Folia biologica (Kraków), vol. **52** (2004), No 3-4

Effect of Prolactin on Estradiol and Progesterone Secretion by Isolated Chicken Ovarian Follicles

Anna HRABIA, Helena PACZOSKA-ELIASIEWICZ and Janusz RZĄSA

Accepted September 6, 2004

HRABIA A., PACZOSKA-ELIASIEWICZ H., RZĄSA J. 2004. Effect of prolactin on estradiol and progesterone secretion by isolated chicken ovarian follicles. Folia biol. (Kraków) **52**: 197-203.

In nonbroody birds, participation of prolactin in the reproductive functions is still unknown and its role in the local regulation of ovarian activity has had little attention. Therefore, the aim of the present study was to determine whether in the domestic hen prolactin influences *in vitro* steroid secretion by white and yellow chicken ovarian follicles. Small white (1-4 mm), medium white (4-6 mm), large white (6-8 mm) and 3 largest yellow preovulatory follicles (F3-F1; F3<F2<F1; 25-36 mm) were isolated at stage 22 h and 3 h before ovulation of the largest (F1) follicle. From the preovulatory follicles, granulosa and theca layers were separated and divided into 4 pieces. Whole white follicles (6 small/dose/ovary; 1 medium or 1 large/dose/ovary) or parts of the granulosa or theca layers were randomly assigned to 1 ml of Eagle's medium containing 0 (control), 1, 10 or 100 ng/ml ovine prolactin and were incubated for 24 h at 38°C. Following incubation, the medium was collected for estradiol and progesterone determination (RIA), and tissues of the follicular wall for protein assay by the method of Lowry. It was found that prolactin affects steroid secretion by chicken ovarian follicles. In white follicles prolactin inhibits estradiol secretion, whereas in yellow preovulatory follicles it stimulates or inhibits steroid secretion and its activity depends on: (1) the dose of prolactin, (2) the type of the follicular layer secreting steroids, (3) the position of the follicle in the hierarchy and 4) the stage of the ovulatory cycle.

Key words: Prolactin, ovary, steroid secretion, chicken.

Anna HRABIA, Helena PACZOSKA-ELIASIEWICZ, Janusz RZĄSA, Department of Animal Physiology, Agricultural University of Cracow, Mickiewicza Al. 24/28, 30-059 Kraków, Poland.
E-mail: rzhrabia@cyf-kr.edu.pl

In the ovary of a laying hen, numerous small white follicles with a diameter 8 mm, five to seven yellow follicles with a diameter 8-36 mm and several postovulatory follicles are present. Yellow follicles are arranged in a hierarchy with the largest preovulatory follicle (F1) destined to ovulate on the next day, the second largest (F2) on the day after, and so on (GILBERT 1971; GRIFFIN *et al*. 1984; JOHNSON 1996).

Unlike mammals, in the chicken ovary the main source of progesterone are cells of the granulosa layer, whereas for estradiol – cells of the theca layer (HUANG *et al*. 1979; BAHR *et al*. 1983; ETCHES & DUKE 1984). Steroidogenic activity of a particular layer changes during follicular growth and maturation. In white, nonhierarchical follicles, the granulosa layer is steroidogenically incompetent (DAVIDSON *et al*. 1979; KACIŃSKA & RZĄSA 1988; NITTA *et al*. 1991; TILLY *et al*. 1991a, b), while steroidogenically active theca synthesises androgens and estrogens (NITTA *et al*. 1991; RODRIGUEZ-MALDONADO *et al*. 1996; GOMEZ *et al*. 1998). White follicles and ovarian stroma with numerous cortical follicles contain more than 50% of ovarian aromatase activity (ARMSTRONG 1984) and produce more than 80% of ovarian estrogens (SENIOR & FURR 1975). During maturation of yellow preovulatory follicles, the production of estrogens by the theca layer gradually decreases while synthesis of progesterone by the granulosa layer dramatically increases, hence the largest F1 follicle during the final hours before ovulation produces mainly progesterone (HUANG *et al*. 1979; BAHR *et al*. 1983; MARRONE & HERTELENDY 1983; ETCHES & DUKE 1984), which is responsible for triggering the preovulatory LH surge and ovulation (WILSON & SHARP 1976a; JOHNSON *et al.* 1985).

It is well known that prolactin plays the most important role in timing and duration of incubation behaviour in broody birds (RIDDLE *et al.* 1935; EL HALAWANI *et al*. 1986; YOUNGREN *et al.* 1991; EL HALAWANI & ROZEINBOIM 1993; MARCH *et al.* 1994), and prolonged elevated levels of prolactin occurring during the incubation period have an antisteroidogenic effect on the ovary (CAMPER &

BURKE 1977; BURKE & DENNISON 1980; BEDRAK *et al*. 1981; ZADWORNY & ETCHES 1988; ZADWORNY *et al*. 1989) in part *via* inhibition of steroidogenic enzyme gene expression (TABIBZADEH *et al*. 1995). In nonbroody birds, the participation of prolactin in reproductive functions is still unknown and its role in the local regulation of ovarian activity has had little attention. So far, only an inhibitory effect of prolactin on the stimulatory action of FSH and LH on theca cells function *in vitro* has been shown in a short communication (LI & YANG 1995). Moreover, it was evidenced that the chicken ovary is a target tissue for prolactin by showing expression of prolactin receptor mRNA (OKHUBO *et al*. 1998). In the present study the *in vitro* response of isolated white and yellow ovarian follicles to prolactin was examined in commercial egg-producing hens. To answer the question whether the effect of prolactin on steroid secretion changes during the ovulatory cycle, the examined follicles were isolated at two stages of the ovulatory cycle: 22 h and 3 h before ovulation of the largest (F1) follicle.

Material and Methods

Animals

The experiment was carried out on Hy-Line laying hens (n=8) at the age of 27 weeks, caged individually under a photoperiod of 14L:10D (light on at 0800 h) with free access to food and water. Time of oviposition was recorded daily at 15 min intervals between 0800 h and 1500 h, and once at 1700 h. Birds used in the experiment had regular sequences of at least 20 eggs per sequence and were decapitated at two stages of the ovulatory cycle: 22 h and 3 h before predicted time of F1 follicle ovulation. The following follicles were isolated from the ovaries: small white (1-4 mm in diameter), medium white (4-6 mm), large white (6-8 mm) and the 3 largest yellow preovulatory follicles (F3-F1; F3<F2<F1; 25-36 mm). The granulosa and theca layers were separated from preovulatory follicles according to the procedure of GILBERT *et al*. (1977) and divided into 4 equal pieces.

Incubation procedure

Whole white follicles and parts of granulosa and theca layers of the preovulatory yellow follicles were randomly assigned to 1 ml of Eagle's medium containing recombinant ovine prolactin prepared according to LEIBOVICH *et al.* (2001) at a dose of 0 (control), 1, 10 or 100 ng/ml and 0.05 g/ml BSA and 2 μl/ml antibiotic-antimycotic solution (10000 units penicillin, 10 mg streptomycin and 25 μg amphotericin B/ml). From each ovary, either 6 small follicles pooled together, 1 medium white follicle, 1 large white follicle or a piece of granulosa or theca were incubated in a 24-well multidish at 38^{o}C for 24 h at each dose level. After incubation, the medium was collected for progesterone and estradiol assays (radioimmunologically) and tissues of the follicular wall for protein determination by the Lowry method (LOWRY *et al*. 1951). The secretion of hormones was expressed per milligram of protein.

Steroid assays

Progesterone and estradiol concentrations in medium were measured radioimmunologically using Spectria kits (Orion Diagnostica, Finland) supplied by Polatom (Świerk, Poland). The detection limit was 90 pg/ml for progesterone and 5.45 pg/ml for estradiol. Recoveries were 98.2% for progesterone and 99.5% for estradiol. Cross reactivity of progesterone antiserum with progesterone, pregnenolone, and corticosterone were 100%, 3.9%, and 0.3%, respectively. Cross reactivity of estradiol antiserum for estradiol, estron, and estriol were as follows: 100%, 0.97%, and 0.44%. The intra- and inter-assay coefficients of variation for progesterone and estradiol were 4.3%, 4.9% and 5.7%, 6.4%, respectively.

Statistical analysis

Data were analysed statistically by two-way ANOVA followed by Duncan's multiple range test. Values are expressed as the mean ± SEM from 8 determinations and considered significantly different at $P<0.05$.

Results

Effect of prolactin on *in vitro* estradiol secretion by whole white (1-8 mm) ovarian follicles

In the control group, estradiol secretion by small (1-4 mm) and medium (4-6 mm) white follicles did not differ between the examined stages of the ovulatory cycle, whereas secretion by large (6-8 mm) white follicles was significantly higher at stage 3 h than 22 h before ovulation (Table 1).

The addition of prolactin into the incubation medium at a dose of 1 ng/ml and 10 ng/ml did not affect estradiol secretion by white follicles, while at a dose of 100 ng/ml it significantly decreased secretion of this steroid by each class of white folli-

Table 1

Effect of prolactin on *in vitro* estradiol secretion (pg/mg protein/24 h) by whole white ovarian follicles (6 small/dose/ovary, 1 medium or large/dose/ovary) isolated at two stages of the ovulatory cycle: 22 h and 3 h before ovulation. Each value represents the mean ± SEM of 8 determinations. Values in the same row with different superscript letters (a-d) are statistically different (P<0.05)

White follicles	Isolated 22 h before ovulation				Isolated 3 h before ovulation			
	Prolactin ng/ml medium							
	0	1	10	100	0	1	10	100
Small 1-4 mm	937 ±101^{ab}	783 ±86^{bc}	767 ±70^{bc}	621 ±51^{c}	1031 ±74^{ab}	984 ±96^{b}	830 ±43^{bc}	738 ±43^{c}
Medium 4-6 mm	586 ±45^{a}	554 ±46^{ab}	429 ±39^{ab}	390 ±31^{b}	581 ±64^{ac}	764 ±97^{ac}	737 ±73^{c}	703 ±54^{c}
Large 6-8 mm	459 ±60^{bc}	435 ±41^{bc}	381 ±43^{cd}	250 ±33^{d}	599 ±43^{a}	532 ±52^{ab}	553 ±46^{ab}	444 ±49^{b}

cles at both examined stages of the ovulatory cycle except medium follicles at stage 3 h before ovulation (Table 1). The decrease in estradiol secretion by small, medium and large follicles at stage 22 h before ovulation was 39%, 66% and 45%, respectively, while at stage 3 h before ovulation the decrease by small and large follicles was 28% and 26%.

Effect of prolactin on *in vitro* estradiol secretion by the theca layer of the three largest yellow preovulatory follicles

In the control group estradiol secretion by the theca layer of the three largest preovulatory F3-F1 follicles did not differ significantly between the examined stages of the ovulatory cycle (Fig. 1). During passage of the follicle from the F3 to the F1 position, estradiol secretion decreased from 2.37±0.20 to 0.32±0.04 ng/mg protein/24 h at stage 22 h before ovulation and at stage 3 h before ovulation from 2.44±0.24 to 0.23±0.03 ng/mg protein/24 h.

The addition of prolactin into the incubation medium at a dose of 1 ng/ml had no effect on estradiol secretion by the theca layer of all examined follicles at both stages of the ovulatory cycle. At a dose of 10 ng/ml, prolactin significantly increased estradiol secretion by the theca layer of F3 and F2 follicles, both at stage 22 h (37% and 53%, respectively) and at stage 3 h (35% and 71%, respectively) before ovulation. This stimulatory effect was slightly, not significantly attenuated by prolactin at a dose of 100 ng/ml. In the case of the theca layer of the F1 follicle, prolactin at a dose 10 ng/ml and 100 ng/ ml significantly increased estradiol secretion at stage 22 h before ovulation (43% and 59%, respectively) and had no effect at stage 3 h before ovulation (Fig. 1).

Effect of prolactin on *in vitro* progesterone secretion by the granulosa layer of the three largest yellow preovulatory follicles

In the control group, progesterone secretion by the granulosa layer of the three largest preovulatory F3-F1 follicles was significantly higher at stage 3 h than 22 h before ovulation (2.5-fold, 2.8-fold and 4.5-fold for F3, F2 and F1 follicle, respectively) (Fig. 2). During passage of the follicle from the F3 to F1 position, progesterone secretion at stage 22 h before ovulation increased from 0.67±0.08 to 1.51±0.25 μg/ mg protein/24 h, whereas at stage 3 h before ovulation from 1.64±0.12 to 6.76±0.32 μg/ mg protein/24 h.

The addition of prolactin into the incubation medium at a dose of 1 ng/ml had no effect on progesterone secretion by the granulosa layer of all examined follicles at both stages of the ovulatory cycle. The effect of higher doses of prolactin on progesterone secretion was dependent on the stage of the ovulatory cycle. At stage 22 h before ovulation, prolactin at a dose of 10 ng/ml and 100 ng/ml significantly increased progesterone secretion by the granulosa layer of F3 (49% and 80%, respectively) and F2 (62% and 62%, respectively) follicles, and had no effect on progesterone secretion by the granulosa of the F1 follicle. At stage 3 h before ovulation, prolactin at a dose of 10 ng/ml and 100 ng/ml had no effect on progesterone secretion by the granulosa layer of the F3 follicle and significantly decreased progesterone secretion by the granulosa of the F2 (47% and 37%, respectively) and F1 (29% and 21.5%, respectively) follicles (Fig. 2).

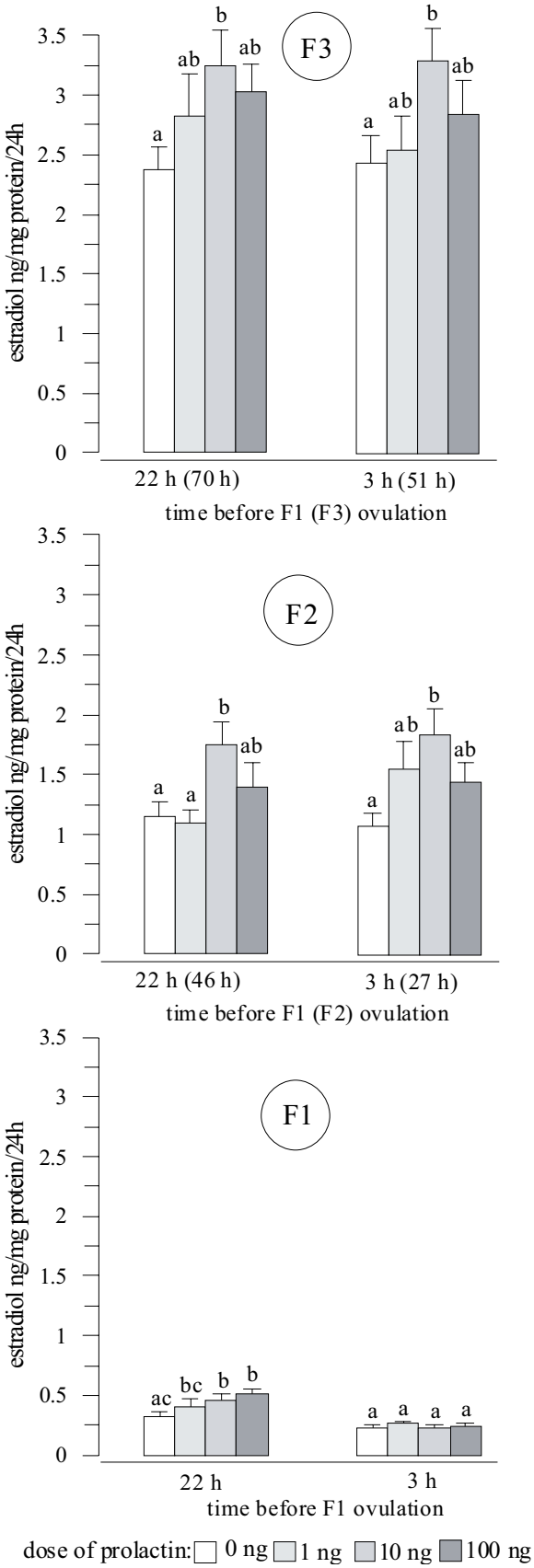

Fig. 1. Effect of prolactin on *in vitro* estradiol secretion (ng/mg protein/24 h) by the theca layer of preovulatory F3-F1 follicles isolated at two stages of the ovulatory cycle: 22 h and 3 h before ovulation. Each value represents the mean ± SEM of 8 determinations. Values with different superscript letters (a-c) differ significantly at $P<0.05$.

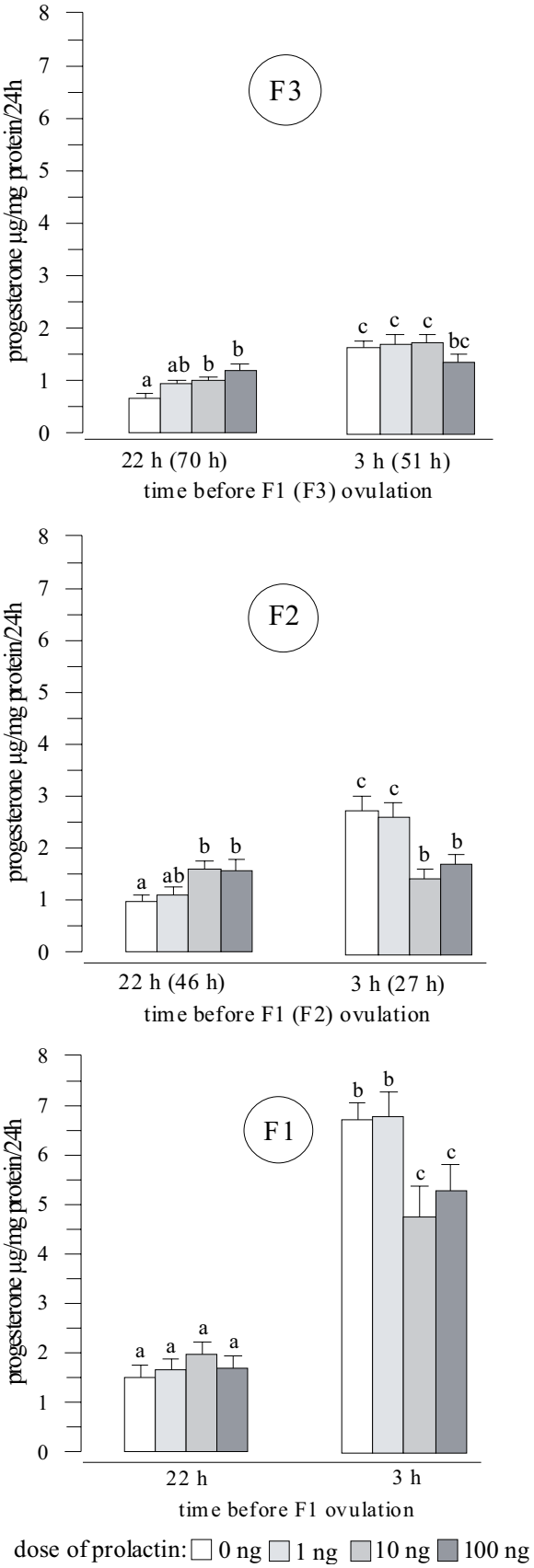

Fig. 2. Effect of prolactin on *in vitro* progesterone secretion (μg/mg protein/24 h) by the granulosa layer of preovulatory F3-F1 follicles isolated at two stages of the ovulatory cycle: 22 h and 3 h before ovulation. Each value represents the mean ±SEM of 8 determinations. Values with different superscript letters (a-c) differ significantly at $P<0.05$.

Discussion

In vertebrates, prolactin exerts multiple effects on diverse physiological processes. Among these are the regulation of mammary gland development, initiation and maintenance of lactation, immune modulation, osmoregulation, and behavioural modification. At the cellular level, prolactin exerts mitogenic, morphogenic, or secretory activities (BEN-JONATHAN *et al*. 1996).

Although the presence of prolactin receptor mRNA was shown in the laying chicken ovary (OHKUBO *et al*. 1998), and a possible role of prolactin in laying performance and steroid hormone secretion in the domestic hen has been suggested (SCANES *et al.* 1977; REDDY *et al.* 2002), the direct action of prolactin on the ovary in nonbroody birds has not been examined. To the authors' knowledge, this is the first study that demonstrated the effect of prolactin on ovarian steroidogenesis in chickens. The results are based on an *in vitro* experiment in which the intact white ovarian follicles and separated theca and granulosa layers of the three largest yellow preovulatory F3-F1 follicles were incubated in the presence and absence of ovine prolactin. Steroid secretion was determined in the incubation medium.

In the population of white follicles, irrespective of the stage of the ovulatory cycle, along with an increase of dose of prolactin, stronger suppression of estradiol secretion was observed by each class of follicles except for medium follicles at stage 3 h before ovulation. However, a statistically significant effect was caused only by the highest (100 ng/ml) dose of prolactin. An inhibitory effect of prolactin on gonadotropin-stimulated estradiol secretion *in vitro* by white follicles was previously shown in laying and out-of-lay Gifujidori hens (ZADWORNY *et al.* 1989). In turkey, a reduction of the cytochrome P450 aromatase mRNA gene expression level in small white follicles in response to exogenous ovine prolactin, and in consequence, suppression of the circulating estradiol level was found (TABIBZADEH *et al*. 1995).

Analysis of *in vitro* steroid secretion by the isolated theca and granulosa layers of yellow preovulatory F3-F1 follicles showed that during the final phase of follicular maturation proceeding ovulation, estradiol secretion by the theca layer decreased, whereas secretion of progesterone by the granulosa layer increased. These results correlate with changes of the examined steroids levels in the theca and granulosa layers of F3-F1 follicles observed during the ovulatory cycle (BAHR *et al.* 1983; KATO *et al.* 1995). The addition of prolactin at a dose of 10 and/or 100 ng/ml stimulated estradiol secretion by the theca layer of F3-F1 follicles both at stage 22 h and 3 h before ovulation except for the F1 follicle at stage 3 h before ovulation. In the case of the granulosa layer, prolactin stimulated the increase of progesterone secretion by F3 and F2 follicles at stage 22 h before ovulation and inhibited progesterone secretion by the granulosa layer of the F2 and F1 follicles at stage 3 h before ovulation. These results taken together indicate that prolactin is involved in the regulation of steroidogenesis in the chicken ovary, and its effect changes during the ovulatory cycle. It was shown previously that during the ovulatory cycle of the domestic hen the plasma level of prolactin significantly changes (SCANES *et al.* 1977). These authors suggested that the decrease in prolactin level prior to the ovulatory LH surge occurring 6-4 h before ovulation of the F1 follicle (WILSON & SHARP 1976b) might facilitate the secretion of ovarian steroids which then exert a positive feedback effect on LH release, whereas an increase in prolactin concentration after the LH peak might be involved in the inhibition of steroidogenesis. The decrease in progesterone secretion by the granulosa layer of the F2 and F1 follicles at stage 3 h before ovulation found in the present study may support this hypothesis. On the other hand, the increase in estradiol secretion as a response to prolactin by the theca layer of F3 and F2 follicles at stage 3 h before ovulation is unclear. Contrary to the present authors' observations, LI and YANG (1995) showed the inhibiting effect of prolactin on gonadotropin-induced steroidogenesis of the theca cells from the domestic hen. The two divergent actions of prolactin on the chicken ovary, both stimulating and inhibiting, observed in the present study are in accordance with prolactin action in the ovaries of mammals reviewed by MC NEILL *et al.* (1987).

The ovarian response to prolactin measured by changes in the concentration of steroid hormones in the culture medium presented in this work demonstrates that prolactin is involved in the regulation of steroidogenesis in the chicken ovary. Prolactin activity may be associated with its effect on the synthesis and/or activity of steroidogenic enzymes. TABIBZADEH *et al.* (1995) demonstrated that administration of prolactin to turkey hens caused suppression of aromatase gene expression. In the rat ovary, the inhibiting action of prolactin on steroidogenic enzyme activity was also shown (DORRINGTON & GORE-LANGTON 1981).

To sum up, the results obtained indicate that *in vitro* prolactin affects steroid secretion by chicken ovarian follicles. In white follicles, prolactin inhibits estradiol secretion, whereas in the largest yellow preovulatory follicles it stimulates or inhibits steroid secretion and its activity depends on: (1) the dose of prolactin, (2) the type of the follicular layer secreting steroids, (3) the position of the

follicle in the hierarchy and (4) the stage of the ovulatory cycle.

Acknowledgements

The authors wish to thank Dr. Arieh GERTLER from the Institute of Biochemistry, Food Science and Nutrition, Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot, Israel, for a generous gift of recombinant ovine prolactin. This work was supported in part by grant DS 3243/KFZ/04.

References

ARMSTRONG D. G. 1984. Ovarian aromatase activity in the domestic fowl (*Gallus domesticus*). J. Endocr. **100**: 81-86.

BAHR J. M., WANG S.-C., HUANG M. Y., CALVO F. O. 1983. Steroid concentrations in isolated theca and granulosa layers of preovulatory follicles during the ovulatory cycle of the domestic hen. Biol. Reprod. **29**: 326-334.

BEDRAK E., HARVEY S., CHADWICK A. 1981. Concentration of pituitary, gonadal and adrenal hormones in serum of laying and broody white rock hens (*Gallus domesticus*). J. Endocrinol. **89**: 197-204.

BEN-JONATHAN N., MERSHON J. L., ALLEN D. L., STEINMETZ R. W. 1996. Extra pituitary prolactin: distribution, regulation functions, and clinical aspects. Endocr. Rev. **17**: 639-669.

BURKE W. H., DENNISON P. T. 1980. Prolactin and luteinizing hormone levels in female turkeys (*Meleagris gallopavo*) during a photoinduced reproductive cycle and broodiness. Gen. Comp. Endocrinol. **41**: 92-100.

CAMPER P. M., BURKE W. H. 1977. The effects of prolactin on the gonadotropin-induced rise in serum estradiol and progesterone of the laying turkey. Gen. Comp. Endocrinol. **32**: 72-77.

DAVIDSON M. F., GILBERT A. B., WELLS J. W. 1979. Activity of ovarian Δ^5-3β hydroxysteroid dehydrogenase in the domestic fowl (*Gallus domesticus*) with respect to age. J. Reprod. Fert. **57**: 61-64.

DORRINGTON J. H., GORE-LANGTON R. E. 1981. Prolactin inhibits estrogen synthesis in the ovary. Nature **290**: 600-602.b

EL HALAWANI M. E., ROZENBOIM I. 1993. The ontogeny and control of incubation behavior in turkeys. Poult. Sci. **72**: 906-911.

EL HALAWANI M. E., SILSBY J. L., BEHNKE E. J., FEHRER S. C. 1986. Hormonal induction of incubation in ovariectomized female turkeys (*Meleagris gallopavo*). Biol. Reprod. **35**: 59-67.

ETCHES R. J., DUKE C. E. 1984. Progesterone, androstendione and oestradiol content of theca and granulosa tissue of the four largest ovarian follicles during the ovulatory cycle of the hen (*Gallus domesticus*). J. Endocr. **103**: 71-76.

GILBERT A. B. 1971. The ovary. (In: The Physiology and Biochemistry of the Domestic Fowl. D. J. Bell & B. M. Freeman eds. Academic Press, London): **3**: 1163-1208.

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

GRIFFIN H. D., PERRY M. M., GILBERT A. B. 1984. Yolk formation. (In: The Physiology and Biochemistry of the Domestic Fowl. B. M. Freeman ed. Academic Press, London): **5**: 345-380.

GOMEZ Y., VELAZQUEZ P. N., JUAREZ-ORAPEZA M. A., PEDERNERA E. 1998. Steroid metabolism in granulosa and theca interna cells from preovulatory follicles of domestic hen (*Gallus domesticus*). Anim. Reprod. Sci. **52**: 81-91.

HUANG E. S.-R., KAO K. J., NALBANDOV A. V. 1979. Synthesis of sex steroids by cellular components of chicken follicles. Biol. Reprod. **20**: 454-461.

JOHNSON A. L. 1996. The avian ovarian hierarchy: a balance between follicle differentiation and atresia. Poult. Avian Biol. Rev. **7**: 99-110.

JOHNSON P. A., JOHNSON A. L., VAN TIENHOVEN A. 1985. Evidence for a positive feedback interaction between progesterone and luteinizing hormone in the induction of ovulation in the hen Gallus domesticus. Gen. Comp. Endocrinol. **58**: 478-485.

KACIŃSKA M., RZĄSA J. 1988. Activity of hydroksysteroid dehydrogenase in the ovarian follicles of the domestic hen. 21st Int. Conf. Poult. Physiol., Praga. Abstr. 19-20.

KATO M., SHIMADA K., SAITO N., NODA K., OHTA M. 1995. Expression of $P450_{17\alpha\text{-hydroksylase}}$ and $P450_{aromatase}$ genes in isolated granulosa, theca interna, and theca externa layers of chicken ovarian follicles during follicular growth. Biol. Reprod. **52**: 405-410.

LEIBOVICH H., RAVER N., HERMAN., GREGORASZCZUK E. L., GOOTWINE E., GERTLER A. 2001. Large-scale preparation of recombinant ovine prolactin and determination of its *in vitro* and *in vivo* activity. Protein Exp. Purif. **22**: 489-96.

LI Y. H., YANG C. R. 1995. Effect of prolactin on the steroidogenesis and proliferation of cultured theca cells from domestic hens. Poult. Avian Biol. Rev. **6**: Abstract 295.

LOWRY O. H., ROSENBROUGH N. J., FARR A. L., RANDALL R. J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**: 265-275.

MARCH J. B., SHARP P. J., WILSON P. W., SANG H. M. 1994. Effect of active immunisation against recombinant-derived chicken prolactin fusion protein on the onset of broodiness and photoinduced egg laying in bantam hens. J. Reprod. Fertil. **101**: 227-233.

MARRONE B. L., HERTELENDY F. 1983. Steroid metabolism by avian ovarian cells during follicular maturation. Biol. Reprod. **29**: 953-962.

MC NEILLY A. S., GLASIER A., JONASSEN J., HOWIE P. W. 1987. Evidence for direct inhibition of ovarian function by prolactin. J. Reprod. Fert. **65**: 559-569.

NITTA H., OSAWA Y., BAHR J. M. 1991. Immunolocalization of steroidogenic cells in small follicles of the chicken ovary: anatomical arrangement and localization of steroidogenic cells change during follicular development. Domest. Anim. Endocrinol. **8**: 587-594.

OHKUBO T., TANAKA M., NAKASHIMA K., TALBOT R. T., SHARP P. J. 1998. Prolactin receptor gene expression in the brain and peripheral tissues in broody and nonbroody breeds of domestic hen. Gen. Comp. Endocrinol. **109**: 60-68.

REDDY I. J., DAVID C. G., SARMA P. V., SINGH K. 2002. The possible role of prolactin in laying performance and steroid hormone secretion in domestic hen *(Gallus domesticus)*. Gen. Comp. Endocrinol. **127**: 249-255.

RIDDLE O., BATES R. W., LAHR E. L. 1935. Prolactin induces broodiness in fowl. Am. J. Physiol. **111**: 352-360.

RODRIGUEZ-MALDONADO E., VALAZQUEZ P. N., JUAREZ-OROPEZA M. A., PEDERNERA E. 1996. Steroid metabolism in the theca externa cells from preovulatory follicles of domestic hen (*Gallus domesticus*). Gen. Comp. Endocrinol. **101**: 173-179.

SENIOR B. E., FURR B. J. A. 1975. A preliminary assessment of the source of estrogen within the ovary of the domestic fowl, *Gallus domesticus*. J. Reprod. Fert. **43**: 241-247.

SCANES C. G., SHARP P. J., CHADWICK A. 1977. Changes in plasma prolactin concentration during the ovulatory cycle of the chicken. J. Endocr. **72**: 401-402.

TABIBZADEH Ch., ROZENBOIM I., SILSBY J. L., PITTS G. R., FOSTER D. N., EL HALAWANI M. E. 1995. Modulation of ovarian Cytochrome P450 17α-hydroxylase and cytochrome aromatase messenger ribonucleic acid by prolactin in the domestic turkey. Biol. Reprod. **52**: 600-608.

TILLY J. L., KOWALSKI K. L., JOHNSON A. L. 1991a. Cytochrome P450 side-chain cleavage (P450scc) in the hen ovary. II. P450scc messenger RNA, immunoreactive pro-

tein, and enzyme activity in developing granulosa cells. Biol. Reprod. **45**: 967-974.

TILLY J. L., KOWALSKI K. L., JOHNSON A. L. 1991b. Stage of ovarian follicular development associated with the initiation of steroidogenic competence in avian granulosa cells. Biol. Reprod. **44**: 305-314.

WILSON S. C., SHARP P. J. 1976a. The effect of progesterone on oviposition and ovulation in the domestic fowl *(Gallus domesticus)*. Brit. Poult. Sci. **17**: 167-173.

WILSON S. C., SHARP P. J. 1976b. Induction of luteinizing hormone release by gonadal steroids in the ovariectomized domestic hen. J. Endocrinol. **71**: 89-98.

YOUNGREN O. M., EL HALAWANI M. E., SILSBY J. L., PHILLIPS R. E. 1991. Intracranial prolactin perfusion induces incubation behavior in turkey hens. Biol. Reprod. **44**: 425-443.

ZADWORNY D., ETCHES R. J. 1988. The effect of PMSG on plasma prolactin, LH, estradiol and ovarian growth in incubating and out-of-lay turkey hens. Poult. Sci. **67**: 319-326.

ZADWORNY D., SHIMADA K., ISHIDA H., SATO K. 1989. Gonadotropin-stimulated estradiol production in small ovarian follicles of the hen is suppressed by physiological concentrations of prolactin *in vitro*. Gen. Comp. Endocrinol. **74**: 468-473.