韓國家畜繁殖學會誌 24(4) : 347~356 (2000)
Korean J. Animal Reprod.

In Vitro Growth and Development of Mouse Preantral Follicles

Kim, D. H.
Medical Science Institute, Eulji General Hospital

Ⅰ. INTRODUCTION

There are thousands of follicles present in the ovaries of mammals. But, most follicles fail before ovulation, and the largest numbers are lost early in life. At birth, more potential oocytes have degenerated than remain in the ovary; moreover, most of the follicles surviving at birth degenerate even before puberty. Very few, highly selected follicles will reach ovulation. The ovarian follicle management system is therefore highly inefficient and has evolved for intense competition between gamates (Hartshorne, 1997).

Procedures for IVM and IVF of oocytes have progressed enormously over the past decade. *In vitro* maturation of oocytes isolated from antral follicles of ovaries has been throught to be potentially useful of oocytes that are lost owing to atresia of follicles (Eppig et al., 1990). The fully grown mammalian oocytes isolated from antral follicles can mature, be fertilized and developed into live young mice (Schroeder and Eppig, 1984), rat (Vanderhyden and Armstrong, 1989), sheep (Staigmiller and Moor, 1984), cattle (Goto et al., 1988), pig (Mattioli et al., 1989) and human (Cha et al., 1991). However, harvesting of developmentally competent oocytes only from large antral follicles limits the number of available oocytes per animal and thus optimal exploitation of the female genetic material and propagation of valuable animal stocks.

The ovarian preantral follicles are a large and potentially valuable source of oocytes that could be used for clinical, agricultural and zoological purposes. If oocytes are harvested in greater numbers from preantral follicles and grown *in vitro*, this would have various advantages. However, for use of oocytes from preantral follicles, the ability to bring preantral follicular stage with immature oocytes to maturity *in vitro* is prerequisite. Previous studies for culture of preantral follicles have focused primarily on the development and function of follicular somatic cells rather than on oocyte development (Mouse: Boland et al., 1994a,b; Nayudu and Osborn, 1992; Hamster: Roy and Greenwald, 1989; Cow: Figueiredo et al., 1993; Pig: Hirao et al., 1994; Human: Roy and Treacy, 1993). Thus, the maturational or embryonic developmental competence of the oocytes in these studies was not determined at either the beginning or end of the culture periods. Nevertheless, these studies have demonstrated dramatic development of an antrum-like structure in the follicles.

Murine has been used for developing a culture system for preantral follicles and studying growth of follicles and oocytes, since they are small, readily available and grow to preovulatory follicles within a short life-span. Particularly, studies for culture of mouse preantral follicles have demonstrated growth and maturation as well as fertilization and development of oocytes from follicles cultured *in vitro* (Cortvrindt et al., 1996; Eppig and Schroeder, 1989; Spears et al., 1994). Furthermore,

live youngs were obtained by the transfer of embryos derived from *in vitro*-grown oocytes (Eppig and Schroeder, 1989; Kim, 2000; Spears et al., 1994), but these studies showed that mouse oocytes grown, matured and fertilized *in vitro* were low developmental capacity when compared to the *in vivo* counterparts. Thus, further studies are needed to improve the culture system for preantral follicles of mammals.

Ⅱ. ISOLATION OF PREANTRAL FOLLICLES

Enzyme treatment and manual dissection have been used to isolate preantral follicles in ovary of mammals. Preantral follicles are readily dissociated from the ovaries of many small mammals, such as mice, by enzyme digestion, usually collagenase assisted by DNase (Eppig and Schroeder, 1989). However, the enzymatic dissociation of follicles from coarser tissue, such as adult human ovaries, is less easily accomplished. Enzyme treatment isolates large numbers of small follicles that lack theca cells and the basement membrane may be damaged.

Spears et al. (1994) isolated small preantral follicles from mouse ovaries by microdissection using 28 gauge needle. Because of the tough fibrous matrix, isolation of preantral follicles from ovaries of adult livestock animals and human is difficult. With respect to cows, it is easier to make slices of the tissue with a scalpel before attempting manual dissection (Figueiredo et al., 1993). With this mechanical method, primary follicles, and mainly secondary follicles with two granulosa cells layers can be isolated. Mechanical method has the disadvantage that smaller number can be collected; however, enzyme exposure is avoided, ensuring an intact theca layer. The combination of collagenase digestion and mechanical isolation resulted in more follicles than mechanical isolation alone.

Ⅲ. *IN VITRO* CULTURE OF PREANTRAL FOLLICLES

According to the experimental purpose, various culture methods of growing preantral follicles to maturity *in vitro* have been developed, such as agar or collagen gel embedding (Carroll et al., 1991; Roy and Treacy, 1993; Torrance et al., 1989), collagen impregnated membranes (Eppig and Schroeder, 1989), 96-V-well microtitre plates (Hartshorne et al., 1994a,b; Spears et al., 1994) and micro droplets covered with mineral oil (Cortvrindt et al., 1996). Table 1 summarizes the culture methods for preantral follicles according to their isolation methods.

Basic concept of these culture methods is to maintain three-dimentional structure between the oocyte and granulosa cells as a complex. In general culture method, three-dimentional structure between the oocyte and granulosa cells easily collapses and then follicles degenerates. Particularly, preantral follicles isolated by enzyme treatment are more difficult to maintain three-dimensional structure between the oocyte and granulosa cells, because theca cells and basal lamina were partially damaged by enzyme, such as collagenase.

Ⅳ. *IN VITRO* GROWTH AND MATURATION OF MOUSE PREANTRAL FOLLICLES

Mouse preantral follicles were grown in the various media, such as Waymouth (Eppig et al., 1992), αMEM (Cortvrindt et al., 1996; Nayudu and Osborn, 1992), DMEM (Liu et al., 1998), F12-DMEM (Li et al., 1995) and TCM 199 (Christmann et al., 1994; Harada et al., 1997). Kim (2000) reported that αMEM was an optimal culture medium for mouse preantral follicles when compared to other medium. It is known that α

Table 1. Culture methods for preantral follicles

Isolation	Culture method	Uses	Species	References
Enzyme	Gel (collagen, agar)	Follicle growth	Mouse	Carroll et al., 1991
		Endocrine study	Human	Roy and Treacy, 1993
		Transplantation	Cow	Hulshof et al., 1995
			Pig	Hirao et al., 1994
	Collagen coated membrane	Production of large numbers oocytes	Mouse	Eppig et al., 1992
Mechanical	V-shape plate	Follicle metabolism	Mouse	Spears et al., 1994
		Steroidogenesis	Sheep	Cecconi et al., 1999
		Oocyte development	Cow	Gutierrez et al., 2000
	Micro drop	Hormonal influence	Mouse	Cortvrindt et al., 1996
		Oocyte development		

MEM medium includes precursors of DNA and is suitable for rapidly dividing cell types (Hartshorne, 1997).

The medium was usually supplemented with a protein source, and this may affect the growth of preantral follicles (Hulshof et al., 1995). Homologous mouse serum (5%) is suitable, as is serum from hypogonadal mice (Boland et al., 1993). Fetal calf serum and fetal bovine serum were successful in supporting the development of follicle and oocyte maturation of attached cultures (Cortvrindt et al., 1996; Kim, 2000). But, human follicular fluid and bovine or human serum albumin were resulted in poor growth of mouse preantral follicles (Kim, 2000; Nayudu and Osborn, 1992).

It is known that addition of FSH to the 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), promoting the survival follicles. 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., 1994b) by stimulating proliferation of granulosa cells (Kim et al., 1999), increases GVBD and extrusion of the first polar body of the *in vitro* grown oocytes (Cortvrindt et al., 1997; Eppig and Schroeder, 1989). Moreover, FSH stimulates the production of estrogen and lactate by isolated preantral follicles (Boland et al., 1993; Nayudu and Osborn, 1992). LH is also an important factor for the growth of granulosa cells (Yong et al., 1992). Qvist et al. (1990) reported that FSH was effective in producing large antral follicles only when adequate amounts of LH were present. Studies in the culture of preantral follicles have shown that an optimal LH:FSH ratio has an impact on follicle survival and oocyte's meiotic maturation (Cortvrindt et al., 1998). Particularly, the addition of 10 mIU/ml LH and 100 mIU/ml FSH (1:10) was more effective for the survival of follicles and the maturation of oocytes (Kim et al., 1999).

Growth factors play an important role in the *in vitro* growth of preantral follicles. EGF inhibits FSH-mediated LH receptor expression, oestradiol production in granulosa cells and follicular growth (Almahbobi et al., 1995; Hsueh et al., 1984; Mason et al., 1990). However, the addition of EGF in preantral follicle culture is known to stimulate oocyte maturation and promotes development to

blastocyst after fertilization (De La Fuente et al., 1999). It is reported that IGF-1 had a stimulatory effect on follicular growth *in vitro* when administered with FSH (Liu et al., 1998). And, insulin shows frequently beneficial to cultures, increasing uptake of metabolic precursors such as amino acids and glucose (Hurwitz et al., 1987). Moreover, it is known that growth differentiation factor-9 (GDF-9), oocyte-derived factor, is an important factor for the *in vitro* growth and differentiation of early ovarian follicles (Hayashi et al., 1999).

Activin is known to stimulate the differentiation and proliferation of granulosa cells, and promotes the development of mouse and rat preantral follicles cultured *in vitro* (Li et al., 1995; Liu et al., 1999). Particularly, Liu et al. (1998) reported that the combination of FSH and activin acted synergistically on the *in vitro* growth of follicles.

Nuclear maturation inhibiting factors are known to influence the culture of preantral follicles. Hypoxanthine has been shown to stimulate antrum formation (Hartshorne et al., 1994b) and increases the proportion of oocytes able to resume meiosis at the end of preantral follicles culture (Carroll et al., 1991; Eppig and Down, 1987). It is known that the addition of dibutyryl cyclic AMP (dbcAMP) yields oocytes which are larger and more competent to resume meiosis (Carroll et al., 1991) and increases steroidogenesis (Carroll et al., 1991; Hsueh et al., 1984).

Ⅴ. DEVELOPMENT OF THE IN VITRO GROWN OOCYTES

It is reported that oocytes grown and matured *in vitro* reduced the capacity to be fertilized (Spears et al., 1994) and showed the higher rate of abnormal fertilization (Hirao et al., 1990) as compared with oocytes grown and matured *in vivo*. The cause of this phenomenon was probably due to incomplete cytoplasmic maturation of oocytes (Hirao et al., 1990). And, it is assumed that long-term *in vitro* culture results hardening of the zona pellucida, thereby prevents the penetration of the sperm into the oocyte (Kim, 2000).

According to the previous studies about developmental capacity of oocytes grown and matured *in vitro*, less than 50% of fertilized oocytes underwent development to the blastocyst stage (Eppig and Schroeder, 1989; Kim, 2000; Spears et al., 1994). It has been several reports available for the production of live youngs after transfer of embryos derived from the *in vitro*-grown, matured and fertilized mouse oocytes. Eppig and Schroeder (1989) and Kim (2000) reported the production of live youngs from oocytes grown *in vitro* from preantral follicles by oviduct transfer of 2-cell embryos. Spears et al. (1994) showed the production of live youngs from oocytes grown *in vitro* by uterine transfer of blastocysts. However, these results showed that below 5% of the transferred embryos developed to the live youngs.

Ⅵ. COMPARISON OF THE *IN VITRO* GROWN AND *IN VITRO* GROWN OOCYTES

The diameter of oocytes is important index for cytoplasmic maturation and developmental competence of oocytes. In the culture of preantral follicles, it is reported that none of the *in vitro*-grown oocytes grew as large as the oocytes isolated from the antral follicles (Eppig and O'Brien, 1998; Hirao et al., 1990; Kim, 2000). However, studies on nuclear maturation are reported that in cultured mouse ovarian follicles, the chromatin organization in the oocytes was normally changed from NSN (non-surrounded nucleolus), meiotic incompetence type, to SN (surrounded nucleolus) configuration, meiotic competence type (Hartshorne et al. 1994a),

and the transition rates of oocyte chromatin organization from NSN to SN were similar to the *in vivo*-grown oocytes (Kim, 2000).

Oocytes grown and matured *in vitro* showed the lower fertilization (Spears et al., 1994) and the higher abnormal fertilization (Hirao et al., 1990). This phenomenon might be due to incomplete maturation of oocyte cytoplasm and the inappropriate activation with sperm penetration (Hirao et al., 1990). It is already assumed that long-term culture results hardening of the zona pellucida preventing the sperm penetration into the oocyte. This assumption is supported by the observation that the time required for digestion of zona pellucida of oocytes grown and matured *in vitro*, using α-chymotrypsin, was longer than *in vivo*-grown oocytes (Kim, 2000).

It is shown that development of oocytes grown and matured *in vitro* to the blastocyst stage was less than 50% which was significantly lower compared to that of oocytes grown and matured *in vivo* (Eppig et al., 1989; Kim, 2000; Spears et al., 1994). In addition, it is reported that total cell number, which regarded as an important index for developmental capacity of blastocyst, is less in the *in vitro* grown oocytes than *in vivo* counterparts (Kim, 2000).

During oocyte growth, gene expression is highly active in order to accumulate the maternal products that are required, after fertilization, to sustain the first steps of embryonic development (Telford et al., 1990). Kim (2000) was compared the expression of specific genes, such as β-actin, GDF-9 and insulin-like growth factor-Ⅱ (IGF-Ⅱ), in mature oocyte grown *in vitro* and *in vivo*. In the β-actin which is an housekeeping gene and a key component of the cytoskeleton, *in vitro*-grown oocyte was significantly lower when compared to the *in vivo* counterpart. GDF-9 that an important factor for the growth and differentiation of early ovarian follicles was also reduced in the *in vitro* grown oocyte. Meanwhile, IGF-II that stimulates growth and metabolism of early embryos and protects cells from apoptosis was not found in the *in vitro*-grown oocyte.

Ⅶ. *IN VITRO* CULTURE OF PREANTRAL FOLLICLES IN OTHER THAN MOUSE

In culture studies of various species, preantral follicles were isolated with or without enzyme treatment of the ovarian tissue. Enzymatic treatment of preantral follicles was demonstrated for cow (Figueiredo, et al., 1993), sheep (Newton et al., 1999), pig (Hirao et al., 1994) and human (Roy and Treacy, 1993). Human (Abir et al., 1997) and cow (Ralph et al., 1995) preantral follicles were also isolated mechanically without enzymatic treatment.

Roy and Treacy (1993) enzymatically isolated human preantral follicles, cultured them in agar gels. The cultured preantral follicles developed antral stage. In the cow, Gutierrez et al. (2000) used microdissection to isolate preantral follicles and cultured them in micro plate. These follicles reached the antral stage and this occurred between Days 10 and 28 of culture. In the study of pig, Hirao et al. (1994) enzymatically isolated preantral follicles and these follicles were cultured in collagen gels. The cultured follicles reached preovulatory size after 16 days. After aspiration of the oocyte its granulosa cell and further culture, some oocytes reached the metaphase Ⅱ. When oocytes grown and matured *in vitro* were inseminated, sperm penetration was observed, but deconde nsation of the sperm was incomplete. The progress of the culture of preantral follicles in mammals is showed in the Table 2.

Table 2. *In vitro* culture of preantral follicles in mammals

Animal	Isolation	Growth	Antrum	Maturation	Embryo	Birth	References
Baboon	●	●					Fortune et al., 1998
Human	●	●	●				Roy & Treacy, 1993
Cow	●	●	●				Gutierrez et al., 2000
Sheep	●	●	●				Cecconi et al., 1999
Pig	●	●	●	●			Hirao et al., 1994
Rat	●	●	●	●	●		Daniel et al., 1989
Mouse	●	●	●	●	●	●	Eppig & Schroeder, 1989 Kim, 2000

Ⅷ. CONCLUSION

Studies on the culture of mouse preantral follicles have demonstrated growth and maturation as well as fertilization and development of oocytes from follicles cultured *in vitro*. Finally, live youngs were obtained by the transfer of embryos derived from *in vitro*-grown oocytes. However, the *in vitro* production efficiency of embryos from preantral follicles is still very low. Therefore, further studies to improve the culture system for preantral follicles are demanded.

In human and farm animals, such as cow and pig, to grow oocytes from preantral follicles until meiotic competence stage, dramatic improvement of present culture system or development of new culture system are probably needed. The visible progress in the establishment of the *in vitro* culture of preantral follicles of human and farm animals could help to enlarge the populations of valuable agricultural, phamaceutical product-producing and endangered animals, and to rescue the oocytes of women about to undergo clinical procedures that jeopardize oocytes.

Ⅸ. REFERENCES

1. Abir, R., Franks, S., Mobberley, M. A., Moore, P. A., Margara, R. A. and Winston, R. M. L. 1997. Mechanical isolation and *in vitro* growth of preantral and small antral human follicles. Fertil. Steril., 68:682-688.
2. Almahbobi, G., Nagoadavthane, A. and Trounson, A. O. 1995. Effects of epidermal growth factor, transforming growth factor alpha and androstenedione on follicular growth and aromatization in culture. Human Reprod., 10:2767-2772.
3. Boland, N. I. and Gosden, R. G. 1994. Effects of epidermal growth factor on the growth and differentiation of cultured mouse ovarian follicles. J. Reprod. Fertil., 101:369-374.
4. Boland, N. I., Humpherson, P. G., Leese, H. J. and Gosden, R. G. 1993. Pattern of lactate production and steroidogenesis during growth and maturation of mouse ovarian follicles *in vitro*. Biol. Reprod., 48:798-806.
5. Carroll, J., Whittingham, D. G. and Wood, M. J. 1991. Effect of dibutyryl cyclic adenosin monophosphate on granulosa cell proliferation, oocyte growth and meiotic maturation in isolated mouse primary ovarian follicles cultured in collagen gels. J. Reprod. Fertil., 92:197-207.
6. Cecconi, S., Barboni, B., Coccia, M. and Mattioli, M. 1999. *In vitro* development of sheep preantral follicles. Biol. Reprod., 60:594-601.
7. Cha, K. Y., Choi, D. H., Koo, J. J., Han, S. Y.,

Ko, J. J. and Yoon, T. K. 1991. Pregnancy after *in vitro* fertilization of human follicular oocytes collected from nonstimulated cycles, their culture *in vitro* and their transfer in a donor oocyte program. Fertil. Steril., 55:109-113.

8. Christmann, L., Jung, T. and Moor, R. M. 1994. MPF components and meiotic competence in growing pig oocytes. Mol. Reprod. Dev., 38:85-90.
9. Cortvrindt, R., Hu, Y. and Smith, J. 1998. Recombinant luteinizing hormone as a survival and differentiation factor increases oocyte maturation in recombinant follicle stimulating hormone-supplemented mouse preantral follicle culture. Human Reprod., 13:1292-1302.
10. Cortvrindt, R., Smitz, J. and Van Steirteghem, A. C. 1996. *In-vitro* maturation, fertilization and embryo development of immature oocytes from early preantral follcles from prepuberal mice in a simplified culture system. Human Reprod., 11:2656-2666.
11. Cortvrindt, R., Smith, J. and Van Steirteghem, A. C. 1997. Assessment of the need for follicle stimulating hormone in early preantral follicle *in vitro* culture. Human Reprod., 12:759-768.
12. Daniel, S. A., Armstrong, D. T. and Gore-Langton R. E. 1989. Growth and development of rat oocytes *in vitro*. Gamete Research, 24:109-121.
13. De La Fuente, R., O'Brien, M. J. and Eppig, J. J. 1999. Epidermal growth factor enhances preimplantation developmental competence of maturing mouse oocytes. Human Reprod., 14:3060-3068.
14. Eppig, J. J. and Downs, S. M. 1987. The effect of hypoxanthine on mouse oocyte growth and development *in vitro*; maintenance of meiotic arrest and gonadotrophin-induced oocyte maturation. Dev. Biol., 119:313-321.
15. Eppig, J. J. and O'Brien, M. J. 1998. Comparison of preimplantation developmental competence after mouse oocyte growth and development *in vitro* and *in vivo*. Theriogenology, 49:415-422.
16. Eppig, J. J. and Schroeder, A. C. 1989. Capacity of mouse oocytes from preantral follicles to undergo embryogenesis and development to live young after growth, maturation and fertilization *in vitro*. Biol. Reprod., 41:268-276.
17. Eppig, J. J., Schroeder, A. C. van de Sandt, J. J. M., Ziomek, C. A. and Bavister, B. D. 1990. Developmental capacity of mouse oocytes that grow and mature in culture; the effect of modification of the protocol. Theriogenology, 33:89-100.
18. Eppig, J. J., Wigglesworth, K. and O'Brien, M. J. 1992. Comparison of embryonic developmental competence of mouse oocytes grown with and without serum. Mol. Reprod. Dev., 32: 33-40.
19. Figueiredo, J. R., Hulshof, S. C. J., Van den Hurk, R., Bevers, M. M., Nusgens, B. and Beckers, J. F. 1993. Development of a new mechanical method for the isolation of intact preantral follicles from fetal, calf and adult bovine ovaries. Theriogenology, 40:789-799.
20. Fortune, J. E., Kito, S., Wandji, S. and Srsen. A. 1998. Activation of bovine in baboon primordial follicular *in vitro*. Theriogenology, 49:441-449.
21. Goto, K., Kajihara, Y., Kosaka, S., Koba, M., Nakanishi, Y. and Ogawa, K. 1988. Pregnancies after co-culture of cumulus cells with bovine embryos derived from *in-vitro* fertilization of *in-vivo* matured follicular oocytes. J. Reprod. Fertil., 83:753-758.
22. Gutierrez, C. G., Ralph, J. H., Telfer, E. E., Wilmut, I. and Webb, R. 2000. Growth and antrum formation of bovine preantral follicles

in long-term culture *in vitro*. Biol. Reprod., 62:1322-1328.

23. Harada, M., Miyano, T., Matsumura, K., Osaki, S., Miyake, M. and Kato, S. 1997. Bovine oocytes from early antral follicles grow to meiotic competence *in vitro*: effect of FSH and hypoxanthine. Theriogenology, 46:743-755.
24. Hartshorne G. M. 1997. *In vitro* culture of ovarian follicles. Rev. Reprod., 2:94-104.
25. Hartshorne, G. M., Sargent, I. L. and Barlow, D. H. 1994a. Meiotic progression of mouse oocytes throughout follicle growth and ovulation *in vitro*. Human Reprod., 9:352-359.
26. Hartshorne, G. M., Sargent, I. L. and Barlow, D. H. 1994b. Growth rates and antrum formation of mouse ovarian follicles *in vitro* in response to follicle-stimulating hormone, relaxin, cyclic AMP and hypoxanthine. Human Reprod., 9:1003-1012.
27. Hayashi, M., McGee, E. A., Min, G., Klein, C., Rose, U. M., Van Duin, M. and Hsueh, A. J. 1999. Recombinant growth differentiation factor-9 (GDF-9) enhances growth and differentiation of cultured early ovarian follicles. Endocrinology, 140:1236-1244.
28. Hirao, Y., Miyano, T. and Kato, S. 1990. Fertilization of *in vitro* grown mouse oocytes. Theriogenology, 34:1071-1077.
29. Hirao, Y., Nagai, T., Kubo, M., Miyano, T., Miyake, M. and Kato, S. 1994. *In vitro* growth and maturation of pig oocytes. J. Reprod. Fertil., 100:333-339.
30. Hsueh, A. J. W., Adashi, E. Y., Jones, P. B. C. and Welsh, T. H. 1984. Hormonal regulation of the differentiation of cultured ovarian granulosa cells. Endocr. Rev., 5:76-126.
31. Hulshof, S. C. J., Figueiredo, J. R., Beckers, J. F., Bevers, M. M., Van der Donk, J. A. and Van den Hurk, R. 1995. Effects of recombinant human FSH, 17β -oestradiol, and their combination on bovine preantral follicles *in vitro*. Theriogenology, 44:217-226.
32. Hurwitz, A., Laufer, N., Keshet, I., Rabinowitz, R., Lewin, A., Palti, Z. and Schenker., J.G. 1987. The effect of insulin on progesterone production and cellular growth in long-term cultures of human granulosa lutein cells. Fertil. Steril., 48:791-795.
33. Kim, D. H. 2000. Studies on *in vitro* growth and development of mouse preantral follicles. PhD Thesis, Konkuk University.
34. Kim, D. H., Chi, H. J., Kang, H. G., Han, S. W., Lee, H. T., Chung, K. S. and Lee, H. J. 1999. Effects of gonadotrophins on *in vitro* growth and maturation of mouse preantral follicles. Korean J. Animal Reprod., 23:53-61.
35. Li, R. H., Phillips, D. M. and Mather, J. P. 1995. Activin promotes ovarian follicle development *in vitro*. Endocrinology, 136:849-856.
36. Liu, X., Andoh, K., Abe, Y., Kobayashi, J., Yamada, K., Mizunuma, H. and Ibuki, Y. 1999. A comparative study on transforming growth factor-β and activin A for preantral follicles from adult, immature and diethylstilbestrol-primed immature mice. Endocrinology, 140: 2480-2485.
37. Liu, X., Andoh, K., Yokota, H., Kobayashi, J., Abe, Y., Yamada, K., Mizunuma, H. and Ibuki, Y. 1998. Effects of growth hormone, activin and follistatin on the development of preantral follicle from immature female mice. Endocrinology, 139:2342-2347.
38. Mason, H. D., Margara, R., Winston, R. M. L., Beard, R. W., Reed, M. J. and Franks, S. 1990. Inhibition of oestradiol production by epidermal growth factor in human granulosa cells of normal and polycystic ovaries. Clin. Endocrinol., 33:511-517.
39. Mattioli, M., Bcci, M. L., Galeati, G. and Seren, E. 1989. Developmental competence of pig

oocytes matured and fertilized *in vitro*. Theriogenology, 31:1201-1207.

40. Nayudu, P. L. and Osborn, S. M. 1992. Factors influencing the rate of preantral and antral growth of mouse ovarian follicles *in vitro*. J. Reprod. Fertil., 95:349-362.
41. Newton, H., Picton, H. and Gosden, R.G. 1999. *In vitro* growth of oocyte-granulosa cell complexes isolated from cryopreserved ovine tissue. J. Reprod. Fertil., 115:141-150.
42. Qvist, R., Blackwell, L. F., Bourne, H. and Brown, J. B. 1990. Development of mouse ovarian follicles from primary to preovulatory stages *in vitro*. J. Reprod. Fertil., 89:169-180.
43. Ralph, J. H., Wilmut, I. and Telfer, E. E. 1995. *In vitro* growth of bovine preantral follicles and the influence of FSH on follicular and oocyte diameters. J. Reprod. Fertil., 15 (Abstr):6.
44. Roy, S. K. and Greenwald. G. S. 1989. Hormonal requirements for the growth and differentiation of hamster preantral follicles in long-term culture. J. Reprod. Fertil., 87:103-114.
45. Roy, S. K. and Treacy, B. J. 1993. Isolation and long-term culture of human preantral follicles. Fertil. Steril., 1993, 59:783-790.
46. Schroeder, A. C. and Eppig, J. J. 1984. The developmental capacity of mouse oocytes that matured spontaneously *in vitro* is normal. Dev. Biol., 102:493-497.
47. Spears, N., Boland, N. I., Murray, A. A. and Gosden, R.G. 1994. Mouse oocytes derived from *in vitro* grown primary ovarian follicles are fertile. Human Reprod., 9:527-532.
48. Staigmiller, R. B. and Moor, R. M. 1984. Effect of follicle cells on the maturation and developmental competence of ovine oocytes matured outside the follicle. Gamete Research, 9:221-229.
49. Telford, N. A., Watson, A. J. and Schultz, G. A. 1990. Transition from maternal to embryonic control in early mammalian development: A comparison of several species. Mol. Reprod. Dev., 26:90-100.
50. Tilly, J. L. and Tilly, K. I. 1995. Inhibitors of oxidative stress mimic the ability of follicle stimulating hormone to suppress apoptosis in cultured rat ovarian follicles. Endocrinology, 136:242-252.
51. Torrance, C., Telfer, E. and Gosden, R. G. 1989. Quantitative study of the development of isolated mouse pre-antral follicles in collagen gel culture. J. Reprod. Fertil., 87:367-374.
52. Vanderhyden, B. C. and Armstrong, D. T. 1989. Role of cumulus cells and serum in the *in vitro* maturation, fertilization and subsequent development of rat oocytes. Biol. Reprod., 40:720-728.
53. Yong, E. L., Baird, R., Yates, L. E., Jr. Reichert, L. E. and Hillier, S. G. 1992. Hormonal regulation of the growth and steroidogenic function of human granulosa cells. J. Clin. Endocrinol. Metab., 74:842-849.

요 약

생쥐 Preantral Follicles의 체외성장 및 발달

김 동 훈

을지병원 의과학연구소

포유동물의 난소 내에는 많은 수의 primordial follicles과 preantral follicles이 존재하고 있으며, 이것은 수정란을 체외생산하기 위한 잠재적인 난자의 공급원이 될 수 있다. 생쥐 preantral follicles 내에 존재하는 난자의 체외성장과 발달을 위하여 몇몇 배양체계가 개발이 되었으며, 적당한 배양조건에서 감수분열 능력이 없는 preantral follicles 내의 난자가 체외배양을 통하여 난자 직경이 증가하고 완전한 핵성숙을 하는 것으로 나타났다. 또한, 체외성장 및 성숙된 난자로부터 생쥐 산자의 성공적인 생산은 preantral follicles 내의 난자가 체외배양을 통해서도 완전한 발달능력을 얻을 수 있음을 입증하였다. 그렇지만, 생쥐 preantral follicle로부터 수정란의 체외생산능력은 매우 낮은 것으로 보고되고 있다. 한편 사람을 비롯한 돼지, 소와 같은 중·대가축의 경우에는 preantral follicle의 체외배양을 통하여 감수분열 능이 있는 단계의 난자로까지의 발달이 아직 보고가 되지 않고 있다. 따라서 지금까지의 연구결과들을 종합해 볼 때, preantral follicles의 체외배양조건을 개선하거나 새로운 배양체계를 개발에 대한 많은 연구가 요구되고 있다. 사람과 중·대가축의 preantral follicles의 체외배양체계의 확립은 우수한 형질을 가진 동물의 확장, 희귀동물 혹은 멸종위기 동물의 보존에 이용될 수 있을 것이며, 그리고 암치료를 위하여 화학적, 방사선 치료를 받아야 하는 여성에게서 향후에 불임치료를 위한 방법으로 활용될 수 있을 것으로 기대된다.

(접수일자 : 2000. 10. 10. / 채택일자 : 2000. 10. 20.)