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SERUM PROGESTERONE AND OESTRADIOL CONCENTRATION IN THE CYCLIC BUFFALO

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Levels of serum progesterone and oestradiol were measured on days -1, 0 (day of oestrus), 1 and 11 of the oestrus cycle of 8 buffalo heifers and 3 buffalo cows during both the hot season (March - May) and the cold season (October - November). Daily variation in the hormonal levels were monitored during October and November. Levels of oestradiol were measured in two-hourly samples taken during the period from the onset of heat to ovulation.

A low level of progesterone (< 0.1 ng/ml) was maintained during the period from day -1 to day +1. It started to increase gradually during the luteal phase and reached a peak level of 5.5 ± 1.4 ng/ml on day 17. This was followed by a rapid decline to a low level (< 1.0 ng / ml) within two days. Oestradiol level started to increase from day 3 and a peak level (20pg/ml) was recorded during the follicular stage.

During the heat period, oestradiol level fluctuated in a pulse fashion with the highest peak level (13.8 pg/ml) recorded at about 6 h after the onset of heat.

Significantly (P < 0.05) higher oestradiol levels were recorded on the day of oestrus in the cold as compared to the hot season. Progesterone levels on day 11 were higher in the hot than in the cold season.

Key words: buffalo, hormones, reproduction

Improvement of the reproductive performance of female farm animals depends on the understanding of the physiology of the reproductive cycle and the hormonal changes associated with it. Progesterone and oestradiol are the main gonadal hormones that reflect the various events occurring throughout the oestrous cycle. There have been several reports on circulating levels of progesterone and oestradiol during the oestrous cycle in cattle (Chenault et al 1975; Dobson 1978). Reports on their levels in buffalos , however, are scant (Pandey 1979; Bachlaus et al 1979). During the hotter months of the year, many buffalos exhibit lower oestrous and ovarian activity (Shafie et al 1982). We have therefore examined circulating levels of progesterone and oestradiol in buffaloes at various stages of the oestrous cycle during both the hot and cold seasons. The transitory changes in oestradiol level during the heat period were also studied.

Materials and Methods

Animals and their management: Eight buffalo heifers and three buffalo cows (*Bubalis bubalus*) were used in this study which was carried out in the Animal Physiology Laboratory, Faculty of Agriculture, Cairo University.

The animals were housed in a semi-open yard and tied under a shed . They were given a maintenance diet according to their live body weight ,

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and were checked for the onset of oestrus three times daily at 0800,1400, and 2000 h using two aproned fertile bulls during the months of March , April and May.

During October and November the animals were checked for oestrous activity every four hours during the expected period of oestrus and until the cessation of the manifestation of heat.

Blood sampling: Blood samples were taken from the jugular vien and serum was separated and stored at -18°C until assayed. During March,April and May daily samples were collected on day -1, day 0 (day of oestrus), day +1 and day 11 post oestrus. Samples were taken on four consecutive days before the expected day of oestrus but only those taken on the day preceding the detected oestrus were analysed.

During October and November, blood samples were taken from the two heifers and three cows on the day of heat (day 0), days 1,2,3,4,7,10,13 , 16 post oestrus. This was followed by daily samples until the onset of the next oestrus. Two-hourly samples were also taken from these five animals starting from the time of onset of heat until ovulation was detected by rectal palpation, performed at times of blood sampling.

Hormonal assays: Progesterone: Serum progesterone level was measured using a double antibody radioimmunoassay method (Garza et al 1980). At 50% displacement,the antiserum obtained (Radioimmunoassay Systems Laboratories Inc., USA) had values of percentage cross reaction of 100% with progesterone, 6.25% with 20 α-dihydroprogesterone, 3.20% with desoxycorticosterone, 0.42% with corticosterone and 0.15% with 17 α-hydroprogesterone. The assay of 5.0, 10.0, 20.0 and 30.0 ng progesterone standard added to a progesterone-free serum (previously treated with charcoal) gave mean recoveries of 95, 100, 90 and 88% respectively. Progesterone standard was used at a range from 0.1 to 100 ng/ml. Sensitivity when assaying 0.05 ml aliquots was 0.1 ng/ml and was calculated as the lowest standard concentration that differed significantly in number of counts from a blank. The inter-assay and intra-assay coefficients of variation were 11.3 and 9.7%.

Oestradiol: A radioimmunoassay method (Radioassay Systems Laboratories, USA 1980) involving the use of double antibody was employed . The antiserum supplied had been checked for cross reactivity, and at 50% displacement the cross reaction percentages were reported to be 100% with oestradiol 17β, 6.5% with Oestriol, 5.20% with Oestradiol 17β and 0.5 % with Oestrone. The assay of 30, 45, 50, 100 and 200 pg oestradiol added to aliquots of oestradiol-free plasma gave mean recoveries of 116,98,103, 110 and 95% respectively. Oestradiol standard was used as a range of 2.5 to 250 pg/ml.

Serum samples were extracted using a mixture of ethyl acetate hexane (3:2 by volume), shaken for 60 seconds and dried under a current of air . In the calculation of results adjustment was made for the efficiency of extraction (96%).

Sensitivity when assaying 0.6 ml aliquots of serum was 2.5 pg, calculated as the lowest standard concentration that differed significantly in the number of counts from a blank.

The inter-assay and intra-assay coefficients of variation were 9.8 and 13% respectively.

Results and Discussion

A. Hormonal levels at different stages of the oestrous cycle: The overall mean concentrations of serum progesterone and oestradiol at different stages of the cycle are presented in Table 1.

Table 1:

Serum progesterone and oestradiol concentrations ($\bar{x} \pm SE$) measured at different days of the oestrous cycle.

	Days of the cycle			
	-1 pre-oestrus	0 day of oestrus	+1 metoestrus	+ 11 dioestrus
Progesterone (ng/ml)	0.3 ± 0.2	< 0.1	< 0.1	3.7 ± 0.9
Oestradiol (pg/ml)	25.1 ± 3.9	25.9 ± 2.8	23.8 ± 3.4	28.7 ± 8.5

1. Progesterone: On day -1 (pre-oestrus) the average progesterone level was 0.3 ± 0.2 ng/ml. In most cases the level was not detectable (below 0.1 ng/ml). This value is similar to that reported by other workers(Pandey 1979; Bachlaus et al 1979; Aboul-Ela 1982). In one case, however, a high value of 2.0 ng/ml was found. Ayalon and Shemesh (1974), found that the concentration of progesterone in cattle increased to a peak of 2.28 ng/ml at 1600h before the onset of heat. They added that the peak could be missed if blood samples were collected at intervals less frequent than 4 hours. Aboul-Ela (1980) postulated that the presence of progesterone during(pro-oestrus)is preparatory for ovulation and heat manifestation.

During both oestrus and met-oestrus periods (days 0 and +1, respectively), the levels remained below 0.1 ng/ml in all animals. This is in accord with other reports in cattle (Chenault et al 1975) and in buffalos (Kamonpatana et al 1979; Bachlaus et al 1980; Aboul-Ela 1982).

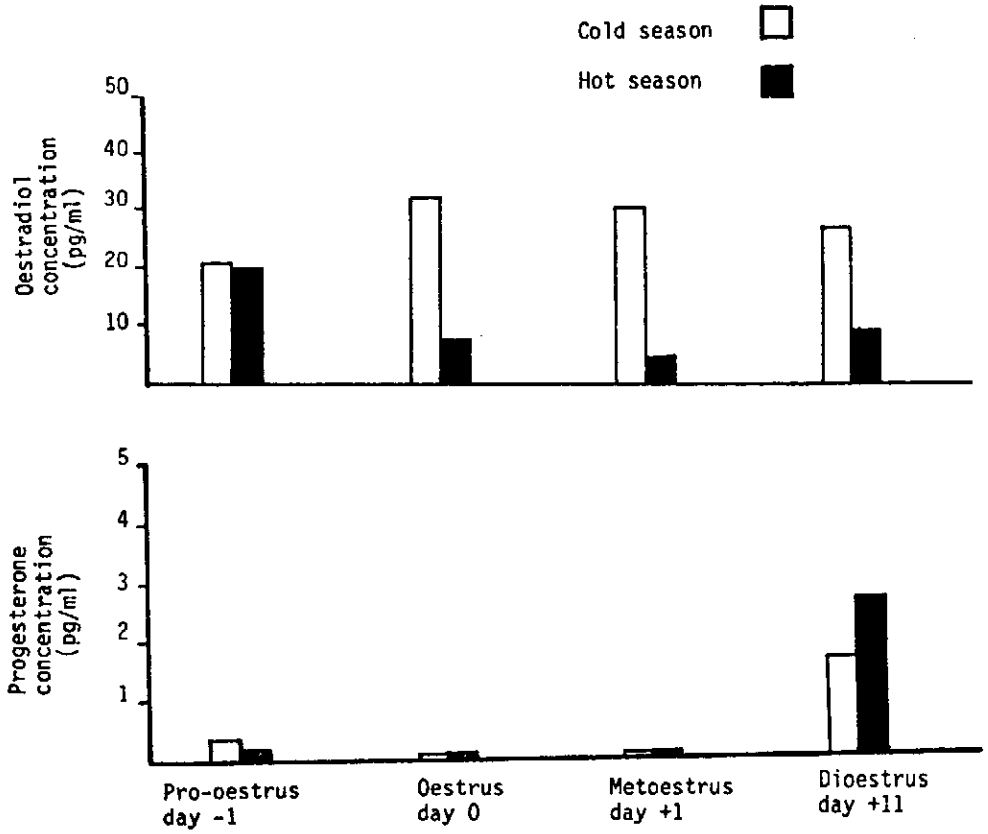
Average concentration on day +11 was 3.7 ± 0.9 ng/ml which is close to that reported by Pandey (1979).

2. Oestradiol: On days -1 and 0 the average serum ostradiol concentrations were almost identical, being 25.1 ± 3.9 and 35.9 ± 2.8 pg/ml, respectively. The level decreased slightly on day +1 (23.8 ± 3.4 pg/ml) with relatively large variation among individual animals. Such variation was even more noticeable on day +11 on which the average oestrdiol concentration was 28.7 ± 8.5 pg/ml. This high level was caused by two high individual values of 83.0 and 58.3 pg/ml, without which the average concentrations would drop to 16.7 ± 2.8 pg/ml.

Effect of season on serum hormonal levels: Levels of both serum progesterone and oestradiol measured at different stages of the oestous cycle during the hot season (March-May) were compared with their levels measured at the corresponding cycle stages during the cold season (October-November), Figure 1. It is of interest to note that levels of oestradiol in the cold season were significantly higher ($P < 0.01$)at all stages of the cycle, apart from day -1, than those measured at the corresponding

Figure 1:

Levels of serum oestradiol and progesterone measured on different days of the cycle during either the cold or the hot season



cycle stages in the hot season. On the other hand, progesterone levels were higher in the hot than in the cold season when measured at dioestrus (day +11). Its concentration remained at the lower limits of detection at the other stages of the cycle in both seasons.

These results indicate a possible role of the climatic conditions on the levels of sex hormone. The decrease of oestradiol level in the hot season, particularly on the day of oestrus may depress the intensity of heat manifestation leading to a higher incidence of silent heat. Lower oestrous activity during the hot season has been reported previously (El-Fouly et al 1976; Shafie et al 1982). The relationship between levels of sex hormones and the pre-ovulatory behaviour have been investigated in the cow (Glencross et al 1981). Detailed trials are needed, however, to investigate such relationships in the buffalo.

B. Daily variation in hormonal changes throughout the oestrous cycle: Figure 2 illustrates the levels of both progesterone and oestradiol measured throughout the cycle in the cold season. Progesterone level remained non-detectable (below 0.1 ng/ml) during the period from the day of oestrus (day 0) to day +3, before it started to increase gradually, reaching a peak level of 5.5 ± 1.4 ng/ml (range 3.3 - 9.8 ng/ml) usually on day + 17 (ranging between days + 17 and +19). This was followed by a rapid decrease to reach a minimum value within two days and remained low until the next heat. On some individuals a second but smaller peak occurred on the day preceding the day of oestrus, as discussed earlier.

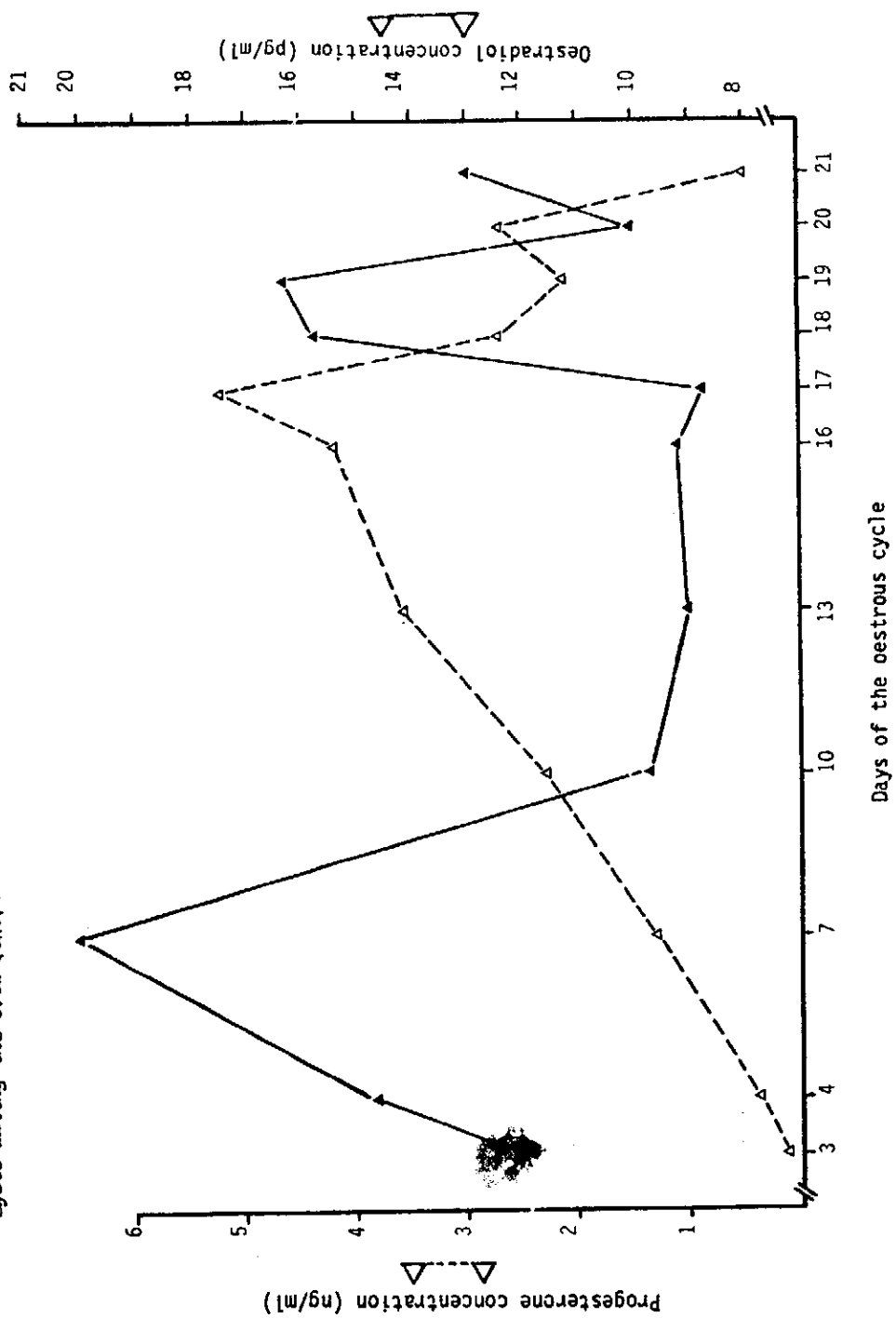


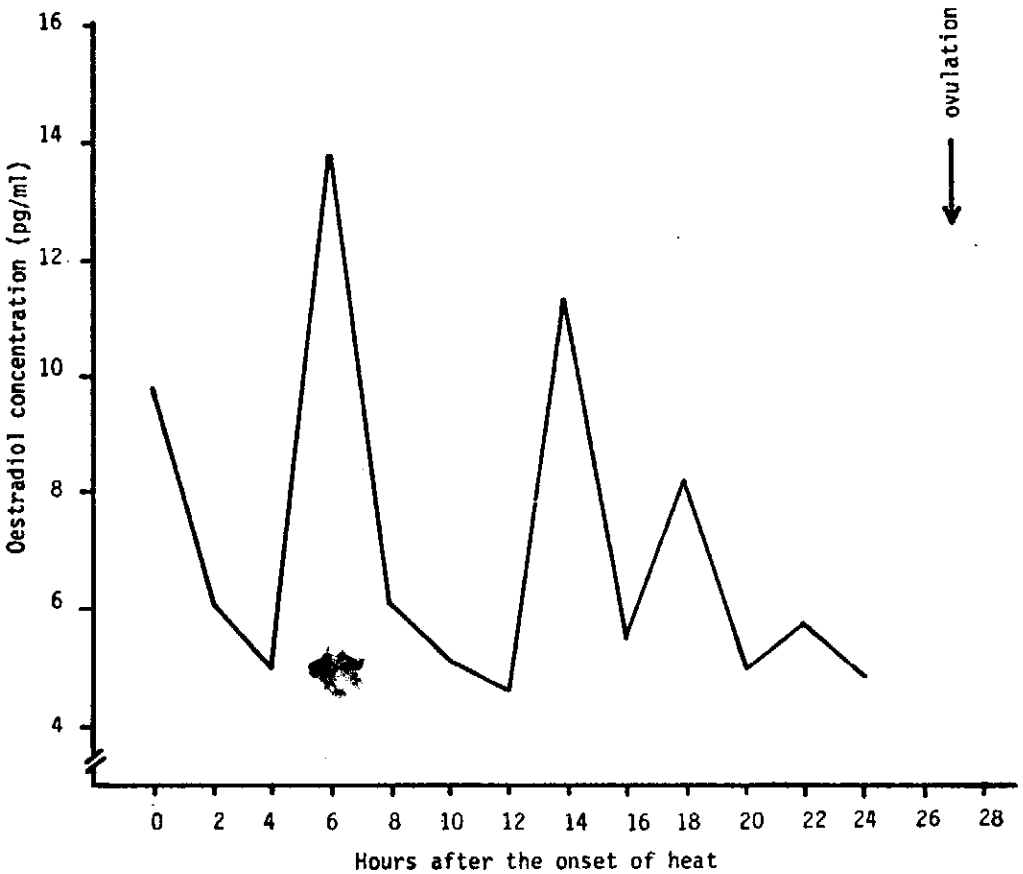
Figure 2:
Changes in the levels of serum progesterone and oestradiol measured throughout the oestrous cycle during the cold season

The nature and timing of changes in progesterone level throughout the cycle observed in the present study is generally similar to those reported by other authors (Pandey 1979; Bachlaus et al 1979, 1980; Aboul-Ela 1982). The levels measured during the luteal phase are similar to those reported by Bachlaus et al (1980). Higher levels were reported by Aboul-Ela (1982). The timing of peak progesterone at a later stage in the cycle (days 14-16) might be a reason for the longer oestrous cycle length reported in the buffalo (Shafie et al 1982) than that observed for cattle.

Oestradiol concentration fluctuated throughout the oestrous cycle (Figure 2). The average concentration on day +3 was 12.0 ± 1.5 pg/ml and increased gradually to reach a peak level of 20.0 pg/ml on day 7. This was followed by a rapid decline and remained at a low level from day 10 to day 17. Another peak occurred prior to the day of oestrus. The mid-cycle oestradiol peak is similar to that reported in cattle by Glencross et al (1973) who attributed it to mid-cycle follicular growth.

Oestradiol changes during the heat period: Average values of oestradiol concentration taken at two-hourly intervals during the period from the onset of heat to ovulation are illustrated in Figure 3.

Figure 3:
Mean serum oestradiol concentration measured during the heat period



At the time of onset of heat average oestradiol level was 9.8 ± 2.9 pg/ml. The peak level (13.8 ± 3.8 pg/ml) occurred about six hours after

the onset of heat. A second peak was seen 8 - 12 hours later after which concentration returned to a low level which was maintained until ovulation. Chenault et al (1975) and Dobson (1978) reported that peak oestradiol level, in the cow, coincided with the LH peak at the middle of the heat period. Variation was also noticeable between successive heat periods within individual animals.

Conclusions

It could be concluded from this study that the nature of changes in progesterone and oestradiol in the buffalo is similar to that of the cow. The interval from ovulation to maximum progesterone level is longer in the buffalo and may be a reason for its longer oestrous cycle as compared to cattle. Season of the year exerts a significant effect on the oestradiol level around oestrus. This finding needs further investigation to distinguish between the various environmental factors involved and to elucidate the control mechanism(s).

References

Aboul-Ela M B 1980 A study of the vaginal electrical resistance in cattle as related to hormone changes and its application in the detection of oestrus PhD Thesis Aberdeen University UK

Aboul-Ela M B 1982 Changes in the intravaginal electrical resistance in relation to oestrus and ovulation in the buffalo Proceedings of seminar on Buffalo Reproduction and Meat held at Tanuku India 14-17 January 1982

Ayalon N and Shemesh M 1974 Pro-oestrus surge in plasma progesterone in the cow Journal of Reproduction and Fertility 36:239-243

Bachlaus N K, Arrora R A, Prassad A & Pandey R S 1979 Plasma levels of gonodal hormones in cycling buffalo heifers Indian Journal of Experimental Biology 17:823-825

Bachlaus N K, Arrora R C, Prassad A & Pandey R S 1980 Effect of prostaglandin $F_{2\alpha}$ -Thamsalt and estrumate (ICI 80996) on plasma progesterone levels in Indian water buffalo (*Bubalus bubalis*) Theriogenology 13:297-303

Barkawi A 1981 Ovarian activity and oestrus detection in Egyptian buffalos MSc Thesis Cairo University Egypt

Chenault J R, Thatcher W W, Kaira P S, Abrams R M & Wilcox C J 1975 Transitory changes in plasma progestins, estradiol, and luteinizing hormone approaching ovulation in the bovine Journal of Dairy Science 58:709-717

Dobson H 1978 Radioimmunoassay of FSH in the plasma of postpartum dairy cows Journal of Reproduction and Fertility 52:45-49

El-Fouly M A, Fotby E A & El-Sobhy H E 1976 The functional reproductive peak in Egyptian cows is related to day length and ambient temperature Archivio Veterinario Italiano 27:123- 129

Garza R, Chow N, Perrin R A L & Rao P N 1980 Radioassay Systems Laboratories Inc Unpublished data

Glencross R G, Esslemont R J, Bryant M J & Pope G S 1981 Relationship between the incidence of pre-ovulatory behaviour and the concentrations of oestradiol - 17β and progesterone in bovine plasma Applied Animal Ethology 7:141-148

Glencross R G, Munro I B, Senior B E & Pope G S 1981 Concentrations of oestradiol 17β, oestrone and progesterone in jugular venous plasma of cows during the oestrous cycle Acta Endocrinologica (KBn.) 73:374-384

Kamonpatana M, Kunawongkrit A, Badhipaksha P & Luvira Y 1979 Effect of $PGF_{2\alpha}$ on serum progesterone levels in the swamp buffalo (*Bubalus bubalis*) Journal of Reproduction and Fertility 56: 445-449

Pandey R S 1979 Hormonal stsus of the female and induced breeding in Murrah buffaloes In: Buffalo Reproduction and Artificial Insemination FAO Animal Production and Health Paper 13:185

Radioassay Systems Laboratories 1980 Procedure for the radioimmunoassay of serum or plasma estradiol - 17β Radioassay Systems Laboratpries Inc California USA

Shafie M M, Mourad H, Barkawi A & Aboul-Ela M B 1982 Characteristics of the oestrous and ovulation cycles in buffalo heifers Zuchthygiene (in press)

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