Autotransplantation of frozen–thawed ovarian tissue in a young woman: ultrastructure and viability of grafted tissue

This is the first report of the presence of ultrastructurally normal primordial and primary follicles, 13 months after autotransplantation of frozen–thawed human ovarian tissue. The stroma contained numerous viable and ultrastructurally normal blood vessels but showed poor cellular density. (Fertil Steril® 2008;90:1215–8. ©2008 by American Society for Reproductive Medicine.)

Transplantation of cryopreserved human ovarian cortical strips allows recovery of endocrine and reproductive function in women who experience iatrogenic menopause after radiotherapy and/or chemotherapy. Recently, two live births (1, 2), one clinical pregnancy (3), and one biochemical pregnancy (4) were reported in patients after transplantation of frozen–thawed human ovarian tissue.

Despite this success, many unresolved issues remain. Human ovarian grafts presently show a reduced life span, probably as a result of ischemic damage and endocrine stress (5). A decrease in follicular number (6, 7) and in the proportion of morphologically normal follicles (8) was reported after both autografting and xenografting of ovarian tissue in animals, suggesting that temporary post-transplantation ischemia may affect follicular survival and structure. Studies by Baird et al. (9) suggest deregulation of follicular growth after transplantation, with premature follicular activation and accelerated depletion of the follicular reserve in the graft. We were able to demonstrate, by using transmission electron microscopy (TEM), developmental uncoupling between the oocyte and follicular cells after short-term transplantation of human ovarian tissue to nude mice (10).

In a recent study, Deng et al. (8) observed well-preserved follicular integrity at the histological and ultrastructural level after cryopreservation and transplantation of rabbit ovarian tissue. However, no ultrastructural studies have been performed to evaluate the impact of orthotopic autotransplantation of human ovarian tissue.

Our aim was to evaluate, by using TEM and vital fluorescent staining, the structural and ultrastructural morphology and viability of frozen–thawed human ovarian tissue that was orthotopically autografted for 1 year. We considered it crucial to establish whether ovarian follicles and oocytes growing in grafts are ultrastructurally normal.

The clinical history of the patient was described elsewhere by Donnez et al. (11). Briefly, a right oophorectomy was performed in a 21-year-old woman before chemotherapy and bone marrow transplantation for sickle cell anemia, and the ovarian cortex was cryopreserved. After chemotherapy and bone marrow transplantation, the patient developed premature ovarian failure. Orthotopic autotransplantation of frozen–thawed ovarian cortical tissue was performed by laparoscopy. After 4 and 1/2 months, restoration of ovarian endocrine function was achieved.

A second laparoscopy was performed 13 months later to reimplant more frozen–thawed ovarian tissue fragments, because FSH and LH values had returned to castrated levels for >6 months. A biopsy was taken from the grafted tissue to analyze its viability and ultrastructure. Approval was obtained from the Institutional Review Board of the Catholic University of Louvain to perform this procedure, and the patient gave her written informed consent. The biopsy was cut into two equal parts for [1] TEM analysis and [2] vital fluorescent staining.

For ultrastructural analysis, the ovarian tissue was fixed by using 2.5% glutaraldehyde for 5 days at 4°C. After fixation, the samples were washed and postfixed with 1% osmium tetroxide for 2 hours. The samples were rinsed again and were dehydrated through an ascending series of ethanol. They were immersed in propylene oxide overnight and subsequently embedded in Epon 812 resin (Fluka Chemie AG, Buchs, Switzerland). Resin blocks were sectioned by using an Ultracut E ultramicrotome (Reichert-Jung, Vienna, Austria). Semithin sections (1 μm thick) were stained with toluidine blue and examined by light microscopy (Zeiss Axioskop, Germany). Ultrathin sections

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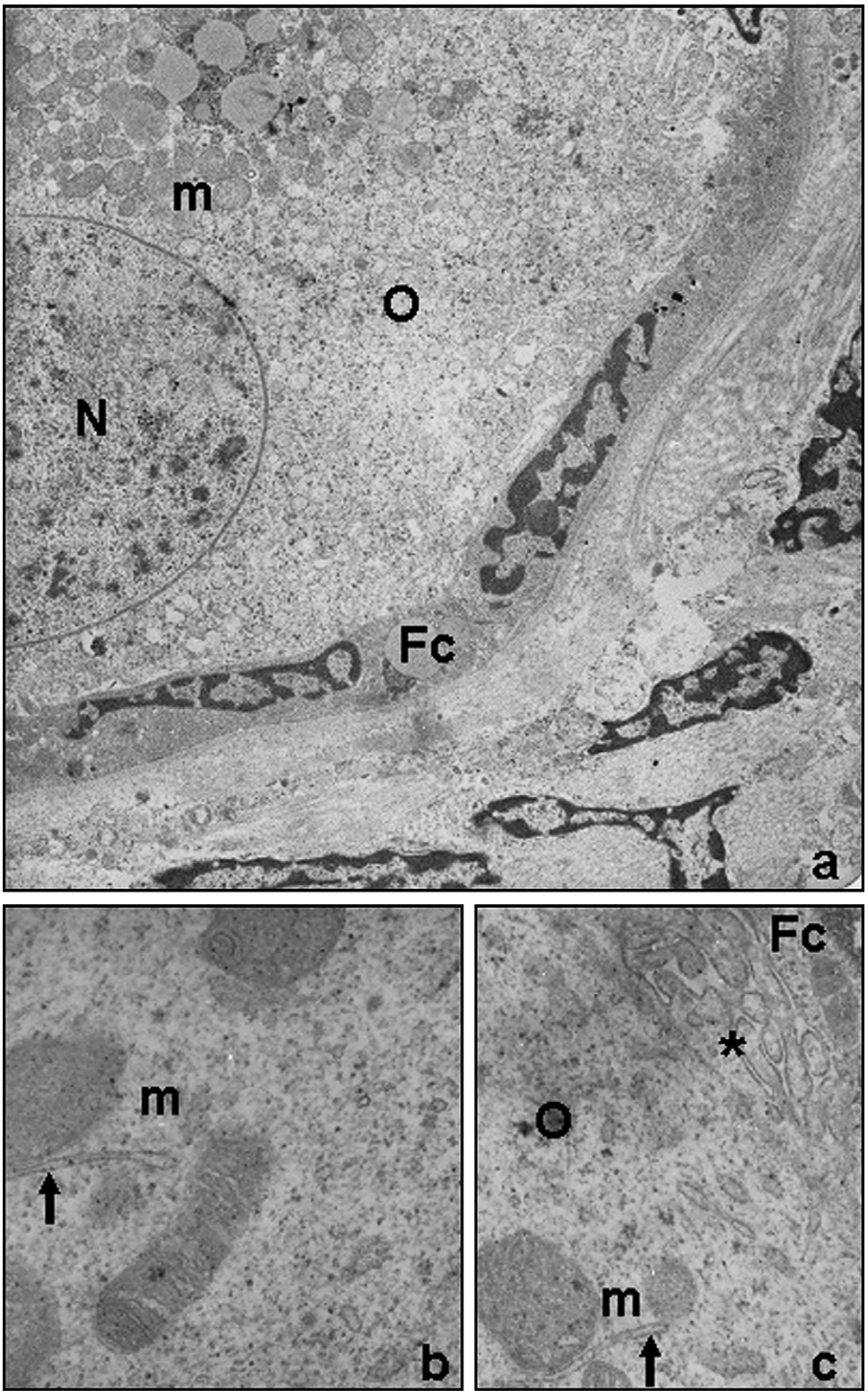
FIGURE 1

Well-preserved frozen–thawed human follicles autografted for 13 months. (**a**) Follicle at the primary stage with the oocyte (O) surrounded by a single layer of cuboidal follicular cells (Fc). m = mitochondria; N = nucleus. (**b**) Follicular cells show rounded mitochondria (m) intermingled with numerous microtubules (*arrow*) in the oocyte cytoplasm. (**c**) Close interdigitations (*asterisk*) are observed between Fc projections and O microvilli. Original magnification, ×3,000.



Camboni. Autografted ovarian tissue: TEM and viability. Fertil Steril 2008.

(60–80 nm) were cut with a diamond knife, mounted on copper grids, and contrasted with saturated uranyl acetate and lead citrate. They were examined and photographed by using a Zeiss EM10 electron microscope operating at 80 kV.

For viability assessment, the ovarian tissue was cut into 200-μm-thick strips by using a tissue sectioner (McIlwain Tissue Chopper; Campden Instruments, Loughborough, United Kingdom), transferred to a solution of phosphate-buffered saline containing 2 μmol/L of calcein AM (Molecular Probes, Leyden, the Netherlands) and 5 μmol/L of ethidium homodimer-I (Molecular Probes), and then incubated with the fluorescent dyes for 45 minutes at 37°C in the dark (12). After exposure to these dyes, the ovarian fragments were washed in phosphate-buffered saline and observed under an inverted fluorescence microscope (Leica). Green fluorescence was visualized in live cells (excitation and emission, 495 and 515 nm), and red fluorescence in dead cells (excitation and emission, 495 and 635 nm).

In the fragments analyzed by TEM, three primordial or primary follicles were detected. Two of them looked perfectly healthy (Fig. 1a–c), with oocytes presenting numerous rounded mitochondria associated with microtubules (arrow in Fig. 1b and c). Close interdigitations were observed between oocyte microvilli and follicular cell projections (Fig. 1c). Follicular cells showed a voluminous nucleus, containing a well-developed nucleolus and isolated peripheral patches of heterochromatin (Fig. 1a). The third follicle, at the primordial stage, displayed irregularities in its nuclear profile and multivesicular bodies in the oocyte cytoplasm. However, cellular membranes were intact, and mitochondria, as well as interdigitations between oocyte microvilli and follicular cell projections, looked well preserved. No follicles were detected in the samples that were destined for viability assessment.

The stromal compartment showed good viability, as proved by vital fluorescent staining, despite the presence of marked fibrosis and poor cellularity detected by TEM. Numerous blood vessels were observed in the biopsy, exhibiting a normal ultrastructure, as assessed by TEM, and good viability, as assessed by vital fluorescent staining.

These observations prove, for the first time, that ultrastructurally well-preserved follicles are present in human ovarian tissue after cryopreservation and autotransplantation. These findings corroborate those of a previous study conducted in our laboratory on xenotransplantation of human ovarian tissue to nude mice (10), in which no significant histological or ultrastructural alterations were observed in primordial and primary follicles after cryopreservation and xenografting for 3 weeks.

The main challenge of transplantation of frozen–thawed ovarian tissue is ensuring a pool of well-preserved, small, nongrowing follicles to increase the graft life span. In the present study, we found well-preserved primordial and primary follicles after cryopreservation and 13 months' grafting, maintaining the integrity of subcellular structures. Primordial and primary follicles are probably more resistant to freeze-thawing procedures (13) and less susceptible to ischemic stress (8, 14) than are larger follicles. We only observed one primordial follicle with slight ultrastructural alterations (irregularities in its nuclear profile and multivesicular bodies in the oocyte cytoplasm), which may be a consequence of both freeze–thawing and grafting procedures.

The presence of viable and ultrastructurally normal blood vessels and stromal tissue proved the functional vascularization of the transplanted tissue after 13 months, ensuring survival of the graft. The poor cellularity and fibrosis observed in stromal tissue may be a consequence of the ischemic damage occurring before the establishment of neovascularization.

In conclusion, our study demonstrated, for the first time, ultrastructurally well-preserved human primordial follicles at 13 months after autotransplantation of cryopreserved ovarian cortex.

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