

**Method:** Survivin gene expression in bovine pre-implantation embryo was analyzed at all stages from day 1 to day 8. Messenger RNA was extracted using the Dynabeads mRNA Direct Kit and the survivin cDNA gene from bovine embryo was cloned by RT-PCR. We produced a recombinant bovine survivin protein corresponding to amino acid sequence 143 of the mature protein in *Escherichia coli*. And survivin protein was purified and injected into two rabbits to produce the antisera. Antibody production and titration were analyzed by dot and western blots. Also, the expression of survivin protein in bovine embryos was investigated by immunocytochemistry with rabbit anti-bovine survivin antibody.

**Results:** RT-PCR revealed that survivin mRNA was expressed in all stages of preimplantation embryos and the expression pattern was increased in later stage embryos. After third inoculation, survivin antibody titer (1;1,000) was sufficient to retrieve the sera and the antibody was proved specific both in dot and western blots. Expression level of survivin protein was similar to level of mRNA in all stage. Also, we confirmed the survivin protein was expressed in bovine embryos by immunocytochemistry.

**Conclusions:** This result demonstrated that survivin protein was expressed in bovine pre-implantation embryos and its distribution range can be indentified using survivin antibody.

## P-8                      Effects of Electro-acupuncture on the Estradiol Valerate-induced Polycystic Ovaries in Rats

Park HJ<sup>1</sup>, Na CS

<sup>1</sup>*Department of Oriental Medicine, Dongshin University*

**Background & Objectives:** A form of polycystic ovary (PCO) resembling some aspects of the human PCO syndrome (PCOS) can be induced in rats by a single injection of estradiol valerate (EV). The PCOS still remains as one of the most common causes of anovulation in women of reproductive age. Therefore, seeking the pathogenesis of PCOS is important for controlling fertility. In traditional Oriental Medicine, acupuncture has been used for function of ovaries. Electro-acupuncture (EA) stimulation affect the female endocrine function. The present study was designed to evaluate if EA could affect the experimentally induced polycystic ovary (PCO) in the rat.

**Method:** Two acupoints of Samumgyo and Hangan were stimulated for the efficacy in the regulation of endocrine levels that is known to participate in the hypothalamic-pituitary-ovarian axis (HPOA) function. The administration of EA was done every other day for 30 and 60 days. The morphological changes of ovaries from EA treatment were compared to those from oil-treated control group and EV group at each days.

**Results:** PCO was fully developed in the rat with a single intramuscular injection of EV at 60 days. Significant development of the healthy growing follicle and corpus luteum was observed in EA group. Plasma progesterone and estradiol levels increased significantly in 8 weeks of treatment with EA. Insulin level was decreased EA group. The present study shows that EA might improve the dysfunction of HPOA in EV-induced rat with PCO.

**Conclusions:** Our data indicate a possible role of EA in the regulation of the female endocrine function and therapeutic approach to overcoming anovulation in women with PCOS.

## **P-9      The Effect of Tumor Necrosis Factor on the Microtubule and Chromosomal Alignment in the Mouse Oocytes**

**Choi WJ, Park JK, Shin JG, Lee JH, Paik WY**

*Department of Obstetrics & Gynecology, College of Medicine, Gyeongsang National University*

**Background & Objectives:** Endometriosis, even in a mild stage, may have a direct negative effect on oocyte development, fertilization and embryogenesis. Tumor necrosis factor is a pleiotropic cytokine with immune-regulating properties. It appears to act as the switch point in the cascade of immunologic processes during endometriosis. Poor quality of oocyte from endometriosis patient may be affected by the presence of tumor necrosis receptors are reported to be present on factor in the peritoneal fluid. TNF- $\alpha$  the oocyte. The objective of our study was to examine the dose-dependent effect on microtubule morphology and chromosomal alignment in cryopreserved metaphase II oocytes.

**Method:** Mature metaphase II oocytes were divided into 5 groups and exposed to mouse TNF- $\alpha$  concentrations prepare in human tubal fluid (HTF): 100, 200, 400 and 600 ng/mL. Controls consisted of an equal volume of HTF. For microtubule staining, fixed oocytes are incubated in anti-tubulin monoclonal Ab followed by incubation in FITC labeled anti-mouse IgG Ab. For chromosome staining, oocytes were incubated in propidium iodide. Stained oocytes were scored for alterations in microtubule morphology and chromosomal alignment under a Fluorescent (Leica, Germany) and scanning Confocal microscope (Leica Lasertechnik GmbH, Heidelberg, Germany).

**Results:** Tumor necrosis factor resulted in alterations in both microtubule morphology and chromosomal alignment. The effect was visible at 200 ng/mL concentration of tumor necrosis factor. Some of affected oocytes displayed microtubules with typical helmet-like appearance and the characteristic barrel shape was lost. Chromosomal alignment was significantly disorganized with higher concentration of tumor necrosis factor.

**Conclusions:** Tumor necrosis factor can damage spindle structure and cause alterations in microtubule and chromosomal alignment in mouse oocyte. The varying response to tumor necrosis factor may be due to the presence of different amounts of tumor necrosis factor receptors. This may be one of the many causes of poor oocyte quality obtained from endometriosis patients. Use of anti-tumor necrosis drugs may be beneficial in reducing/reversing these changes.