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## Comparative Changes in the Serum Concentrations of Inhibin-B, Prolactin, Gonadotropins and Steroid Hormones at Different Reproductive States in Domestic Turkey Hens

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**Abstract.** The present study was undertaken to compare the changes in circulating levels of inhibin-B, prolactin, follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol-17 $\beta$ , progesterone and testosterone during the different reproductive states of turkey hens. Blood samples were collected during different reproductive states, at laying, incubating and out of lay. Inhibin-B was measured by ELISA, while other hormones were determined by Chemiluminescent Microparticle Immunoassay (CMIA). The results revealed highly significant differences among the hen's states for all serum hormone concentrations. The highest levels of inhibin-B and prolactin were observed in incubating hens, while the lowest values were observed in laying hens. In contrast, the highest levels of FSH, LH, estradiol-17 $\beta$ , progesterone and testosterone were found in the laying group, while the lowest values were found in the incubating group. The progesterone level was higher in the laying group compared with the other groups. These results clearly demonstrate that negative correlation was found between both the inhibin-B and prolactin levels and the gonadotropin and steroid hormone concentrations during the different reproductive states of the turkey hens. In addition, the results suggest that inhibin-B may be involved in control of FSH and LH secretion.

**Key words:** Turkey, Inhibin-B, Gonadotropins, Prolactin, Steroid hormones

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Turkey hens can rapidly shift from a laying condition to one characterized by ovarian regression, incubation behavior, hyperprolactinemia and/or out of lay [1]. Inhibin is a glycoprotein hormone consisting of two dissimilar subunits,  $\alpha$  and  $\beta$ , that are linked by disulfide bonds and selectively suppresses follicle-stimulating hormone (FSH) secretion [2]. The existence of bioactive inhibin in testis preparations [3, 4] and ovarian granulosa cells [5, 6] has been reported. The effect of induction of ovarian regression and removal of ovarian follicles on plasma inhibin indicate that the ovary is the major source of inhibin in the hen [7, 8]. Prolactin secretion from the avian anterior pituitary gland is principally maintained by tonic stimulation from hypothalamic prolactin-releasing factors [9]. Prolactin secretion markedly changes during the reproductive cycle of the turkey hen. The hyperprolactinemia associated with incubation behavior (broodiness) induces ovarian regression, resulting in a substantial loss of egg production in commercial breeder flocks [10]. Administration of exogenous prolactin suppresses plasma gonadotropins necessary for egg production in domestic fowl [11]. However, there is additional evidence that suppression of gonadotropin secretion in incubating birds also involves a mechanism independent of increased prolactin secretion [12, 13]. In turkey hens, the changes in the concentrations of inhibin-B, prolactin, gonadotropins and progesterone have been poorly documented. Therefore, in the present study, we investigated the changes in inhibin-B, prolactin, FSH, luteinizing hormone (LH), estradiol-17 $\beta$ , progesterone and testosterone hor-

mones during the different reproductive states.

### Materials and Methods

#### Animals and experimental design

Domestic turkey hens (local Egyptian strain) were used in the present study. The turkeys were in the first year of production. The hens received a stimulatory photoperiod of 14 h of light:10 h of dark throughout the experimental period and were maintained in floor pens with trap nests. They were fed commercial diets available *ad libitum* and had free access to water.

The hens were used at three different physiological stages (laying, incubating, and out of lay) during the experimental period. In the 1<sup>st</sup> stage (laying), the hens laid eggs for 4 months from December to March, and 20 blood samples were collected from 5 laying hens starting on the first day of lay with a monthly interval over the next three months. In the 2<sup>nd</sup> stage (incubating), the hens incubated eggs naturally for 4 weeks, and 25 blood samples were collected from 5 incubating hens starting on the first day of incubation with a weekly interval. In the last stage (out of lay), the hens, after natural incubation, were in rest from lay for 2 months, and 10 blood samples were collected from 5 hens during this period (one sample after 1 month and the other after 2 months from the end of natural incubation). All blood samples were taken from the wing vein in the morning.

Serum was separated and frozen at  $-20$  C until assayed for hormones. Samples from the different reproductive states were assayed for inhibin-B, prolactin, FSH, LH, estradiol-17 $\beta$ , progesterone and testosterone.

### Hormonal assay

Serum samples were assayed by either ELISA for inhibin-B or Chemiluminescent Microparticle Immunoassay (CMIA) for FSH, LH, prolactin, estradiol-17 $\beta$ , progesterone and testosterone using commercially available kits on an Architect system (Abbott diagnostic division, Abbott laboratories, Abbott Park, IL USA) [14].

Inhibin-B ELISA kits were purchased from Diagnostic Systems Laboratories (Webster, TX, USA). The level of inhibin-B was determined using a two-site ELISA that employs monoclonal antibodies (mAbs) raised against synthetic peptide fragments of the human  $\beta$ -subunit [15]. This assay has been validated for use in the chicken as described previously [16, 17]. Recombinant human inhibin-B was used as the assay standard and the detection limit was 0.06 ng/ml. The percentage of recovery was 91%. The within- and between-plate coefficients of variation (CV) were 8.6 and 7.9%, respectively.

The Architect FSH, LH, prolactin and estradiol-17 $\beta$  assays are two-step immunoassays using CMIA technology with flexible assay protocols, referred to as Chemiflex.

In the first step of FSH estimation, the sample and anti- $\beta$  FSH-coated paramagnetic microparticles are combined. The FSH present in the sample then binds to the anti- $\beta$  FSH-coated paramagnetic microparticles. After washing, anti- $\alpha$  FSH acridinium labeled-conjugate is added as the second step [18, 19]. In the first step of LH estimation, the sample and anti- $\beta$  LH-coated paramagnetic microparticles are combined. The LH present in the sample then binds to the anti- $\beta$  LH-coated paramagnetic microparticles. After washing, anti- $\alpha$  LH acridinium-labeled conjugate is added as the second step [18, 19]. In the first step of prolactin estimation, the sample and anti-prolactin-coated paramagnetic microparticles are combined. The prolactin present in the sample then binds to the anti-prolactin-coated paramagnetic microparticles. After washing, anti-prolactin acridinium-labeled conjugate is added as the second step [20]. Pre-Trigger and Trigger solution are then added to the reaction mixture; the resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of FSH, LH and prolactin in a sample and the RLUs detected by the Architect optical system. The sensitivity of the assay was 0.06, 0.05 and 0.6 ng/ml and the percentage of recovery was 89.2–99.1, 90–100 and 92.4–101.1% for FSH, LH and prolactin, respectively. The intra- and interassay coefficients of variation were 2.8 and 3.3%, 3.4 and 4.1% and 3.2 and 3.8%, for FSH, LH and prolactin, respectively.

In the first step of estradiol estimation, the sample, specimen diluent, assay diluent and anti-estradiol-coated paramagnetic microparticles are combined. The estradiol present in the sample then binds to the anti-estradiol-coated paramagnetic microparticles. After incubation, estradiol acridinium-labeled conjugate is added to the reaction mixture. After a second incubation and washing, Pre-Trigger and Trigger solution are added to the reaction mixture; the resulting chemiluminescent reaction is measured as RLUs. An inverse relationship exists between the amount of estradiol in the sample and the RLUs detected by the Architect optical system [21]. The sensitivity of the assay was <10 pg/ml. The percentage of recovery was 88.1–98.2%. The intra- and interassay coefficients of variation were 5.5 and 4.8%, respectively.

The Architect progesterone and testosterone assays are one-step immunoassays using CMIA technology with flexible assay protocols, referred to as Chemiflex.

The sample, antifuoresceine (mouse, monoclonal) fluorescein-progesterone complex-coated paramagnetic microparticles and anti-progesterone (sheep, monoclonal) or anti-testosterone (mouse, monoclonal) acridinium-labeled conjugate are combined to create the reaction mixture. The progesterone or testosterone present in the sample competes with the antifuoresceine (mouse, monoclonal) fluorescein-progesterone complex-coated microparticles for binding with the anti-progesterone (sheep, monoclonal) or anti-testosterone (mouse, monoclonal) acridinium-labeled conjugate to form antibody-antigen complexes [22, 23]. After second washing, pre-Trigger and Trigger solutions are added, and the resulting chemiluminescent reaction is measured as RLUs. An inverse relationship exists between the amount of progesterone or testosterone in the sample and the RLUs detected by the Architect optical system. The sensitivity of the assay was 0.1 ng/ml and 50 pg/ml and the percentage of recovery was 90–110% and 97–104% for progesterone and testosterone, respectively. The intra- and interassay coefficients of variation were 5.4 and 5.6% and 4 and 4.5%, for progesterone and testosterone, respectively.

### Statistical analyses

Values are presented as means  $\pm$  SEM. Statistical analysis was computed using the General Linear Model (GLM) procedure of SAS [24], and significant differences between means were detected using Duncan's multiple range test [25]. A probability value (P) of less than 0.05 was considered to be significant.

## Results

### Dose response curves

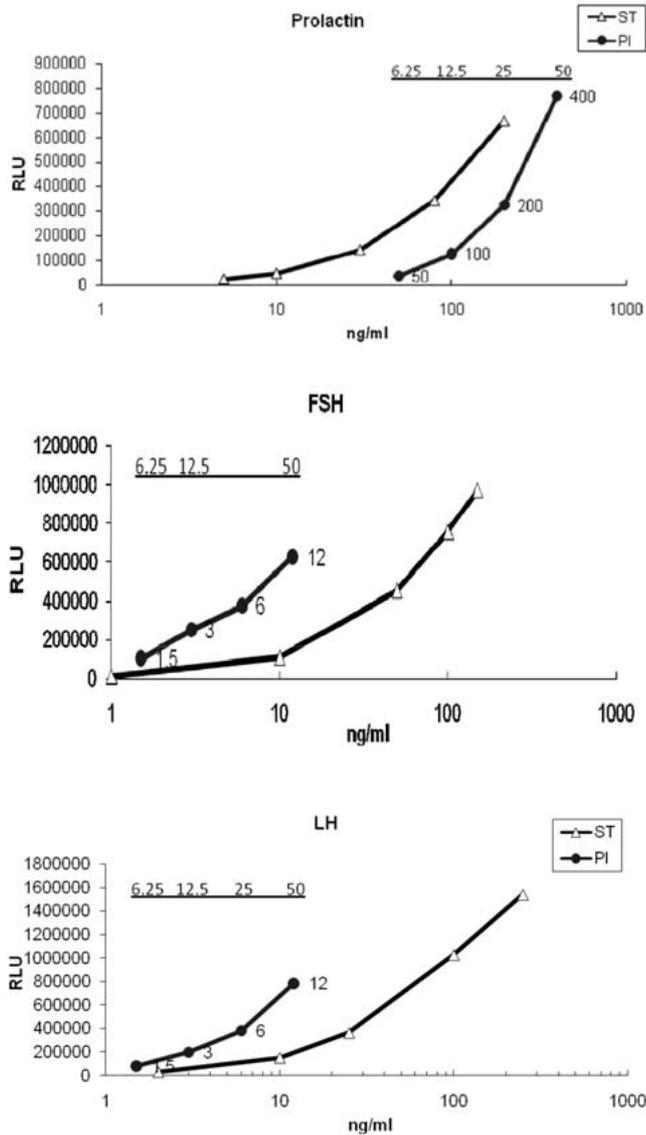
Serial dilutions of serum from adult laying turkey hens produced excellent dose-response curves (Fig. 1). The slopes of these curves were parallel with the human FSH, LH and prolactin standard curves when analyzed by one-way ANOVA ( $P \leq 0.05$ ,  $n=3$ ). These results indicate that FSH, LH and prolactin were present in the circulation of the turkey hens. Hence, the CMIA system is applicable to the turkey.

### Serum concentrations of inhibin-B, prolactin, FSH and LH during different reproductive states of turkey hens

The serum concentrations of inhibin-B, prolactin, FSH and LH during the different reproductive states of turkey hens are shown in Fig. 2. The concentrations of these hormones markedly and significantly changed during the different reproductive stages. The highest inhibin-B and prolactin concentrations were obtained in the serum of incubating hens, while the lowest levels were obtained in the serum of laying hens. In contrast, the highest concentrations of FSH and LH were observed in the serum of laying hens; however, the lowest levels were observed in the serum of incubating hens.

### Serum concentrations of estradiol-17 $\beta$ , progesterone and testosterone during different reproductive states of turkey hens

The serum concentrations of estradiol-17 $\beta$ , progesterone and

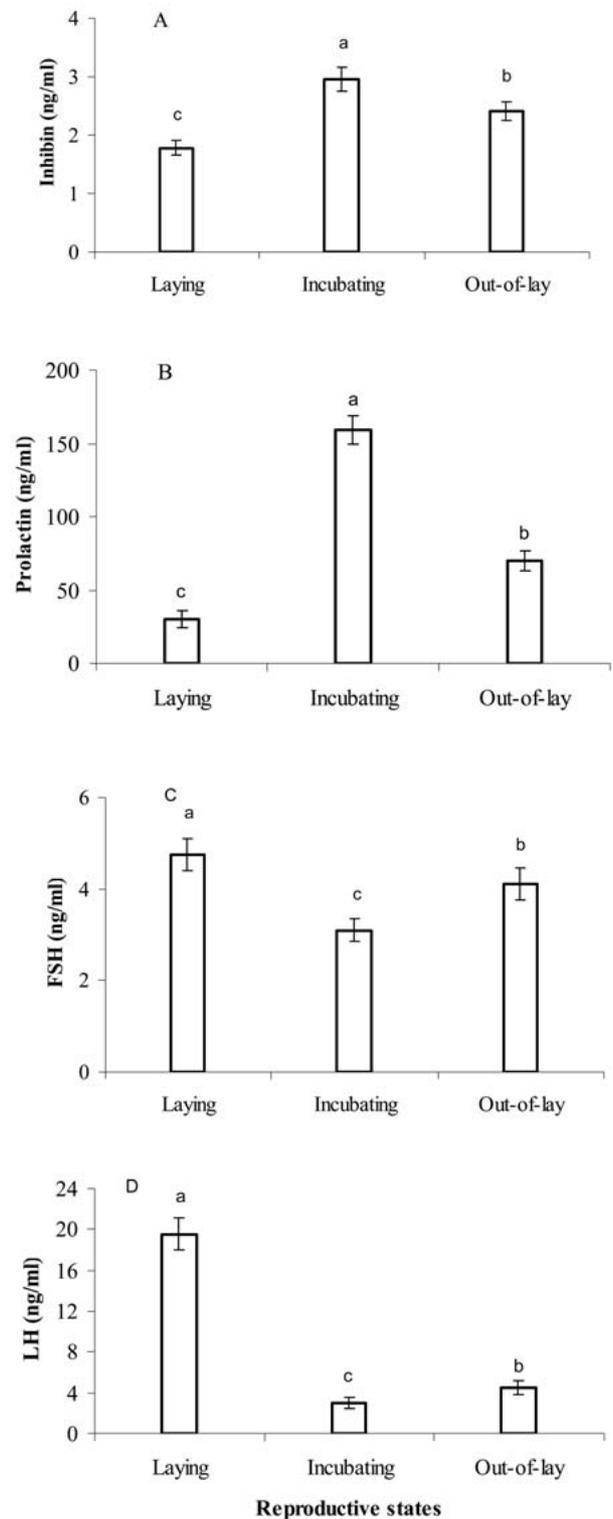


**Fig. 1.** Dose-response curves for the two-site CMIA for prolactin, FSH and LH, showing parallelism between the respective standards ( $\Delta$ ) and serial dilutions of pooled laying turkey serum ( $\bullet$ ). Values are the means of triplicate determinations.

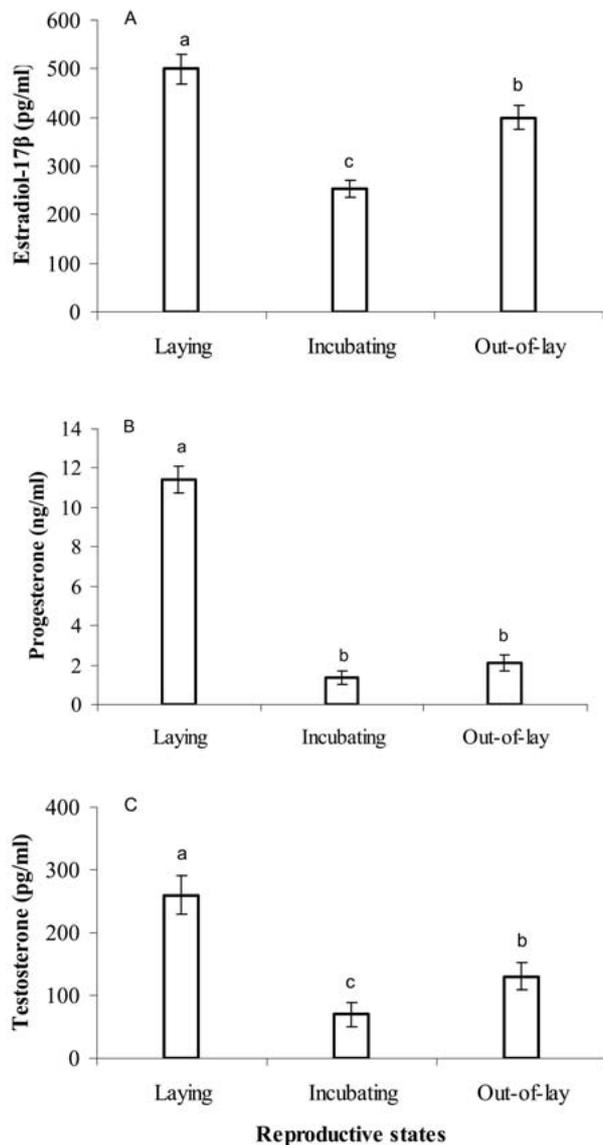
testosterone during the different reproductive states of the turkey hens are shown in Fig. 3. There were highly significant differences in the serum concentrations of these hormones during the different reproductive states. The highest levels of estradiol-17 $\beta$ , progesterone and testosterone concentrations were obtained in the serum of laying hens, while the lowest levels were obtained in the serum of incubating hens.

*Correlations among hormones during the different reproductive states of turkey hens*

The correlation coefficients for the hormone concentrations during the different reproductive states of the turkey hens are



**Fig. 2.** Serum concentrations of inhibin-B (A), prolactin (B), FSH (C) and LH (D) during laying (n=20), incubating (n=25) and out of lay (n=10) in turkey hens. Values are means  $\pm$  SEM. Values without common characters differ significantly ( $P \leq 0.05$ ).



**Fig. 3.** Serum concentrations of estradiol-17 $\beta$  (A), progesterone (B) and testosterone (C) during laying (n=20), incubating (n=25) and out of lay (n=10) in turkey hens. Values are means  $\pm$  SEM. Values without common characters differ significantly ( $P \leq 0.05$ ).

presented in Table 1. The results revealed that there were prevalent highly significant negative correlations between both inhibin-B and prolactin and both the gonadotropin and steroid hormone concentrations. In contrast, highly significant positive correlations were found between the gonadotropin and steroid hormone concentrations during the different reproductive states of the turkey hens.

### Discussion

In the present study, we investigated the changes in circulating levels of inhibin-B, prolactin, FSH, LH, estradiol-17 $\beta$ , progesterone and testosterone during the different reproductive states of

turkey hens. This study clearly demonstrates that inhibin-B, prolactin, gonadotropins and steroid hormones were secreted throughout laying, incubating and out of lay, but the concentrations of these hormones differed significantly ( $P \leq 0.0001$ ) according to reproductive states of the turkey hens. Our findings demonstrated that the laying period was characterized by higher serum concentrations of FSH, LH, estradiol-17 $\beta$ , progesterone and testosterone and lower concentrations of inhibin-B and prolactin than the other reproductive states. In contrast, the incubating period was characterized by higher serum concentrations of inhibin-B and prolactin and lower concentrations of gonadotropins and steroid hormones than the other reproductive states. In the chicken ovary, the main source of progesterone is cells of the granulosa layer, whereas the main source of estradiol is cells of the theca layer [26, 27]. The steroidogenic activity of a particular layer changes during the different physiological states of the ovary [28–31]. 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 [27, 32], which is responsible for triggering the preovulatory LH surge and ovulation [33].

Inhibin, a dimeric glycoprotein hormone, is primarily produced by the follicular granulosa cells of female mammals [34]. In the chicken, the granulosa cells of the largest follicles of the laying hen are the primary source of inhibin [35]. Chen and Johnson [36] reported that small preovulatory follicles produce the primary ovarian inhibin, which plays an autocrine or paracrine role in hen preovulatory follicles. In a subsequent report, Inhibin-B mRNA was also shown to be expressed in the hierarchical follicles, with the highest expression in the granulosa layers of small yellow follicles and undetectable in the largest follicles [37, 38]. Previous results indicate that intact inhibin-B is secreted in the greatest quantity from the small follicles of the hen, which suggests that these follicles could be a primary source of inhibin-B [39, 40]. Furthermore, the plasma inhibin-B levels are highest early in the cycle of the rat in the presence of small growing follicles [41]. Consistent with the previous results, higher serum levels of inhibin-B during the incubation period cannot be excluded. Our results showed that the levels of serum inhibin-B rose and serum FSH fell during the incubation period of the turkey hens. These data imply that small follicles are an important source of inhibin-B and, at least, may suggest a dominant endocrine role for inhibin-B in the turkey hen [8].

A negative correlation was found between inhibin-B and both gonadotropins and steroid hormones. The results demonstrated that the highest level of serum inhibin-B coincided with the lowest levels of gonadotropins and steroids hormones. These results are in agreement with those of previous studies. Culler and Negro-Vilar [42] reported a negative relationship between FSH and inhibin in the postnatal rat. Also, the relationship between pituitary gonadotropins and gonadal inhibin has been characterized from hatching to maturity in chickens [43, 44]. Both studies found a negative relationship between inhibin and FSH in female chickens that appeared to become functional at sexual maturity. Removal of large follicles leads to a rise in plasma the FSH level, indicating a possible endo-

**Table 1.** Correlation coefficients between the hormone levels during different reproductive states

Hormone	Prolactin	FSH	LH	Estradiol-17 $\beta$	Progesterone	Testosterone
Inhibin-B	0.945***	-0.933***	-0.944***	-0.924***	-0.923***	-0.965***
Prolactin	1.000	-0.932***	-0.898***	-0.982***	-0.895***	-0.961***
FSH		1.000	0.873***	0.908***	0.881***	0.906***
LH			1.000	0.827***	0.995***	0.979***
Estradiol-17 $\beta$				1.000	0.817***	0.921***
Progesterone					1.000	0.971***
Testosterone						1.000

\*\*\* P $\leq$ 0.0001.

crine role for inhibin in FSH regulation in the chicken [8]. Inhibin plays an important role in control of FSH secretion during the ovulatory cycle in the duck [45]. It has been shown to block binding of FSH to its receptor on ovarian granulosa cells [46]. Recently, Huang *et al.* [47] reported a negative correlation between inhibin and LH during the reproductive cycle in Magang geese.

It is well known that prolactin plays the most important role in the timing and duration of incubation behaviour in broody birds [48–51]. In the present study, prolactin secretion markedly changed during the reproductive cycles of the turkey hens. The hyperprolactinemia associated with incubation behavior (broodiness) induces ovarian regression [10, 47, 52], resulting in a substantial loss of egg production and inhibition of reproductive activities in many avian species. Prolactin secretion in birds is under the stimulatory control of vasoactive intestinal peptide (VIP). Active immunization with VIP results in a substantial reduction in the plasma prolactin concentration and an increase in flock egg production due to the elimination of incubation behavior [53].

The results show a highly significant negative correlation between prolactin and both gonadotropins and steroid hormones, with the highest level of serum prolactin coinciding with the lowest levels of gonadotropins and steroid hormones. These results are in agreement with other findings reported previously [42, 50, 54, 55].

Prolactin decreases reproductive activity by acting on the hypothalamus and inhibiting gonadotropin-releasing hormone release, acts on the pituitary to reduce LH- $\beta$  subunit mRNA expression and LH release [56] and acts directly on the ovary, reducing steroidogenic enzyme mRNA expression, thus inhibiting steroid hormone production [57]. The prolonged elevation of the levels of prolactin during the incubation period has an antisteroidogenic effect on the ovary [58–61] in part *via* inhibition of steroidogenic enzyme gene expression [57].

In a short communication, prolactin was shown to have an inhibitory effect on the stimulatory action of FSH and LH on theca cell function *in vitro* [62]. Moreover, expression of prolactin receptor mRNA is evidence that the chicken ovary is a target tissue for prolactin [54, 63, 64]. Finally, prolactin has been shown to have an inhibitory effect on gonadotropin-stimulated estradiol secretion *in vitro* by white follicles in laying and out-of-lay Gifujidori hens [61].

In summary, this study provided a detailed endocrine profile during different physiological situations in turkey species and demonstrated that reproductive hormones are involved in regulation of

the reproductive activities of turkey hens. The rise in inhibin-B is correlated with the non-laying period of female turkey hens. Furthermore, inhibin-B may contribute to regulation of FSH and LH secretion during the reproductive cycles of turkey hens. However, further studies are needed to investigate the rise of inhibin-B and its role during incubation behavior.

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