Tissues, Pathology, and Diagnostic Microscopy

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Ultrastructural study on vitrification and slow freezing of the ovarian tissue

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Infertility can occur as a result of cancer therapy (kemotherapy and radiotherapy) and the other reasons [1]. Oocyte, embryo and ovarian tissue freezing which is still experimental are developed options for fertility preservation [2]. There are two main ways of cryopreserving biologic tissue: slow freezing or vitrification [3] [4]. Copper grids were used as carrier when we studied method of vitrification. We examined slow freezed and vitrified tissues with electron microscopy.

Eighteen pairs of ovaries were collected from eight-week-old female BALB-C type mice. There were three groups in our groups; vitrification, slow freezing, and control. SF was performed in dimethyl sulfoxide (DMSO) and sucrose with the help of a controlled freezer (SY-LAB, ICECUBE 14S, Austria). Tissues were equilibrated in solution of DMSO, ethylene glycol and sucrose before loading on copper electron microscope grid in vitrification group. After ultimate thawing, tissues transferred through the series of gradually decreasing CPAs for 5 minutes each (20% - 10% - 5% - 0% DMSO and EG with 0.4 M sucrose) for the removal of CPAs osmotically. Eventually, all thawed tissues and six fresh ovarium tissues as the control group were washed in L-15 and fixed in glutaraldehyde for electron microscopy.

In control group, oocyte nuclei, distribution of cytoplasm and the junctions between oocyte-granulosa were intact in non-degenerating primordial and primary follicles in the thin sections (figure 1-A, 1-B, 1-C, 2-A, 2-B). In slow freezing group the linkage of follicle cells between both each other and oocytes were preserved. ZP and the basal lamina between granulosa and theca cells were regular. Spaces and vacuolizations inside the cytoplasm of granulosa cells were noticed (figure 1-D, 1-E, 1-F, 2-C, 2-D). In vitrification group, shrinkage in granulosa cells and an increase in condensation were noticed. Junctions of oocyte and degenerated granulosa cells were disappeared and plazmalemma was not noticed (figure 1-G, 1-H, 1-I, 2-E, 2-F).

The distinction of experimental groups was that the number of degenerate follicles was more than that of normal follicles. Both vitrification on EM grids and slow freezing seem to preserve primordial follicles effectively. This study is supported by Ankara University BAP 10B 3330003

1. S.S. Kim, Fertility Preservation in female cancer patient: Current developments and future directions, Fertil Steril (2006) 85:1-11.
2. A. Revel, In vitro maturation and fertilization oocyte from an intact ovary of surgically treated patient with endometrial carcinoma: A case report. Hum Reprod (2004) 19: p. 1608-1611.
3. S.S. Kim, D.E. Battaglia, and M.R. Soules, The future of human ovarian cryopreservation and transplantation: Fertility and beyond. Fertil Steril (2001) 75: p. 1049-1056.
4. V. Isachenko, Human ovarian tissue vitrification versus conventional freezing: morphological, endocrinological, and molecular biological evaluation, Reproduction (2009) 138(2), p. 319-27.

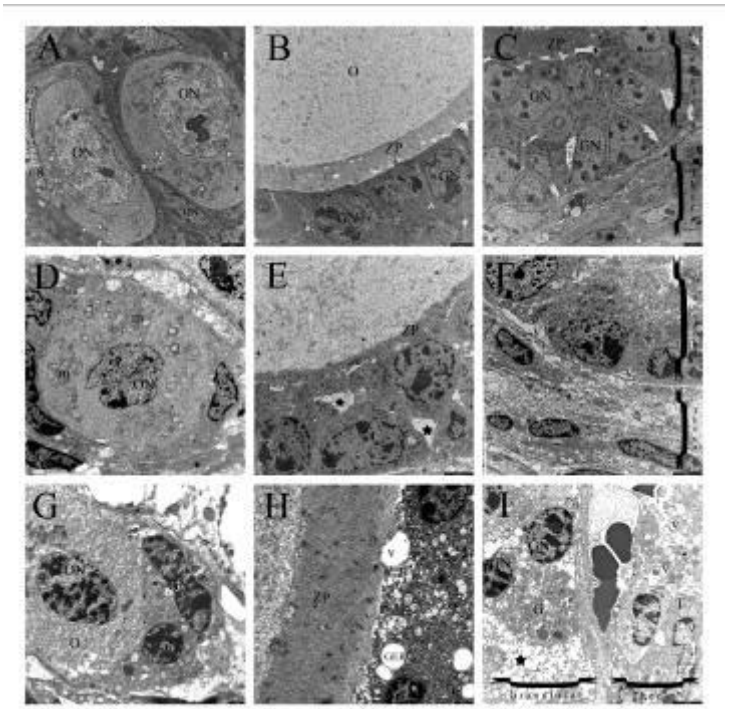

Figure 1. A, B, C: control group, D, E, F: slow freezing group, G, H, I: vitrification group. ON: oocyte nucleus, GN: granulosa nucleus, O: oocyte, ZP: zona pellucida, T: theca, m: mitochondrion, v. vacuole, GER: rough endoplasmic reticulum, star: intercellular spaces.

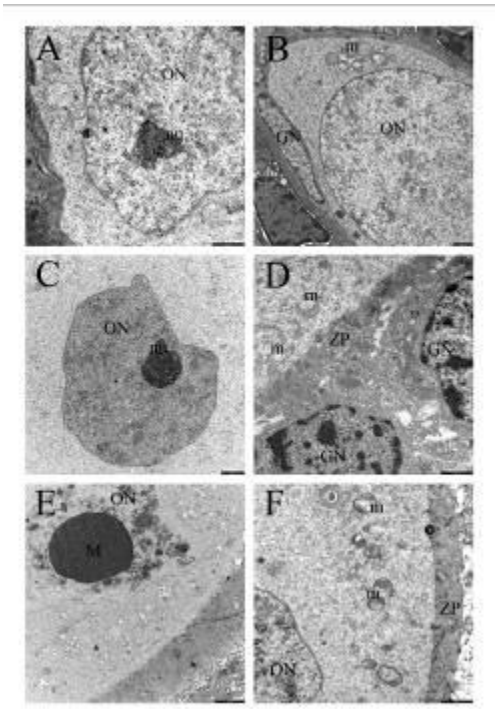

Figure 2. A, B: control group, C, D: slow freezing group, E, F: vitrification group. ON: oocyte nucleus, GN: granulosa nucleus, nu: nucleolus, GN: granulosa nucleus, O, m: mithocondrion, ZP: zona pellucida, M: dark mass of nuclear remnant.