Ovarian Tissue Vitrification

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Surgeries and gonadototoxic therapy during cancer and non-cancer treatment result in loss of fertility in reproductive age. Preservation of fertility potential in young women is an urgent task for reproductive medicine today.

The research objective was to develop the procedure for ovarian tissue vitrification with the subsequent control of its efficiency by xenotransplantation to laboratory animals.

There were vitrified 14 fragments of ovarian cortical layers of women aged of 21 to 30 years with normal ovarian reserve who underwent the surgeries due to oncogynecological pathologies. Penetrating and non-penetrating cryoprotectants, protein substitute, buffer solution (Sigma Aldrich, USA) were used for vitrification. Histological examination using haematoxylin eosin staining was performed before vitrification and after warming; ovarian tissue was cultured after warming and estradiol content was measured in culture medium after 24 and 72 hrs. In 4 female rabbits with baseline estradiol of 40 ± 5 pg/ml; following surgical ovariectomy the estradiol level was less than 1.5 pg/ml, and this was considered as a sign of simulated menopause. Post surgery the animals were heterotopically xenotransplanted with frozen-thawed cortical fragments of women ovarian tissue (to broad ligament of uterus, peritoneum of anterior abdominal wall, and into subcutaneous fat pockets). Hormonal monitoring in animals included assessment of estradiol and progesterone levels.

Total number of preantral follicles assessed histologically was 155: 146 follicles (93.5%) with normal morphology; 9 (6.5%) with signs of damage (rupture of the follicle, oocyte shrinkage). Characteristics of stroma were similar as in ovarian cortical samples before vitrification. Estradiol level in the culture medium after 24 and 72 hrs was (32.4 ± 5.4) and (240.7 ± 30.5) pg/ml, respectively. Stable estradiol indices were found in animals after 3.5 months. Animals underwent a 10-day treatment with Puregon gonadotropin (6 days with 25 IU, 4 days with 50 IU, totally 350 IU). Laparotomy on the peritoneum of anterior abdominal wall revealed the growth of follicles, which were of 3 to 11 mm. No follicle growth was found in other sites of ovarian tissue grafting.

Thus, the xenotransplantation of devitrified ovarian tissue to laboratory animals allowed to preserve the function of follicles survived during ovarian cortical layer vitrification.