Evaluation of the ovarian reserve in women transplanted with frozen and thawed ovarian cortical tissue

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Objective: To investigate ovarian reserve and ovarian function in women transplanted with frozen/thawed ovarian tissue.
Design: Retrospective cohort study.
Setting: University hospital.
Patient(s): 18 women transplanted with their own frozen/thawed ovarian tissue.
Intervention(s): None.
Main Outcome Measure(s): Levels of antimüllerian hormone (AMH), duration of function of the transplanted ovarian tissue, outcome of assisted reproduction.
Result(s): Of the 18 women who received transplanted ovarian tissue, levels of AMH were measured in 12 women; AMH never exceed a concentration of 1 ng/mL, and in several cases they were below the detection limit of the assay in combination with regular menstrual cycles. Two women with AMH below the detection limit conceived spontaneously. The duration of function of the transplants was between 9 months and 7 years and still functioning. Twelve women received assisted reproduction therapy; in 72 cycles, 65 oocytes were retrieved. The pregnancy rate and live-birth rate per cycle were 6.9% (5 of 72) and 2.8% (2 of 72), respectively.
Conclusion(s): The relatively poor outcome of assisted reproduction in women transplanted with frozen/thawed ovarian tissue may reflect reduced follicular selection rather than defective or aged oocytes. In normal women, reduced follicular selection with age may be part of explaining the decline in female fecundity with increasing age. (Fertil Steril® 2012;97:1394–8. ©2012 by American Society for Reproductive Medicine.)
Key Words: AMH, antimüllerian hormone, cryopreservation, fertility preservation, ovarian tissue, transplantation

In girls and women suffering from a malignant disease, the ovarian pool of follicles may become severely compromised as a consequence of their treatment (1). Depending on the age of the patient and the actual gonadotoxic treatment, some women run a risk of becoming infertile and experience primary ovarian insufficiency (2, 3). In an attempt to preserve fertility, some of these women choose to have ovarian tissue cryopreserved (4–9). In case the ovarian pool of follicles does become depleted, they can request a reimplantation of their tissue. The freezing and transplantation procedure only allow a fraction of the frozen follicles to survive and start to work in the grafted tissue. In humans, there is no precise information on the percentage of surviving follicles in the grafted tissue, as there are no nondestructive methods to measure the number of follicles in a given piece of cortex before and after the freezing/thawing and transplantation procedure. In sheep, it has been estimated that the cryopreservation process is relatively effective, but the transplantation procedure may cause a loss of up to 70% of the follicles due to ischemic damage (10). Xenografting to immunodeficient mice has shown surviving human follicles but has been unable to provide quantitative information on the number of viable follicles (11–14).

In a clinical setting, around 25% to 50% of one ovary is normally transplanted (15). As the remaining ovary is considered to be postmenopausal without follicles, only a small fraction of the original ovarian reserve will be present in the transplanted tissue. Despite this, we and others have observed that some of these women actually show ovarian activity and regular menstrual cycles for several years after transplantation (15–21).

We evaluated the ovarian reserve of women transplanted with frozen/thawed ovarian tissue by measuring antimüllerian hormone (AMH) levels

Received June 10, 2011; revised and accepted February 24, 2012; published online March 15, 2012.
T.G. has nothing to disclose. K.T.S. has nothing to disclose. S.G.K. holds an unrestricted grant from the Danish Cancer Foundation. E.E. has nothing to disclose. C.Y.A. has nothing to disclose.
Supported by the Danish Cancer Society (DP05112/R2-A41-09-S2), the Danish Medical Research Council (271-07-0452; 09-072265), the Novo Nordic Foundation, Sophus Carl Emil Friis and Olga Doris Friis Foundation, the Lundbeck Foundation, and the University Hospital of Copenhagen.
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Fertility and Sterility® Vol. 97, No. 6, June 2012 0015-0282/$36.00

doi:10.1016/j.fertnstert.2012.02.036

and assessed the women's chances of achieving menstrual cycles and becoming pregnant. In this context, we also evaluated the oocyte quality from follicles aspirated in connection with conventional in vitro fertilization and intracytoplasmic sperm injection (IVF-ICSI) treatment in these women.

MATERIALS AND METHODS

Cryopreservation of the ovarian tissue was performed as previously described elsewhere (22–25). In brief, one whole ovary was excised laparoscopically, and the cortical tissue was isolated. The tissue was cut into fragments of approximately 5 × 5 × 1 mm that were frozen in individual ampules.

All 18 women who underwent transplantation had initially one entire ovary removed laparoscopically for cryopreservation except one patient, who due to a previously unilateral oophorectomy for benign reasons only had part of the remaining ovary removed (patient 5). Transplantation of ovarian tissue to each of the 18 women was performed as previously described elsewhere (23) and followed the same procedure in all cases. (Patients details are provided in Table 1). In brief, immediately after thawing, the pieces of ovarian cortex were replaced to the remaining postmenopausal ovary except for one patient in whom neither ovary remained. In the latter case, the ovarian tissue was positioned in subperitoneal pockets corresponding to the pelvic wall. Additionally, six other patients had ovarian tissue transplanted subperitoneally corresponding to the pelvic wall and/or the anterior abdominal wall.

The amount of tissue transplanted to a woman varied from around 20% to 60% of one ovary. The remaining ovary was the primary site of transplantation, and only in cases in which there were pieces of cortex in excess were they transplanted to heterotopic sites. The number of cortex pieces transplanted varied from 6 to 12. Thus, all women had enough tissue for a total of two to three individual transplantations.

All patients had high levels of follicle-stimulating hormone (FSH, median 74 IU/L; range: 42 to 200 IU/L), amenorrhea, and clinical signs of menopause at the time of transplantation, except for patients 14 and 17. Patient 14 was 38 years old at the time of cryopreservation; she was 40 years old at the time of transplantation and wanted to increase her chances of conceiving. Patient 17 had tissue reimplanted to increase her chance of conceiving as she did not develop antral follicles when stimulated and her FSH level on day 2 of her cycle was 19 IU/L.

In the present study, the concentration of AMH was evaluated at different time intervals after transplantation in 12 women. Antimüllerian hormone levels were measured by use of a commercially available AMH enzyme-linked immunosorbent assay (ELISA) kit as previously described elsewhere (26).

Of the 18 women transplanted, a total of 12 women underwent IVF-ICSI after transplantation using standard procedures. Oocyte retrieval was normally performed after a modified natural cycle protocol (15).

The Danish public healthcare system covers the cost of fertility preservation, storage, transplantation, and the use of possible assisted reproduction afterward. The cryopreservation procedure has been approved by the Regional Committee on Biomedical Research Ethics (J. no. H-2-2011-044). All women gave written informed consent.

RESULTS

Duration of Transplantation

The 18 Danish women who received transplantation regained ovarian function in each single case and maintained ovarian function for up to 7 years after transplantation with 20% to 60% of the cortical tissue from one ovary (Table 2). In 12 patients, the transplants remained functioning, but in six

TABLE 1

Characteristics of 18 women undergoing transplantation of frozen/thawed ovarian tissue.

Patient	Diagnosis	Age at cryopreservation (y)	Age at first/second transplantation (y)	Transplanted tissue at first/second transplantation (% of one ovary)	Graft site of first/second transplantation
1	Non-Hodgkin	32	34/39	18/35	O/O+A
2	Hodgkin	28	30/35	41/28	O+A+P/O+A
3	Hodgkin	25	27/30	60/30	O+A+P/O+A
4	Hodgkin	26	28/29	31/38	O/O
5	Ewing sarcoma	27	28	54	O
6	Breast cancer	35	37/38	53/47	O/O
7	Hodgkin	31	34/36	40/40	O+P/O
8	PNH	19	21	33	O+A
9	Non-Hodgkin	35	37/38	46/30	O/O
10	Aplastic anemia	25	28	41	O+A
11	Cervical cancer	25	30	50	P
12	HUS, ovarian cysts	33	38	100 (of retrieved tissue)[a]	O
13	Ewing sarcoma	9	13	20	O
14	Breast cancer	38	40	43	O
15	Autoimmune vasculitis	23	27	42	O
16	Breast cancer	31	34	29	O
17	Breast cancer	33	36	37	O
18	Hodgkin	29	31	47	O

Note: A = anterior abdominal wall; HUS = hemolytic urinary syndrome; O = remaining ovary; P = pelvic wall; PNH = paroxysmal nocturnal hemoglobinuria.
[a] Three pieces of cortex.

Greve. Reduced follicle pool and fertility. Fertil Steril 2012.