EFFECTS OF GONADOTROPHINS ON FOLLICULAR STEROID HORMONE PRODUCTION IN HYPOPHYSECTOMIZED HENS (*Gallus domesticus*)

Z. D. Li[1], O. Koga[2], K. Tanaka and N. Fujihara[3]

Department of Animal Sciences, College of Agriculture
Kyushu University, Hakozaki, Fukuoka 812, Japan

Summary

We assessed effects of ovine luteinizing hormone (oLH) and follicle-stimulating hormone (oFSH) on the granulosa and theca layers from the four largest follicles, F_1-F_4 of hens which had been hypophysectomized 12 h before expected ovulation. Ovine LH (0.4 mg), oFSH (0.4 mg) or oLH in combination with oFSH (0.4 mg each) was injected intravenously 6 h after hypophysectomy. Progesterone, testosterone and estradiol-17 β levels of the granulosa and theca layers which were removed 6 h after hormone injection, were measured by radioimmunoassay. Progesterone contents of F_1-F_4 granulosa layer at 12 h after hypophysectomy were much lower than those of control hens. This reduced progesterone level was restored partially by the injection of oLH alone for F_1, while no follicles responded to oFSH treatment. In contrast, the injection of oLH in combination with oFSH resulted in high progesterone content of the granulosa layer from all four follicles. Progesterone content of the theca layer was negligible in all treatments. Simultaneous injection of oLH and oFSH also elevated estradiol-17 β level accumulating in the theca layer from all follicles, of which much higher concentrations of estradiol-17 β were observed when comparison were made to each of their corresponding controls. No appreciable change in testosterone contents of two layers was observed in the present experiments. These results suggest that oFSH augments function of oLH to stimulate the production of progesterone in the granulosa layer and estradiol-17 β in the theca layer.
(Key Words: Hens, Follicles, GTH, Steroid Hormones, Hypophysectomy)

Introduction

In the domestic fowl, high plasma concentrations of plasma progesterone, testosterone, and estradiol-17 β occur in coincidence with increased concentrations of these gonadal steroids in the ovary, and progesterone level rises only in the largest follicle and estrogen in the smallest one (Shahabi et al., 1975). Preovulatory surge of LH in plasma of laying hens is associated with increased plasma concentration of steroid hormones, being possibly due to a positive feedback action of LH (Furr et al., 1973; Senior and Cunningham, 1974; Johnson and van Tienhoven, 1984). Exogenous progesterone or testosterone, but not estrogen, has been reported to cause increased secretion of LH from the pituitary (Wilson and Sharp, 1975, 1976a; Etches and Cunningham, 1976). Extensive studies on the role of these steroids revealed that testosterone may not participate in preovulatory surge of LH (Wilson and Sharp, 1976b; Luck, 1982). On the other hand, Wilson and Sharp (1976b) suggested that, in the hen, the release of LH is facilitated by positive feedback effect of a combination of estrogen and progesterone. Thus, it is still not clear whether any particular steroid has a temporal relationship with preovulatory LH release mechanism and whether FSH participates in this event.

The present study was conducted to clarify effects of GTH on the production of gonadal steroid hormones in hypophysectomized hens.

[1]Present address : Laboratory of Animal Biochemistry, College of Biological Science, Beijing Agricultural University, Beijing, China.

[2]Present address : Department of Animal Sciences, Kyushu Tokai University, Choyo, Aso, Kumamoto 869-14, Japan.

[3]Address reprint requests to Dr. N. Fujihara, Department of Animal Sciences, College of Agriculture, Kyushu University 46-06, Hakozaki, Fukuoka 812 Japan.

Received March 24, 1993
Accepted June 10, 1993

Materials and Methods

Birds

Birds from commercial hybrid egg-laying stock (Shaver) were used throughout the present study. They were maintained in individual laying cages fitted with automatic oviposition recorders and were exposed to a 14 h-photoperiod (lights on from 05:00-19:00 h). Feed and water were available at all times. All hens selected for these studies were 9 months old and producing more than 5 eggs with a pause of only a single day between clutches.

Experimental procedures

Hens were anesthetized by an intravenous injection of Nembutal (40 mg/hen; Abbot Laboratories, U.S.A.) 12 h before expected ovulation and anchored on a stereotaxic instrument, followed by removal of the anterior pituitary (transbuccal approach) according to the method of Tanaka and Nobukuni (1977). Sham operation was done to each hens of the control group by drilling the palate until the tip of burr reaches just anterior to dural sheath of the pituitary. A total of 50 birds were hypophysectomized.

Six hours after hypophysectomy, each hens were injected intravenously with 0.4 mg of oLH, 0.4 mg of oFSH or a combination of the two hormones (0.4 mg each). Treated hens were killed by cutting the jugular vein 6 h following administration of GTH. Ovine LH (NIAMDD-oLH-22, 2.3 μ/mg) and oFSH (NIAMDD-oFSH, 15 μ/mg) were generously provided by the National Pituitary Agency. The four largest follicles (F_1- F_4) of ovarian hierarchy were cut from their respective stalks and placed immediately into a 0.15 M-NaCl solution which had been cooled previously with the ice water. The granulosa and theca layers were isolated following Gilbert et al. (1977) and homogenized separately using a glass homogenizer placed in ice. Homogenates were stored at −20℃ until use.

Granulosa and theca homogenates from each follicle were mixed respectively with ethyl ether (3:1 V/V) and allowed to stand for a few minutes. Ether phase was set aside and water phase re-extracted with ethyl ether. This extraction procedure was repeated 10 times. Pooled extracts were evaporated to dryness in a water bath at 40℃.

Radioimmunoassay

For radioimmunoassay, each dried extract was dissolved in 1 ml of absolute ethanol and two aliquots of 0.4 ml were transferred to a 12 × 73 mm disposable glass tube. Progesterone, testosterone and estradiol-17β were measured according to the method described by Tanaka et al. (1986) except that different antisera were used. Cross-reactivity of antisera against various steroid hormones has been summarized in table 1. Minimum detectable levels of progesterone, testosterone and estradiol-17β were 0.75 pg, 1.5 pg and 1.5 pg per ml, respectively (n=3). Intra assay coefficient of variability was 1.55%.

Protein content in homogenized granulosa and theca layers was measured by the Lowry method (Lowry et al., 1951), and the amount of each steroid hormone was expressed as ng or pg per mg protein.

Statistical analysis

All experiments were statistically analysed by using analysis of variance, followed by Newman-Kuels test to assess the significance of difference among the means (Snedecor and Cochran, 1967).

TABLE 1. CROSS-REACTIVITY OF ANTISERA AGAINST VARIOUS HORMONES

Antisera to:	Percent (%) cross-reactivity of hormones								
	Progesterone	Testosterone	Estradiol-17β	Pregnenolone	17α-hydroxyprogesterone	Dehydrotestosterone	Androstenedione	Estrone	Estriol
Progesterone	–	0.02	0.01	0.03	0.08	. .	–	–	–
Testosterone	0.01	–	0.01	0.01	–	11.60	0.70	–	–
Estradiol-17β	0.01	0.06	–	0.01	–	–	–	0.20	0.61

Results

Progesterone concentration of the granulosa layer in controls was 34 ng per mg protein for F_1 follicle. However, it decreased as follicular size decreased, while all of four follicles (F_1-F_4) removed 12 h following hypophysectomy showed markedly low level (figure 1). When oLH alone was given 6 h following hypophysectomy, only F_1 follicle showed progeterone-producing ability at 50 percent (17 ng/mg protein) of controls and no other follicles showed any significant response to oLH treatment. Ovine FSH administration had no effect on four follicles. In contrast, administration of oLH in combination with oFSH after hypophysectomy resulted in a high progesterone level in the granulosa layer from all follicles.

Negligible concentration of progesterone was detected in the theca layer from F_2-F_4 follicles, whereas appreciable amount of progesterone was observed in F_1 follicle when treated with oLH alone or oLH in combination with oFSH following hypophysectomy (figure 1).

Estradiol-17β content of the granulosa layer was much lower than that of the theca layers from any of the follicles. On the other hand, the concentration of estradiol-17β of the theca layer in control hens was lowest (6 pg) for F_1 follicle and highest (25 pg) for F_4 follicle.

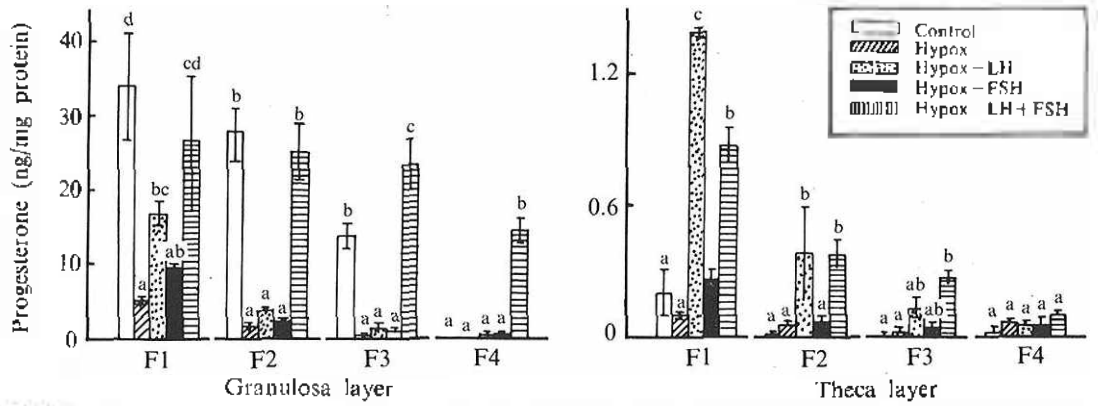

Figure 1. Progesterone concentrations of isolated granulosa and theca layers of the four largest follicles removed 12 h after hypophysectomy. Hormone was given 6 h after hypophysectomy. Each datum point is the mean ± SEM. Values with no common superscripts are significantly different ($p < 0.05$).

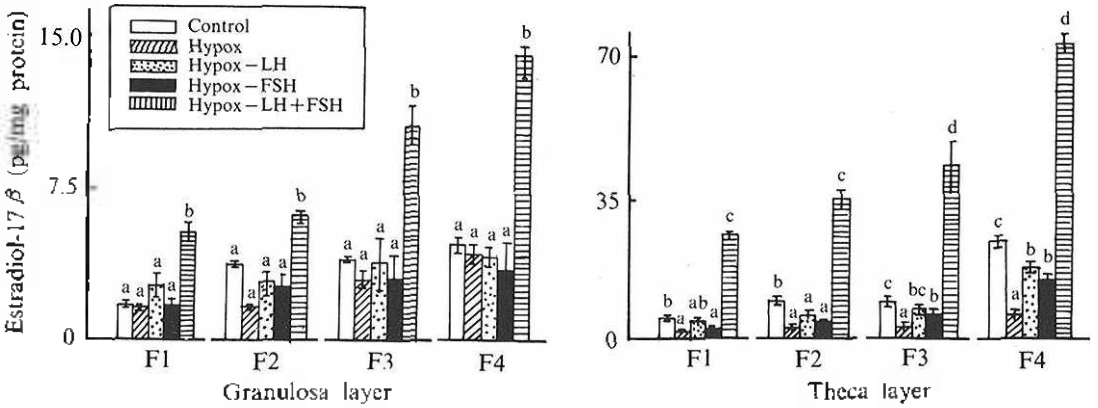

Figure 2. Estradiol-17β concentrations of isolated granulosa and theca layers of the four largest follicles removed 12 h after hypophysectomy. Hormone was given 6 h after hypophysectomy. Each datum point is the mean + SEM. Values with no common superscripts are significantly different ($p < 0.05$).

A low or non-detectable concentration of estradiol-17β was observed in F_1-F_4 granulosa layers regardless of hypophysectomy (figure 2). In the theca layer, the production of estradiol-17β was completely inhibited by hypophysectomy. Administration of oLH or oFSH alone 6 h after hypophysectomy failed to stimulate the production of estradiol-17β for all four types of follicle. In contrast, simultaneous administration of oLH and oFSH led to increased estradiol-17β content in the theca layer and response was increased as follicular size decreased. There was a significant difference in estradiol-17β content in the theca layer between controls and combined administration of oLH and oFSH for all four follicles (figure 2).

On the contrary, there was no difference in testosterone level in the granulosa and theca layers between hens that had with or without the GTH treatment post the hypophysectomy (figure 3).

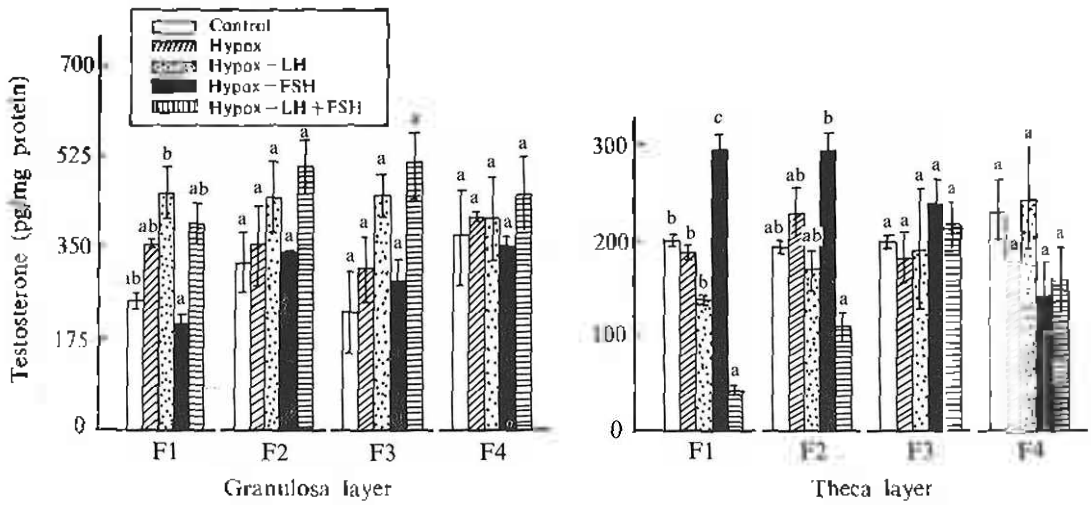

Figure 3. Testosterone concentrations of isolated granulosa and theca layers of the four largest follicles removed 12 h after hypophysectomy. Hormone was given 6 h after hypophysectomy. Each datum point is the mean ± SEM. Values with no common superscripts are significantly different ($p < 0.05$).

Discussion

Hypophysectomized hens were used in the present study, because it has been considered that the elimination of the pituitary factor other than GTH is necessary to clarify effects of exogenous LH and FSH on ovarian steroidgenesis. The choice of time interval of 6 h between hypophysectomy and administration of GTH seemed reasonable as there is evidence that 100 percent of follicles of hypophysectomized hens can be ovulated after 15 h of GTH administration (Opel and Nalbandov, 1961). Hypophysectomy showed an inhibitory effect on progesterone synthesis in the granulosa layer and estradiol-17β in the theca layer. Such lowered progesterone-producing ability was restored by exogenous adminstration of oLH in combination with oFSH for all four follicles, while oLH or oFSH alone were partially effective only for F_1 follicle. Action of oFSH, when combined with oLH, may not be due to additive effect of oLH contamination in oFSH preparation, since a dose of 0.4 mg FSH used in the present experiment contains only about 3 mg activity of NIH-LH-S1. On the other hand, the finding that the theca layer did not respond to FSH agrees with the results of Wells et al. (1985) and Hammond et al. (1981) who used intact hens and isolated granulosa cells, respectively. In view of the fact that FSH augments action of LH to induce ovulation *in vivo* (Kamiyoshi and Tanaka, 1972) and *in vitro* (Tanaka et al., 1987), this facilitated ovulation might be attributed to accelerated synthesis of progesterone. The present results also imply that endogenous FSH participates in preovulatory surge of pro-

gesterone in addition to action of follicular maturation. Estradiol-17 β production by follicles was also abolished by hypophysectomy. However, simultaneous administration of oLH and oFSH resulted in a very siginificant elevation of the estradiol-17 β contained in their theca cells rather than from the control birds, and the response of smaller follicles was much greater than that of the later ones. On the other hand, no appreciable change in testosterone concentrations of either cell layer was observed in hypophysectomized hens with or without GTH treatment. The cause for this phenomenon is not clear at the present time.

Steroid production in theca cells from preovulatory follicles is thought to be primarily under the stimulatory control of LH, because highly purified chicken follicle-stimulating hormone (FSH) and recombinant human FSH stimulate steroid production *in vitro* only at high doses (100 to 200 ng/ml) (Tilly and Johnson, 1989; Kowalski et al., 1991). On the one hand, shortly after a follicle has been selected into the preovulatory hierarchy, the granulosa layer acquires the ability to respond to LH (Tilly et al., 1991) and loses responsiveness to FSH (Ritzhaupt and Bahr, 1987). Subsequent steroid production from granulosa cells of preovulatory follicles appears to be predominantly under the stimulatory control of LH, and results in the formation of progesterone, which is required for initiating or potentiating the preovulatory LH surge or both (Johnson, 1993).

Under the present experimental conditions, it is suggested that FSH augments action of LH to produced progesterone in the granulosa layer and estradiol-17 β in the theca layer. Further work, however, is required to determine the exact mechanism of action of GTH to follicular steroidgenesis in laying hens.

Acknowledgements

We are grateful to Dr. Y. Hasegawa, Medical School, Gunma University, Japan, for the gift of antiserum used in the present study, and the National Pituitary Agency, NIAMDD, for the gift of ovine LH and FSH. We also thank Mr. Y. Tsuzuki for preparing the manuscript. This study was supported by a Grant-in-Aid to K. T. from the Ministry of Education, Science and Culture (61560309).

Literature Cited

Etches, R. J. and F. J. Cunningham. 1976. The interrelationship between progesterone and luteinizing hormone during the ovulation cycle of the hen (*Gallus domesticus*). J. Endocrinol. 71:51-58.

Furr, B. J. A., R. C. Bonney, R. J. England and F. J. Cunningham. 1977. Luteinizing hormone and progesterone in peripheral blood during the ovulatory cycle of the hen *Gallus domesticus*. J. Endocrinol. 57:159-169.

Gilbert, A. B., A. J. Evans, M. M. Perry and M. H. Davidson. 1977. A method for separating the granulosa cells, the basal lamina and the theca of the preovulatory ovarian follicle of the domestic fowl (*Gallus domesticus*). J. Reprod. Fert. 50:179-181.

Hammond, R. W., W. H. Burke and F. Hertelendy. 1981. Influence of follicular maturation on progesterone release in chicken granulosa cells in response to turkey and ovine gonadotrophins. Biol. Reprod. 24:1048-1055.

Johnson, A. L. 1993. Regulation of follicle differentiation by gonadotrophins and growth factors. Poultry Sci. 72:867-873.

Johnson, A. L. and A. van Tienhoven. 1984. Effects of aminoglutethimide on luteinizing hormone and steroid secretion, and ovulation in the hen, *Gallus domesticus*. Endocrinology. 114:2276-2283.

Kamiyoshi, M. and K. Tanaka. 1972. Augmentative effect of FSH on LH-induced ovulation in the hen. J. Reprod. Fert. 29:141-143.

Kowalski, K. L., J. L. Tilly and A. L. Johnson. 1991. Cytochrome P_{450} side chain cleavage (P_{450scc}) in the hen ovary. I. Regulation of P_{450scc} messenger RNA levels and steroidgenesis in theca cells of developing follicles. Biol. Reprod. 45:955-966.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265-275.

Luck, M. R. 1982. Effects of an anti-androgen in the laying hen (*Gallus domesticus*). J. Reprod. Fert. 64:381-385.

Opel, H. and A. V. Nalbandov. 1961. Ovulability of ovarian follicles in the hypophysectomized hen. Endocrinology. 69:1029-1035.

Ritzhaupt, L. and J. M. Bahr. 1987. A decrease in FSH receptors of granulosa cells during follicular maturation in the domestic hen. J. Endocrinol. 115:303-310.

Senior, B. E. and F. J. Cunningham. 1974. Oestradiol and luteinizing hormone during the ovulatory cycle of the hen. J. Endocrinol. 60:201-202.

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

Snedecor, G. and G. W. Cochran. 1967. Statistical

Methods (6th ed.). Iowa State Univ. Press, Iowa.

Tanabe, Y., N. Saito and T. Nakamura. 1986. Ontogenetic steroidogenesis by testes, ovary, and adrenals of embryonic and postembryonic chickens (*Gallus domesticus*). Gen. Comp. Endoc. 63:456-463.

Tanaka, K. and K. Nobukuni. 1977. A stereotaxic device for hypophysectomy of the domestic fowl. Jap. J. Zootech. Sci. 48:532-537.

Tanaka, K., Z. D. Li and Y. Ataka. 1987. Studies of ovulation in the perfused ovary of the fowl (*Gallus domesticus*). J. Reprod. Fert. 80:411-416.

Tilly, J. L. and A. L. Johnson. 1989. Regulation of androstenedione production by adenosine 3', 5' monophosphate and phorbol myristate acetate in ovarian thecal cells of the domestic hen. Endocrinology. 125:1691-1699.

Tilly, J. L., K. I. Kowalski and A. L. Johnson. 1991. Stage of follicular development associated with the initiation of steroidgenic competence in avian granulosa cells. Biol. Reprod. 44:305-314.

Wells, J. W., M. A. Walker, J. Culbert and A. B. Gilbert. 1985. Comparison of the response *in vivo* to luteinizing hormone and follicle stimulating hormone of the granulosa of six follicles from the ovarian hierarchy in the chicken (*Gallus domesticus*). Gen. Comp. Endoc. 59:369-374.

Wilson, S. C. and P. J. Sharp. 1975. Changes in plasma concentrations of luteinizing hormone after injection of progesterone at various times during the ovulatory cycle of the domestic hen (*Gallus domesticus*). J. Endocrinol. 67:59-70.

Wilson, S. C. and P. J. Sharp. 1976a. Effects of androgens, oestrogens and deoxycorticosterone acetate on plasma concentrations of luteinizing hormone in laying hens. J. Endoc. 69:93-102.

Wilson, S. C. and P. J. Sharp. 1976b. Induction of luteinizing hormone release by gonadal steroids in the ovariectomized domestic hen. J. Endoc. 71: 87-98.