amniotic membrane for the cell therapy.

Method: Mesenchymal stem cell (MSC)-like cells were obtained from the human amnion after a Caesarean section. The phenotypic characteristics of these amnion-derived cells (AMC) were examined using RT-PCR, immunocytochemistry and telomerase activity assay. To examine their differentiation potential, AMC were cultured in specific induction medium for the osteogenesis, chondrogenesis, adipogenesis and neurogenesis. After culture, cell differentiation was assessed by Von Kossa, Oil red O, Alcian Blue and Neu N stainings.

Results: Human AMC were successfully isolated and maintained through 9 passages. RT-PCR analyses of the AMC at 3rd passage showed the prominent expression of Oct-4, SCF, nestin, PAX-6, vimentin, N-CAM, CK18, BMP-4, GATA-4, AFP and HNF-4a genes. Immunocytochemical study after 6 passages demonstrated the distinct expression of collagen I, II, III and XII, fibronectin, HCAM, a-smooth muscle actin, desmin, SSEA-3 and -4. Results of the telomerase activity assay indicated that AMC at 3rd passage possess the activity. AMC cultured in the specific differentiation induction medium exhibited positive staining with each stain, implying that they could differentiate into osteocyte, adipocytes, chondrocyte and neuronal cells under appropriate conditions.

Conclusions: Profiles of gene expression, protein localization and telomerase activity assay of human AMC showed typical features of known adult stem cells. Considering their multi-differentiation potential, human AMC could be an excellent alternative source for the human cell therapy, replacing MSC and other fetal stem cells.

P-26 Comparison of Freezing Methods (Slow-freezing vs Vitrification) in the Aspect of Early Follicular Development (Morphology and Development Associated Genes)

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Background & Objectives: Cryopreservation of ovarian tissues has been performed using slow-freezing method. Recently, a rapid, simple and economical cryopreservation method, vitrification, has been newly applied to ovarian tissues. But, there are still controversies on the efficacy of these two methods. In this study, in order to compare the efficacy of two freezing methods, we evaluated the morphological development and also analyzed the development associated genes of ovarian primordial follicle after slow-freezing or vitrification.

Method: Slow cryoconserved or vitrified ovaries and fresh control from 1-day-old female mice were in-vitro cultured for 5 days. During culture, the ovaries were examined histologically to evaluate morphologically abnormalities and development of primordial follicles at 1 and 5 day after culture, respectively. The

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