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Developmental Capacity of Mouse Oocytes within Preantral Follicles Cultured in Medium Supplemented with Gonadotrophins

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ABSTRACT

The present study was conducted to examine the developmental capacity of mouse oocytes within preantral follicles cultured various concentrations of FSH and LH and the expression of cytochrome P450 cholesterol side-chain cleavage enzyme (P450scc) and cytochrome P450 17 α-hydroxylase ($P450_{17\alpha}$) mRNA, as luteinization and atretic marker, in these culture conditions. In addition, we investigated the concentrations of progesterone and testosterone in culture medium. The developmental potential upto blastocyst of the oocytes grown *in vitro* was higher in the FSH alone (30.2%) and 10 mIU/ml LH and 100 mIU/ml FSH treated (28.0%) groups than in the 100 mIU/ml LH and 100 mIU/ml FSH treated group (22.0%). And the mean numbers of cell per blastocyst was higher in the FSH alone (50.9±26.1) and 10 mIU/ml LH and 100 mIU/ml FSH treated (51.0±21.1) groups when compared to the 100 mIU/ml LH and 100 mIU/ml FSH treated group (45.2±15.1). The expressions of P450scc and $P450_{17\alpha}$ mRNA in the oocyte-cumulus complexes were increased with increasing of LH concentration, and also the secretions of progesterone and testosterone were increased. Especially, in the 100 mIU/ml LH and 100 mIU/ml FSH treated group, the expression of P450scc and $P450_{17\alpha}$ were significantly increased, and the secretion of progesterone and testosterone were significantly increased. Therefore, these data show that gonadotrophins are essential for the *in vitro* culture of preantral follicles, but that increasing of LH concentration is reduced the developmental capacity of oocytes. The cause of these findings may be due to increasing of progesterone and testosterone secretion by the enhance of P450scc and $P450_{17\alpha}$ mRNA expressions, as markers of luteinization and atresia. Conclusively, this study suggest that supplementation of 100 mIU/ml FSH or 10 mIU/ml LH and 100 mIU/ml FSH may be optimal condition for the culture of mouse preantral follicles.

(Key words : Preantral follicle, FSH, LH, Developmental capacity, P450scc, $P450_{17\alpha}$)

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Ⅰ. INTRODUCTION

Recent advances in *in vitro* reproductive technologies have opened up new opporzstunities in human assisted reproduction technology (ART) and biotechnology. However, these techniques depend on the predictable production of fully developed oocytes, and currently their availability is limited by the number of antral follicles present in the ovaries. The development of preantral follicle culture system that can potentially produce large quantities of oocytes of uniform developmental status will significantly advance the use of these techniques. Additionally, it may make possible the preservation and long-term storage of the female germ plasm (Gutierrez et al., 2000).

Recently, *in vitro* culture techniques for preantral follicles have been improved to assist investigation of the mechanism of oocyte and follicular development. Under appropriate conditions, meiotically incompetent oocytes from preantral follicles can grow to final size and complete nuclear maturation *in vitro* (Eppig and Down, 1987; Nayudu and Osborn, 1992; Cortvindt et al., 1996; Kim et al., 1999). Furthermore, studies for culture of mouse preantral follicles have demonstrated successful growth and maturation as well as fertilization and development of oocytes from follicles cultured *in vitro*. And then, it was verified that successful culture of preantral follicles are achieved only if the three-dimensional organization of the granulosa cells around the oocyte is maintained throughout culture (Eppig and Schroeder, 1989; Spears et al., 1994). Indeed, the oocyte is dependent on the surrounding granulosa cells from which it receives its nutrients (Heller et al., 1981). Nutrient transport between the granulosa cells and the oocyte is achieved by extensive gap junction communication (Buccione et al., 1990). Granulosa cell contact is essential for maintaining the oocyte in meiotic arrest, since their removal (Eppig and Downs, 1984), or disruption of the gap junction communications between granulosa cells and the oocyte, results in spontaneous germinal vesicle breakdown (Eppig and Downs, 1987).

It has been reported that goandotrophins, FSH and LH, play a central role in the *in vitro* growth and maturation of ovarian preantral follicles (Nayudu and Osborn, 1992). FSH serves as the primary inducer of granulosa cell maturation. Together with estradiol, FSH activates the proliferation of granulosa cells, enhances aromatase-enzyme activity, and promotes the expression of LH receptors (Zeleznik et al., 1974; Carson and Smith, 1986; Xu et al., 1995). LH plays also an important role for the growth of granulosa cells (Yong et al., 1992), the formation of antrum and the production of estrogen (Qvist et al., 1990). Furthermore, Cortvrindt et al. (1998) reported that FSH was more effective in the preantral follicle survival and meiotic maturation of oocyte within preantal follicle when adequate amounts of LH were present. However. developmental ability of the *in vitro* grown oocytes cultured in medium supplemented with gonadotrophins after fertilization have not been evaluated.

The objective of present study was to examine the developmental capacity of mouse oocytes within preantral follicles cultured various concentrations of FSH and LH and the expression of cytochrome P450 cholesterol side-chain cleavage enzyme (P450scc) and cytochrome P450 17α-hydroxylase ($P450_{17\alpha}$) mRNA, as luteinization and atretic marker, in the oocyte-cumulus complexes cultured in these culture conditions. In addition, we investigated the concentrations of progesterone and testosterone in culture medium.

Ⅱ. MATERIALS AND METHODS

1. Isolation of Preantral Follicles

The ovaries were aseptically removed from 12-day-old ICR female mice. The ovaries were immersed into Leibovitz L-15 medium (41300-039, Gibco, U.S.A.) containing 1 mg/ml collagenase (Type 1A; C-2674, Sigma, U.S.A.) and 0.2 mg/ml DNase I (DN-25, Sigma, U.S.A.) for 20 minutes at 37℃ and repeatedly drawn in and out of the pipette until the ovaries were dissociated into individual follicles (Eppig and O'Brien, 1996). The follicles to be cultured were selected by the following criteria: i) intact round follicular structure with two to three layers of granulosa cells; and ii) the oocyte had to be visible, round and centrally located within the follicle. All selected follicles were pooled and randomly divided over the culture condition under study.

2. *In Vitro* Growth and Maturation of Preantral Follicles

The culture medium was α-minimal essential medium (αMEM; 11900-024, Gibco, U.S.A.) supplemented with 5% fetal bovine serum (FBS; 16170-078, Gibco, U.S.A.), and follicle stimulating hormone (FSH; Metrodin-HP, Sereno, Switzerland) and luteinizing hormone (LH; L-5259, Sigma, U.S.A.). The follicles were cultured on Transwell -COL membrane inserts (3.0 μm pore size, 24.5 mm diameter; Costar, U.S.A.) in six well cluster dishes to prevent the loss of structural integrity between the oocyte and granulosa cells (Eppig and Schroeder, 1989). The follicles were cultured for 10 days at 37℃ in 5% CO_2 in air. Half of the medium was changed every 2 days. After 10 days of growth *in vitro*, follicles allowed to mature for 16～18 hours in medium supplemented with 1.5 IU/ml human chorionic gonadotrophin (hCG; Profasi, Sereno, Switzerland).

3. *In Vitro* Fertilization

in vitro-matured oocyte-cumulus complexes (OCCs) were put into 50 μl drops of Whittingham's T_6 medium supplemented with 30 mg/ml bovine serum albumin (BSA; A-3311, Sigma, U.S.A.) under mineral oil. As a control, *in vivo*-matured oocytes were obtained from 23-day-old ICR mice. The mice were induced to superovulate with 5 IU pregnant mare serum gonadotrophin (PMSG) 48 hours before treatment with 5 IU human chorionic gonadotrophin (hCG). Fifteen to 16 hours after the hCG injection, oocytes were recovered from the oviducts. They were put into the drops of fertilization medium.

Sperm was collected from the cauda epididymis of mature ICR male mice, and incubated for 2 hours in fertilization medium before insemination. The final concentration of spermatozoa was 1～2 $\times$ 10^6/ml. After 4 hours, oocytes were washed and cultured for *in vitro* development.

4. *In Vitro* Development

The fertilized oocytes were cultured in 20 μl drops of potassium simplex optimized medium (KSOM; Lawitts and Biggers, 1993) supplemented with 10% (v/v) serum substitute supplement (SSS; Irvine, U.S.A.), 1% (v/v) essential amino acids (M-7145, Sigma, U.S.A.) and 0.5% (v/v) non-essential amino acids (B-6766, Sigma, U.S.A.) under mineral oil. The oocytes were examined the following day, and the number of 2-cell embryos were noted. Two cell embryos were further cultured for 4 days until the blastocyst stage.

Blastocysts were stained with Hoechst 33342 to count the number of cells. The cell number of blastocyst was examined under a fluorescence microscope.

5. Assessment of Steroid Hormone Production

Half of the culture medium, which was sampled and subsequently replaced by fresh medium, the following secretory products were measured: progesterone and testosterone. Steroids were measured with commercially available radioimmunoassay kits made for competition assay method (Europe S.A.). Fifty μl of each samples, standards and controls were mixed with 500 μl of each I^{125} labeled steroids in the polystyrene tube fixed each antibodies on the wall, and incubated in waterbath at 37℃ for 3 hours. After incubation, all reactants except total counts were removed and washed out remnants of reactants with 3ml of washing solution, using automatic tube washer. In order to increase the reproducibility of the assay, all tubes except total counts were placed on the table for 5 minutes and aspirated the remaining drop of liquid carefully and then were counted the radioactivity of I^{125} in γ-ray counter for 60 seconds (RIA-matII, Startek). To construct the calibration curve, the bound radioactivities as a percentage of the binding determined at the zero standard point were calculated and plotted with computer assisted methods using 3 cycle semi-logarithmic or logit-log graph.

The concentration of steroids in culture medium were determined by interpolation of the sample values in the reference curve. The sensitivity for testosterone and progesterone were 0.05 and 0.044 ng/ml, respectively. All experiments were executed with duplicate.

6. Assay for Gene Expression

1) RNA Extraction

Oocyte-cumulus complexes (OCCs) were washed in three changes of PBS containing 0.1% PVP and then transferred in a minimal volume of PBS into 300 μl of TRIzol™ Reagent (GibcoBRL, U.S.A.). RNA was extracted according to the manufacturer's instructions. Samples were lysed by repetitive pipetting. After the incubation of samples for 5 minutes at room temperature, 60 μl of chloroform was added. The sample tubes were shaken vigorously by hand for 15 seconds and were incubated at room temperature for 5 minutes. The samples were centrifuged at 14,000 rpm for 15 minutes at 4℃. Following centrifugation, the upper aqueous phase was transferred into new eppendorf tubes. Isopropyl alcohol (100 μl) was added to the samples and the samples were incubated for 10 minutes at room temperature. The samples were centrifuged at 14,000 rpm for 15 minutes at 4℃ for the RNA precipitation. After the removal of supernatant, the RNA pellet was dried briefly and dissolved in RNase-free water. The RNA was either immediately subjected to RT-PCR or stored at −70℃.

2) Reverse Transcription

Reverse transcription (RT) was performed with total RNA isolated from OCCs. RT was performed using 1st strand cDNA Synthesis Kit (Boehringer Mannheim, Germany). Each RNA sample (8.2 μl) was added into the mixture containing 1X reaction buffer, 5 mM of $MgCl_2$, 1 mM of deoxynucleotide mix, 1.6 μg of Oligo-p$(dT)_{15}$ primer, 50 unit of RNase inhibitor and 20 unit of AMV reverse transcriptase. The mixture was incubated at 25℃ for 10 minutes and then 42℃ for 60 minutes. Following the 42℃ incubation, the mixtures were incubated for 5 minutes at 99℃ for denaturation of AMV reverse transcriptase. Then, the mixture were cooled at 4℃. At this point, the samples were either immediately used for PCR or stored at −70℃.

3) Polymerase Chain Reaction (PCR)

PCR amplification was performed in a 20 μl comprising 2 μl of cDNA product (OCCs or 3 oocytes equivalent), 1 unit of Taq DNA polymerase (Perkin Elmer, U.S.A.), 250 μM dNTP, 10mM

Tris-HCl (pH 9.0), 40 mM KCl, 1.5mM $MgCl_2$, tracking dye, 2 μl of each specific primer (Bioneer, Korea) and 9.8 μl of sterile water. To monitor possible contamination, a sample consisting of the PCR mixture and RNA sample was included during each amplification as a negative control. Primer sequences used in this study are indicated in Table 1. PCR condition for β-actin was 94℃ for 3 minutes; 31 cycles of 94℃ for 30 seconds, 54℃ for 45 seconds, 72℃ for 1.5 minutes. PCR conditions for P450scc and $P450_{17\alpha}$ were 95℃ for 5 minutes; 33 cycles of 95℃ for 1 minutes, 68℃ for 1.5 minutes, and 72℃ for 5 minutes.

After amplification, the RT-PCR products were separated by agarose (2%) gel electrophoresis, stained by ethidium bromide and visualized under UV. The amounts of specific mRNAs were analyed using image analyzing system with computer software (Vilber Lourmat, France).

7. Statistical Analysis

The statistical significance of the data was analyzed using Students t-test and the chi-square test. Statistical significance was considered at the $P<0.05$ level.

Ⅲ. RESULTS

1. Developmental Potential after Fertilization

To determine the developmental potential of theoocytes that *in vitro*-grown and -matured with various FSH and LH concentrations, mature oocytes were fertilized after maturation for 16~18 hours. As shown in Table 2, no significant differences were observed in the cleavage rates among the treatment groups. However, FSH treated group (63.8%) was somewhat higher compared to the FSH100+LH10 (59.3%) and FSH100+LH100 (53.5 %) treated groups. The developmental rates to morular and blastocyst stages were significantly when higher in FSH treated group (40.3 and 30.2%, respectively) compared to the FSH100+LH100 treated group (27.7 and 22.0%, respectively), but there were no difference between the FSH and FSH100+LH10 (35.3 and 28.0%, respectively) treated groups. The mean numbers of cell per blastocytst (Table 3) was somewhat higher in the FSH (50.9±26.1) and FSH100+LH10 (51.0±21.1) treated groups when compared to the FSH100 +LH100 (45.2±15.1), but there was no statistical difference among the treatment groups.

2. Expressions of P450scc and $P450_{17\alpha}$ mRNA

In this experiment, the expression levels of P450scc and $P450_{17\alpha}$ in the OCCs that *in vitro* -grown and -matured to various concentrations of FSH and LH were adopted as markers of luteinization and atresia. Thus, the semi-quantitative analysis of P450scc and $P450_{17\alpha}$ mRNA were conducted. The expression levels of P450scc mRNA (Fig. 1) were not different between FSH alone and FSH100

Table 1. Primers used for RT-PCR and product size

Gene	Primer	Primer sequence	Product size (bp)
β-actin	5'	5'-GTGGGCCGCTCTAGGCACCAA-3'	539
	3'	5'-CTCTTTGATGTCACGCACGATTTC-3'	
P450scc	5'	5'-AGTGGCAGTCGTGGGGACAGT-3'	411
	3'	5'-TAATACTGGTGATAGGCCACC-3'	
$P450_{17\alpha}$	5'	5'-CCCATCTATTCTCTTCGCCTGGGTA-3'	743
	3'	5'-GCCCCAAAGATGTCTCCCACCGTG-3'	

Table 2. Development of mouse oocytes grown and matured with various concentrations of FSH and LH after fertilization

Treatment	No. of oocytes fertilized	No. (%) of embryos developed to			
		2-cell	4-cell	Morular	Blastocyst
In vivo[1]	149	115(77.2)[a]	108(72.5)[a]	95(63.8)[a]	84(56.3)[a]
FSH[2]	149	95(63.8)[b]	76(51.0)[b]	60(40.3)[b]	45(30.2)[b]
FSH100+LH10[3]	150	89(59.3)[b]	71(47.3)[b]	53(35.3)[b]	42(28.0)[b]
FSH100+LH100[4]	159	85(53.5)[b]	59(37.1)[b,c]	44(27.7)[b,c]	35(22.0)[b]

[a,b] $P<0.001$, [a,c] $P<0.05$

[1] Oocytes were collected from the oviducts of 23-day-old mice treated superovulation.

[2] 100mIU/ml FSH

[3] 100mIU/ml FSH and 10mIU/ml LH

[4] 100mIU/ml FSH and 100mIU/ml LH

Table 3. Cell number per mouse blastocyst derived from oocyte grown and matured with various concentrations of FSH and LH

Treatment	No. of blastocysts examined	No. of cells (mean±SEM)	Range
In vivo	39	61.1±12.7[a]	42~ 86
FSH	35	50.9±26.1[b]	12~117
FSH100+LH10	32	51.0±21.2[b]	13~101
FSH100+LH100	30	45.2±15.1[c]	14~118

[a,b] $P<0.05$, [a,c] $P<0.001$

+LH10 treated groups. However, the expression of P450scc mRNA in FSH100+LH100 treated group was significantly higher when compared to FSH alone and FSH100+LH10 treated groups. The expression of $P450_{17\alpha}$ mRNA, as atretic marker of follicle (Fig. 2) was also showed the same pattern as in P450scc expression.

3. Secretions of Progesterone and Testosterone

To compare the secretions of progesterone and testosterone, as markers of luteinization and atresia, in the mouse preantral follicles grown with various concentrations of FSH and LH, culture media were collected on day 10 after culture. The level of progesterone (Fig. 3) was not different between FSH and FSH100+LH10 treated groups. However, the level of progesterone in FSH100+LH100 treated group was significantly higher compared to FSH and FSH100+LH10 treated groups. The level of testosterone (Fig. 4) also showed the same pattern as in progesterone profile. On the other hand, these profiles of progesterone and testosterone secretion were correlated with expression of P450scc and $P450_{17\alpha}$ mRNA as shown in Fig. 1 and 2.

Ⅳ. DISCUSSION

During folliculogenesis *in vivo*, follicle survival depends on the presence of physiological concen-

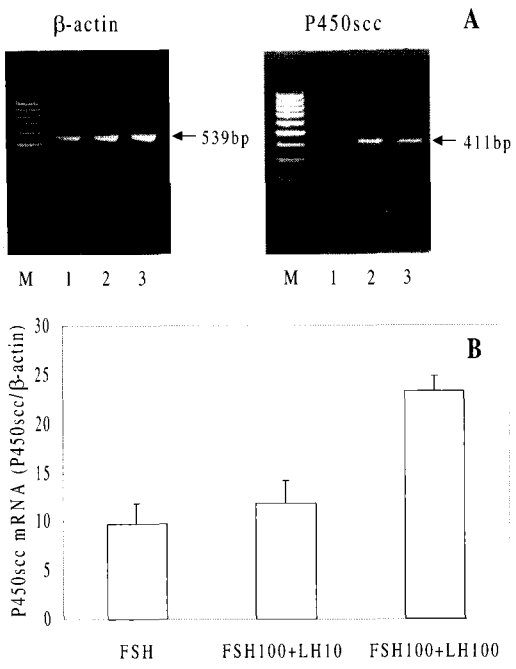

Fig. 1. Relative amounts of P450scc mRNA expression in the mouse oocyte-cumulus complexes within preantral follicles cultured in medium containing various concentrations of FSH and LH. Preantral follicles were cultured for 10 days. A: RT-PCR products. M, molecular size marker; Lane 1, 100 mIU/ml FSH treated OCCs; Lane 2, 100mIU/ml FSH and 10mIU/ml LH treated OCCs; Lane 3, 100mIU/ml FSH and 100mIU/ml LH treated OCCs. B: Relative amount of P450scc mRNA. FSH, 100mIU/ml FSH treated OCCs;FSH100+LH10, 100mIU/ml FSH and 10 mIU/ml LH treated OCCs; FSH100+LH100, 100 mIU/ml FSH and 100 mIU/ml LH treated OCCs.

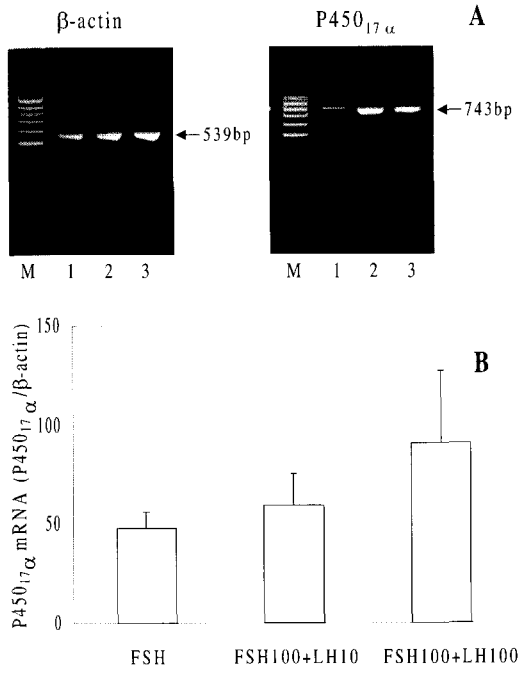

Fig. 2. Relative amounts of $P450_{17\alpha}$ mRNA expression in the mouse oocyte-cumulus complexes within preantral follicles cultured in medium containing various concentrations of FSH and LH. Preantral follicles were cultured for 10 days. A: RT-PCR products. M, molecular size marker; Lane 1, 100mIU/ml FSH treated OCCs; Lane 2, 100mIU/ml FSH and 10mIU/ml LH treated OCCs; Lane 3, 100mIU/ml FSH and 100mIU/ml LH treated OCCs. B: Relative amount of P450scc mRNA. FSH, 100mIU/ml FSH treated OCCs; FSH100+LH10, 100mIU/ml FSH and 10mIU/ml LH treated OCCs; FSH100+LH100, 100mIU/ml FSH and 100mIU/ml LH treated OCCs.

tration of gonadotrophins (Hsueh et al., 1994). The supplement of FSH and LH in a culture system for preantral follicles provides the possibility of assessing the effects of each gonadotrophin separately or in combination on follicular growth, differentiation, secretory activity and on oocyte maturation from early primary stages up to full maturity. FSH serves as the primary inducer of granulosa cells maturation. Together with oestradiol, FSH activates the proliferation of granulosa cells, enhances aromatase-enzyme activity, and promotes the expression of LH receptors (Zeleznik et al.,

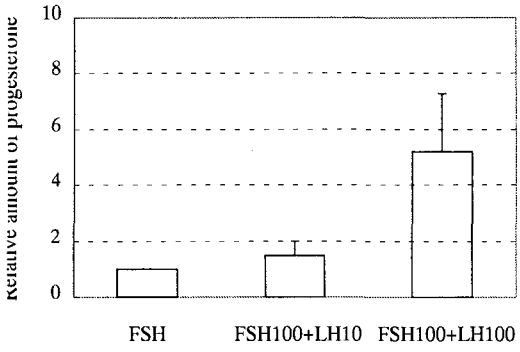

Fig. 3. Relative amounts of progesterone in culture medium collected from mouse preantral follicles cultured in various concentrations of FSH and LH. After 10 days of culture, progesterone concentration was measured from medium. The mean amount of the FSH group was considered as 1. FSH, 100mIU/ml FSH treated OCCs; FSH100+LH10, 100mIU/ml FSH and 10mIU/ml LH treated OCCs; FSH100+LH100, 100 mIU/ml FSH and 100mIU/ml LH treated OCCs.

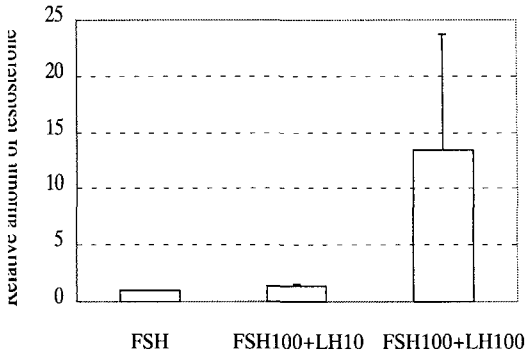

Fig. 4. Relative amounts of testosterone in culture medium collected from mouse preantral follicles cultured in various concentrations of FSH and LH. After 10 days of culture, testosterone concentration was measured from medium. The mean amount of the FSH group was considered as 1. FSH, 100mIU/ml FSH treated OCCs; FSH100+LH10, 100mIU/ml FSH and 10mIU/ml LH treated OCCs; FSH100+LH100, 100 mIU/ml FSH and 100 mIU/ml LH treated OCCs.

1974; Carson and Smith, 1986; Xu et al., 1995). FSH is one of the most important factors for the growth of follicles in ovaries. It is known that addition of FSH in culture medium plays an important role in survival and maturation of follicles and oocytes and also alleviates oxidative stress by enhancing superoxide dismutase and other scavenger systems (Tilly and Tilly, 1995). Previous studies on the culture of mouse preantral follicles have reported that FSH promotes the growth of follicle and the formation of antrum (Hartshorne et al., 1994) by stimulating proliferation of granulosa cells, increases GVBD and extrusion of the first polar body of the *in vitro*-grown oocytes (Eppig and Schroeder, 1989) and significantly increases the production of estrogen and lactate of follicles (Nayudu and Osborn, 1992; Boland et al., 1993). In previous experiment, we confirmed that FSH plays key role in survival and growth of mouse preantral follicles. It was observed that, without FSH, the three-dimensional structure between oocyte and granulosa cells collapses and then follicle degenerates due to poor proliferation of granulosa cells (Kim et al., 1999).

LH is also an important factor for the growth of granulosa cells (Yong et al., 1992), the formation of antrum and the production of estrogen (Qvist et al., 1990). Recent immunohistochemical studies have demonstrated that LH receptor is also initially expressed in cumulus cells during follicular development, suggesting that LH might interfere during the oocyte's entire growth phase (Bukovsky et al., 1993). Addition of LH to FSH throughout the culture period increase antral-like cavity formation of follicles and maturation rates of the *in*

vitro-grown oocytes (Qvist et al., 1990; Cortvrindt et al., 1998), and significantly increase in glucose uptake in cultured follicles. *In vivo* studies in normal rat have been shown that an optimal LH:FSH ratio has an impact on oocyte production (Armstrong et al., 1989). In previous experiment, we showed higher survival rates of follicles and higher maturation rates of oocytes when 10 mIU/ml LH and 100 mIU/ml FSH were added in culture medium (Kim et al., 1999). Steroid hormones might be important regulators of the essential oocyte cytoplasmic changes for normal fertilization (Zhang and Armstrong, 1989). The interfollicular variation in steroid secretion is very large within each culture condition. Cortvrindt et al (1996) reported that addition of LH to FSH remarkably increased the oestrogen and progesterone during the culture period.

On the other hand, in this experiment, the developmental rates of oocytes grown in various concentrations of FSH and LH did not show statistical difference, but 100 mIU/ml FSH and 100 mIU/ml LH concentrations decreased blastocyte development and cell number per blastocyst. To find the cause of this lower developmental capacity, we examined the concentrations of progesterone and testosterone in culture medium, and the expressions of P450scc and $P450_{17\alpha}$ in oocyte -cumulus cell complexes, as luteinization and atretic marker.

The P450scc that converting cholesterol to prognenolone is known as a rate-limiting enzyme in biosynthesis of progesterone which induces the luteinization of granulosa cells (Robel, 1993; Wang et al., 1999). Vanderhyden et al. (1998) showed that progesterone production by oocyte-granulosa cell complexes in both preantral and antral follicles was inhibited and the concentration was not different. Premature luteinization with increased progesterone concentration negatively affects oocytes, leading to a reduction in their fertilizability (Schoolcraft et al., 1991). High concentration of progesterone adversely affects embryo quality and IVF pregnacy rates (Dhawan et al., 2000). A subtle rise in serum progesterone concentration during the follicular phase has also been associated with reduced implantation rates (Harada et al., 1995). The $P450_{17\alpha}$ is known to catalyze the formation of testosterone from pregnenolone or progesterone (Robel, 1993). It is known that the earliest marker of atresia is a reduction of aromatizing ability which induces an accumulation of androgen. Testosterone and 5α-dihydrotestosterone block cell division and inhibit aromatization further (Driancourt et al., 1993). Furthermore, Zelinski-Wooten et al. (1993) reported that high testosterone:oestrogen ratio in rhesus monkeys did not inhibit follicular development, but fertilization rate of the resulting oocytes was decreased. This study demonstrated that increasing of LH concentration in culture medium promoted the biosynthesis of progesterone and testosterone by the increasing of P450scc and $P450_{17\alpha}$ mRNA expression. Especially, these patterns were more augmented when both 100 mIU/ml FSH and 100 mIU/ml LH were added in culture medium.

Therefore, these findings suggest that gonadotrophins such as FSH and LH, are essential for the *in vitro* growth of preantral follicles, but the addition of high concentration LH in medium reduces the developmental rates following fertilization, which may be due to the excessive secretion of progesterone and testosterone by the higher expression of P450scc and $P450_{17\alpha}$ mRNA. Conclusively, this study suggest that supplementation of 100mIU/ml FSH or 10mIU/ml LH and 100mIU/ml FSH may be optimal condition for the culture of mouse preantral follicles.

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요 약

성선자극호르몬이 첨가된 배양액에서 체외배양된 생쥐 Preantral Follicles내 난자의 발생능력

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본 연구는 다양한 농도의 FSH와 LH에서 배양된 생쥐 preantral follicles내 난자의 발생능력을 조사하고, 이러한 조건에서 배양된 난자-난구세포 복합체에서 황체화의 지표인 cytochrome P450 cholesterol side-chain cleavage enzyme (P450scc)와 퇴행화의 지표인 cytochrome P450 17α-hydroxylase ($P450_{17\alpha}$) mRNA의 발현정도를 조사하고, 또한 progesterone과 testosterone의 분비농도를 살펴보기 위하여 실시하였다. 체외성장된 난자의 배반포까지의 발달능력은 100 mIU/ml FSH단독첨가군 (30.2%)과 100 mIU/ml FSH+10mIU/ml LH 첨가군 (28.0%)이 100mIU/ml FSH+100mIU/ml LH 첨가군 (22.0%)보다 높은 결과를 나타냈다. 그리고 배반포의 평균 세포수에 있어서도 FSH 단독첨가군 (50.9±26.1)과 100mIU/ml FSH+10 mIU/ml LH 첨가군 (51.0±26.1)이 100mIU/ml FSH+100mIU/ml LH 첨가군 (45.2±15.1)보다 많은 것으로 조사되었다. 난자-난구세포 복합체에서 P450scc와 $P450_{17\alpha}$의 발현은 LH의 첨가농도가 증가함에 따라서 증가하였으며, 그리고 progesterone과 testosterone의 분비도 증가를 하였다. 특히, P450scc와 $P450_{17\alpha}$의 발현 그리고 progesterone과 testosterone의 분비는 100mIU/ml FSH+100mIU/ml LH 첨가군에서 다른 첨가군들에 비하여 유의하게 증가하였다. 따라서, 이러한 결과들은 성선자극호르몬이 preantral follicles의 체외배양을 위해서는 필수적이지만, LH 첨가농도의 증가는 난자의 발생능력을 감소시킨다는 것을 보여주었다. 그리고 이러한 결과에 대한 원인의 하나는 황체화의 지표인 P450scc와 퇴행화의 지표인 $P450_{17\alpha}$ 발현의 증가에 의한 progesterone과 testosterone의 분비증가에 기인한 것으로 추정된다. 결론적으로, 본 연구는 배양액내에 100mIU/ml FSH 혹은 100mIU/ml FSH+10 mIU/ml LH의 첨가가 생쥐 preantral follicles의 체외배양을 위한 적정조건임을 제시하고 있다.

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