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ALTERATIONS IN CONCENTRATIONS OF INHIBIN IN RELATION TO FSH AND STEROIDS HORMONE DURING ESTROUS CYCLE IN EGYPTIAN BUFFALOES.

(With 1 Figure)

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التغير في تركيزات الأنـهبين وعلاقته بالحافة الجريبية وهرمونات المبيض أثناء دورة الشياع في الجاموس المصري

أجريت هذه الدراسة في محطة بحوث الإنتاج الحيواني بمسطرد التابعة لقسم الإنتاج الحيواني بكلية الزراعة جامعة الأزهر بالقاهرة . استخدم في هذه الدراسة عدد سبع جاموسات أظهرت الشياع المصاحب بالتبويض وتم معرفة ذلك عن طريق الجس المستقيمي وكشف الشياع ثلاث مرات يومياً .

استهدفت الدراسة متابعة التغيرات في بعض الهرمونات التي اشتملت على هرمونات الأنهبين الحافة الجريبية وهرمون الاستروجين وهرمون البروجسترون . تراوحت مستويات هرمون الأنهبين بين ٤٣٩ إلى ٥٨٣ بيكوجرام/ملل أثناء المراحل المختلفة لدورة الشياع ،ولقد كان أعلى تركيز لهرمون الأنهبين يوم الشياع بالنسبة ، الحافة الجريبية فقد كان أعلى تركيز لهذا الهرمون يوم الشياع ٢٥.٤٨ نانوجرام/ملل ثم انخفض وتذبذب مستواه باتجاه نهاية دورة الشياع حيث بدأ في الزيادة مرة أخرى على نهاية دورة الشياع . كان أعلى تركيز لهرمون الاستروجين ١٨.٥٤ بيكوجرام/ملل يوم الشياع بينما كان أعلى تركيز لهرمون البروجسترون ٤.٣٩ نانوجرام/ملل اليوم الثاني عشر من دورة الشياع .

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يمكن أن نستخلص من هذه الدراسة أن كل من هرمون الأنهبين والايستروجين من الممكن أن يكون لهما تأثير رجعى على تنظيم إفراز الحافة الجريبية في الجاموس المصري.

SUMMARY

Seven Egyptian buffaloes were used to study the plasma changes of inhibin, FSH, estradiol-17β and progesterone concentrations during the at estrous cycle. These hormones were determined at estrous and two interval until day twenty two of estrous cycle. Plasma inhibin estimated by enzyme-linked immunoasorbent assay (ELISA). Both FSH, estradiol-17β and progesterone were estimated by radioimmunoassay (RIA). In order to elucidiate the feedback system controlling mentioned hormones during estrous cycle, variations in the levels of hormones and their inter-relationshpis were studied. Plasma inhibin levels ranged between 439.28 and 584 pg/ml during various stages of estrous cycle, peak of inhibin level was at day of estrous by an average of 584 pg/ml. Follicle stimulating hormone levels were higher at day of estrous (25.48 ng/ml), decreased and fluctuated towards end of estrous cycle and then increased at twenty two days of estrous cycle. Peak of estradiol-17β was 18.54 pg/ml at day of estrous, while the peak of progesterone was 4.39 ng/ml at twelfth day from the start of estrous. It could be concluded that each of inhibin and estradiol-17β seems to have a feedback regulatory effect on FSH secretion in buffaloes.

Key words: Inhibin, FSH, estradiol-17β, progesterone, estrous cycle, buffalo.

INTRODUCTION

Inhibin is secreted by granulosa cells in response to FSH and its major action is the negative feedback control of pituitary FSH secretion (Ying, 1988). In mammalian species, ovulation is associated with the morphological, chemical and physiological changes in the ovulatory follicle. All these changes occur in response to the preovulatory LH and FSH (Dhali, et al. 2007).

Inhibin is a glycoprotein with two subunits α and β that belongs to the forming factor-β superfamily (Padilla-Rivas and Holtz 2006). The ovarian follicles have been identified as the major source of inhibin in sheep (Mann, et al. 1989), in goats (Medan, et al. 2005) in cattle (Rodgers, et al. 1989) and buffaloes (Palta, et al. 1996). This hormone have been studied in buffaloes by only a few authors over the past few years. Palta, et al. (1997) observed an increase of inhibin concentrations during the follicular phase with a peak occurring on day zero (estrous day defined by the lowest progesterone value). Inhibin has been implicated in the feedback regulation of FSH in heifers (Turzillo and Fortune 1990), in cows (Kaneko, et al. 1995) and in buffaloes (Singh, et al. 2001).

In cows, plasma FSH surge was significantly higher around estrous (Dhali, et al. 2007). Medan, et al. (2005) reported that inhibin A was negatively correlated with FSH, while it was positively correlated with estradiol-17β in goats. However, few information are available on the inhibin levels during estrous cycle in buffaloes. Therefore, the present study was undertaken to investigate the inhibin levels during different stages of estrous cycle in relation to FSH, estradiol-17β and progesterone in Egyptian buffaloes.

MATERIALS AND METHODS

Seven Egyptian buffaloes, aged 3-4 years, from the experimental farm of the Faculty of Agriculture Al-Azhar University, were used. Animals were fed according to their live body weight and production, on concentrate mixture containing 17% crude protein, 2.5% fat and 15% crude fiber. In addition to rice straw or wheat straw and berssem hay, Egyptian clover, (*Trifolium alexandrinum*) which was offered at 20 to 25 Kg/animal/day.

Blood samples were collected from the jugular vein of buffaloes at day of estrous and at two days intervals thereafter for 22 days. Buffaloes were observed for estrous sings three times daily, at morning, mid day and afternoon. A separate fertile bull in addition to rectal palpation were used for of determination estrous day. Plasma was seprated and stored at-20^0C till determination of the inhibin, follicle stimulating hormone (FSH), estradiol-17β and progesterone.

Inhibin level was determined using enzyme-linked immunoasorbent assay (ELISA) kit, manufactured by Biosource Europe S. A, Rue de I'industrie, 8 B-1400 Nivelles-Belgium, as described by Buckler (1992). The mature form of inhibin has a molecular weight of 32.000 daltons, and consists of one alpha-chain (approx. 18 KDa) and one beta-chain (14 KDa) linked by disulphide bridges (Ying 1988). Plasma FSH, estradiol-17β and progesterone were estimated by direct radioimmunoassay (RIA) using coat A-count kit. (DPC-Diagnostic, Corporation, Los Angles CA 90045, U.S.A.) According to Razdan, et al. (1982), Xing, et al. (1993) the and Abraham (1981) for three hormones receptively. Statistical analysis was carried out using Duncan's multiple range. Correlation coefficient among various hormones in the different phases was evaluated according to GL M procedure of SAS (1988).

RESULTS

The mean concentrations of inhibin, FSH, estradiol-17β and progesterone from day of estrous to day twenty two of estrous cycle are shown in Fig. (1). The highest concentrations of plasma inhibin (584 pg/ml) and of FSH (25.48 ng/ml) were recorded at the day of estrous. A general decrease of both inhibin and FSH concentrations was observed to reach the lowest level (439.28 pg/ml and 13.25 ng/ml) at days 8 and 12 of estrous cycle, respectively. The concentrations of inhibin and FSH were slightly increased to reach 448.14 pg/ml and 18.11 ng/ml, respectively, at the end of estrous cycle. The differences of inhibin and FSH concentrations during the estrous cycle were significant ($P<0.01$).

As shown in Fig (1) the general pattern of secretion of steroids showed that the highest estradiol-17β level was 18.54 pg/ml at day of estrous. At the same time the level of progesterone was low 0.64 ng/ml. Estradiol-17β has drastically dropped (12.07 pg/ml) at day two of the estrous cycle, continual to drop progressing to reach amnoder on day 18 (2.2pg/ml) then fluctuated and increased again 8.17 pg/ml towards the end of the cycle. The statistical analysis of estradiol-17β concentrations among days of the estrous cycle was significant ($P<0.01$). During estrous, the concentrations of inhibin/FSH and inhibin/estradiol-17β were positively correlated (0.88 and 0.40 respectively). It seems that there are a synergistic interaction between inhibin and estradiol-17β in the control of FSH secretion in buffaloes. The plasma progesterone profiles confirmed that all experimental animals have normal estrous cycle and ovulated following the onset of estrous. Progesterone concentrations reached a high level (4.39 ng/ml) at twelfth day of the estrous cycle. A gradual decrease of progesterone concentration was observed (0.52 ng/ml) at the end of estrous cycle.

DISCUSSION

The inhibin levels reported in this study are in coincidence with the levels reported by Singh, et al. (2001) and close to those reported by Palta, et al. (1997). The same trend was observed by Palta, et al. (1996) who found an increase of inhibin concentrations during the follicular phase with a peak occurring on day zero (estrous day). Kaneko, et al. (1992) reported a maximum inhibin concentration in cows two hours after the LH peak. Vanmontfort, et al. (1998) found that inhibin concentration showed no significant variation during the estrous cycle in sheep, however, highest levels were recorded during the follicular phase, which then decreased during the luteal phase to basal levels.

In the present study the inhibin levels declined continuously after the day of estrous and were at their lowest value until twelfth day of the cycle which is corresponded to the absence of follicular growth on the ovary at this time in buffaloes. Singh, et al.(2001) demonstrated that the emergence of follicular waves seems closely tied to the elevation of plasma FSH. They postulated that species possessing large-size follicles, follicles do not develop during the luteal phase and have higher levels of feedback regulators such as inhibin.

A consistent fall of inhibin 1-4 h after LH peak, reaching a nadir at the time of the second surge of FSH has been reported (Findlay, et al 1990). Amounts of inhibin in the follicular fluid and inhibin α subunit mRNA in granulosa cells are greater in healthy follicles compared to atretic follicles at several stages of development (Handerson, et al. 1984 and Ireland and Ireland 1994).

The present study showed that inhibin concentrations slowly increased again from the eighteenth day until the end of estrous cycle, suggesting that this increase was due to development of follicle on the ovary for new the cycle. Singh, et al. (2001) reported that rise of inhibin and setradiol-17β could be attributed to the growing crop of follicles on the ovary, which seems to have started around the eighth day of the cycle in buffaloes.

In general, the level of FSH reported in this study are in agreement with those noticed by Singh et al, (2001) and Dhali, et al (2007). The later authors observed that the concentration of FSH was higher during the first three days of estrous cycle. The FSH level decreased to the basal level on day four of the estrous cycle. The results of the present study were lower than those found by Razdan, et al. (1982) on the day of estrous. However, Janakiraman, et al. (1980) have reported even higher levels during the whole estrous cycle.

The estradiol-17β levels found in this study were at the same order as observed by Barkawi (1984), El-Terbany (1998) and Singh, et al (2001) and lower than reported by Kumar, et al (1991) on the day of estrous. Estradiol-17β levels were found to be higher than those reported by Avenell, et al. (1985) on the day of estrous. The results showed that plasma progesterone concentrations are similar to those found by Barkawi

(1984), Panchal, et al. (1992), Sarvaiya, et al. (1993) and Singh, et al. (2001) .

Medan, et al. (2005) indicated that growing follicles secreted inhibin and estradiol-17β which in turn suppressed FSH secretion in goats. Kaneko, et al. (1995), showed that passive immunization against inhibin during the follicular phase of the cycle in resulted in a transient increase of FSH, in cows thereby showing that inhibin has a definite role in the control of FSH secretion. Bleach, et al. (2001) showed that circulating inhibin A concentrations were positively correlated with levels of estradiol-17β, whereas circulating concentrations of FSH were negatively correlated with inhibin A levels .

Fluctuation of the circulating FSH levels is involved in the recruitment of the follicles (Medan, et al. 2005). Also, they showed that inverse relationship between inhibin and FSH confirms the hypothesis that inhibin inhibits FSH secretion. Medan, et al. (2003) found an inverse relationship between FSH and inhibin A during estrous cycle in goats that confirms the hypothesis that inhibin A contributes to the inhibition of FSH secretion.

The present investigation showed high progesterone levels during mid of estrous cycle which indicates fully developed corpus luteum. There is no positive correlation between inhibin and progesterone (-0.39). From the obtained results, it could be confrmed that corpus luteum is the source of inhibin in buffaloes. Similar observations were reported by Singh, et al. (2001) in buffaloes and Kaneko, et al. (1992) in cattle.

It could be concluded that each inhibin and estradiol-17β seems to have a feedback regulatory effect on FSH secretion in Egyptian buffaloes.

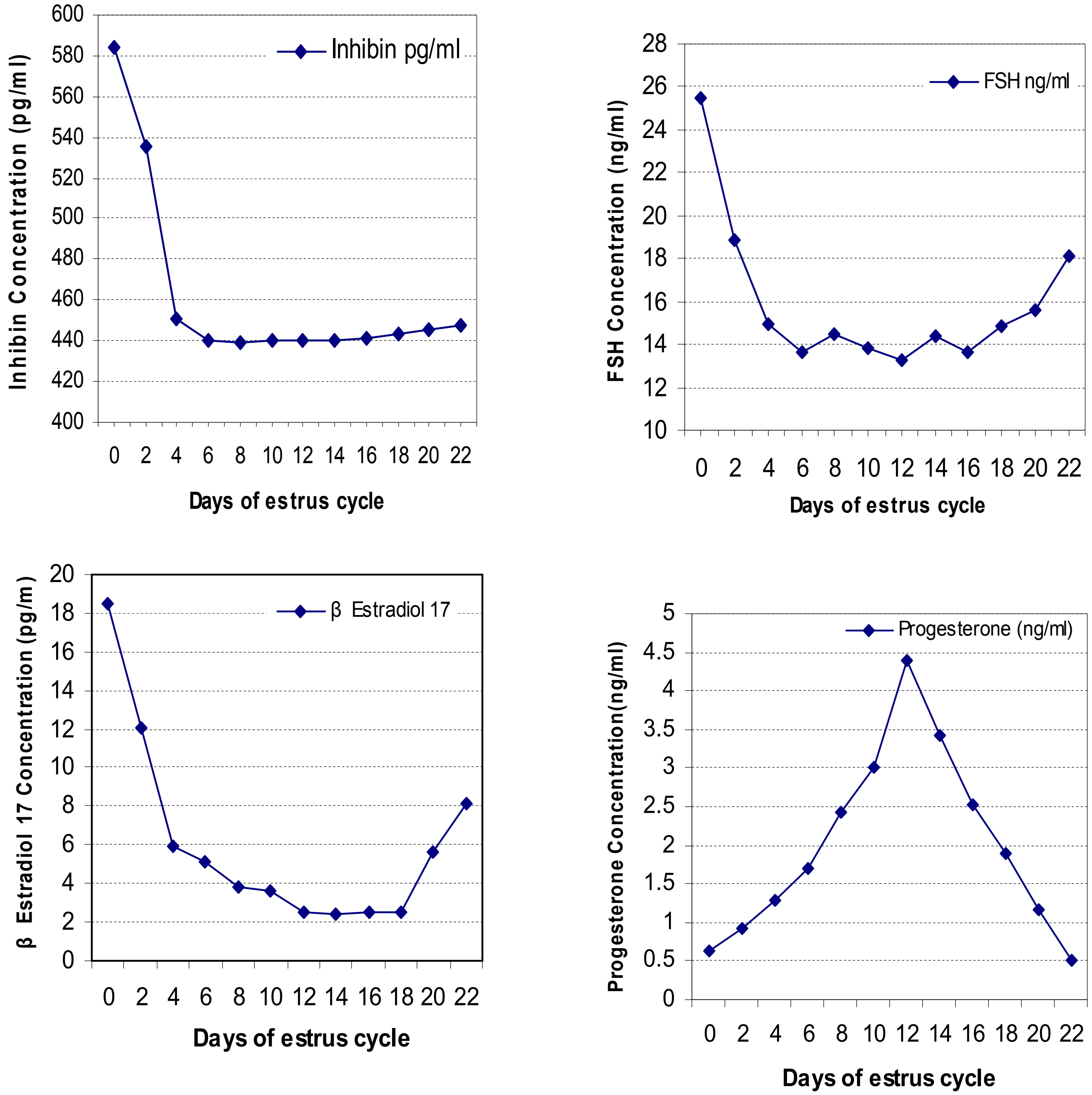

Figure1. Plasma concentrations of inhibin, FSH, estradiol-17β and progesterone during days of estrous cycle.

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