Korean J. Zool. 39:257-265, 1996

Development of Steroidogenic Capacity during Follicle Growth in Amphibian Ovarian Follicles

Ryun Sup Ahn, Jaemog Soh, Wook-Bin Im, Chul Ho Ra, and Hyuk Bang Kwon*

Department of Biology and Hormone Research Center, Chonnam National University, Kwangju 500-757, Korea

Previously, we demonstrated that estradiol (E_2) was produced by medium sized follicles of *Rana nigromaculata* and *Rana dybowskii in vitro*. Present experiments were carried out to determine when the growing follicles have obtained the ability to produce E_2. Follicles in different growth stages were isolated and cultured for 6 hours in the presence or absence of frog pituitary homogenates (FPH, 0.1 pituitary/2ml) or various steroid precursors (200 ng/2ml). Levels of progesterone (P_4), 17 -hydroxyprogesterone (17α-OHP), androstenedione (AD), testosterone (T) or E_2 in the medium were measured by RIA. The smallest follicles failed to produce steroids, whereas the smaller follicles produced considerable amounts of steroids (211 pg/follicle), and the medium sized follicles produced a large amounts of steroids (1653 pg/follicle) in response to FPH. Addition of pregnenolone (P_5) resulted in a marked increase in P_4 but not in other steroids by the smallest follicles whereas the treatment resulted in a marked increase in P_4, 17α-OHP, AD, T and E_2 by the smaller and medium follicles. When the amounts of steroids are calculated on the basis of unit surface area (pg/mm^2), the ability of the smallest follicles to produce P_4 from P_5 was similar to those of smaller and medium sized follicles. However, the smallest follicles failed to metabolize P_4 to other steroids whereas the smaller and medium follicles did. Taken together, the data suggest that the smallest follicles do not response to FPH in terms of steroid production but they have capacity to convert P_5 to P_4 and that the smaller follicles have potential to produce E_2 although much less efficient than medium sized follicles.

KEY WORDS: Amphibians, Frog, Ovarian Follicles, Steroidogenesis

It has been well established that oocyte growth and maturation in amphibians are regulated by steroids produced by somatic components of the follicles in response to gonadotropin (Schuetz, 1967; Masui and Clarke, 1979; Lin and Schuetz, 1985). Among the steroids produced, estradiol (E_2) was known to trigger oocyte growth by stimulating synthesis of yolk protein by liver (Wallace and Bergink, 1974) and progesterone (P_4) was known to induce final oocyte maturation. Thus, two ovarian steroids are physiologically important in the ovarian function of amphibians. Much informations are accumulated about the follicular steroidogenesis in later period of follicle growth. Medium sized follicles were known to produce E_2 while larger follicles produce mainly testosterone (T) and the largest fully grown follicles produce progestins in *Xenopus* (Fortune, 1983), fishes (Nagahama *et al.*, 1986, 1987), and chickens (Bahr *et al.*, 1983; Huang *et al.*, 1979). Likewise, we also found that only medium sized follicles (Class III) produced E_2 while larger follicles

*To whom correspondence should be addressed.

(Class IV, or V) did not in *R. nigromaculata*. These facts suggest that fast growing (medium sized) follicles have capacity to produce E_2 and lose the capacity when they have grown to larger follicles. However, there are few informations about the steroidogenesis during the early stage of follicular development and it is not known when the small growing follicles have capacity to produce E_2 as well as other steroids. Present experiments were carried out to determine when the small follicles obtain steroidogenic capacity to produce various steroids and to get the responsiveness to FPH in terms of steroid production during follicle development.

Materials and Methods

Animals

Frogs (*R. nigromaculata*) were collected from fields in Chonnam area at summer (May - August). Animals were kept in plastic boxes containing tap water and maintained at room temperature and were used for experiments within 3 days of collection as described previously (Kwon *et al.*, 1991).

Classification of the follicles

Isolated follicles from frog ovaries were sorted into different Classes of follicles by size and pigmentation as described previously (Kwon *et al.*,1991). The smallest Class I follicles (0.22-0.40 mm) with transparent oocytes were collected at May, the smaller Class II follicles (0.35-0.70 mm) with yellow colored-oocyte at May or June, and the medium sized Class III follicles (0.70-1.11 mm) with partially pigmented-oocyte at August.

Culture of follicles

Follicles at different growth stages were isolated manually and cultured for 6 hours in amphibian Ringer (AR) in the presence or absence of FPH (0.1 pituitary/2 ml) or various steroid precursors (P_5, P_4, 17α-OHP, AD or T, 200 ng/2 ml, each). After culture, the media were saved in the deep freezer for later assay. FPH was prepared from female frogs which were collected at corresponding experimental periods (Class I in May ; Class II in May or June; Class III in August). Pituitary glands were homogenized in AR at 4°C using a glass homogenizer. Homogenate was centrifuged (15,000 × g, 20 min, 4°C) to remove debris, and the supernatant was frozen (–20°C) in aliquots until needed. All steroids were purchased from Sigma Chemical Co. (St. Louis, MO). The duration of follicle culture and the doses of FPH or exogenously added steroids were chosen on the basis of our previous data from several species of *Rana* (Ahn *et al.*, 1993; Kwon *et al.*, 1993 1994). In vitro culture was carried out at room temperature (22 – 24°C) with 50 follicle (Class I and II) or 20 follicles (Class III) in 2 ml of AR per well (24 wells per dish, Nunc, Roskilde, Denmark). Control and experimental group were cultured in triplicate using follicles from each animal and each experiment was replicated with follicles from three frogs. The surface area of growing follicles was calculated from the diameter of the follicles measured under a dissecting microscope. The area of the smallest Class I follicles were about 0.31 mm^2, those of smaller Class II follicles were 1.06 mm^2 and those of medium sized Class III follicles were 2.01 mm^2.

Steroid radioimmunoassay

P_4, 17α-OHP, AD, T and E_2 secreted by the ovarian follicles into the medium during culture were measured by radioimmunoassay(RIA). Medium samples were kept in a deep freezer (–20°C) until needed and assayed directly without further purification. General assay procedures were adapted from those described by Fortune (1983) and utilized in previous studies (Kwon *et al.*, 1989, 1991, 1993). Labeled P_4 ([1,2,6,7-^{3}H]-progesterone; 99 Ci/mmol). 17α-OHP ([1,2,6,7-^{3}H]-hydroxyprogesterone; 58.5 Ci/mmol), T ([1,2,6,7- ^{3}H]-testosterone, 98 Ci/mmol), and E_2 ([2,4,6,7-^{3}H]-estradiol, 108 Ci/mmol) were obtained from Amersham (Buckinghamshire, England). Labeled AD ([1,2,6,7-^{3}H] - androstenedione, 86.1 Ci/mmol) was purchased from New England Nuclear (Boston, MA). The antisera of the steroids were produced and evaluated by Dr. Y. D. Yoon (Hanyang University, Seoul). To validate the RIA procedure for measurement of specific steroid in the presence of

exogenous precursors, 100 ng/ml of various steroid precursors was added to culture medium (AR) and aliquots (100 μl) were analyzed for specific steroid RIA. Results from such analysis thus provided a means of assessing the effects of cross-reactivity and served as an additional experimental control. These non-specific binding values were subtracted from original steroid assay data (Kwon and Ahn, 1994). The P_4 antiserum cross-reacted 14.0% with 5α-dihydroprogesterone, 0.5% with 17α-OHP and 20α-dihydroprogesterone, 0.2% with T, 0.1% with cortisol, and less than 0.01% with other steroids. The 17α-OHP cross-reacted 1.6% with 17α,21-dihydroxyprogesterone, 0.8% with P_4, 0.03% with cortisol, and less than 0.001% with T and E_2. The T antiserum cross-reacted 14.0% with 5α-dihydrotestosterone, 6.0% with 5α-androstenediol, 0.8% with AD, and less than 0.01% with other steroids. The AD antiserum cross-reacted 1.13% with androsterone, 0.5% with T, 0.32% with 5β-dihydrotestosterone, 0.12% with 5α-dihydrotestosterone, and less than 0.01% with other steroids, Each sample was quantified for tritium using a Packard Tri-Carb 1500 liquid scintillation analyzer. Routinely, duplicate steroid standards were included in each assay (P_4, 12.5-2000 pg; AD, 2.5-500pg). Steroid concentrations were calculated on a microcomputer using SecuRIA software (Packard, Downners Grove, IL). The between- and within-assay coefficients of variation (CVs) for P_4 were 9.2 and 8.5%, respectively. The CVs for 17α-OHP were 8.2 and 6.7%, for T, 9.4 and 7.4%, for E_2, 10.1 and 8.7%, and for AD, 8.4 and 7.2%, respectively. the lower limit of assay sensitivity for P_4 was 12.5 pg, and that for 17α-OHP, T, E_2, and AD were 5 pg/follicle.

Statistical analysis

Statistical analysis of data included one or two-way analysis for variance (ANOVA) or T-test.

Results

Steroid production by growing follicles *in vitro* in response to FPH

Follicles in different growing stages (Class I , II and III) were isolated and cultured for 6 hours in the presence or absence of FPH (0.05 pituitary/ml) and after culture, incubation media were collected and used for steroids assay. The levels of steroids such as P_4, 17α-OHP, AD, T and E_2 secreted by each sized follicles were measured and the data (total of steroids measured) were depicted in Figure 1. The total concentrations of steroids calculated on the basis of unit surface area of follicles were also depicted in the same Figure.

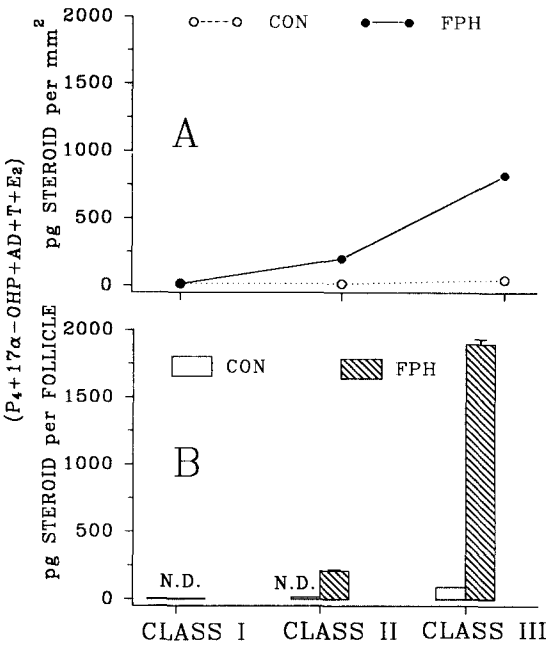

Fig. 1. Steroid production by the smallest (Class I), the smaller (Class II), and the medium (Class III) follicles in response to FPH. The different sized follicles were isolated and cultured for 6 hr in the presence or absence of FPH (0.05 pituitary/ml). After culture, levels of P_4, 17α-OHP, AD, T and E_2 in medium were measured. The amount of steroids was expressed as pg per mm2 of surface area of follicle (A) or pg per follicle (B). Each point in the figure A represents average pg of total steroids/mm^2 of surface area of follicle and bar in the figure B represents average (mean ± SEM) pg of total steroids measured/follicle. (n=9, triplicate incubations, 3 animals).

The smallest Class I follicle produced nearly non-detectable levels of steroids (<10pg/follicle) in response to FPH. The smaller Class II follicles produced low but significant amount of steroids in response to FPH (total amount; 217 pg/follicle, P_4; 21, 17α-OHP; 33, AD; 39, T; 32, E_2; 93 pg/follicle) ($P<0.01$,when compared with Class I follicles). The medium sized Class III follicles produced considerable amounts of steroids in response to FPH (total amount; 1654 pg/follicle, P_4; 487, 17α-OHP; 169, AD; 94, T; 441, E_2; 463 pg/follicle) ($P<0.01$, when compared with Class II follicles). In the absence of FPH, low or non-detectable levels of steroid produced by Class I or II follicles (<20 pg/follicle) whereas low but significant amounts of steroids (total amount; 90 pg/follicle) were produced by Class III follicles ($P<0.01$. when compared with other FPH minus groups).

When the amounts of steroids produced by the growing follicles in response to FPH were calculated on the basis of unit surface area, less than 10 pg/mm^2 were produced by Class I, 100 pg/mm^2 by Class II, and 827 pg/mm^2 by Class III (Fig. 1A).

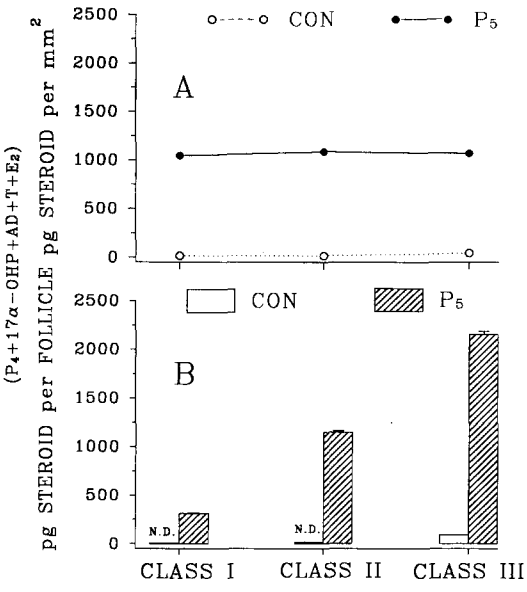

Fig. 2. Conversion of P_5 to other steroids by the different size of follicles. The smallest, smaller and medium follicles were isolated and cultured for 6 hr in the presence of P_5 (100 ng/ml). After culture, P_4, 17α-OHP, AD, T and E_2 in medium were measured. Each point in the figure A represents average pg of total steroids/mm^2 of surface area and bar in B represents average (mean ± SEM) pg of total steroids/follicle (n=9. triplicate incubations, 3 animals).

Conversion of exogenous P_5 to other steroids by follicles of different growing stages

Conversion of exogenously added P_5 to other steroids by different follicles (Class I, II and III) were examined. Exogenous addition of P_5 resulted in a marked increase in P_4 levels by all the three types of follicles (Class I; 301, Class II; 401, Class III; 755 pg/follicle). Interestingly, however, only P_4 was accumulated in medium by Class I follicles whereas all the steroids in delta 4 pathway were accumulated in medium by Class II and III follicles (Fig. 2). The levels of steroids accumulated in medium by Class II were 259 pg/follicle of 17α-OHP, 178 pg/follicle of AD, 103 pg/follicle of T, 217 pg/follicle of E_2 and those by Class III were 311 pg/follicle of 17α-OHP, 216 pg/follicle of AD, 462 pg/follicle of T and 409 pg/follicle of E_2. The total amounts of steroids produced by the follicles were represented in Figure 2A. Interestingly, the total amounts of the steroids produced by those different follicles were not significantly different when the amounts were calculated on the basis of unit surface area ($P>0.05$) (Fig. 2A).

Conversion of exogenous P_4 to other steroids by follicles at different growing stage

Conversion of P_4 to other steroids (17α-OHP, AD, T and E_2) by different Class of follicles was examined (Fig. 3B). Exogenously added P_4 converted to 17α-OHP efficiently by Class II (263.0 pg/follicle) and III (354.0 pg/follicle) follicles but not by Class I follicles (<10 pg/follicle). Likewise, other steroids such as AD, T and E_2 were produced by Class II (AD; 158.0, T; 90, E_2; 224 pg/follicle) and Class III (AD; 249, T; 527, E_2; 493 pg/follicle) follicles in the presence of P_4. It appeared that significantly higher levels of steroids were produced by Class III follicles than that by Class II follicles ($P<0.01$) (Fig. 3B).

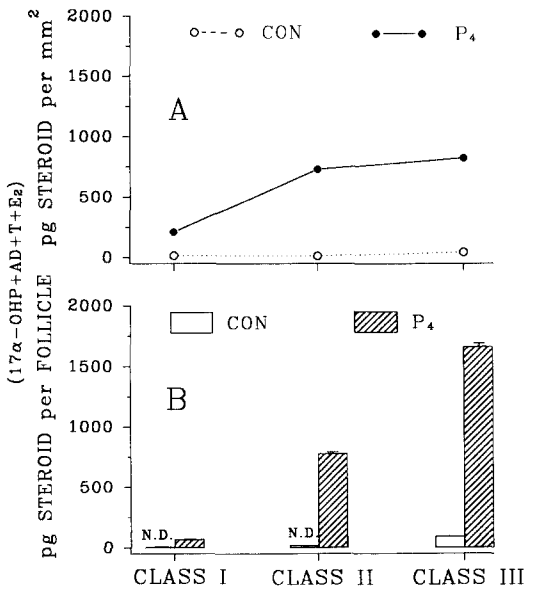

Fig. 3. Conversion of P_4 to other steroids by the different size of follicles. The smallest, smaller and medium follicles were isolated and cultured for 6 hr in the presence of P_4 (100 ng/ml). After culture, 17α-OHP, AD, T and E_2 in medium were measured. Each point in the figure A represents average pg total steroids/mm^2 of surface area of follicle and bar in B represents average (mean ± SEM) pg of total steroids/follicle (n=9, triplicate incubations, 3 animals).

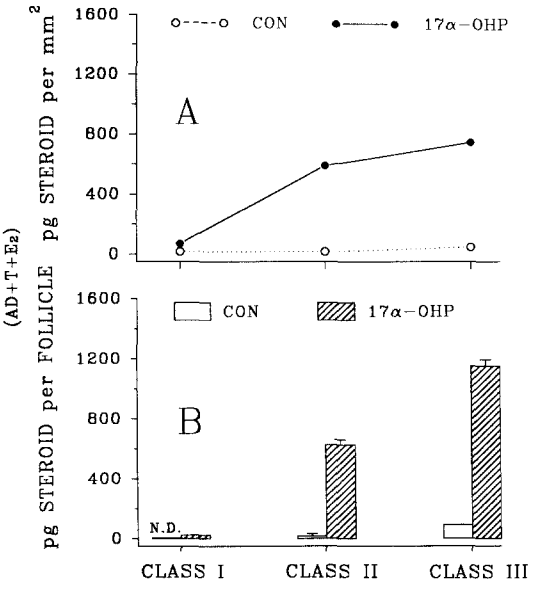

Fig. 4. Conversion of 17α-OHP to other steroids by different size of follicles. The smallest, smaller and medium follicles were isolated and cultured for 6 hr in the presence of 17α-OHP (100 ng/ml). After culture, AD, T and E_2 in medium were measured. Each point in the figure A represents average pg total steroids/mm^2 of surface area of follicle and bar in B represents average (mean ± SEM) pg of total steroids/follicle (n=9, triplicate incubations, 3 animals).

However, the amounts of steroids produced by the unit surface area of the follicles were the same between Class II (693 pg/mm^2) and III (807 pg/mm^2) follicles and the amount was very low in Class I follicles (87 pg/mm^2).

Conversion of exogenous 17α-OHP to other steroids by follicles at different growing stage

Conversion of 17α-OHP to other steroids by the follicles were also examined. Exogenous 17α-OHP converted to AD, T and E_2 efficiently by Class II and III follicles (624 and 1493 pg/follicle, respectively) but not by Class I follicles (22 pg/follicle)(Fig. 4). Although the levels of steroids produced by Class III follicles were much higher than that by Class II follicles in the presence of 17α-OHP (Fig. 4B)($P<0.01$), the amounts of steroids produced by the unit surface area of the follicles were similar between Class II and III follicles ($P>0.05$)(Fig. 4A).

Conversion of exogenous AD to T or E_2 by different stages of follicles

Exogenous AD converted to T or E_2 by Class II and III follicles (811 and 2310 pg/follicle, respectively) but not by Class I follicles (15 pg/follicle)(Fig. 5). T levels produced by Class II and III follicles in the presence of AD were 563 and 1770 pg/follicle, respectively, and E_2 levels were 248 and 628 pg/follicle, respectively. Thus, significantly higher levels of steroids (T plus E_2) were produced by Class III follicles than by Class II follicles in the presence of AD ($P<0.01$)(Fig. 5B). The total amounts of steroids produced by unit surface area of Class III follicles were also higher that by Class II follicles (Fig. 5A) ($P<0.01$).

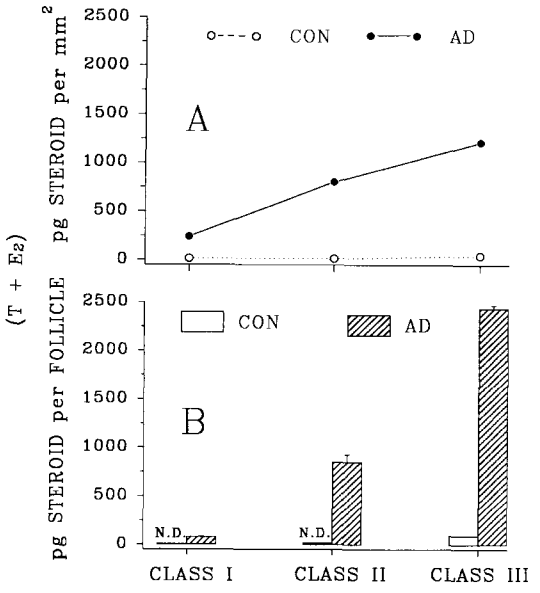

Fig. 5. Conversion of AD to T or E_2 by different size of follicles. The smallest, smaller and medium follicles were isolated and cultured for 6 hr in the presence of AD (100 ng/ml). After culture, T and E_2 in medium were measured. Each point in the figure A represents average pg total steroids/mm^2 of surface area of follicle and bar in B represents average (mean ± SEM) pg of total steroids/follicle (n=9, triplicate incubations, 3 animals).

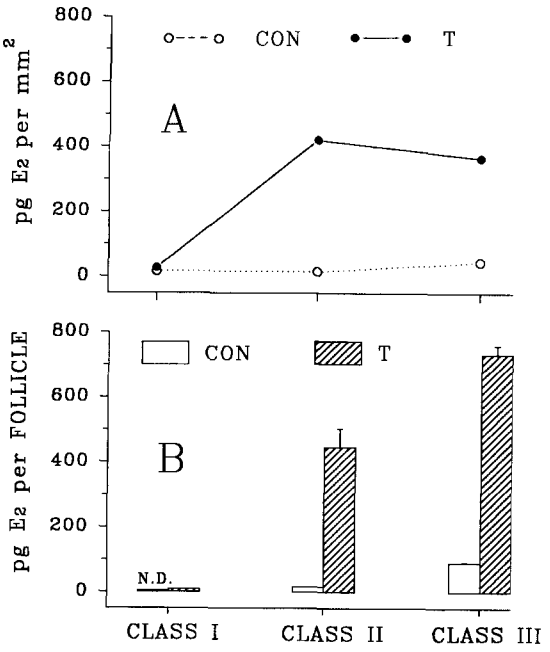

Fig. 6. Conversion of T to E_2 by different size of follicles. The smallest, smaller and medium follicles were isolated and cultured for 6 hr in the presence of T (100 ng/ml). After culture, E_2 in medium were measured. Each point in the figure A represents average pg of E_2/mm^2 of surface area of follicle and bar in B represents average (mean ± SEM) pg of E_2/follicle (n=9, triplicate incubations, 3 animals).

Conversion of exogenous T to E_2 by follicles at different growing stages

Exogenously added T appeared to convert to E_2 efficiently by Class II and III (446, 733 pg/follicle, respectively) but not by Class I follicles (9 pg/follicle) (Fig. 6B). The levels of E_2 produced by Class III follicles were higher than that by Class II follicles in the presence of T, but interestingly, the amount of E_2 produced by unit surface area of Class II were higher than that by Class III ($P<0.05$) (Fig. 6A).

Discussion

The data presented here demonstrate that the smallest Class I follicles of *Rana nigromaculata* ovary have the capacity to produce P_4 in the presence of P_5 but are not able to metabolize P_4 further to other steroids, while the smaller Class II follicles have the capacity to produce E_2, the end product of steroidogenic delta 4 pathway in the presence of P_5 *in vitro*. Thus it is evident that the smallest follicles have the enzyme 3β-hydroxysteroid dehydrogenase (3β-HSD) which is responsible for conversion of P_5 to P_4 and the smaller follicles already have developed all the enzymes responsible for production of E_2 from P_5 in *Rana* ovary.

Previously, we have shown that only the medium sized Class III follicles can produce E_2, but the larger Class IV or Class V follicles can not produce the steroid even in the presence of its immediate precursor T, or in the presence of FPH stimulation (Ahn *et al.*, 1993; Kwon *et al.*, 1993). The data presented here further demonstrated that the smaller Class II follicles have potential to produce E_2 although the smaller follicles produce much less amount of E_2 than the medium sized follicles in response to FPH (Fig. 1). Thus it is

clear that the medium sized follicles have the maximum potential to produce E_2 and they lose the ability rapidly when they have grown larger. The smallest follicles appeared to have capacity to produce P_4 in the presence of its precursor, P_5 very efficiently (Fig. 2) and this data are in accord with those obtained from ovarian follicles of *Rana pipiens* (Petrino and Schuetz, 1986). However, since the smallest follicles can not convert P_4 to other steroids, it seems clear that they have not developed other steroidogenic enzymes in the delta 4 pathway yet. Considering the fact that E_2 is necessary for oocyte growing (Wallace, 1985), it is highly probable that E_2 is responsible for the initiation of oocyte growth in early stage of follicle development and be less important for growing in later stages.

As the size of follicles are different according to the different growing stages, it is not appropriate to evaluate the steroidogenic capacity of individual follicle cells (granulosa) on the basis of steroidogenic capacity of a follicle. If it takes for granted that the absolute size of granulosa cells enclosing the follicles are the same among different follicles, the number of granulosa cells will be proportional to the surface area of the follicle (Petrino and Schuetz, 1986). Thus it seems reasonable to present the amount of steroid on the basis of unit surface area when the activity of granulosa cells are considered. In that sense, it is interesting that activities of some enzymes in granulosa cells do not increase during early growing period. Even the granulosa cells in the smallest follicles have the same capacity to convert P_5 to P_4 as those of granulosa cells in medium sized follicles (Fig. 2). Similarly, granulosa cells in the smaller follicles can convert various precursors to product steroids as efficiently as those in the medium sized follicles (Fig, 2, 3, 4, and 6) except the conversion of AD to T or E_2 (Fig. 5).

Interestingly, although the smaller class II follicles have ability to produce E_2 like the medium sized follicles, they produced much less amount of E_2 than that produced by medium sized follicles in response to FPH (Fig. 1.). Similar phenomenon was observed in *Xenopus* (Fortune, 1983) and in chicken (Nakamura *et al.*, 1991; Nitta *et al.*, 1991). Possibly, the smaller follicles have limited precursors available for production of product steroids or their receptors for gonadotropins are not yet fully active as proposed by other investigators with different animals (Hillier *et al.*, 1978; Kikuchi and Ishii, 1992) Some enzymatic reaction step prior to the formation of P_5 may be involved in FPH-induced steroidogenesis and this step is not ready in the smaller follicles as proposed by Petrino and Schuetz (1986). Further studies are required to answer this critical questions.

In summary, we firstly demonstrated that the smaller Class II follicles have ability to produced E_2 in the presence of precursor steroids while the smallest follicles have ability to produce P_4 from P_5 in *Rana* ovarian follicles *in vitro*. Thus it is evident that most of the enzymes in the delta 4 pathway in ovarian follicles are developed in the early period of follicular development in *Rana*.

Acknowledgements

The present studies were partially supported by the Basic Science Research Institute Program, Ministry of Education, Project No. BSRI-94-4425 and by KOSEF through Hormone Research Center (HRC-95-0103).

References

Ahn, R.S., S.K. Ko., D.G. Bae, Y.D. Yoon, and H.B. Kwon, 1993. Steroidogenic shift by cultured ovarian follicles of Rana dybowskii at breeding season. *J. Exp. Zool.* **267:** 265-282.

Bahr, J. M., S.-C. Wang, M.Y. Huang, and F.O. Calvo, 1983. Steroid concentration in isolated theca and granulosa layer of preovulatory follicles during the ovulatory cycle of domestic hen. *Biol. Reprod.* **29:** 326-334.

Fortune, J.E., 1983. Steroid production by *Xenopus* ovarian follicles at different developmental stages. *Dev. Biol.* **99:** 502-509.

Huang, E.S.-R., K.J. Kao, and A.V. Nalbandov, 1979. Synthesis of sex steroids by cellular components of chicken follicles. *Biol. Reprod.* **20:** 454-461.

Hillier, S.G., A.J. Zeleznik, and G.T. Ross, 1978. Independence of steroidogenic capacity and luteinizing hormone receptor induction in developing granulosa

cells. *Endocrinology* **102:** 937-946.

Kikuchi, M. and S. Ishii, 1992. Changes in luteinizing hormone receptors in the granulosa and theca layer of the ovarian follicle during follicular maturation in the Japanese quail. *Gen. Comp. Endocrinol.* **85:** 124-137.

Kwon, H.B., H.H. Choi, R.S. Ahn, and Y.D. Yoon, 1991. Steroid production by amphibian (*Rana nigromaculata*) ovarian follicles at different developmental stages. *J. Exp. Zool.* **260:** 66-73.

Kwon, H.B., R.S. Ahn, W. K. Lee, W.B. Im, C.C. Lee, and K. Kim, 1993. Changes in the activities of steroidogenic enzymes during the development of ovarian follicles in *Rana nigromaculata*. *Gen. Comp. Endocrinol.* **92:** 225-232.

Kwon, H.B. and R.S. Ahn, 1994. Relative roles of theca and granulosa cells in ovarian follicular Steroidogenesis in the amphibian, *Rana nigromaculata*. *Gen. Comp. Endocrinol.* **94:** 207-214.

Lin, Y-W.P. and A.W. Schuetz, 1985. Spontaneous oocyte maturation in *Rana pipiens* oocyte: estrogen and follicle wall involvement. *Gamete Res.* **12:** 11-28.

Masui, Y. and H.J. Clarke, 1979. Oocyte maturation. *Intl. Rev. Cytol.* **57:** 186-281.

Nagahama, Y., 1987. Gonadotropin action on gametogenesis and Steroidogenesis in teleost gonads. *Zool. Sci.* **4:** 209-222.

Nagahama, Y., F.W. Goetz, and J.D. Tan, 1986. Shift in the ovarian follicles of the goldfish (*Carassius auratus*) during gonadotropin-induced oocyte maturation. *Dev. Growth Differ.* **28:** 555-561.

Nakamura, T., M. Funabashi, and Y. Tanabe, 1991. *In vitro* studies on the Steroidogenesis in the presence of pregnenolone as precursors by the follicular tissue of the domestic fowl (*Gallus domesticus*). *J. Steroid Biochem. Mol. Biol.* **38:** 105-110.

Nitta, H., Y. Osawa, and J.M. Bahr, 1991. Multiple steroidogenic cell population in the theca layer of preovulatory follicles of the chicken ovary. *Endocrinology* **129:** 2033-2040.

Petrino, T. and A.W. Schuetz, 1986. Protein synthesis and Steroidogenesis in amphibian (*Rana pipiens*) ovarian follicles; Studies on the conversion of pregnenolone to progesterone. *Gen. Comp. Endocrinol.* **63:** 411-450.

Schuetz, A.W., 1967. Effect of steroids on the germinal vesicle of oocytes of frog (*Rana pipiens*) *in vitro*. *Proc. Soc. Exp. Biol. Med.* **124:** 1307-1310.

Wallace, R.A., 1985. Vitellogenesis and Oocyte Growth in Nonmammalian Vertebrate, In: Developmental Biology (Browder, L., ed.). Plenum Press, New York, Vol. 1, pp.127-177.

Wallace, R.A. and G.W. Bergink, 1974. Amphibian vitellogenin: Properties, hormonal regulation of hepatic synthesis and ovarian uptake, and conversion to yolk protein. *Am. Zool.* **14:** 1159-1175.

(Accepted June 14, 1996)

성장중인 양서류 여포의 스테로이드 생성능력 획득에 관한 연구

안련섭 · 소재목 · 임욱빈 · 나철호 · 권혁방(전남대학교 자연대 생물학과, 호르몬연구센터)

참개구리를 사용하여 난소내 여포들이 성장시기에 획득하게 되는 스테로이드들의 생성능력을 조사하였다. 개구리로부터 중간(0.7-1.11 mm) 크기 이하의 여포들을 분리한 다음 전구스테로이들이나 뇌하수체추출물(FPH)을 포함한 배양액에서 6시간 배양 후 이들에 의해 산물스테로이드로 전환된 양을 방사면역 측정법으로 조사하였다. FPH의 처리는 중간 크기의 여포만 스테로이드들의 생성을 현저히 촉진하고 작은 여포들(0.35-0.7 mm)에서는 촉진효과가 매우 미약하였다. Pregnenolone의 첨가는 가장 작은 여포(0.22-0.4 mm)들에 의해서도 progesterone(P_4)으로 전환되었다. 그러나 가장 작은 여포에서는 P_4를 첨가했을 때에도 역시 같은 현상이 나타났다. 작은 여포와 중간 여포들에 의해 전환된 스테로이드들의 양을 단위표면적의 전환량으로 계산 비교해 본 결과 전환된 양은 거의 같았다. 이는 작은 여포의 여포세포들(granulosa cells)이 이미 E_2에 이르는 모든 중간 산물들을 생성할 능력을 가졌다는 것을 의미한다. 그러나 이들이 FPH에 의해서는 스테로이드를 조금밖에 생성하지 못하는 것으로 보아 아직 부족한 또 다른 요인들이 있는 것으로 추정된다.