Livebirth after uterus transplantation

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Summary

Background **Uterus transplantation is the first available treatment for absolute uterine infertility, which is caused by absence of the uterus or the presence of a non-functional uterus. Eleven human uterus transplantation attempts have been done worldwide but no livebirth has yet been reported.**

Methods **In 2013, a 35-year-old woman with congenital absence of the uterus (Rokitansky syndrome) underwent transplantation of the uterus in Sahlgrenska University Hospital, Gothenburg, Sweden. The uterus was donated from a living, 61-year-old, two-parous woman. In-vitro fertilisation treatment of the recipient and her partner had been done before transplantation, from which 11 embryos were cryopreserved.**

Findings **The recipient and the donor had essentially uneventful postoperative recoveries. The recipient's first menstruation occurred 43 days after transplantation and she continued to menstruate at regular intervals of between 26 and 36 days (median 32 days). 1 year after transplantation, the recipient underwent her first single embryo transfer, which resulted in pregnancy. She was then given triple immunosuppression (tacrolimus, azathioprine, and corticosteroids), which was continued throughout pregnancy. She had three episodes of mild rejection, one of which occurred during pregnancy. These episodes were all reversed by corticosteroid treatment. Fetal growth parameters and blood flows of the uterine arteries and umbilical cord were normal throughout pregnancy. The patient was admitted with pre-eclampsia at 31 full weeks and 5 days, and 16 h later a caesarean section was done because of abnormal cardiotocography. A male baby with a normal birthweight for gestational age (1775 g) and with APGAR scores 9, 9, 10 was born.**

Interpretation **We describe the first livebirth after uterus transplantation. This report is a proof-of-concept for uterus transplantation as a treatment for uterine factor infertility. Furthermore, the results show the feasibility of live uterus donation, even from a postmenopausal donor.**

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Introduction

Absolute uterine factor infertility is the only major type of female infertility that is still viewed as untreatable. The major causes are congenital absence of the uterus (Rokitansky syndrome, also called Mayer-Rokitansky-Küster-Hauser syndrome), previous hysterectomy, and severe intrauterine adhesions.[1–4] In the UK alone, more than 12 000 women of childbearing age are thought to have absolute uterine factor infertility.[5] The available motherhood options for women with this disorder are adoption (to acquire legal motherhood), or pregnancy in a gestational surrogate carrier to acquire genetic motherhood, followed by adoption to also acquire legal motherhood. However, surrogacy is not allowed in many countries because of ethical, legal, or religious reasons.

We have undertaken preclinical research into uterus transplantation for more than a decade, using a step-by-step logical developmental approach, in which we have used several animal species, ranging from rodents to non-human primates.[6,7] Recently, we initiated the first clinical trial of transplantation, involving nine women who received uteri from live donors. Two of the women had to undergo hysterectomy during the initial months, with the causes being uterine artery thrombosis and severe intrauterine infection.[8] The other seven women began menstruation during the first 2–3 months and the grafts remained viable, with regular menstruations during the first post-transplantation year. Occasional subclinical episodes of mild rejection were detected on cervical biopsies, which were effectively reversed by short courses of increased immunosuppression.

Except for our clinical trial of nine women, only two other human uterus transplantation efforts have been reported. The first case resulted in progressive uterine necrosis during the initial months, and a fully necrotic uterus was removed 3 months after transplantation.[9] The second case involved a uterus from a deceased donor being transplanted into a patient with Rokitansky syndrome.[10] The patient underwent embryo transfer 18 months after transplantation and two pregnancies that miscarried before gestational week 6 have been reported.[11] No further reports exist about this case.

In this report, we describe the clinical course of the first patient in our cohort who achieved a clinical pregnancy resulting in delivery of a baby.

Methods

Patient

In 2013, a 35-year-old patient underwent uterus transplantation at Sahlgrenska University Hospital (Gothenberg, Sweden) as part of our clinical trial of uterus transplantation in nine women with absolute

For **clinical trial** see http://clinicaltrials.gov/show/NCT01844362]

uterine factor infertility (ClinicalTrials.gov number NCT01844362). The study was approved by the regional ethics board of the University of Gothenburg. The donor, recipient, and her male partner had given their written informed consent. The 6-month outcomes of the study have been published.[8]

The recipient (blood group O+) was born with Rokitansky syndrome (Müllerian agenesis). She belongs to the atypical Rokitansky syndrome category[12] since she was also born with only one kidney and has vaginal and uterine aplasia. A functional neovagina had been created by self-dilatation. She was a non-smoker, was not on any medication, and had a body-mass index (BMI) of 21 kg/m². The recipient was, before transplantation and at several times during the post-transplantation period, informed that surgical removal could later be recommended for medical reasons, owing to rejection, surgical complications at caesarean section, or side-effects of immunosuppression. The ethics approval and consent forms state that the uterus should be removed after a maximum of two successful pregnancies.

The donor (blood group O+) is a close family friend of the recipient. At surgery, she was 61 years old. She is two-parous with two previous vaginal deliveries, at 26 years of age (birthweight 3000 g) and at 29 years of age (birthweight 3250 g). Both deliveries were spontaneous and at gestational week 41. She is a healthy non-smoker and her BMI at surgery was 20 kg/m². Menopause occurred around 7 years before transplantation. To ascertain menstrual functionality of the uterus before transplantation and to possibly increase uterine artery blood flow preoperatively, she was treated for 3 months with a sequential oral contraceptive pill, containing ethinylestradiol (30–40 µg daily) and levonorgestrel (50–125 µg daily). Bleedings occurred as expected.

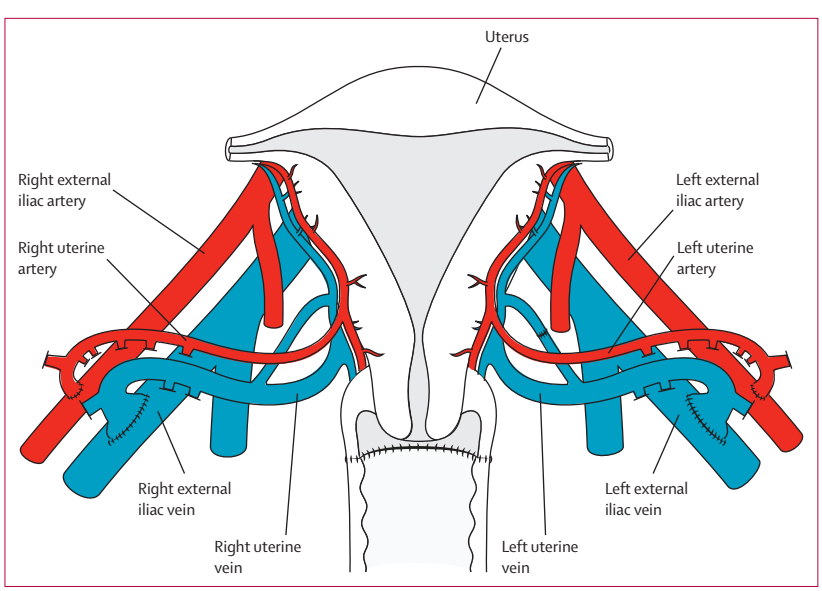

Figure 1: **Schematic drawing of the vessel connections of the transplanted uterus**

The HLA mismatch between donor and recipient was 3/2 and no HLA antibodies were present. Both donor and recipient were seropositive for cytomegalovirus and Epstein–Barr virus infections.

In-vitro fertilisation

In-vitro fertilisation was done during the period from 18 to 6 months before transplantation. The patient's Rokitansky syndrome, with no menstruation to aid in synchronisation of gonadotrophin stimulation and the cranially and laterally positioned ovaries, caused difficulties in initiation and monitoring of gonadotropin stimulation. Her blood concentration of anti-Müllerian hormone was 1·9 ng/mL. She underwent three full cycles of gonadotrophin stimulation. All cycles involved ovarian downregulation for 2–3 weeks by nasal administration three times daily with 300 µg of the gonadotropin-releasing hormone agonist buserelin (Suprecur; Hoechst, Frankfurt, Germany); this treatment began 7–9 days after a positive luteinizing hormone urine test, indicating the spontaneous gonadotrophin surge. We used ultrasound with abdominal probe and blood analysis of oestradiol values to assess follicle maturation. Human menopausal gonadotrophin (Menopur; Ferring, Copenhagen, Denmark) was used as the primary gonadotrophin in the first cycle (150 IU human menopausal gonadotrophin for 11 days) and recombinant follicle-stimulating hormone (Gonal-F; Merck Serono, Darmstadt, Germany) was added to the second and third cycles (225 IU human menopausal gonadotrophin plus 150 IU follicle-stimulating hormone, for 12 days in cycle 2 and for 14 days in cycle 3). Ovulation was triggered by injection of 250 µg recombinant human chorionic gonadotrophin (Ovitrelle; Merck Serono, Darmstadt, Germany). Oocyte pick-up was done transabdominally by abdominal ultrasound guidance. The oocytes were fertilised by intracytoplasmic sperm injection. Single embryo transfer was done around 12 months after transplantation during the natural menstrual cycle according to our local routine for frozen embryo transfer, with a soft embryo transfer catheter under abdominal ultrasound guidance.

Surgery

The surgical procedures of the donor and the recipient, and the anaesthesia, have previously been described in detail.[8] Uterus transplantation surgery entails isolation of the uterus with bilateral, long venous, and arterial vascular pedicles. The complexity of the surgery is mostly related to the extensive vascular dissection that includes the distal parts of the internal iliac veins and arteries. In this specific case, two large uterine veins on each side converged into one major uterine vein that drained into the internal iliac veins. On the patient's left side, one of these veins passed over the ureter and the other went under the ureter, and therefore one of these veins had to be transected to enable removal of the uterus with an intact ureter. After surgical isolation, the

uterus was flushed bilaterally through the arterial ends with cold histidine–tryptophan–ketoglutarate solution (Custodiol-HTK; NordMedica AS, Gentofte, Denmark). The vascular ends of the graft were trimmed and the left-sided vein that had been divided was anastomosed end-to-end by a continuous suture (8-0 polypropylene).

1 h before final graft retrieval from the donor, surgery to prepare the recipient for transplantation was initiated in an adjacent operating theatre. Through a midline incision, the external iliac vessels were dissected and prepared for anastomosis. The vaginal vault was separated from the bladder and rectum. Sutures, to be used for uterine fixation, were placed bilaterally through the round ligaments, sacrouterine ligaments, and the paravaginal connective tissues. The uterus was brought into the pelvis and end-to-side vascular anastomoses were done to connect the uterine veins to the external iliac veins (with 8-0 polypropylene sutures) and the anterior divisions of the internal iliac arteries to the external iliac arteries (with 7-0 polypropylene sutures) on both sides (figure 1). We then opened the blood flow to the uterus and ascertained that good pulses existed distal to the arterial anastomosis sites and that the uterine tissue changed from pale to reddish, which is a sign of peripheral tissue perfusion. Then, we fixed the uterus to the ligaments and sutured the extensive bladder peritoneum on the uterine graft on top of the recipient's bladder to provide extra structural support.

To establish that blood flow through the uterine arteries continued during the first post-transplantation days, we placed a 20-MHz Doppler probe with a silicon cuff (Cook-Schwartz Doppler probe; CookMedical, Bloomington, IN, USA) around the left uterine artery. The signal was transduced through a thin cable, which was exteriorised through the midline incision. The probe could then be easily pulled out after the 3-day observation period.

The surgeries of the donor and recipient proceeded uneventfully. The skin-to-skin durations of surgeries were 10 h 7 min for the donor and 4 h 55 min for the recipient. The anastomoses were created in the sequence of left venous, left arterial, right venous, and right arterial. After unclamping for reperfusion, we placed one extra suture over the arterial anastomosis site on the right side to prevent blood leakage. The estimated (Doppler) blood flow through the right uterine artery was then 40 mL per min. The uterus (figure 2A) was then attached to the orthotopic position (figure 2B). Perioperative blood loss was 0·6 L in the donor and 0·75 L in the recipient. The total ischaemic time of the uterine graft was 2 h 19 min (cold ischaemia: 1 h 6 min; warm ischaemia: 1 h 13 min). A retroperitoneal haematoma was diagnosed in the recipient on the second postoperative day and she was transfused with two units of leukocyte-reduced packed red blood cells. Both the donor and the recipient were discharged from the hospital after 6 days of postoperative care.

Immunosuppression and follow-up

The recipient received induction immunosuppression with intravenous anti-thymocyte globulin (Thymoglobulin; Genzyme, Cambridge, MA, USA) 2·5 mg/kg just before surgery and 12 h later. One dose of 500 mg methylprednisolone (Solu-Medrol; Pfizer, New York, NY, USA) was administered intravenously just before uterine reperfusion.

Maintenance immunosuppression was achieved with oral tacrolimus aiming at trough levels of 5–10 ng/mL (Prograf/Advagraf; Astellas Pharma, Chertsey, UK), and mycophenolate mofetil aiming at trough levels of 40–60 mg·h/L (Cellcept; Roche, Basel, Switzerland) was also administered orally during the first 10 months post-surgery. Azathioprine 2 mg/kg per day (Imurel; Orion Pharma, Sollentuna, Sweden) was then used instead of mycophenolate mofetil after 10 months, to avoid the potentially teratogenic effects of mycophenolate mofetil in the run-up to the embryo transfer attempts. Moreover, prednisolone (Prednisolon; Pfizer, New York, NY, USA) 5 mg daily was added from month 6 post-transplantation because of repeated rejection episodes (see Results section).

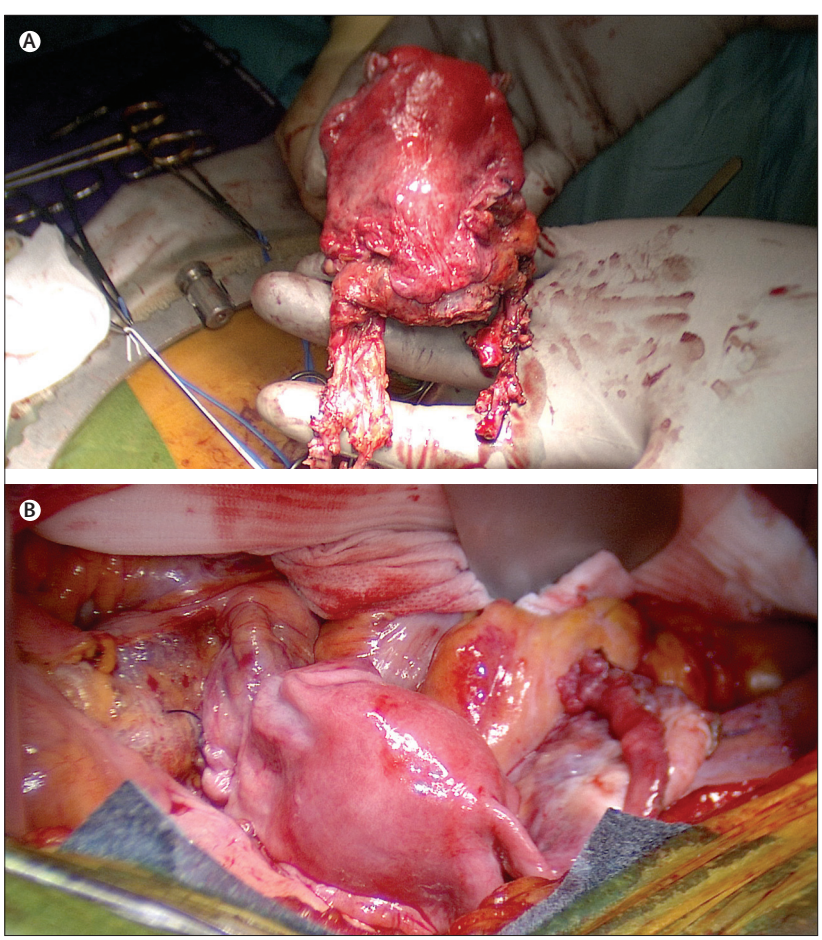

Figure 2: **Uterus transplantation procedure**
(A) The uterus with its long vascular pedicles is removed from the donor. (B) The uterine graft is revascularised and fixed in the pelvis of the recipient.

	Week 8	Week 12	Week 13	Week 15	Week 19	Week 25	Week 27	Week 29	Week 31
Weight (kg)	66	66	65	64	66	69	70	71	74
Cervical length (mm)*	..	..	44	43	45	48	50	49	31
Blood pressure (mm Hg)	100/75	100/75	110/60	110/60	110/70	120/75	110/70	120/80	170/105
Proteinuria (g/L)	0	0	0	0	0	0	0	0	1–3
Haemoglobin (g/L)	84	99	99	99	89	98	100	102	107

*Cervical length was not measured before week 13.

Table: **Maternal characteristics during pregnancy by completed week of gestation**

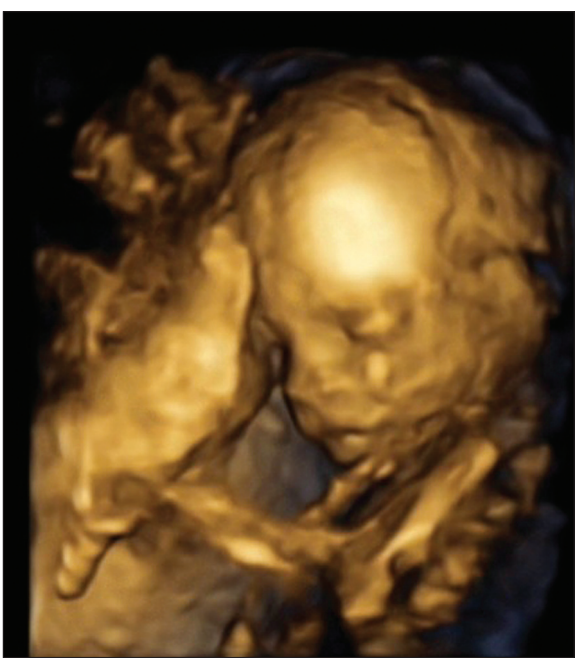

Figure 3: **3D image of the fetus' face at organ ultrasound screening in gestational week 18**

The recipient was followed up by frequent clinical visits and laboratory examinations, initially twice weekly during the first postoperative month and then every 2 weeks in months 2–6. Subsequently, she was seen monthly. The clinical examination involved a gynaecological examination with visual inspection of the transplanted uterine cervix, bacterial culture from the cervical canal, and occasional cervical biopsies. Ultrasound scans with transvaginal and abdominal probes were done to assess uterine size, and endometrial thickness and echogenicity. Uterine artery flow velocity waveforms on both sides were assessed by Doppler ultrasound, with the abdominal probe placed just above the inguinal ligament.

Biopsies of the uterine cervix were obtained at predetermined timepoints (at 1, 2, and 4 weeks, and monthly thereafter) and in the event of pathological signs (abnormal vaginal discharge, fever, discoloured cervix, or abdominal pain) that could be related to local infection or graft rejection. The histological examination of the biopsies used a uterine rejection grading system that was initially developed for the non-human primate uterus.[13] Any presence of cervical intraepithelial neoplasia was followed by tests for human papillomavirus.

Clinical follow-up also included monitoring of blood pressure and bodyweight, and laboratory monitoring of: serum creatinine and liver enzymes; blood haemoglobin, leukocytes, platelets, iron store, and glucose; urine albumin or creatinine; blood tacrolimus concentration (measured in whole blood by an automated chemiluminescent immunoassay; Abbott Diagnostics, Abbott Park, IL, USA) and concentrations of mycophenolate mofetil (measured by an enzyme multiplied immunoassay technique [Horiba ABX Pentra; Thermo Fisher Scientific, Waltham, MA, USA]).

During pregnancy, the patient was monitored according to Sahlgrenska University Hospital's routine programme for pregnant transplant patients, including frequent visits (every 2–3 weeks) to specialists in high-risk obstetrics and transplantation. Ultrasound data relating to the pulsatility index of the uterine arteries and umbilical artery and fetal growth were compared against data from the normal population.[14,15]

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

The first menstruation in the recipient occurred spontaneously 43 days post-transplantation and continued for 4 days. She then had regular menses with a median interval of 32 days and ranging between 26–36 days. The endometrium showed typical changes in median width (maximum 11·3 mm; range 7·9–15·3 mm). The blood flow velocity waveforms of the uterine arteries were similar on the left and right side and were within the low to normal range throughout the observation period. Median pulsatility indices were 1·27 (range 0·89–2·37) on the left side and 1·83 (1·30–2·54) on the right side.

Two mild rejection episodes (one after 9 days and the other at 6 months and 24 days) and one borderline

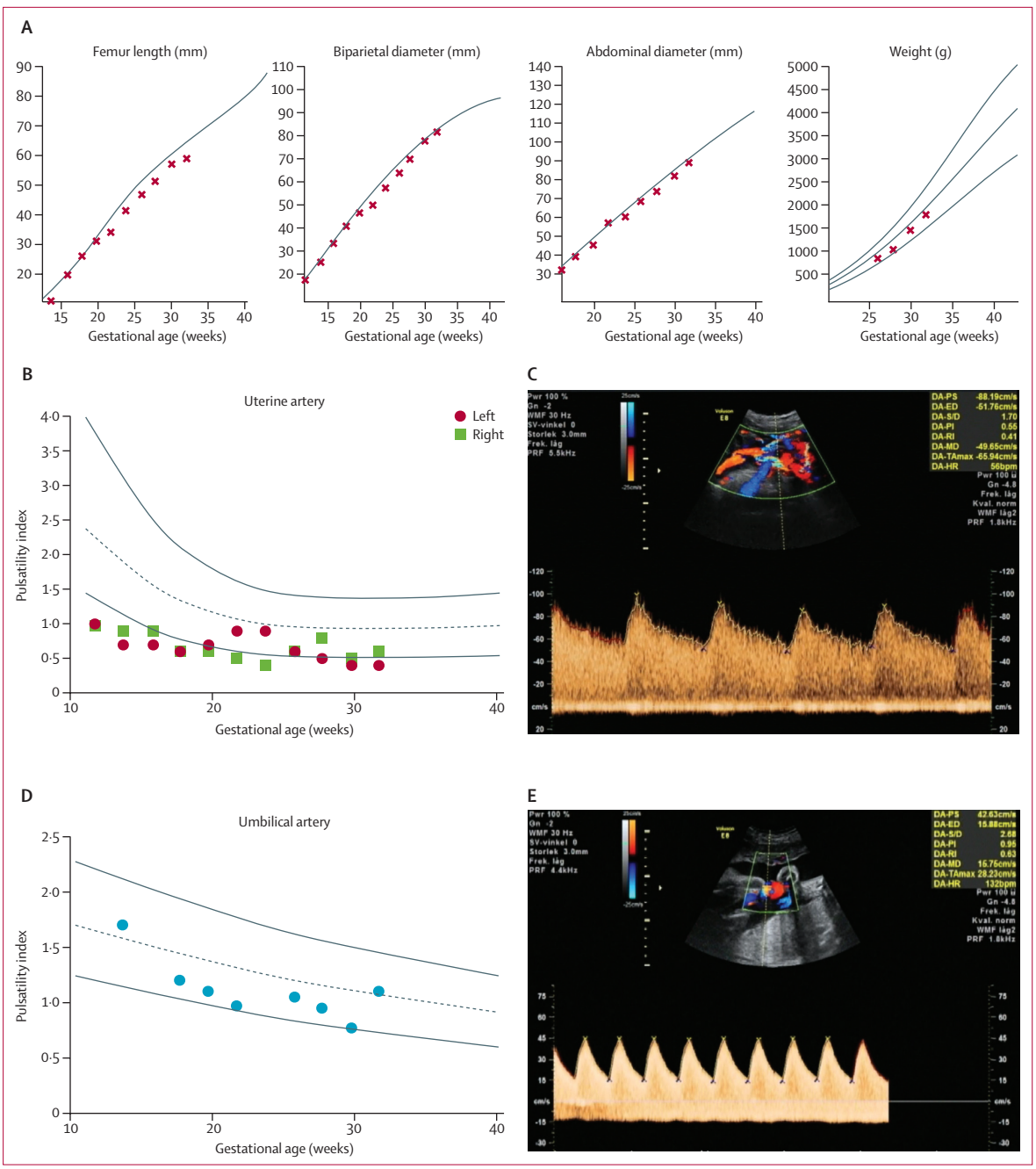

Figure 4: **Ultrasound estimates of fetal growth and flow velocity in maternal uterine arteries and fetal umbilical artery during pregnancy**
(A) The femur length, biparietal diameter, abdominal diameter, and estimated fetal weight were all within the normal range. (B) The uterine artery flow velocity waveform (pulsatility index) was low to normal on both sides; (C) shows a typical recording. (D) The pulsatility index in the umbilical artery was within the normal range during pregnancy; a recording is displayed in (E).

episode (at 2 months and 28 days) were diagnosed by cervical biopsies. These episodes occurred without any clinical symptoms. The rejection diagnosis was based on changed histology with a moderate increase in lymphocyte density in the stroma and the epithelium, with some spongiosis in the basal level of the epithelium. Occasional apoptotic cells were recorded in the basal epithelium together with reactive changes in the surface epithelium. The rejection episodes were successfully reversed by corticosteroid treatment (see later).

A biopsy 8 months and 12 days after transplantation showed moderate squamous epithelial dysplasia (p16 positive) and koilocytosis. Human papillomavirus typing

identified subtype 31. A follow-up mini-conisation 2 weeks later, with inclusion of the biopsy site, was normal and subsequent biopsies displayed no dysplasia or koilocytosis.

The median blood concentrations of tacrolimus were 9·5 ng/mL (range 3·0–12·0) during the first 3 months, 8·9 ng/mL (8·5–9·9) during months 4–6, 12 ng/mL (7·9–12·0) during months 7–9, and 10·0 (9·5–12·0) during months 10–12. The median blood tacrolimus concentration for the entire pre-pregnancy period was 9·8 ng/mL (range 3·0–12·0). The mycophenolate mofetil area under the curve was 51·3 mg·h/L (range 36·5–96·7) during the 10 months of treatment. The first mild rejection episode (on day 9) was treated with methylprednisolone 500 mg intravenously for 3 days, followed by oral prednisolone (starting dose 10 mg twice daily) for 4 weeks in a tapered protocol. The borderline episode (at 2 months and 28 days) was only treated with oral prednisolone in the 4 weeks-tapered protocol. The second mild rejection episode (at 6 months and 24 days) was initially treated in the same way as the previous mild rejection episode (see above) but the patient continued with oral prednisolone (5 mg daily) for the whole pre-pregnancy period. Since the recipient experienced two clear rejection episodes and because one was fairly close in time to the planned omission of mycophenolate mofetil, we decided that the patient would stay on mycophenolate mofetil for a total of 10 months and that azathioprine (2 mg/kg per day) would then be the replacement antiproliferative immunosuppression. Blood pressure and haemoglobin concentration during this initial post-transplantation year were stable at around 120/70 mm Hg and 100 g/L, respectively.

After in-vitro fertilisation, before transplantation, one cryopreserved embryo was obtained from one oocyte in cycle 1, four embryos from nine oocytes in cycle 2, and six embryos from eight oocytes in cycle 3. The embryo transfer was done 1 year after uterus transplantation and took place in the early luteal phase of the menstrual cycle. 3 days after a positive urinary luteinizing hormone test, three embryos were thawed, one of which was acceptable for transfer. The four-cell embryo had three surviving blastomeres. At embryo transfer, the endometrial thickness was 6 mm. The patient was on continuous treatment with 75 mg acetylsalicylic acid once daily since transplantation. She was treated with oral folic acid (250 µg twice daily) from 2 weeks before embryo transfer and vaginal progesterone (Lutinus; Ferring, Copenhagen, Denmark) 100 mg three-times daily for 9 weeks after embryo transfer.

A pregnancy test was positive 3 weeks after embryo transfer and 2 weeks later the intrauterine location and heartbeat of the fetus was detected by ultrasound. The pregnancy proceeded normally between 8 and 31 weeks of gestation—ie, the pregnant woman gained 8 kg in weight, cervical length was between 43 mm and 50 mm, haemoglobin was around 100 g/L (except for week 20 [see later]), blood pressure and blood glucose concentrations were in the normal range, and no proteinuria occurred (table). 3D ultrasound organ screening in gestational week 18 was normal (figure 3). Creatinine concentrations, in this patient with a single kidney, were somewhat raised during the pre-pregnancy period (median 94 µmol/L [range 80–111]) and were further elevated during pregnancy (106 µmol/L [86–147]), with creatinine concentrations constantly higher than 100 µmol/L from gestational week 27. Ultrasound examination then showed a slight hydronephrosis in the single right kidney. She was working full time until the day before delivery.

During pregnancy, growth in fetal femur length, biparietal diameter, abdominal diameter, and estimated weight were normal (figure 4A). The blood flow velocity waveform of the uterine arteries remained within the normal to low range during pregnancy (figure 4B, C). The pulsatility index of the umbilical artery was normal throughout pregnancy (figure 4D, E).

The patient was admitted to the obstetrics division at Sahlgrenska University Hospital (Gothenburg, Sweden) at 31 weeks and 5 days because of pre-eclampsia, with a blood pressure of 180/120 mm Hg, mild headache, proteinuria (urine albumin 18 mg/L), and lowered platelet count (96×10^9/L). She was given labetalol (200 mg three times daily) and nifedipine (10 mg twice daily), both administered orally, to reduce the raised blood pressure. Betamethasone (12 mg intravenously) was administered as respiratory distress syndrome prophylaxis. The patient had an increasing number of uterine contractions and cardiotocography showed occasional variable decelerations from around 10 h after admission. We applied continuous cardiotocography surveillance and because of ongoing repeated episodes of an abnormal cardiotocography

Figure 5: **The newborn baby just after birth**

pattern, a caesarean section was done 16 h after admission. The caesarean section was undertaken in spinal analgesia through a midline incision. Only mild adhesions existed. After careful dissection of the bladder peritoneum, with localisation of the major uterine vessels, a low-transverse uterine incision was made. The child was in breech position and was delivered 26 min after skin incision. The placenta (weight 375 g) was delivered immediately afterwards. Histological examination of the placenta showed a normal umbilical cord with tree vessels and no inflammation. The placental villi showed pre-eclampsia-like changes with villi of small calibre in relation to gestational length. Increased fibrin deposits and signs of fibrin thrombi were recorded in villous capillaries. Inflammation was not seen. After delivery, the uterus contracted well on intravenous oxytocin (10 IU). The uterine incision was sutured with a standard two-layered technique. A small myometrial biopsy was taken from the fundus uteri and the histology of this was normal.

The birthweight of the neonate was 1775 g, length was 40 cm, and head circumference 28·5 cm (figure 5). APGAR scores were 9, 9, 10 and the umbilical artery pH was 7·21. The first postnatal week was uneventful and the baby was in good condition, requiring only phototherapy and room air. The mother was in a good condition the day after delivery and her blood pressure was normalised spontaneously, with no further treatment. She was discharged from the hospital 3 days after caesarean section and is followed up in regular outpatient visits. The creatinine concentrations had decreased from 143 µmol/L on the day of delivery to 98 µmol/L 5 days later. The baby was discharged in good health from the neonatal unit 16 days after birth and the weight 21 days after delivery was 2040 g.

During pregnancy, the recipient continued with triple immunosuppression consisting of tacrolimus, azathioprine, and prednisolone. Tacrolimus concentrations tended to decrease during the first trimester and the tacrolimus dose was gradually increased to 40% of the pre-pregnancy dose (which was 10 mg daily) to achieve intended blood concentrations between 8 and 10 ng/mL. The median tacrolimus concentration during the entire pregnancy period was 7·3 ng/mL (range 4·6–13·0). During the first trimester, the patient presented with increasing leucopenia and anaemia, and the dose of azathioprine was consequently lowered from 2 mg/kg per day to 1·2 mg/kg per day during a period of 4 weeks. After 18 full weeks and 2 days of gestation, the protocol cervical biopsy showed mild rejection but the patient was asymptomatic. Inflammation was seen both in the subepithelial stroma and in the basal parts of the squamous cell epithelium (figure 6A). Apoptosis was also evident in the epithelium. No donor-specific HLA antibodies were detected at that time. She was treated with intravenous methylprednisolone for 3 days (250 mg on day 1, and 125 mg on days 2 and 3). The control biopsy after 20 full gestational weeks and 1 day (figure 6B) and the per-protocol biopsy after 30 full gestational weeks and 6 days were normal. The dose of azathioprine was then increased to its original dose (2 mg/kg per day) and the patient continued on this dose until delivery. The blood concentrations of tacrolimus during pregnancy were similar to those during the initial post-transplantation year (median 8·1 ng/mL [range 6·1–13·0]).

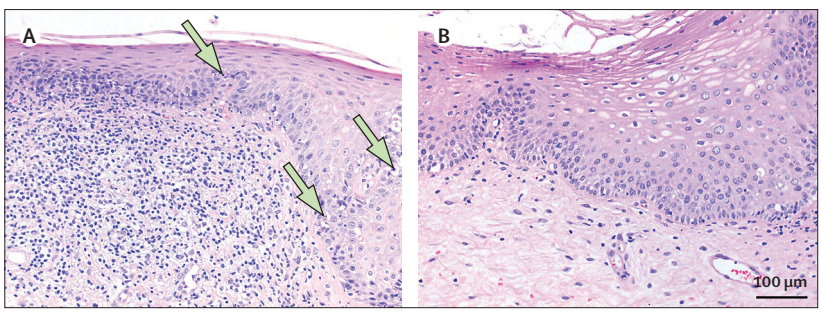

Figure 6: **Light micrographs from cervical biopsies showing rejection and normalisation 2 weeks after treatment**
(A) Biopsy showing mild rejection. A dense infiltrate of leukocytes, mainly lymphocytes, exists in the stroma and infiltrates into the basal layers of the epithelium, where occasional apoptotic cells can be seen (arrows). (B) 1 week later, after anti-rejection treatment, the leukocyte infiltration is almost completely reversed. The slides are stained with haematoxylin and eosin.

The haemoglobin concentration 3 days before embryo transfer was 101 g/L and the patient started treatment with oral ferrous sulphate 100 mg twice daily. In gestational week 8, haemoglobin fell to 84 g/L despite the ferrous sulphate treatment. One dose of 500 mg intravenous ferric carboxymaltose (Ferinject; Orifarm AB, Stockholm, Sweden) was administered and treatment with 60 µg weekly subcutaneous darbepoetinalfa (Aranesp; Amgen, Thousand Oaks, CA, USA) was initiated. Table 1 provides the blood haemoglobin concentrations throughout the pregnancy.

Discussion

Despite remarkable advances in infertility treatment, since the birth of the first in-vitro fertilisation baby in 1978[16] major forms of uterine factor infertility have remained untreatable. Our demonstration of a livebirth after uterus transplantation in a woman born with no uterus has eradicated the diagnosis of absolute uterine factor infertility.

This livebirth after human uterus transplantation comes after more than a decade of intensive animal research in this specialty by several groups worldwide. The ethical issues surrounding uterus transplantation are complex in their specific facets of non-maleficence, autonomy, beneficence, justice, and dignity. Modern uterus transplantation research began shortly after the first human hand transplantation in 1998,[17] which in many ways opened up the field of transplantation surgery to also include non-vital tissues or organs that after transplantation would have the chance to substantially increase an individual's quality of life. Thus, face and larynx transplantation have also now reached the stage as established clinical procedures.

Most types of organ and tissue transplantation that are done today use graft obtained from a deceased donor. The live donor concept is established for renal transplantation and for liver transplantation, but rates of live renal and liver donation vary greatly between countries. In the present study, the live donor was a close family friend of the recipient, by contrast with the other donors of our study cohort who were all family members. Our patient's first choice of donor was her mother, but blood group incompatibility prevented her from taking part in the study.

Uterus donation from live donors adds another key element into a risk–benefit analysis concerning uterus transplantation.[18] Uterine donation from a deceased donor would obviously substantially reduce the overall risks and complexity of the surgical procedure. In the uterus transplantation that was done in Turkey in 2011, the uterus was from a heart-beating, brain-dead, 22-year-old female, donor who had never been pregnant. Naturally, the young age of that uterus and its extensive vasculature would offer a benefit but this has to be balanced against the advantage of a uterine graft that has proved its functionality in terms of normal pregnancies. Moreover, the live donor concept allows for meticulous diagnostic workup of the uterine graft to exclude pathologies that could interfere with fertility potential, such as adenomyosis and endometrial polyps.

A specific concern with uterine donation is of course to exclude pathological disorders of the uterus that might be related to precancerous disorders. Both the donor and the recipient were human papillomavirus negative at our pretransplantation examinations and the presence of cervical dysplasia with human papillomavirus at 9 months after transplantation was unexpected. The reason for the temporary human papillomavirus positivity and secondary dysplasia is unknown.

A major reason to do in-vitro fertilisation before transplantation was that we needed to ascertain that fertility, in terms of fertilisation and initial embryo development, existed within the couple. Moreover, an in-vitro fertilisation procedure after transplantation might be more difficult than one before transplantation because of the abnormal uterine vascular pedicles and anastomosis sites that might increase the risk of bleeding at oocyte pick-up and because the immunosuppressed patient may have an increased risk of pelvic infection after the pick-up procedure. The first in-vitro fertilisation cycle of the patient only generated one embryo for cryopreservation and she was subsequently stimulated with very high gonadotrophin doses to obtain the normal oocyte yield. A previous report on surrogate in-vitro fertilisation outcome of patients with Rokitansky syndrome,[19] shows that women with the atypical Rokitansky form—as was the case with our patient—are poor responders and have a lower fertilisation rate than do women with the typical form of Rokitansky syndrome.

The patient became pregnant at her first transfer of a frozen-thawed embryo. The chance of that occurring in our setting and in her age group is around 16%.[20] Naturally, we did a single embryo transfer to avoid the risk of any multiple pregnancy, which would be an unnecessary additional obstetrical risk factor.

We diagnosed one episode of mild rejection during pregnancy. The histological rejection signs of the uterine cervix were reversible with a short course of increased corticosteroid treatment. The pregnancy itself induces a local immunosuppressed state and the myometrial and endometrial tissue of the uterine body might well have not shown the type of rejection-related inflammation that we diagnosed in the uterine cervix.

The pregnancy of our patient proceeded essentially normally for the first 31 weeks. The growth curves and the blood velocity waveforms of the umbilical cord were normal throughout pregnancy. In autologous uterus transplantation in sheep—which have pelvic vessels sizes similar to those in human beings—normal birthweights were also recorded.[21] Pulsatility indices of uterine arteries were within the normal to low range. A lower pulsatility index indicates decreased resistance of the blood vessels, which might well be caused by the denervated nature of the uterine graft and the absence of normal nerve-mediated vasocontrictive mechanisms. The normal to low pulsatility index of the uterine arteries would suggest a low, rather than high, risk of pre-eclampsia development. However, this specific situation of vascular supply from the external iliacs and with a transplanted uterus might ultimately reduce the predictive value of changes in uterine artery waveforms.

***Panel:* Research in context**

Systematic review

We searched PubMed for all publications with the search terms "uterus" AND "transplantation" AND "human", and we also ran a search for "uterine" AND "transplantation" AND "human". The searches included all papers, published in English language only, from 1956 up until Sept 30, 2014. The first search yielded 998 published papers and the second search provided 2228. All titles and abstracts, when available, were read to find out if they contained information about any human cases. Published data on 11 human uterus transplantation cases were found. The first human case (transplantation done in 2000) was reported in one research paper and the second case (in 2011) was reported in three research papers, covering surgery with 12-month follow-up, in-vitro fertilisation, and embryo transfer attempts with two early miscarriages, and one video presentation of surgical technique. The other nine cases were reported in our study detailing the surgery and 6-months outcome. Additionally, four reports existed on the surgical techniques of uterus retrieval from human deceased donors. The searches found nine articles about the ethics of uterus transplantation and 48 general reviews that at least partly covered the topic of human uterus transplantation.

Interpretation

Our present study is the first report of a livebirth after uterus transplantation and is thereby a proof of concept for this treatment of absolute uterine factor infertility. The efficiency of uterus transplantation as an infertility treatment is unclear and remains to be established. The livebirth rate of the complete cohort of the first clinical trial of uterus transplantation, which our patient belongs to, and results of future cohort studies will shed light on the efficiency of the procedure but also about what medical and psychological risks are involved.

The reason for the development of pre-eclampsia in this specific case is not known, but several plausible explanations exist. Immunosuppression might increase the risk of pre-eclampsia, and after kidney transplantation the pre-eclampsia rate is as high as 22%.[22] This situation is similar to the patient in our study, who also had one kidney and was on immunosuppression. The fact that a single kidney by itself could be an underlying factor of pre-eclampsia development is indicated by the fact that the rate of pre-eclampsia is about two-times higher in kidney donors than in their pregnancies before donation.[23] Other factors that might underlie the development of pre-eclampsia are the old age of the uterus, as indicated by the sevenfold increased rate of pre-eclampsia in women 50–60 years of age undergoing oocyte donation.[24] However, whether or not this increased rate is due to uterine factors is unclear since it can also be caused by age-related changes in other organs. Moreover, pre-eclampsia is more common in in-vitro fertilisation pregnancies[25] than after natural conception, and the total allogeneic situation, with a donated uterus or a donated oocyte, can increase the risk of pre-eclampsia.[26]

Our patient had periods of anaemia, leucopenia, and increased creatinine concentrations during pregnancy, which were probably immunosuppression-related side-effects that became apparent during pregnancy, with its added demands on several systemic functions. Thus, the potential side-effects of immunosuppression—such as nephrotoxicity (tacrolimus), bone marrow toxicity (azathioprine), diabetogenic effect (corticosteroids and tacrolimus)—should be taken in account in the planning of a pregnancy attempt after uterus transplantation, to decide the best possible time for embryo transfer in relation to immunosuppressive medication.

Uterus transplantation is the first ephemeral type of transplantation that has been introduced in which the graft is not intended for lifelong use. The uterus can be removed after one or two babies have been born, which would reduce the long-term side-effects caused by the immunosuppressive drugs. The patient of our present study had been informed that we can recommend surgical removal of the uterus before a second pregnancy attempt in the case of any major side-effects of immunosuppression. Such a decision should not be taken during the first few months after delivery, to allow for spontaneous reversion of side-effects that might have been aggravated by the pregnancy. Moreover, this delay would provide further observation time to ensure that the delivered baby is healthy and allow the uterus to return to its normal size, which would simplify any hysterectomy surgery. The autonomy of the patients should be respected and any future decision to surgically remove the uterus needs to be made in consensus with the recipient and her partner.

In conclusion, our demonstration of the first livebirth after uterus transplantation opens up the possibility to treat the many young women with uterine factor infertility worldwide.

Contributors

MB initiated the study, did surgery, followed up the patients, and wrote the report. LJ did surgery, followed up the patients, obtained data, and wrote the report. HB and HH followed up the pregnancy, obtained data, and wrote the report. NK did surgery, followed up the patients, and obtained data. JM did histological analyses, obtained data, and wrote the report. PD-K and MG did surgery, followed up the patients, and obtained data. AE did anaesthesia and obtained data. MM did in-vitro fertilisation and obtained data. JE followed up the patients, obtained data, and wrote the report. CD-G did surgery, obtained data, and wrote the report. AH did surgery and wrote the report. MO did surgery and wrote the report. LN did in-vitro fertilisation, obtained data, and wrote the report.

Declaration of interests

We declare no competing interests.

References

1 Marshall LM, Spiegelman D, Barbieri RL, et al. Variation in the incidence of uterine leiomyoma among premenopausal women by age and race. *Obstet Gynecol* 1997; **90:** 967–73.

2 Al-Inany H. Intrauterine adhesions. An update. *Acta Obstet Gynecol Scand* 2001; **80:** 986–93.

3 Grimbizis GF, Camus M, Tarlatzis BC, Bontis JN, Devroey P. Clinical implications of uterine malformations and hysteroscopic treatment results. *Hum Reprod Update* 2001; **7:** 161–74.

4 Saravelos SH, Cocksedge KA, Li TC. Prevalence and diagnosis of congenital uterine anomalies in women with reproductive failure: a critical appraisal. *Hum Reprod Update* 2008; **14:** 415–29.

5 Sieunarine K, Zakaria FB, Boyle DC, et al. Possibilities for fertility restoration: a new surgical technique. *Int Surg* 2005; **90:** 249–56.

6 Brannstrom M, Wranning CA, Altchek A. Experimental uterus transplantation. *Hum Reprod Update* 2010; **16:** 329–45.

7 Diaz-Garcia C, Johannesson L, Enskog A, Tzakis A, Olausson M, Brannstrom M. Uterine transplantation research: laboratory protocols for clinical application. *Mol Hum Reprod* 2012; **18:** 68–78.

8 Brannstrom M, Johannesson L, Dahm-Kahler P, et al. First clinical uterus transplantation trial: a six-month report. *Fertil Steril* 2014; **101:** 1228–36.

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

10 Ozkan O, Akar ME, Ozkan O, et al. Preliminary results of the first human uterus transplantation from a multiorgan donor. *Fertil Steril* 2013; **99:** 470–76.

11 Erman Akar M, Ozkan O, Aydinuraz B, et al. Clinical pregnancy after uterus transplantation. *Fertil Steril* 2013; **100:** 1358–63.

12 Strubbe EH, Willemsen WN, Lemmens JA, Thijn CJ, Rolland R. Mayer-Rokitansky-Kuster-Hauser syndrome: distinction between two forms based on excretory urographic, sonographic, and laparoscopic findings. *AJR Am J Roentgenol* 1993; **160:** 331–34.

13 Johannesson L, Enskog A, Molne J, et al. Preclinical report on allogeneic uterus transplantation in non-human primates. *Hum Reprod* 2013; **28:** 189–98.

14 Nicolaides KH, Rizzo G, Hecher K, Ximenes R. Doppler in obstetrics. London: The Fetal Medicine Foundation, 2002.

15 Marsal K, Persson PH, Larsen T, Lilja H, Selbing A, Sultan B. Intrauterine growth curves based on ultrasonically estimated foetal weights. *Acta Paediatr* 1996; **85:** 843–48.

16 Steptoe PC, Edwards RG. Birth after the reimplantation of a human embryo. *Lancet* 1978; **2:** 366.

17 Dubernard JM, Owen E, Lefrancois N, et al. First human hand transplantation. Case report. *Transpl Int* 2000; **13** (suppl 1)**:** S521–24.

18 Olausson M, Johannesson L, Brattgard D, et al. Ethics of uterus transplantation with live donors. *Fertil Steril* 2014; **102:** 40–43.

19 Raziel A, Friedler S, Gidoni Y, Ben Ami I, Strassburger D, Ron-El R. Surrogate in vitro fertilization outcome in typical and atypical forms of Mayer-Rokitansky-Kuster-Hauser syndrome. *Hum Reprod* 2012; **27:** 126–30.

20 Thurin A, Hausken J, Hillensjo T, et al. Elective single-embryo transfer versus double-embryo transfer in in vitro fertilization. *N Engl J Med* 2004; **351:** 2392–402.

21 Dahm-Kahler P, Wranning C, Lundmark C, et al. Transplantation of the uterus in sheep: methodology and early reperfusion events. *J Obstet Gynaecol Res* 2008; **34:** 784–93.

22 Kallen B, Westgren M, Aberg A, Olausson PO. Pregnancy outcome after maternal organ transplantation in Sweden. *BJOG* 2005; **112:** 904–09.

23 Reisaeter AV, Roislien J, Henriksen T, Irgens LM, Hartmann A. Pregnancy and birth after kidney donation: the Norwegian experience. *Am J Transplant* 2009; **9:** 820–24.

24 Paulson RJ, Boostanfar R, Saadat P, et al. Pregnancy in the sixth decade of life: obstetric outcomes in women of advanced reproductive age. *JAMA* 2002; **288:** 2320–23.

25 Sazonova A, Kallen K, Thurin-Kjellberg A, Wennerholm UB, Bergh C. Obstetric outcome in singletons after in vitro fertilization with cryopreserved/thawed embryos. *Hum Reprod* 2012; **27:** 1343–50.

26 Levron Y, Dviri M, Segol I, et al. The 'immunologic theory' of preeclampsia revisited: a lesson from donor oocyte gestations. *Am J Obstet Gynecol* 2014; published online March 19. DOI:10.1016/j.ajog.2014.03.044.