Preliminary study on water buffaloes (*Bubalus bubalis*) in marshes of Iraq

(Defining some values of reproductive hormones)

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Summary :

The present study aimed to determine some of reproductive values of water buffaloes in marshes of Iraq which may be used as a primary values for assaying the reproduction and production of water buffaloes. The study performed on immature male and female water buffaloes. LH, FSH and Prolactin hormones were determined. The results showed effect of sex and age on FSH and Prolactin levels. The sex was effective in LH level, while the age was no effective on LH level of male water buffaloes, whereas it was effective in LH level of females. Results were discussed from physiological aspect as compared to previous scientific studies, especially these values, as to Iraqi buffaloes, are regarded new preliminary values.

Key words: Reproductive hormones , Water buffaloes , Marshes of Iraq

الخلاصة :

هدفت الدراسة الحالية تحديد بعض القيم التكاثرية في جاموس مناطق اهوار العراق التي يمكن ان تعتمد كقيم اولية لتقييم تكاثرية وانتاجية هذا الحيوان الاقتصادي ، حيث درست مستويات الهرمون المحفز للجريب والهرمون اللوتيني وهرمون الحليب لذكور واناث الجاموس وضمن مجموعتين عمريتين (اقل من اويساوي 6 اشهر ، اكثر من 6 اشهر). اظهرت نتائج الدراسة الحالية تاثير الجنس والعمر في مستوى الهرمون المحفز للجريب وهرمون الحليب . لم يكن للجنس تأثير معنوي في مستوى الهرمون اللوتيني، بينما كان للعمر تاثيرا في مستوى الهرمون اللوتيني لذكور الجاموس فيما لم يؤثر في مستوى الهرمون اللوتيني للإناث. وقد نوقشت النتائج من الناحية الفسيولوجية مقارنة مع دراسات علمية سابقة لاسيما وان هذه القيم بالنسبة للجاموس العراقي تعتبر قيم اولية جديدة.

Introduction:

Buffaloes contribute more than one-third of total milk production in Asia and are the second largest producer of milk in the world. Buffaloes are also valued for meat and draught purposes (Bandyopadhyay *et al.* , 2003). The world population of buffaloes is approximately 170 million heads (FAO, 2003).

Asian buffalo includes two subspecies known as the River and Swamp types, the morphology and purposes of which are different as are the genetics. The River buffalo has 50 chromosomes of which five pairs are submetacentric, while 20 are acrocentric: the Swamp buffalo has 48 chromosomes, of which 19 pairs are metacentric. The difference in the diploid number is only apparent. In fact, the large Swamp buffalo chromosome 1 originated from tandem fusion translocation between the River buffalo chromosome 4 (telomeres of p-arm) and 9 (centromere) (Di Berardino and Iannuzzi, 1981). During this phenomenon, the nucleolus organizer regions (NORs) present in the River buffalo chromosome 4p were lost and the centromere of chromosome 9 inactivated (Di Berardino and Iannuzzi, 1981). Swamp buffaloes are mostly found in south east Asian countries. A few animals can also be found in the north eastern states of India (Sethi, 2003). Buffaloes are known to be better at converting poor-quality roughage into milk and meat. They are reported to have a 5 percent higher digestibility of crude fibro than high-yielding cows; and a 4-5 percent higher efficiency of utilization of metabolic energy for milk production (Mudgal, 1988).

In Iraq, according to data provided by Borghese (2004), there where 98 000 total River Khuzestani or Iraqi buffaloes, 40 000 adult females with kg 1 320 as medium lactation milk yield, in a 270 day lactation period. Buffaloes are bred in the marshes and swim far and wide for feeding on papyrus, reeds, common ash and other plants. When the flood water is high their owners have to go out and collect these plants in order to feed the buffaloes on platforms. Rice hulls are also given when available. Buffaloes in towns rarely graze on natural pasture; they are fed mostly on concentrates, green forage, straw and agricultural by-products(Magid ,1996).

Follicle stimulating hormone (FSH) is responsible for the early development of the ovarian follicle in the female and for the initial steps of spermatid maturation in the male. The luteinizing hormone (LH) stimulates testosterone synthesis by the interstitial cells of Leydig of the testes. The action of LH is dependent on FSH induction of Leydig cell LH receptors. Indirectory LH stimulates spermatogenesis by way of its effect on testosterone biosynthesis, which is require for maturation of the germ cell. In female LH induces ovulation and is necessary for the initial development of the corpus luteum. (Hadley, 2000). Prolactin synergizes with LH by increasing LH receptor sites in the corpus luteum. It also has a stimulating effect on the development of the mammary gland and the synthesis of milk (Terzano *et al.* , 2005).

According to rare or decrease of the studies related with water buffaloes in marshes of Iraq, the importance of the levels of hormones in the reproduction of production, and in order to observe and differences between the gonadotropin hormones and prolactin in immature male and female water buffaloes aged less than 6 months and above 6 months, the present study has been done.

Materials and Methods:

Animals: Immature male and female water buffaloes at marshes of south of Iraq were used during the period between October 2007 – May 2008. The animals were divided into two groups of age, the first one was equal or less than 6 month and the other was more than 6 months. Blood samples were collected from jugular vein under possible minimal stress. These samples were put in a tube with out any anticoagulant to separate serum by using a centrifuge with 2500 for 15 minutes for assaying the levels of hormones.

Measurements of hormones: Levels of FSH, LH and Prolactin were determined by Enzyme Linked Immuno Sorbent Assay (ELISA) (Amballi *et al.*, 2007) by using of Elisa reader type BioElisa ElX800, Germany, with kits, BioCheck, Inc, Germany. .

Statistical analysis:

Statistical analysis was performed using one way analysis of variance (ANOVA). If a significance was found, differences among individual group means were tested by the least significant difference (LSD) test. Values were considered statistically significant at P≤0.05. Data are presented as Mean±Standard error.

Results:

Effect of sex and age on FSH level :

The results in table (1) showed a significant differences in FSH level in the two age groups of male water buffalo. The male aged equal or less than 6 months was high in FSH level than male age mare than 6 months. Also, there was a significant differences in FSH level between groups of female ages. The female aged more than 6 months was high in FSH level than female aged less than 6 months. The results indicated a significant increase in FSH levels in two age groups of female water buffalo compare with male water buffalo at ≤ 6 months and these at > 6 months old.

Table (1) : Effect of correlation between sex and age on FSH level of water buffaloes.

Sex	**Age**	**N of samples**	**FSH level (Mean ± S.E.)**
Male of water buffaloes	6 months ≤	10	2.92 **c** ±0.20
	> 6 months	10	1.51 **d** ±0.08
Female of water buffaloes	6 months ≤	10	37.30 **b** ±3.03

The basic data related with the level of FSH in two age periods of male and female water buffalo explained in figure (1).This figure contain the results of all samples of male and female water buffaloes.

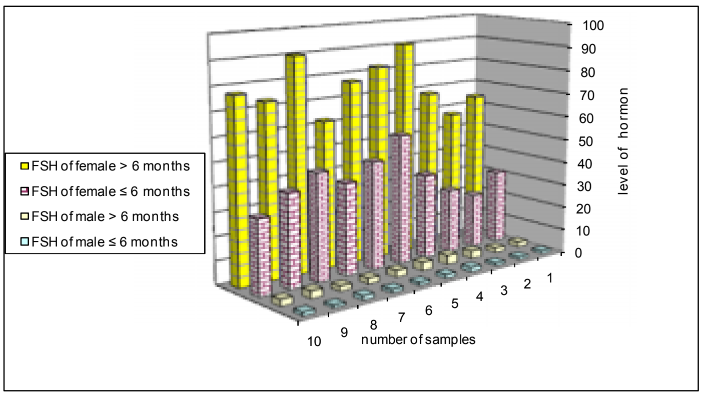

Figure (1) : The basic data of FSH level in male and female water buffaloes.

Effect of sex and age on LH level :

The results of the effect of correlation between sex and age of the level of LH in male and female water buffaloes were presented in table (2). These results showed a non significant effect of age on LH level in male water buffaloes, whereas there was a significant in LH level of female water buffalo at age equal or less than 6 months compare with these female at age more than 6 months. Too, the results indicated a significant increase in LH levels in two age groups of female water buffalo compare with male water buffalo at ≤ 6 months and these at > 6 months old.

Sex	Age	N of samples	LH level Mean ± (S.E.)
Male of water buffaloes	6 months ≤	10	0.19 **c** ±0.01
	> 6 months	10	0.20 **c** ±0.008
Female of water buffaloes	6 months ≤	10	1.64 **a** ±0.07

Table (2) : Effect of correlation between sex and age on LH level of water buffaloes

The basic data of LH level in two age periods of male and female water buffalo explained in figure (2). This figure contain the results of all samples related with LH level in male and female water buffaloes.

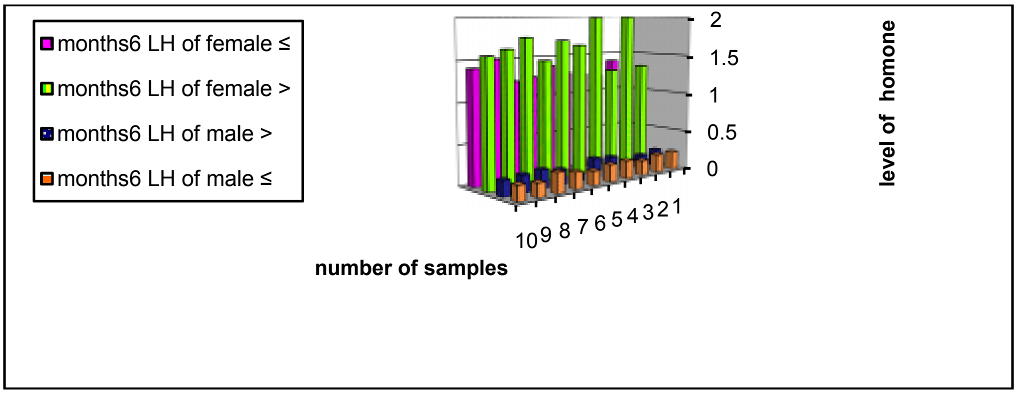

Figure (2) : The basic data of LH level in and female water buffaloes.

Effect of sex and age on Prolactin level :

The results of the effect of sex and age of the level of Prolactin in male and female water buffaloes were presented in table (3). The results was shown a significant differences between the male aged groups. The prolactin level of males aged equal or less than 6 months was higher than males aged more than 6 months. Also, the results indicated a significant differences between the female aged groups. The prolactin level of females aged more than 6 months was higher than females aged equal or less than 6 months. The results indicated a significant increase in FSH levels in two age groups of female water buffalo compare with male water buffalo at ≤ 6 months and these at > 6 months old.

Table (3) : Effect of correlation between sex and age on Prolactin level of water buffaloes

Age	N of samples	Prolactin level (Mean ± S.E.)
6 months ≤	10	2.67 **c** ±0.12
> 6 months	10	1.71 **d** ±0.10
6 months ≤	10	237.13 **b** ±10.13
> 6 months	10	334.00 **a** ±6.50

The basic data related with the level of Prolactin in all samples of water buffalo illustrated in figure (3).

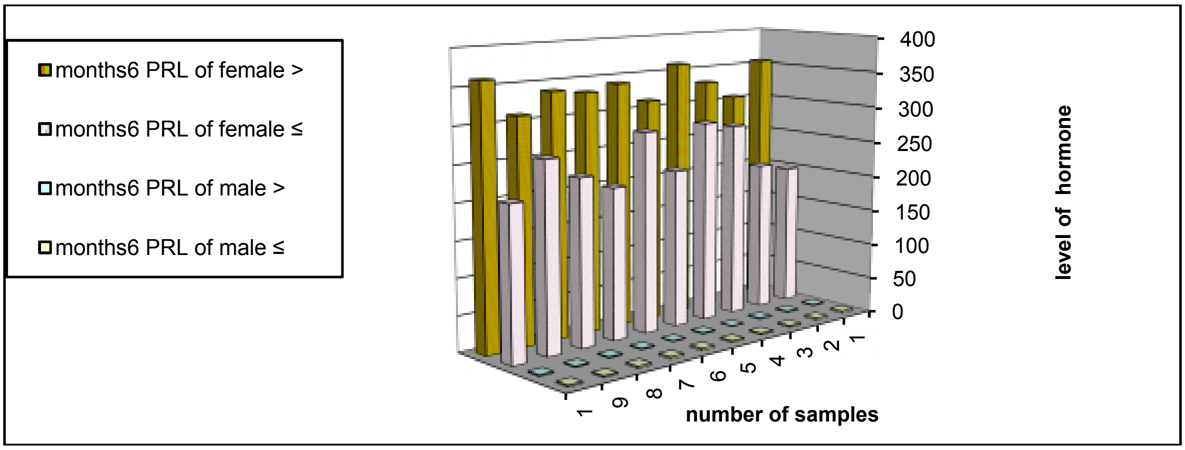

Figure (3) : The basic data of Prolactin level in male and female water buffaloes.

Discussion :

The results of the present study showed the influence of sex and age of the level of FSH and Prolactin, while there is non significant effect for sex and age on LH level. It is very important for study of the hormonal parameters in water buffalo, but one of the problem that we faced in this study is the rare literature dealing with the subject of the present study.

The follicle stimulating hormone (FSH) promotes follicle growth and estrogen production through granulosa cells in the ovarian follicles. This hormone has been studied by several authors in buffalo cows over the past few years. Seren *et al*. (1994) found the FSH surge (1.6-6.0 ng/ml) to be coincident with the LH peak and to have a duration time of six to nine hours, with a lack of evident additive peaks during the luteal phase of the oestrus cycles, when the hormone levels ranged between 0.2-1.5 ng/ml. Singh et al. (2001) also found the FSH surge to be coincident with the LH peak, averaging near 25 ng/ml, but reported the major surge of this hormone on the tenth day after oestrus and a minor increase on days 4 and 15 after oestrus. Palta and Madan (1995, 1997) recorded FSH peak means of 70 - 80 ng/ml and a duration time of 5.8 ± 0.07h (during pregnancy) and 5.5 ± 0.02h (during postpartum) after GnRH stimulation. It has now been established also in buffalo that a transient rise in serum concentration of FSH begins each follicular wave (Baruselli *et al.* , 1997; Singh *et al.* , 2001; Presicce *et al.* , 2003) and a decreased episodic secretion of LH is associated with loss of dominance and with the end of a nonovulatory follicular wave. Palta and Madan (1995, 1997) found alterations of the gonadotrophins release by hypophysis in pregnant and postpartum buffalo cows. The basal level of FSH presented significant differences during pregnancy; in fact it was higher at day 60 than at day 240 of pregnancy. During postpartum the basal levels of LH and FSH increased significantly from day 2 to day 20, but only the LH levels were higher at day 35 in comparison to day 20. The authors pointed to a progressive reduction of the hypophysial responsiveness to the GnRH during pregnancy and this could be due to the chronic negative feedback exerted by gonadal steroids, together with a probable reduction of GnRH receptors in the pituitary, both verified in other ruminants (Nett, 1987; Schoenemann *et al*., 1985). After delivery a progressive reestablishment of the positive feedback by estrogens was observed and a rise of the basal gonadotrophins levels was recorded.

The luteinizing hormone (LH) is important in studies of ovarian activity since its preovulatory surge is responsible for the rupture of the follicle wall and ovulation. The peak values are always well defined in respect to the basal blood levels; the duration of LH surge was estimated by different authors and was calculated at 6 to 12 hours(Seren et al., 1994) or 6 to 9 hours (Maurel *et al*., 1995) or 8 to 12 hours(Barile *et al*., 1998) in Mediterranean buffalo cows. Buffalo cows are characterized by the occurrence of two peaks of the hormone during the oestrus time; this phenomenon was recorded by Seren *et al.* (1994) in25 percent of the 24 observed oestruses. The double LH peak was frequently followed by double ovulation that also occurred after a single peak in an additional 8 percent of the animals. LH peak time (in relation to oestrus symptoms and to ovulation time) is more important than the LH peak. Seren *et al*., (1994), monitoring ovulation time by ultrasound, found that the mean time of LH peak-ovulation was 35.5 hours (in buffalo cows with single ovulation) and 60 hours (in buffalo cows with double ovulation). Moioli *et al.* (1998), in spontaneous oestruses of buffalo cows, found a mean interval LH peak-ovulation (this latter detected by rectal palpation considering follicle changes from turgid to flaccid) of 25.2 ± 13.1h and of 46.1 ± 18.8h respectively in oestruses followed by pregnancy and in those not followed by pregnancy.

In buffalo cows prolactin blood concentrations during the oestrus cycle have not been extensively studied. Seren *et al.* (1994) reported a pulsatile secretion starting before the luteolysis and lasting during the oestrus, ending near the ovulation time. The plasma levels were very variable, ranging from 10-20 to 150-200 ng/ml.

Owing to such rarity in studies related with water buffaloes in Iraq, especially on reproductive hormones, we suggest that future studies should be geared to tackle with such subject with different ages of animals for its importance in reproductivity and productivity.

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