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A technique for laparoscopic transplantation of frozen-banked ovarian tissue

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Objective: To describe a laparoscopic technique for transplanting frozen-banked ovarian tissue.

Design: Case study.

Setting: Academic medical center.

Patient(s): A patient whose ovarian tissue was previously frozen with the slow-freeze technique.

Intervention(s): Microsurgical reconstruction of ovarian cortex and its laparoscopic transplantation to the ovarian fossa.

Main Outcome Measure(s): Blood flow to the grafts by doppler, follicle development and ovulation by ultrasound, E_2 and progesterone production, and resumption of spontaneous menses.

Result(s): The patient ovulated and menstruated 4 months after the transplant in response to ovarian stimulation with menopausal gonadotropins.

Conclusion(s): Laparoscopic transplantation of frozen-banked ovarian tissue beneath the pelvic peritoneum can restore ovarian function. (Fertil Steril® 2001;75:1212–6. ©2001 by American Society for Reproductive Medicine.)

Key Words: Ovary, cryopreservation, transplantation, laparoscopy

Transplantation of cryopreserved autologous ovarian tissue has been successfully performed in many animal models (1). These studies proved that frozen ovarian tissue could resume its cyclical function after transplantation without vascular reanastomosis. In the rodent, when dimethyl sulfoxide (DMSO) and a storage temperature of −196°C were used, cyclicity was restored by grafting in 75% of the mice—a similar percentage to that observed in controls carrying fresh ovarian grafts (2). Even more remarkably, frozen–thawed fetal mouse ovaries restored cycles to 100% of syngeneic adult ovariectomized mice, many of which became fertile (3). These models used isografts or autografts because neither the ovary nor its normal location is immunologically privileged (4).

Studies of the sheep ovary have shown that ovarian tissue cryopreservation is feasible in a large species. Because the whole ovary is too large to be cryopreserved, cortical slices were prepared from one organ for freezing to liquid nitrogen temperatures using a medium containing DMSO and a slow-freezing, rapid-thawing protocol (5). When the contralateral ovary was removed 3 weeks later, frozen-thawed and fresh slices of tissue were grafted to opposite sides with 5–0 delayed-absorbable suture to the ovarian pedicles. The first ovulations occurred approximately 4 months after the second operation. In a subsequent study, a further set of eight sheep was grafted with bilateral frozen-thawed autografts. The ovaries regained cyclicity and functioned similarly to the controls for 22 months, until they were removed (6).

Laboratory experience with frozen-banked human ovarian tissue has been consistent with findings from animal studies. In a quantitative study, human ovarian tissue was cryopreserved in four different cryoprotectants. They were then thawed and grafted in severe combined immune deficiency (SCID) mice (7). Survival rates for the primordial follicles frozen in DMSO, ethylglycol (EG) and propanediol (PROH) ranged from 44%–84%. The follicles

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cryopreserved with glycerol did poorly (10%). In a recent study in which DMSO was used as a cryoprotectant, we showed that frozen-thawed primordial follicles in ovarian xenografts continued to initiate growth after more than 6 months of grafting. At the end of the experiment, a significant number of healthy primordial follicles still remained uninitiated (8).

Because of the accumulating evidence from the aforementioned animal and human laboratory studies, our center, like many other assisted reproduction centers, began banking ovarian tissue before chemotherapy and radiotherapy and, in some cases, after oophorectomy for benign ovarian disease. Despite the increasing use of ovarian tissue freezing, however, no procedure has been described in humans to reimplant frozen-banked ovarian tissues yet.

With the aim of developing a human ovarian transplantation procedure, we initiated a clinical trial and performed the first laparoscopic ovarian transplantation in the human using frozen-banked autologous ovarian tissue. We recently reported the outcome of this case (9). The purpose of this article is to describe the laparoscopic technique of transplantation of frozen-banked human ovarian tissue.

MATERIALS AND METHODS

Preoperative Evaluation

The ovarian tissue was from a 29-year-old woman and had been removed at another institution, reportedly to control intractable dysfunctional bleeding, with the patient's consent. Ovarian tissue was grossly normal at the time of removal, and cortical strips were cryopreserved in 1.5-M propanediol with the slow-freeze protocol. The patient contacted us 6 months after she had her ovarian tissue cryopreserved. After numerous consultations, the 72 vials of tissue were transported to New York. The study was approved by the institutional review board at New York Methodist Hospital, and the patient signed an informed consent indicating her desire to participate in this experimental protocol.

One of the ovarian pieces (6 × 6 × 2 mm) was thawed, and half of it was histologically sectioned. The other half was cultured for 6 days, and E_2, P_4, and testosterone production were measured daily. Histology revealed few one- to two-layer follicles and showed that 50% of the stroma retained normal cellularity. Ovarian pieces produced increasing amounts of E_2 (baseline vs. in culture: 21.6 ± 1.2 pg/mL vs. 222.6 ± 33.0, $P<.0001$), progesterone (0.1 ± 0.005 ng/mL vs. 0.6 ± 0.2 ng/mL, $P<.03$), and testosterone (11 ± 0.5 ng/dL vs. 15 ± 0.4, $P<.0001$) in culture. On the basis of these results, a decision was made to thaw 60 vials that contained 80 pieces of ovarian cortex measuring 2–15 mm by 2–10 mm by 1–3 mm. Before the transplant and on 0.1 mg of transdermal estradiol, the patient's E_2, FSH, and LH were 339 pg/mL, 1.3 mIU/mL, and 1.4 mIU/mL, respectively.

Procedure

Ovarian pieces were thawed by holding the vials in room air for 30 seconds and then plunging them into a 37°C water bath for 2 minutes. Then the contents of each vial was emptied in the first-wash media at 4°C, which contained 1.5 M PrOH, 0.1 M sucrose, and 20% of the patient's own serum in phenol-free α-modified minimal essential medium (MEM). The tissues were then taken through decreasing gradients of the cryoprotectant (1 M for 5 minutes, 0.5 M for 5 minutes, and 0 M for 10 minutes) and finally transferred to the transport media on ice which consisted of 10 μg/mL cefotetan, 10 IU of hMG (Serono, Norwell, MA) and 20% autologous serum in phenol-free α-MEM (Sigma, St Louis, MO). Thawing was completed 40 pieces at a time to avoid prolonged waiting time before transplant.

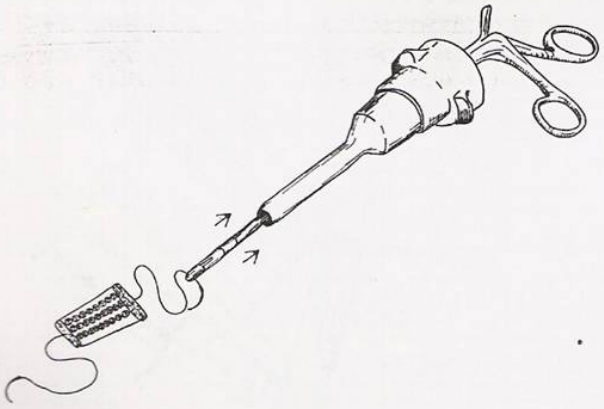
In the operating room, the pieces were put on ice, and several pieces were removed at a time from the main Petri dish to be placed in a second Petri dish, which was placed on a cold stage. The second Petri dish also contained the transport medium. The cold stage was created by turning a surgical basin upside down and placing ice underneath the basin. The tissues, while immersed in culture medium in the second Petri dish, were then strung with 6–0 Vicryl by passing the needle between the cortex and stroma under a surgical microscope. Three strings were formed, and they were then anchored to a frame made from Surgicel (Ethicon, Somerville, NJ). This frame was further strengthened with 1–0 Vicryl on the sides. 0-Vicryl sutures were then tagged to the apex and base of the frame. In the mean time, the patient was anesthetized, and three trocars were inserted: an 11-mm one in the umbilicus, a 5-mm one in the right lower quadrant, and a 13-mm one (with fascia anchor) suprapubically.

First, a pocket was created by sharp and blunt dissection in the pelvic sidewall (ovarian fossa), posterior to the broad ligament, superior to ureters, and inferior to iliac vessels in the supine position. Then the graft was loaded retrogradely, into a 13-mm trocar (Fig. 1). The trocar was then reinserted in the fascia anchor suprapubically, and the graft was dropped in the pelvis by pulling on the leading suture. The leading suture was then placed in the most dependent portion of the pocket, approximately 1 cm above the ureter, and the needle was passed through the peritoneum in to the pelvic cavity (Fig. 2). By pulling on this suture, the graft was wedged in the pelvic pocket (Fig. 2).

Next, the base suture was dropped in, and this was placed through the upper edge of the peritoneal pocket. When this suture was pulled from the intraperitoneal site, it stretched and flattened the graft against the pelvic sidewall (Fig. 3). A second graft was prepared similarly and was placed superior and caudal to the first one. Then the peritoneum was approximated with interrupted sutures, using an extracorporeal knot placement technique (Fig. 4). Several peritoneal sutures were also included the base of the grafts (Surgicel) to further secure them in place.

FIGURE 1

Retrograde loading of the graft that was reconstructed by stringing the ovarian tissue between two strips of Surgicel.



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The patient received aspirin (80 mg) for 1 week, starting 1 day before the transplant. She also received daily injections of hMG (75 IU) for 1 week, starting immediately after the transplant. hMG was given based on the results of previous animal studies indicating that the gonadotropins enhance revascularization of autografts (10).

FIGURE 2

Placement of the leading suture in the pelvic pocket and through the lower peritoneal edge.

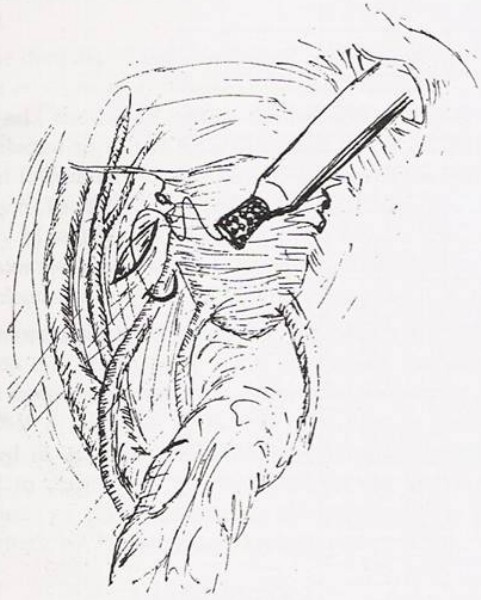

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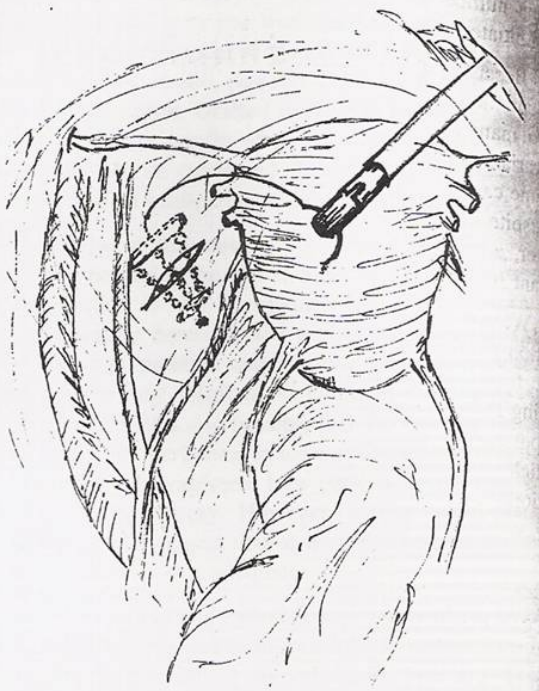
FIGURE 3

Placement of the base suture through the upper peritoneal edge. By pulling on this suture, the graft is flattened against the vascular pelvic wall.



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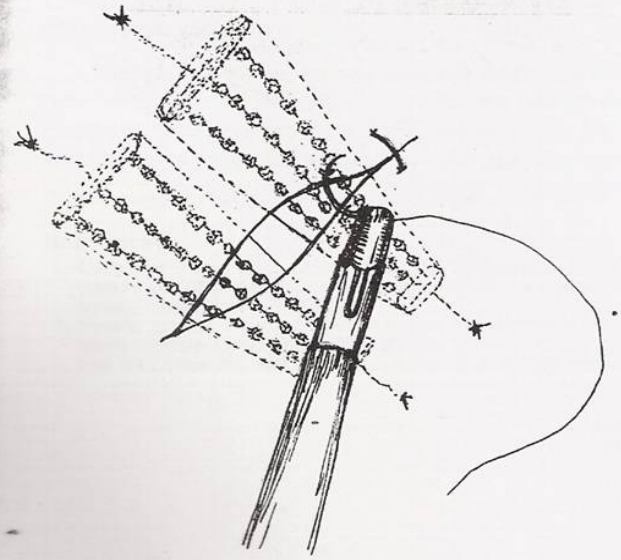
RESULTS

Grafts were visualized immediately after the surgery, and the blood flow to the grafts could be distinguished 3 weeks later by Doppler-ultrasound examination. Fifteen weeks after the procedure, the patient was stimulated with hMG (Pergonal; Serono). After 11 days of stimulation, a dominant follicle emerged in the ovarian graft, and hormone replacement was discontinued. Serial ultrasounds demonstrated continual follicle growth, but the hMG dose had to be gradually increased from 150 IU/d to 675 IU/d to sustain this.

After 24 days of stimulation, the average follicle diameter reached 17 mm. Serum E_2 peaked at 93 pg/mL (normal midcycle range: 69–364 pg/mL), and the hMG dose was increased to 675 IU. But because E_2 level dropped to 75 pg/mL the next day, 10,000 IU of hCG was given to trigger ovulation. Progesterone and E_2 levels peaked at 13 ng/mL and 53 pg/mL, respectively, 7 days after the first hCG injection. Ovulation was confirmed by sonographic demon-

FIGURE 4

Closure of peritoneum with interrupted sutures. Note the placement of two grafts side by side.



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stration of a collapsed follicle, the appearance of free fluid in the cul de sac, and endometrial transformation. The testosterone levels also increased in response to ovarian stimulation (from 18.3 ± 3 ng/dL to 35.6 ± 2, $P=0.0002$). The patient had spontaneous menses 16 days after the first hCG injection.

On the third day of her next menstrual cycle, the patient's hormone levels were as follows: FSH was 16 mIU/mL; LH, 12 mIU/mL; and E_2, 17 pg/mL. On her fifth cycle day, the patient elected to restart E_2 replacement at 0.1 mg/day. A dominant follicle measuring 4 mm was noted in the graft on cycle day 6. The follicle reached 6 mm but ceased growth on cycle day 11 and became atretic on cycle day 16. The range of testosterone levels was 43–50 ng/dL. At this stage, the patient elected to suspend further monitoring for 6 months.

Ten months after the transplant, the patient was stimulated with 300 IU of hMG for 8 days while she was on estrogen replacement treatment. Ultrasound monitoring suggested that a dominant follicle of 5–6 mm in diameter developed in the graft. Unfortunately, because the patient declined to discontinue hormone replacement and further stimulation, we could not confirm the presence of a functional follicle by E_2 and progesterone measurements.

DISCUSSION

Here, we reported a laparoscopic technique for transplantation of frozen-banked ovarian tissue. An ovarian-remnant syndrome is ruled out by visualizing the grafts immediately after the transplantation and demonstrating follicle development and ovulation from these grafts. The luteal phase appeared to be normal, although large doses of hMG were needed to sustain ovarian follicle growth, and the follicular-phase peak-E_2 levels were less than usual. There was spontaneous follicle development in the patient's next cycle, but the follicle became atretic, possibly because of exogenous estrogen administration. Grafted tissue was suspected to be functional nearly 10 months after the transplant, but these findings could not be confirmed. Therefore, the long-term duration of ovarian transplants still remains in question.

There may be several reasons why large doses of gonadotropins were needed in the first stimulation cycle. The most likely explanation is that the patient had a low ovarian reserve and simply demonstrated a poor response. Differences in tissue preparation and freezing methods between different centers may result in variation in the quality of tissue preservation. It is also possible that the vascularization of a graft in the retroperitoneal space was limited and that the tissue perfusion was restricted. Low peak-E_2 levels may be explained with this theory. However, normal luteal-phase progesterone levels suggest that at least the vascularization of the corpus luteum was adequate.

Because the patient had hypothalamic amenorrhea, ovarian stimulation was needed to induce follicle maturation. In patients with a functioning hypothalamic–pituitary axis, however, there is no reason to believe that spontaneous ovulatory cycles cannot be achieved. Previous animal studies have clearly shown that the spontaneous fecundity can be restored with ovarian transplantation (1, 3, 5).

Even though the feasibility of harvesting oocytes for in vitro fertilization is already apparent, we have not yet tested the feasibility of conception with oocytes derived from transplanted frozen-thawed tissue. Further studies will focus on the quality of embryos generated from transplanted ovarian tissue, and if healthy embryos can be generated, embryo transfer will be attempted. Until then, no claims can be made about the fertility restoration potential of this procedure.

Appearance of free fluid in the cul de sac after ovulation is intriguing because the graft was placed retroperitoneally. Whether there was a peritoneal defect through which the follicular fluid could permeate cannot be determined. It is also possible that the high doses of gonadotropins used in this case might have caused fluid transudation from the peritoneal surfaces through a mechanism similar to that seen in ovarian hyperstimulation syndrome. Nevertheless, it is unlikely that a retroperitoneally placed graft can result in a spontaneous pregnancy, and a transvaginal oocyte retrieval and in vitro fertilization will always be needed to achieve pregnancies with this technique.

It needs to be added that other potential sites exist for ovarian transplantation. Transplantation of fresh ovarian tis-