Endocrine Function and Oocyte Retrieval After Autologous Transplantation of Ovarian Cortical Strips to the Forearm

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In women, one of the common and distressing adverse effects of chemotherapy and radiotherapy is premature ovarian failure.1 Previously, ovarian transposition was performed with varying degrees of success but the scatter radiation and vascular compromise limited its effectiveness.2,3 Moreover, this treatment could not protect against the gonadotoxicity of chemotherapy. Recently, a multitude of laboratory, animal, and human xenograft studies has described an emerging alternative for these patients: autotransplantation of fresh or frozen-banked ovarian cortical strips.4 In addition, many women experience premature menopause due to oophorectomy performed for benign indications.5,6

We have reported the first case of laparoscopic transplantation of frozen-thawed ovarian tissue to the pelvic sidewall with subsequent ovulation.5,6 However, when there is a possibility that a patient may receive pelvic radiotherapy, or when close surveillance of ovarian tissue is required, a heterotopic location may be more desirable. 

Context In reproductive-age women, one of the common adverse effects of chemotherapy and radiotherapy is premature ovarian failure. In addition, a significant number of women experience early menopause due to oophorectomy performed for benign indications.

Objective To develop an ovarian transplantation technique to preserve endocrine function in women undergoing sterilizing radiotherapy and/or chemotherapy, or oophorectomy.

Design and Setting Case study of 2 patients in New York who received autologous ovarian transplantation (patient A, November 1999; patient B, April 2000) to the forearm prior to pelvic radiotherapy or after oophorectomy.

Participants Patient A is a 35-year-old woman with stage IIIB squamous cell cervical carcinoma and patient B is a 37-year-old woman with recurrent benign ovarian serous cysts.

Main Outcome Measures Follicular development evident by ultrasound examination; cyclical production of estradiol and progesterone; restoration of serum follicle-stimulating hormone, luteinizing hormone, and testosterone levels to nonmenopausal range; and disappearance of menopausal symptoms.

Results Menopause was confirmed immediately after the transplantation in both patients by serum follicle-stimulating hormone measurements (patient A, 47 mIU/mL; patient B, 50.7 mIU/mL). In patient A, follicle development was noted by physical and ultrasound examinations approximately 10 weeks after the transplantation. The mean (SE) follicle-stimulating hormone and luteinizing hormone levels decreased to 8.6 (0.4) mIU/mL and 12.8 (0.8) mIU/mL, respectively. The peripheral estradiol levels showed cyclical variation (mean [SE], 115 [9.2] pg/mL [422 [33.8] pmol/L]), and during the 18-month follow-up, a dominant follicle developed each month. The estradiol levels from the right cubital vein were consistent with ovarian vein measurements (mean [SE], 1069 [269] pg/mL [3924 [987.5] pmol/L]). Percutaneous oocyte aspirations yielded a mature oocyte. In patient B, ovarian function was demonstrated by ultrasound visualization of a 9-mm follicle by 6 months after transplantation. Thereafter, the patient had spontaneous menstruation every 25 to 28 days. Ovulation was further confirmed by midluteal progesterone measurements (range, 7-10.1 ng/mL; mean [SE], 8.5 [0.9] ng/mL). Patient B’s ovarian graft was still functional 10 months after the transplantation.

Conclusions Subcutaneous ovarian transplantation appears to be a relatively simple, novel technique to preserve endocrine function in women undergoing sterilizing cancer therapy or surgery.
We chose the forearm, based on previous studies demonstrating that both fresh and frozen-thawed parathyroid tissues have been successfully transplanted in this location.7,8

METHODS
The study was approved by the institutional review board committees at the New York Methodist and New York Presbyterian hospitals.

Patient A
A 35-year-old woman was diagnosed with stage IIIB squamous cell cervical carcinoma. She consented to fresh transplantation prior to pelvic radiotherapy to preserve her ovarian function. After frozen section biopsies showed no metastasis, both ovaries were removed laparoscopically, and their cortices were prepared in 16 strips of 5×50 × 1 to 3 mm.9 A 1-cm vertical incision was made over the brachioradialis muscle, 5 cm below the antecubital fossa. Ovarian strips were wedged subcutaneously, using a suture pull-through technique. The patient was started on 1 mg of micronized estradiol on postoperative day 2.

The patient was radiosensitized with cisplatinum (50 mg/m² × 1) and 5-fluorouracil (1000 mg/m² per day on days 3-6). This course was repeated 4 weeks later. During weeks 1 through 5, the patient received external beam radiotherapy to the pelvis for a total dose of 5040 cGy. During this radiotherapy, the patient’s arm was raised above her head and shielded. Two weeks later, 2 sessions of brachytherapy were performed with cesium.

Patient B
A 37-year-old woman developed a recurrent benign serous cyst in her only ovary. Her 1 ovary had been previously removed due to a serous cystadenoma. The patient had a “frozen-pelvis” due to dense pelvic adhesions and had multiple laparotomies for cystectomies on the remaining ovary. After the third recurrence, a decision was made by her gynecologist to perform an oophorectomy. After the removal of the ovary, healthy ovarian tissue was harvested from the specimen. The transplantation technique was similar to that in the first patient, but for aesthetic reasons the tissue was transplanted more medially in the forearm.

RESULTS
Patient A
Six weeks after the transplantation, the patient’s follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were 47 and 35 mIU/mL, respectively. Approximately 10 weeks after the transplant, she reported the presence of a painless bulge at the site of the transplant (FIGURE 1). Ultrasound examination showed a 15-mm dominant follicle (FIGURE 2A) and 4 other antral follicles, measuring 5 to 7 mm (Figure 2B), at which point estrogen replacement was discontinued. Repeat hormonal analyses showed estrogen production, as well as normalization of FSH and LH concentrations. The testosterone levels were also restored to the nonmenopausal range (TABLE). Serial ultrasound examinations showed continual development of new antral follicles as large as 15.5 mm.

Within 78 to 117 days after the transplantation, mean (SE) FSH was 13.6 (0.54) (range, 11-18.1 mIU/mL), however, between 120 and 227 days it decreased to 8.6 (0.4) (range, 6.2-12.4 mIU/mL) (P<.001). Similarly, LH decreased from 18.3 (1.5) (range, 9-29 mIU/mL) to 12.8 (0.8) (range, 6-26 mIU/mL) (P=.002).

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Table. Hormone Measurements 78 to 140 Days After Transplantation Comparing Peripheral and Central Values in Patient A

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Mean (SE) Right Hand</th>
<th>Mean (SE) Right Cubital Fossa</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol, pg/mL</td>
<td>115 (9.2)</td>
<td>1069 (269)</td>
<td>.005</td>
</tr>
<tr>
<td>Progesterone, ng/mL</td>
<td>0.4 (0.06)</td>
<td>0.77 (0.12)</td>
<td>.001</td>
</tr>
<tr>
<td>Testosterone, ng/dL†</td>
<td>37 (2.1)</td>
<td>51 (5.5)</td>
<td>.008</td>
</tr>
<tr>
<td>Follicle-stimulating hormone, mIU/mL</td>
<td>13.9 (1.8)</td>
<td>14.2 (0.54)</td>
<td>.30</td>
</tr>
<tr>
<td>Luteinizing hormone, mIU/mL</td>
<td>18.1 (1.8)</td>
<td>19.0 (1.6)</td>
<td>.07</td>
</tr>
</tbody>
</table>

*To convert to pmol/L multiply by 3.671. †To convert to nmol/L multiply by 0.0347.
To determine the cyclical hormone production, the patient was monitored every 1 to 5 days during postoperative days 78 through 117. The right hand (RH) estradiol measurements representing peripheral hormone levels showed 2 surges (FIGURE 3A). Estradiol levels from the right cubital fossa (RCF), which represent the immediate output of the graft, showed a significantly higher average (Figure 3B) compared with the peripheral levels (RH). Although serum progesterone levels fluctuated between 0.13 and 0.38 ng/mL, they did not reach ovulatory levels.

The Table reflects extended monitoring between 78 and 140 days after the transplantation. There was a statistically significant gradient in estradiol, progesterone, and testosterone between the RH and RCF measurements. The values from the RCF resembled those of the ovarian vein, indicating that the veins in that area had assumed ovarian drainage.

Because the longevity of grafted tissue could not be predicted, the patient wished to cryopreserve embryos for future use with a gestational surrogate. A percutaneous oocyte retrieval was performed on postoperative day 216, when the RH estradiol level was 157 pg/mL (576 pmol/L). An immature oocyte containing a germinal vesicle was obtained from an 11-mm follicle.

To synchronize follicle development in the subsequent attempt, the patient’s pituitary gonadotropin production was blocked with a gonadotropin-releasing hormone antagonist (250 µg/d of Antagon for 2 days) (Serono, Norwell, Mass). Ovarian stimulation was performed with 150 to 225 IU of recombinant FSH (Follistim; Organon Inc, West Orange, NJ). After 11 days of stimulation, 4 follicles ranging in size from 11.5 to 15.5 mm were visualized on ultrasound. The estradiol level for RCF was 3482 pg/mL (12782 pmol/L) and was 264 pg/mL (969 pmol/L) for RH.

Thirty-six hours after the administration of human chorionic gonadotropin, 3 oocytes were recovered percutaneously. Two oocytes from 15.5-mm follicles were postmature; an 11.5-mm follicle did not yield an oocyte; and an oocyte from a 14-mm follicle was in metaphase I (FIGURE 4A). This oocyte was matured in vitro overnight (FIGURE 4B); intracytoplasmic sperm injection with donor sperm did not result in fertilization.

In response to the human chorionic gonadotropin injection, the patient’s RH and RCF progesterone levels reached mean (SE) ovulatory levels of 3.08 (1.43) and 19.2 (12), respectively. Culture of granulosa cells from the 14-mm follicle confirmed progesterone production in vitro (data not shown).

Approximately 10 months after the transplantation, the patient was diagnosed with local recurrence in the pelvic sidewall and was treated with 3 courses of 650 mg of carboplatinum over 9 weeks. Despite this treatment, the patient’s FSH levels remained near the normal mean (SE) of 10.2 (1.5) mIU/mL at the 18-month follow-up.

**Patient B**

A postoperative FSH level of 50.7 mIU/mL confirmed menopause. Five months later, the patient felt a lump growing at the transplant site. One month later, an ultrasound showed a 7.5-mm follicle in the forearm, which grew to 9 mm in 2 days. Hormone replacement (1 mg of micronized estradiol and 10 mg of medroxyprogesterone acetate) was discontinued. During the subsequent month the patient reported spontaneous menstruation. On day 13 of that cycle, a 9-mm follicle was noted by ultrasound and her hormone measurements indicated a mid-cycle surge (FSH, 40 mIU/mL; LH, 90 mIU/mL; estradiol, 254 pg/mL [932 pmol/L]; progesterone, 2.1 ng/mL). The patient menstruated spontaneously 2 weeks later, and every 25 to 28 days thereafter. Ten months after the transplant the patient was monitored again.
Six to 11 days after an LH surge (62 mIU/mL), progesterone ranged from 7 to 10.1 ng/mL (mean [SE], 8.5 [0.9] ng/mL), confirming spontaneous ovulation. Levels of FSH, LH, and estradiol on the second day of menstruation indicated normal ovarian reserve (15.4 mIU/mL, 6.6 mIU/mL, and 47 pg/mL, respectively).11 Probably because the tissue was transplanted more medially, no gradient was noted between the RH and RCF.

Neither patient complained of discomfort, but indicated that they could "feel" the follicle during mid cycle. Their moliminal symptoms returned and both expressed a sense of well-being with endogenous estrogen. Patient A preferred to wear long-sleeve shirts mid cycle, while patient B did not have any cosmetic concerns.

**COMMENT**

This is the first report of endocrine function and oocyte retrieval after autologous grafting of ovarian cortical strips to the forearm. This implantation site affords easy access for grafting, monitoring, and ultrasound examination.

The lack of spontaneous ovulation in patient A can be attributed to the fact that estradiol levels never exceeded 250 pg/mL (918 pmol/L), which is a prerequisite for induction of an LH surge.12 However, in vivo and in vitro progesterone production in response to human chorionic gonadotropin indicated that the granulosa cells were capable of luteinization. In patient B, peak estradiol levels exceeded 250 pg/mL (918 pmol/L) and spontaneous ovulation and menstruation occurred. A recent primate study also showed restoration of spontaneous ovulation after transplantation of fresh and frozen-thawed strips to the forearm.13 Ovarian endocrine function was restored despite the radiosensitizing chemotherapy. This can be explained by the fact that the dose was small and because relatively nongonadotoxic drugs were used compared with the alkylating agents. In addition, while the revascularization was in progress during the first week, tissue diffusion of these drugs was probably restricted. Exposure to chemotherapy, however, could be completely avoided by ovarian cryopreservation, and by delaying the transplantation after the treatment. In the present cases, this could not be done because the age limit for cryopreservation was set at 34 years in the research protocol.

In conclusion, ovarian transplantation to the forearm results in endocrine function. With optimization of ovarian stimulation and percutaneous oocyte retrieval techniques, this procedure may also restore fertility in the near future when used in conjunction with in vitro fertilization.

**Author Contributions:** Study concept and design: Oktay, Kan, Rucinski. Acquisition of data: Oktay, Economos, Kan, Rucinski, Veeck, Rosenwaks. Analysis and interpretation of data: Oktay. Drafting of the manuscript: Oktay. Critical revision of the manuscript for important intellectual content: Oktay, Economos, Kan, Rucinski, Veeck, Rosenwaks. Statistical expertise: Oktay, Economos. Obtained funding: Oktay, Rosenwaks. Administrative, technical, or material support: Oktay, Kan, Rucinski, Veeck, Rosenwaks. Study supervision: Oktay, Rosenwaks.

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**REFERENCES**