Heterotopic uterine transplantation by vascular anastomosis in the mouse

R Rachoo El-Akouri, G Kurlberg, G Dindelegan, J Mölne, A Wallin and M Brännström

Department of Obstetrics and Gynecology, The Sahlgrenska Academy at Göteborg University, Göteborg, Sweden
1Department of Surgery, The Sahlgrenska Academy at Göteborg University, Göteborg, Sweden
2Department of Pathology, The Sahlgrenska Academy at Göteborg University, Göteborg, Sweden

Requests for offprints should be addressed to R Rachoo El-Akouri, Department of Obstetrics and Gynecology, Sahlgrenska University Hospital, S-413 45 Göteborg, Sweden; Email: randa.racho@obgyn.gu.se

Abstract

A method of heterotopic uterine transplantation was developed in the mouse as a model system for studies of uterine function and transplant immunology of the uterus. The model involved transplantation of the right uterine horn and the cervix by vascular anastomosis to a donor animal with the intact native uterus remaining in situ. F1-hybrids of inbred C57BL/6 X CBA/ca (B6 CBAF1) mice of 6–8 weeks of age (n=42) were used. The specific pelvic vascular anatomy of these mice was first examined by intra-aortal injection of a two-component silicon-rubber curing agent. The surgery of the donor animal involved microsurgical isolation of the right uterine horn and the cervix, with preserved vascular supply from the aorta through the right uterine artery. After isolation of the uterine horn with vascular supply and venous drainage, including approximately 3 mm of the inferior vena cava and aorta, the organ was put on ice. The recipient animal was prepared by exposing and mobilizing the infrarenal part of the aorta and the vena cava. The grafted uterus was placed in the abdomen on the left side and the aorta and vena cava of the graft were anastomosed end-to-side to the aorta and vena cava of the recipient animal with 11–0 sutures. The total time for these procedures declined with time and was 125 ± 4 min for the last 28 operations. Viability of the uterus was confirmed, several days later, by demonstrating a blood flow similar to that of the native uterus, and histology of the grafted uterus demonstrated normal morphology, including intact ultrastructure of the endothelial cells. The overall survival rate of the recipient animals increased with learning from approximately 40% in animals 1–21 to 71% in animals 22–42. The proportion of viable grafts, as judged by normal blood flow and histology among the surviving mice was 25% in animals 1–21 and 87% in animals 22–42. An undisturbed function of the transplanted uterus horn was finally demonstrated by its ability to implant inserted blastocysts and to carry pregnancy with fetal weight being similar to that of fetuses in the native uterus and controls. In conclusion, this is the first report of successful transplantation of the uterus with proven functionality in the mouse. The model should be useful for many aspects of research in uterine physiology and pathophysiology.


Introduction

The mouse is considered to be the most important animal within many medical research fields, including endocrinology. The increasing availability of inbred mice strains with typical features (Wiktor-Jedrzejczak et al. 1990, Pollard et al. 1991, Cohen et al. 1997) as well as of specific transgenic and knock-out mice (Stewart et al. 1992, Kauma 2000, Zoubina & Smith 2001) has led to a tremendous advance in attempts to understand complex physiological processes, including reproductive events. The data accumulated from experiments in these animal models has so far been from studies of transgenic or gene-deleted mice, mostly carrying global upregulations or deletions of specific genes. It is not known from these animal models whether a specific uterine phenotype is attributable to secondary hormonal or other systemic imbalances or is a result of the increased or deleted expression of the gene in the uterus. Furthermore, there are no uterine tissue-specific promoters known today to allow for construction of conditional uterine-specific knock-out mice. To be able to study the effects of a specific mediator in the uterus it would be of great benefit to develop techniques for transplantation of the uterus in a mouse model.

During the last decades there have also been tremendous developments in the field of organ transplantation.
The clinical work in this field has mainly been concentrated on the development of transplantation of central organs, such as the heart, the kidney and the liver. Animal models have generally been developed prior to introduction of transplantation of specific organs in the human to investigate important issues such as tissue rejection and the specific immunology involved (Lee et al. 1973, Blom & Orloff 1998, Hoyerstrup et al. 2000). With the recent developments of more effective immunosuppression to control tissue rejection, the research and clinical fields of transplantation have also come to involve studies on transplantation of other organs such as the pancreas and the small intestine (Kort et al. 1973, Di Cataldo et al. 1989, He et al. 1998). Transplantation of reproductive organs has been considered in reproductive medicine for both scientific and clinical purposes. Among the female reproductive organs, a majority of studies have been conducted to study various aspects of ovarian transplantation, first attempted as a potential treatment for the menopause (Davidson 1912, Nattrass 1915). The animal models used for ovarian transplantation were generally autotransplantation models, with only the ovary replanted (Baird et al. 1976) or the ovary replanted together with the oviduct (Winston & Browne 1974) within the same animal. There are fewer reports in the literature concerning transplantation of the uterus. The uterus was transplanted together with the oviduct and ovary as shown in the ewe (Baird et al. 1976), the dog (Yonemoto et al. 1969, Mattingly et al. 1970) and the macaque monkey (Scott et al. 1971). The technique for uterine transplantation was either by direct vascular anastomosis (Yonemoto et al. 1969, Mattingly et al. 1970) or by omentopexy to obtain revascularization (O’Leary et al. 1969, Barzilai et al. 1973). In the more commonly used small experimental animals, there are early reports on non-vascular procedures for transplantation of the uterus as presented in the guinea pig (Bland 1972) and in the rabbit (Confino et al. 1986). Lately, an en-bloc vagino-uterino-ovarian vascular anastomosis transplantation technique in the rat has been presented (Lee et al. 1995). The function of these transplanted uteri has not been further assessed.

One major obstacle involved in any organ transplantation in mice is the small size of the animal. Thus, it is necessary to use delicate microsurgical techniques for vascular anastomosis. The purpose of the present study was to describe the surgical technique which we have developed for heterotopic uterine transplantation in the mouse and to evaluate the transplanted organ in terms of tissue blood perfusion and histology. In addition, we present initial results demonstrating successful implantation and pregnancy in the transplanted uterus.

Materials and Methods

Animals

F1-hybrids of inbred female C57BL/6 × CBA/ca (B6 CBAF1) mice of 6–8 weeks of age (weight 20–25 g) were purchased from M&B A/S (Ry, Denmark). Animals were housed with up to ten in each cage and they had free excess to water and pelleted food. The temperature in the animal quarter was between 21 and 23 °C, with a relative humidity of 50–60%. The animal room was illuminated between 0700 and 1900 h and the mice were adapted to these conditions for at least 5 days prior to the experiments. The experiments were approved by the local animal ethics committee and were carried out according to the principles and procedures outlined in the NIH Guide and Use of Laboratory Animals.

Vascular anatomy determination by microdissection and Microfil injection

Initial experiments were designed to obtain a detailed characterization of the abdominal and pelvic vascular anatomy in relation to the uterus on both the arterial and venous side in the mouse strain used. The purpose of this was to achieve the background information necessary for further development of the microsurgical techniques involved in the transplantation surgery. To obtain this information, microdissection and Microfil injection (Flow Tech, Inc., Carver, MA, USA) were used. Microfil is a two-component silicon-rubber curing agent that has been used extensively for visualization of the vasculature of other sites of the body, such as the rat kidney glomeruli (Norlen et al. 1978) and also in the human for studies of the vascular supply to the thenar area of the hand (Omokawa et al. 1997).

The mouse was anesthetized by continuous administration of 2% isoflurane (Baxter, Kista, Sweden) in a mixture of air (500 ml/min) and O₂ (500 ml/min) and placed in a supine position. A mid-line laparotomy was then performed and the aorta and the vena cava were exposed and mobilized. An operating stereomicroscope (Leica M 651 with a DC 300 digital camera connection; Leica microsystems AB, Sollentuna, Sweden) with magnifications from 6 × to 40 × was used. The inferior mesenteric vessels were cauterized and cut followed by retraction of the colon to the left side of the animal. The aorta and vena cava were then separated by gentle dissection between the vessels at a point just below the branching of the renal vessels. The ovarian vessels on each side were ligated by 8–0 suture (nylon monofilament; S&T, Neuhausen, Switzerland). The external iliac vessels were also ligated at a point 5–6 mm distal to the branching of the inferior epigastric vessels. The aorta and vena cava were then ligated just below the branching of the renal vessels. Just inferior to the ligation the aorta and vena cava were catheterized with polyethylene tubing (diameter 0·6 mm; Intramedic; Becton-Dickinson, Parsippany, NJ, USA), fixed by an 8–0 silk thread and connected to a 27 gauge cannula. A 5 ml syringe containing 0·154 M NaCl supplemented with heparin sulfate (100 IU/ml; Leo Pharma AB, Malmö, Sweden) and xylocaine (0·2 mg/ml;
Astra Zeneca, Göteborg, Sweden) was gently injected through the aortic tubing. The injection continued until transparent fluid flowed from the vena cava.

A two-step procedure was performed to enable visualization of both the arteries and veins. The blue Microfil compound (high viscosity; 350–450 cps) was flushed through the cannula on the venous side until all veins in all organs of the lower part of the abdomen appeared blue. The cannula was thereafter closed with a titanium clip. At that point, red Microfil compound (high viscosity; 350–450 centipoise (cps)) was injected through the aortic catheter. This high viscosity Microfil liquid is too viscous to pass through capillaries and will then just fill up the arterial side of the vascular system. The cannula was closed with a titanium clip. The mouse was killed by cervical dislocation immediately after the procedures, and the abdominal and pelvic parts of the animal were cut out and placed in a plastic bag at 4°C overnight. To achieve transparency of tissue, the specimen was then placed for 24-h periods in 50% glycerine, 75% glycerine and 85% glycerine and finally preserved in 100% glycerine.

**Donor operation**

The animal was anesthetized by isoflurane (see above), placed on its back and the abdomen was shaved and cleaned with 70% ethanol. A laparotomy was performed via a mid-line incision from the symphysis pubis to the xiphoid process.

The surgical procedure described below was performed to isolate and transplant the right horn of the uterus including the upper part of the cervix. To be able to perform this and to identify all vascular structures an operating stereomicroscope (see above) was used. The transplantation specimen and the vascular anatomy with sites of ligation is presented in Fig. 1. The small intestines were initially retracted to the left side of the animal and packed in moistened gauze. The inferior mesenteric artery (Fig. 1) was then cauterized using bipolar diathermy (Cox-Comp Bikoagulator; Instrumenta AB, Billdal, Sweden) at a maximum effect of 2 W. The colon was divided just above the rectum and retracted to the side to obtain a free operating field. Both ureters were also cauterized and cut.

To be able to fully expose the external iliac vessels on the right side, the inferior epigastric vessels were cauterized and cut just above the ilioinguinal ligament. A suture (10–0 nylon monofilament; S&T) was placed under the external iliac vessels 3–4 mm distal to the branching of the inferior epigastric vessels and the vessels were ligated proximally and cauterized distally followed by severance in between. A thorough dissection with removal of extravascular tissue and cauterization of small vessels surrounding the external iliac vessels was then performed up to a point just distal to the branching of the external pudendal vessels (Fig. 1). The pudendal vessels were cauterized and cut.

The vessels supplying the lower portion of the cervix and the bladder (superior vesical, inferior vesical, cervical and vaginal vessels) were cauterized to prevent bleeding. The bladder was then removed.

The right uterine horn was then isolated from the left uterine horn at a site close to the common uterine cavity by ligations (8–0 suture; S&T). The left uterine horn was removed and immediately fixed in Kidney Biopsy Fixation Solution (Bie & Berntsen A-S, Rødovre, Denmark) to be used for later histological examination and comparison with the transplanted right uterine horn.

The cervix was then transected at the level just below the attachment to the bladder. Special care was taken to avoid the vascular branches from the right-sided hypogastric trunk. The cervix could now be lifted up somewhat to more easily identify the uterine artery and veins and to attach a titanium clip (Weck Closure Systems Ltd, Research Triangle Park, NC, USA) on the superior gluteal vessels. The left common iliac vessels were then ligated with 10–0 suture on the proximal side and a clip on the distal side followed by severance in between. These vessels were then freed from the underlying tissue up to the caudal vessels by cauterizing some small dorsal vessels. The lumbar vessels (2–4), which branch dorsally from the
aorta and vena cava, were then cauterized and cut. The aorta and the vena cava were separated from each other by gentle upward traction on the aorta and blunt dissection in between from a point just below the branching of the renal vessels and 3–4 mm in a distal direction. The right uterine horn was ligated by 8–0 suture at a site between the tip of the right uterine horn and the oviduct. On the proximal side of the oviduct, a clip was attached and severance was performed in between. The right uterine horn was then dissected free from its attachment to the dorsal peritoneum and folded over to expose the right hypogastric trunk.

The aorta and the vena cava were tied off inferior to the ovarian vessels by 8–0 suture to create a complete segment including the inferior aorta and the inferior vena cava with the vascular connection to the right uterine horn. Small incisions were then made in the vena cava and in the aorta just below the ligation. A 30 gauge needle connected to a 1 ml syringe was then inserted into the incision of the aorta to gently flush the specimen with 0·4–0·6 ml ice-cold 0·154 M NaCl supplemented with heparin sulfate and xylocain (see above) to expand vessels and prevent thrombosis. During this procedure it could easily be seen whether the donor uterus was properly perfused by assessing that the uterus blanched and that clear liquid flowed through the incision of the vena cava. The donor uterus was then placed in and kept in ice-cold 0·154 M NaCl. During this ‘back-table’ preparation the aorta and vena cava stumps were separated, cut straight at their ends and cleaned from extravascular tissue to await the surgical preparation of the recipient.

**Recipient operation**

The animal was anesthetized in the same manner as the organ donor (see above) and then injected s.c. with 15 IU heparin sulfate. The abdomen was shaved and cleaned with 70% ethanol and a laparotomy was performed through a mid-line incision. The intestines were gently packed in a saline-moistened gauze and placed to the left. Loose connective tissue overlying the infrarenal aorta and vena cava were removed to expose these areas of the major vessels. Two to three lumbar vessels were cauterized and cut to enable further mobilization of the aorta and the vena cava. Hemostatic clamps (width 1 mm; S&T) were placed around both aorta and vena cava at sites just above the branching of the inferior mesenteric vessels and just below the branching of ovarian vessels (Fig. 2). To be able to perform end-to-side anastomosis of the graft aorta to the recipient aorta a longitudinal cut (about 1 mm length) was made in the anterior aortic wall to create an opening. The lumen was flushed with 0·154 M NaCl and 1 ml glucose (50 mg/ml) were then administered s.c. The animal was placed under a heat lamp until fully awake. The mouse received heparin sulfate (5 IU) s.c. at 24 h after the operation. General condition and the laparotomy wound were assessed every day for the first week and then twice weekly.

**Embryo transfer**

In one of the transplanted mice (8 weeks old), the ability of the transplanted uterus to implant transferred embryos was tested. Six blastocysts were retrieved from a naturally mated B6 CBAF1 female 4 days after mating with a vasectomized male. The blastocysts were transferred to the uterine-transplanted mouse (Hogan et al. 1994) 3 days after mating with a vasectomized male. The
embryo transfer was made during anesthesia (see above) by a midline laparotomy. Direct entrance through the uterine wall was accomplished with a 30 gauge needle and a glass pipette with the blastocysts was introduced through the hole. Three blastocysts were placed in the native uterus and three blastocysts were placed in the graft. The mouse was killed by cervical dislocation on day 10 after embryo transfer and the number of fetuses was counted.

Laser Doppler flowmetry
Laser Doppler flowmetry represents a new method for estimation of tissue perfusion (Oberg 1990). The flow-meter used in this study (Periflux 5000; Perimed, Järfälla, Sweden) consists of a solid-state diode laser with a wavelength of 780 nm and a maximum power output at the probe tip of 1 mW. The probe used (PF 407) contains optic fibers for transmission of laser light. The principle of laser Doppler flowmetry is that a laser light is transmitted to the tissue via a fiber optic probe. When this light hits moving blood cells, it undergoes a change in wavelength (Doppler shift). The magnitude and frequency distribution of these changes are directly related to the number and velocity of blood cells. Measurements presented as arbitrary perfusion units (PU) are viewed and recorded in a Perisoft software computer program (Perisoft, Järfälla, Sweden).

At 15 and 30 days after the operation the mouse was anesthetized by isoflurane (see above). The abdomen was opened through a mid-line incision and an adhesive mini probe (Perimed) was placed on the surface of the graft uterus and on the native uterus. The probe was also placed on the surfaces of the small intestine, liver and the kidney for recordings. The blood flow was recorded for periods of 2–3 min and the average blood flow, expressed in PU, was then calculated in the Perisoft computer program.

Light and electron microscopy
The recipient mice were killed and the native and donor uteri were quickly removed and fixed in Kidney Biopsy Fixation Solution. Tissue pieces were then dehydrated and embedded in paraffin for light microscopy (Nikon light microscope and Coolpix MDC camera) after staining with hematoxylin and eosin. Small pieces of tissue were cut and fixed by immersion in 3% glutaraldehyde in 0·1 M sodium cacodylate buffer, pH 7·4, postfixed in 2% osmium tetroxide in the same buffer and, after dehydration in alcohol and propylene oxide, embedded in Agar 100 epoxy resin. Thin sections were cut and contrasted with uranyl acetate and lead citrate, on the grids, and examined in a Philips CM12 electron microscope.

Results
Vascular anatomy
During the development of the microsurgical technique it was apparent that the vascular anatomy varied somewhat from that described in text books. It was also difficult to localize all vessels through the microscope. The MicrofilR technique enabled us to characterize the pelvic vascular network in detail (Fig. 3), which was of importance for the further development of the surgical operative procedure.
Modifications of surgery and transplantation

A total of 42 animals was included and out of these 23 animals (55%) regained good health. Some animals \((n=8)\) died due to hemorrhage during surgery or during the first 48-h post-operative period. The other excluded animals \((n=11)\) were killed since they were found to be paraplegic after the operation. These paraplegic animals were all among the first 14 animals operated (Fig. 4). The survival rate increased from 38% among the first 21 animals to 71% in the last series (animals 22–42). The rate of successful transplantation, as judged by a well-preserved graft with normal blood flow, increased from 25% for animals 1–21 up to 87% for animals 22–42 (Fig. 4). The time for surgery including donor operation, graft preparation and recipient operation gradually decreased with experience of the procedures (Fig. 5). In the animals in which blastocysts were transferred (three blastocysts to native uterus, three blastocysts to transplanted uterus), three and one normal fetuses were seen in the native and transplanted uterus (Fig. 6) respectively. In the transplanted uterus, one absorbed pregnancy was seen.

Morphology

On visual inspection, a normal color, tone and consistency of transplanted uteri were seen in successful grafts at several time-points during the observation period of 8 weeks after operation. Light microscopic examination of the graft (Fig. 7) after the transplantation demonstrated a normal structure with all layers of the uterine wall and the epithelial layer of the uterine glands exhibiting normal morphology when compared with donor and native uteri.

Electron microscopy

Grafted tissue as well as controls were examined ultrastructurally after 4 weeks. No apparent differences between the groups were observed in the glandular epithelium, muscle wall or blood vessels. Special attention was given to the endothelium in capillary walls (Fig. 8), but no inflammatory injuries or structural changes were observed.

Blood flow

The blood flow recordings demonstrated a similar flow in the graft uterus as compared with the native uterus (Fig. 9). The liver, small intestine and the kidney...
Figure 7 Light microscopic examination of native (a, c, e) vs graft uterus (b, d, e) at 2 weeks (a, b), 4 weeks (c, d) and 8 weeks (e, f) after operation: magnification × 40. The epithelial cells and the stroma are similar in the graft compared with the native uterus. Scale bar represents 30 μm.
exhibited higher blood flow as compared with the uterus (Fig. 9).

Discussion

The aim of the present study was to develop a method for transplantation of the uterus which could be used for further studies of various aspects of both uterine physiology and the immunology of uterus rejection. Although uterine transplantation has been achieved in other animals such as the dog (Yonemoto et al. 1969, Mattingly et al. 1970), the ewe (Baird et al. 1976) and the rat (Lee et al. 1995) a murine model would have advantages, in large part because of the great availability of defined inbred and genetically engineered mouse strains. Moreover, important research tools such as recombinant proteins, monoclonal antibodies and specific assays are extremely well developed for the mouse as compared with other experimental animal species.

We chose to develop a transplantation method with vascular anastomosis between the graft and the host. Some previous reports on uterine transplantation in other species used an omental–pedicle graft as a source to obtain uterine revascularization (O’Leary et al. 1969, Scott et al. 1970). A comparative study of this omentopexy method and direct vascular anastomosis techniques in autotransplantation of the uterus of the dog (Barzilai et al. 1973) showed that the new blood supply obtained through revascularization from the omentum may only be sufficient for a short time-period after transplantation, since a large proportion of the transplanted animals had their uterus replaced by a solid fibrous mass (Barzilai et al. 1973). However, it must be emphasized that the microsurgical technique used in the present study is extremely delicate. There is a requirement for a high magnification operating microscope, since many of the vessels that are isolated and severed have a diameter well below 500 µm. The diameters of the aorta and vena cava that were anastomosed end-to-side are about 700 µm and 1500 µm respectively in mice of around 25 g body weight (Holt et al. 1981).

The surgical procedure which took the longest time was the isolation of the graft, where great caution had to be taken to preserve the blood supply to the uterus throughout the operation and also to avoid leakage from the vessels branching from the vasculature of the graft. It is evident from the learning curve of the present study that the times for this and also the other procedures were substantially reduced over time.

The surgery for uterine transplantation seems to be more complicated than other transplantation procedures used in modern research involving gene-deleted mouse models. Transplantation of ovaries from wild-type to knock-out mice or vice versa is a widely used technique. This technique is, compared with uterine transplantation, rather simple. The removal of ovaries is performed from the bursal cavity by sharp scissors and the grafted ovaries can then simply be put into the bursa for subsequent revascularization with a subsequent satisfactory return to fertility (Yun et al. 1990).

In this first report on uterine transplantation in the mouse we decided to develop a heterotopic model, where
In the present study, we have demonstrated pregnancy achieved in a uterus transplanted as an isograft. This is an advance in comparison with previous reports of pregnancies in the autografted uterus or of the dog (Eraslan et al. 1966). This is, to our knowledge, the first pregnancy reported in a model not involving autotransplantation (replantation). In the autotransplantation experiments on dogs (Eraslan et al. 1966), successful pregnancy occurred in three out of eighteen females that were studied.

In these initial experiments we chose a syngeneic transplantation model to avoid rejection. Knowledge about the immunologic response in uterus transplantation in allogeneic models is very limited. Studies with allogenic transplantation of the uterus in rhesus monkeys, using the omental–pedicle technique, showed rejection of the transplanted uterus during a 2- to 3-week period after the transplantation (Scott et al. 1971). The histologic appearance was that of progressive infiltration of mononuclear immune cells. Furthermore, transplantation of the uterus in the rhesus monkey revealed that the rejection of the endometrium was more rapid than that of the myometrium (Scott et al. 1971). In a recent study involving vagino–utero–ovarian allogeneic transplantation with end-to-side aortic–aortic, vena cava–vena cava vascular anastomosis (Lee et al. 1995) the findings were that of edema on day 5 and hemorrhagic edema on day 10.

In future attempts to accomplish uterine transplantation in the human, for fertility purposes, in patient groups such as those with uterine agenesis or patients who have undergone hysterectomy in early years because of benign or malignant diseases, a thorough study of the rejection phenomenon is needed. An allogeneic mouse model would be the necessary experimental model to accumulate knowledge and it would be feasible to extrapolate these results of experiments in the mouse to possible clinical situations in the human.

In studies of functions due to altered expression of a specific gene in an organ, conditional knock-out mouse models have been used with the expression being coupled to tissue-specific promoters. For example, to achieve selective altered expression in the pancreas or the liver, use can be made of insulin and albumin promoters respectively (Postic et al. 1999). Concerning the uterus, there is to date no tissue-specific promoter that can be used and the uterus transplantation model presented in this study may thus offer novel opportunities for investigators to test the contribution of a specific gene to the physiology of the organ. It would, for example, be of great interest to study the importance of specific gene transcription within the uterus in processes such as decidualization, implantation and parturition.

References


Davidson HS 1912 Transplantation of the ovary in the human being: record of three cases. Edinburgh Medical Journal 3 441–449.


Accepted 16 April 2002

Received 8 March 2002