CEOG Clinical and Experimental Obstetrics & Gynecology
Original Research

The auto-transplantation of uterus in swine: surgical techniques and long-term graft survival

Xiao-Li Zhang[1,3*], Wei-ping Li[1*], Zhong-Yu Liu[1*], Yuan-Qing Yao[1], Hong-Xu[2]

[1]Department Gynecology and Obstetics, [2]Department Ultasonography, PLA General Hospital, Beijing [3]Department Gynecology and Obstetics, CAPF General Hospital, Beijing (China)

Summary

Objective: Uterus transplantation (UTx) provides a available treatment for women with absolute uterus-related infertility. The aim of this study was to develop surgical techniques for UTx and to observe the long-term graft survival in the swines. *Materials and Methods:* Ten female swines received uterus auto-transplantation surgery after general anesthesia. The operative time, ischemic time, and vascular injury in each swine were recorded. The bicornual uterus with vaginal canal and main vessel were removed, bake-table preparation was performed with cooled at 4 °C, and perfused with heparin saline with continuous flushing. The uterus was placed in original pelvic position. The uterus arteries and veins were end-side anastomosed with external iliac arteries and veins in the host swines. *Results:* One case was randomly selected as the preliminary experiment. The remaining nine cases showed that the uterine arteries and veins were anastomosed successfully and only eight cases attained long-term survival. The host in another case died four days after surgery from suspicious embolism postoperation. The blood flow of uterus arteries was observed by Doppler ultrasonography at four days after the operation and no congestion was found. The second-look laparotomy at six months after surgical was subsequently performed and the uterus showed a normal morphology and size. However, the pregnancy was unsuccessful due to the shorter observation time, and the severe adhesion and tubal obstruction after surgery may have affected pregnancy of those swines. *Conclusion*: These preliminary experiences suggest that the uterine auto-transplantation using the end-side vascular anastomosed method is feasible. The accumulation of basic data and experiences from swine experiments can provide important guidance for establishment of UTx in humans.

Key words: Uterine transplantation; Swine; Uterine factor infertility.

Introduction

With the progress of assisted reproductive technology (ART), more and more infertility patients receive effective treatment. However, these patients with absolute uterine factor infertility (UFI) remain untreatable. Women with UFI due to the absence of the uterus or a non-functional uterus currently have no opportunity of having children. Recent studies suggest that these patients accounted for about 3%-5% of the general population [1-3]. UFI can be divided into the congenital and acquired types. The congenital type includes the Mayer–Rokitansky–Küster–Hauser syndrome (MRKHs) [4, 5] with the total absence of the uterus and the upper part of the vagina, which occurs in 1/4,500 in women, corresponding to around 1.5 million patients worldwide [6, 7] of uterus hypoplasia and uterus malformations. The acquired type includes hysterectomy due to malignant uterine tumor such as cervical cancer and benign diseases such as leiomyoma and postpartum hemorrhage. Particularly, leiomyoma is likely to be the most frequent cause of the benign tumor, and the incidence of leiomyoma in the reproductive-aged women is as high as 10% [8]. These women could not deliver a child and resort to gestational surrogacy. However, gestational surrogacy is not allowed in many countries due to ethics or religion, such as China. Uterine transplantation (UTx) is an important option for these women with UFI [9, 10]. It could graft a functional uterus for delivering children. Based on recent developments in transplantation techniques and immunology, the transplantation field has expanded to life-enhancing tissue transplant, such as hand, face, and uterus [11]. In total 11 cases of UTx have been performed in humans and Fageeh *et al.* reported the first human UTx attempt in Saudi Arabia in 2000 [12]. The transplantation recipient was a 26-year-old female who lost her uterus six years earlier due to postpartum hemorrhage, and the donor was a 46-year-old patient with multiloculated ovarian cysts, underwent a modified hysterectomy to preserve tissue, and vascular integrity, but the uterus only survived for 99 days after transplantation, Several reasons may been revealed such as acute thrombosis in vessels which resulted infarction in the uterine body. Furthermore, the acute vascular occlusion appeared to be caused by inadequate uterine structure support, which led to probable tension, torsion, or kinking of the connected vascular uterine grafts. The second case of uterus transplantation was performed in Turkey in 2011 from a multiorgan donor [13, 14]. The recipient subsequently un-

*Contributed equally.

Revised manuscript accepted for publication July 20, 2017

derwent two pregnancies, with early miscarriages occurring both times. The causes of pregnancy failures may be ascribed to the decreased vascular plasticity, placentation defects, and the loss of innervations. These factors may negatively affect the pregnancy potentials in the uterine allograft. The first two single cases were unsuccessful and were not based on the animal experiments preparations. Subsequently, nine transplantations were performed within a clinical UTx trial in Sweden between 2012–2013 [15–17]. The first live birth after UTx was reported in 2014, and so far, in total, four healthy babies have been born from this cohort [18]. Thereby human UTx has become a proof-of-concept as a therapeutic reality for women with absolute uterine factor infertility.

Over the past 15-years, multiple uterus transplant studies have been performed in different animal species, such as rabbits [19-20], swine [21-25], and non-human primates [26-28], and sheep [29]. Pregnancy and delivery after allograft with the use of immunosuppressive agents and autotransplantation have been shown in some animals, such as sheep, cynomolgus and monkeys [30, 31]. Study on swine as an animal model of uterine transplants is rarely reported, as the operations for vascular anastomosis in swine are more difficulty. However, the size of uterine vessels and the deep pelvis swine model are similar to the human one and it would be beneficial to simulate the clinical conditions. In the present study, the authors explored surgical techniques for uterus auto-transplantation and observed the long-term survival of the transplanted organ after operation. They focused on the surgical procedures and ischemic preservations during the uterus auto-transplantation. The swine model is needed to enrich our knowledge and experience for the future clinical application of UTx in humans.

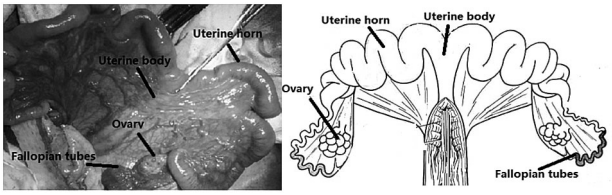

Figure 1. — The anatomical of the swine′s internal genital organs, Swine UTx model has larger bicornuate.

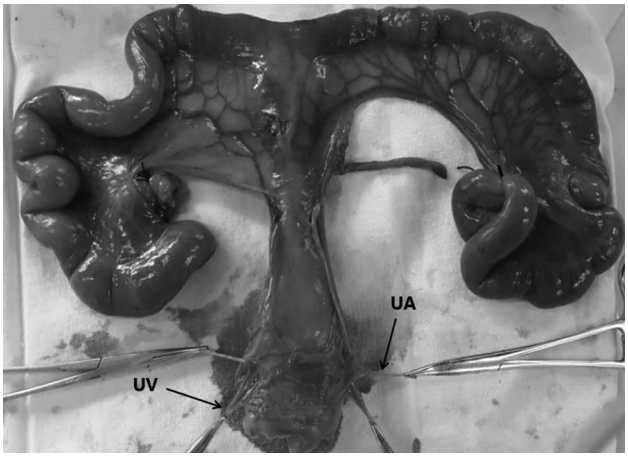

Figure 2. — Excised uterus. F: fallopian tube; UA: uterus artery; UV: uterine vein.

Materials and Methods

Animals: The study was approved by the animal ethics committee of PLA General Hospital (NO. 2015-D10-09). Ten healthy adult female swines were used in this study. They were 8-24 months of age, weighing between 30-60 kg, and sexually mature for several months before the experiments. The swines were fed with granulated full-fodder twice a day and the water is free available.

Anethesia: Ten swines were anesthetized by intramuscular injection (i m). Injection of midazolam (5-10 mg) and ketamine (4 mg/kg). The swines received an i.m of 3% mebumalnatrium (5 mg/kg). The anesthesia was maintained using halothane inhalation. An oxygen mixture was provided to secure the haemoglobin saturation above 97%. The abdominal regions were shaved and the animals were positioned in the supine position. The abdominal region and vagina were disinfected with iodine tincture and covered with sterilized drapes. The swine received an i.v. infusion of 0.9% Nacl 500 mL to compensate the fluid losses and an i.v. infusion of penicillin 250 mL to prevent infection during the experimental procedure.

Retrieval of the uterus: All surgical procedures were performed under sterile conditions, and the surgeons used auxiliary magnifying glass microscope for vascular anastomosis operation. A midline incision from the pubic bone up to the level of the second mammary gland was performed by scalpel and monopole diathermy (40 W power), and then the abdominal cavity was entered. The intestines were packed into the upper abdomen with used wetted gauze, and the laparotomy incision was held open by a self-retaining retractor. The anatomical characteristics of the internal genital organs and vascular of the swine differed from these of the humans (as shown in Figure 1).

The uterus and fallopian tube with ovaries were exposed. The bicornual uterus was funicular and tortuous with the size of approximately 26 cm. The size of the bilateral ovaries was approximately 1.5×2.0 cm. The vessels associated with blood flow in the uterus were the uterine arteries, deep uterine veins, ovarian arteries, and a large number of microvascularity. The initial steps of the retrieval surgery included the dissection of the bladder peritoneum from the anterior portion of the cervix and the mobilization of the bilateral uterine artery and related vein. These deep uterus veins were found in the deeper region of pelvis below the uterine artery and vein. The uterine arteries and veins were selected as vascular grafts in this experimental procedure. The uterus and both fallopian tubes were cut off from the ovaries and the fallopian tubes fascia (Figure 2). All minor vessels which should be divided were coagulated by monopole diathermy (40 W power), and the vaginal canal was incised. The vascular pedicles of the uterus arteries and veins were prepared via vascular clamp. The uterus was removed from the abdominal cavity with connected blood vessel pedicles and vaginal-canal and both fallopian tubes.

Figure 3. — The uterus was removed from the swine, placed in a sterile basin with -4°C cold saline about 250 ml for cold ischemic storage.

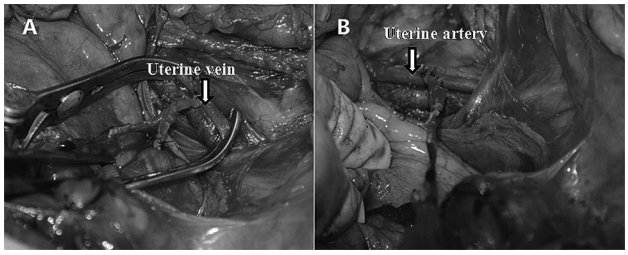

Figure 4. — Vascular anastomosis of the uterus. Bilateral vein and the artery of the uterus with external iliac vein were anastomosed by end-to-side in three cases.

Back-table preparation: The uterus was removed from the pelvis of the swine, and then was put in a sterile basin with -4 °C cold saline about 250 mL for cold ischemic storage (Figure 3). The ischemia time was classified into warm ischemia time (organ retrieval and vascular anastomosis) and cold ischemia time (organ storage). Ischemia time and perfusion time were recorded. The fluorinated plastic outer core (outer diameter: 0.74 mm, inner diameter: 0.52 mm) of a 26 G needle was inserted into the distal ends of the uterus artery divisions of the internal iliac arteries. Approximately 20 ml heparinized saline (2 mL:12,500 IU) was used to flush the residual blood from organ to avoid thrombus formation. The distal ends of the uterus veins were loosen vascular clamp to allow drainage. All operation procedures were performed using the magnifying glass microscope (magnification × 20). The uterus was kept in ice-cold saline until transferred to the original pelvic.

Uterus auto-transplantation: The uterus was repositioned in situ after the back-table preparation. The vaginal canal was sutured to the stump by interrupted (1–0 polyglactin) sutures. Vascular anastomosis of the uterus was performed under magnifying glass microscope (magnification × 20) using microscopic instruments. The external iliac artery and vein were dissected between a distance of about 2.5-3.0 cm. The vascular clamps were placed on both ends of the isolated segments of the external iliac vein and artery. The external iliac vessels (the vein and artery) of the uterus were anastomosed end-to-side (Figure 4) by interrupted sutures (7-0 polyglactin). After the bilateral arterial and venous were anatomized, the uterine blood flow was evaluated based on the pulsations of the uterine arteries. The uterine color changed from whitish to reddish, the blood flow past the venous and arterial anastomosis sites at the end of the experiment by bisection or after operation. Both sides were removed from the vascular clamps and the anatomized sites were observed carefully. Any larger leakage was subdued by placement of additional sutures. The authors fixed vaginal canal in post pelvic wall to prevent uterus prolapsing from the mesosalpinx and residual suture (1-0 polyglactin).

Table 1. — *Surgical parameters result.*

	N=9
Surgery time (min)	365±38.1
Vascular dissectional time (min)	125±12.9
Backtable preparation time (min)	46.5±3.1
Vascular anastomosis time (min)	117.5±23.7

Data are expressed as median±SD

Results

Surgical parameters result: The uterine auto-transplantations of the ten swines were performed at different times in the animal laboratory of the PLA general hospital. Nine swines received uterus auto-transplantation except the first case which was set as the preliminary experiment. The surgical time, ischaemic time. and vascular injury of each swine were examined and recorded (Table 1). The mean-time of surgery of the auto-transplanted swines was 365 ± 38.1 minutes. The time of vascular dissectional was 125 ± 12.9 minutes. The back table preparation time included the cold ischemia time and perfusion time. The time of back-table preparation and vascular anastomosis were 46.5 ± 3.1 min and 117.5 ± 23.7 minutes, respectively. All nine cases showed that the uterus arteries and veins vascular were anastomosed successfully and only eight cases attained long-term survival. The host in another case died four days after surgery from suspicious embolism postoperation.

Assessment of the transplanted uterus using Doppler ultrasonography: four days after surgery, the Doppler ultrasonography revealed that the uterine arterial and venous blood flows were stable in all the eight cases. The bilateral uterine arteries can transmit blood flow and the animal survived for more than six months after surgery. The peak systolic velocity (PSV) of the bilateral uterine arterial blood flow was 28 cm/s (left) and 36.3 cm/s (right) (Figure 5).

Secondary laparotomy: In all the eight cases, the secondary laparotomy was performed around six months after primary surgery as severe adhesion was found in the pelvis and uterus. The size of the uterus was normal. The uterus was adhered to the bladder fascia and the wall of pelvis. Bilateral cornual uterus was adhered to the pelvic sidewalls. Both sides of the ovaries have been wrapped on the fallop-

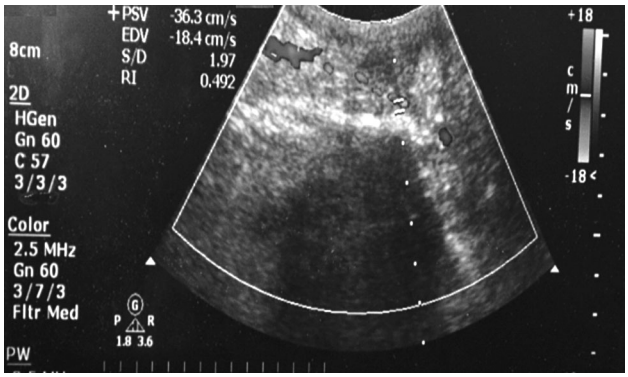

Figure 5. — Postoperative Doppler ultrasonography. Color and pulse Doppler methods. The PSV of the right uterine artery is 36.3 cm/s.

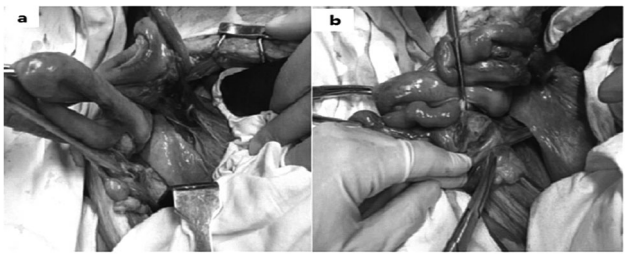

Figure 6. — Laparotomy findings six months after surgery. (a) Uterus of a normal size. Severe adhesion at a site surrounding the bilateral cornual uterus. (b) The size of the ovary and fallopian tube were also normal, but bilateral fallopian tube circuity, and both sides of the ovaries are wrapped on the fallopian tube.

ian tube. It was difficult to separate the uterine artery from the external iliac vessels due to the adhesion or scar (Figure 6).

Overall results: Ten cases uterus transplants were performed including the first case as the preliminary experiment. In nine cases, the uterus arteries and veins were anatomized successfully. However, only eight cases attained long-term survival. The host in another case died four days after surgery from suspicious embolism postoperation. The laparotomy exploration found ascites and intestinal necrosis. Therefore, the cause of death could be ascribed to the uterine necrosis. Six months after transplantation surgery, secondary laparotomy suggested that the uterus in the eight cases had normal morphology and size. However, the pregnancy was unsuccessful due to the shorter observation time, and the severe adhesion and tubal obstruction after surgery may affect pregnancy of those swines.

Discussion

The present study was limited to ten cases of auto-uterus transplantation in the swines. However, the establishment of animal model is significant in swine. The uterus is located in the pelvic in humans, and the vascular anastomosis is difficult. Rejection may be serious as the transplant organ contains mucosal tissue. The application of immunosuppressant post-operation is crucial and the perinatal need to be monitoring. Furthermore, UTx remains to be controversial. As the UTx sought only to improve human quality-of-life rather than to prolong life [32-34], it would be necessary to assess the risk and benefits of UTx procedure as well as social needs. The first live birth from the patient who underwent uterine transplantation occurred on September 4, 2014, in Sweden. The researcher also initiated an animal-based UTx research project in 1999 [16]. The animal experimental research in UTx had been performed 15-years prior. These data from animal studies provided a reference for clinical applications in others species other than humans.

In this study, the authors performed uterus auto-transplantation in swine. As the operational procedure and anatomical architecture in the pelvis of swine are similar to humans, and the animal costs is economic. Further accumulation of data is needed to improve the safety and stability of the technology for clinical applications. Therefore, the results would be meaningful in humans. However, there are also several disadvantages that because the swine breeding cycle is long and postoperative due to adhesive tubal obstruction would impair pregnancy. Thus, UTx experiments are still poor in swine model, Avison *et al.* [22] performed the only study on allogeneic UTx, in which considered this animal model is feasible. Evidences in swines is needed for establishment of surgical procedures such as microvascular anastomosis, cold ischemic storage, and perfusion.

Firstly, UTx requires removal of a pedicle of sufficient length and width, and removal of the uterine artery as an important vessel in UTx. It is the main blood flow feeding artery to the uterus. The venous system prevents congestion by drainage of uterine blood flow including the superficial uterine vein, deep uterine vein, and ovarian vein [35, 36]. The present authors used uterine and deep uterus veins. The size of the superficial uterine vein is smaller than uterine deep vein, but sometimes the deep uterine vein is unavailable owing to different anatomical structures. Moreover, its position in the pelvic floor and parallel with cardinal ligament makes vascular anastomosis difficult to perform. Therefore, the hemorrhage and ureteric injuries may cause disadvantages and extend the surgical time [37]. However, the uterine vein is a tiny vessel in which the thrombosis would developed over anastomosis lines. Thus, the present authors were unable master this model in the first two cases. They did not use the ovarian vein, because of its inappropriateness in premenopausal women with ovarian function in human uterus transplantation, as the ovarian vein is a thick vessel which is simple to separate

and has a long pedicle. Furthermore, the maintenance of the venous circulation is very important to avoid uterine thrombus formation, congestion, and uterine necrosis after transplantation. Uterine arteries were cleaved from vessels with the pedicle length of about 5-6 cm. The vein was about 5.5 cm, which was the most appropriate length for re-anastomosis of vessels. The arteries and veins with a wide diameter were reconstructed by end-to-side anastomosis of these vessels with the unilateral external iliac artery and vein, respectively. Additionally, with the improved surgical procedure, vascular anastomosis methods could improve the survival of the uterus. In the non-human primate, the artery reaching the internal iliac artery and the vein reaching the inferior vena cava/renal vein are removed. Furthermore, the end-to-end anastomosis of the unilateral internal iliac artery and end-to-side anastomosis of the external iliac vein are performed. In autografting in cynomolgus monkeys, end-side anastomosis to the external iliac artery is performed if the vascular pedicle of the uterine artery had sufficient length. Meanwhile, the end-to-end anastomosis to the internal iliac artery is used if the vascular pedicle length is insufficient. The present authors used the end-to-side method to anatomize the external iliac vessels and artery and vein of the uterus of woven clip by interrupted sutures. This suture method can decrease the formation of thrombosis, decrease the separation of uterine arteriovenous length, and simple to suture.

Fixed uterine ligaments in pelvis is essential for strengthening the support structures of the uterus. If uterus is not fixed in the pelvis, the it may cause prolapse of the anastomosed blood vessels. The first clinical UTx failed as the inadequate uterine structure support and tangle, torsion, and tension of the vasculature, which subsequently resulted in uterine embolization. Fixations of the round ligament to pelvic sidewalls, uterus ligaments to the lower posterior part of the uterus, suture of the bladder peritoneum, and reconstruction of cardinal ligaments are necessary. Because swine has a bilateral uterus with large size, the present authors fixed vaginal canal in post pelvic wall. With the increased anatomy knowledge about the uterus in swine, the stabilization of the learning curve for transplantation operation from retrieval of the uterus to auto-transplantation could improve the operation process and the isolation of the uterine artery/vein.

Conclusions

In conclusion, the uterine auto-transplantation experiments were performed in swine with long uterine horns and the tiny size of the vessels, as the operational procedure was similar to humans because the anatomy in the pelvis. Further studies are needed to perform the allogeneic UTx in others species combined with effective immunosuppression and to explore the post-transplantation immune tolerance.

References

[1] Grimbizis G.F., Camus M., Tarlatzis B.C., Bontis J.N., Devroey P.: "Clinical implications of uterine malformations and hysteroscopic treatment results". *Hum. Reprod Update*, 2001, *7*, 161.

[2] Kisu I., Banno K., Mihara M., Suganuma N., Aoki D.: "Current status of uterus transplantation in primates and issues for clinical application". *Fertil. Steril.*, 2013, *100*, 280.

[3] Erman Akar M., Ozkan O., Aydinuraz B., Dirican K., Cincik M., Mendilcioglu I., *et al.*: "Clinical pregnancy following uterus transplantation". *Fertil. Steril.*, 2013, *100*, 1358.

[4] Saravelous S.H., Cocksedge K.A., Li T.C.: "Prevalence and diagnosis of congenital uterine anomalies in women with rproductive failure: a critical appraisal". *Hum. Reprod. Update*, 2008, *14*, 415.

[5] Bryan A.L., Nigro J.A., Counseller V.S.: "One hundred cases of congenital absence of the vagina". *Surg. Gynecol. Obstet.*, 1949, *88*, 79.

[6] Oppelt P., Renner S.P., Kellermann A., Brucker S., Hauser G.A., Ludwig K.S., *et al.*: "Clinical aspects of Mayer-Rokitansky-Kuester-Hauser syndrome: recommendations for clinical diagnosis and staging". *Hum. Reprod.*, 2006, *21*, 792.

[7] Cheroki C., Krepischi-Santos A.C., Szuhai K., Brenner V., Kim C.A., Otto P.A., *et al.*: "Genomic imbalances associated with mullerian aplasia". *J. Med. Genet.*, 2008, *45*, 228.

[8] Borgfeldt C., Andolf E.: "Transvaginal ultrasonographic findings in the uterus and endometrium: low prevalence of leiomyoma in a random sample of women age 25-40 years". *Acta Obstet. Gynecol. Scand.*, 2000, *79*, 202.

[9] Brannstrom M.: "Uterine transplantation: a future possibility to treat women with uterus factor infertility?" *Minerva Med.*, 2007, *98*, 211.

[10] Brannstrom M., Racho El-Akouri R., Wranning C.A.: "Uterine transplantation". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 2003, *109*, 121.

[11] Devauchelle B., Badet L., Lengel B., Morelon E., Testelin S., Michallet M., *et al.*: "First human face allograft: early report". *Lancet*, 2006, *368*, 203.

[12] Fageeh W., Raffa H., Jabbad H., Marzouki A.: "Transplantation of the human uterus". *Int. J. Gynaecol. Obstet.*, 2002, *76*, 245.

[13] Ozkan O., Erman Akar M., Ozkan O., Erdogan O., Hadimioglu N., Yilmaz M., *et al.*: "Preliminary results of the first human uterus transplantation from a multiorgan donor". *Fertil. Steril.*, 2013, *99*, 470.

[14] Erman Akar M., Ozkan O., Aydinuraz B., Dirican ., Cincik M., Mendilcioglu I., *et al.*: "Clinical pregnancy after uterus transplantation". *Fertil. Steril.*, 2013, *100*, 1358.

[15] Brannstrom M., Johannesson L., Dahm-Kahler P., Enskog A., Molne J., Kvarnstrom N., *et al.*: "First clinical uterus transplantation trial: a six-month report". *Fertil. Steril.*, 2014, *101*, 1228.

[16] Brannstrom M., Johannesson L., Bokstrom H., Kvarnstrom N., Molne J., Dahm-Kahler P., et al.: "Live birth after uterus transplantation". *Lancet*, 2015, *385*, 607.

[17] Johannesson L., Kvarnstrom N., Molne J., Dahm-Kahler P., Enskog A., Diaz-Garcia C., et al.: "The uterus transplantationtrial:1-year outcome". *Fertil. Steril.*, 2015, *103*, 199.

[18] Brannstrom M.: "Uterus transplantation". *Curr. Opin. Organ Transplant.*, 2015, *20*, 621.

[19] Confino E., Vermesh M., ThomasW. Jr., Gleicher N.: "Nonvascular transplantation of the rabbit uterus". *Int. J. Gynaecol. Obstet.*, 1986, *24*, 321.

[20] Sieunarine K., Doumplis D., Kuzmin E., Corless D.J., Hakim N.S., del Priore G., *et al.*: "Uterine allotranspiantation in the rabbit model using a macrovascular patch technique". *Int. Surg.*, 2008, *93*, 288.

[21] Sieunarine K., Zakaria F.B., Boyle D.C., Corless D.J., Noakes D.E., Lindsay I., *et al.*: "Possibilities for fertility restoration: a new surgical technique". *Int. Surg.*, 2005, *90*, 249.

[22] Wranning C.A., El-Akouri R.R., Lundmark C., Dahm-Kahler P., Molne J., Enskog A., *et al.*: "Auto-transplantation of the uterus in the domestic pig (Sus scrofa): Surgical technique and early reperfusion events". *J. Obstet. Gynaecol. Res.*, 2006, *32*, 358.

[23] Avison D.L., DeFaria W., Tryphonopoulos P., Tekin A., Attia G.R., Takahashi H., *et al.*: "Heterotopic uterus transplantation in a swine model". *Transplantation*, 2009, *88*, 465.

[24] Sieunarine K., Boyle D.C., Corless D.J., Noakes D.E., Ungar L., Marr C.E., *et al.*: "Pelvic vascular prospects for uterine transplantation". *Int. Surg.*, 2006, *91*, 217.
[25] Hurst S.A., Smith J.R., del Priore G.: "Experiences in uterine transplantation". *Transplantation*, 2010, *89*, 769.
[26] Priore G., Schlatt S., Malanowska-Stega J.: "Uterus transplant techniques in primates: 10 years' experience". *Exp. Clin. Transplant.*, 2008, *6*, 87.
[27] Enskog A., Johannesson L., Chai D.C., Dahm-Kahler P., Marcickiewicz J., Nyachieo A., *et al.*: "Uterus transplantation in the baboon: methodology and long-term function after auto-transplantation". *Hum. Reprod.*, 2010, *25*, 1980.
[28] Johannesson L., Enskog A., Dahm-Kahler P., Hanafy A., Chai D.C., Mwenda J.M., *et al.*: "Uterus transplantation in a nonhuman primate: long-term follow-up after autologous transplantation". *Hum. Reprod.*, 2012, *27*, 1640.
[29] Ramirez E.R., Ramirez D.K., Pillari V.T., Vasquez H., Ramirez H.A.: "Modified uterine transplant procedure in the sheep model". *J. Minim. Invasive Gynecol.*, 2008, *15*, 311.
[30] Ramirez E.R., Ramirez Nessetti D.K., Nessetti M.B., Khatamee M., Wolfson M.R., Shaffer T.H., *et al.*: "Pregnancy and outcome of uterine allotransplantation and assisted reproduction in sheep". *J. Minim. Invasive Gynecol.*, 2011, *18*, 238.
[31] Mihara M., Kisu I., Hara H., Iida T., Araki J., Shim T., *et al.*: "Uterine autotransplantation in cynomolgus macaques: the first case of pregnancy and delivery". *Hum. Reprod.*, 2012, *27*, 2332.
[32] Benagiano G., Landeweerd L., Brosens I.: "Medical and ethical considerations in uterus transplantation". *Int. J. Gynaecol. Obstet.*, 2013, *123*, 173.
[33] Lefkowitz A., Edwards M., Balayla J.: "The Montreal criteria for the ethical feasibility of uterine transplantation". *Transpl. Int.*, 2012, *25*, 439.
[34] Arora K.S., Blake V.: "Uterus transplantation: ethical and regulatory challenges". *J. Med. Ethics*, 2013, *40*, 396.
[35] Fujii S., Takakura K., Matsumura N., Higuchi T., Yura S., Mandai M., *et al.*: "Precise anatomy of the vesico-uterine ligament for radical hysterectomy". *Gynecol. Oncol.*, 2007, *104*, 186.
[36] Yabuki Y., Asamoto A., Hoshiba T., Nishimoto H., Nishikawa Y., Nakajima T.: "Radical hysterectomy: an anatomic evaluation of parametrial dissection". *Gynecol. Oncol.*, 2000, *77*, 155.
[37] Yoo S., Terai Y., Tanaka T., Tanaka Y., Tsunetoh S., Kanemura M., *et al.*: "Role of the two-point pull-up technique for treating the uterine arteries during radical hysterectomy and trachelectomy". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 2013, *170*, 544.

Corresponding Author:
YUANQING YAO, M.D.
The Department Gynecology and Obstetrics
of PLA General Hospital
28 Fuxing Road
Beijing 100853 (China)
e-mail: yqyao301hp@163.com