Preliminary results of orthotopic en bloc uterus and ovary transplantation in the laboratory rat

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Summary

A new experimental model of whole uterus and ovary transplantation in the laboratory rat was achieved. The main goals of this study were concerned with developing and standardizing the microsurgical technique of uterus transplantation in rats and observing the particular cellular patterns of acute allograft rejection at the level of the transplanted graft. Thirty-five orthotopic uterus transplantations were performed. An additional 20 female rats were used for dissection training sessions. Recipients were euthanasied at 24 hours, 48 hours and 72 hours. Immediate postoperative survival was 100%. Patency of the microsurgical anastomoses, checked at 24 hours, was 100%. At 72 hours thrombosis occurred in all anastomoses. The explanted uterine grafts were fixed in formaline and analyzed under light microscopy and specific imunohistochemical analysis.

The acute allograft rejection has a particular cellular reaction pattern, probably due to the unique diversity of the tissues that compose it. Inflammatory cells like LTCD8+, LBCD20+ and mastocytes tend to agglomerate in the vicinity of nervous and vascular structures, showing no signs of lymphoid tissue disposition like in typical acute rejection.

Uterus transplantation in rats has proven to be a valid experiment that allows us to express hope that by further research on transplantation of the uterus gynecologists will be able to introduce an adapted technique in the treatment of specific cases of human female infertility.

Key words: Uterus and ovary transplantation; Experimental model in rats.

Introduction

Transplantation is one of the most remarkable success stories of modern medicine. The first successful kidney transplant in humans occurred in 1954 between identical twins. A cadaveric kidney transplant had been attempted in 1951, but immunosupressants were not available and the patient died on postoperative day 37. In the seventies heart transplantation was followed by liver and lung transplantation. In the past decade there has been an explosion of innovative developments in transplant procedures.

Clinical transplantation of the genital organs is still shadowed with doubt, obviously because the uterus is a life generating organ rather than life sustaining. Nonetheless, there are various pathological conditions that may be amendable with uterus transplantations in the future, such as Rokitansky-Kuster-Hauser or the ARD (androgen resistant disease) syndrome. At present women suffering from similar conditions (uterine agenesia, hysterectomy after traumatic or pathologic circumstances in nullipara, malformations) can use a surrogate mother (usually a close relative) to carry out a pregnancy if ovarian function is unaffected and healthy ovules can be produced and fecundated in vitro and then transferred into the carrier mother's uterus.

Revised manuscript accepted for publication January 9, 2003

Of course before we think about genitalia clinical transplantation, there are some fundamental problems, such as immunosuppression during pregnancy and psychological factors that demand extended scientific multidisciplinary research.

The present paper describes an experimental model of uterus and ovary transplantation providing an effective tool for further in vivo research in future studies.

Materials and Methods

Adult female Brown Norvegian rats with an average weight of 215 grams were used for the experiments. All animals were chosen from the Biobasis in the Center for Laparoscopic Surgery and Microsurgery "Pius Branzeu", University of Medicine and Pharmacy "Victor Babes" Timisoara. Rats were housed at 22ºC with food and water given ad *libitum*. The study was analyzed and granted by the Local Committee of Ethics in Animal Experiments. Anaesthesia was of inhalatory type with ether. A total of 100 animals were used, 70 for transplantation procedures and 30 for preliminary dissections of the pelvic region. Standard microsurgical instruments and the operative Leica MD-650S microscope was used for the microsurgical procedures. The transplant recipients were euthanasied at 24, 48 and 72 hours. The explanted grafts were fixed in 10% formalin for 24 hours and then included in paraffin. Standard hematoxilin eosin and Masson staining were used to prepare the tissue samples for examination in light microscopy. For the immunocytochemistry studies specific monoclonal antibody trays were used. All procedures were performed in clean but not sterile conditions.

The anaesthetized animal used as a donor is laid down on the operating table abdomen up. Via a xifopubian laparotomy the peritoneal cavity is entered, followed by gut isolation in the right side of the animal. Phreno-ovarian ligaments are identified and cut. Then the whole graft together with the ovaries is mobilized from cranial to caudal by cutting the posterior peritoneum along the uterine tubae. The external iliac axis is identified and ligated bilaterally. Pudendal vessels are managed in the same way. The vagina is carefully freed from the superior vesical wall and its venous plexus. The urethers are dissected and separated by the tubal vessels bilaterally. Finally the pedicle of the graft, aorta and vena cava, are mobilized and dissected free from the surrounding tissues. A single tie is put around the aorta and vena cava just below the renal vessels, and then both vessels are cut immediately beneath the sutures. The blood is flushed out from the graft with cold 4ºC Ringer solution with a catheter via the aorta-common iliac-uterine arteries. Irrigation is stopped only when clear fluid emerges from the venous segment of the graft. The abdominal cavity is entered in the receptor in a similar way as in the donor. Hysterectomy is performed. The remnant vaginal stump is anchored with two stay sutures. The aorta and vena cava are dissected free from the subrenal level to the common iliac bifurcation. Both vessels are clamped cranialy and caudaly. A longitudinal arteriotomy and subsequent venotomy is performed. The uterine graft is placed into the receptor's abdominal cavity, and two end-to-side anastomoses are performed – the graft's caval segment to the receptor's vena cava and the graft's aortic segment to the receptor's abdominal aorta – using a continuous technique with 10-0 Nylon.

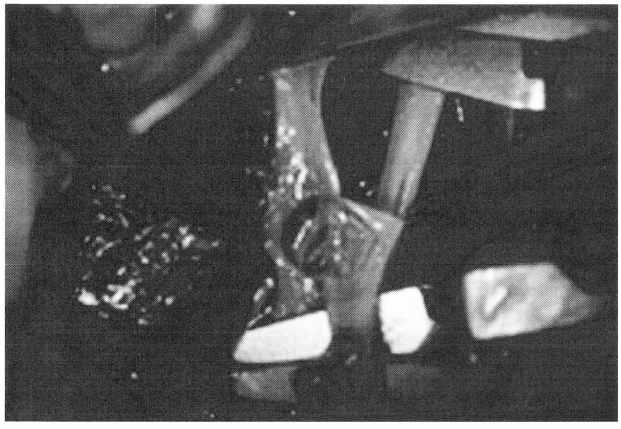

Figure 1. — End-to-side anastomosis in the aorta.

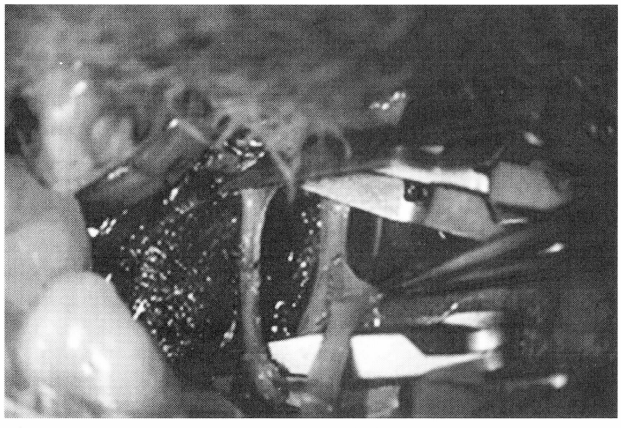

Figure 2. — End-to-side anastomosis in the vena cava.

The vaginal end of the graft is anastomosed end-to-end with the vaginal stump of the recipient with 7-0 monofilament suture.

The vessels are declamped as follows: cranial caval clip, caudal caval clip, caudal aortic clip and finally cranial aortic clip. Graft myometrial revascularisation peristalsis can be observed 10-12 minutes after declamping. During this period the graft must be kept warm with gauze soaked in warm Ringer's solution. The abdominal cavity is closed in two layers after carefully washing the peritoneal cavity with serum. Prior to putting the animal in its cage, 4 ml of physiologic serum are administered with a subcutaneous injection.

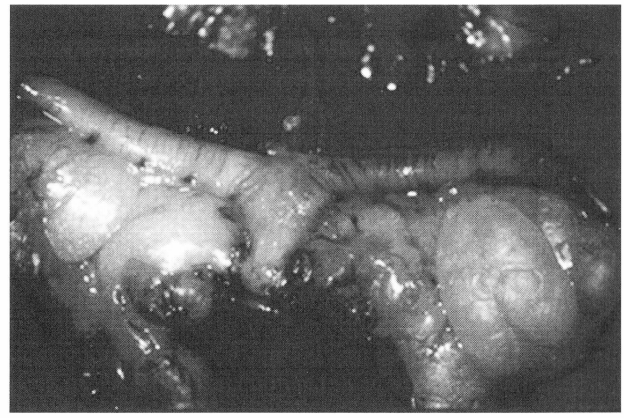

Figure 3. — Revascularized uterine graft before orthotopic transplantation.

Results and Discussion

Microsurgical procedures had an average duration of three hours. Average cold ischaemia time was 25 minutes. Postoperative survival was 100% at 24 hours. At 48 hours two rats died of extensive post-anaesthetic lung damage. Subsequently at 72 hours two more animals died of peritonitis. All dead animals were considered technical failures and excluded from the study. Vascular anastomoses stayed permeable 24 hours, but after that they gradually became obstructed. At 72 hours all anastomoses were blocked by expressive thrombus. The lumbar vascular pedicles, which are vessels encountered during graft dissection and receptor preparation, showed in 2.5% of the rats a constant anomaly - the transcaval path of the right iliolumbar arteries.

Host versus graft disease has a hyperacute rejection pattern which begins to be illustrated in the tissues prepared from the 48-hour grafts. By entering the peritoneal cavity of the donor 48 hours after transplantation, the uterine graft appears blue in color with intestinal loops and fibrinous tissue glued around it, thus demonstrating the host's reaction against the graft. The presence in the tissue of CD_{34}, Von Willebrandt vascular markers and inflammatory type cells, mostly marked eosinophilia, suggests an anaphylaxis phenomenon with a granular cell distribution rather than the classical lymphoid pattern.

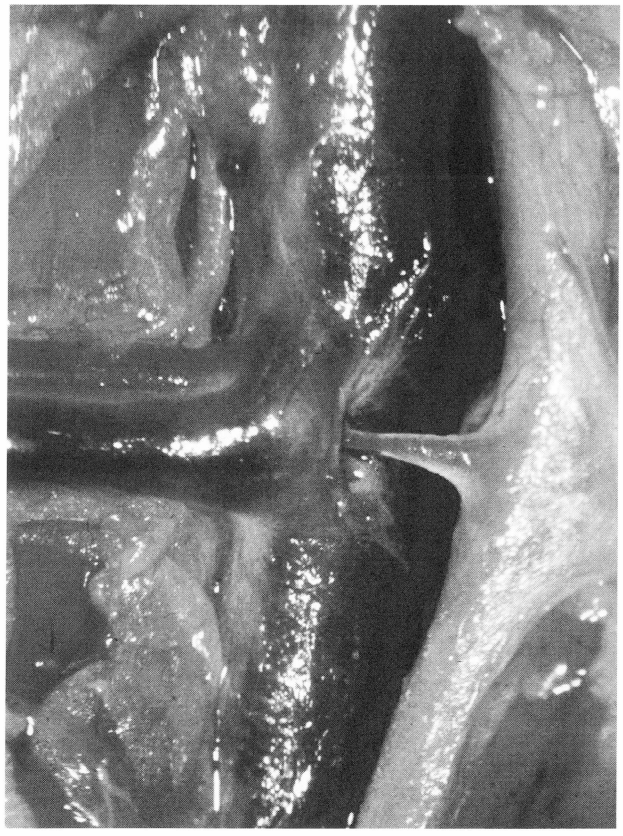

Figure 4. — Transcaval path of the right iliolumbar artery.

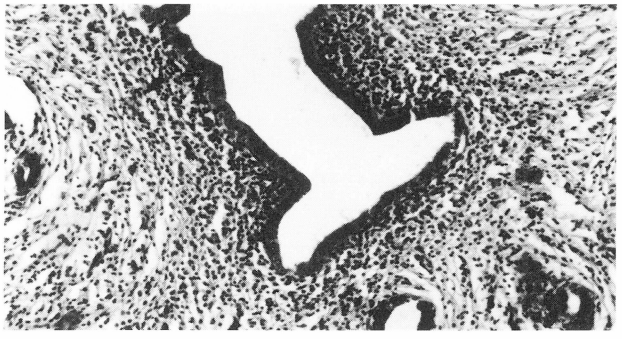

Figure 5. — Graft endometrial necrosis after 72 hours (Masson stain).

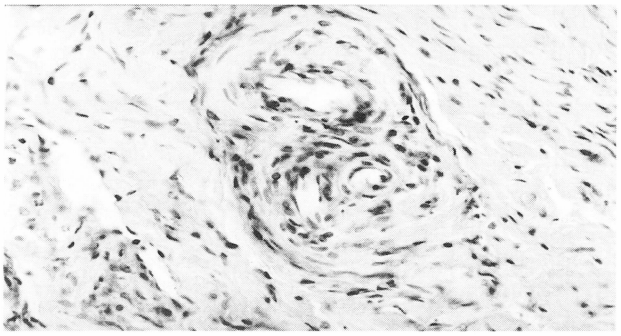

Figure 6. — Marked eosinophilia around myometrial arteriola and venula.

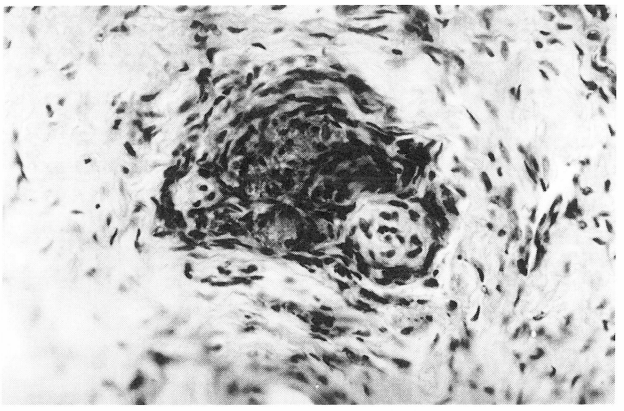

Figure 7. — Perivascular and perineuritis intense cellular reaction.

Observable also is a particularly intense perinevritis reaction at 72 hours with a large number of lymphoid cells, also probably due to the fact that nervous fibers were resutured.

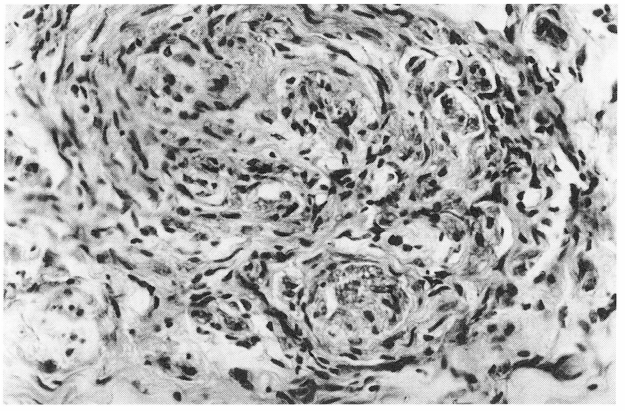

Figure 8. — Local inflammatory intense perineuritis with large numbers of lymphoid cells.

Conclusions

Gynaecologists should be part of the relatively new transplantation chapter of modern medicine and be able, together with interested patients, to change the classical concept that there is no necessity for uterus transplantation.

On our part we are firm believers that uterus transplantation is going to be adopted for rare, specific cases of gynaecological disease as *the solution.*

Consequently, we have started a multidisciplinary project aimed at studying experimental models in animals in order to try to develop the necessary complicated procedures for future uterus transplantation in humans.

In our opinion, the presented experimental model in rats is a valid first step towards the desired final achievement and of course future studies including immunological aspects have to resume this beginning.

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Flemish Gynaecological Oncology Group (FGOG)

Endocrine treatment and prevention of breast and gynaecological cancers

Brussels, Belgium - KBC Building
January 15-17, 2004

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Scientific and Organizing Committee: F. De Prins, Antwerp; C. De Rop, Bonheiden; W. Tjalma, Antwerp; P. van Dam, Antwerp; J. Van Ginderachter, Ghent

PRELIMINARY PROGRAMME OVERVIEW

Thursday afternoon, January 15th

Endocrine prevention of breast and gynaecological malignancies.

Oestrogens/tamoxifen and the endometrium.

Friday, January 16th

Growth factors and female steroids, their receptors and interfering factors.

Adjuvant therapy for early hormone-dependent breast and gynaecological malignancies.

SERMs and SEEMs.

Advanced and metastatic disease.

Saturday, January 17th

Review sessions on breast and gynaecological cancers.

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