follicles of morphologically abnormalities were classified as follows: [1] oocyte with pyknotic nucleus; [2] oocyte with cytoplasm damage; [3] oocyte with nucleus and cytoplasm damage combined. Total RNA was extracted from the fresh and frozen-thawed ovaries cultured for 5 day. The expression of transcripts for follicle development associated genes (Kit ligand; KL, basic fibroblast growth factor; bFGF, Leukemia inhibitory factor; LIF) and markers (growth and differentiation factor 9; GDF9, and inhibin-a subunit.) of follicle differentiation were analyzed using RT-PCR.

Results: In the frozen ovaries, the proportion of all kind of morphologically abnormal follicles was significantly higher in both freezing methods compared with the fresh control. The primordial follicles with cytoplasm or double (nucleus + cytoplasm) abnormalities were not difference between slow-freezing and vitrification methods, but the proportion of primordial follicles with abnormal nucleus was higher in slow-frozing method. The development rate of primordial follicles from slow-frozen and vitrified ovaries was significantly lower than that from fresh control, but there was no difference between two cryopreservation methods. There was no difference in mRNA expression patterns for growth factors (KL, bFGF, LIF) and follicle differentiation markers (GDF-9, inhibin-a subunit) between fresh and frozen-thawed ovaries by slow-freezing or vitrification method.

Conclusions: The vitrification is very simple, easy and economical method that could replace the expensive and time consuming slow freezing method for ovarian primordial follicles cryopreservation.

P-27 Clinical Characteristics of Vaginal Bleeding During GnRH Agonist Treatment with Tibolone in Endometriosis Patients

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Background & Objectives: Unexpected vaginal bleeding during GnRH agonist treatment with tibolone in endometriosis patients might cause fears and discomforts, and also reduce the compliance of therapy. This study was undertaken to evaluate the incidence and the factors associated with vaginal bleeding in endometriosis patients during GnRH agonist treatment and add-back therapy with tibolone.

Method: One hundred eighty-eight consecutive patients with moderate to severe endometriosis were recruited, who undertook pelviscopic surgery and postoperative GnRH agonist treatment with tibolone for 6 months. Patients were divided into two groups; Group A, patients without episode of vaginal bleeding (n=137) and Group B, patients with vaginal bleeding (n=51). And clinical features were analyzed and compared between two groups using the Chi-square or Wilcoxon's two-sample test.

Results: Dermographic profile including age, BMI, parity, menstrual pattern, timing of 1st GnRH agonist injection and endometriosis stage was not different between two groups. The incidence of vaginal bleeding was 27.1% and irregular spotting (62.7%) was the most frequent bleeding pattern. The proportion of patients who undertook ovarian surgery was higher in Group B (p=0.0162). Preexisting uterine pathologies

(myoma, endometrial abnormalities) did not influence on the incidence of vaginal bleeding.

Conclusions: The incidence of vaginal bleeding was 27.1% and the ovarian involvement of endometriosis might influence on the incidence of vaginal bleeding during postoperative GnRH agonist and add-back therapy with tibolone.

P-28 Cryoprotection of VEGF is Mediated by Antiapoptotic Effect in Rat Granulosa Cells

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Background & Objectives: Vascular endothelial growth factor (VEGF) has been known as a cytoprotective agent in hematopoietic cells, neurons, myocytes, which protects these cells from apoptosis. And recently, VEGF also has shown to have cytoprotective effect in granulosa cells, which are vulnerable to apoptosis. Although the mechanism of cell death during cryopreservation is incompletely understood, apoptosis through caspase activation was suggested as a possible mechanism of cell death in cryopreserved cord blood hepatopoietic cells and hepatocytes. This study was conducted to evaluate the involvement of apoptosis in the freezing and thawing process and the possible protective effect of VEGF in rat ovarian granulosa cells.

Method: Granulosa cells were obtained from PMSG stimulated Sprague Dawley rats by needle puncture under microscope. Granulosa cells were cultured in DMEM/12 medium with or without VEGF 50 ng/ml for 24 hours. Then the granulosa cells were frozen and thawed, and finally cultured for an additional 24 hours. Cell viability was determined using Trypan blue exclusion test and Annexin-V/ propidium iodide (PI) staining was performed for each step to distinguish viable, early, and late apoptotic, and necrotic cells. DNA degradation was evaluated by PI/RNase staining. Apoptotic cell death was confirmed by caspase-3 colorimetric assay. For the evaluation of dose-dependency, different doses of VEGF treatment (25, 50, 100, 200 ng/ml) were also applied. Statistical analysis was done using two-way ANOVA and Bonferroni multiple comparison test.

Results: After freezing-thawing process with additional 24-hour culture, VEGF treated group showed significantly higher number of viable granulosa cells ($p=0.0036$) and a significant decrease of the percentage of cells with degraded DNA (subdiploid DNA content) ($p=0.001$) than untreated group. VEGF treatment reduced late apoptosis induced by freezing and thawing process ($p=0.008$) without any effect to early apoptosis rate, suggesting that VEGF might delay apoptosis progression. Confirming this effect of VEGF, the expression of caspase-3 was significantly decreased in VEGF treated group ($p=0.0078$). Furthermore, VEGF treatment reduced early apoptosis during subsequent 24-hour culture after thawing ($p=0.0001$) resulting in higher rate of viable granulosa cells after cryopreservation ($p=0.001$). Among the different doses of VEGF treatment, 50 ng/ml of VEGF showed the highest protective effect against the