Allograft En Bloc Vagino-Utero-Ovarian Avascular Transplant Versus Autograft Implantation in Rats

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Abstract

Objectives: **The aim of this study was to compare the results of an allograft en bloc vagino-utero-ovarian avascular transplant with those of autograft implantation in rats.**

Materials and Methods: **Thirty-four inbred adult virgin female Albino rats (age range, 10 - 12 weeks) were divided into 2 groups: the control group (autograft, n=11) and the study group (en bloc vagino-utero-ovariectomy, n=23). In the study group, the uterus and adnexa and the ovaries of the donor rat were transplanted to the recipient animal. Twenty-five to 30 days after that procedure, all rats were killed, and the samples were assessed histopathologically. No immunosuppressive drugs were used.**

Results: **Ten rats died during the postoperative period. In 16 rats, the transplanted system had survived completely at the conclusion of the study. In each of the study groups, complete survival of the uterus and ovaries was noted in 8 rats (34.8% in the study group and 72.8% in the control group). In all rats except 1, histopathologic examination did not reveal any signs of the classic criteria for tissue rejection reaction. The lack of revascularization, nonspecific signs of inflammation, and the presence of large granular lymphocytes and natural killer cells were reported.**

Conclusions: **Our data indicated that the outcome of both allograft and homograft avascular en bloc transplant of vagino-utero-ovariectomy in rats was successful, and that immunologic rejection did not seem to have an important role in those procedures.**

Key words: *Uterus transplant, Rat, Rejection*

There are few reports of animal experiments involving transplants of the uterus (1). In 1964, Zhordania and Gotsiridze (2), using omentopexy for revascularization, successfully reimplanted the uterus and its appendages in sheep, a procedure that resulted in several subsequent pregnancies in the study subjects. En bloc reimplantation (transplanting the uterus, ovaries, and the fallopian tubes) has been reported in a few studies. In those reports, 2 techniques (vascular and avascular transplant) were most often used.

Eraslan and colleagues (3) were the first authors who reported an en bloc vagino-utero-ovarian vascular transplant, a procedure duplicated by other investigators (4, 5). Other researchers (6) tried a transplant approach that used the aorta and vena cava. Using the vascularization of an omental pedicle, Scott and colleagues (7) showed that an autotransplant involving subtotal abdominal hysterectomy and bilateral salpingectomy was successful in adult rhesus monkeys. Other studies have shown that pregnancy has occurred after a vascularized contralateral autotransplant of the fallopian tube in the ewe (8). Lee and colleagues (9), who studied en bloc vagino-utero-ovarian vascular transplant between syngeneic Lewis rats, showed that this procedure was successful without antirejection medication, but the study subjects experienced no subsequent pregnancies which represents the survival of a uterus transplanted from another animal that is closely related to the donor and recipient (1).

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Because of the ease of operation and the promising results, a nonvascularized technique for transplanting the uterus and its appendages became a focus of research. La Sala and colleagues (10) showed that nonvascularized 1-cm pieces of uterus that were reimplanted into the opposite horn of the uterus (an autograft) of rats resulted in pregnancy. Also, evidence suggests that there may be advantages to the avascular (as opposed to vascular) transplant of organs. For example, experimental models have shown that oviduct tissue in a vascularized bed is associated with rejection. (11).

To our knowledge, no study has assessed the outcome of nonvascularized transplant of the uterus and its appendages. The aim of our study was to compare the results of an allograft en bloc vagino-utero-ovarian avascular transplant with that of an autograft implantation in rats.

Materials and Methods

Thirty-four inbred adult virgin female Albino rats (age range, 10-12 weeks) were procured from the Pasteur Institute (Tehran, Iran). Rats were allowed free access to water and rat chow. All protocols were approved by the animal welfare regulatory committee of Shahid Beheshti University of Medical Sciences. The experimental procedures were performed in accordance with the Guide to the Care and Use of Experimental Animals, Canadian Council on Animal Care, 2nd edition, 1993 (12).

Rats were divided into 2 groups: the control group (autograft, n=11) and the study group (en bloc vagino-utero-ovariectomy, n=23). Two rats with an abnormality of these organs were excluded from the study and were replaced with other subjects. To anesthetize the animals, we used an intraperitoneal injection of ketamine 50 mg/kg and chlorpromazine 10 mg/kg. After the insertion of a 20-gauge venous catheter into the tail of each rat, we injected 1000 IU/kg of heparin to prevent blood coagulation in the small vessels. Normal saline was infused (1 to 2 drops per minute) throughout the operation. To prevent vascular spasm and thrombosis, the rats were sprinkled every 30 minutes throughout the procedure with papaverine that had been diluted with normal saline.

After each rat had been anesthetized, its abdomen was opened by means of a middle laparotomy incision, and the intestines were retracted to the left side of the abdomen. In the donor rat, all aortic branches were ligated with 5-0 silk sutures. To provide sufficient blood supply to the uterus and adnexa, the tubo-ovarian and uterine branches of the internal iliac arteries were spared. After the vessels had been detached, the rat received an infusion of heparinated normal saline through the aorta to remove all blood from the uterus and all vessels. Then, the utero-ovarian ligaments were cut from above, and the vagina was transected at the midline. The uterus and adnexa and the ovaries and their corresponding blood vessel stumps were removed and preserved in sterile conditions at 4°C in normal saline.

The same procedure was performed in the recipient rat. Then, in the study group, the uterus and adnexa and the ovaries of the donor rat were transplanted to the recipient animal. To accomplish this, the edges of the vaginal cuff were sutured to the recipient vaginal cuff (Figure 1). On each side, in the lower third of the rectus abdominis muscle and lateral to the middle laparotomy, 2 incisions were made. Through those incisions, the horns of the transplanted uterus and adnexa and the ovaries were removed from the peritoneal cavity and were fixed to the aponeurosis of the rectus abdominis (Figures 2 and 3). After skin closure, the subject was transported to the recovery unit and then to the ward. In the control group, that procedure was performed with each donor rat's own tissue.

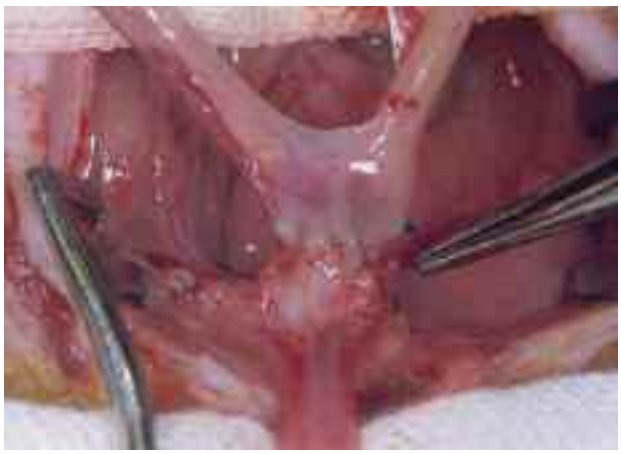

Figure 1. The edges of the vaginal cuff were sutured to the recipient vaginal cuff.

After 25 to 30 days, all rats underwent exploratory surgery after having received a general anesthetic. Those that died immediately from surgical complications during the postoperative period were

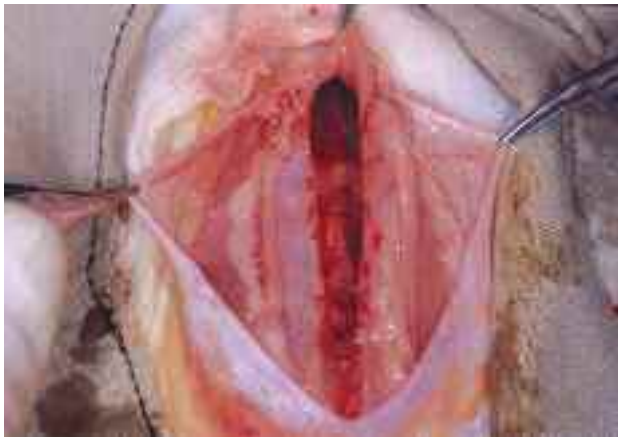

Figure 2. On each side, in the lower third of the rectus abdominis muscle and lateral to the middle laparotomy, 2 incisions were made. Through those incisions, the horns of the transplanted uterus and adnexa and the ovaries were removed from the peritoneal cavity.

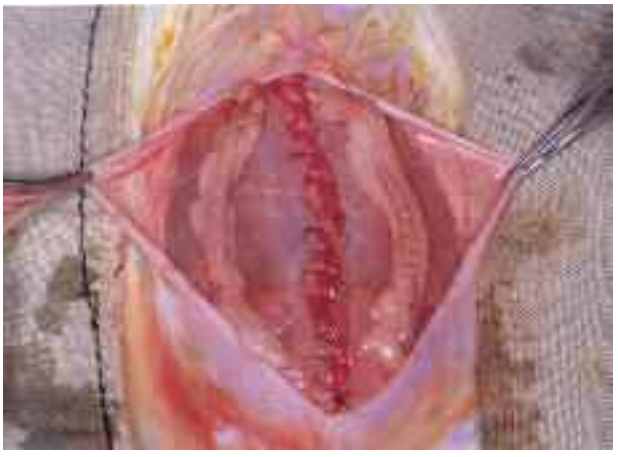

Figure 3. The horns of the transplanted uterus and adnexa and the ovaries were fixed to the aponeurosis of the rectus abdominis.

excluded from the study. In rats that completed the study, neovascularization and signs of rejection (including necrosis and atrophy) were reported, and the survival of the transplanted system was histopathologically assessed for the presence of large granular lymphocytes in the uterus, atrophy, necrosis, fibrosis, and other signs of rejection (such as lymphocyte infiltration) in the transplanted tissues. Throughout the study, no rat received an immunosuppressive drug.

Data are expressed as the mean ± SD or the number of patients. Parametric data were analyzed with the Mann-Whitney *U* test. The chi-square test and the Fisher exact test were used for categorical data analysis. Statistical calculations were performed with SPSS software (Statistical Product and Services Solutions, version 15.0, SPSS Inc, Chicago, IL, USA). Differences were considered statistically significant at a *P* value of less than .05.

Results

Ten rats died during the postoperative period. The mean of duration of surgery was statistically significantly shorter in the control group than in the study group. However, the duration of the postoperative period was comparable in the 2 groups. In 16 rats, the transplanted system had survived completely at the conclusion of study (Figures 4 and 5). In each of the study groups, complete survival of the uterus and ovaries was confirmed in 8 rats (34.8% in the study the group and 72.8% in the control group) (Table).

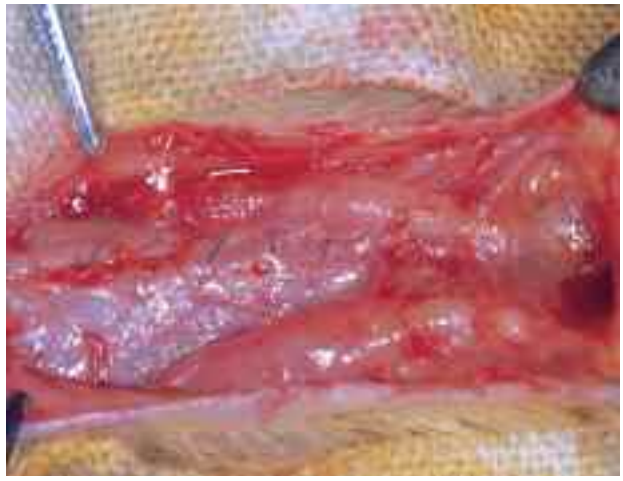

Figure 4. Complete survival of the transplanted system. Note the excellent neovascularization.

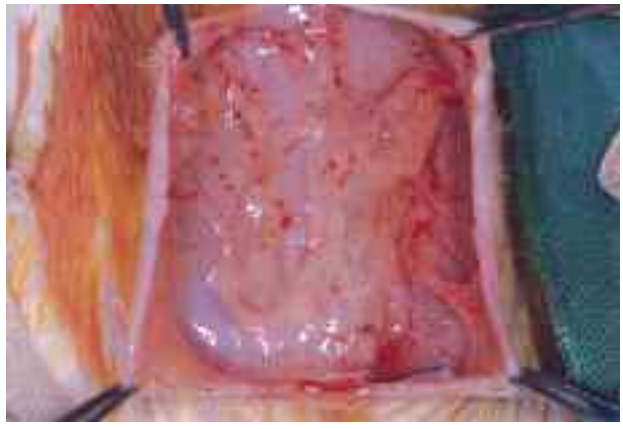

Figure 5. A necrotized transplanted system.

In all rats (even those with necrotized organs) except 1, histopathologic examination did not reveal any signs of the classic criteria for tissue rejection reaction. The lack of revascularization, nonspecific signs of inflammation, and the presence of large granular lymphocytes and natural killer cells were

reported. The classic signs of humoral rejection were noted in only 1 rat.

Table. The results of allograft en bloc vagino-utero-ovarian avascular transplant versus autograft implantation in rats

Variables	Study group (n=23)	Control group (n=11)	*P* Value
Died (No.)	9	1	.101
Duration of surgery (min)	91.3 ± 22.3	71.0 ± 23.0	.028
Postoperative period (d)	27.5 ± 11.1	33.1 ± 21.2	.388
Neovascularization			.162
Excellent	4	3	
Moderate	7	2	
Poor	3	4	
Transplanted ovaries			.312
Survived	7	0	
Partial necrosis	1	4	
Necrosis	6	1	.195
Transplanted uterus			
Survived	4	4	
Partial necrosis	2	0	
Necrosis	8	5	
Survival of transplanted system	8	8	.1243.

Discussion

To our knowledge, this is the first study reporting the results of avascular transplant en bloc vagino-utero-ovariectomy. The results revealed that allografts and homografts of en bloc clinical uterine-ovarian transplants were effective and resulted in the complete survival of the transplanted system in 34.8% of the rats in the study group and 72.8% of the rats in the control group. Furthermore, there were no signs of the classic criteria for tissue rejection, even in rats in which the transplanted organs did not survive and despite the lack of treatment with immunosuppressive drugs.

The postoperative mortality rate was 29.4%. The long duration of anesthesia and surgery, which exceeded 2 hours in some cases, might have contributed to the postsurgical mortality rate. Applying protamine sulfate would have led to thrombosis at the site of anastomosis, which would have caused the avascularization and necrotization of the grafted uterus. Those events would hasten the death of the animal. Also, owing to the fewer number of procedures needed, the duration of surgery in control group was shorter. We decided not to negate the effects of heparin by applying protamine sulfate. In addition, transplanted organs experience a period of ischemia before implantation and are therefore more vulnerable to ischemia-reperfusion injury. The shorter duration of surgery in the control group resulted in a shorter duration of ischemia and ischemic injury and, in turn, a relatively weaker inflammatory response. Those factors explain the higher survival rate of the transplanted system in the control group; however, the survival rate in the control group did not differ with statistical significance from that in the study group.

High rates of success with this transplant technique have been reported in other studies as well. Eraslan and colleagues (3) removed en bloc the uterus, ovaries, and fallopian tubes of dogs, reimplanted those organs in their original position, and reanastomosed the hypogastric arteries and the hypogastric or common iliac veins. The ovarian vessels were not reanastomosed. The results of that study revealed that the reimplanted structures functioned normally. Of the 8 dogs studied, 3 had subsequent successful pregnancies. Rats are excellent models for the study of reproductive organs and organ transplant. Unless encouraging results are obtained with that model, the use of primates in such research and conducting relevant clinical trials are not justifiable (13).

Another finding of this study was the lack of immunologic response that may have been driven by large granular lymphocytes and natural killer cells. Natural killer cells are usually under both inhibitory and activation control to prevent the lysis of healthy cells, but they participate in an immune response when necessary. Some authors have suggested that natural killer cells have an important role in the suppression of autoimmunity and tissue graft rejection (14).

A limitation of this study was the nonassessment of the function of the transplanted organs after the rats had been killed; however, to our knowledge, no impregnation has been reported in rats after a procedure like that described in this report. For example, Lee and colleagues (9) noted that although an en bloc vagino-utero-ovarian transplant in the rat is possible, impregnation has not yet been achieved after that procedure in rats. Scott and associates (7) showed that there was no pregnancy in primate because of tubal fibrosis and occlusion after uterus transplant. Later, the same group of researchers reported that using cyclosporine in syngeneic rats that had received an en bloc vascularized adnexal isograft or allograft resulted in pregnancy (15). Other reports of successful pregnancy following the use of cyclosporine after tubal and ovarian allografts in rats (16) and microsurgical en bloc vascularized tubo-ovarian allografts in rabbits (17) have also been published.

We suggest that the function of en bloc vagino-utero-ovariectomy in rats should be examined in future studies. Furthermore, it is important to assess the ovaries to determine whether a drastic reduction in the total number of primordial follicles occurs after transplant. Much additional research is necessary to improve the results of this type of grafting (whether autograft or allograft) before it can be used in women.

In summary, our data indicate that the outcome of both the allograft and homograft avascular en bloc transplant of vagino-utero-ovariectomy in rats is successful and that immunologic rejection does not seem to have an important role in surgical success. The good results from this technique are due to the ease of operation and the reduction in immunologic problems that arise from allografts.

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