Hormonal Changes During Reproductive Stages in Turkey Hens

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Abstract: The present study was undertaken to compare changes in circulating levels of inhibin-B, prolactin, FSH, LH, estradiol-17β, progesterone and testosterone during different reproductive stages of turkey hens. Blood samples were collected during different reproductive stages: at laying, incubating and out of lay. The results revealed that, highly significant differences ($P \leq 0.001$) among hen's states on all serum hormones concentrations were found. The highest levels of inhibin-B and prolactin were observed in broody hens, while the lowest values were observed in laying hens. In contrast, the highest levels of FSH, LH, estradiol-17β, progesterone and testosterone hormones were found in laying hens, while the lowest values were found in broody hens. Also, the results clearly demonstrated that, negative correlation was found between both inhibin-B and prolactin levels with gonadotropins and steroid hormones concentrations during different reproductive states of turkey hens. In addition, the results suggest that inhibin-B may contribute to the regulation of FSH and LH secretion during the reproductive stages of turkey hens. However, further studies will be needed to investigate the relationship between the rise of inhibin-B and its role during incubation behavior.

Keywords: gonadotropins, inhibin-B, prolactin, steroid hormones, turkey.

INTRODUCTION

Turkey hens can rapidly shift from a laying condition to one characterized by ovarian regression, incubation behavior, hyperprolactinemia, and/or out of lay (Ramachandran *et al.,* 1996). Inhibin is a glycoprotein hormone consisting of two dissimilar subunits, α and β, which are linked by disulfide bonds, and selectively suppresses follicle-stimulating hormone (FSH) secretion (Vale *et al.,* 1988). The existence of bioactive inhibin in testis preparations (Bandivdekar *et al.,* 1982; Sedqyar *et al.,* 2008) and ovarian granulosa cells (Akashiba *et al.,* 1988; Sedqyar *et al.,* 2008) has been observed. The effect of induction of ovarian regression and removal of ovarian follicles on plasma inhibin indicates that the ovary is the major source of inhibin in the hen (Johnson *et al.,* 1993; Vanmontfort *et al.,* 1994). Prolactin secretion from the avian anterior pituitary gland is principally maintained by tonic stimulation from hypothalamic prolactin-releasing factors (Bern and Nicoll, 1968). Prolactin secretion markedly changes during the reproductive cycle of the turkey hen. The hyperprolactinemia associated with incubation behaviour (broodiness) induces ovarian regression, resulting in a substantial loss of egg production in commercial breeder flocks (El-Halawani, 1988). Administration of exogenous prolactin suppresses plasma gonadotropins necessary for egg production in domestic fowl (Lea *et al.,* 1996). However, there is additional evidence that the suppression of gonadotropins secretion in incubating birds also involves a mechanism independent of increased prolactin secretion (Sharp *et al.,* 1988; Sharp et al, 1989). In turkey hens, changes in the concentrations of inhibin-B, prolactin, gonadotropins, and progesterone are poorly documented. Therefore, in the present study, we investigated the changes in inhibin-B, prolactin, FSH, LH, estradiol-17β, progesterone, and testosterone hormones during different reproductive stages.

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. Hens received a stimulatory photoperiod of 14 hr of light: 10 hr of dark throughout the experimental period and were maintained in floor pens with trap nests. They were fed on commercial diets available *ad libitum* and had free access to water.

Hens were used at three different physiological stages (laying, incubating and out of lay) during the experimental work. The 1st stage (laying), the hens laid eggs for 4 months from December to March and 20 blood samples were collected from 5 laying hens at staring of lay and at monthly intervals. The 2nd stage (incubating), the hens incubated eggs naturally and 25 blood samples were collected from 5 broody hens at starting of incubation and at weekly intervals. The last stage (out of lay), hens after natural incubation were in rest from lay for 1 to 2 months and 10 blood samples were taken from 5 hens after 1 and 2 months from the terminal of natural incubation. All the blood samples were taken from wing vein in the morning. Serum was separated and kept frozen at -20°C until assayed for hormones. Samples from the different reproductive stages were assayed for inhibin-B, prolactin, FSH, LH, estradiol-17β, progesterone and testosterone.

Hormonal assay:

Inhibin-B was assayed by ELISA. It is determined using two-site ELISAs that employ monoclonal antibodies (mAbs) raised against synthetic peptide fragments of the human β B-subunit (Groome, 1996). This assay was validated for use in the chicken as described previously (Lovell *et al.,* 2000; Lovell *et al.,* 2001). Recombinant human inhibin-B is used as assay standards and detection limits were 0.06 ng/ml. The percentage of recovery was 91%. Within-and between-

plate coefficients of variation (CV) were 8.6 and 7.9 %, respectively.

FSH, LH, prolactin, estradiol-17β, progesterone and testosterone were assayed by Chemiluminescent Microparticle Immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex, using commercially available kits on Architect system by Abbott diagnostic division (AIDD, 2006). In the first step of FSH estimation, sample and anti-β FSH coated paramagnetic microparticles are combined. FSH present in the sample binds to the anti-β FSH coated paramagnetic microparticles. After washing, anti-α FSH acridinium labeled conjugate is added in the second step (Pierce and Parsons, 1981; Beastall, 1987). Moreover, in the first step of LH estimation, sample and anti-β LH coated paramagnetic microparticles are combined. LH present in the sample binds to the anti-β LH coated paramagnetic microparticles. After washing, anti-α LH acridinium labeled conjugate is added in the second step (Pierce and Parsons, 1981; Beastall, 1987). While, in the first step of prolactin estimation, sample and anti-prolactin coated paramagnetic microparticles are combined. Prolactin present in the sample binds to the anti-prolactin coated paramagnetic microparticles. After washing, anti-prolactin acridinium labeled conjugate is added in the second step (Friesen *et al.*, 1972). Pre-Trigger and Trigger solution are then added to the reaction mixture; the resulting chemiluminescent reaction was measured as relative light units (RLUs). A direct relationship exists between the amount FSH, LH and prolactin in the sample and 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 inter-assay coefficients variations for FSH were 2.8 and 3.3%, respectively, for LH were 3.4 and 4.1%, respectively and for prolactin were 3.2 and 3.8%, respectively.

Furthermore, in the first step of estradiol-17β estimation, sample, specimen diluent, assay diluent and anti-estradiol-17β coated paramagnetic microparticles are combined. Estradiol-17β present in the sample binds to the anti-estradiol coated paramagnetic microparticles. After incubation, estradiol acridinium labeled conjugate was added to the reaction mixture. After second incubation and washing, Pre-Trigger and Trigger solution were added to the reaction mixture; the resulting chemiluminescent reaction was measured as relative light units (RLUs). An inverse relationship exists between the amount of estradiol in the sample and RLUs detected by the Architect optical system (Whitley *et al.*, 1994). The sensitivity of the assay was <10 pg/ml. The percentage of recovery was 88.1-98.2%. The intra- and inter-assay coefficients variations were 5.5 and 4.8%, respectively.

The Architect progesterone and testosterone assays are one-step. Sample, antifluorescine (mouse, monocleonal) fluorescene-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. Progesterone or testosterone present in samples competes with antifluorescine (mouse, monoclonal) fluorescine-progesterone complex coated microparticles for binding with anti-progesterone (sheep, monoclonal) or anti-testosterone (mouse, monoclonal) acridinium labeled conjugate to form antibody-antigen complexes (Abraham *et al.*, 1972; Burtis and Ashwood, 1994). After second washing, pre-Trigger and Trigger solutions were added and the resulting chemiluminescent reaction was measured as relative light units (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 0.1ng/ml and 50 pg/ml and the percentage of recovery was 90-110% and 97-104% for progesterone and testosterone, respectively. The intra- and inter-assay coefficients variation for progesterone 5.4 and 5.6 %, respectively and for testosterone 4 and 4.5 %, respectively.

Statistical analysis:

Values are presented as means ± SEM. Data were analyzed using the General Linear Model (GLM) procedure of SAS (SAS Institute Inc., 1998), and the significant differences between means were detected according to Duncan's multiple range test (Duncan, 1955). A probability value (P) of less than 0.05 was considered to be significant.

RESULTS

Serum concentrations of inhibin-B, prolactin, FSH and LH during different reproductive stages in turkey hens:

Serum concentrations of inhibin-B, prolactin, FSH and LH during different reproductive stages in turkey hens are shown in Fig.1. Concentrations of these hormones markedly (P<0.05) changed during different reproductive stages. The highest levels of inhibin-B and prolactin concentrations were obtained in serum of broody hens, while the lowest levels were obtained in serum of laying hens. In contrast, the highest concentrations of FSH and LH were observed in serum of laying hens, however, the lowest levels were observed in serum of broody hens.

Serum concentrations of estradiol-17β, progesterone and testosterone during different reproductive stages in turkey hens:

Serum concentrations of estradiol-17β, progesterone and testosterone during different reproductive states of turkey hens are shown in Fig. 2. There were significant (P<0.05) differences in serum concentrations of these hormones during different reproductive stages. The highest levels of estradiol-17β, progesterone, and testosterone concentrations were obtained in serum of laying hens, while the lowest levels were obtained in serum of broody hens.

Correlation coefficients between hormones levels during different reproductive stages in turkey hens:

Results of correlation coefficients between hormones concentrations during different reproductive

stages in turkey hens are presented in Table 1. Results revealed that there were prevalent significant ($P<0.001$) negative correlation between both inhibin-B and prolactin with both gonadotropins and steroids hormones concentrations. In contrast, significant ($P<0.001$) positive correlations were found between gonadotropins and steroids hormones concentrations during different reproductive stages of turkey hens.

Table (1): Correlation coefficients between hormones levels during different reproductive states of turkey hens.

Hormone	Prolactin	FSH	LH	Estradiol- 17β	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-7β				1.000	0.817***	0.921***
Progesterone					1.000	0.971***
Testosterone						1.000

***= *(P≤0.0001)*

DISCUSSION

In the present study, we investigated the changes in circulating levels of inhibin-B, prolactin, FSH, LH, estradiol-17β, progesterone and testosterone during different reproductive stages of turkey hens. It clearly demonstrated 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 ($P\leq0.0001$) according to reproductive stages of turkey hens. Our findings indicated that the laying period was characterized by higher serum concentrations of FSH, LH, estradiol-17β, progesterone and testosterone and lower concentrations of inhibin-B and prolactin than the other reproductive stages. In contrast, the incubation period was characterized by higher serum concentrations of inhibin-B and prolactin and lower concentrations of gonadotropins and steroids hormones than those found in the other reproductive stages. In the chicken ovary, the main source of progesterone are cells of the granulosa layer, whereas for estradiol-cells are present in the theca layer (Bahr *et al.*, 1983; Etches and Dukec, 1984).

Steroidogenic activity of a particular layer changes during different physiological states of the ovary (Kacinska and Rzysa, 1988; Nitta *et al.*, 1991; Rodriguez-Maldonado *et al.*, 1996; Gomez *et al.*, 1998). 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 follicle during the final hours before ovulation produces mainly progesterone (Etches and Dukec, 1984; Marrone and Hertelendy, 1983), which is responsible for triggering the preovulatory LH surge and ovulation (Johnson, 1985). Inhibin, a dimeric glycoprotein hormone, is produced primary by the follicular granulosa cells of the female mammals (Erickson and Hsueh, 1992). In chicken the granulosa cells of largest follicles of laying hen are the primary source of inhibin (Vanmontfort, 1992). Moreover, removal of large follicles leads to a rise in plasma FSH levels, indicating a possible endocrine role of inhibin in FSH regulation in the chicken (Johnson *et al.*, 1993).

Another finding, reported that the small preovulatory follicles produce the primary ovarian inhibin which plays an autocrine or paracrine role in hen preovulatory follicles (Chen and Johnson, 1996). In a subsequent report, inhibin-B mRNA is also expressed in the hierarchical follicles with highest expression in the granulosa layer of small yellow follicles and undetectable in the largest follicles (Davis and Johnson, 1998; Lovell *et al.*, 2002). Also, Hecht *et al.* (2000); Johnson *et al.* (2005) detected that the intact inhibin-B was secreted in a greatest quantity from the small follicles of the hen, which suggested that these follicles could be a primary source of inhibin-B. Furthermore, plasma inhibin-B levels are highest early in the ovarian cycle of the rats (Woodruff *et al.*, 1996). Consistent with the previous results, higher serum levels of inhibin-B during incubation period can not be excluded. Our results showed that serum inhibin-B was higher and serum FSH and LH were lower during incubation period of turkey hens. These data imply that the small follicles are important source of inhibin-B and may suggest that dominant endocrine role for inhibin-B in the turkey hen (Johnson *et al.*, 1993).

On the other hand, a negative correlation was found between inhibin-B and both gonadotropins and steroids hormones. Our results demonstrated that the highest level of serum inhibin-B was coincided with the lowest levels of gonadotropins and steroids hormones. These results are in agreement with previous studies.

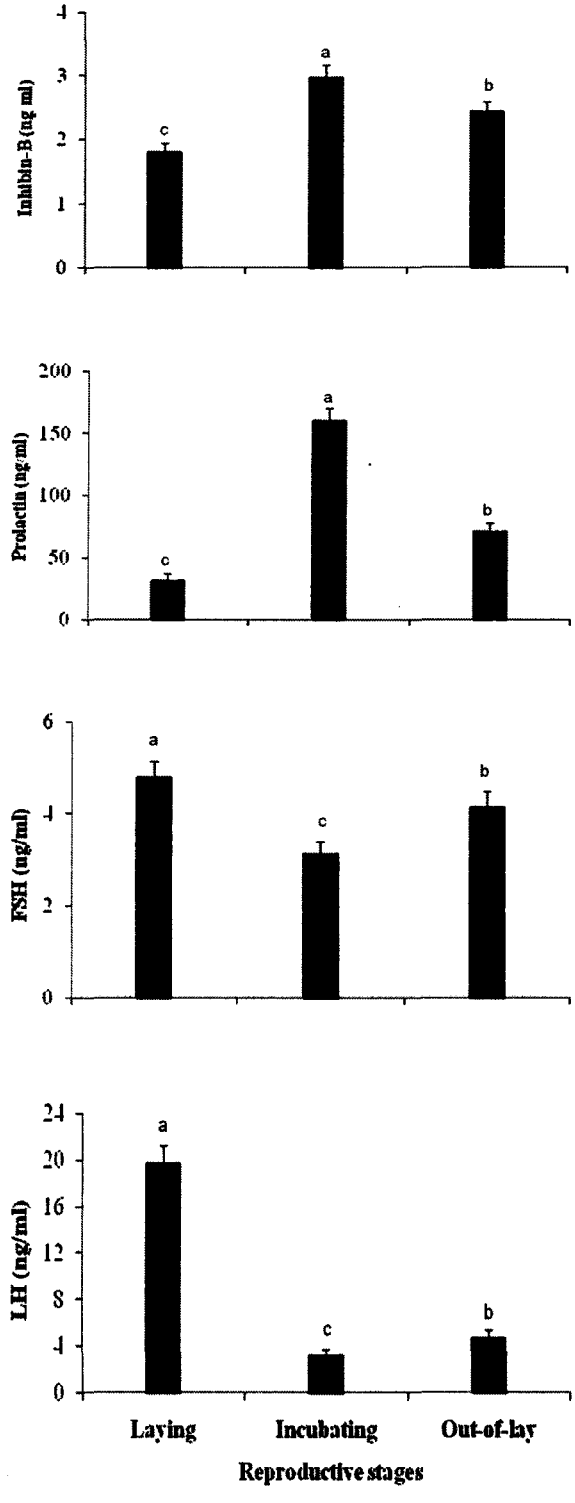

Figure (1): Serum concentrations of inhibin-B, prolactin, FSH and LH during laying (n=20), incubating (n=25) and out of lay (n=10) in turkey hens. Means ± SE without common letters differ significantly ($P<0.05$).

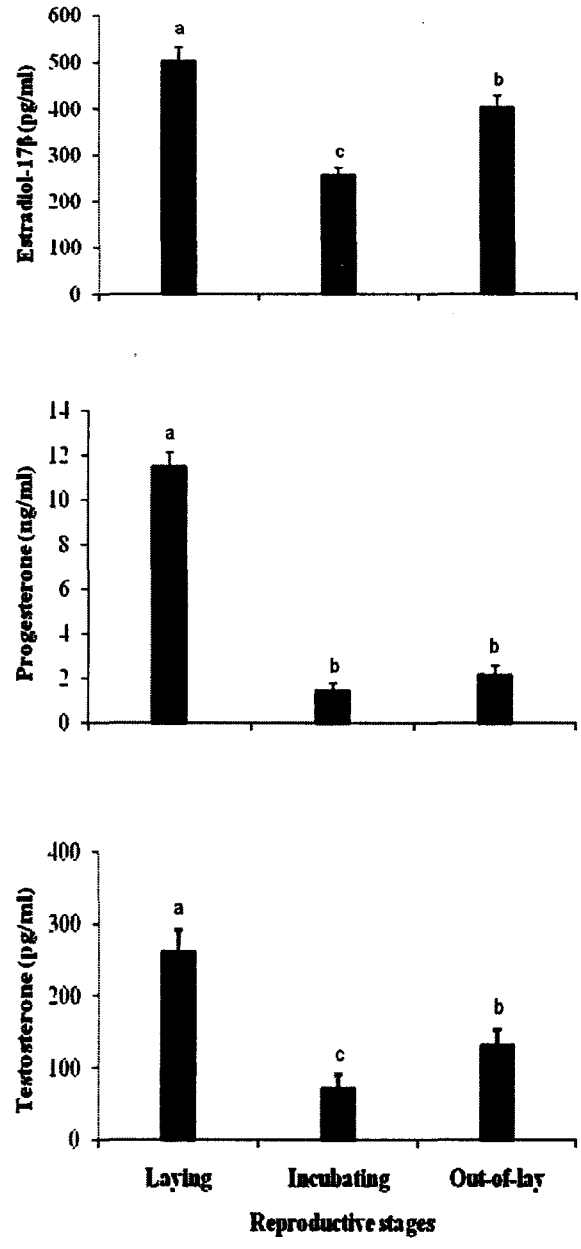

Figure (2): Serum concentrations of estradiol-17β, progesterone and testosterone during laying (n=20), incubating (n=25) and out of lay (n=10) in turkey hens. Means±SE without common letters differ significantly ($P<0.05$).

Culler and Negro-Vilar (1982) reported that a negative relationship between FSH and inhibin was found in the postnatal rat. Also, Vanmontfort *et al.* (1995); Johnson and Brooks (1996) found a negative relationship between inhibin and FSH in the female chickens at sexual maturity. Also, Yang *et al.* (2005) reported that the inhibin plays an important role in the control of FSH secretion during the ovulatory cycle in duck. It has been shown to block the binding of FSH to its receptor on ovarian granulosa cells (Schneyer *et al.*, 1991). Recently, Huang *et al.* (2008) reported that a negative relationship between inhibin and LH was found during reproductive cycles in the Magang geese.

It is well known that prolactin plays the most important role in timing and duration of incubation behaviour in broody birds (El-Halawani *et al.*, 1982; Youngren *et al.*, 1991; El-Halawani *et al.*, 1993; March *et al.*, 1994). In the present study, prolactin secretion markedly changes during the reproductive stages of the turkey hens. The hyperprolactinemia associated with incubation behavior (broodiness) induces ovarian regression (El-Halawani *et al.*, 1988; Shi *et al.*, 2007; Huang *et al.*, 2008), resulting in a substantial loss of egg production and inhabiting 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 plasma prolactin concentration and increase in flock egg production due to the elimination of incubation behavior (El-Halawani, 1995).

Our results showed a highly significant ($P \leq 0.0001$) negative correlation between prolactin and both gonadotropins and steroids hormones, the highest level of serum prolactin was coincided with the lowest levels of gonadotropins and steroids hormones. These results are in agreement with other findings reported previously (Culler and Negro-Vilar, 1988; El-Halawani and Rozenboim, 1993; Anna *et al.*, 2004; Rozenboim *et al.*, 2004).

Prolactin decreases reproductive activity by acting on the hypothalamus and inhibiting gonadotropin-releasing hormone release, also it acts on the pituitary to reduce LH-β subunit mRNA expression and LH release (You *et al.*, 1995), and directly on the ovary, reducing steroidogenic enzyme mRNA expression, thus inhibiting steroid hormone production (Tabibzadeh *et al.*, 1995). The prolonged elevated levels of prolactin occurring during the incubation period have an antisteroidogenic effect on the ovary (Burke and Dennison, 1980; Bedrak *et al.*, 1981; Zadworny and Etches, 1988; Zadworny *et al.*, 1989) in part *via* inhibition of steroidogenic enzyme gene expression (Tabibzadeh *et al.*, 1995). Also, 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 and Yang, 1995). Moreover, it was evidenced that the chicken ovary is a target tissue for prolactin by showing expression of prolactin receptor mRNA (Ohkubo at al., 1998; Reddy *et al.*, 2002; Anna *et al.*, 2004). An inhibitory effect of prolactin on gonadotropin-stimulated estradiol-17β secretion *in vitro* by white follicles was previously shown in laying and out-of-lay of Gifujidori hens (Zadworny *et al.*, 1989).

In summary, this study provided detailed endocrine profile during different physiological situations in turkey. Also it demonstrated that reproductive hormones are involved in regulation of reproductive activities of turkey hens. The rise in inhibin-B is correlated with non-laying period of female turkey hens. Inhibin-B may contribute to the regulation of FSH and LH secretion during the reproductive stages of turkey hens. However, further studies will be needed to investigate the relationship between the rise of inhibin-B and its role during incubation behavior.

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التغيرات الهرمونية خلال المراحل التناسلية فى إناث الرومى

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تهدف هذه الداسة إلى مقارنة التغيرات الهرمونية التى تحدث خلال المراحل التناسلية المختلفة: مرحلة الإنتاج, مرحلة الرقاد, مرحلة الراحة فى إناث الرومى. جُمعت عينات الدم من الإناث خلال المراحل التناسلية السابقة. تم تقدير الهرمونات التالية فى سيرم الدم: (inhibin-B, prolactin, FSH, LH, estradiol-17β, progesterone and testosterone).
وكانت أهم النتائج المتحصل عليها مايلى:
١- أظهرت النتائج تغيراً معنوياً واضحاً ($P\leq0.001$) فى مستوى تركيز كل الهرمونات المدروسة تبعاً لتغير المرحلة التناسلية لإناث الرومى.
٢- لوحظ أن أعلى تركيز لمستوى هرمونى inhibin-B and prolactin كانا فى سيرم دم الإناث خلال مرحلة الرقاد، بينما لوحظ أن أقل تركيز لهذين الهرمونيين كانا فى سيرم الدم للإناث خلال مرحلة الإنتاج وكانت الفروق عالية المعنوية ($P\leq0.001$).
٣- وجُد أن أعلى تركيز لهرمونات: FSH, LH, estradiol-17β, progesterone and testosterone كان فى سيرم الدم للإناث خلال مرحلة الإنتاج بينما أقل التركيزات فقد وجدت فى سيرم الدم للإناث خلال مرحلة الرقاد وكانت الفروق عالية المعنوية ($P\leq0.001$).
٤- أظهرت النتائج وجود ارتباطاً عالياً وسالباً ومعنوياً بين تركيز هرمونى inhibin-B and prolactin مع باقى الهرمونات المدروسة خلال المراحل التناسلية المختلفة.
أوضحت هذة التجربة صورة للتغيرات الهرمونية خلال المراحل الفسيولوجية المختلفة لإناث الرومى. و يُستنتج من هذه الدراسة أن هرمون inhibin-B قد يكون له دور فى تنظيم إفراز كلٌ من هرمونى FSH & LH خلال المراحل التناسلية المختلفة فى إناث الرومى. كما توصى هذه الدراسة بإجراء المزيد من الأبحاث لتحرى العلاقة بين زيادة تركيز هرمون inhibin-B ودوره خلال مرحلة الرقاد.