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STEROIDOGENESIS IN THECA CELLS OF CHICKEN FOLLICLES

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ABSTRACT

The aim of this study was to assess and compare the steroidogenic response to luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in cells of the theca interna and theca externa isolated from follicles at different stages of maturation. Cells of the theca interna and theca externa isolated from the largest, second largest and third-fifth largest preovulatory follicles of the chicken (*Gallus domesticus*) were incubated at the concentration of 2×10^5 cells/ml/tube in Medium 199 containing 10mM Hepes and 0.2% bovine serum albumin with increasing doses (0, 0.1, 1.0, 10 and 100 ng/ml/tube) of ovine LH (oLH) or ovine FSH (oFSH). After the 4 hr-incubation, progesterone (P_4), testosterone (T), and estradiol-17 β (E_2) in the medium were measured by RIA. P_4 and T were detected in cultured medium of theca interna cells but were undetectable (< 25 pg/tube) in that of theca externa cells, while E_2 was detected in cultured medium of theca externa cells but not (< 25 pg/tube) in that of theca interna cells. The productions of P_4 and T were stimulated in a dose dependent manner by oLH and oFSH. The responses to oLH and oFSH were greater in smaller follicles, although the response to oFSH was less than that to oLH. E_2 production was stimulated in a dose-dependent manner by oFSH with greater response in smaller follicles but not by oLH. The present results support a three-cell theory for estrogen production in avian follicles and indicate that, in the chicken, the smaller follicles may play a conspicuous role in gonadotropin-induced steroidogenesis.

KEYWORDS

Chicken, gonadotropin, steroidogenesis, theca interna and externa cells.

RESUMEN

El propósito de este estudio fue de ensayar y comparar la respuesta esteroideogénica a la hormona luteinizante (LH) y la hormona del folículo estimulante en las células de la teca interna y externa, aisladas desde folículos en diferentes estados de maduración. Las células de la teca interna y externa aisladas de primeros largos y segundo y tercero-quinto largos folículos preovulatorios de la gallina (*Gallus domesticus*), fueron incubados a concentración de 2×10^5 células/ml/tubo, en medio 199 conteniendo 10mM Hepes y 0.2% de suero de albumina de bovino, con incremento de dosis (0, 0.1, 1.0, 10 y 100 ng/ml/tubo), de ovino LH (oLH) o ovinoFSH (oFSH). Después de 4 horas de incubación, progesterona (P_4) Testosterona (T), y estradiol 17β (E_2), fueron medidos por RIA, P_4 y T fueron detectados en el medio de cultivo de la teca interna, pero indetectable (< 25 pg/tubo) en células de teca externa, mientras que E_2 fue detectado en medios de cultivo de células de la teca externa, pero no (< 25 pg/tubo), en células de la teca interna. La producción de P_4 y T fue estimulado en dosis dependiente por oLH y oFSH. La respuesta a oLH y oFSH fue grande en los folículos menores, aunque la respuesta a oFSH fue menor que oLH. La producción de estradiol fue estimulada en dosis dependiente por oFSH, con gran respuesta en pequeños folículos pero no por oLH. El presente resultado soporta la teoría de tres células para la producción de esteroides en folículos de ave e indica que en la gallina, los pequeños folículos juegan un papel conspicuo en que la gonadotropina induce la esteroideogénesis.

PALABRAS CLAVES

Gallina, gonadotropina, esteroideogénesis, células de teca interna y externa.

INTRODUCTION

In avian, the major hormone being secreted from the ovarian follicle are progesterone (P_4), testosterone (T) and estradiol 17β (E_2) (Gilbert, 1977). It has well been established that, with follicular maturation, there is a marked increase in steroidogenic activity of the granulosa cells which produce mainly P_4 whereas steroidogenic activity in the theca cells, which produce T and E_2 , is decreased (Shahabi et al., 1975; Huang & Nalbandov, 1979; Nakamura et al., 1979; Bahr et al., 1983; Marrone & Hartelendy, 1983). A three-cell theory for estrogen production in avian species has recently been proposed (Porter et al., 1989; Pedernera et al., 1989; Nitta et al., 1991; Velazquez et al., 1991; Kato et al., 1995; Caicedo, 1997); this theory is based on the facts that at least two kinds of steroidogenic cells are present in the theca layer of preovulatory follicles, and P_4 produced by granulosa cells is converted by theca interna cells to androgens, which is subsequently metabolized to estrogen by theca externa cells. The production of P_4

in granulosa cells is stimulated by gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). As the follicles mature, the granulosa cells sensitivity to LH is increased while the sensitivity to FSH is decreased; by the last day before ovulation, the steroidogenic response to FSH disappears and the granulosa cells solely under the regulatory influence of LH (Huang et al., 1979; Scanes & Fagioli, 1980; Hammond et al., 1981; Robinson et al., 1988). In contrast to the granulosa cells, the role gonadotropins in the control of steroidogenesis in cells of the theca interna and theca externa of chicken preovulatory follicles has not been clearly defined, although the production of T and E₂ in the theca cells of avian follicles has been shown to be influenced by gonadotropins hormone (Huang & Nalbandov, 1979; Onagbesan & Peddie, 1989; Porter et al., 1989, 1991; Nitta et al., 1991).

The purpose of the present studies was to evaluate and compare the steroidogenic activity in response to gonadotropins hormone, LH and FSH, in cells of the theca interna and theca externa isolated from follicles at different stages of maturation.

MATERIALS AND METHODS

Animals:

White Leghorn hens laying at least four eggs in a sequence were used. Seven to ten hen 1-2 hr after oviposition were killed for each of at least three replications, and the largest (F₁), second largest (F₂) and third-fifth(F₃-F₅) largest preovulatory follicles were removed from the ovary of each hen.

Isolation of theca interna and theca externa cells

After removing the outer fibrous tissues and the separation of the granulosa layer according to the method of Gilbert et al. (1977), the remainder of each follicle (*theca folliculi*) was inverted, and incubated for 30 min in medium 199 containing 10mM Hepes and 0.2% collagenase. The theca interna layer was gently scraped off with a scalpel blade until the color of tissue changed from pink to near white, and returned to Medium 199 containing 10mM Hepes and 0.2% collagenase, then further incubated for 20 min. after the incubation, mechanical dispersion was performed for 5 min with a syringe. The remaining theca externa layer was minced into about 2 mm square

pieces, and incubated for 60 min in Medium 199 containing 10mM Hepes and 0.2% collagenase with mechanical disruption of tissue at 15 min interval with a syringe. The cell suspensions of theca interna and theca externa were filtered through a nylon gauze (60 μ m mesh) and centrifuged at 400 X g for 10 min at 4°C. The cell pellet was resuspended in Medium 199 containing 40% Percoll and centrifuged at 400 X g for 20 min. Top layer containing theca cells was removed and pelleted by centrifugation at 400 X g for 10 min. The cell pellet was suspended in culture medium containing 10mM Hepes and 0.3% bovine serum albumin (BSA) in Medium 199, and centrifugated at 400 X g 10 min. This cell-washing procedure was repeated three times.

Culture of theca cells

After the cell count on a hemocytometer following a trypan blue dye exclusion, living cells of the theca interna and theca externa were diluted to 2×10^5 cells/0.5 ml with culture medium. oLH (NIDDK oLH-S26) and oFSH (NIDDK oFSH-S20) were diluted in culture medium at the concentrations of 0.1, 1, 10 and 100 ng/0.5 ml and added to the cell suspensions. The cells with the incubation volume of 1ml were incubated for 4 hr at 37°C. after the incubation, the cultured medium was stored -20°C until assayed for steroid hormones.

Assays of steroid hormones

P₄, T, and E₂ in the medium were measured in duplicate without extraction by a routine radioimmunoassay. The antiserum of P₄, T and E₂ were donated by Dr. Wakabayashi (Gunma University, Japan). Radioactive steroids [1, 2, 6, 7 - ³H] P₄, [1, 2, 6, 7 - ³H] T and [2, 4, 6, 7, 16, 17- ³H] E₂, were purchased from Amersham Company. In brief, in glass tubes (10 X 75 mm), 0.1ml of the cultured medium diluted with 1% BSA-0.1M phosphate buffered saline, 0.1 ml of P₄, T or E₂ anti-serum diluted with 1% normal rabbit serum-0.05 M EDTA-0.1M phosphate buffered saline and 0.1 ml of 1% BSA-0.1M phosphate buffered saline containing ³H-P₄, ³H-T or ³H-E₂ of about 20,000-25,000 cpm were added, and incubated for 24 hr at 4°C. For the separation of bound and unbound steroids, 0.2ml of dextran-coated charcoal suspension (6.5g charcoal Nori A and 0.625g dextran T-70 per liter of 0.01M phosphate saline) was added, and the tubes were kept in an ice bath for 30 min. After the centrifugation at 3,000 rpm for 15 min at 4°C, the supernatant was added to a vial containing about 4 ml of ASC-II scintillator and vortexed for 10 sec. On the following

day, the radioactivity was counted for 5 min. The assay sensitivity of P₄, T and E₂ (more than 2 SD difference from zero bound) was 2.5 pg per tube in all steroids. The intra-and inter-assay coefficients of variation of P₄, T and E₂ were 6.0 and 12.5, 10.2 and 15.2 and 8.5 and 11.2% respectively.

Statistical analysis

The data were analyzed by analysis of variance and followed by Duncan's new multiple range test to determine significant difference among treatment means.

RESULTS

In a few cases of the present experiments, small amounts of P₄, T, E₂ were detected in the cultured media of theca externa cell and theca interna cells, respectively. However, in most experiments E₂ was not detected (P<25 pg/tube) in the cultured medium of theca interna cells while P₄ and T were undetectable in the culture medium of theca externa cells. Therefore, because the detections of E₂ in theca interna cells and of P₄ and T in theca externa cells, the data obtained in the experiment which the separation of thecal cells was deemed to be incomplete, were thus eliminated from the present results.

The results of the measurement for P₄, T and E₂ in the cultured medium are shown in Figs. 1, 2, and 3, respectively. As shown in Figs. 1 and 2, P₄ and T were detected in the cultured medium of theca interna cells but below the measurement limit for these hormones (< 25 pg/tube) in the cultured medium of theca externa cells. E₂, however, was detected in the cultured medium of theca externa cells but not in the cultured medium of theca interna cells (Fig.3). Basal productions of P₄ and T by cells of the theca interna and of E₂ by cells of the theca externa, determined in untreated cells during the 4 hr incubation period, were 44.3-64, 232- 450 and 113-151 pg/2X10⁵ cells/4 hr in the cells of F₁, 110-124, 280-326 and 254- 482 pg/2X10⁵ cells / 4hr in the cells of F₂, and 130-136, 222-4429 and 352-483 pg/2 X10⁵ cells/4hr in the cells of F₃₋₅ respectively. The production of P₄ and T by cells of theca interna was stimulated by both oLH and oFSH in a dose-related manner. However, the extent of the stimulation was different not only between the treatments of oLH and oFSH but also among follicles with different stage of development. Thus, the response to oLH in both production of P₄ and T was greater

than the response to oFSH. Moreover, there was a greater response in smaller follicles. The production of E₂ by cells of the theca externa was stimulated only by oFSH in a dose-dependent manner, but not by oLH. Also the E₂ production in response to oFSH was greater in smaller follicles.

DISCUSSION

The present studies, confirm a three-cell theory for estrogen production by preovulatory follicles of the domestic hen in which at least two thecal cells subpopulations are involved in estrogen production in the theca layer; the theca interna cells produce androgens, which are subsequently metabolized to estrogen by theca externa cells (Porter et al., 1989; Pedermera et al., 1989; Nitta et al., 1991; Velazquez et al., 1991; Kato et al., 1995; Rodriguez-Maldonado et al., 1996), because P₄ and T were detected in cells of the theca interna but not in cells of theca externa, while E₂ was detected in cells of the theca externa but not in those of the interna. In addition, the presents study provides a profile of steroidogenesis in response to LH and FSH in cells of the theca interna and theca externa during the development of preovulatory follicles of the chicken.

As has been already known by the presence of cytochrome P₄₅₀ cholesterol side chain cleavage (Kowalsky et al., 1991; Nitta et al., 1991) and 3β-hydroxysteriod dehydrogenase (Nitta et al., 1993) in the theca interna, the present study confirm that P₄ is produced in theca interna cells, although the production of P₄ is very little compared to that in cultures of granulosa cells reported by many investigators (Johnson, 1990). In addition, the present study demonstrated that theca interna cell production of P₄ with T in all follicles examined is stimulated by not only LH but also FSH, although less by FSH than LH. Moreover, the theca interna cell response to these gonadotropins was greater in smaller preovulatory follicles. To the best of our knowledge, only Porter et al. (1989) had examined the effect of both LH and FSH on steroidogenesis by theca interna cells. They reported that in the turkey, the production of P₄ and androgen in theca interna cells was stimulated by oLH in the smaller F₅ but not in the larger F₁ and also not by porcine FSH at the concentration of 1,000 ng/ml in both F₁ and F₅. Although the distinctions between our and their results on the response of theca interna cells to LH and FSH are unclear, they used trypsin while we

used collagenase for the isolation of theca cells. As the damage to cells by enzymatical dispersion is generally known to be greater by trypsin than by collagenase, the difference in enzyme used may explain the discrepancy for the theca interna cell response to gonadotropins between our and their results. The presence of receptors for LH and FSH in avian theca layer has been reported by Kikuchi & Ishii (1992) & Masuda et al. (1984), respectively, who found that these receptors are more numerous in smaller than larger follicles. Therefore, the higher sensitivity of theca interna cells to gonadotropins in smaller preovulatory follicles may be attributed to the greater number of gonadotropin receptors in smaller follicles.

In the present experiments, the production of E₂ in theca externa cells was not stimulated by LH. The present results are in agreement with those of Porter et al. (1989) and Nitta et al. (1991), who also demonstrated that estrogen production is stimulated by the coincubation of theca interna and theca externa cells and enhanced in the presence of oLH. Porter et al. (1989) also reported that E₂ production by theca externa cells of F₅ was not enhanced by oLH in spite of the addition of T (10⁻⁷ or 10⁻⁶ M) as substrate of estrogen, whereas E₂ production by the addition of 10⁻⁶ M of T was greater than with the addition of 10⁻⁷ M of T. The present results, together with all of the above mentioned results, indicate that LH does not stimulate the activity of aromatase which converts androgens into estrogens. However, we found that FSH, with its greater response in smaller follicles, enhances the production of E₂ in cultures of theca externa cells alone despite the undetectable level of T as a precursor of estrogens. Nitta et al. (1991), had reported that fibroblast in the theca externa may function as an additional site of conversion of P₄ to androtenedione as a substrate to produce estrogens. Therefore, the increase in E₂ production by FSH in cultures of theca externa cells alone indicates that FSH may enhance the aromatase activity in cells of the theca externa of the chicken.

As described above, the activity for steroidogenesis in avian theca layer is greater in smaller preovulatory follicles, so the steroidogenic capability in the theca layer is decreased with follicular development. This decrease has been explained by the decrease in the enzyme activity for steroidogenesis (Nakamura et al., 1979; Marrone & Hertelendy, 1985; Mori et al., 1985; Nitta et al., 1993), the responsiveness to LH (Porter et al., 1991), the number of receptors for gonadotropins (Masuda

et al., 1984; Kikuchi & Ishii, 1992), the mRNA levels of cytochrome P450_{17 α} hydroxylase and P450_{aromatase} (Kato et al., 1995) in the theca layer, and by the interaction of the granulosa and theca layers (Huang & Nalbandov, 1979; Marrone & Hertelendy, 1983; Mori et al., 1984; Johnson et al., 1987). Based on the responsiveness of isolated theca interna or theca externa cells to LH and FSH for steroidogenesis, we conclude that the smaller preovulatory follicles exert a profound influence on gonadotropin-induced steroidogenesis in the theca layer of the chicken. Also exert a very important influence in the production of steroid hormone in ovarian development follicles.

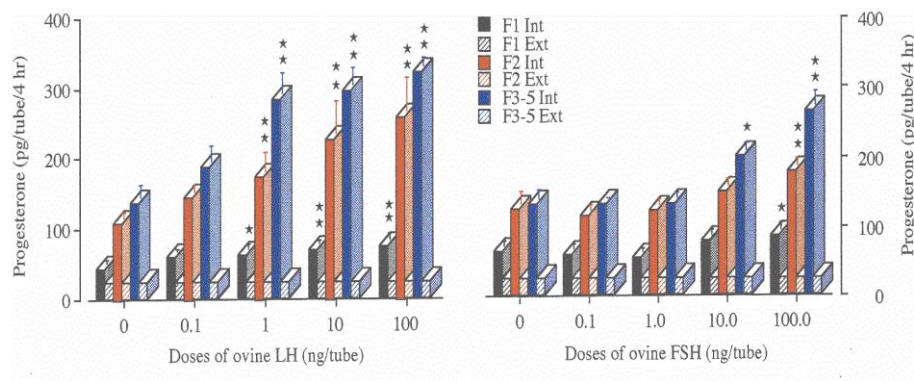


Fig.1. Effect of ovine LH and FSH on progesterone by cells of theca interna and theca externa, isolated from the largest, second largest and third-fifth preovulatory follicles. Cells of theca interna (Int), theca externa (Ext), isolated from the largest (F₁), second largest (F₂) and third-fifth (F₃₋₅) largest preovulatory follicles were incubated for 4 hr with or without increasing of doses of (0.1-100 ng/tube) of ovine LH or FSH. After the incubation, progesterone in the médium was measured. Results are expressed as mean±SEM of at least 3 replicates. Asterisks indicate significantly a difference (*P < 0.05, ** P < 0.01), from basal production (Without ovine LH and FSH) of each follicle.

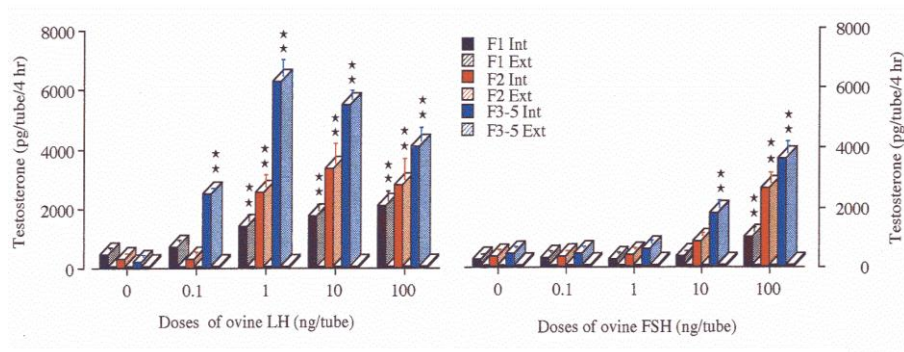


Fig. 2. Effect of ovine LH and FSH on testosterone by cells of theca interna and theca externa from the largest, second largest and third-fifth preovulatory follicles. Cells of theca interna (Int), theca externa (Ext) from the largest (F_1), second largest (F_2) and third-fifth (F_{3-5}) largest preovulatory follicles were incubated for 4 hr with or without increasing of doses of (0.1-100 ng/tube) of ovine LH or FSH. After the incubation, testosterone in the medium was measured. Results are expressed as mean \pm SEM of at least 3 replicates. Asterisks indicate significantly a difference (* $P < 0.05$, ** $P < 0.01$) from basal production (without ovine LH and FSH) of each follicle.

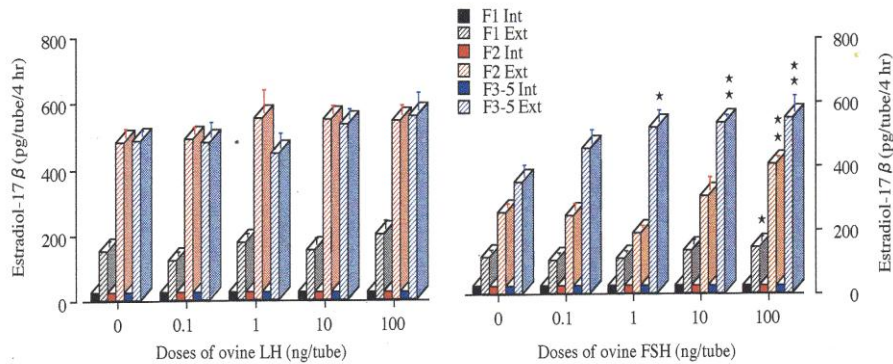


Fig. 3. Effect of ovine LH and FSH on estradiol-17 β by cells of theca interna and theca externa isolated from the largest, second largest and third-fifth preovulatory follicles. Cell of theca interna (Int), theca externa (Ext), isolated from the largest (F_1), second largest (F_2) and third-fifth (F_{3-5}) largest preovulatory follicles were incubated for 4 hr with or without increasing of doses of (0.1 -100 ng/tube) of ovine LH or FSH. After the incubation, estradiol-17 β in the medium was measured. Results are expressed as mean \pm SEM of at least 3 replicates. Asterisks indicate significantly a difference (* $P < 0.05$, ** $P < 0.01$) from basal production (withouth ovine LH and FSH) of each follicle.

REFERENCES

- Bahr, J.M., S.C. Wang, M.Y.Huang & F.O. Calvo. 1983. Steroid concentration in isolated theca and granulosa layers of preovulatory follicles during the ovulatory cycle of domestic hen. *Biology of Reproduction*, 29: 326-334.
- Caicedo, R.E. 1997. Interection of Gonadotropins and steroids hormone in provulatory follicles in domestic hen. Doctoral Thesis. Gifu University. Japan.
- Gilbert, A.B. 1977. The endocrine ovary in the production, In "Physiology and Biochemestry of the Domestic Fowl (Bell, D.J., & Freedman, B.B. eds)", Vol. 3, pp 1449-1468. Academic Press Incorporation, London.
- Gilbert, A.B., A.J. Evans, M.M. Perry & M.H. Davidson. 1977. A Method for separating the granulosa cells, the basal lamina and the theca of the preovulatory ovarian follicles of the domestic fowl (*Gallus domesticus*). *Journal of Reproduction Fertility*, 50:178-181.
- Hammond, R.W., W.H. Burke & F. Hertelendy.1981. Influence of follicular maturation on progesterone release in chicken granulosa cells in response to turkey and ovine gonadothopins. *Biology of Reproduction*, 24: 1045-1055.
- Huang, E.S.R., K.J. Kao & A.V. Nalbandov.1979. Synthesis of sex steroids by cellular components of chicken follicles. *Biology of Reproduction*, 20:454-461.
- Huang, E.S.R. & A.V. Nalbandov.1979. Steroidogenesis in chicken granulosa and theca cells: *in vitro* incubation system. *Biology of Reproduction*, 20:442-453.
- Johnson, A.L. 1990. Steroidogenesis and actions of steroids in the hen ovary. *Critical Reviews of Poultry Biology*, 2:319-346.
- Johnson, P.A., Stoklosowa & J.M. Bahr. 1987. Interaction of granulosa and theca layers in the control of progesterone secretion in the domestic hen. *Biology of Reproduction*, 37:1149-1155.

Kato, M.K., Shimada, N. Saito, K. Noda & M. Ohta. 1995. Expression of P450 17α hydroxylase and P450 aromatase genes in insolated granulosa theca interna and theca externa layers of chicken ovarian follicles during follicular growth. *Biology of Reproduction*, 52: 405-410.

Kikuchi, M. & S. Ishii. 1992. Changes in luteinizing hormone receptors in the granulosa and theca layers of the ovarian follicle during follicular maturation in the Japanese quail. *General and comparative endocrinology*, 85: 124-137.

Kowalsky, K. I., J. Tilly & A. L. Johnson. 1991. Cytochrome P450 side-chain cleavage (P450SCC) in the hen ovary. I. Regulation of P450SCC messenger RNA levels and steroidogenesis in theca cells of developing follicles. *Biology of Reproduction*, 45:955-966.

Marrone, B.L. & F. Hertelendy. 1983. Steroidogenesis by avian ovarian cells: effects of luteinizing hormone and substrate availability, *American Journal of Physiology*, 244:E487-E493.

Marrone, B.L. & F. Hertelendy. 1985. Decreased androstenedione production with increased follicular maturation in theca cells from the domestic hen (*Gallus domesticus*). *Journal of Reproduction and Fertility*, 74: 543-550.

Masuda, S., K. Wakabayashi & O. Koga. 1984. Changes of properties of follicle stimulating hormone like gonadotropin binding sites in the follicles of the hen during follicular growth. *Japanese Journal of Zootechnical Science*, 55:399-408.

Mori, M., K. Kohmoto & Y. Shoda. 1984. Role of granulosa and theca cells on *in vitro* progesterone production in preovulatory follicles of the Japanese quail. *Japanese Poultry Science*, 21: 206-214.

Mori, M., F. Aoki, K. Kohmoto & Y. Shoda. 1985. Metabolism of steroid hormones *in vitro* by follicular tissues of the Japanese quail. *Biology of Reproduction*, 33: 11-20.

Nakamura, T., Y. Tanabe & H. Hirano. 1979. Progesterone and estradiol levels in follicles and *in vitro* metabolism of steroid hormones

by hens during the ovulatory cycle (in Japanese). Japanese poultry Science, 16: 15-25.

Nitta, H., Y. Osawa & J.M. Bahr. 1991. Multiple steroidogenic cell populations in the thecal layer of proevulatory follicles of the Chicken ovary. Endocrinology, 129: 2033-2040.

Nitta, H., J.I. Mason & J.M. Bahr. 1993. Localization of 3 β -hydroxysteroid dehydrogenase in the chicken ovarian follicles shifts from the theca layer to granulosa layer with follicular maturation. Biology of reproduction, 48:110-116.

Onagbesan, O.M. & M.J. Peddie. 1989. Calcium-dependent stimulation of estrogen secretion by FSH from theca cells of the domestic hen (*Gallus domesticus*). General and Comparative Endocrinology, 75: 177-186.

Pedernera, E., P. Velázquez, Y. Gómez & G. del Pliego. 1989. Isolation of steroidogenic cell subpopulations in the follicular theca of the ovary in the domestic fowl. In 'Growth factors among the Ovary (Hirshfield, A.N. ed)' pp. 351-355, Sero Symposium Plenum, New York.

Porter, T.E., B.M. Hargis, J.L. Silsby & M.E. El Halawani. 1989. Differential steroid production between theca interna and theca externa cells: a Three-cell model for follicular steroidogenesis in avian species. Endocrinology, 125: 109-116.

Porter, T.E., B.M. Hargis, J.L. Silsby & M.El Halawani. 1991. Characterization of dissimilar steroid production by granulosa, theca interna and theca externa cells during follicular maturation in the turkey (*Meleagris gallopavo*). General and Comparative Endocrinology, 84: 1-8.

Robinson, F.E., R.J. Etches, L.C. Anderson, W. H. Burke, K.W. Cheng, F.J. Cunningham, S. Ishii, P.J. Sharp & R.T. Talbot. 1988. Steroidogenic relationships of gonadotrophin hormones in the ovary of the hen (*Gallus domesticus*). General and Comparative Endocrinology, 69:455-466.

Rodriguez-Maldonado, E., P.N. Velázquez, N.A. Juárez-Oropeza, & E. Pedernera. 1996. Steroid metabolism in theca externa cells from preovulatory follicles of domestic hen (*Gallus domesticus*). *General and Comparative Endocrinology*, 101: 173-179.

Scanes, C.G., & J.H. Fagioli. 1980. Effects of mammalian and avian gonadotropins on in vitro progesterone production by avian ovarian granulosa cells, *General and Comparative Endocrinology*, 41: 1-7.

Shahabi, N.A., H.W. Norton & A.V. Nalbandov. 1975. Steroid levels in follicles and the plasma of hens during the ovulatory cycle. *Endocrinology*, 96: 962-968.

Velázquez, P., Y. Gómez, G. del Pliego & E. Pedernera. 1991. Steroidogenic cells subpopulations obtained from the theca of preovulatory follicles in the ovary of the domestic fowl. *General and Comparative Endocrinology*, 83: 243-248

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