Transplantation of the uterus

Mats Brännström *, Caiza Almén Wranning, Randa Račho El-Akouri

Department of Obstetrics and Gynecology, The Sahlgrenska Academy at Göteborg University, Gothenburg, Sweden

Abstract

Most women with uterine factor infertility have today no prospect of carrying a pregnancy to term. The development of a method for transplantation of the human uterus would be a means for many of these women to become both genetic and gestational mothers. In this article we review the literature concerning the history and recent development in the area of uterine transplantation. We describe our newly developed model for heterotopic uterine transplantation in the mouse, which we are using for studies of pregnancy outcome and rejection mechanisms. We also address some of the specific questions that need to be solved before attempts to transplant the human uterus should be performed.

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1. Introduction

During the last decades there have been considerable advancements in the treatment of both female and male infertility. Depending on the exact definition of infertility, it is estimated that between 8 and 30% of couples of reproductive age in the western world are infertile (Gray, 1990; Marchbanks et al., 1989; Thonneau et al., 1991). In approximately 40% of couples seeking medical consultation for infertility, the cause is designated to the woman (Hull et al., 1985). The introduction of IVF has made many causes of female infertility such as ovulatory dysfunction and tubal factor treatable. However, most abnormalities underlying uterine factor infertility are not treatable.

Uterine factor infertility could be either congenital Müllerian anomalies such as uterine agenesis or hypoplasia, or acquired. Müllerian anomalies are estimated to be present in about 1/200-girls (Nahum, 1998) and even minor Müllerian anomalies such as septate or unicornuate uterus could result in infertility or subfertility (Nickerson, 1977; Raga et al., 1997). In Rokitansky–Kuster–Hauser syndrome with congenital absence of the uterus and vagina there is an experience with gestational surrogacy, and in a follow up of 17 live born children females, no congenital anomalies were found (Petrozza et al., 1997). This indicates that although congenital absence of the uterus is a genetic disease, it is not necessarily inherited in a dominant fashion.

The largest subgroup of women with acquired uterine factor infertility are those with leiomyoma. Leiomyomas may cause infertility when they are submucous or otherwise situated at anatomical sites or of a size that distort the uterine cavity or interfere with implantation. Leiomyomas may also directly induce endometrial abnormalities (Verkauf, 1992) or increase uterine contractility to cause spontaneous abortion (Buttram and Reiter, 1981). A minority of the patients with infertility due to leiomyoma can be successfully treated by myomectomy to regain fertility.

Another group with acquired uterine factor infertility are those with intrauterine adhesions, also named Asherman’s syndrome. This group is estimated to constitute between 1.5 and 3% of all infertile women (Dmowski and Greenblatt, 1969; Sugimoto, 1978). The causes are often iatrogenic after curettage or due to infections such as tuberculosis. Patients who have undergone hysterectomy due to benign diseases such as leiomyomas or post partum hemorrhage or due to malignant diseases such as early stage of cervical cancer...
constitute another large group with acquired uterine factor infertility.

Eventhough the groups of patients mentioned above usually have well functioning ovaries with a preserved follicle reserve, they have in most countries no possibility to become genetic mothers. In some nations (Canada, the UK, Hungary, the Netherlands, Brazil, Israel, South Africa and some states of the USA and Australia) gestational surrogacy is approved by legal authorities and this would be a means for the patient with an uterine factor infertility to become a genetic mother. Gestational surrogacy is defined as treatment by which the gametes of the genetic couple are used to produce embryos, which are transferred to a woman who has agreed to act as a host for these embryos. It is also named IVF-surrogacy or full surrogacy. Due to its complicated ethical nature, gestational surrogacy is outlawed in many countries of the world where it conflicts with religious beliefs, general ethical views or national law. A study of legislation concerning assisted reproductive techniques in 40 nations found that most European countries and all Muslim and Asian nations included in the study have legislation or guidelines that do not allow IVF surrogacy. In Argentina, Belgium, Finland, Greece and India gestational surrogacy is practiced without legislation or guidelines (Cohen and Jones, 2001).

The ethical considerations concerning gestational surrogacy are many even in countries where it is approved and it raises fundamental questions about parenthood, autonomy of the surrogate mother, the potential risks for both embryo and surrogate carrier and—maybe most important—the psychological implications for the prospected child (Ber, 2000; Brinsden, 2001; Shuster, 1992). A successful method for human uterus transplantation would minimize the impact of these difficult ethical questions along with some of the ethical dilemmas concerning international adoption. The advantages of a model for a successful uterine transplantation compared to gestational surrogacy are obvious for the infertile couple—apart from the joy of experiencing a pregnancy, they would not be dependent on a third party during gestation and would have full control over maternal lifestyle-influences on their offspring. Furthermore, the genetic mother, instead of the surrogate, would take the physiological risks involved with any pregnancy. Issues such as maternal bonding during gestation, the definition of motherhood and the risk of economic pressure being a factor in recruitment of the surrogate carrier, would be abolished. Also, the prospected child would not have to deal with the possible conflict of having two mothers.

Below we will give a brief orientation of the current state of research of relevance to the issue of uterine transplantation and present some of the questions we intend to address through our own research.

2. Uterine transplantation models

The history of experimental transplantation of the uterus in animals spans most of the 20th century, with the majority of studies being performed during the 1960’s and 1970’s (Table 1). The two major issues of concern have been—and are still—the surgical method for vascular supply of the transplanted uterus and the rejection of the graft. To isolate the two issues from each other, autotransplantation—when the organ is taken out from the animal and then transplanted back to the same individual—have been utilized for the study of revascularisation.

The complex vascular anatomy of the pelvic region invited the development of alternative methods to vascular anastomosis, where omentopexy has been the most commonly used. The omentum is then wrapped and sutured around the transplant and creates a milieu that favors spontaneous revascularisation. Autotransplantation of the uterus, with or without ovaries and oviducts, by omentopexy in the dog (O’Leary et al., 1969; Scott et al., 1970) and Macaque monkey (Scott et al., 1971) yielded satisfactory revascularisation of the uterus in more than 70% of the animals. Moreover, studies in the ewe demonstrated 12 deliveries after autotransplantation by omentopexy (Zhordania and Gotsiridze, 1964). In another method for revascularisation by fixation of the uterus to the broad ligament, satisfactory results were also seen in the rabbit (Confino et al., 1986). There exists one study in dogs where the omentopexy method is compared with vascular anastomosis of autotransplanted uteri, and the results were that all grafts by omentopexy showed massive necrotic degeneration at examination by day 90, while all grafts with vascular anastomosis were viable (Barzilai et al., 1973). Two pregnancies by normal mating and one successful delivery were reported in this group.

Eventhough technically more difficult than any non-vascular method, transplantation of the uterus by vascular anastomosis has been used in some animal models since the 1960s. Autotransplantation by this technique in the dog (Barzilai et al., 1973; Eraslan et al., 1966; Mattingly et al., 1970) proved successful on a long term basis in more than 80% of the animals. The losses were mainly due to direct post-operative complications such as intra-abdominal bleeding. In these three studies pregnancies were achieved after normal mating and deliveries with healthy pups were reported. The vascular anastomosis techniques used were end-to-end anastomosis between the uterine, hypogastric, or inferior vena cava vessels of the graft to either of the external iliac or hypogastric vessels of the recipient.

During the first half of the last century, the idea of the uterus as an immunologically privileged organ prevailed eventhough not undisputed. The matter was settled in the early 1970’s through a number of homotransplanta-
Table 1
A selection of animal models used for the study of transplantation of the uterus

<table>
<thead>
<tr>
<th>Reference</th>
<th>Species</th>
<th>Type</th>
<th>Vascular supply</th>
<th>Transplant</th>
<th>Immuno-suppression</th>
<th>No. of animals</th>
<th>Results</th>
<th>Viable grafts</th>
<th>Pregnancies/deliveries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhordania and Gotsiridze, 1964</td>
<td>Sheep</td>
<td>a</td>
<td>Omentopexy</td>
<td>Uterus and ovaries en bloc</td>
<td>–</td>
<td>20</td>
<td>Yes</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>Eraslan et al. (1966)</td>
<td>Dog</td>
<td>a</td>
<td>Anastomosis</td>
<td>Uterus and ovaries en bloc</td>
<td>–</td>
<td>18</td>
<td>10 normal function</td>
<td>10</td>
<td>Not tested</td>
</tr>
<tr>
<td>Yonemoto et al. (1969)</td>
<td>Dog</td>
<td>h</td>
<td>Anastomosis</td>
<td>Uterus and uterus and ovaries en bloc</td>
<td>Azathioprine &amp; prednisone</td>
<td>14</td>
<td>7 rejections by day 17–45</td>
<td>7</td>
<td>Not tested</td>
</tr>
<tr>
<td>O’Leary et al. (1969)</td>
<td>Dog</td>
<td>a</td>
<td>Omentopexy</td>
<td>Uterus and ovaries en bloc</td>
<td>–</td>
<td>32</td>
<td>23 normal function</td>
<td>23</td>
<td>Not tested</td>
</tr>
<tr>
<td>Mattingly et al. (1970)</td>
<td>Dog</td>
<td>a/h</td>
<td>Anastomosis</td>
<td>Uterus and ovaries en bloc</td>
<td>Azathioprine</td>
<td>7 autot.</td>
<td>6 autot. normal function</td>
<td>6</td>
<td>2 (autot.)</td>
</tr>
<tr>
<td>Scott et al. (1970)</td>
<td>Dog</td>
<td>a/h</td>
<td>Omentopexy</td>
<td>Segmented uterus</td>
<td>Azathioprine and prednisone (5 homot.)</td>
<td>50 holot. 40 autot.</td>
<td>13 homot. rejection by day 6–21</td>
<td>13</td>
<td>Not tested</td>
</tr>
<tr>
<td>Scott et al. (1971)</td>
<td>Primate</td>
<td>a/h</td>
<td>Omentopexy</td>
<td>Uterus and tubes</td>
<td>–</td>
<td>10 holot. 4 autot.</td>
<td>10 homot. non-viable by day 28</td>
<td>10</td>
<td>None</td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>4 autot. normal uterine function, tubal occlusion</td>
<td>4</td>
<td>Normal menstruation and mating</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4 homot.</td>
<td>4 homot. rejection from day 4</td>
<td>4</td>
<td>Normal menstruation</td>
</tr>
<tr>
<td>Barzilai et al. (1973)</td>
<td>Dog</td>
<td>a</td>
<td>Omentopexy</td>
<td>Uterus and ovaries en bloc</td>
<td>–</td>
<td>13 oment.</td>
<td>9 by omentopexy normal by dat 21, total nectosis by day 90</td>
<td>9</td>
<td>1 (anastomosis)</td>
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<td></td>
<td></td>
<td></td>
<td>Anastomosis</td>
<td></td>
<td></td>
<td>12 anast.</td>
<td>10 anastomosis normal by day 90</td>
<td>10</td>
<td>1 (anastomosis)</td>
</tr>
<tr>
<td>Confino et al. (1986)</td>
<td>Rabbit</td>
<td>a/h</td>
<td>Sutured to the broad ligament</td>
<td>Unilateral uterus and ovary en bloc</td>
<td>Cyclosporine</td>
<td>8 autot.</td>
<td>3 autot. preserved by day 3, 7, 30</td>
<td>3</td>
<td>Not tested</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10 homot.</td>
<td>4 homot. (no cyclosp.) rejection from day 3</td>
<td>4</td>
<td>Not tested</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3 homot. (cyclosp.) preserved by day 3, 7 and 30</td>
<td>3</td>
<td>Not tested</td>
</tr>
<tr>
<td>Lee et al. (1995)</td>
<td>Rat</td>
<td>h</td>
<td>Anastomosis</td>
<td>Uterus and ovaries en bloc</td>
<td>–</td>
<td>24 synngic</td>
<td>Syngenic normal from day 1–180</td>
<td>24</td>
<td>Not tested</td>
</tr>
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<td></td>
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<td></td>
<td>20 allogenic</td>
<td>Allogenic rejection from day 5</td>
<td>20</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>42 syngenic</td>
<td>22 viable grafts</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Racho El-Akouri et al. (2002)</td>
<td>Mouse</td>
<td>h</td>
<td>Anastomosis</td>
<td>Unilateral uterus</td>
<td>–</td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

a, Autotransplantation; h, homotransplantations.
tions in the dog by either omentopexy or vascular anastomosis (Mattingly et al. 1970; Scott et al., 1970; Wingate et al., 1970; Yonemoto et al., 1969) and by omentopexy in the Macaque monkey (Scott et al., 1971). The rejection pattern recorded by all these investigators was similar to what had been observed concerning the rejection of other organs. Regimens for immunosuppression were, when used, experimental doses of azathioprine and prednisolone but close immunosuppression were, when used, experimental concernings the rejection of other organs. Regimens for immunosuppression and also because the IVF-procedure that would circumvent tubal factor infertility as a cause to transplant the uterus was developed. However, the growing knowledge of immunological mechanisms involved in graft rejection, advancements of efficient immunosuppressive regimens, the development of inbred and gene-deleted strains of mice along with the advances in microsurgery and IVF offers a great advantage for today’s scientists. Only one report on uterine transplantation in an animal model is reported in the literature after 1990. There is a brief report of results from both syn- and allogenic-transplantations of the uterus in the rat, using end-to-side vascular anastomosis of the aorta and inferior vena cava (Lee et al., 1995). The syngenic transplantations showed normal gross appearance and histology at examination up to 6 months after transplantation, while allografts showed hemorrhagic edema of the entire graft by day 10.

We recently developed a transplantation model in the mouse to study the implantation and pregnancy process in transplanted uteri as well as transplant immunology of the uterus (Racho El-Akouri et al., 2002). During the development of the transplantation model we decided to use a heterotopic model, where we placed the uterine horn and the cervix inside a recipient with the native uterus preserved. The advantage of this heterotopic approach would be to have a native uterus to compare the results related to pregnancy outcome within the same animals. We used 6–8 weeks old mice and the surgery of the donor involved microsurgical isolation of the right uterine horn and the cervix with preserved vascular supply from the aorta, through the iliac vessels and the uterine vessels. The uterine horn with the vascular supply and venous drainage up to and including 3 mm of the inferior vena cava and aorta was isolated. This procedure took about 40–60 min. The recipient animal was operated to expose and mobilise the inferior part of the aorta and the vena cava and the graft was anastomosed end-to-side. In these first experiments a syngenic model was used so that the uteri were transplanted from donors to recipients belonging to the same inbred strain. The viability of the uterus was examined at intervals for a period up to 8 weeks after transplantation. The morphology, both on the electron and the light microscopy levels, did not show any differences between the native and graft uterus. Moreover, the tissue blood flow was similar in the native and graft uterus. Finally, the function of the transplanted uterus was evaluated in one animal where a transplanted mouse was made pseudo-pregnant and blastocysts were implanted into the native and graft uterus. Pregnancies were achieved in the grafted uterus and fetal weights were similar to that of fetuses from the native uterus at day 13 of gestation, when the animal was sacrificed.

In ongoing experiments, we have continued to test the results of embryo transfer. It was evident that pregnancy rate was fairly low in the previous animal model where the cervix was left intra-abdominally. The model was further developed to create a cervical–cutaneous stoma on the grafted uterus to achieve drainage of the fluid that accumulated inside the uterus and the cervix (Fig. 1). The pregnancy results in this model were better and comparable to that of the native uterus. Post-natal growth seemed to be similar in offspring from this model and that from controls.

3. Transplantation of the human uterus

There has recently been one attempt to transplant a human uterus. In April year 2000 a 26-year-old female, who had lost her uterus due to post partum hemorrhage, underwent a uterus transplantation (Fageeh et al., 2002). The donor was a 46-year-old patient operated with hysterectomy for benign ovarian disease. The uterus was removed from the donor and segments of the great saphenous vein were anastomosed to each of

![Fig. 1. The two models of heterotopically transplanted right uterine horn with cervix. The left panel illustrates the method with the cervix left intra-abdominally and the right panel shows the method with the cervix attached to the abdominal wall to create a cervical–cutaneous stoma.]()
the three uterine veins and the uterine artery on each side. These were anastomosed to the external iliac vein and arteries of the recipient. The fixation of the uterus was done only to the vagina of the recipient. Immunosuppression was managed by cyclosporine, azathioprine and prednisolone. After 99 days, the recipient was operated with hysterectomy because of signs of massive uterine necrosis possibly due to vascular thrombosis. Interestingly the Fallopian tubes remained viable and there was no evidence of rejection of those. It was concluded that there may have been an inadequate uterine structural support which had lead to tension and torsion of the vessels and thereby decreased perfusion (Fageeh et al., 2002).

Eventhough this first transplantation of the human uterus presents a feasible surgical technique for vascular supply to the transplanted human uterus, we strongly believe that several other issues need to be resolved before a safe and successful transplantation of a human uterus that has the ability to implant an embryo and harbour a growing fetus to term is possible. It is of vital importance not to raise any false hopes for women and couples with untreated uterine factor infertility, by rushing the development of a method before as many relevant issues as possible have been evaluated. These issues would include:

- assessment of the tolerance of the uterus to cold ischemia and reperfusion injuries to establish a clinically safe method for organ-storage between procurement and transplantation
- optimization of surgical techniques for vascular anastomosis and prevention of prolapse or torsion of the transplanted uterus
- evaluation of rejection mechanisms of the mammalian uterus
- assessment of the efficiency of different immunosuppressive regimens and their impact on both patient and fetus
- the performance of a thorough evaluation of and program set-up for donor and recipient selection criteria and physical and psychological care in accordance with international medical ethics and national law

4. Preservation of the uterus

When the first heart transplantation was performed 1967, continuous machine perfusion with cooled, oxygenated Ringer lactate buffer was used during transfer between donor and recipient (Barnard, 1967). Today cold ischemic storage with preservation through suppression of metabolism by hypothermia after flushing of the vascular system with a suitable buffer to prevent hypoxic and reperfusion injuries is used (Southard and Belzer, 1995). Depending on how central the organ is and its main functional aspect, different organs show different tolerance to cold ischemic storage. The clinically used time limit for the human heart and lung is around 6 h (Fischer et al., 2001; Wildhirt et al., 2000) and for kidney and liver up to 24 h (Muhlbacher et al., 1999).

The injuries to an organ caused by cold ischemia manifest themselves at reperfusion as formation of edema, vasoconstriction with subsequent infarction and oxidative injuries to different enzymes. These changes in turn cause inflammation and cell death and—if the degeneration is severe—loss of function of the organ (Grinyo, 2001; Laskowski et al., 2000; Maroszynska and Fiedor, 2000).

In experiments on the human uterus involving physiological studies, warm, continuous flow perfusion with oxygenated Krebs-Ringer solution for 48 h maintained viability of the uterus, while the first signs of degeneration in the control samples (warm storage, nonperfused) were seen after 6 h (Bulletti et al., 1986). This indicates that the human uterus is fairly robust. The fact that the uterus is not a central organ also means that no immediate, energy consuming activity is required and the uterus will have time to recover in situ after the transplantation.

In evaluating the preservation of the human uterus the two main aspects of interest are the time limit and most suitable type of preservation solution. Possible means to study the effects of cold ischemia on uterine tissue and reperfusion injuries on whole organ is outlined in Fig. 2. Our aim is to study several functional, histological and biochemical parameters on uterine tissue after different times of cold ischemia using different types of preservation solutions. We also intend to study the same parameters after cold ischemia/reperfusion of whole organ, using the method developed by Bulletti et al. (1986). Through this we believe that it
will be possible to establish a clinically safe time limit and method for preservation of the human uterus.

5. Immunosuppression and rejection

Since the purpose of a transplantation of the human uterus would be to restore fertility, the effect of immunosuppressive drugs on implantation and embryonic and fetal development need to be investigated before a successful transplantation can take place.

A meta-analysis of the outcome of pregnancies in transplanted patients or patients with auto-immune diseases treated with cyclosporine showed a slight elevation of pre-term deliveries, fetal growth retardation and congenital malformation compared to the general population (Bar Oz et al., 2001). This cannot be considered conclusive evidence for a negative effect of cyclosporine on fetal development though, since the study could not exclude the effects of the underlying maternal disease or of other immunosuppressive drugs such as azathioprine often administered in combination with cyclosporine. A study concerning the teratogenic effects of several immunosuppressive drugs in the rat, found no such effects of cyclosporine (Schmid, 1984) while a smaller study on tubal-ovarian transplanted rats showed a teratogenic effect of cyclosporine in high doses, but a reduced dose, still efficient for prevention of rejection, had no negative effect on the offsprings (Scott et al., 1987). Azathioprine is considered being a mild teratogen as demonstrated in the rat (Schmid, 1984) while corticosteroids such as prednisone are not considered teratogenic but can be associated with a slightly higher risk for cleft palate (Park-Wyllie et al., 2000).

It is clear that for the purpose of a safe immunosuppression regimen in a uterine transplantation program, a more elaborate study on these effects must be done. By utilizing the mouse model, which we have described above, it would be possible to study this and also other aspects relevant for the issue such as molecular mechanisms of rejection of the mammalian uterus and the effects on implantation and fetal development of denervation of the uterus, which would be a result of transplantation.

6. Recipient and donor — criteria and care

There are several considerations to be made when evaluating whether a woman with uterine factor infertility is suitable for uterine transplantation. It is important to consider the age of the recipient so that she has a satisfactory ovarian reserve and can undergo IVF treatment with good chances of achieving fertilization. She should of course have no genetic children from before and the social and family situation should be stable. The donor in a uterine transplantation program would be a live, volunteering woman who has at least one child and no desire for another. The donation would of course be completely altruistic. Preferably the donor would be related to the infertile woman—perhaps her mother or sister since the chance for a good HLA-tissue and blood group match then would be higher. When related donors are considered, care must be taken that no family emotional coercion exist. It is important that the parties involved are cared for by separate counselors and physicians to guarantee the autonomy of both donor and recipient.

Selection-criteria concerning the donor should include normal uterine anatomy at laparoscopy and hysterosalpingography, endometrial biopsy without atypia and no history of cervical dysplasia. The age of the donor seems to be of minor concern as long as she is cycling or under hormone replacement treatment that retains a receptive uterus. Several studies concerning the outcome of oocyte donation from younger to older women show that the causes of age-related infertility is rather due to aging oocytes than to uterine senescence (Abdalla et al., 1993; Borini et al., 1996; Navot et al., 1994; Sauer, 1997).

Thorough information about procedures and risks involved as well as of the chances to actually conceive would be given according to already existing procedures concerning live donors in transplantation and IVF-patients.

7. Conclusion

It’s clear that human uterine transplantation may be a feasible treatment for uterine factor infertility in the future. In this review we are showing that there exist a fairly limited number of animal studies on techniques for uterine transplantation and that these studies have mostly been concerned with methods to obtain vascularisation of the uterus. There is now an available mouse model to study several aspects of mechanisms involved in uterine transplantation. With the use of the mouse model the rejection phenomenon can be studied in detail and there are also possibilities to use gene deleted or transgenic mice to study specific aspects on this mechanism. It is important to study the effects of immunosuppression on the transplanted uterus and to find the most suitable combination of immunosuppressions to be used in a clinical setting. Moreover, detailed studies on effects on the fetuses of immunosuppressions have to be performed. Since there have been several major ethical issues concerning gestational surrogacy we think that the treatment option of uterine transplantation also should be evaluated with respect to ethical issues before being performed. It should at last be stated that treatment for uterine factor infertility by uterine trans-
plantation would be a suitable treatment option for a limited number of patients. Thus, clinical practice of this method should be limited to a small number of centers with expertise in all aspects of this area.

References


