

treatments as it can allow the establishment of bank of oocytes for women with cancer or problems with embryo cryopreservation. Attempts to improve the freezing of oocytes have been tested and specially vitrification. The aim of our study was to determine the effectiveness of trehalose as intracellular cryoprotectant in the vitrification of bovine oocytes matured in vitro.

DESIGN: Animal, experimental study.

MATERIALS AND METHODS: A total of 166 bovine oocytes were included in the study and were allocated in six groups. Three groups were control: TC (vitrification - 10), TP (needle insertion - 10) and TM (trehalose injection - 15) and three groups were submitted to vitrification: TMV (microinjection with trehalose and vitrification - 79), TVDE (ethylenoglycol and DMSO and vitrification - 47) and TEV (electroporabilization with trehalose and vitrification - 15). After two weeks, the oocytes were thawed. The straws containing the oocytes were placed at 41°C in solution one containing TCM 199 with HEPES supplemented with 20% bovine fetal serum and 5mol/l of sucrose for rinsing. For step oocytes were kept in the same solution for 5 minutes. For step 3 oocytes were kept in the same solution with 2.5mol/l of sucrose for 5 minutes and for step 4 oocytes were kept in solution without sucrose for 5 minutes. After thawing we evaluated the cytoplasmic re-expansion capacity, membrane integrity using propidium iodide and glycoprotein alterations of the zona pellucida marked with lectins conjugated with fluorescein 5- isotiocianato (FITC).

RESULTS: The evaluation of the return to the isotonic volume after thawing showed a higher rate in group TEV (80%) when compared to group TMV (55%) and TVDE (68%). Oocytes of group TEV presented lower cytoplasmic extrusion (13%). The evaluation of membrane integrity showed membrane damage in 36%, 64% and 29% of the oocytes of the treatment groups TMV, TVDE and TEV, respectively. The presence of manose and sialic acid at the zona pellucida surface, after thawing, did not present difference among treatment groups (100% for all groups).

CONCLUSIONS: The obtained results demonstrate that intracellular injection of trehalose has an important crioprotectant effect for vitrification of bovine oocytes matured in vitro, however more studies are necessary in order to minimize cellular damages before its application for human oocytes.

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THE IMPACT OF OOCYTE VITRIFICATION ON LABORATORY AND CLINICAL OUTCOMES IN 30 TO 38 YEARS OLD IVF PATIENTS. C.-C. Chang, D. P. Bernal, C. W. Elsner, A. A. Toledo, S. M. Slayden, Z. P. Nagy. Reproductive Biology Associates, Atlanta.

OBJECTIVE: Cryopreservation is known to have significant effects on oocyte physiology, which may compromise the viability of resulting embryos. Vitrification has been successfully applied to human oocyte cryopreservation recently. However, the impact of oocyte vitrification has not been evaluated in the regular IVF patient population. Thus, our objective was to investigate the impact of oocyte vitrification on laboratory and clinical results in typical IVF patients aged between 30 and 38 years.

DESIGN: Prospective study.

MATERIALS AND METHODS: Inclusion criteria were: 1, patients consented to the IRB study; 2, maternal age between 30-38 years-old; 3, >10 MII oocytes obtained. MII oocytes from every patient were randomly divided into two groups (50%/50%): vitrified/warmed (A) and fresh sibling egg control group (B). Cryopreservation on oocytes was performed by the vitrification with 15% ethylene glycol, 15% DMSO and 0.5 M sucrose. Oocyte warming was performed immediately after vitrification by serial dilutions in three steps using 1.0M, 0.5M, and 0M sucrose solutions. Oocytes were fertilized by ICSI 2-3 h after warming, and fresh sibling oocytes were also microinjected at the same time. On day 5, embryos were assessed and only embryos derived from egg freezing were transferred.

RESULTS: A total of 104 MII oocytes were retrieved from 7 study participants. Fifty-five oocytes were allocated to group A, and 49 oocytes were assigned to group B. Forty-nine oocytes survived after vitrification and warming (89.1%). The fertilization rate was 93.9% (46/49) in group A and 89.8% (44/49) in group B. Thirty-one of 46 embryos (67.4%) in group A developed to blastocyst stage until day 6, and 30 out of 44 embryos (68.2%) in group B. Embryo transfer was performed on day 5; 15 blastocysts were transferred to 7 patients; a total of 9 FCAs were detected in 6 patients (implantation: 9/15, 60.0%). Six pregnancies are still ongoing.

CONCLUSIONS: In the present study, we demonstrated that the clinical efficiency of oocyte cryopreservation is high, and both fertilization and de-

velopment rates are comparable to fresh sibling oocytes. These results indicate that the impact of vitrification can be reduced to minimal making it possible to achieve high pregnancy and implantation rates in this age group of IVF patients. In addition, these results open a wide range of possibilities for a similar age group of general fertile population, such as fertility preservation.

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THE EFFECT OF NERVE GROWTH FACTOR ON THE DEVELOPMENT OF HUMAN PRIMORDIAL FOLLICLES IN VITRO CULTURE. Q. Zhang, C. Wei, Y. Li, W. Wang, D. Yang. The Center of Reproductive Medicine, Second Affiliated Hospital Sun Yat-sen University, Guangzhou, China; Second Affiliated Hospital Sun Yat-sen University, Guangzhou, China.

OBJECTIVE: To investigate the actions of NGF on human early follicle development.

DESIGN: Case-controlled experiment.

MATERIALS AND METHODS: Ovarian biopsies from 21 patients were cut into ovarian pieces(opt) of 1mm thickness which distributed into 5 groups: groupI (non-culture group),groupII (NGF group),groupIII (basic culture group),groupIV (anti-NGF antibody group),groupV (control IgG group). After culture, all the tissue samples were fixed for histological analysis, immunohistochemistry analysis,TUNEL test.

RESULTS: Here were 33.83%±31.73%, 53.51%±39.41%, 39.30±31.73% primordial follicles among groupII, groupIII and groupIV, significantly lower than the proportion of primordial of groupI, which was 82.16%±13.63%, P<0.01; the percentage of developing follicles in groupII was 54.14%±26.16%, significantly higher than that of groupI, groupIII and groupIV, which were 14.63%±15.00%, 23.39%±28.66%, 31.44±32.24% respectively, P<0.01. The percentage of abnormal follicles in groupI was 3.22%±7.48%, significantly lower than that of groupIII, groupII, groupIV, which were 23.1%±30.94%, 12.03%±11.75%, 29.26±28.61% respectively, P<0.05. The Positive Unit value of groupI, groupIII, groupII, groupIV were 6.63±3.77, 5.66±4.74, 12.48±6.61, 6.67±4.58, 6.92±6.66 respectively, the PU value of groupII was significantly higher than that of the other groups, p<0.05. The PCNA positive follicles in groupI, groupIII, groupII, groupIV, groupV were 28.45%, 37.25%, 59.79%, 22.72%, 33.33% respectively, the rate of positive follicles in groupII was higher than that of groupIII and groupIV, p<0.005. The apoptotic index of stroma cells of groupII, groupIII, groupIV, groupV were 0.06±0.06, 0.12±0.07, 0.14±0.09, 0.12±0.09 respectively. The apoptotic index of stroma cell in groupII was significantly lower than that of groupIII, groupIV, p<0.01.

CONCLUSIONS: NGF can help to promote the transition of primordial-to-primary follicles and their development in vitro culture of human ovarian tissues; By decreasing the apoptosis and increasing proliferation of stroma cells, NGF can help to promote the survival and development of early follicles in vitro culture of human ovarian tissues.

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VEGF LEVEL IN OVARIAN TISSUES AFTER HETEROTOPIC AUTOTRANSPLANTATION IN ICR MICE. W. J. Choi, S. A. Lee, J. H. Lee, J. Han, A. Agarwal, W. Y. Paik. OB/GY, School of Medicine, Gyeongsang National University, Jinju, Korea; School of Medicine, Gyeongsang National University, Jinju, Korea; Physiology, School of Medicine, Gyeongsang National University, Jinju, Korea; Center for Reproductive Medicine, Cleveland Clinic, Cleveland, OH; School of Medicine, Gyeongsang National University, Jinju, Korea.

OBJECTIVE: Ovarian tissue banking is a promising technique for the preservation of fecundity in young female cancer patients. Revascularization plays a critical role in successful ovarian tissue transplantation, and vascular endothelial growth factor (VEGF) is a principal factor that promotes neovascularization. This study was designed to assess the VEGF levels in cryopreserved ovarian tissue after heterotopic autotransplantation in ICR mouse.

DESIGN: Prospective study.

MATERIALS AND METHODS: The ovarian tissues were obtained from 5 or 6 weeks aged ICR mouse. Ovarian tissues were divided into three