

method of pre-antral follicles from ovary by means of mechanical or enzymatical method in mice.

Method : Mouse (ICR, 3~4 weeks old) ovarian pre-antral follicles of 70~130 μm (A: =90; B: 91~110; C: 111~130 μm) in diameter were isolated by mechanical method (group I) using syringe needles or by enzymatical method (group II) using 600 IU collagenase and DNase and cultured individually in 20 μl droplet of media under mineral oil on culture dish for 8 days.

Results: On 8th day after culture, disruption rate ($84.4 \pm 18.8\%$ vs. $9.4 \pm 18.8\%$) was significantly higher ($p < 0.05$) in group II than in group I. Survival rate ($88.0 \pm 28.9\%$ vs. 0%) was significantly higher ($p < 0.0000$) in group I than in group II. There was no differences in survival rate among different initial sized pre-antral follicles in group I (A: $86.7 \pm 12.5\%$, B: 100% , C: $69.2 \pm 30.0\%$, NS) or group II (A: 0% , B: 0% , C: 0% , NS) respectively.

Conclusions: In conclusion, compared to mechanical dissection, enzymatical isolation of pre-antral follicles resulted in higher follicular disruption rate. Survival rate was not affected by different initial sizes of pre-antral follicles in mice.

P-6 The Clinical Efficacy of Low-dose Aspirin and Prednisolone in IVF-ET Patients Undergoing COH with GnRH Agonist Long Protocol

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Background & Objectives: To determine whether the use of low-dose aspirin and prednisolone improves the outcomes in in vitro fertilization and embryo transfer (IVF-ET) patients undergoing controlled ovarian hyperstimulation (COH) with GnRH agonist long protocol.

Method: Two hundred and forty IVF-ET cycles were assigned to four groups: control group (aspirin(-) & prednisolone(-), n=59), low-dose aspirin group (aspirin(+)) & prednisolone(-), n=43, Group 1), prednisolone group (aspirin(-) & prednisolone(+), n=80, Group 2), and low-dose aspirin and prednisolone group (aspirin(+)) & prednisolone(+), n=58, Group 3). The COH and pregnancy outcomes were retrospectively compared among the four groups.

Results: Group 1 showed a significantly higher fertilization rate compared to control group (73.6% vs. 64.1% , $p=0.050$). Serum estradiol (E2) level on hCG day was 995.5 ± 767.5 pg/ml in control group, $1,550.7 \pm 1,254.5$ pg/ml in group 1, $1,469.2 \pm 1,206.6$ pg/ml in group 2 and $1,796.0 \pm 1,548.0$ pg/ml in group 3 and higher in the three treatment groups compared to control ($p=0.012$, $p=0.006$, $p < 0.001$, respectively). Cumulative embryo score (CES) per number of embryo transferred was also higher in Group 1 (17.4 ± 8.4 , $p=0.001$) and 3 (16.0 ± 6.1 , $p < 0.001$) compared to control (12.2 ± 5.1). There were no significant differences in the implantation and the pregnancy rates among the four groups.

Conclusions: The use of low-dose aspirin or prednisolone may be beneficial in IVF-ET patients undergoing COH with GnRH agonist long protocol. Further larger-scale prospective randomized investigations are necessary to confirm these findings.

P-7 Involvement of Fas and FasL System and Active Caspase-3 in Apoptotic Signaling of Spermatogenic Cells after Prepubertal Exposure to 4-tert-Octylphenol

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Background & Objectives: 4-tert-octylphenol (OP) is known to disrupt testicular development and reduce male fertility. The purpose of the present studies was to investigate the effects of chronic exposure of OP on the apoptosis in spermatogenic cells. To delineate further the pathways involved, we examined the changes of the expression of FasL, Fas and Caspase-3.

Method: Prepubertal male rats (F344) were injected with estradiol valerate (EV; 0.4 µg) or OP (0.4, 4 or 40 mg) for 14 or 28 days. The frequency of apoptosis of testicular cells was demonstrated by the in situ 3'-end-labelling method. Serum testosterone concentration was measured by radioimmunoassay and the expressions of FasL, Fas and Caspase-3 mRNAs and proteins were determined by RT-PCR analyses and immunohistochemistry, respectively.

Results: Decreased sizes and weights and adversely impaired histological structure of testis were observed in the OP and EV treated groups. Serum testosterone concentration was markedly decreased in all of the experimental animals treated for 28 days. Apoptotic germ cells in the testis, visualized by in situ 3' end labeling, were increased and it coincided with the increased gene expressions of FasL. Immunohistochemistry demonstrated that the markedly increased both FasL and Fas expressions in the spermatogenic cells, especially in degenerating spermatocytes. Moreover, increased immunoreactivity of active caspase-3 was detected in spermatogenic germ cells in the OP-exposed testis and the sites of the expression were also consistent with those of Fas and FasL.

Conclusions: Taken together, the present studies demonstrate that OP causes the impairment of spermatogenesis leading to an increase in apoptosis of testicular germ cells. Especially, it is suggested that up-regulation of Fas and FasL system and the elevated activity of caspase-3 may be involved in apoptosis of the spermatogenic cells.

This study was supported by grants from Korea Science and Engineering Foundation, ABRL; R14-2003-036-010020.