Auto uterine transplantation: an experimental clinical trial in female dog model

Thesis
Submitted for Fulfillment of Master Degree
in Obstetrics and Gynecology

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2016

INTRODUCTION

Infertility has a negative quality-of-life aspect for most women ***(Aarts et al., 2011)***. Infertility is categorized by WHO as a disease; a couple has met the criteria of being infertile if the woman has not become pregnant after 2 years of regular intercourse with no use of contraception. In many societies, including most parts of the western world, the term infertility usually refers to a 12 months involuntarily childlessness after contraceptive-free intercourse ***(Akhi, 2012)***. Global estimates suggest that nearly 72.4 million couples experience fertility problems ***(Boivin et al., 2007).***

Infertility is generally divided into primary infertility, when there has never been a child born within a couple, and secondary infertility, when there is a failure to conceive following a previous pregnancy within the couple. It is problematical to exactly estimate the prevalence of infertility, but involuntarily childlessness is usually estimated to be present among around 15% of all couples ***(Forti et al., 1998).*** It is stated that around 30% of the infertility causes are due to male factor and 30% to female factor. Around 10-15% of causes are due to combined factors in both male and female and the rest are still categorised as unexplained infertility ***(Akhi, 2012)***.

Female infertility can be due to dysfunction on either the hypothalamic/pituitary level, the ovarian level or the uterine/cervical/ vaginal level ***(Akhi, 2012).*** Uterine-factor infertility includes cases of absence of the uterus or those with

nonfunctional uterus in terms of pregnancy capability and occurs due to congenital or acquired causes such as uterine agenesis, hysterectomy, uterine hypoplasia, arcuate uterus and intrauterine adhesions ***(Brännström et al., 2012).***

The overall prevalence of uterine-factor infertility is approximately 3%-5% in the general population ***(Ramirez et al., 2011).***

A possible treatment of uterine infertility is uterus transplantation ***(Altchek, 2003; Brannstrom et al., 2003; Brannstrom, 2007),*** providing an alternative to adoption or gestational surrogacy to acquire motherhood.

Uterus transplantation (UTx) has been conducted in several animal models including dog ***(Eraslan et al., 1966)*** , *mouse* ***(El-Akouri et al., 2002, 2003a,b),*** rat ***(Wranning et al., 2008a,b, 2010a,b)***, sheep ***(Dahm-Kahler et al., 2008; Wranning et al., 2010)***, pig ***(Avison et al., 2009)***, baboon, and macaque ***(Del Priore et al., 2008).***

Autologous transplantation is transplantation of the uterus removed from an animal and transplated again to the same animal. The first mention about uterine transplantation in the scientific literature is from 1927 ***(Eraslan et al., 1966)***.

The experimental work on uterine transplantation was reinitiated in the 1960s and the 1970s, with the research primarily involving auto transplantations (replantations), where the uterus with its appendages was isolated from the animal, taken out for a short period of ischemia and then reintroduced into the same animal. Two principally different modes of

securing blood flow to the transplanted uterus were tested, vascular anastomosis to achieve immediate reperfusion, and attachment of the uterus to an abdominal surface, to acquire a gradual revascularization through outgrowth of new blood vessels, were examined and compared. Vascular anastomosis, at the level of the internal iliac vessels, was first used in en bloc auto transplantations of the uterus, oviducts and ovaries in the dog ***(Yonemoto et al., 1969).***

Auto-uterine transplantation in non-human primate model (cynomolgus macaque) with anastomosis of two uterine arteries to the external iliac artery was performed and achieved pregnancy for the first time in this model ***(Mihara et al., 2012).***

A technique for isolation of the bicornuate dog uterus and adnexae with blood vessels for re-anastomosis was described several decades ago ***(Eraslan et al., 1966).*** The common part of the internal iliac artery was dissected from its origin and freed caudally with ligations of all branches except the uterine and superior vesicle arteries. The uterine veins were dissected cranially from the uterus to the fusion of the internal and external iliacs. The ovarian vessels were divided, followed by clamping of the vagina. The blood flow to the specimen was then interrupted by division of the common internal iliac artery. Noteworthy is that the vaginal connection, although clamped, was intact during flushing and re-anastomosis and the uterus was never disconnected from the body. A similar dissection method was later used by another group ***(Truta et al., 1969)*** but with the difference that the vagina was at no time transected. Later experiments were proper auto-transplantations ***(Barzilai***

et al., 1973; Paldi et al., 1975) since the vagina was divided at an early stage. None of these reports describe dissection of the ureters, most likely since the arteries were dissected in a caudal direction from the common iliacs and freed only to a level before crossing the ureters. The utero-tubal-ovarian specimen could then not be removed from the body.

After auto-transplantation of utero-tubal-ovarian grafts in dogs, five pregnancies (11% pregnancy rate) with live births were seen, in the studies where pregnancy was one end-point ***(Eraslan et al., 1966; Truta et al., 1969; Barzilai et al., 1973)***. However, these experiments involved only short ischemic periods and the uterus was never outside the abdomen.

A decade ago a Saudi Arabian team, with minimal preparatory animal studies, performed the first human UTx attempt ***(Fageeh et al., 2002).*** The graft was removed after 99 days due to prolapse and necrosis.

The Brannstrom group of uterus transplantation has been active in the field since 1999 and the first publication in a scientific journal was in 2002. 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 fertilization treatment of the recipient and her partner had been done before transplantation, from which 11 embryos were cryopreserved. 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). One 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 hours later a caesarean section was done because of abnormal cardiotocography. A male baby with a normal birth weight for gestational age (1775 g) and with APGAR scores 9, 9, 10 was born ***(Brännström et al., 2014).***

Hypothesis:

In animals undergoing experimental auto uterine transplantation, isolation of uterine vessels especially veins needs good training.

AIM OF WORK

The aim of this study is training for isolation of uterine vessels and assesses the viability of the transplanted uterus after experimental auto- uterine transplantation in the female dog and examination for a gradual revascularization through outgrowth of new blood vessels.

Materials and Methods

Study design:

Twelve outbreed non-pregnant female dogs of proven fertility were divided into two groups. Animals were in apparent general condition and were kept in medical research center, Ain Shams University Hospitals and were left to acclimatize to their surroundings for at least 1 week before surgery to reduce stress. All animals were kept for 4 weeks then 2nd look laparotomy was done for assessment of transplanted uterus and for histopathological examination.

Control group:

Consisted of two normal animals subjected to sham operation and they underwent general anaesthesia and laparotomy without uterus transplantation. The animals were kept under care for 2nd look laparotomy 4 weeks later to be compared with the study group.

Study group:

Consisted of ten animals under went laparotomy as a model for the auto uterine transplantation. UTx was performed using a previously described surgical technique by **Eraslan et al. (1966); Wingate et al. (1970); Mattingly et al. (1970); Barzilai et al. (1973 and Paldi et al., 1975).** The blood vessels from the uterus were isolated up to and including the internal iliac arteries and veins. Reanastomosis was performed end-to-end anastomosis of the common branch of the internal iliac artery and the common branch of the internal iliac vein.

Inclusion criteria:

-Age of animal between (fertile age 1-2 years).

Exclusion criteria:

- Extremes of age (too young<1 year or old age).

- Medical problem e.g diabetes mellitus or nutritional disease.

- Gynecological problem e.g congeital malformations.

Type of study:

The study is considered as exploratory descriptive animal study.

Site of study:

Medical research center, Ain Shams University Hospitals

Technique:

On the day of the operation, the animals will be brought to the operating room and will be positioned in a supine position. The anterior abdominal wall will be shaved and cleaned. The region will be disinfected with povidone iodine 10% solution and draped.

Anesthesia:

The dog will be anesthetized with ketamine hydrochloride through intravenous injection (50 mg/kg) and xylazine (7 mg/kg). The urinary bladder will be catheterized through the urethra with a balloon catheter (outer diameter, 4.7 mm).

Surgical technique

All surgical procedures will be performed using the surgical procedure described by ***Eraslan et al. (1966); Wingate et al.(1970); Mattingly et al.(1970); Barzilai et al. (1973) and Paldi et al., (1975).*** A midline incision in the skin from the pubic bone up to the level of umbilicus will be performed. Then the rectus sheath then muscles of anterior abdominal wall will be opened. The intestines will be packed into the upper abdomen. The round ligament will be processed, and the broad ligaments of the uterus and retroperitoneum will be exposed.

Hysterectomy will be started with opening the broad ligament and the ureters were identified. Hysterectomy will include the uterus with the connected blood vessel pedicles, cervix, upper part of vagina and the proximal portion of two uterine horns leaving small portion of distal part for reanastomosis. The vessels, except for the ovarian arteries and veins, will be selected as vascular grafts because the utero-ovarian ligaments will be cutted and tied bilaterally to preserve the ovaries and tubes.

Eight vascular clamps will be applied to each uterine and utero-ovarian artery and vein, securing the proximal and distal ends of the uterine vasculature. Heparin is then administered. Finally, all the vessels will be transected using microsurgical scissors, and total abdominal hysterectomy will be achieved.

Graft implantation:

The uterus will be transferred immediately again to the same animal after flushing any residual blood from all the uterine vessels by Sodium heparin (12,500 U) and irrigation of uterus by a 23-gauge intravenous catheter with histidine-tryptophan ketoglutarate.

All vascular uterine pedicles will be identified; the uterus is positioned anatomically into the pelvic cavity. The vagina will be anastomosed first to ensure proper orientation and positioning of the specimen, the vaginal tissue is approximated with 2-0 Vicryl sutures in a continuous, non-interlocking fashion to avoid tissue ischemia followed by end-to-end vascular re-anastomosis, ensuring vascular integrity, hemostasis and patency.

Vascular anastomosis:

All vessels will include in vascular pedicle were approximated using the end-to-end anastomosis technique ***(Eraslan et al., 1966; Truta et al., 1969; Yonemoto et al., 1969; Mattingly et al., 1970 and Paldi et al., 1975)*** with continuous, non-interlocking sutures. The internal iliac veins will be first approximated with 7-0 polypropylene sutures. The clamps on the veins will be released to evaluate venous patency. After documenting venous patency, the clamps will be applied again till doing arterial anastomosis. The internal iliac arteries will be approximated with 7-0 polypropylene sutures. The vascular clamps on arteries will be released. The uterine horns will be rejoined. The uterus will be attached to the

peritoneum on the pelvic side in order to immobilize the uterus and avoid poor fixation of the uterus. The intestines will be repositioned into the abdominal cavity and the abdominal wall was closed in two layers with 0 vicryl sutures. The abdomen will be closed after ensuring an absence of problems in the abdominal cavity.

Postoperative care and follow-up:

All animals will be managed under sterile precautions and kept in medical research center in Ain Shams University hospitals for approximately 4 weeks. To prevent postoperative blood clotting, low-molecular-weight heparin (20 mg/day) will be given. Vital signs, food and water intake, vaginal discharge and rectal temperatures will be recorded daily. Moxifloxacin hydrochloride (400 mg/day orally) will be administered for 7 days as preventive antibiotic therapy.

Outcome measures:

1-Primary outcome:

During the operation**,** Successful reperfusion of the graft will be judged to have occurred when the color of the uterus changes during reperfusion from its blanched appearance after flushing to a pink color (within 1–2 min). Inspection of the patency of the vascular anastomosis sites, pulsation through the transplanted vessels and occurrence of spontaneous and oxytocin induced uterine contractions will be recorded.

The healing of the vaginal anastomosis will be assessed by manual distension of the vaginal-vaginal anastomosis site

and incisions to detect tissue blood flow will be made into the vagina.

2- Secondry outcomes:

Histopathology:

A total abdominal hysterectomy will be Performed 4 weeks after the transplantation procedure for histologic studies. Biopsy specimens from the uterus, cervix will be embedded in paraffin, sectioned and stained with hematoxylin-eosin. All the sections will be analyzed by light microscopy for signs of ischemic injury, infection, thrombosis, edema, stasis and necrosis and apoptosis.

Results:

Viable uterine tissue and vascular patency will be observed during operation and second-look laparotomy. Histological analysis of the graft tissue will reveal tissue architecture. Results will be tabulated and represented in images of histological analysis.

Ethical considerations:

Ethics in medicine is based on the moral, religious and philosophical principles as well as the values of the society. Uterus transplantation would need to satisfy, as any surgical innovation, criteria as defined by FD Moore, i.e. laboratory background, field strength and institutional stability ***(Moore, 2000)***.

We think it is essential that uterus transplantation is discussed from an ethical perspective, in parallel with the animal UTx research. The ethics around UTx will naturally be

influenced by the active debates surrounding both the field of transplantation surgery and that of reproductive medicine and in particular assisted reproduction ***(Dondorp et al).*** UTx would need to satisfy accepted bioethical principles (respect for autonomy, beneficence, non-maleficence and justice) and their applications (informed consent, appropriate assessment of risk and benefit and fair selection of individuals). The laboratory background of UTx should involve extensive experience of the procedure in several species and with excellent success rate, taking into account the learning curve of UTx surgery ***(Wranning et al., 2008a).*** The animal models should involve non-human primates, to include experimental circumstances which would be similar to human UTx. Studies of human pelvic vascular anatomy could also be considered, if these could be carried out at elective surgery for clinical reasons. Satisfactory field strength implies expertise and long tradition of the institution in all clinical fields that may be involved. Institutional stability implies that an innovative surgical procedure, such as UTx, should only be carried out in a hospital where there is sufficient support for the experimental procedure and with team members that have worked together for a long time. Evaluation of stability of the institution and the team should include assessment of resources and commitment to care for a living donor, the recipient and her partner as well as for the future child for many years. The important ethical principle of a dominance of benefits over risks should thoroughly be analyzed also for UTx, where four subjects may be involved ***(Brannstrom et al., 2010).***

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