärlförsörjning till livmodern (3 timmar) var den kirurgiska överlevnaden hos babianerna hög (90 %). De transplanterade livmödrarnas funktion var dock dålig (20 %) varför den kirurgiska metoden genomgick ändringar. En andra studie av autolog transplantation av livmodern hos babianer påbörjades och initialt ökades endast genomspolningen av den uttagna livmodern. Då fortsatt dålig funktion av de transplanterade livmödrarna uppvisades gjordes på förslag av och med deltagande av en transplantationskirurg, ytterligare justeringar såsom medtagande av grövre och längre kärl och ökad genomspolning av den uttagna livmodern. Efter dessa förändringar ökade funktio-

nen hos de transplanterade livmödrarna avsevärt (60 % återfick menstruation). Den utvecklade metoden för autolog transplantation av livmoder överfördes till att genomföra även allogena försök i en babianmodell. Livmödrarna i den allogena modellen kom från antingen levande eller avlidna givare. När levande givare var involverade användes den ovan beskrivna tekniken med sammanfogande av kärlen. Hos de avlidna givarna kunde större kärl användas och transplantatets stora kroppspulsåder och nedre hålven användes för kärlsammankoppling till mottagarens motsvarande kärl. Mottagarna fick efter transplantationen olika behandlingsregimer, de var antingen utan medicinering, alternativt fick ett eller flera immundämpande läkemedel. Resultaten visade god överlevnad (100 %) men uteblivna menstruationer. Möjligheter till uttag av livmodern med långa tillhörande kärl undersöktes hos kvinnor som genomgick ett omfattande uttag av sin livmoder p.g.a. cancer. De uttagna kärlen, i en situation som i mycket liknar ett möjligt uttag av livmodern hos en levande givare, uppvisade längder av 50-70 mm utan att patientens tillfrisknande efter operationen påverkades. Hos avlidna givare simulerades ett uttag av livmodern och de medtagna kärlen omfattade antingen nedre kroppspulsådern och nedre hålvenen eller de stora bäckenkärlen. Att döma av de genomförda studierna på uttag av livmoder med tillhörande kärl hos människa, bör transplantation av livmoder från en levande givare, med sammankoppling av kärl till mottagarens bäckenkärl, eller från en avlidna givare, med sammankoppling av kärl till mottagarens kroppspulsåder och hålven alternativt bäckenkärl, vara genomförbar.

Sammantaget har autolog och allogen transplantation av livmodern kunnat visas hos babian med stor säkerhet för både givare och mottagare. Dessutom har möjligheten att genomföra uttag av livmodern med tillhörande kärl, i transplantationssyfte, hos människa undersökts och visats vara ett för donatorn säkert ingrepp. Det är sannolikt att transplantation av livmodern, baserat på mångårig forskning, idag har goda möjligheter att genomföras vid en enhet med stor erfarenhet av och väl uppbyggd struktur kring transplantation.

List of papers

This thesis is based on the following studies, referred to in the text by their Roman numerals. Reprints were made with permission from the publishers.

I. Uterus transplantation in the baboon: methodology and long-term function after auto-transplantation

Enskog A, Johannesson L, Chai D.C, Dahm-Kähler P, Marcickiewicz J, Nyachio A, Mwenda J.M, Brännström M

Human Reproduction, 2010; 25:1980-7

II. Uterus transplantation in a non-human primate: long-term follow-up after autologous transplantation

Johannesson L, Enskog A, Dahm-Kähler L, Hanafy A, Chai D.C, Mwenda J.M, Diaz-Garcia C, Olausson M, Brännström M

Human Reproduction 2012 Mar 27. [Epub ahead of print]

III. Preclinical nonhuman primate report on allogeneic uterus transplantation in baboon

Johannesson L, Tekin A, Enskog A, Mölne J, Diaz-Garcia C, Hanafy A, Dahm-Kähler P, Tryphonopoulos P, Morales P, Rivas K, Ruiz P, Tzakis A, Olausson M, Brännström M

Submitted

IV. Vascular pedicle lengths after hysterectomy: toward future human uterus transplantation

Johannesson L, Diaz-Garcia C, Leonhardt H, Dahm-Kähler P, Marcickiewicz J, Olausson M, Brännström M.

Obstetrics & Gynecology, 2012;119:2219-25.

V. Uterus recovery from deceased donor.

Brännström M, Johannesson L, Moon J, Tekin A, Tryphonopoulos P, Olausson M, Tzakis A.

In manuscript

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Abbreviations

ATG	antithymocyte globulin
AUFI	absolute uterine factor infertility
bw	body weight
CNI	calcineurin Inhibitor
FDA	Food and Drug Association
FIGO	International Federation of Gynecology and Obstetrics
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HPV	Human Papilloma virus
HTK	histidine tryptophan ketoglutarate
IACUC	Institutional Animal Care and Use Committee
ICSI	intracytoplasmic sperm Injection
im	intramuscular
IPL	infundibulopelvic ligament
IPR	Institute of Primate Research
IS	Immunosuppression
ISERC	Institutional Scientific Evaluation and Review Committee
iv	intravenous
IVC	Inferior vena cava
IVF	In vitro fertilization
MHC	major histocompatibility complex
MMF	mycophenolate mofetil
MRA	magnetic resonance angiography

MRI	magnetic resonance Image
MRKH	Mayer-Rokitansky-Küster-Hauser syndrome
NTPR	National Transplantation Pregnancy Registry
sc	subcutaneous
SEM	standard error of the mean
SET	single embryo transfer
TBST	Tris-buffered NaCl and Tween 20
UFI	uterine factor Infertility
UTx	uterus transplantation
UW	University of Wisconsin solution
WHO	World Health Organization

Definitions in short

Allogeneic transplantation	transplantation of tissue or organ from an individual to a genetically non-identical recipient
Autologous transplantation	transplantation of tissue or organ from one part of the body to another in the same individual
Heterotopic transplantation	a transplanted organ/tissue placed in an abnormal location
Intracytoplasmic sperm injection	method of in vitro fertilization in which a sperm is injected into an oocyte for implantation within the womb
Monoclonal antibody	antibody produced by a single clone of cells
Orthotopic transplantation	a transplanted organ/tissue placed in its normal position
Polyclonal antibody	antibody derived from different cell types

Introduction

To become a parent is unquestionably one of the most common and wanted expectations in adulthood. A majority of adults includes formation of a family and having children in their life plan/dream. For many of these adults, however, the dream doesn't so easily come true and the feeling of loss can have profound negative consequences for the affected person and/or couple. Infertility is a worldwide public health issue with remarkably similar prevalence between more and less developed countries [1]. While many infertile couples overcome their situation and become parents, some after a successfully treated infertility and others through adoption or surrogacy, there is still a substantial amount of couples that are left with no possible option to become parents.

Transplantation of organs is nowadays no longer restricted only to those with life-threatening illness. In recent years, the so-called life-quality enhancing transplants, such as face, hand or leg, have become a medical reality. As of lately, interest have also been raised in the possibility of transplanting the uterus as a treatment for special cases of infertility. Still classified as experimental, uterus transplantation (UTx), although tested in animal studies and attempted in two human cases, stands to prove that it is sufficiently developed, concerning safety and technique, before further human cases are conducted.

Infertility

Infertility is clinically defined as unwanted non-conception following one year of unprotected intercourse, during the fertile phase of the menstrual cycle in women not using contraception [2, 3]. Around 80 % of pregnancies occur within the first six menstrual cycles of unprotected intercourse, in women aged ≤ 30 years [4]. The prevalence of current infertility in women aged 20-44 years has been estimated to be approximately 9 % (range 3.5–16.7 %) whereas lifetime infertility in the same study, including more than 170 000 women, ranged

from 6.6 % to 26.4 % [1]. These calculations would correspond to more than 70 million women (or couples) worldwide currently experiencing infertility [1].

There is usually a differentiation between primary infertility (absence of conception) and secondary infertility (where the couple has previous pregnancies/children) and the causes of both types can be divided in four major categories; male factor, female factor, combined factor or unexplained infertility. Although difficult to exactly determine the individual impacts of these categories, it is commonly reported that female factors correspond to approximately 35% of the infertility while the percentage of male factors, combined factors and unexplained infertility are 30, 20 and 15 % respectively [5]. Male factor infertility is usually related to poor sperm quantity and/or quality, or obstruction of the reproductive duct, preventing ejaculation. Concerning female factor infertility more than half of the cases can be ascribed to ovulatory disorders, such as late menarche, abnormal cycle length, premenstrual syndrome and abnormal bleedings [6]. The World Health Organization (WHO) has identified the most common female factors of infertility, beside ovulatory disorders, to be tubal abnormalities (26%), endometriosis (4%), hyperprolactinaemia (4%), mucous abnormalities (4%) and genital tract disorders (4%) [7].

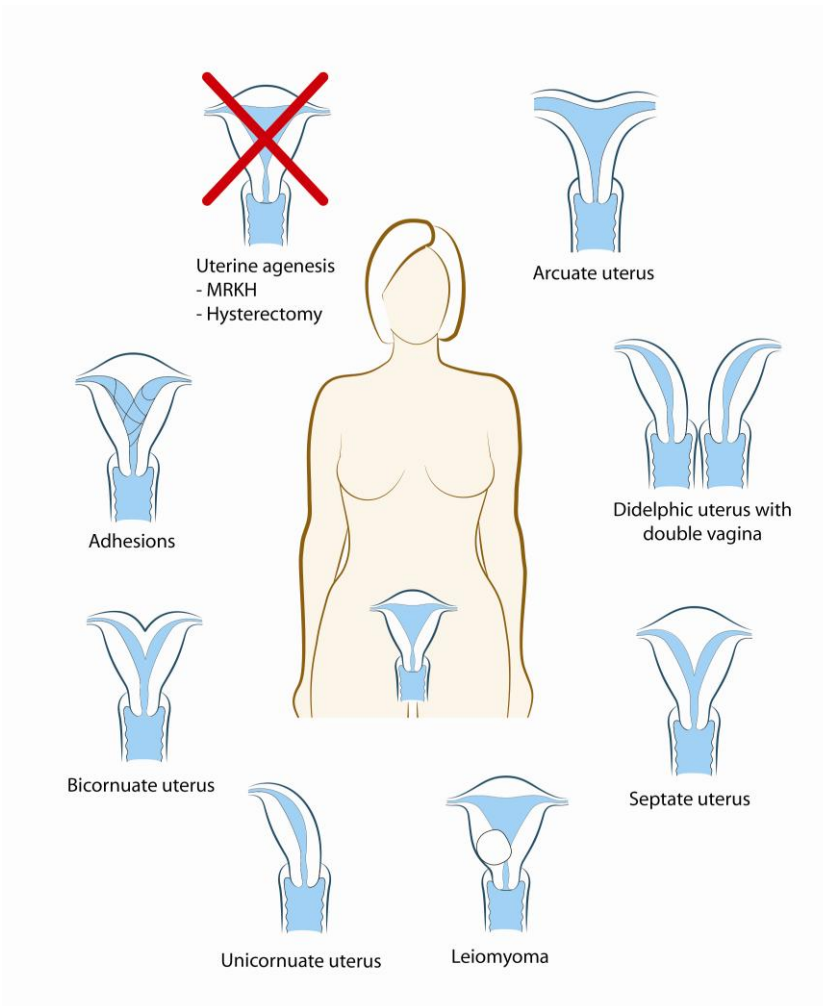
The collaboration between Doctors Steptoe and Edwards, resulting in the development of in vitro fertilization (IVF) [8] and the introduction of intracytoplasmic sperm injection (ICSI) [9], have given many infertile couples a significant hope of achieving pregnancies. Almost all couples with infertility due to a female tubal factor or male factor, secondary to low sperm count, can undergo attempts at IVF and ICSI with satisfactory results. However, despite this magnificent progress and constant development of new assisted reproductive technologies there are still couples who remain unconditionally infertile.

Uterine factor infertility

Uterine factor related infertility (UFI) is either congenital or acquired and can be either absolute (absolute uterine factor infertility, AEFI) or relative (Table 1, Fig 1). The group of women suffering from UFI has been estimated to make up approximately 3-5 % of the infertile female population [10] or, expressed in

absolute numbers, around 15 000 women of fertile age in the UK alone [11]. This group of women with UFI may in the future benefit from a properly and scientifically introduced UTx procedure. However, before UTx is considered for the group of relative UFI cases, all other possible treatments, such as corrective surgery, should naturally have been ruled out.

Fig 1. Schematic drawing of different malformations of the uterus.



Congenital uterine malformations have a prevalence of around 7 % among females [12], and the majority of these malformations present themselves either as septate within the uterus, result of a failed absorbance of the partition between the two fused Mullerian ducts, or a bicornuation of the uterus (Fig 1). Although, these two conditions can often be corrected by surgery, other forms of malformations, like unicornuation and a didelphic uterus, accounting for 20 % of the malformations, cannot be surgically cured [13]. Even more severe congenital malformations are the total absence of a uterus or the presence of only small remnants of uterus like tissue along the pelvic sidewalls seen in the Mayer–Rokitansky–Küster–Hauser (MRKH) syndrome. This rare (1:4000) condition is characterized by Müllerian duct aplasia, resulting in lack of the upper two-thirds of the vagina and the uterus but presence of normal ovaries and thus normal hormone levels [14, 15]. These females are usually identified when presenting with primary amenorrhea as teenagers and generally undergo surgical creation of a neovagina when past puberty. In a study of 17 biological daughters to females with the MRKH-syndrome, no congenital malformations of the uterus could be seen that would indicate a strong genetic inheritance [16].

Myoma is the most common cause of acquired UFI, both absolute and relative UFI included. Around 10 % of the female population between 33 and 40 years of age have myomas [17], and the incidence increases with age. An estimated 1 % of the females with myoma in the age group 30-34 years will undergo hysterectomy as treatment and the corresponding percentage in the age group 35-39 years of age is 2.5 % [18]. Corrective surgery, in the form of myomectomy, is usually considered as a primary option for a woman of fertile age, who has yet to form a family. The effectiveness of the surgical treatment is dependent on the position, number and size of the myomas.

Intrauterine adhesions (Asherman's syndrome) can cause recurrent pregnancy loss and infertility. These adhesions can occur secondary to intrauterine infections, surgical abortions or genital tuberculosis, with the latter cause being more common in third-world countries [19, 20]. If the uterine adhesions are left untreated an infertility rate of >50 % can be expected [19]. Hysteroscopic

lysis of adhesions can restore the size and shape of the uterus cavity but nevertheless more than two-thirds of women with severe adhesions remain infertile in spite of the attempts to cure [21].

Table 1. Overview of causes of uterus factor infertility and estimated prevalence. Data from [12, 13, 18, 21-28]. Numbers in %.

Cause	Prevalence	Cause-specific infertility
AQUIRED		
Myoma	21-26	40
Intrauterine adhesions	1-2	70
Hysterectomy		
Myoma	1-1.5	100
Peripartal	0.04-1.25	100
Cervical cancer	0.00004-0.0001	100
CONGENITAL		
Malformations		
Arcuate uterus	1.3-6.2	17.3
Bicornuate uterus	0.7-1.3	37.5
Septate uterus	0.8-1.4	38
Unicornuate uterus	0.3-0.5	56.3
Didelphic uterus	0.1-0.3	40
Uterine hypoplasia	0.038	100
Uterine aplasia	0.0002	100

Cervical cancer is the second most common form of cancer in women worldwide [29], with around 50 % of the affected women being under the age of 40 [23], and with a non-negligible group of patients under the age of 30 [30]. It has been estimated that around 50 % of women diagnosed with cervical cancer under the age of 40 are suitable for fertility-sparing surgical interventions (radical trachelectomy), where the cervix is resected but the uterus is spared [31]. Trachelectomy is restricted to tumours of stage 1A2-1B with an invasion of less than 10 mm and a diameter not exceeding 20 mm [32, 33]. The remaining group of women, who are non-eligible for fertility-sparing surgery, are treated

with conventional radical hysterectomy with preservation of the ovaries and are then included in the group of AUI. Nair and co-workers estimate that the annual prevalence of hysterectomy, due to benign or malign causes, in the U.S is 0.2/1000, in women below 24 years of age, or expressed in absolute numbers about 5 000 [34].

Although all efforts are made not to perform radical hysterectomies in fertile women with a non-complete family it may still be indicated and performed as an emergency peripartum intervention in case of severe haemorrhage that is resistant to other forms of treatment [35].

The history of organ transplantation

In the beginning of the last century, the innovative work of French doctor Carrel and his developments of new surgical techniques laid the foundation for transplantation surgery. Carrel received the Nobel Prize of Physiology and Medicine in 1912 “in recognition of his work on vascular suture and the transplantation of blood vessels and organs”. Although the surgical skills of transplantation were perfected early, the results of organ transplantations were still poor and the breakthrough only came with the introduction of immunosuppressive drugs. During the Second World War, the immunologist Peter Medawar and fellow plastic surgeon Thomas Gibson started to look into mechanisms leading to rejection of foreign tissue. Their research was triggered by the difficulties of allografting skin for reconstruction of severe burn deformities, suffered by allied Navy personnel and British fighting pilots. The duo of Medawar and Gibson were the first to suggest that the immune system was responsible for the destruction of transplanted skin grafts [36]. Eventually Peter Medawar was rewarded a Nobel Prize in 1960 and later a Knighthood.

In 1954, the first kidney transplantation between identical twins was performed by the American plastic surgeon Joseph Murray [37]. When Ronald Herrick donated one of his kidneys to his twin brother, it was the first time in modern medical history a normal healthy person was subjected to a major surgical operation not for his or her own direct benefit. Three years later, the first

successful human allograft, and the first composite tissue allograft, followed. It was an *en bloc* transplantation of a digital flexor tendon mechanism by American plastic surgeon Erle Peacock [38].

Fig 2: Ronald and twin brother Richard Herrick 10 months after Richard received his transplanted kidney.



In 1960, the British surgeon Roy Calne, advised by Medawar, travelled to Boston for a fellowship. Calne teamed up with Doctors Moore and Hitchings and together they developed the experimental drug, BW-322, that dramatically lowered the rejection frequency of allografts [39]. This antiproliferative agent is today known as azathioprine, and was commonly used throughout the world in organ transplantation for many years. Encouraged by the introduction of this new drug, some years later in 1962, Murray and colleagues transplanted a patient with a kidney from an unrelated deceased donor and treated the patient with azathioprine [40]. The patient was the third that the team transplanted with organs from deceased donors and he survived more than one year, becoming the world's first patient with a successful unrelated renal allograft. This event marked a new era, where an organ transplantation from an unrelated/deceased donor no longer were a science fiction scenario but recognized as a realistic and available treatment for a severely ill patient.

The development of immunosuppression (IS) treatments progressed and in 1964 it was shown in a canine model that an episode of acute rejection could be treated successfully with large doses of corticosteroids [41]. Corticosteroids were later added to the azathioprine therapy also for maintenance IS. Antilymphocyte globulins were introduced both to treat episodes of rejection that

could not be repressed by corticosteroids, and soon after as part of an induction protocol [42, 43]. The era of azathioprine as the most important component in IS lasted until the 1980s when cyclosporine, a calcineurin inhibitor (CNI), was introduced. Addition of this agent in the IS protocols significantly reduced the rate of kidney loss due to episodes of rejection and cyclosporine has been the backbone of IS ever since. It was not until the last decade, other IS agents, such as the modern CNI tacrolimus, the antiproliferative agent mycophenolate mofetil (MMF) and the interleukin-2 inhibitor sirolimus became available, as tools to further hamper acute and chronic rejection

Rejection

The immune system is a complex collaboration of cells, tissues and organs, sharing the delicate task of defending the body from a wide variety of intruders, such as viruses, bacteria and fungi, destructing harmful own tissue, such as tumours, and repairing damaged tissue. The foundation of the immune system is the ability of self/nonself discrimination. Two different pathways are usually identified, the innate immune system, characterized by a nonspecific quick response, and the adaptive immune system, that triggers a slower but specific response and contains an individual pool of receptors corresponding to previous life-time exposure. The innate immune system includes key-players like granulocytes, macrophages, dendritic cells, natural killer cells and the complement system, while the adaptive immune system mostly functions through actions of T-and B-cells. In transplant immunology, the focus of attention has traditionally been in mechanisms of the adaptive immune system, and especially T-cells.

The transplantation of an organ triggers the mechanisms of the immune system in several ways. The surgical trauma and exposure of ischemia and reperfusion inevitably leads to tissue damage that result in cellular swelling and release of pro-inflammatory products. The inflammation activates the innate system and can also promote initiation of T-cells and antigen presenting cells to induce graft rejection and destruction. The damage from ischemia and reperfusion injury may be sufficient to lead to both acute and chronic rejection.

Rejection is the recipients' complex response to transplanted tissue. The main target of this response is the major histocompatibility complex (MHC) antigens, in humans called human leukocyte antigen (HLA), that are expressed on the cell surface. The HLAs mediate interactions of the immune cells and determine compatibility of donor and recipient. Rejection can be categorized into three groups depending on when it occurs; hyperacute, acute or chronic rejection.

Hyperacute rejection

The onset of hyperacute rejection is immediate upon reperfusion and destroys the organ within minutes to hours. It is caused by preexisting antibodies against donor HLA, endothelial-cell antigens, and ABO blood-group antigens that bind to the endothelium. Complement activation is an important component followed by interstitial haemorrhage, oedema, platelet aggregation and coagulation resulting in immediate capillary thrombosis formation and irreversible graft loss. The production of the preexisting antibodies may be induced by previous transplants or blood transfusions. With preoperative antibody detection, preventing incompatible transplantations; the majority of hyperacute rejections can be avoided.

Acute rejection

An acute rejection usually occurs within a couple of weeks to months after transplantation. The process can either be cellular or humoral. The acute cellular rejection is mediated by the recipients T-lymphocytes that recognize the donor MHC-antigens presented by the dendritic cells (also called antigen presenting cells), leading to recruitment of T killer cells as well as natural killer cells attacking the graft. Acute humoral rejection is primarily mediated by antibodies and complement mechanisms. The antibodies are either preexisting, like the preformed antibodies against HLA in patients with previously failed transplants, or developed after transplantation (anti-donor antibodies). The acute rejection process is mainly mediated by the binding of antibodies to the endothelium of the graft.

Chronic rejection

The phenomenon of chronic rejection usually does not occur the first months after transplantation, but develops months to years after episodes of acute rejection have resolved. Chronic rejection is mediated by both humoral and cellular mechanisms. It usually appears as fibrosis and scarring and is a progressive deterioration of transplant function due to inflammatory processes that eventually will lead to graft destruction. There are factors known to increase the risk of chronic rejection, such as, previous episodes of acute rejection, initial delayed graft function, ischemia/reperfusion damage and inadequate IS. In most cases chronic rejection, cannot be prevented.

Immunosuppression

Immunosuppression used in organ transplantation can be categorized as induction, maintenance, or rescue therapies.

The induction therapy strives towards depletion of circulating T-lymphocytes, thus attempting to avoid early acute cellular rejection (usually beginning within ten days of transplantation). The therapy is usually intense and long-term use of induction therapy might be potentially toxic to the exposed patient. Induction therapy may consist of high doses of maintenance drugs (CNI, corticosteroids) and also include specialized induction agents such as polyclonal (antithymocyte globulin, ATG) or monoclonal (basiliximab, daclizumab) antibodies. Antithymocyte globulin causes t-cell lysis. The monoclonal antibodies bind to the interleukin-2 receptor of activated T-cells, preventing proliferation without myelosuppression or renal impairment. Immunomodulating methods such as donor specific transfusion or irradiation can also be a part of induction therapy.

Since the need for IS decreases as time passes from the event of transplantation the less potent but also less toxic, maintenance therapy can take on. The maintenance IS aims to avoid both episodes of acute rejection and to minimize chronic rejection and is usually given for the rest of the recipient's life, provided that the transplanted organ is still functioning. The maintenance therapy

is commonly designed as a triple IS protocol consisting of CNI, corticosteroids and an antiproliferative agent. The protocol strives to combine drugs with different mechanisms to achieve maximal effect at the same time as the adverse effects will be kept minimal, with low dose-related toxicity.

If an episode of rejection occurs, the rescue therapy enters, with usually increased doses of the maintenance therapy (usually consisting of CNI, corticosteroids) and/or corticosteroids and/or ATG, characterized by efficacy and potency, but also if used chronically, intolerable for the patient.

Uterus transplantation

Research in the field of UTx have through the years been conducted in several different animal models including rodents (mouse, rat) [44-47], large domestic species (sheep, pig) [48-51] and nonhuman primates (baboon, macaque) [52-54]. Excluding the early experiments, when the dog was a common animal model, initial studies of UTx have usually been done in rodents and small animals. The findings and conclusions of these experiments have subsequently been used to perform UTx in larger animals, where the setting is more human-like when it comes to anatomy, size and physiology. As of lately, also nonhuman primates have been used in animal UTx, this being considered as the last step before clinical introduction, and to make the transition to human UTx as safe as it possibly can be.

Early animal studies

Already in 1927, Bykow and associates successfully performed avascular autologous transplantation of the uterus wrapped in omentum in three dogs, and at eight months follow-up, the uteri were viable [55]. The introduction of IS opened up new horizons in the transplantation community and thus started a new era of extensive research and progressive treatments. Forty years later than Bykow, in the late 60's and early 70's, several studies were published reporting different attempts of UTx using both vascular anastomosis and an avascular technique where the uterus transplants were often wrapped in omen-

tum, this latter method triggered by the complexity of the vascular anatomy of the pelvic region. Eraslan and colleagues made, in 1965, one of the first attempts at vascular autologous *en bloc* utero-ovarian transplantation in a dog model [56]. However, in this study the uterus was not actually removed from the abdominal cavity. The graft vessels were dissected and transected in the approximate level of the common origin of the internal iliac arteries. The uterus, still attached to the clamped vagina, was subsequently reimplanted end-to-end in the original position. Thereafter the vagina was transected and reattached. During approximately 30 min of ischemia, the graft was perfused with physiological saline. Out of 18 transplanted animals, there was evidence of patency of the bloodvessels and reported ovarian function and oestrus in at least six of the ten long-term surviving animals. Pregnancy was ensured in three dogs and delivery of two litters of nine and three puppies each was reported. Rejection patterns of allogeneic transplanted uteri was reported four years later, in 1969, when 14 dogs were transplanted in the U.S. from female, to female (n=9) or male (n=5) dogs [57]. After retrieval, the uterus was flushed with chilled heparinized Ringer's lactate solution and vascular anastomosed end-to-end with the internal iliac artery of the graft to the common internal iliac artery of the recipient, and bilateral end-to-side with the internal iliac vein of the graft and the recipient. Azathioprine was given postoperatively to all recipients (3-5 mg / day and kg bodyweight). Out of eight long-term survivors, a viable uterus was found in five animals at termination after 45 days. A Canadian follow-up study carried out one year later looked at the use of azathioprine and cortisone to mediate graft rejection after UTx in dogs, and as comparison the study included untreated animals and one animal treated with antithymocyte serum [58]. In this study, vascular thrombosis was a major finding both in treated and untreated dogs, and it was stipulated as a primary manifestation of uterus rejection. A similar study, looking at uterus rejection after avascular UTx, showed no difference in rejection patterns between untreated and azathioprine treated animals [59]. In a comparative study in dogs, the avascularly transplanted uteri showed massive necrotic degeneration after 90 days whereas the grafts with vascular anastomosis were viable [60].

In 1971, Scott and colleagues used a nonhuman primate model (rhesus macaque) for UTx when performing avascular auto- and allogeneic transplanta-

tions [61]. The grafts were retrieved with a subtotal hysterectomy and the retrieval time of 20 minutes did not include vascular dissection. Blood flow was re-established by neoangiogenesis from the omentum but full rejection was reported on day 14.

Modern animal studies

During the late 70s, 80s and 90s there was little attention paid to UTx with the successful introduction of IVF and ICSI as major infertility treatments being possible causes. In the early 2000s, the research field was rediscovered and have since then been a highly debated topic, but nevertheless evolving rapidly.

Rodents and small animals

Rodents are commonly used in medical research. Although far from a human-like setting, the knowledge concerning reproductive physiology, immunology and genetics in rodents is thorough which makes them good as an experimental model and at a low cost. In addition, syngeneic transplantations experiments can readily be done in rodents, since large numbers of inbred strains are available. In early 2000s, initial studies describing successful UTx after syngeneic transplantations in mouse [44] were published. The common uterus cavity was retrieved along with one uterus horn, and the vascular pedicle including the ipsilateral uterine/iliac vessels all the way up to the aorta and the vena cava. Anastomoses were done end-to-side with the aortal and caval ends to the recipient's infrarenal aorta and vena cava. The cervix of the transplanted uterus was left unattached in the abdomen and the native uterus was left in situ. The surgical learning-curve was elegantly illustrated by the survival rate of the animals which was increased from 40 to 70 % after the first 20 transplantations. A successful long-term graft-function was demonstrated in 90 % of the survivors of the animals undergoing surgery, and one pregnancy after embryo transfer was reported. Since the intraabdominally placed cervix did not drain cervical secretion accurately, the UTx mouse model was modified and the cervix instead placed as a cervical-cutaneous stoma on the abdominal wall [45].

Transmyometrial embryo transfer showed similar pregnancy rates in both the native and the transplanted heterotopic uteri. Live-birth was reported and the birth weight, postnatal development and fertility of the pups were normal. The same modified syngeneic UTx model was also used to evaluate the influence of cold ischemia on the viability and function of the transplanted uterus [62]. The uterus graft was prior to transplantation kept in cold preservation solution for 24 or 48 hours wherein the uteri preserved for 24 hours showed normal morphology and blood flow two weeks post UTx while grafts preserved for 48 hours presented with decreased blood flow and necrosis. In five out of six animals that had been transplanted with a uterus preserved for 24 hours, embryo transfer resulted in pregnancy and normally developed offspring. The initial experiments in rats used a similar method as described in mice [44, 45] and the graft included one uterus horn and a vascular pedicle with uterine vessels together with aorta/vena cava [63] or the common iliacs [46, 64]. The first rat UTx model was syngeneic and the uterus graft was placed heterotopically and anastomosed end-to-side to the native aorta and vena cava whilst the native uterus was kept in situ [63]. The study presented a good animal survival (> 95%) but struggled with formation of thrombi that caused loss of transplanted uteri in one third of the animals. Some years later, an orthotopic rat UTx model, allowing spontaneous mating, was developed [65]. The common iliac vessels were used for vascular anastomoses in both graft and recipient and the cervix with a vaginal rim was attached to the native vagina after a hysterectomy had been performed. Efforts at spontaneous mating resulted in pregnancy rates of 50 % in both control animals and in transplanted animals and there was no difference between the groups regarding number of pups and postnatal development. The same year the first pregnancy after allogeneic UTx was reported [46]. Similar rates of pregnancies could be seen between control animals and transplanted animals. In follow-up studies with the same strain combinations and IS (tacrolimus) the pregnancies were allowed to go to term and the pups developed normal well into adulthood (C. Díaz-García, personal communication).

Apart from rodents, the feasibility of UTx has also been tried in other small animals like the rabbit. In 1986, Confino and co-workers published a report on

allogeneic UTx in a rabbit model [66]. The uterus graft, retrieved through a sub-total hysterectomy, was transplanted avascularly after normothermic flushing with Ringer's lactate solution. The animals received cyclosporine and after one month a few animals showed preserved myometrium and endometrium. In another study in rabbits, the uterus with a vascular pedicle up to and including the aorta and vena cava, was recovered from deceased donors, and after one hour transplanted with end-to-side anastomosis to the recipient [67]. Surgical survival was good and a short-term follow-up presented viable uteri with no signs of thrombosis formation but several postoperative complications, like paraplegia, intraperitoneal haemorrhage and pulmonary embolism occurred later.

Large animals

Models of UTx in large animals include, apart from the previously described early experiments in dogs [56, 57], also trials in pigs [11, 48, 50] and sheep [68-72]. Autologous transplantations of the bicornuate uterus of the pig was initially reported in 2005 by Sieunarine and co-workers [11]. The graft was after one hour of cold ischemia, perfused with cold University of Wisconsin (UW) or Celsior solutions, and then reintroduced. The graft survival was followed for several days with signs of gradual formation of vascular thrombosis at the anastomosis sites of the uterine vessels. In another study of autologous UTx, the uterus was flushed with cold Ringer's Acetate solution and replanted after 1-2 hours of cold ischemia with anastomosis of the uterine vessels end-to-end to their origin just above the ureters [48]. The model was, due to the size of the pig uterus in correlation to the size of the vessels, considered difficult. Noticeable was also the time of vascular anastomosis, during which time the graft was subjected to gradual warming, that was around two hours. The results accordingly showed satisfactory reperfusion only in four out of 19 animals [48]. Histological changes indicating possible ischemia-reperfusion damage was seen in some grafts.

Some years later heterotopic allogeneic UTx was performed in miniature swine, where the vascular pedicles were dissected for inclusion in the graft all the way up to the insertion of the renal vessels in the aorta and vena cava [50]. The

transplanted uterus was following flushing *in situ* with chilled UW, placed retroperitoneally behind the ascending colon and the cervix and vaginal vault exteriorized as a cutaneous stoma through the abdominal wall. The native uterus was kept in situ. The IS therapy consisted of induction with iv tacrolimus followed by maintenance with oral cyclosporine after 12 days. After one year, half the population of animals (n=5) was alive and healthy and episodes of rejection, occurring during the second and third months, had been successfully treated with modified IS (increased doses of cyclosporine and steroids).

The sheep has, in comparison to the pig, a larger pelvis, a relatively small uterus with larger vessels, more comparable to the proportions of a human female. In 2008 attempts at autologous UTx in sheep were performed and the grafts were orthotopically placed with vascular anastomosis of the uterine vessels, including the anterior part of the internal iliac vessels end-to-side to the external iliac vessels [69]. Blood reperfusion was seen in five out of seven animals and after three hours there was visible blood flow in the tissue and spontaneous uterus contractility. The same research team published studies of early changes of reperfusion of the sheep uterus after one hour of cold followed by warm ischemia [70] and it was concluded that that the uterus of the sheep has the capacity to tolerate at least one hour of tissue damaging warm ischemia. In 2010 the technique previously used by Dahm-Kähler and co-workers [69] was modified to also include one uterus horn and the associated oviduct and ovary to the graft to enable test of fertility after natural mating [68]. The graft was replanted and the surgical time was around eight hours. Animal survival rate was 50 % and in 60 % of the transplanted mated ewes pregnancy occurred [68]. The lambs were comparable in size to lambs from control ewes.

Concerning allogeneic UTx in sheep, Ramirez and colleagues developed a minimally-invasive technique of graft retrieval and the transplants uterine vessels were anastomosed end-to-end with their native peers after a recipient hysterectomy was performed, a technique that is only applicable when a recipient hysterectomy is part of the UTx procedure [71]. After six months, during which time the animals were on a cyclosporine therapy, hysterectomies revealed viable uterus tissue and vascular patency in 60 % of the animals. In a follow-up study, in

2010, 12 ewes were allogeneically transplanted with a uterus graft, using the same surgical technique as previously described [51]. Immunosuppression was increased compared to the previous experiment by higher doses of cyclosporine. Four months post-transplantation, five ewes were subjected to embryo transfer [51]. Three pregnancies occurred with the outcome of one ectopic pregnancy, one miscarriage and one live birth by caesarean section. No follow-up was done of the lamb. In a recent publication by a French research group, orthotopic allogeneic UTx was performed in ewes with end-to-side anastomosis with the donor aorta and cava unilateral to the recipient external iliac vessels [73]. Immunosuppression was maintained by cyclosporine and mycophenolate mofetil and rejection monitored by vaginoscopy, magnetic resonance imaging (MRI) and second look laparotomy. All uterus transplants showed thrombosis of vessels and signs of necrosis after 10 weeks with poor fixation of the graft, rejection and insufficient achieved vessel lengths speculated to be the causes.

Nonhuman primates

No species of animals has such a resemblance to the human when it comes to anatomy and physiology of the reproductive organs as the nonhuman primates. Two species of nonhuman primates have been subjected to research involving UTx, cynomolgus macaque and baboon.

Prior to the so far sole published human UTx case, the Saudi Arabian team used the baboon as a model for autologous orthotopic UTx [74]. In the 16 animals used in the preparatory study, the first eight were performed with vascular anastomosis of the uterine vessels end-to-end. Vascular thrombosis was revealed in 75 % of the vascular connections and subsequently the technique was altered to instead include anastomoses between the uterine vessel and the internal iliac vessels in an end-to-side fashion. In the second set of animals vascular patency was demonstrated in 90 % of the anastomosis. The study was terminated after 6-12 weeks and demonstrated animal and graft survival. However exact data of this experiment is not provided.

In 2011, a Japanese team performed a small study of autologous UTx in two cynomolgus macaques [75]. During a 6-8 hours long retrieval, the uterus and

the uterine vessels were recovered. Vascular anastomosis was done bilaterally with the uterine vessels to the external iliac vessels. Postoperatively one animal died after two days with acute renal failure assumed as probable cause. The surviving animal resumed menstruation postoperatively.

In a another study from the same research group, a unilateral anastomosis of one uterine artery and one uterine vein is proposed to provide sufficient blood supply following UTx [76].

Recently the same group reported pregnancy after autologous UTx and natural mating in a cynomolgus macaque (Kisu, personal communication). The uterine arteries and one ovarian vein were used for anastomosis.

Human studies

In the year 2000, a trial UTx in humans was undertaken in Saudi Arabia [74]. A 26-year-old woman, who had previously undergone an emergency peripartum hysterectomy due to extensive bleeding, received a uterus transplant including oviducts from an unrelated 46-year-old live donor. The donor hysterectomy was performed following elective surgery because of bilateral benign ovarian cysts. As the vascular pedicles of the uterine vessels that were obtained at the retrieval surgery were short, the team of surgeons had to elongate the vascular pedicles by vascular segments of the saphenous veins. The anastomoses were performed with the extended pedicles of the uterine arteries and veins bilaterally end-to-side to the external iliac vessels of the recipient. Initially, the surgery and the postoperative care of the donor and the recipient were without major complications. Immunosuppressive treatment followed a standard triple therapy regimen and one episode of acute rejection could be successfully treated with ATG. The uterus was responsive to oestrogen and progesterone treatment with endometrial proliferation and withdrawal bleeding. After three months, necrosis and thrombosed vessels of the graft were seen and the uterus was removed without incident. The exact cause of these events is not entirely clear but the authors suggest that prolapse of the uterus, as a result of inadequate structural support, with secondary thrombosis of the supplying vessels led to the uterus necrosis.

In 2007, Del Priore and co-workers attempted to retrieve uterus grafts including complete internal iliac vessels bilaterally as part of a multi-organ donation [77]. The total length of the procured vessels was only achieved in two out of seven cases.

In 2011, a second human UTx attempt was performed at Akdeniz University, Turkey (Ö. Özkan, personal communication). The recipient was a young female suffering from uterus aplasia and she was transplanted with a uterus from a young multi-organ donor. This second case is yet to be published in a scientific journal.

Pregnancy after transplantation and immunosuppression during pregnancy

A common concern in UTx research, and especially when a human implication is considered, is the potential effects on the developing foetus/child both concerning the transplantation procedure itself and also that of the IS drugs that are essential during the pregnancy.

The first case of pregnancy following organ transplantation was reported already in 1956. A 21 year old woman had received a kidney transplant from her identical twin sister, being the third twin transplant ever performed, and the first in a female [78]. Menstruation was resumed after six months and after three further months the transplanted woman became pregnant. The pregnancy was reported to be without any complications and a healthy baby boy of 3300 g was delivered by caesarean section. Three years later the woman was pregnant again and a healthy baby girl was delivered by caesarean section following an uncomplicated pregnancy. No adverse effect was reported on the kidney graft following either of the pregnancies. Pregnancy after kidney transplantation and during use of IS (azathioprine and prednisone) was first reported in 1967, when a 24 year old women, transplanted three years previously with the kidney of her mother, came into the hospital for a routine check-up and revealed a seven months pregnancy [79]. During the third trimester the

mother to be, had increased blood pressure ($\approx 140/110$ mmHg), weight gain of about 1 kg/week and ankle oedema. After spontaneous membrane rupture, a healthy baby girl was born vaginally, with an Apgar score of 10 at one minute of age and weighing 2610 g.

As of today there is extensive data on pregnancies of organ recipients with concurrent IS. Since the first reported pregnancy of a transplant patient, more than 14 000 births have been reported in a 2006 paper [80] and surely several thousands more have occurred after that and it is likely that many of these pregnancies are not reported at all. There are three large registers that offer data about the outcome of pregnancies in transplant recipients; the American National Transplantation Pregnancy Registry [81] and the two British based, European Dialysis and Transplant Association Registry [82] and the UK Transplant Pregnancy Registry [83]. All three registers

shows similar trends of increased occurrence of complications, such as ectopic pregnancy, miscarriage, preterm birth, low birth-weight, stillbirth and neonatal death, during pregnancy. In 2005, Källén and co-workers, presented a Swedish population study including all pregnancies of transplanted mothers and it was shown that the reported incidence of miscarriage preeclampsia, premature birth and growth inhibition were equal in pregnancies prior to and



after the organ transplantation in the same individual [84]. Thus it was concluded that the increased risks of pregnancy-related complications were more likely related to the underlying disease rather than to the transplantation and IS themselves. In line with this assumption, is the fact that many recipients of organ transplants already prior to the transplantation have hypertension and renal dysfunction, both related with considerably increased risks of pregnancy complications [85]. The incidence rates of hypertension and preeclampsia has been reported to be increased in transplant recipients when compared to the non-transplanted population. In a review from 2006 it was suggested that the incidence rate vary depending on which type of organ is transplanted, exemplified by the reported hypertension-rate of 28-72 % of kidney recipients whilst pancreas and liver recipients have corresponding percentages of 75% and 22-44%, respectively [80]. Preeclampsia is reported to affect one-third of the recipients of sole kidney or kidney and pancreas whilst it affects only around one-tenth of the heart and lung recipients [80]. Preterm birth and low birth-weight is reported in half of all organ transplant recipients [80]. Regarding risk of graft rejection and loss during pregnancy, recipients of lung transplants have the highest reported risk, within 2 years of the delivery (31 and 23 %), but it should be noted that this group contains a small number of patients and uncertainty regarding data accuracy exists [80]. Other reports however indicate that the rejection rates in pregnant recipients of organ transplants are similar to those in non-pregnant recipients [86].

Conception following UTx will be preceded by IVF, which also is associated with some increase in obstetric complications. In a recently published study by Sazanova and co-workers, the risk of preterm delivery, growth inhibition and preeclampsia was increased with a single embryo transfer (SET) compared to natural conception (8.5, 4.0, 4.5 % (SET) and 6.0, 2.7, 2.9 % (natural conception) respectively) [87].

Intake of IS to prevent organ rejection is vital throughout pregnancy. All common medications used to prevent episodes of rejection, and thereby to prevent from organ loss, crosses the placenta barrier and subsequently reaches the foetal circulation [88]. The foetus/child is unavoidably exposed to potential toxic and teratogenic agents during important developmental stages. However the

exact distribution to the foetus has been difficult to determine [89] and the effects of the medications may not be obvious at birth. The potential side-effects of IS have a broad spectrum, ranging from major severe malformations to delicate hardly detectable neurocognitive defects. The American Food and Drug Administration (FDA) has categorized immunosuppressive medications using a scale ranging from A-D and X, where A represents no human risk and X an absolutely contraindication [80].

Corticosteroids, used both as maintenance and rejection therapy, simply cross the placenta barrier, however around 90 % of the given dose is metabolized in the placenta before reaching foetal circulation [90]. The FDA categorizes the drug as B (animal studies showing risk, but no evidence of human risk). The placental metabolism of corticosteroids may in the majority of cases protect the foetus from unwanted side-effects like adrenal suppression. Prednisone has been reported to be associated with birth defects, particularly when given in high doses, exceeding 20 mg per day [91].

Calcineurin inhibitors, commonly used as maintenance therapy, easily cross the placenta barrier and reaches the foetal circulation but the blood levels have been reported to be around 50 % of the blood levels of the mother [92, 93]. The FDA categorizes these drugs as C (human risk not ruled out). The use of CNI is reported to be associated with birth defects in humans [94]. The NTPR reported in 2003, prevalence data on miscarriage (22 %), still-birth (3 %), prematurity (55 %) and low birth weight (53 %) [95]. Kainz and co-workers reported 100 pregnancies, exposed to tacrolimus, whereof 68 % ended in live births, 2 in neonatal deaths, and 1 in stillbirth [96]. The remaining pregnancies ended either in abortion (spontaneous 12 % or induced 12 %), were lost in follow-up (3 %) or ongoing at termination of the study (2 %). Of the deliveries 59% were premature and the most common child complications were hyperkalemia, hypoxia and temporary renal dysfunction. Congenital malformations were seen in four children but without any pattern.

Mycophenolate mofetil, used as maintenance therapy in organ transplantation, rheumatoid arthritis and lupus nephritis, was by the FDA categorized as C (human risk not ruled out). Animal models have shown malformations in offspring

exposed to MMF and the European transplantation community discourage use during pregnancy [91]. There is limited data on human exposure of MMF during pregnancy but in a report from Le Ray and colleagues, multiple malformations, specifically, facial dysmorphism and midline anomalies, including agenesis of the corpus callosum was shown in foetus following use of MMF during pregnancy [97]. The NTPR examined the outcomes of pregnancies with exposures to MMF and in kidney recipients a total of 26 pregnancies resulted in 11 spontaneous abortions and 15 live births [98]. Structural malformations, such as hypoplastic nails and short fifth fingers, cleft lip and palate and neonatal death with multiple malformations, were reported in 26.7% of the 15 children. The European Best Practice Guidelines recommend that the use of MMF should be terminated six weeks prior to attempts of conception [99].

The 10th of March 2012, the first child born to a transplant recipient turned 54 years. The collected data with over 50 years of successful pregnancies with the concurrent use of IS after organ transplantation proves the possibility to achieve pregnancy with healthy offspring and is considered one of the benefits offered to women through organ transplantation.

Ethical aspects of uterus transplantation

Uterus transplantation is a complicated scientific and medical procedure but, even so, it may be the ethics surrounding UTx that represent the greatest challenge towards general medical acceptance. The ethics regarding UTx involves essential issues concerning reproduction, parenthood and medical advancements. As moral, religious values and beliefs differ between cultures and societies and in the light of those circumstances; UTx may be accepted under some conditions whilst others might find it unacceptable. Although the ability to reproduce is not essential in life, many affected couples consider it to be of vital importance. As UTx constitutes both a new surgical procedure, proposed as a treatment for UFI, but at the same time being a non-life-saving organ transplantation procedure, the ethical analysis of the procedure should be assessed by stringent and thorough criteria. A few independent attempts to analyze the ethical issues and draw up guidelines for UTx have been published.

In 2009, the International Federation of Gynecology and Obstetrics (FIGO) committee for the ethical aspects of human reproduction and women's health presented a report on the background and ethical aspects of UTx and guidelines for the procedure [10]. This publication cites that uterine infertility (relative and absolute) is to be expected in 3-5 % of the female population and that the current options for this group of women to overcome their infertility, in an international perspective, are either adoption or surrogate motherhood. There are large groups of women for whom adoption or surrogate motherhood is not possible or allowed, for personal, social or religious reasons. This report states that UTx should reach a human clinical stage only after considerable research in appropriate large animal models, including nonhuman primates. Critical issues, such as emotional pressure on doctors from potential uterus transplant recipients, which could possibly lead to premature human clinical introduction, were identified. In this official report it was also stated that it would be clearly unethical to remove the uterus for the purpose of transplantation from a young woman who has not completed childbearing, or to use a malformed uterus for transplantation purposes.

Recently, the Montreal criteria for the ethical feasibility of UTx were published, with a viewpoint to facilitate the transition of UTx from an experimental to a clinical phase [100]. The report proposes criteria required for a woman to be ethically considered as a candidate for UTx, and it is stated that the desire to experience gestation is sufficient to justify the potential negative consequences of UTx. This is valid if UTx is performed in an appropriate clinical setting after thorough research has found the procedure reasonably safe.

Since the introduction of modern assisted reproductive techniques, many infertile couples have been able to form a family; however, for others, the only possibilities to achieve parenthood have been through gestational surrogacy or adoption. Even if one of the basic foundations of modern society is to secure a stable population growth, many Western world countries suffer from an ageing population with a reversed population pyramid. In spite of this, widespread acceptance of gestational surrogacy or adoption is not a matter of course. While these two alternatives provide an excellent option for a large number of infertile couples, they may in some cultural, societal, legal or religious settings be severely restricted or considered inappropriate.

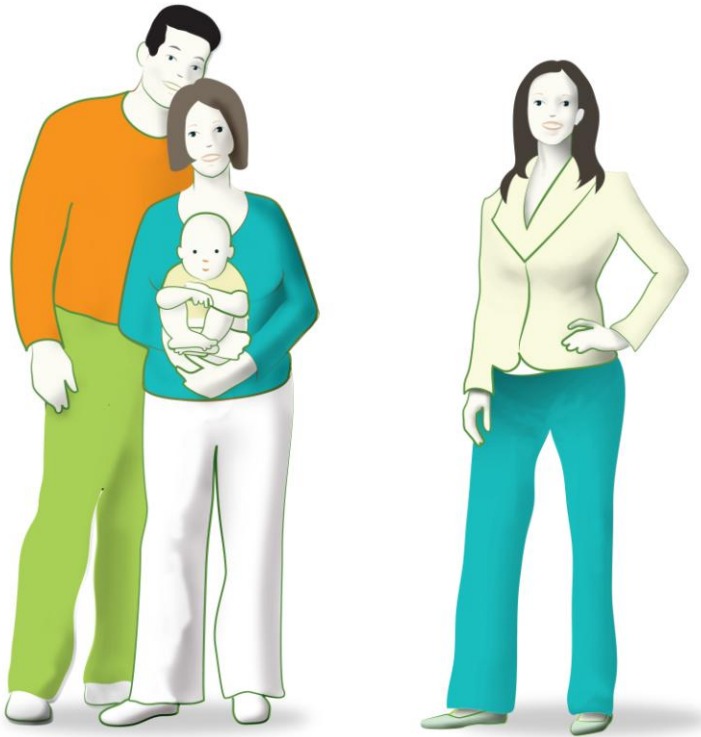
In surrogacy; either the surrogate mother's own oocyte is fertilized by artificial insemination with the sperms of the intended father (traditional surrogacy) or the surrogate mother carries the intended parent's genetic child conceived through IVF (gestational surrogacy). The intentions of becoming a host of surrogacy can either be of an altruistic or a commercial nature and in some countries the legitimacy is different between these two alternatives [101]. The Catholic Church is opposed to assisted conception; in particular, if associated with the donation of gametes and/or surrogacy [102], while the Anglican Church takes a less rigid view of the matter and does not specifically forbid surrogacy [103]. In the Jewish religion, it is considered a duty to reproduce and multiply (first commandment; Old Testament, Genesis 1:18) [103] and surrogacy is not forbidden. The main issue, however, will be to whom the child belongs; from a religious point of view, a child born as a result of surrogacy will belong to the father who donated the sperm and to the woman who gave birth [104]. In the Islamic interpretations, artificial reproduction is not specifically mentioned but the importance of marriage, family formation, and procreation is emphasized and when reproduction fails, Islam encourages attempts to cure infertility [105]. In vitro fertilization is not prohibited as long as it involves husband and wife [105], but contribution by a third party as a provider of oocyte, sperm or embryo is not acceptable [103]. Surrogacy was previously allowed in Saudi Arabia, if between wives of the same husband, but in Mecca in 1985 the Islamic Council withdrew its earlier permission [103]. Additionally, adoption is generally not accepted in the Islamic world. With the above mentioned religious aspects in mind, UTx would not specifically be prohibited according to Jewish, Catholic, Islamic or Anglican beliefs.

An ethical analysis of UTx has to include not only the donor and the recipient, as in many other organ transplantations settings, but also the partner of the recipient (the father of a child born from a transplanted womb) and their future child (born from a transplanted uterus).

The uterus recipient naturally carries the risk associated with the surgical procedure and anaesthesia, although in the case of UTx the surgical intervention is considered relatively simple and is performed on a young and healthy (apart

from her UFI) woman. The surgical and anesthesiological risks include postoperative infections, bleeding during surgery, thromboembolism, aspiration and allergic reactions. The recipient will also have to use IS, the risks of which are fairly well known after many years of experience of long-term use by patients. It is common knowledge that the use of CNI inhibitors somewhat increases the risk of renal impairment. In reports of hand, leg and face transplantation, where relatively young and healthy patients are transplanted, no signs of renal impairment have been shown during a follow-up period for almost a decade [106]. With long-term use of IS there may be a slightly increased risk of squamous cell carcinoma and lymphoma. After UTx, IS will be given to the recipient for a limited time period (2-3 years). Furthermore, during the first year after UTx, the recipient will run a greater risk of opportunistic infections (viral, fungal) that do not normally occur in a healthy person and, for that reason recipients will also be treated with viral prophylaxis. These potential risks need to be balanced against the benefits that the recipient will experience with the possibility of carrying and giving birth to their own biological child.

The donor of the uterus (in case of a live donor) is naturally also affected by the surgical and anesthesiological risks previously mentioned. Hysterectomy is the most common major surgical procedure that women of today undergo and only in Sweden, around 10 000 hysterectomies are performed annually on benign indications [107]. As the UTx donor will be of normal weight, non-smoking, healthy, drug-free and well prepared for surgery, the risks for the donor must be considered minimal. The psychological well-being of the donor may be increased as she may be able to help a friend, next of kin or an unknown female to cure infertility and achieve motherhood. Another possible benefit of the donor is the hysterectomy itself which eliminates the risk of endometrial and cervical cancer.



The partner of the uterus recipient, although suffering no direct surgical or medication-related risks, is expected to experience a potential increase in psychological discomfort initially, related to concern for their partner/wife before, during and after the transplantation and additionally during a future pregnancy and delivery. The partner may, on the other hand, experience increased psychological well-being due to the possibility of future parenthood with a biological child with the current partner/wife (uterus recipient).

The last party is the potential child conceived by IVF, born from a transplanted uterus of a mother using immunosuppressive therapy. The current state of knowledge of IS and pregnancy is previously summarized (see Pregnancy after transplantation and IS during pregnancy).

The major concerns with UTx can be summarized in a few unique issues: Are the technical aspects; that is the surgical procedure, refined enough to perform further UTx attempts in humans (bearing in mind that no animal model can predict the actual human experience)? Is the knowledge of IS and the effects on the foetus adequate and accurate? Is there a readiness and acceptance for UTx in modern society?

The ultimate decision to proceed with human UTx will be taken by researchers and clinicians in cooperation with the institution and the ethical establishment where they work and, naturally, by the woman who will be the patient, the partner as well as the donor. Because of the delicate ethical issues surrounding UTx, the treatment should only be considered for those women with AUI for whom no other alternative treatment is available. The intention of UTx should not exclusively be to allow the woman to be pregnant and deliver per se, but to allow a couple to have a child. Additionally, open access for the public to the ethical issues and discussions is of great importance.

Aim of the study

The primary aim of this thesis, Uterus transplantation: An experimental study in primates, was to develop a surgical technique for autologous UTx in nonhuman primates and to make further use of this technique to include allogeneic UTx in nonhuman primates. The additional aim was to study the surgical feasibility of UTx in humans from both live and deceased donors.

Specific aims:

Nonhuman primates

- To extend UTx research, technically previously developed in rodents and domestic animals, to a non-human primate species by developing surgical techniques for uterus retrieval and autologous transplantation in the baboon;
- To further develop a surgical technique for autologous UTx in a non-human primate species and to assess long-term graft function, including tests of fertility after natural mating;
- To extend the technique of autologous UTx developed in non-human primates to explore allogeneic UTx in non-human primates with simultaneous use of immunosuppression;

Humans

- To evaluate the lengths and diameters of uterine vessels recovered at radical hysterectomy to assess the prospects for direct vascular anas-

tomosis bilaterally to the external iliacs in UTx, and thereby the feasibility of live uterus donation;

- To examine the feasibility of uterus retrieval with the lower parts of the aorta and vena cava included in the graft at human multi-organ recovery from a deceased donor.

Material and Methods

Study protocol

The study protocol of Papers I, II and the part of Paper III concerning live donor UTx was approved by the Institutional Scientific Evaluation and Review Committee (ISERC) and the Animal Care and Use Committee of the Institute of Primate Research, Nairobi, Kenya. The study protocol concerning deceased donor UTx in Paper III was reviewed and approved by the University of Miami Institutional Animal Care and Use Committee (IACUC).

The study protocol of Paper IV was approved by the human ethics committee of Sahlgrenska Academy, University of Gothenburg. The participants of the Study group in Paper IV additionally signed a written informed consent prior to inclusion. The protocol of Paper V was approved by the Institutional Review Boards of University of Miami.

Designs

All Papers (I-V) followed an experimental and prospective design.

Settings

The non-human primate surgeries of Papers I and II took place at the Institute of Primate Research (IPR), located in Karen, Kenya. In Paper III, the surgeries were conducted both at the IPR (live donor UTx) and at the Mannheimer Foundation, Homestead (deceased donor UTx) located in the Miami metropolitan area, FL, USA. All human surgeries of Paper IV were conducted at the Gynaecology Division of Sahlgrenska University Hospital, Göteborg, Sweden. The human surgical procedures of Paper V took place at the Department of Surgery,

Division of Liver and Intestinal Transplantation, Miller School of Medicine, University of Miami, Miami, FL, USA.

Study populations

Animals

Papers I, II and III (live donor UTx)

Included in the studies were healthy adult female olive baboons (*Papio Anubis*; 9.5–16.6 kg) with regular menstrual cycles over the last (≥ 4) months (Paper I $n=15$, Paper II $n=16$, Paper III $n=36$) (Table 2). The age and parity of the animals were not known since they were not born in captivity. However inspecting their dental status gave an estimation of their age and all were found to be of reproductive age, well before ovarian senescence. Following transplantation, the uterus recipients were held in single cages and fed commercial monkey diet with additional fruits and vegetables. The uterus donors were kept for inclusion in other studies.

Paper III (deceased donor UTx)

Four healthy female hamadryas baboons (11–14 kg) with regular menses for ≥ 4 months were included (Table 2). The age of the animals was 5–6 years and all were nulliparous. Following transplantation, the uterus recipients were held in single cages and fed commercial monkey diet with additional fruits and vegetables. The uterus donors were euthanized at the end of the surgical procedures.

Humans

Paper IV

The study group included 19 women all undergoing a Piver III radical hysterectomy [108] with pelvic lymph node dissection due to uterus/cervical cancer

(Table 2). The surgical procedure was modified to also include dissection of the uterine vessels. Additionally, 76 women that during the study period underwent a similar surgical procedure (excluding the modified separate uterine vessel dissection) formed the control group.

Paper V

Seven female, deceased multi-organ donors were included (Table 2). Preoperative testing included screening for human immunodeficiency virus (HIV) and hepatitis. There were no other specific criteria for exclusion apart from previous major gynaecological surgery.

Table 2: Overview of the study populations of Papers I-V

	Total number of subjects	Species
Paper I	15	Baboon (<i>Papio Anubis</i>)
Paper II	16	Baboon (<i>Papio Anubis</i>)
Paper III	40	Baboon (<i>Papio Anubis</i> , <i>Papio Hamadryas</i>)
Paper IV	95	Human
Paper V	7	Human

Statistical analysis (Papers I-IV)

In Paper I, results are expressed as mean \pm SEM (standard error of the mean). In Papers II, III and IV the results are expressed as medians and ranges. Statistical analyses were conducted with PASW Statistics (version 18.0 for Windows; SPSS Inc, Chicago, IL, USA). In Paper II the nonparametric Mann-Whitney U test was used since normal distribution could not be assumed. In Paper IV, differ-

ences between groups were analyzed with the Mann-Whitney U test or the Chi square test. Correlation analyses were performed by Rho Spearman's correlation test. A P-value of <0.05 was considered significant.

Animal studies (Papers I-III)

Determination of blood type

In Paper III (live donor UTx) buccal cell samples were taken with cotton swabs and transferred to two glass slides per animal. The smears were air dried and fixed in ice-cold acetone. Two cell areas on each slide were marked with a hydrophobic marker pen and the slides were subsequently rinsed in TBST (Tris-Buffered NaCl and Tween 20). Unspecific binding was blocked with Background Sniper for 20 min. Mouse monoclonal anti-human antibodies towards blood group A and B were used in 1:10 dilution and anti-A (021 anti-A type 2, Clone A005, Isotype: IgG3) and anti-B (035 anti-B, Clone No.15, Isotype: IgG3) were applied in one encircled area each of the slides. The other area was left with only TBST as negative control. The slides were incubated for 1 hour at room temperature. Detection was performed with a MACH3 polymer detection kit with Vulcan Fast Red. The slides were subsequently counterstained with hematoxylin and covered with Aqua Pertex. Human buccal smears from blood-typed individuals were used as positive control cells for blood group A, B and O. In Paper III (deceased donor UTx), blood samples were taken preoperatively. The samples were processed as plasma and a reverse typing agglutination test were performed using suspensions of A and B cells. The majority of the animals included in the study that were born and bred in Karen, Kenya (live donor UTx) were B (39 %) or AB (42 %) with a minority being of type A (19 %). The tested animals from the colony, born and bred in Miami, FL, USA (deceased donor UTx) were either B (50 %) or AB (50 %). Out of all the donor/recipient combinations, 55 % were ABO identical and additionally 45 % were ABO compatible.

Anaesthesia

In Papers I-III, the anaesthetic procedure was similar and the procedure is in detail described in the Papers. The animals were fasted during the night before surgery (water not withheld). Prior to surgery, the animals were given an im injection of ketamine [100 mg (body weight, bw <10 kg) or 200 mg (bw >10 kg)] combined with 10 mg xylazine to induce anaesthesia. After administration of lidocaine spray orally, the animal was intubated. Anaesthesia was maintained using halothane (Papers I, II and III (live donor UTx)) or isoflurane (Paper III (deceased donor UTx)) to minimal alveolar concentration of 1.3 and an oxygen/nitrous oxide mixture to secure the haemoglobin saturation to above 97%. Normothermia and adequate hydration were maintained throughout the surgery using heating pads/blankets and continuous iv infusion (3 ml/kg bw/preoperative fasting hour and surgical hour) of warm (37°C) physiological saline. Before cessation of anaesthesia, the animals received meloxicam (0.3 mg/kg bw) and the corticosteroid betametason (1 mg im) as postoperative pain relief. After extubation, the animal was kept in a single cage to enable administration of medication and for further observation. Anaesthesia during sampling of biopsies and second-look surgery was identical to that of the primary surgery.

Uterus graft retrieval

Papers I, II and III (live donors)

The different surgical techniques of UTx used in this thesis are described in the Papers in detail. In brief, the vascular dissections started by separation of the infundibulopelvic ligaments (IPL) from the pelvic sidewall and mobilization of the ureters to enable bilateral dissection of the internal iliac artery. The uterine arteries were dissected bilaterally from a point of around 10 mm cranially to the crossing of the ureters and towards the pelvic walls to the point of the branching between the anterior and posterior portions of the internal iliac artery (Papers I and II (initial group)). In subgroups of Paper II (modified group) and of Paper III (live donors) the main trunk of the internal iliac artery was included in the graft bilaterally. In these latter groups, the two main posterior branches of the internal iliac artery were divided and the largest branch on

each side was included (length of ≥ 5 mm) in the graft to allow end-to-end anastomosis of one of the internal iliac artery ends to the largest posterior branch on the contra lateral side. The ovarian veins were dissected either to the crossing of the IPL and the ureter (Papers I and II (initial group)) or up to the vena cava on the right side and the kidney vein on the left side (Papers II (modified group) and III (live donors)), to, in the latter groups, acquire venous ends with reasonable thick vessel walls and aiming to simplify the back-table preparation and anastomosis surgery. The ureters were dissected free from their attachments to the cervix and the uterine vessels, all the way to their inflows into the bladder. The rectum was separated from the upper vagina. The large vaginal veins and arteries, which run on the lateral aspects of the vagina and anastomoses with the descending branches of the uterine vessels, were mobilized from the vagina and then severed at a level around 15 mm caudal to the portion of the cervix. The bladder peritoneum was detached from the cervix and the cranial portion (≈ 15 mm) of the vagina, and the vagina was subsequently divided. The IPLs and the anterior portions of the iliac arteries were then clamped and severed. The uterus graft, including the uterus with a vaginal rim, was removed from the abdominal cavity.

Paper III (deceased donors)

The purpose of the surgical procedure was to recover the vault of the vagina, the uterus, the Fallopian tubes and the ovaries *en bloc* with their arterial inflow (including the abdominal aorta and the iliac vessels) and venous outflow up to and including a large portion of the caval vein. The vascular pedicles were accessed through careful dissection. To enable mobilization of the colon the inferior mesenteric artery (IMA) was subsequently ligated and transected. A bilateral transection of the ureters was performed at the level of the crossover of the uterine arteries and the aim was to include the upper portion of the bladder in the specimen to facilitate suspension of the uterus graft at transplantation.

The external iliac vessels were tied and transected at the level of the inferior epigastric vessels bilaterally, after which the uterine vessels were mobilized. Vesical, pudendal and sacral branches of the internal iliac artery were ligated

and transected. All lumbar branches of the abdominal aorta and inferior vena cava, up to the level of the renal vessels, were ligated and transected. After the graft was mobilized further, it was transected at the level of the vagina and inspected to assure maintained blood flow and hemostasis. Flushing of the graft was done in situ (see section below). The abdominal aorta and vena cava were subsequently to flushing transected and the uterus graft was removed from the abdomen.

Table 3: Overview of the different preservation techniques used in the animal studies (Papers I-III).

	Preflushing	Preservation solution	Volume (ml) /time (min) of flushing	Arteries used for flushing
Paper I	-	Ringer-Acetate	40/-	Uterine arteries
Paper II				
initial group	-	HTK	100/10	Uterine arteries
modified group	Heparinized saline (5-10 ml)	HTK	300/30	Anterior portion of internal iliac arteries
Paper III				
LD	Heparinized saline (5-10 ml)	HTK	300/30	Anterior portion of internal iliac arteries
DD	-	Heparinized UW	Until blanched	Aorta

LD = Live donor uterus transplantation, DD = Deceased donor uterus transplantation, HTK = Histidine Tryptophan Ketoglutarate, UW = University of Wisconsin solution

Preparation of the graft at back-table and flushing

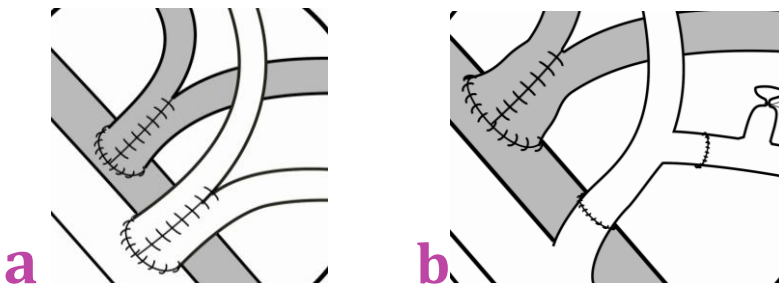
Papers I-III

In Papers I and II (initial group) Teflon catheters (inner diameter 0.64 mm; Terumo, Leuven, Belgium) were inserted through the distal ends of the anterior divisions of the internal iliac arteries and secured with haemostatic clamps or ligatures. The uterus graft was then flushed with either ≈40 ml Ringer-Acetate

through each uterine artery (Paper I), histidine tryptophan-ketoglutarate (HTK) of 4°C for 10 minutes (Paper II (initial group)) or preflushed with 5-10 ml of cold heparinised saline supplemented with lidocaine, followed by HTK flushing (80 mm Hg) for 30 min with a large volume (≈300 ml) at 4°C (Papers II (modified group) and III (live donors)). In Paper III (deceased donors), the flushing was done in situ and a large vascular catheter was inserted in the abdominal aorta and the graft was perfused with cold heparinized (20.000 IU) UW solution until the organ was totally blanched (Table3).

Following flushing, the largest ovarian vein on each side was identified and the venous ends were joined side-to-side by interrupted sutures (9-0 nylon) to create a larger vessel. In Papers I and II (initial group) a similar procedure was performed on the uterine artery using interrupted suture (8-0 nylon or prolene). In Paper II (modified group) an end-to-end anastomosis (interrupted 8-0 prolene) of one of the internal iliac artery ends to the largest posterior branch on the contra lateral side was performed (Fig 3).

Fig 3. Schematic drawing of the vessel anastomoses between **a)** the side-to-side unified ovarian veins and uterine arteries to the external iliac vessels in the animals of Paper I and II (initial group) and **b)** the side-to-side unified ovarian veins and the end-to-end unified uterine arteries to the external iliac vessels in the animals of Paper II (modified group).



Recipient surgery

Papers I, II and III (live donor UTx)

Prior to transplantation of the uterus the live donor UTx recipients of Paper III, underwent a standard hysterectomy. The uterine vessels and the superior vaginal vessels were carefully dissected and ligated close to the body of the uterus, and the vaginal vault was left open with marking sutures at its corners. In Papers I and II, this procedure was not necessary since the transplantation was autologous.

In order to prepare for vascular anastomosis, the external iliac vessels on the left side of the animal were dissected free and separated from each other over a distance of about 30 mm. Vascular clamps were placed on both ends of the isolated segments of the external iliac vein and artery followed by longitudinal incisions into the anterior vascular walls to accommodate anastomoses with the venous and arterial ends of the graft vessels. The venous anastomosis was done end-to-side (9-0 nylon). The arterial anastomosis was performed end-to-side (8-0 prolene) to the external iliac artery by two gynaecology surgeons (Papers I and II (initial group)) or end-to-end to the internal iliac artery by a transplant-surgeon with the assistance of one gynaecology surgeon (Paper II (modified group)). The vaginal cuff of the graft was then reattached/attached to the vagina by continuous suture (4-0 Vicryl) and the same kind of suture was used to attach the uterus body to the round ligaments. Adequate uterus blood flow after anastomosis was judged by pulsations of the uterine arteries and capillary refill of the serosal surface of the uterus. The abdomen was closed by continuous sutures of the fascia (1-0 polydioxanone) and the skin (3-0 Vicryl).

Paper III (deceased donor UTx)

The native uterus was dissected from its ligaments close to the uterus body and transected at the level of the vagina and removed from the operative field. The vaginal vault was left open with marking sutures at its corners. Prior to clamping the infra-renal aorta and the inferior vena cava to implant the graft, heparin (3000 IU) was given iv. The infra-renal aorta was cross-clamped and a longitudinal arteriotomy was made to fit the aortic conduit in the graft. The

graft was brought to the field and an end-to-side running anastomosis was performed on the aorta. The inferior vena cava (IVC) was also cross-clamped and a longitudinal venotomy was performed for the venous anastomosis in the same manner as described for the aorta. After completion of the two anastomoses, the vascular clamps were released and the graft was reperfused. The graft and anastomoses were inspected for optimal positioning and hemostasis. The upper part of the vagina, which was attached to the graft, was subsequently anastomosed with the vaginal vault of the recipient. The abdominal wall and skin was closed with separate running sutures.

Recordings of ischemia

In Paper I, only total warm and total cold ischemia were recorded. In Papers II and III (live donors) three ischemic periods were recorded according to established criteria by Halazun and co-workers in 2007 [109]. The criteria describe initial warm ischemia (Warm ischemia part 1) as the time between vascular clamping and flushing, cold ischemia as the time elapsing from the start of cold flushing to the start of anastomosis surgery and the second period of warm ischemia (Warm ischemia part 2) as the time of anastomosis surgery until graft perfusion.

Monitoring of cyclicity

The characteristics of the menstrual cycle in baboons are very similar to those of women. The cycle of the baboon is slightly longer, generally 33 ± 2 days compared to 28 ± 2 days in woman. The length of pregnancy is on the other hand one-third shorter, i.e. six months. The perineal skin, or sex skin, in the baboon changes its appearance in a cyclic manner that is related to the hormonal changes of the menstrual cycle. These changes are clearly visible and provide a dependable and non-invasive bioassay for determination of cyclic activity and cycle stage. The length of the menstrual cycle is determined by counting the days from the first day when perineal turgescence appears of one cycle, until the day of reappearance in the following cycle [110]. The cycle can be divided into four phases consisting of nine stages; the menstrual phase (Stage 0), the

follicular phase (Stages 2–6), the luteal phase (Stages 7 and 1) and the pregnancy phase (Stage 8). During the postmenstrual period (Stage 2), the perineum is at rest (deflated) and subsequently increasingly inflates during the following two stages. Post-ovulation/secretory period (Stage 5) is characterized by maximal perineal inflation and is followed by the luteal/secretory phase with increasing turgescence. The perineal area starts to deflate during the premenstrual phase (Stage 1). These typical changes in perineal appearance were determined by daily observations by experienced (> 10 years experience) animal technicians using the numerical grading system, as described above, and noted on a chart for each baboon (Papers I-III).

Mating

Papers I and II included trials of natural mating. Animals showing resumed menstruation after UTX were introduced in a one-to-one fashion to males with proven fertility through previous fatherhood and semen analysis. The male and female of a mating couple was placed in adjacent single cages, separated by a movable barrier. Once the female was in the late follicular phase (stage 4-5), according to the perineal skin status, the barrier was removed to allow for mating. The barrier was removed between 8.30 AM and 15.00 PM with a short separation break for feeding in the middle of the day. Every month mating was allowed for 7-10 days, depending on the appearance of the perineal skin. The males were replaced every third cycle when pregnancy did not appear.

Biopsies and histologic analysis

Papers I and II

In Paper I, biopsies were taken from the uteri and ovaries after four months (five animals) and after 17-19 months (two animals). In Paper II, sampling of biopsies was obtained from the ovary, cervix, uterus corpus and Fallopian tubes, if present, after 6-7 months and after 12-18 months. The biopsies were fixed in 4 % buffered formaldehyde, dehydrated, embedded in paraffin, sec-

tioned and stained with haematoxylin and eosin followed by examination under light microscopy.

Table 4: Suggested classification of acute uterus rejection in endocervical biopsies after uterus transplantation, developed by Mölne, Ruiz and Johansson.

<i>Grade</i>	<i>Rejection</i>	<i>Biopsy findings</i>
0	No	Normal morphology
1	Mild	Mild diffuse mixed inflammatory cell infiltrate (mainly lymphocytes). Occasional epithelial apoptotic bodies, focal distribution. Surface epithelium intact. No necrosis
2	Moderate	Moderate, diffuse mixed inflammatory cell infiltrate (mainly lymphocytes). Increased amount of epithelial apoptotic bodies. Reduced thickness surface epithelium, possible focal erosion. No necrosis
3	Severe	Significant, diffuse and aggregate, mixed inflammatory cell infiltrate (mainly lymphocytes; neutrophils and eosinophils may be present). Frequent apoptotic bodies. Epithelial erosions, focal to total. Focal necrosis.
4*	Total necrosis	Necrotic tissue only

*Assumed to represent end stage rejection

Paper III

Biopsies were taken transvaginally, from the cervix once weekly (live donor UTx, 18 recipients) or from the cervix and the endometrium twice weekly (deceased donor UTx, 2 recipients). The biopsies were obtained under general an-

aesthesia and in the deceased donor UTx recipients using a hysteroscope (Gyrus ACMI IDH-4 flextip, Olympus America Inc, Center Valley, PA, USA). The biopsies were fixed in formaldehyde, embedded in paraffin, sectioned and stained with haematoxylin and eosin. Histologic severity of acute cellular graft rejection was scored, from none to severe, according to a subjective scale based on experience of rejection characteristics of other organs and previous uterus rejection of several animal species (Table 4)

Second-look laparoscopy and laparotomy

In Paper I, laparoscopies were performed after 2-4 months in the animals that showed cyclicity. Insufflation was performed through a Verres needle (intra-abdominal pressure of 10 mmHg) and the laparoscope was introduced via an umbilical incision. Laparotomies were done in Paper I after four months (all animals) and after 17-19 months (menstruating animals).

Euthanization

The animals of Papers I-III were euthanized during anaesthesia by iv phenobarbiturate.

Human studies (Papers IV and V)

Imaging techniques

In Paper IV, four of the study patients underwent a preoperative MRI by a 1.5-Tesla scanner (Intera; Philips Medical Systems, Best, The Netherlands) with a four-channel phased-array coil. The examination was conducted according to a routine protocol for cervical cancer, including pelvic axial, coronar and sagittal multi-slice T2-weighted acquisitions, axial T1-weighted acquisitions, and axial and sagittal T1-weighted acquisitions with fat saturation after iv administration of 8-10 ml gadolinium contrast medium (Gadovist®, 1 mmol/ml; Bayer, Leverkusen, Germany). In two of the patients, MRA was performed (flip angle 30°, repetition time/echo time 5.1/1.8 msec, matrix 352 x 352) and 3D maximum-intensity-projection images were generated from the source images. In

addition, several acquisitions were tested in order to optimally visualize the uterine veins, for instance time of flight and balanced fast field echo, but the acquisitions that proved to be most useful were the sagittal T2-weighted turbo spin-echo (flip angle 90°, repetition time/echo time 4493-4595/100-120 msec, field of view 230 mm, slice thickness 4 mm, gap 1 mm, matrix 352 x 352) and sagittal T1 high-resolution isotropic volume excitation (flip angle 7-10°, repetition time/echo time 3.6-5.7/1.8-2.6 msec, overlapping slice thickness 1.5-4 mm, matrix 400 x 400) with gadolinium contrast enhancement. The evaluation was performed by a radiologist (H. Leonhardt), with 16 years experience of vascular imaging and 10 years experience of MRI for gynaecologic diseases. This observer was blinded to the operative results. The uterine arteries were measured on each side, from the exit at the anterior branch of the internal iliac artery to the cervix. The dominant vein trunk was measured bilaterally, from the ostium in the internal iliac vein to the first large branching towards the uterus. The internal diameters of the arteries and veins were measured in the mid section.

In Paper V, the retrieved uterus grafts were placed on a back-table and flushed with preservation fluid. Subsequently an iodine based contrast medium was injected in the procured arterial pedicle of one side and the route of the uterine artery and the collateral blood flow was studied with angiography.

Surgical procedures

All women in Paper IV, regardless whether belonging to the Study or the Control group underwent a modified (uterosacral ligaments not excised at the sacral attachments) abdominal Piver type III hysterectomy [108] through a subumbilical midline incision and with wide radical excision of the parametrial and paravaginal tissues. Briefly, the pararectal and paravesical spaces were defined by blunt dissection after entering the broad ligaments. Bilateral lymphnode dissections, with removal of pelvic lymph nodes along the external iliac vessels, the common iliac vessels and in the obturator fossa, were performed prior to hysterectomy. The ureters were dissected free bilaterally to the bladder. The uterine arteries were isolated from surrounding tissue from the branching of the umbilical artery and up to approximately 10 mm cranially to the ureter. This procedure in general identified 2-3 larger uterine veins on each side, whereof the largest of the veins on each side were carefully dissected free

(also from the uterine artery), starting cranially of the ureter and moving towards the pelvic sidewalls. Typically, this dissection identified four large venous branches of the major uterine vein and these branches led to the other uterine veins or the vaginal vein. The venous branches were ligated with 2-0 sutures. The uterine veins were gently dissected as far to their orifice in a common trunk before the internal iliacs. The uterosacral ligaments were resected midway between the uterus and the sacral attachments and only the upper part (20-30 mm) of the vagina was removed, allowing a preserved blood and nerve supply to the bladder without compromising the radicality of resection of paracervical tissue. The parametrium was subsequently divided and the uterus was removed from the abdominal cavity. The vaginal vault was sutured. The distance between the external iliac arteries, at a point 1/3 from the bifurcation of the internal/external iliac artery to the branching of the circumflex artery just proximally to the inguinal ligament, was measured as an indication of the width between potential anastomosis sites in UTx. The surgery was then finished by conventional sutures of the fascia and skin.

In Paper V, the women were multi-organ donors and were kept on life supporting ventilation throughout the organ retrieval procedure. A midline incision was performed from the pubic bone and to include a complete sternotomy. The dissection of the uterus was done at the same time as the dissection of thoracic and abdominal organs. The round ligaments were divided and followed by opening of the paravesical and pararectal spaces and dissection on the lateral aspects of the uterus. The bladder peritoneum and the rectum were gently separated from the frontal and dorsal part of the cervix, respectively. The sacrouterine ligaments were then exposed and could be transected. The IPLs were severed and the ovaries and oviducts were included in the specimen. Prior to clamping the blood flow (aorta), heparin was given iv through a central venous catheter. The distal aorta was clamped for abdominal organ recovery and at the same time the external iliac arteries were cannulated for perfusion of the uterus. The low aortic cross-clamp was placed just caudal to the inferior mesenteric artery in case 1 and at the level of the aortic bifurcation in the other cases. The uterus was then flushed with 1 L of UW solution. Further dissection was subsequently performed to ligate and sever major branches

from the internal iliacs and also to cut and ligate the external iliacs just distal to the cannula.

Measurements of procured vessels

In Paper IV, the uterus was taken from the abdominal cavity and then placed on the back-table. Using a 1 mm calibrated measuring tape, the lengths of the uterine arteries and veins were measured from their attachments to the uterus body to the ends with the diameters of these ends were also being measured. Care was taken not to apply any stretching of the vessels.

Assessment of postoperative recovery

In Paper IV, the postoperative recovery of the women were noted both considering lengths of hospital stay and rate of surgery-related complications at discharge from hospital and at six months control.

Results and comments

In the Results and comments section, a selection of the results of the five Papers are commented and to some extent discussed. Major issues are commented and discussed in the Discussion part of the thesis.

Paper I

Initial trials of autologous uterus transplantation in a baboon model

Prior to this paper, experimental animal research in the field of UTx had primarily been done using rodent models [63, 111], to some extent larger animal models, such as sheep [70, 71] and pig [48, 50] and sporadically nonhuman primates [61, 112]. The large dissimilarities between rodents/large domestic animals and humans, in body size, anatomy, immunology, length of pregnancy and menstruation cycle, made it a prerequisite to extend the research to a nonhuman primate model to establish a sufficient knowledge base, and not to proceed to an immature human attempt of UTx. Nonhuman primate models have previously been used in scientific studies in the fields of transplantation and in reproductive physiology but ethical and financial issues often make the use of these types of animals scarce. Baboons exhibit similar physiological and anatomical characteristics as humans and their reproductive system is well known. Baboons and humans, as well as other higher primates, have a simplex uterus in which no separation between the horns is present and thus a single cavity exist. The arterial inflow of the uterus is, both in humans and baboons, mainly through the uterine arteries and the venous outflow through the uterine and ovarian veins. The ageing baboons also exhibit a natural menopause, not characteristic for other animals [113].

Previous research in nonhuman UTx has involved both avascular [61] and vascular [74, 112] techniques. The published study on avascular UTx was done already in 1971 and included both autologous and allogeneic trials in rhesus macaques [61]. A subtotal hysterectomy, without vascular retrieval, was performed and the graft was wrapped in the omentum to achieve vascularisation. Neoangiogenesis was reported but full graft rejection was shown, after allogeneic, but not autologous transplantations, on day 14. The only previously published report of vascular allogeneic UTx in rhesus macaques, used a first set of animals to study the pelvic anatomy and to perform sham retrieval and transplantation of the uterus [112]. Subsequently five allogeneic transplantations of the uterus including vascular pedicles of aorta and vena cava were performed. The anastomosis site of the recipient was chosen depending on the individual animals' anatomy following a preferred hierarchy of internal iliac vessels, external iliac vessels, common iliac vessels and aorta together with vena cava. In another report with the published human UTx case, there is also some experiences from vascular autologous UTx in the nonhuman primate species of the baboon and these are reported as preparatory experiments prior to the human trial [74]. The baboon uterus was herein retrieved with the uterine vessels included. The grafts were reimplanted end-to-end to the uterine vessels in the initial group or end-to-side to the internal iliac vessels in the following group. In the first set of animals, there was vascular thrombosis reported in 75 % of the anastomosis sites and this rendered the investigators to change the surgical technique. Thus, in the second set of animals, involving anastomosis on the external iliacs, 90 % of the anastomosis sites showed patency. However, results in terms of graft function or animal survival was not provided. The aim of Paper I were to develop a surgical technique for uterus retrieval and autologous transplantation in the baboon.

Results

The initial dissection study, that was included in Paper I, revealed that the ovarian veins of the baboon have a notably larger diameter than the uterine veins. Based on this finding, it was decided to include the uterine arteries/anterior portions of internal iliac arteries and the ovarian veins in the graft for vascular

anastomosis to the external iliac vessels. The duration of surgery (retrieval, organ preparation and transplantation) was around six hours whereof the retrieval was almost 3 hours. Flushing of the transplant resulted in outflow of blood through the ovarian veins and blanching of the specimen. The duration of anastomosis surgery, when the graft gradually was warmed, was > 1 hour and the total ischemic period was almost 3 hours. The animal survival rate presented in Paper I was 90 %. One animal out of the ten included in the experimental series, died within the first 12 postoperative hours, possibly due to cardiomyopathy. A hormonal cyclic pattern was resumed in 50 % of the animals, in two cases after 1-2 months and in three cases after 3-4 months. Menstruation with hormonal cyclicity was resumed in 20 % of the animals. Visual and histological confirmation of the graft function was done in the animals and showed two normal uterus grafts with normal ovaries. These two animals were mated but no pregnancy occurred.

Comments

Comparison of the results of Paper I to previously published reports of vascular UTx can to some extent be done to the rhesus macaque model described by Del Priore [112]. However, the vascular anastomosis in Paper I differs from the previously performed report and in the present study the graft included vessel pedicles of the ovarian veins and the uterine arteries extended to include also the anterior branches of the internal iliacs. The pedicles were unilaterally anastomosed end-to-side to the external iliac vessels, after unification of the two arterial ends side-to-side and with a similar procedure on the venous ends of the transplant. The result of graft function or animal survival cannot be compared to the previous studies since data of that kind was difficult to extract from these reports. However, the animal survival proved to be higher when compared to other autologous UTx studies in large animal models, such as the sheep (50 %) [68] and the cynomolgus macaque (50 %) [75]. The graft survival and function was rather low in Paper I (20 %) and the exact cause of this is impossible to determine. However, it may be speculated that relative stenosis of the anastomosis sites may have been a likely cause. Interestingly, in Paper I, graft outcome seemed related to the hormonal cyclicity if it was resumed im-

mediately (within two months) or after a lag phase of two to three months. The two cases where menstruation reappeared were also the ones where the hormonal cyclic pattern was resumed quickly. This shows the greater capacity of the ovarian tissue than of the uterus to tolerate ischemia. The effect of warm ischemia to ovaries has been studied in sheep following avascular transplantation of strips of ovarian cortex and a great loss of primordial follicles were reported [114, 115]. The results of a lag phase between transplantation and cyclicity in three animals suggests that the follicles which were well into folliculogenesis died after transplantation but that the pool of small follicles would survive. It would then take some months before the small and surviving follicles had matured into antral follicles with high steroid output.

Paper II

Further development of the autologous uterus transplantation in a baboon model with focus on the surgical procedure and long-term outcome

Given the results in the first report on autologous UTx in a nonhuman primate (Paper I), where only 20 % of the animals at termination of the study had a preserved and menstruating uterus, it was concluded that a refinement of the surgical technique was needed. Poor results were also shown in similar studies of autologous UTx in the cynomolgus macaque, with animal survival rates at 3 months reported to be 50 % [75] or 25 % [76], and with a functioning graft in 25 % of the latter study [76]. A uterus graft with poor function, even if not rejected, will most likely not be able to implant an embryo or withstand a pregnancy. Since the initial study (Paper I) was by an autologous design there is reason to believe that the cause of the poor graft function lies within the surgical technique/procedure. Nevertheless, there would be several critical surgery-related steps where the procedure could be changed/improved, such as inclusion of larger graft vessels to be retrieved for anastomosis and an alteration of the anastomosis site. Due to the limited space in the pelvis it may be easier to perform unilateral anastomosis rather than bilateral and to perform these to external or common iliac vessel instead of internal iliac vessels. A shorter ischemia time and better preservation to minimize the ischemia-reperfusion damage will likely generate a better graft function. The aim of the present

study was to further develop the surgical procedure in an autologous UTx model in the baboon and to enable evaluation of long-term function.

Results

The short-time survival of the animals was initially 66 % and all of the surviving animals resumed a hormonal cyclic pattern (Table 5). However, no animal resumed menstruation. Due to unsatisfactory results in the initial group of six animals, further alterations of the surgical technique were decided upon. Vessel dissection was altered such as to include the main trunk of the internal iliac artery to allow an end-to-end anastomosis of one of the two arterial ends prior to transplantation (Fig 3b). An extended dissection of the venous pedicle, up to vena cava/kidney vein level, was performed and the graft was flushed with a larger volume of preservation fluid, following preflushing with heparinized saline. Moreover, the unilateral anastomosis was changed from end-to-side to end-to-end. These modifications were suggested by the transplant surgeon, who now actively participated in the experiments. Subsequently, the short-time survival in the modified group was 100 % and 80 % of the animals resumed a hormonal cyclic pattern. Importantly, 60 % of the animals presented with resumed regular menstruation. No pregnancy occurred after natural mating of the menstruating animals. Adhesions and tubal blockage were seen in the post-mortem analysis.

Comments

The long-term function of the uterus graft improved considerably after modifications of the surgical technique in the animals included in the second group of animals, with menstruating animals 3-fold higher than that of Paper 1, with the original technique (Table 5). In spite of the more extensive retrieval surgery in the modified group the time of organ recovery was decreased compared the initial group (156 min compared to 214 min). This decrease in time may be related to that an experienced transplant surgeon joined the team but also to that the long durations of surgery during the initial learning phase were abolished. Both the arterial and venous anastomoses were altered by the modifica-

tions after the initial group of the study. The baboon ovarian vein has thin walls and the difficult anastomosis surgery may lead to partial constriction of the anastomosis site and subsequent poor graft perfusion and thrombosis. The use of larger veins in the modified group simplified the anastomosis surgery and may have had a protective effect regarding thrombi formation.

Attempts of mating were performed in the menstruating animals. However, even if naturally mated for more than a total of 60 cycles, there was no evidence of pregnancy. A likely explanation for lack of conception was the frequent and severe adhesions that were seen at termination of the study (Fig 4). Peritoneal adhesions are a commonly known outcome of peritoneal irritation such as infection or abdominal surgery.

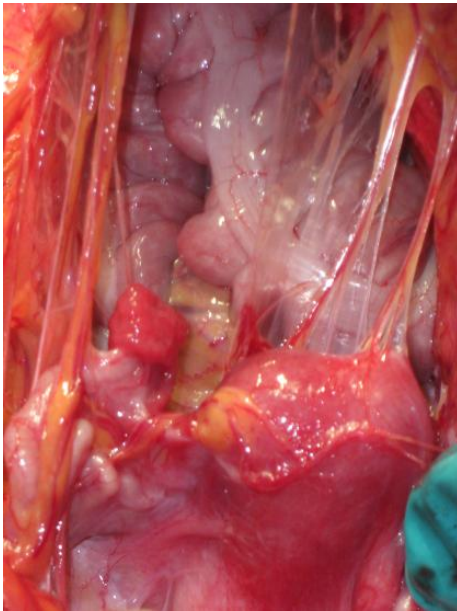


Fig 4. Severe adhesions seen during second-look surgery in an animal of the modified group of Paper II.

No signs of patency of the Fallopian tubes and thus passage of neither sperm nor oocyte would be possible, due to the intra-abdominal adhesions or possibly intra-Fallopian adhesions (that we were unable to detect on histological section). One way of bypassing the tubal occlusion may of course be the use of IVF, commonly used in humans for tubal factor infertility. Although IVF has been used since many years in the rhesus macaque [116, 117], attempts to develop a similar method for IVF in baboons have proved to be more difficult. Nyachieo and co-workers showed an ICSI fertilization rate of 23-54 % in retrieved baboon oocytes but no pregnancy [118]. Recently a single case of live offspring, following IVF in baboons were reported [119]. One at-

tempt of natural mating after autologous UTx in a nonhuman primate (cynomolgus macaque) has been successful (Kisu, personal communication). No live birth has as of today been reported following UTx in a nonhuman primate.

Table 5: Short term survival, resumed hormonal activity and menstruation in recipients of a uterus transplant from a live donor (Paper I, II and III) or a deceased donor (Paper III). Results are shown in %.

	Short-term survival	Hormonal cyclicity	Menstruation
Paper I	90	50	20
Paper II			
Initial group	66	66	0
Modified group	100	80	60
Paper III			
live donor			
Untreated	100	50	0
CNI -monotherapy	100	0	0
Triple therapy	100	50	0
deceased donor	100	100	0

Untreated = No immunosuppression, CNI-monotherapy = slow release oral or intramuscular tacrolimus, Triple therapy = Induction with antithymocyte globulin and corticosteroids. Maintenance with tacrolimus, corticosteroids and mycophenolate mofetil.

Paper III

Initial trials of allogeneic uterus transplantation in a baboon model

Although the studies of autologous transplantations in nonhuman primates (Paper I and II) provided the opportunity of developing surgical techniques and to evaluate graft function after reimplantation without interference of immunologic rejection, the aim in UTx is naturally a successful allogeneic transplantation. Intending to prevent rejection following organ transplantation, IS is a necessity although the balance between optimal dose and over-dose, resulting

in side-effects such as infections or malignancies, may be difficult in a nonhuman primate setting. In both solid organ and composite tissue transplantation IS usually consists of induction therapy, to avoid acute rejection, and life-long maintenance therapy, to avoid episodes of acute and chronic rejection. In non-human primate models, the administration of drugs in general and of IS in particular, is difficult and the preference of route is often dependant on the duration and frequency of the drug-administration [120]. Oral administration is limited by taste and unwillingness of intake. Gavage, with or without sedation, may if used long-term induce esophagitis. Intravenous administration requires the animals to be repeatedly sedated and im administration, although handy, may cause muscle granuloma if repeated frequently [120]. In 1988, Todo and co-workers evaluated the effect of, at the time of the study new IS, tacrolimus, following renal transplantation in baboons [121]. Graft rejection was seen in untreated animals and animals treated with im doses of < 3 mg/kg/day. In animals treated with 6 mg/kg/day, renal failure was detected but the findings of graft rejection were mild. Since then, several publications exist on tacrolimus based IS in primates, whereof some advocate monotherapy as sufficient [122], and others that a full-triple IS regimen [123] is needed to prevent organ rejection. The aim of this paper was subsequently to extend the UTx baboon model to also explore allogeneic UTx with concurrent use of IS.

Results

Allogeneic UTx was performed following recipient hysterectomy either using a graft from a live or a deceased donor. In the live donation UTx, anastomoses were done with fused ends of internal iliac arteries and fused ends of the ovarian veins unilaterally to the internal iliac vessels of the recipient. In the deceased donation, UTx anastomoses were performed with aorta and vena cava of the graft to the recipient's aorta and vena cava. The transplanted animals received either oral slow release CNI (tacrolimus) as monotherapy or induction with ATG followed by triple therapy (tacrolimus, mycophenolate, corticosteroids) and were compared to transplanted untreated controls. The short-term survival rate (i.e. the surgical survival) was both in the recipients and the live donors' total (100 %). The live donor surgery lasted for around three hours

while the recipient surgery had a duration of 2.5-3.5 hours (both live and deceased donor transplantation). The ischemic time that the grafts were exposed to, was longer in the live donor group (3 hours) than in the deceased donor group (2.5 hours). Following transplantation all recipients showed episodes of rejection which could, when treated properly, be resolved. Although hormonal cyclicity reappeared in some animals (40 %) as a sign of well-being, none showed resumed menstruation during the study period.

Comments

Historically, the uterus has been suggested to be immune-privileged with reference to the capacity it possesses to harbour a semi-allogeneic pregnancy. On the contrary, several studies have shown that the transplanted uterus triggers a similar rejection that other transplanted solid organs do and that this process can only be repressed by the use of immunosuppressive medication [50, 74]. This was also shown in this paper, where we were able to study the rejection patterns of the primate uterus following allogeneic transplantation. An early diagnosis of rejection and subsequently an early start of rejection therapy is a major challenge in the field of transplantation. Traditionally blood samples, imaging techniques like ultra sound and magnetic resonance imaging have played major parts in monitoring rejection as complimentary methods to the use of invasive methods such as biopsies. Lately also the possibilities of “transplantomics” (genomics, transcriptomics, proteomics and metabolomics) have been investigated to potentially play a future role in detection of transplant rejection [124]. For each type of transplanted organ there are specific ways of monitoring rejection. The Kidney Disease Improving Global Outcomes (KDIGO) recommends that a protocol for monitoring kidney transplant function should include measuring urine volume, urine protein excretion, serum creatinine, glomerulofiltration rate and ultrasound examination [125]. In liver transplantation a protocol for monitoring rejection usually includes serum aminotransferases and alkaline phosphatase, Doppler-ultrasound, endoscopic retrograde cholangiopancreatography, magnetic resonance cholangiopancreatography and percutaneous transhepatic cholangiogram [126]. However, the golden standard for definite determination of rejection in solid organ transplantation remains

histological analysis of tissue material obtained by biopsy [125]. There is often an organ-specific grading systems like the internationally accepted standard for grading acute liver and kidney rejection, such as the Banff schema [127] used to evaluate the severity of the rejection .

There is no organ-specific type of blood sample that may be helpful to reveal early dysfunction of a uterus graft. In the only published human UTx, rejection of the uterus was monitored by Doppler-ultrasound to study pulsatility and resistance in blood flow, and magnetic resonance imaging (MRI) [74]. Cervical biopsies were in this study avoided in fear of disruption of the anastomosis. In Paper III, the rejection was primarily monitored through biopsies of the cervix and in some cases the endometrium. The biopsies were generally consistent to presence or lack of hormonal activity and menstruation presented by the individual animal. Additionally the biopsies correlated well to the efforts of treating episodes of rejection with an increase of IS. In future human UTx, Doppler-ultrasound will probably have a prominent position in detecting rejection as well as visual inspection of the cervix and endometrium by hysteroscopic examination. Biopsies of the myometrium, cervix or even the transplanted vaginal rim will be useful. In the Turkish human UTx case, a skin-graft was transplanted to potentially provide information of uterus rejection (Özkan, personal communication). This method has previously been tested in intestinal transplantation, where a linear correlation was seen between the histological grading of the skin graft and the intestinal graft in pigs [128].

Paper IV

Uterus recovery from live donors

In a potential human UTx setting, the uterus graft may be donated from either a live or a deceased donor. Live donation of organs is as of today common practise with many reported benefits for the recipient that receives an organ from a live donor, such as a planned surgical procedure at an optimal timing for donor and recipient, short ischemic duration and possibility to preoperative

extensive evaluation of the graft. The difficult issue of an increasing demand and the current deficit of available organs from deceased donors naturally raise interest in live donation.

The proportion of live vs. deceased donation differs largely between countries. In Sweden 2011, 42 % of the transplanted kidneys were from live donors whereas the corresponding percentage of liver transplantation was 3 % [129]. In the US, 36 % of the kidney transplantations in 2009 were with grafts from a live donor [130]. Although the first human UTx was performed with an organ from a live donor [74] no other publication has aimed to estimate the surgical achievability of live donation of the uterus for transplantation purpose. This paper aimed to evaluate the feasibility of human live uterus donation by to some extent simulating a retrieval operation and estimate the uterine vessels recovered during a radical hysterectomy. It should be pointed out that a standard total hysterectomy with vascular dissections should be the situation most similar to live uterus donation. However, for ethical reasons it is not justified to do extensive vascular dissections in such cases. In a radical hysterectomy procedure for cervical cancer the parametrium are included in the uterus specimen and the uterine artery is dissected as part of the procedure. Thus, venous dissection with isolation of these vessels was the only added in vivo procedure in this study that also included some back-table preparation

Results

This paper demonstrates that long vascular pedicles of the uterine arteries and veins, suitable for UTx, can be obtained without compromising post-operative recovery in a live uterus donor situation. The dissection of the vessels added around half an hour to a radical hysterectomy procedure. No differences were detected regarding peri- or postoperative morbidity. The free ends of the procured uterine arteries were around 65-70 mm in length and the corresponding free ends of the uterine veins had lengths of 50-55 mm. Preoperative evaluation of the vascular anatomy provided useful information of the arteries. The inter-external-iliac-artery distance (median) was 90 mm.

Comments

In a human UTx situation, with bilateral anastomoses of veins and arteries, certain lengths of the vascular pedicles are compulsory. The recovered vascular pedicles in the paper should, considering the distance of the potential anastomosis site of external iliacs (90 mm), be enough to enable UTx without using elongating grafts. The lengths of the veins were somewhat shorter than that of the arteries, but this fact may be of less clinical importance since the external iliac veins are situated somewhat deeper and more medial in the pelvis than the external iliac arteries. In the published human case, the uterine vessels of the donor was transected 25 mm from the uterus and grafts from the great saphenous vein of 60-80 mm were used to extend the vascular pedicles [74]. The use of multiple, as in the study by Fageeh - eight, grafts not only adds surgical and ischemic time but may also increase the risk of thrombi formation.

One of the most prominent issues in live donor transplantation is donor safety. An extensive presurgical evaluation of the potential donor will minimize not only the risk for the donor but also to maximize the chance of achieving an optimal graft function [131]. In a Norwegian study from 2003 including nearly 2000 kidney donors, the mortality was reported to be none and the risk of major peri-operative complications 2.1 % [132]. In more than 10,000 American live kidney donors, the peri-operative mortality was 0.02 % [133]. The same study showed that the risk of complications in the live kidney donors was less than or equal to one percent. Regarding the safety of the patients of Paper IV, where the surgical procedure, with the addition of vessel dissection, had similarities to uterus retrieval from a live donor, the postoperative recovery of the study population was not comprised. The described complications were similar in the Study group and the Control group. It is noteworthy that the women in this paper were not healthy donors, but rather women with a cancer diagnosis, and therefore the rate of complications (around 20 %) is expected to be lower when surgery is performed in preoperatively healthy individuals.

Paper V

Uterus recovery from deceased donors

Whilst the advantages in live donation of organs possibly will render a graft of superior quality there is obvious advantages also with transplantation with organs from a deceased donor. The total absence of a third party risk is maybe the most important issue but deceased donation also provides the opportunity to include large-size vessels in the transplant which will make the anastomosis procedure easier and faster. The aim of the present paper was to examine the feasibility of uterus retrieval, including the lower parts of the aorta and vena cava in the graft at multi-organ recovery from deceased donor.

Results

Vascular pedicles including both venous outflow and arterial inflow up to the levels of lower aorta and vena cava were obtained in one out of seven retrievals and in the other six cases the vascular pedicles included around 20 mm of the bilateral common iliacs. This paper demonstrates the achievability of uterus retrieval from multi-organ donors with vascular pedicles including the aorta and vena cava, indicating possibility of aorta/aorta and cava/cava anastomosis in human UTx.

Comments

One previous report, aiming to determine the possibility of uterus retrieval for UTx, was published by Del Priore and co-workers in 2007 [77]. During a period of six months, there were almost 150 multi-organ procurements in females that were identified as potential donors of a uterus. Donor inclusion criteria included age (16-45 years), recent normal Pap test (screening for human papilloma virus, HPV), regular menses, no previously known infertility and a sonogram or MRI. Exclusion could be on basis of infectious disease (HIV, hepatitis), myoma larger than 10 mm, gynaecologic cancer, endometriosis or malforma-

tions of the uterus. The intention was to secure the complete internal iliac vessels, up to the bifurcation of the common iliacs for possible anastomosis to the internal iliac vessels of the recipient. In the seven organ retrievals made, the intended vessel pedicles were procured in two women and in the remaining five cases the anterior portion of the iliacs were included in the graft. In two of the cases a unilateral loss of the uterine vessels led to use of the ovarian vein. The results of Paper V are in accordance with the report by Del Priore and co-workers [77] but with greater success of retrieving bilateral vessels that also included the common iliacs. One reason for this greater success rate may be that all retrievals in our study were done by well-experienced transplant surgeons. The ideal situation would be one with uterus retrieval performed by a well-trained team of a pelvic surgeon (gynaecologist) together with a transplant surgeon. The pelvic surgeon would be the expert on dissection deep down in the pelvis and the transplant surgeon would have greater knowledge of retrieval surgery of the internal iliacs and up to and including the vena cava/aorta.

The ovarian veins can be considered as a vascular out-flow option in both live donation (given that the donor is postmenopausal) and deceased donation, provided that other abdominal organs are not recovered simultaneously. Possible anastomosis sites may in a human situation be in case of a deceased donor; aorta/cava, common iliacs, external iliacs or the internal iliacs. Consideration in this matter will have to be taken to the individual pelvic and vessel anatomy of the recipient and the procured vascular pedicles and a consensus in this matter might be difficult to introduce.

The availability of organs and donors is an essential issue. In the paper from Del Priore and co-workers only nine out of 150 potential donor families consented to donation of the uterus specifically [77]. The low consent rate can naturally be explained by the fact that it was a research study rather than actual donation and that donation of a uterus is new and largely unheard of. However it may also be related to that the uterus carries a symbolic feature of woman/mother-hood and people may regard uterus donation more questionable than donation of other types of organs. It may well be that unwillingness

to include a uterus on the list of consented organs from deceased young females may in the future lead to shortage of uterus grafts. In both Papers IV and V, postmenopausal donors were included in contradistinction to the study by Del Priore where the upper age limit for inclusion was 45 years [77]. However, it might be suboptimal to include postmenopausal deceased uterus donors where the possibility to pretransplatory evaluation of the hormonal response of the endometrium is limited. In a report published in 2005, the characteristics of all deceased organ donors in US during 2002 were reported [134]. Among the more than 6000 donors 38 % were female and in the age group of 17-40 years old, 36 % were females. Given that the gender ratio is stationary in the different age groups this would render an estimation of that only 14 % of the deceased donors are female in the age 17-40 years and 12 % in the age 41-59 years. If there is an increased demand of human UTx, a lack of organ supply will likely, as in most other organ transplantation, be present.

Discussion

In the Discussion section, a few key issues of this thesis, of UTx in general and future concerns that would profit from a broader discussion, have been put forward. Other results are discussed, to some extent, in the Results and Comments part of the thesis.

Nonhuman primate models in development of solid organ transplantation and uterus transplantation

Extension of the research on UTx to include a nonhuman primate model was one of the issues stated in the FIGO ethical guidelines, as desirable before human implementation [10]. We considered the use of a nonhuman primate model vital to the studies; in particular as UTx would be a non-vital type of organ transplantation and given that there is no other animal model that provides such a resemblance to the human regarding reproductive anatomy and physiology [135] (Table 5). All of the animal studies, included in the thesis, were performed in a baboon model. From an evolutionary point of view, the baboon is closer to the human than the macaque, which is the most widely used animal for research in reproductive biology [136]. The baboon also has the advantage of a relatively large body size.

Regarding research prior to, and during the first years after, the introduction of transplantation procedures in other solid organs, different nonhuman primate models have been commonly used, as will be discussed below. Following the first human renal transplantations between identical twins [37], and the initially reported poor results of the human allogeneic attempts, interest was directed towards developing a test model with great resemblance to the human.

Table 5. A comparison of uterus anatomy and reproductive characteristics in human and different animal species, commonly used in medical research

Species	Body size of a fertile female	Female sexual maturity	Shape of uterus	Oestrous/ Menstruation cycle (days)	Gestation length (days)
Human	65-70 kg	12-15 years	simplex	28	273
Rat	100 g	5 weeks	bicornuate	4 or 5	22
Mouse	30 g	4 weeks	bicornuate	4 or 5	20
Sheep	45-100 kg	6-8 months	bicornuate	17	147
Dog	varies by breed	8-18 months	bicornuate	150	63
Pig	130-200 kg	5 months	bicornuate	21	114
Baboon	10–15 kg	4-4.5 years	simplex	33	183

The rhesus macaque was considered suitable and extensive evaluations of surgical techniques [137], renal function after transplantation [138] and haematology and immunology [139] were performed. The transplantation procedure in the rhesus macaque followed essentially that of the human renal transplantations by Murray [140] and was initialized with the dissection of the left common iliac vessels. The left kidney was mobilized and the renal vessels transected. Subsequently, the kidney was transplanted to the pelvis with end-to-end anastomosis of the renal vessels to the common iliacs and reimplantation of the ureter into the bladder. The mean ischemic time was 26 minutes and the 24-hour survival was 73 %. Vascular thrombosis was shown in 11 of 16 surviving rhesus macaques, and this complication was attributed to technical difficulties such as short (<15 mm) renal vessel and size discrepancy of the vessels used for the anastomosis.

In liver transplantation, most of the initial studies were performed in allogeneic models of the dog [141] and the pig [142, 143] but in 1970, Calne and co-workers published a study of allogeneic liver transplantation in a rhesus macaque model [144]. The six liver recipients were untreated and five died within 15 days of surgery. In 1971, Myburgh and co-workers presented a series of almost a hundred allogeneically liver-transplanted baboons with and without IS [145-147]. The animals were divided into subgroups depending on immunosuppressive treatment (untreated or with combinations of anti-baboon lymphocyte globulin, azathioprine, prednisolone, donor liver antigen and donor bone marrow). The results showed that 75 % of the untreated controls died within the first two weeks and the grafts showed typical features of cellular rejection. The animals that survived more than two weeks eventually died from chronic rejection, dominated by progressing intrahepatic cholestasis.

In the history of heart transplantation, many studies in the 1960s showed feasibility in dog models [148-150], but the research teams struggled with the immunological response of the dog to the immunosuppressive treatments [151]. For this reason, the research field expanded to include studies of nonhuman primates, and in 1969, Willman and co-workers performed 18 autologous and two allogeneic transplantations of the heart in baboons [151]. The transplantation procedure included division of the aorta and pulmonary arteries close to the valves, division of the left atrium and the caval vein. The heart was arrested by cold Ringer's solution and the coronary arteries were not perfused for 40-50 min. The animal was kept on extracorporeal circulation during the arrest and the heart and vessels were implanted in their original positions. The allogeneic transplantations used IS with azathioprine and, additionally, methyl prednisolone was used at signs of diminished heart function (when depression of R wave amplitude was detected). The team found the baboon well suited as a model for heart transplantation, and was able to show satisfactory autologous graft function; however, they also describe difficulties of adequately dosing the immunosuppressive treatment. Difficulties in drug-administration of IS are also described in Paper III, where we struggled to obtain adequate blood levels of

IS. There is very little knowledge of, uptake and metabolism of immunosuppressive medication in nonhuman primates, and dosage is largely unknown. Thus, we experienced difficulties, not only with administration and dosing, but also with interpretation of the blood levels. These difficulties have been described by others. Regarding hand transplantation in nonhuman primates, although technically feasible, several reports describe problems to foresee timing of transplant rejection and survival despite similar immunosuppressive therapies given to the animals [123, 152, 153].

In 1972, combined heart and lung autotransplantations were carried out in 25 baboons [154, 155]. The rate of resumed spontaneous rhythmic respiration was 92 % and 20 % of the animals survived for > six months. In 1980, Reitz and co-workers used a cynomolgus macaque model to confirm the ability of primates to withstand cardiopulmonary auto- and allo-transplantation [156]. The retrieval included transections of the aorta, the inferior and superior vena cava and the trachea cranial to the carina. The graft was either preserved in ice-cold physiological saline solution, experiencing circulatory arrest, or aided by cardiopulmonary bypass, and reimplanted with anastomoses to the vena cava, aorta and trachea. The allogeneic transplantations were performed with concurrent IS with cyclosporine and azathioprine. The success rate was higher in the animals that were transplanted with the aid of the cardiopulmonary bypass, where 100 % of the auto-transplanted and 42 % of the allo-transplanted animals were long-term survivors.

The development of UTx in animal models warrants a comparison to the development of classic organ transplantations. With no consideration of the early experiments of UTx experiments before the 1980s, the majority of initial studies in UTx have been performed in mouse [111, 157] and rat [63] [63] models. Nonetheless, large animals remain the best model to ensure that human studies are feasible, and later studies have extended the research field to include large animals, such as pig [48, 50] and sheep [69, 72]. When the era of solid organ transplantation began, the dog was common in medical research; thus

many early solid organ transplantation studies were performed in a dog model [148, 158]. The dog, frequently used as a pet, is not often chosen today as an animal model in medical research. Although no animal model can foresee all different aspects of human trials, they stand a better chance when the models have high resemblance to the human situation. For this reason, nonhuman primates have been used lately in UTx. The trials have initially been autologous (Papers I and II) and subsequently, to mimic a human UTx situation, also allogeneic (Paper III). The nonhuman primate models are considered to be the final step prior to clinical introduction aiming to make the initial human UTx attempts as safe as possible.

Animal survival following uterus transplantation

Papers I and II were both on autologous UTx in baboons. Prior to Paper I, only a few experiences with UTx in non-human primates had been reported [61, 74, 112], of which only two used a vascular technique [74, 112]. The exact surgical techniques used were not described in detail in the reports, why we considered the initial dissection study (Paper I) essential in order to retrieve knowledge of the anatomy of the pelvic area and vessels of the baboon. The initial dissections showed that the uterine veins, that parallel the uterine arteries, were thin and narrow in the baboon. The ovarian veins proved to have a larger diameter than the uterine veins and were also prominent and easily accessible for dissection. The findings led to the use of the large ovarian vein rather than the uterine vein for the anastomosis procedure.

The duration of the surgery was around 6 hours (Papers I and II), which must be considered fairly long. Even so, the short-term animal survival rate, immediately correlated to the duration and other aspects of the surgery, was around 90 % (Papers I and II). This indicates that the baboon tolerates these long interventions well. Other studies of autologous UTx in large animals, including both recovery and transplantation surgery in the same individual, have presented an animal survival rate of 50 % in cynomolgus macaques [75] and sheep [68]. An autologous approach, involving both uterus recovery and transplantation in the

same individual, would naturally never take place in a human setting; thus, the surgical duration is expected to be shorter.

In the recipients of the allogeneic UTx (Paper III), the time of uterus retrieval was around 2.5-3.5, almost half of the total surgical time with the animals in the autologous UTx studies (Papers I and II). All allotransplant recipients (Paper III) survived the surgery. In a sheep UTx model, the allotransplant recipients showed a survival rate of 67 % [72] and 100 % [71]. In the two human attempts performed so far, both recipients have experienced an uneventful recovery [74] (Özkan personal communication). In kidney transplantation, the recipient survival rate the first year after transplantation was 95.9 %, reported by the U.S. Department of health and human services, based on more than 40 000 transplantations during the period 2002-2004 [159]. It should be kept in mind when comparisons are made between UTx and other solid organ transplantations that the recipients of UTx will be healthy young females, a privilege unfortunately not shared by recipients of other organs.

Uterus tolerance to ischemia

One of the most important aspects in all transplantation surgery is to keep the duration of ischemia at a minimum, to obtain adequate organ preservation and to provide a viable graft for transplantation with intact graft function. It is known that injuries to the transplant, induced by ischemia and reperfusion, may result in delayed function [160, 161] or non function of grafts [162], episodes of acute rejection [163], and chronic rejection [164]. A large study of almost 6500 kidney recipients showed a significant negative correlation between the duration of cold ischemia and graft survival (>6 years) [165]. Traditionally, the foundation in preservation of organs for transplantation purpose has been cold storage with specific preservation solutions to reduce cellular swelling and metabolism. Lately, there has also been increasing interest in normothermic preservation, especially concerning transplants from older deceased donors, where the organ function is suspected to be less than optimal [166, 167]. The idea of normothermic preservation is to sustain the cellular metabolism of the graft and the rationale is to combine ATP regeneration (from a circulating supply of nutrients and oxygen) and remove waste products. Normothermic pres-

ervation generally includes perfusion with an oxygenated and blood-based perfusion solution.

In the animal studies included in this thesis (Papers I, II and III), total ischemic time was around 2.5-3 hours, regardless of whether the UTx was autologous or allogeneic. Since the sensitivity to ischemia differs between different types of organs, the value of a comparison between the uterus and other transplanted organs may be questionable, but should at least offer some direction as to what might be expected. In a study of nearly 400 kidney transplants followed for more than six years, it was shown that ischemia during cold storage less than 18 hours did not seem to negatively affect the survival or function of the graft [168]. In a report on graft function and graft survival after heart transplantation, no difference in outcome was noted between ischemia times of 2-4 hours [169]. Another report on paediatric heart recipients showed no difference in patient or graft outcome when cold ischemia times of 90 min were compared with those of eight hours [170]. Thus it may be concluded that ischemia time of less than eight hours does not compromise the outcome of the transplanted heart or patient. In a meta-analysis of liver recipients it was shown that both graft and patient survival were best when the grafts cold ischemia time was between 7.5 and 12.5 hours [171]. Cold ischemia time <12 hours combined with warm ischemia time < 45 min has been shown to generate liver allograft losses in 5.4 %, whereas prolongation of both warm and cold ischemia time (>45 min and >12 hours respectively) increased the allograft loss to 42.9 % [163].

A few studies aiming to evaluate the tolerance of the uterus to ischemia have been published. Pregnancy and delivery of healthy normal pups were reported in a syngeneic mouse model where the uterus grafts were transplanted after cold ischemia for 24 hours [62]. In ewes, pregnancy was reported following three hours of warm ischemia [68]. To my knowledge, there are only three publications on the tolerance of ischemia of the human uterus. In a study of the tolerability of human uterine tissue to cold preservation small tissue sam-

ples of the myometrium were subjected to cold ischemia for six or 24 hours [172]. No major histological changes in tissue preserved in Perfadex or UW were reported. Del Priore and co-workers reported normal histological findings in a uterus graft retrieved from a multi-organ donor and subjected to twelve hours of cold ischemia [77]. Another study from the same team, where small tissue samples of human uterus were evaluated after up to 48 hours of cold preservation, revealed no morphological changes when examined histologically [173].

It is difficult to extrapolate the conclusions regarding ischemic duration and graft survival in the animal studies of this thesis. The main reason for this is that there are many confounding factors, such as surgical technique and IS. However, the restored menstruation rate (60 %) in Paper II, following a total ischemia time of three hours, indicates that the uterus tolerates ischemia for a period of at least three hours.

Uterus graft function following uterus transplantation

Since the Fallopian tubes and ovaries were included in the autologous transplant (Papers I and II) of the thesis, the hormonal cyclicity presented a non-invasive method of follow-up of the animals, both regarding general well-being and as a marker of the graft survival. Thus, resumed perineal skin-changes and hormonal cyclicity would indicate re-established ovarian function and a successful transplantation procedure. Hormonal cyclicity was seen in 50 % (Paper I) and in 75 % of the animals (Paper II). In the surviving animals, that failed to prove signs of cyclicity, second-look surgery showed total elimination of the entire graft. It was also shown during the second-look surgery that the animals with late onset of the resumed cyclic pattern (normally following a lag-phase of 2-4 months) had intact and viable ovarian tissue but lacked the uterus (Papers I and II). It is, however, well known that ovarian tissue can survive if transplanted avascular in humans and in 2011, Donnez and co-workers reported 13 live births after transplantation of cryopreserved ovarian cortex, both in previous cancer patients and in patients treated with high doses of chemotherapy

for microscopic polyangiitis and sickle cell anaemia. [174]. The results of Papers I and II show the greater ability of the ovaries, compared with the uterus, of survival after ischemia and the capacity for revascularization. In Paper III, the ovaries were only included in the deceased donor grafts. This resulted in the recipients of deceased donor grafts having a total of four ovaries after transplantation, while the recipients of living donor grafts kept their native ovaries. In Paper III, hormonal activity only indicated the presence of well-being and not graft survival. Menstruation, as a sign of uterus graft function and well-being, was resumed only in Paper I (20 %) and Paper II after modification of the surgical technique (60%). However, the long-term-surviving animal in the deceased donor UTx experiments of Paper III resumed menstruation after the study was terminated and currently has regular cycles (A. Tzakis, personal communication).

Live or deceased organ donation in human uterus transplantation

Since the first solid organ transplantations were performed, the field of transplantation surgery has developed immensely and as a result of this, an increasing shortage of organs world-wide has become more and more obvious and problematic. Interest in transplanting organs from live donors has escalated and today, it is common in kidney, liver and lung transplantation. In the US, 37 % of all the renal transplants today are retrieved from live donors [175]. Lately, the field of tissue engineering technologies has been expanded and successful reconstruction of several different tissues, such as the trachea and the oesophagus, have been presented [176]. This technique of engineering new organs/tissues from synthetic materials or decellularized natural tissues may be one solution to organ shortage in the future.

In a potential human UTx situation, both live and deceased donation can be considered and advantages can be found with each approach. In the published human UTx case, the uterus was transplanted from a live donor [74], whereas in the second human case, the uterus was donated from a deceased multi-organ donor (Özkan, personal communication). In Paper III where organs were

procured from both live and deceased donors, no difference could be seen between groups regarding survival of the transplanted animals. The small sample size and the prevalence of many confounding factors make it impossible to draw any conclusions regarding graft survival from live or deceased donors.

The potential benefits of live donation are the possibility to plan the surgical procedure in advance, with both parties of the transplantation in the best possible physical condition, and that the organ, being exposed to minimum ischemia time, will be in a favourable state [177]. Other possible advantages of live UTx would be that the transplant will undergo a preoperative evaluation providing an opportunity to choose an organ with no underlying disease/malfunction, such as myoma, HPV infection and intrauterine adhesions, or to avoid long waiting time for available organs. A requirement of a donor uterus from a live donor should be previous pregnancy and confirmed live birth, to demonstrate a favourable potential for normal pregnancy. A retrospective report of recipients of liver grafts published in 2007 identified an increased survival rate in patients receiving grafts from live donors compared with grafts from deceased donors [178]. Another report of 868 potential liver recipients, 712 of whom actually ended up with a transplant, showed a significant increase in survival associated with transplantation with a live allograft compared with continued waiting for a deceased allograft [179]. These studies consider the potentially prolonged waiting time for a transplant from a deceased donor when determining survival potential.

On the contrary, if the uterus is procured from a deceased donor there is the advantage that the transplanted organ may make it possible to create more favourable sites for vessel anastomosis, as large vessels, such as the aorta and cava, can be included in the graft (Fig 5). In 2007, Del Priore and co-workers, published a study of procurements of uterus grafts from deceased donors [77]. The complete bilateral internal iliac vessels were successfully recovered together with one-third of the uterine grafts and in the remaining cases, the anterior portion of the iliac vessels of lengths that the authors considered adequate for uterine vascular transplantation purposes were procured. In Paper V, the vascular pedicles, including the infrarenal aorta and vena cava, were ob-

tained in one case and in the remaining six cases the vascular pedicle instead included the bilateral common iliacs.

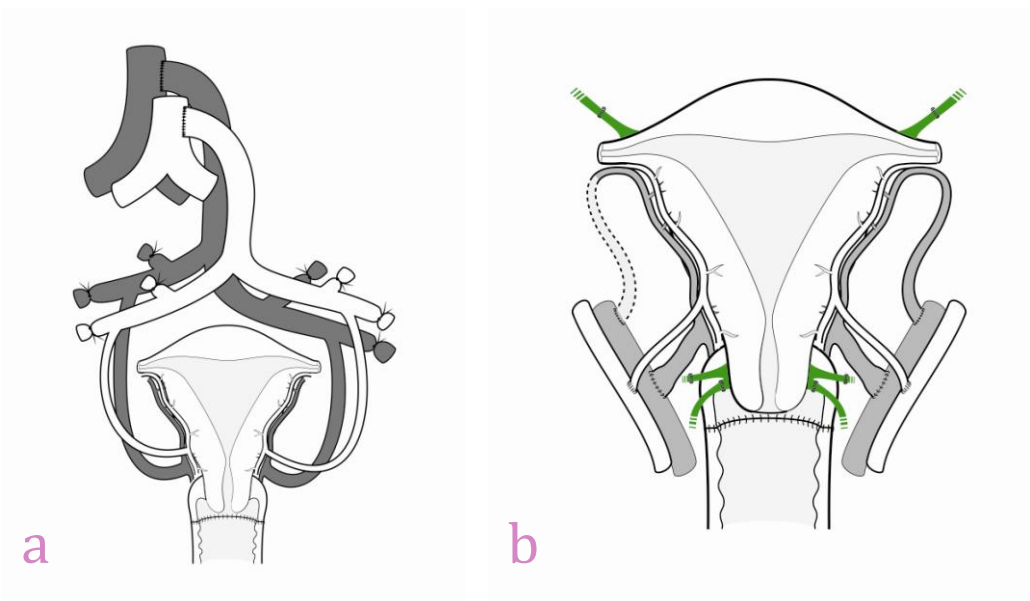


Fig 5. Schematic drawings of the possible anastomosis sites in uterus transplantation with graft from a) deceased donor, with procured vascular pedicles including aorta and vena cava and, b) live donor with procured vascular pedicles including the uterine vessels and the unilateral ovarian vein. Green markings shows potential points of fixation, round ligaments and sacrouterine ligaments.

Adequate vessel length for UTx was also procured in Paper IV from live donors. A major benefit of deceased donor transplantation is that the surgical risk of the donor is diminished (see Results and Comments). The same requirement of a previous normal pregnancy in the uterus before donation should also apply to deceased uterus donation.

Timing of pregnancy following uterus transplantation

Pregnancy subsequent to organ transplantation has become common in the past decades. The optimal timing of a potential pregnancy is of crucial impor-

tance but it has to be kept in mind that the treating doctors are often left to rely on case reports or reports of small series of data to give adequate information to the patient. Renal transplant recipients have traditionally been advised to wait 18-24 months before attempting conception [180]. By that time, it is known that the dosage of IS has decreased and is usually stabilized. The limited supply of available organs in some countries leads to long waiting times, resulting in a “loss of fertile years” in some women. As the risk of rejection decrease considerably the first year after transplantation, secondary to the introduction of more potent immunosuppressive treatment, the American Society of Transplantation suggests that the traditional waiting time of up to two years before conception may be unnecessary restrictive [181]. It is rather suggested that the question of the timing of pregnancy should rely on individual factors such as the expected risk of rejection or infection (for example cytomegalovirus) in correlation with current graft function and concurrent use of potentially harmful medication [181]. In 2003, a consensus meeting was held by the Women's Health Committee of the American Society of Transplantation, to guide physicians when asked the questions mentioned above [181]. It was concluded that pregnancy is usually safe after the first year, provided there is stable graft function and that no or few rejection episodes have occurred in the year before conception [181]. This consensus has also been supported by others [125]. Regarding UTx in humans, the answer to when pregnancy should be introduced is, of course, not known. In the animal studies with reported pregnancy, conception following UTx, was achieved after 35-38 days in the rat model [46, 64], after two [68] or two to three months [72] in the sheep model, and after three months in a nonhuman primate model (Kisu, personal communication). All these animal models are still far from the complexity of the human and it is not possible to directly extrapolate conclusions from one model to another. In the second case of human UTx, performed in Turkey in 2011, no attempts of conception, have, wisely, been made so far, nine months post UTx (Özkan, personal communication). This is in line with the recommendations of the consensus of the American Society of Transplantation.

The knowledge of medical science today is limited concerning the effects of IS and pregnancy and there may be effects on the foetus exposed to the immu-

nosuppressive treatments that we cannot predict, although the collected data show good outcomes. There is, however, more to the question of pregnancy after transplantation than the strictly medical issues. In transplantation of solid organs (not the uterus), the couple concerned may have no other option in order to form a family than to go through with a pregnancy, due to the restrictions of the adoption services in some countries. The choice of pregnancy naturally lies with the affected couple, but they should be aware of the risk that they may have a child with a disability or special needs and that the pregnancy might induce parental disability.



Concluding remarks

This thesis has proved, autologous and allogeneic UTx in a nonhuman primate model, to be a donor- and recipient safe surgical procedure, regardless of whether the graft is from a live or a deceased donor. Additionally, feasibility of human uterus retrieval was shown in both deceased donor and in a potential live donor setting without comprising donor safety.

It is concluded that UTx, based on solid experimental research, today stand a good chance of a successful outcome if performed in a facility with experienced expertise following a strict management protocol.

For some infertile women and couples suffering from UFI, that today do not stand a possible chance of cure, the research in UTx have certainly been given hope. Maybe one day in the near future, UTx will have the potential of helping these women and their partners to fulfil their dream of forming a family and have children.

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