First clinical uterus transplantation trial: a six-month report

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Objective: To report the 6-month results of the first clinical uterus transplantation (UTx) trial. This type of transplantation may become a treatment of absolute uterine-factor infertility (AUFI).
Design: Prospective observational study.
Setting: University hospital.
Patient(s): Nine AUFI women and their live uterine donors, the majority being mothers.
Intervention(s): Live-donor UTx and low-dose induction immunosuppression.
Main Outcome Measure(s): Data from preoperative investigations, surgery and follow-up for 6 months.
Result(s): Durations of donor and recipient surgery ranged from 10 to 13 hours and from 4 to 6 hours, respectively. No immediate perioperative complications occurred in any of the recipients. After 6 months, seven uteri remained viable with regular menses. Mild rejection episodes occurred in four of these patients. These rejection episodes were effectively reversed by corticosteroid boluses. The two graft losses were because of acute bilateral thrombotic uterine artery occlusions and persistent intrauterine infection.
Conclusion(s): The results demonstrate the feasibility of live-donor UTx with a low-dose immunosuppressive protocol.
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Key Words: Infertility, human, transplantation, uterus

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Uterine-factor infertility is caused by absence or dysfunction of the uterus. This untreatable condition, which is either congenital or acquired, affects 1 in 500 fertile-age women (1–4), corresponding to ~200,000 women in Europe. Surrogacy or adoption may be satisfactory options for many women with uterine-factor infertility but may be unacceptable for others owing to ethical, legal, or religious concerns (5). In Sweden, surrogacy is not legally approved.

We have examined uterus transplantation (UTx) as a possible infertility treatment. During the past decade, we have stepwise studied and optimized the procedure in animals, with the aim of clinical implementation (6–8). The ethics around UTx are unquestionably complex and have been the focus of several publications (9, 10). A controlled trial on human UTx would shed some light on important questions, such as medical and psychologic risks and benefits. Unlike any other transplantation currently performed, UTx is ephemeral; it is not intended for

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lifelong duration. The graft is removed after one or two healthy babies have been born, to limit the immunosuppression period.

Since the first birth in a woman transplanted with a solid organ half a century ago (11), more than 15,000 babies have been born to transplanted and immunosuppressed mothers, with no reported increased risk of fetal malformation (12). This indicates that human UTx and accompanying immunosuppression are compatible with normal pregnancy and progeny.

There have been two previous UTx attempts, both by teams with no preceding research records in the field. The first case, in 2000, resulted in graft failure with hysterectomy after 3 months (13). The reason for failure was not clear, but the authors stated that the likely cause was prolapse of the organ due to poor pelvic fixation, which led to compression of blood vessels and vascular thrombosis. The second case, in 2011, involved a uterine graft from a deceased female multiorgan donor (14). This case has also presented two pregnancies but with early miscarriage (15).

Our research on UTx started more than 10 years ago (16) and we have, by a scientific step-by-step approach, examined and optimized the UTx procedure. This animal-based research has been carried out in rodents, large domestic animals, and a nonhuman primate (7, 8). In 2012, we were granted ethical permission to conduct a clinical trial on human UTx at Sahlgrenska University hospital with uteri from live donors. At the time of writing, the entire cohort of nine recipients, with their donors, has been monitored for 6 months after transplantation. In the present paper, we report the perioperative and 6-month postoperative outcome of the nine donor-recipient pairs entering the UTx trial.

METHODS

Patients and Pretransplantation Investigations

The Regional Human Ethics Committee approved a study of up to ten live-donation UTx procedures. We performed two initial cases in September 2012, and after an extended observation period of these cases we later decided to perform a prospective observational study, with the trial being registered at ClinicalTrials.gov (registration no. NCT01844362). A preliminary screening process of 30 prospective recipients ended in selection of ten suitable women. The reasons for exclusion were medical or psychologic risk factors in recipients or planned donors. All of the recipients and their partners had been thoroughly counseled about their national and international options to gain parenthood through adoption or surrogacy. Multiple visits to psychologists and independent doctors (Table 1) assured that they were fully aware of the research nature of the trial. In vitro fertilization was performed to exclude any sterility factor related to fertilization failure and to cryopreserve embryos for transfer more than 12 months after transplantation, according to international transplantation recommendations (17). An independent Transplantation Board finally assessed each donor-recipient pair and excluded one pair because the recipient was found to have bilateral pelvic kidneys. Written informed consents were obtained from all donors, recipients, and their partners. All investigation procedures, surgeries, and follow-up visits were at the Sahlgrenska University hospital.

TABLE 1

Preoperative medical investigations of recipients and donors.

Radiology	MRI (abdominal and pelvic)
	Chest x-ray
	Vaginal ultrasound scan
Clinical	Electrocardiography (ECG)
	Exercise ECG[a]
	Pap smear
Blood chemistry	
Liver function	Alanine transaminase (ALT)
	Aspartate transaminase (AST)
	Alkaline phosphatase (ALP)
	Albumin
	Total protein
	Bilirubin
Kidney function	Creatinine
	Urea
	Electrolytes
	Dissolved salts
General	Hemoglobin
	White blood cells (WBC)
	Prothrombin time (PT)
	Activated partial thromboplastin time (APTT)
	Total particle concentration (TPC)
	C-Reactive protein (CRP)
Microbiology	Cytomegalovirus (CMV)
	Epstein-Barr virus (EBV)
	Human immunodeficiency virus (HIV)
	Hepatitis A, B, C
	Chlamydia
	Human papilloma virus (HPV)
	Gonorrhea
	Syphilis
Assessment by specialist in	Gynecology
	Transplantation surgery
	Psychology
	Clinical immunology
	Anesthesiology
	Internal medicine
	Radiology

Note: MRI = magnetic resonance imaging.
[a] Donors only.

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The characteristics of recipients and donors are presented in Table 2. Five of the donors were mothers of recipients, and four of these were postmenopausal. Eight patients had the Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome with congenital absence of the uterus and vagina (18), and one recipient had undergone radical hysterectomy for cervical cancer. All recipients had been in a steady relationship with their partners for ≥ 3 years.

Immunology and Microbiology

Donors' and recipients' human leukocyte antigen (HLA) A, B, C, DRβ1, and DQβ1 loci were typed with the use of polymerase chain reaction (PCR); reverse sequence-specific oligonucleotides (LABtype; One Lambda) and their degrees of HLA mismatch (at the A, B, and DRβ1 loci) were determined. HLA antibodies were detected with the use of the Luminex-based LABscreen panel reactive antibodies assay (One Lambda). A mean fluorescence intensity (MFI) value >1,000

TABLE 2

Subjects' characteristics.

	Recipients	Donors	Partners
n	9	9	9
Age (y)	31.5 ± 3.9	53.0 ± 7.0	34.3 ± 4.0
BMI	22.4 ± 1.5	25.6 ± 4.2	
Previous smoking, n (%)	3 (33)	4 (44)	
Previous abdominal surgery, n (%)			
Laparotomy	2 (22)	3 (33)	
Laparoscopy	6 (67)		
Urogynecologic characteristics, n (%)			
Kidney malformation	4 (44)		
Single kidney	3 (33)		
Unilateral pelvic kidney	1 (11)		
Cause of absent uterus, n (%)			
MRKH	8 (89)		
Cervical cancer	1 (11)		
Type of vagina, n (%)			
Normal	1 (11)	9 (100)	
Self-dilated	3 (33)		
Therapeutically dilated	1 (11)		
Skin	4 (44)		
Pregnancies		3.3 ± 1.3	
Live births		3.0 ± 0.9	
Deliveries, n (%)			
Vaginal		25 (93)	
Cesarean section		2 (7)	
Menopausal state, n (%)			
Premenopausal		4 (44)	
Postmenopausal			
<5 y		2 (22)	
>5 y		3 (33)	

Note: Plus-minus values are mean ± SD. BMI = body mass index; MRKH = Mayer-Rokitansky-Küster-Hauser syndrome.

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above the negative control was considered to be positive. Both standard complement-dependent cytotoxicity and flow-cytometric crossmatch tests with the use of T and B lymphocytes were performed. Flow cytometry was performed on a FACScalibur cytometer using a 1,024 linear scale and the Cellquest Pro software (BD Biosciences). They were also tested for relevant viral and bacterial infections (Table 1) with standard techniques. The partners were tested for HIV, hepatitis B and C, and syphilis.

Anesthesia and Postoperative Medication

Anesthesia was similar in donors and recipients. Before the start of general anesthesia, a mixture of 10 mg bupivacaine (Marcain Spinal; AstraZeneca) and 0.1 mg morphine (Morfin Special; Biophausia) was given intrathecally at the L3–4 or L4–5 level. Subsequent induction of anesthesia started with intravenous (IV) infusions of remifentanil (Ultiva; GlaxoSmithKline) at a rate of 0.25 mg/kg/min, together with a bolus (2–3 mg/kg body weight [bw]) of propofol (Fresenius), followed by 40–50 mg rocuronium bromide (Esmeron; MSD) intravenously. The patient was then intubated and maintenance anesthesia administered with sevoflurane (Sevofluran Baxter; Baxter) and remifentanil infusion (Ultiva). Anesthesia depth was monitored and adjusted, with a target minimal alveolar concentration of 0.5–0.7, and further monitored by maintaining electroencephalographic and frontal electromyographic signal activity (Entropy; GE Healthcare) at a level of ~25%–35%.

Up to 3 L Ringer acetate and 0.5 L dextran-70 (Macrodex; Meda) were given perioperatively. To maintain fluid balance, 500 mL hydroxyethyl starch (Tetraspan; Braun,) or 20 g albumin (Albumin Baxter; Baxter Medical) was administered as needed. Dopamine (Giludop; Abcur) infusion was used to keep mean arterial pressure at a level of >65 mm Hg. An autologous blood recovery system (Cell Saver; Haemonetics) was available during surgeries. The surgical duration for each patient was defined as the period from the first skin incision to completed skin closure.

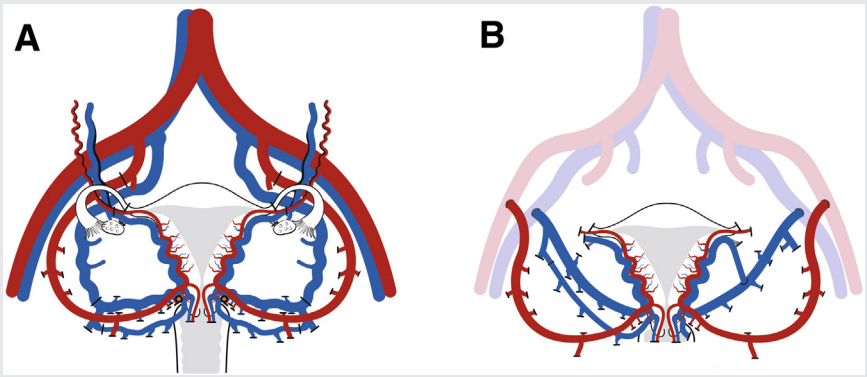
To reduce postoperative nausea, 4–8 mg ondansetron (Ondansetron; Braun), 4 mg betamethasone (Betapred; Sobi), and 0.5–1 mg droperidol (Dridol; Postrakan) were administered intravenously at the end of surgery. Paracetamol (Perfalgan; Bristol-Myers Squibb), parecoxid (Dynastat; Pfizer), tramadol (Tradolan; Nordic Drugs), and morphine (Morfin Meda; Meda) were used as initial postoperative pain relief.

Antibiotics (4 g; Piperacillin/Tazobactam; Farmaplus) were given once preoperatively and three times daily for 1 day in donors and 3 days in recipients. Thrombosis prophylaxis for donors was with dalteparin (5,000 IU; Fragmin; Pfizer) during postoperative days (PODs) 1–21. Thrombosis prophylaxis for recipients was with acetylsalicylic acid (75 mg; Trombyl; Pfizer) once daily throughout the 6-month follow-up period and with dalteparin (5,000 IU; Fragmin) during PODs 1–42.

Donor Surgery

Donor surgery involved a midline incision from the pubic bone to the umbilicus, followed by isolation of the uterus with long vascular pedicles consisting of the bilateral uterine arteries and veins up to and including parts of the internal iliac vessels (Fig. 1A). Substantial parts of the round ligaments and the sacrouterine ligaments, as well as an extensive sheet of the bladder peritoneum, were preserved on the graft side to enable stable fixation of the uterus in the recipient. Bilateral salpingectomy was performed, preserving the uterine branch of the utero-ovarian vein (Fig. 1A). Dissections on the pelvic sidewalls included dissection of the ureters from their passages over the iliac vessel bifurcations distally to their inlets into the bladder. This included meticulous dissection of the uterine veins and uterine arteries from their firm attachments to the ureters. When the ureters had been completely mobilized from the cervix and uterine vessels, vascular dissection aimed at mobilizing the internal iliac arteries and veins started from the bifurcations of the internal and external iliac vessels and proceeded distally (Fig. 1A). This dissection included severance of multiple major vascular branches. The vagina was transected 10–15 mm caudal to the vaginal fornix, and the uterus was eventually attached to the donor by only its two arterial and two venous vascular pedicles. The major feeding arteries and veins were then clamped and severed, before the uterus was removed from the pelvis to a back-table setting.

FIGURE 1



Overview of transections, ligations, and anastomosis lines at uterus transplantation. (A) Schematic drawing of the arteries (*red*) and veins (*blue*) connected to the uterus. Transection lines are indicated by *black lines*. (B) The uterus in place in the pelvis of the recipient with bilateral end-to-side anastomoses on the recipient's external iliac vessels.

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The clamping points of the donor's internal iliac vessels were closed with continuous polypropylene (6-0) sutures and the abdomen was closed in standard three-layered manner. A suprapubic bladder catheter was inserted to avoid urinary retention secondary to the extensive dissection around the bladder. The suprapubic catheter was removed on day 4 or 5 after surgery, when the residual urinary volume had decreased to <150 mL. Anesthesia was discontinued and the donor was brought to the postoperative recovery unit.

Graft Preparation

The uterus was quickly brought to the back-table and initially flushed with 10 mL heparinized saline solution through each artery, followed by flushing for 10–20 minutes with cold preservation solution (Custodiol HTK-solution; Nordmedica), until the organ was blanched and the venous effluent was clear. The graft was kept on ice until transplanted into the recipient. In some patients, back-table preparation involved anastomosing (7-0 polypropylene) one ovarian vein end-to-side to the uterine vein (Fig. 1B) to achieve greater venous drainage, whereas this branch of the ovarian-uterine vein was subsequently directly anastomosed to the external iliac vein of the recipient in others (see below). Organ ischemia was divided into three distinct periods. The first warm ischemia period was from cross-clamping at organ retrieval to commencement of cold flushing. The cold ischemia period was from initiation of organ flushing to as long as the organ was kept on ice. The second warm ischemia period was the time from removal of the organ from ice to blood reperfusion. Because the first warm ischemia period was <2 minutes in all cases, this period was added to the longer second warm ischemia period and data on warm ischemia are presented as the sum of these periods.

Recipient Surgery

To synchronize donor and recipient surgeries, thereby avoiding a long cold ischemia period for the uterine graft, a second team of surgeons started the preparatory surgery of the recipient in an adjacent operating room well before the anticipated procurement of the organ. This laparotomy also was performed through a subumbilical midline incision. First, the vaginal vault was dissected free from the bladder and the rectum. In the eight MRKH patients, the uterine rudiment had to be cleaved to reach the top of the vagina. Sutures (1-0 polypropylene) for subsequent organ fixation were attached to the round ligaments, the sacrouterine ligaments, and the two lateralized parts of the uterine rudiment (MRKH patients) or the paravaginal connective tissue (cervical cancer patient). The surgery was then directed toward preparation of the external iliac vessels for subsequent anastomosis. The external iliac artery and vein were bilaterally separated from each other and from adjacent tissue to a distance of ~60 mm.

The uterine graft was then brought, still on ice, into the recipient's operating room. It was placed in its normal position in the pelvis and bilateral end-to-side vascular anastomoses were performed between the graft vessels and the external iliac vessels with the use of continuous 7-0 (arterial anastomosis) or 8-0 (venous anastomosis) polypropylene sutures. After each venous anastomosis had been sutured, the vascular clamp over the external iliac vein was opened. In some cases, single sutures were used to seal any leakage from the anastomosis line. In six patients, the uterine branch of the ovarian-uterine vein was used and anastomosed to the ipsilateral uterine vein or prepared for direct anastomosis to the external iliac vein (Fig. 1B). Mannitol (30 g; Mannitol Baxter Viaflo; Baxter Medical) was given as an IV bolus just before the arterial clamps were removed, and systolic blood pressure was carefully monitored and maintained at >100 mm Hg. After completed vascular anastomosis surgery, adequate blood flow through the uterine arteries of the vessels was verified and quantified with a vascular Doppler probe (Vascular TTFM Probe; Medistim) placed around the uterine artery.

The recipient's vaginal vault was then opened by a longitudinal incision of ~40 mm. The vaginal rim of the graft was anastomosed to the top of the recipient's vagina with a

continuous absorbable 2-0 suture. The uterus was fixed in its pelvic location by attaching the uterine ligaments to their pelvic counterparts with the previously placed fixation sutures, and by overlaying the extensive bladder peritoneum of the graft over the recipient's bladder fundus. A Doppler probe with a silicon cuff (Cook-Schwartz Doppler probe; Cook Medical) was then placed around one uterine artery to ascertain that pulsatile blood flow was maintained during the initial 72-hour postoperative period, with the signal transduced from a 20-MHz crystal on the tip of a thin cable through the midline incision. The cable, with its crystal, was then gently pulled out from its intra-abdominal position, leaving the silicon cuff in situ. The surgery and anesthesia of the recipients were terminated identically to the donors.

Immunosuppression and Long-Term Medication

The recipients followed a standardized protocol of induction and maintenance immunosuppression. Preoperatively, the recipient had received 1g mycophenolate mofetil (MMF; Cellcept; Roche). During transplantation, before reperfusion, she was given 500 mg methylprednisolone (Solu-Medrol; Pfizer) and antithymocyte antibodies, to deplete T lymphocytes, either thymoglobulin (IV, 2.5 mg/kg bw; Genzyme Aps) or antithymocyte globulin (ATG; IV, 5 mg/kg bw; ATG-Fresenius; Fresenius), with identical doses of each drug repeated 12 hours later.

Maintenance immunosuppressive therapy was continued with tacrolimus (Prograf,-Astellas Pharma) twice daily (adjusted to trough levels of 10–15 ng/mL during weeks 1–5 and 5–10 ng/mL during week 6 and thereafter) and MMF twice daily with MMF area under the curve trough levels of 40–60 mg·h/L. Oral glucocorticosteroids (Prednisolon; Pfizer) were administered once daily on the day of surgery and during the first 4 postoperative days.

Antiviral prophylaxis consisted of a daily oral dose (450 mg) of valganciclovir (Valcyte; Roche), which was given for 3 or 6 months, depending on cytomegalovirus status (3 months when both donor and recipient were positive; 6 months when donor was positive and recipient negative). When both donor and recipient were negative, prophylaxis was not given.

Follow-up

Surgical complications were registered with the use of Clavien-Dindo classification (19). The recipients were monitored by clinical examination twice weekly during the 1st month, once weekly during months 2–3, and thereafter every other week. The uterus and endometrium were examined with vaginal and abdominal two-dimensional ultrasonography (Flex Focus 400; BK Medical). Color Doppler ultrasound was used to assess that blood flow was maintained through the uterine arteries. Clinical examination included visual inspection of the uterine cervix as well as cervical cultures and biopsies. The biopsies were obtained at predetermined time points (1, 2, and 4 weeks, and monthly thereafter) as well as on suspicion of graft rejection based on clinical examination (discolored cervix, abnormal vaginal discharge, enlarged uterus, fever, abdominal pain). Drug levels (tacrolimus, MMF) were monitored by standard assays, and routine blood tests were performed to assess liver and kidney function and to detect infection.

Rejection was diagnosed based on histopathologic examination of cervical biopsies and graded according to the proposed rejection classification for the primate uterus (6).

Statistics

Values are presented as individual values and as mean ± SD.

RESULTS

Matching and Immunology of Patients

Seven donor/recipient pairs were ABO identical, and two were compatible. Both cytotoxic and flow-cytometric crossmatch tests were negative in all patients, and no patients had HLA antibodies. The degree of HLA mismatch among the donor and recipient pairs varied from 1/0 to 3/2 (Table 3).

Surgery

Surgical parameters are presented in Table 3. Donor surgery lasted 10–13 hours. Recipients 1 and 2 underwent prolonged anesthesia because recipient and donor surgeries were improperly synchronized, so that 9- to 10-hour waiting periods occurred before graft anastomosis. Blood transfusion was not required during any surgery, although volumes <0.6 L were returned by the autologous blood recovery system in seven cases. Cold ischemia periods lasted 1 to 2 hours. Major interindividual variation in dominant uterine artery blood flow, recorded by Doppler, was observed (Table 3).

Postoperative Period and Complications: Donors

The initial postoperative hospital stay of the donors was 6 days (Table 3), with no need for intensive care. One grade IIIb surgical complication occurred. Donor 2 presented on POD 16 with watery vaginal discharge due to a ureterovaginal fistula. A pyelostomy catheter was inserted and her ureter was reimplanted on POD 134, after which recovery was uneventful.

Postoperative Period and Complications: Recipients

The first two recipients experienced nausea and dyspnea on POD 1, with chest x-rays revealing pleural fluid (grade I complications). They were observed at an intermediary care unit until the symptoms ceased and the pleural fluid resorbed spontaneously (POD 2–3). No other recipient experienced these symptoms. A grade II complication (blood transfusion) occurred in one recipient on POD 2 (Table 3), and a retroperitoneal hematoma was visualized on computerized tomographic (CT) scan.

Two recipients developed complications necessitating surgical removal of the graft (grade IIIb complications). Recipient 2 was readmitted on POD 33 with abdominal pain, fever, and vaginal discharge. Gynecologic examination revealed signs of cervical/uterine infection, confirmed by positive

TABLE 3

Surgical, anesthesiologic, and hospitalization parameters.

Pair	Age (y)	Donors' relation to recipients	HLA mismatch	Cytomegalovirus Ig	Epstein-Barr virus Ig	Duration: Anesthesia	Duration: Surgery	Duration: Back-table Preparation	Duration: Anastomoses Veins	Duration: Anastomoses Arteries	Duration: Ischemia Warm	Duration: Ischemia Cold	Arterial blood flow (mL/min)[a]	Blood loss (L)	Intraop. autoblood (L)	Blood transfusion (L)[b]	Highest grade of complication[c]	Postop. hospitalization (d)
Recipient 1	33	Mother	2/0	pos	pos	15 h 0 min	4 h 10 min	1 h 30 min	39 min	35 min	1 h 18 min	1 h 30 min	63	0.4	0	0	Grade II	8
Donor 1	52			neg	pos	12 h 4 min	10 h 54 min							0.3	0	0		6
Recipient 2	38	Mother	2/1	neg	neg	13 h 57 min	4 h 17 min	1 h 47 min	43 min	29 min	1 h 38 min	1 h 47 min	50	1.6	0.49	0	Grade IIIb	9
Donor 2	58			pos	pos	13 h 46 min	12 h 37 min							2.4	0.49	0	Grade IIIb	6
Recipient 3	28	Mother's sister	3/1	pos	pos	10 h 50 min	4 h 50 min	1 h 4 min	31 min	25 min	1 h 34 min	1 h 4 min	50	0.8	0.2	0		6
Donor 3	54			neg	pos	13 h 37 min	12 h 53 min							0.8	0	0		6
Recipient 4	27	Mother	2/1	neg	pos	6 h 5 min	5 h 4 min	0 h 57 min	32 min	30 min	1 h 17 min	0 h 57 min	43	0.25	0	0		6
Donor 4	50			neg	neg	11 h 11 min	10 h 34 min							0.6	0	0		6
Recipient 5	35	Family friend	3/2	pos	pos	5 h 45 min	4 h 55 min	1 h 6 min	32 min	30 min	1 h 13 min	1 h 6 min	40	0.75	0	1.2	Grade II	6
Donor 5	61			pos	pos	11 h 6 min	10 h 17 min							0.6	0	0		6
Recipient 6	27	Mother	1/1	neg	pos	8 h 17 min	4 h 30 min	2 h 0 min	60 min	23 min	1 h 24 min	2 h 0 min	75	0.7	0.18	0		3
Donor 6	53			pos	pos	11 h 50 min	10 h 52 min							0.7	0.11	0		6
Recipient 7	28	Mother	1/0	pos	pos	6 h 35 min	4 h 44 min	0 h 54 min	20 min	30 min	1 h 15 min	0 h 54 min	30	0.3	0	0	Grade II	7
Donor 7	50			pos	pos	11 h 35 min	10 h 17 min							0.4	0	0		6
Recipient 8	33	Sister	1/1	pos	pos	7 h 53 min	5 h 56 min	0 h 56 min	25 min	24 min	1 h 14 min	0 h 56 min	30	0.75	0.2	0	Grade II	8
Donor 8	37			pos	pos	11 h 55 min	11 h 23 min							0.4	0	0		6
Recipient 9	35	Mother-in-law	3/2	neg	neg	8 h 14 min	4 h 31 min	1 h 28 min	42 min	47 min	1 h 32 min	1 h 28 min	10	0.5	0	0	Grade IIIb	7
Donor 9	62			pos	pos	14 h 5 min	13 h 8 min							2.1	0.52	0		6
All recipients	31.5 ± 3.9					9 h 4 min ± 3 h 14 min	4 h 46 min ± 30 min	1 h 18 min ± 23 min	36 ± 11 min	30 ± 6.9 min	1 h 23 min ± 9 min	1 h 18 min ± 23 min	44 ± 18	0.67 ± 0.38	0.12 ± 0.15	0.13 ± 0.36		6.7 ± 1.6
All donors	53.0 ± 7					12 h 13 min ± 60 min	11 h 37 min ± 1 h 5 min							0.92 ± 0.73	0.12 ± 0.21	0 ± 0		6.0 ± 0

Note: Plus-minus values are mean ± SD.
[a] Peak value of the dominant artery.
[b] Packed red cells.
[c] According to the Clavien-Dindo classification of surgical complications (17).

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Enterococcus faecalis culture. After 4 days of intravenous antibiotics, the symptoms resolved; the recipient was discharged on POD 38 with oral antibiotics. She was kept on oral antibiotics owing to persisting *Enterococci* in cervical cultures and a modest increase in C-reactive protein (CRP), except during one febrile episode (POD 78–83) when IV antibiotics were temporarily reintroduced. She was readmitted on POD 98 owing to aggravated symptoms, and an intrauterine abscess measuring 3×4 cm was seen on CT scan. Despite two surgical drainage attempts, the abscess persisted and the patient developed initial signs of septicemia. Hysterectomy was performed on POD 105. Morphologic examination revealed extensive areas of necrosis, with some neutrophil-dominated inflammation, but no signs of rejection. Subsequent recovery was uneventful.

Recipient 9 had sudden cessation of the uterine artery Doppler signal on the morning of POD 3, and gynecologic examination revealed a blood-congested cervix. Acute laparotomy revealed a congested uterus and uterine arteries without palpable pulses. The entire graft was removed and morphologic examination revealed focal necrosis and moderate ischemic myometrial damage, but no signs of rejection. Occluding thrombi were found in both major arteries and veins. The patient was discharged from the hospital 5 days after hysterectomy, with frequent initial follow-up visits and contacts with the team's doctors and psychologist. At 6-month follow-up, she was back at work and had experienced no physical sequelae.

Rejection and Uterine and Renal Function

Three (numbers 1, 5, and 7) of the seven recipients with viable uteri throughout the observation period, suffered single episodes of mild rejection during the 1st month. One recipient (number 8) had two episodes of mild rejection (month 1 and month 3). Morphologic analysis of the cervical tissue showed mild infiltration of lymphocytes and neutrophils, mainly in the basal squamous epithelium of the ectocervix. All rejection episodes (grade II complications) were successfully treated with corticosteroids for 7–10 days. Spontaneous menstruations resumed within 2 months in the seven patients with regular menstrual patterns (interval 27–32 days) and with a mean maximum endometrial thickness of 14 ± 3 mm.

Preoperative creatinine levels, mirroring renal function, were 7.35 ± 9.1 μmol/L, and a reversible increase (maximal increase 37.1 ± 20.6 μmol/L) occurred during the initial 1–2 months of immunosuppression.

DISCUSSION

This study represents the first clinical trial of human UTx, after two solitary human UTx attempts in 2000 (13) and 2011 (14). In contrast to those single cases, which were not preceded by any internal UTx research, our team has been involved in animal-based UTx research for several years (6–8). In the rodent models, we have studied rejection patterns in a uterine allograft, immunosuppression to avoid uterine graft rejection, and, importantly, pregnancies and offspring after UTx. Large animal models, including primates, have mostly been used to optimize the surgery at UTx and to evaluate suitable combinations of immunosuppressants. Our introduction of human UTx to the clinical arena complies with the recently released IDEAL recommendations for clinical introduction of major surgical procedures (20).

Uterus transplantation, as a non–life-saving transplantation and a technically complicated infertility treatment, raises several ethical questions (21–23) regarding priorities in the health care sector, as well as the risks and benefits of the procedure. A clinical trial such as ours generates important data on the risks and benefits, which will also be advantageous in an ethical analysis of UTx.

We recovered the uterine grafts from live donors. Live donation of organs is most common in renal transplantation; ~40% of transplanted kidneys are from live donors in the United States (24). Because our prior UTx research in sheep (25) and baboons (6, 26) followed the live donor concept, our team was well prepared for that particular surgical procedure. All donors had undergone at least one normal pregnancy. In the recent UTx case in Turkey (14), the uterus was obtained from a nulliparous deceased donor and the local team assembled on short notice for surgery. The recipient has subsequently undergone two pregnancies, with early miscarriages occurring both times (15). The cause of these pregnancy failures may be related to intrinsic factors in the particular uterus or to the triple immunosuppression still administered. As recently outlined by Donnez (27), there may exist specific factors of a transplanted uterus, such as decreased vascular plasticity, placentation defects, and loss of innervation, that may negatively affect the pregnancy potential in a uterine allograft.

Our team, with surgeons from three continents, had to plan the exact dates of the surgeries well in advance. Furthermore, our hospital board required that all surgeries be done during weekends in operating rooms that were usually closed on weekends. Deceased-donor UTx would not be a realistic option under these conditions.

Before the first UTx procedure, we had anticipated considerably easier and faster retrieval surgery than was actually the case, because uterus retrieval in our baboon UTx studies took ~2.5 hours (6, 26). Therefore, and based on our feasibility study of uterine vessel dissection at radical hysterectomy, we had predicted a duration of 3–4 hours (28). In that study, the median surgery duration was ~5 hours, when uterine vessel dissections were included in the hysterectomy and lymph node dissection procedure. The most time-consuming surgical step in retrieval surgery in the present study was ureter and uterine vessel dissection, which took ~4–6 hours. In a radical hysterectomy, the uterine arteries are transected at their inlets into the anterior division of the internal iliac arteries, and ureteric dissection is fairly easy. In the present cases, all uterine vessels were preserved, and extremely meticulous dissection is unavoidable in this surgically inaccessible area of the funnel-shaped pelvis. In all novel types of surgery, procedure duration tends to decrease as it becomes more standardized. This would most likely be the case for UTx live donor surgery in the future.

Naturally, as in all live organ donations, our surgery involved a person, with no direct health benefit from the donation, being exposed to a surgical risk. In live kidney

and liver donation, the risks of major surgical complications (grade III) are ~4% (29, 30). In the present study, one donor developed a ureterovaginal fistula which necessitated hysterectomy (grade IIIb complication). The late appearance of the fistula indicates that the cause was partial damage, possibly by diathermy, followed by a gradual weakening of ureteral wall. This particular donor surgery, spanning >12 hours, was extremely difficult, owing to the absence of natural dissection planes, and involved an older donor. This ureteric complication in the present study and the perioperative ureteric laceration, which occurred in the original case in year 2000 (13), indicates that extremely gentle surgery should be used in close proximity to the ureters.

Two of the nine recipients lost their grafts during this 6-month period reported here. The cause of graft loss was escalating uterine infection in one case and acute thrombosis in the other. The lengthy procurement procedure (12 hours) and poor synchronization of donor and recipient surgeries, with a resulting very long anesthesia time for the recipient, may have been other predisposing factors behind uterine infection. Moreover, the vaginal-vaginal anastomosis sutures were placed in close proximity to the cervix in that case, for anatomic reasons. This may have negatively affected the normal cervical barrier to ascending infections, especially in an immunosuppressed recipient.

The recipient with acute uterine artery thrombosis was heterozygous for protein C deficiency, which does not exclude a patient from any type of clinical transplantation surgery. However, this mutation is associated with 3–6-fold increased risk of venous thromboembolism (31). It is unclear whether the complication in this case of increased risk for thromboembolism might have been avoided if more intense or prolonged anticoagulants had been administered (31) or if the total surgical time of >16 hours that the uterus and its vasculature are exposed to could have been considerably shortened. Other factors, which may have predisposed to thrombosis of this case, are that this transplantation involved the oldest donor and that the uterine artery blood flow, after anastomosis, was the lowest of all cases. The total failure rate of 2/9 should be viewed in the light of this being a novel type of transplantation, with major potential for further optimization.

Our immunosuppression protocol was milder than in the two previous cases of human UTx (13, 14) as well as in those initially used for hand and face transplantations (32). Nowadays, the general understanding is that less immunosuppression is sufficient in any type of composite tissue transplantation (33). The fact that we observed only mild rejection episodes in four of the seven recipients indicates that it is unlikely that immunosuppression was suboptimal.

Effective monitoring of each specific organ to detect any rejection at an early stage, when it is reversible and has not caused organ damage, is a major challenge in organ transplantation. Because UTx is a novel type of transplantation, the ideal mode for monitoring the organ is not known. In the first human UTx case in 2000, repeated Doppler examinations and CD4/CD8 ratios in blood were used to detect rejection (13). One rejection episode was detected and effectively reversed, but the uterus had to be removed later. In the more recent case from 2011, Doppler and biopsies of the graft's vaginal portion were used and no rejection episodes were reported (14).

The mild rejection episodes in our patients were asymptomatic, with normal results on ultrasound and gynecologic examination. We based our rejection diagnosis on the assumption that a human uterine allograft would react similarly to that of a baboon, in which we observed the wide spectrum from mild to severe rejection (6). The fact that corticosteroids were effective in normalizing the histologic pattern in our human cases indicates that these were true rejection episodes.

The plan for this cohort is to start embryo transfer 12–18 months after UTx if the clinical course has been uneventful, with no rejection episodes for ≥4–6 months. The uterus will be removed after one or two successful pregnancies.

In summary, this study shows that a live-donor UTx procedure has a low risk despite extended surgery duration. The report of the first successful human UTx case, defined as a live birth from a transplanted human uterus, has yet to be published.

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