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CONCURRENT CHANGES OF PLASMA PROGESTERONE, OESTRADIOL- 17β, OESTRONE SULPHATE, CORTICOSTEROIDS AND A METABOLITE OF PGF2α THROUGHOUT PREGNANCY, AROUND PARTURITION AND DURING POSTPARTUM IN BUFFALO COWS

By

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SUMMARY

Concentrations of plasma progesterone, oestradiol-17β, oestrone sulphate, corticosteroids and 13, 14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ were measured in 12 buffalo cows during the whole length of gestation, around parturition and 15 days postpartum. Concentrations of progesterone and oestradiol-17β increased slightly during the first 2 (3.5 ± 0.9 ng/ml) and 4 (14.8 ± 2.1 pg/ml) months of pregnancy. Their walues remained fairly constant until the end of pregnancy period when progesterone concentrations decreased significantly (P<0.05) at day 7 (0.9 ± 0.1 ng/ml) and oestradiol-17β increased markedly (P<0.01) at day 10 (26.3 ± 2.6 pg/ml) prior to parturition. Progesterone showed basal values (<0.5 ng/ml) from day 4 prepartum to day 15 postpartum while oestradiol-17β concentrations were maximal (82.8 ±3.6 pg/ml) during labour reaching basal values (<12 pg/ml) at day 5 postpartum. Concentration of oestrone sulphate remained low (< 140 pg/ml) during the first half of gestation period increasing sharply (P<0.01) thereafter to a peak of 5920 ±116.5 pg/ml by 30 hours prepartum and declined to about 50% of its concentrations afterwards reaching basal levels (<80 pg/ml) at day 2 postpartum. Concentrations of corticosteroids fluctuated narrowly (1.7 ± 0.3 ng/ml) throughout the whole gestation length, increasing significantly (P<0.05) at day 12 prepartum (5.3 ± 1.8 ng/ml) and peaking to 16.8 ± 3.2 ng/ml at the moment of delivery. Their values declined below 3 ng/ml from day 3 postpartum onwards. PGFM concentrations were at basal levels (200-600 pg/ml) throughout the first 9 months of pregnancy, beginning to increase progressively 9 days prepartum (2.2 ± 0.2 ng/ml) and reaching maximal concentrations (13.8±2.3 ng/ml) during delivery of the foetus. The postpartum PGFM concentrations remained in excess of 5 ng/ml during the first week and reaching basal values at day 12 postpartum.

We concluded that progesterone, in particular 3α-dihydroprogesterone, is essential for maintaining pregnancy in buffaloes and that maturational events leading to parturition are linked with both the luteolytic effect of corticosteroids and the oestrogen-stimulated increassed synthesis and release of $PGF_{2\alpha}$.

INTRODUCTION

Buffaloes contribute a major share in the dairy and meat production in Egypt and several south as well as south-east Asian countries. However, one of the major factors limiting more efficient utilization of the buffalo is its poor reproductive performance. In order to realize the buffalo's full reproductive potential, its reproductive endocrinology needs to be studied in detail.

Most reports on buffaloes were directed for the study of two or three hormones mainly progesterome and oestrogens, using small number of animals with insufficient blood sampling in many cases. Moreover, these studies were confined to either the first 30-50 days postmating (Batra et al., 1979; Suri et al., 1980; Chantaraprateep, 1987; Kamonpatana, 1987; Monika Gupta and Prakash, 1990), the first 8-10 months of pregnancy (Kamonpatana et al., 1983; Kamonpatana, 1984; Virakul, 1987; Hung and Prakash, 1990), around parturition (Perera et al., 1981; Kamonpatana, 1984; Prakash and Madan, 1986; El-Belely et al., 1988) or the 15-60 days postpartum (Perera et al., 1981; Jainudeen et al., 1983; El-Belely et al., 1988).

The present attempt was, therefore, undertaken to give a detailed information on the pattern of changes in plasma progesterone, oestradiol-17β , oestrone sulphate, corticosteroids and the major circulating prostaglandin F2α metabolite (PGFM) throughout the whole gestation period and around parturition as well as during the early postpartum period in a group of 12 buffalo cows.

MATERIALS AND METHODS

Animals:

Twenty-one multiparous and non-pregnant buffalo cows, which calved since 4-6 months and aged betweeen 6-9 years, were chosen from Turra farm near Cairo. All animals had been kept under the same feeding and management conditions. In each animal, the uterus and ovaries were palpated per rectum 3 times at 10 days interval to confirm the regularity of their oestrous cycles. Each cow received a double intramuscular injection (25 mg) of Lutalyse (Dinoprost Thromethamine, Belgium) at 11 days interval to synchronize oestrus and mated twice 12 and 24 hours following the induced oestrus by buffalo bulls of known high fertility. Rectal examination was performed thereafter at 2 weeks interval until the end of the experimental period. Seven animals returned to oestrus within 21 to 33 days postmating and 2 animals showed purulent vaginal discharges 64 and 82 days postmating. These 9 cows were disqualified from the results of this investigation.

Blood sampling:

Blood was collected from the 12 pregnant buffalo cows by jugular venepuncture once every 30 days throughout the whole gestation period. A siliconized catheter (Vasofix, B. Broun Melsungen AG, Germany) was inserted percutaneously into the jugular vein of each animal during the last month of gestation. Blood was collected daily from day 15-21 until day 7-10 and then every 12 hours up to 3-5 days before the expected day of parturition followed by sampling at 6 hours intervals until parturition was completed (0 hour). Blood was collected daily thereafter until 15 days postpartum and the catheter was then removed. Blood was drawn into vials containing EDTA, chilled in ice and centrifuged at 2000 g for 15 minutes. Plasma was stored frozen at -20°C until assayed.

Hormonal ansays:

Plasma steroid hormone concentrations were assessed in duplicates on toluene:petroleum ehter (4:5v/v) for progesterone and toluene:ether 94:5 v/v) for oestradiol-178 using procedures described by El-Belely (1993). The antibody used to assay progesterone cross-reacted considerably (61%) only with 3a-dihydroprogesterone. The sensitivity of the assay was 0.1 ng/ml tube. The average intra-and inter-assay coefficients of variation were 6.8% (n=10) and 8.2% (n=14), respectively. The specific antibody for oestradiol-17β cross-reacted with 100% oestradiol-17β, 84.6% oestrone, 18.5% oestradiol-17α and 10% oestriol. The sensitivity of the assay was 10 pg/ml tube. Intra-and inter-assay coefficients of variation averaged 7.9% (n=10) and 8.3% (n=16), respectively.

Concentrations of plasma oestrone sulphate were assessed as reported by Wright et al., (1978) using a specific anti-oestrone-3-hemisuccinate-BSA antiserum. Cross reactions were 100% oestrone sulphate, 25% oestrone, 70 % glucuronide, 5% oestradiol-17α and < 1% oestriol, androstenedione and cortisol. The sensitivity of the assay was 15 pg/ml tube. The average intra-and inter-assay coefficients of variation in 12 replicates were 18.6% and 21.2%, respectively.

Corticosteroids plasma concentrations were measured by a compelitive protein binding asay employed by Eissa & El-Belely (1990). The sensitivity of the assay was 0.1 - 0.2 ng/ml tube. The average intra-and inter-assay coefficients of variation were 11.2% (n=22) and 11.6% (n=30), respectively.

PGFM concentrations were assayed in unextracted plasma by radiolimmunoassay according to Kindahl et al., (1976). The antiserum cross-reacted approximately 16 % against 13, 14-dihydro-15-keto-$PGF_{2\alpha}$, 4% against 13, 14-dihydro-$PGF_{2\alpha}$ and 0.4% against $PGF_{2\alpha}$. A cross-reaction of <0.1 % was detected against other prostaglandins. The detection limit of the assay was 40 pg/ml tube. The intra-and inter-assay coefficients of variation averaged 16 and 21%, respectively.

Statistical analyses:

Variation in plasma hormonal concentrations was testd by least-squares analysis of variance using the general linear models (GLM) procedures of the Statistical Analysis System (SAS< 1990).

RESULTS

BACKGROUND INFORMATION:

The mean gestation length of the 12 buffalo cows under investigation was 314 ± 1.6 days 9range:310-318 days). Parturition was asiated in 3 cows while the other 9 animals had delivered normally. Eight female and 4 male calves were delivered healthy with weights considered normal (22-26kg). Complete expulsion of the foetal membranes in all animals was observed between 6 and 22 hours (mean 12.3 ± 2.1 hours) postpartus. The corpus luteum of pregnancy regressed very rapidly and was palpable as a hard small protuberance (<5 mm diameter) by day 10-15 postpartum.

Hormonal concentrations:

Plasma concentrations of progesterone, oestradiol-17β, oestrone sulphate, corticosteroids and PGFM are shown in Figs. 1,2 and 3, plotted as means ±SE.

Plasma concentrations of progesterone fluctuated between 1.9 and 3.8 ng/ml (3.5 ± 0.9 ng/ml) during the first 2 months of pregnancy decreasing slightly to 2.9 ± 0.8 ng/ml during the 3rd month and declined gradually until day 8 prepartum when it decreased (0.9 ± 0.1 ng/ml) significantly

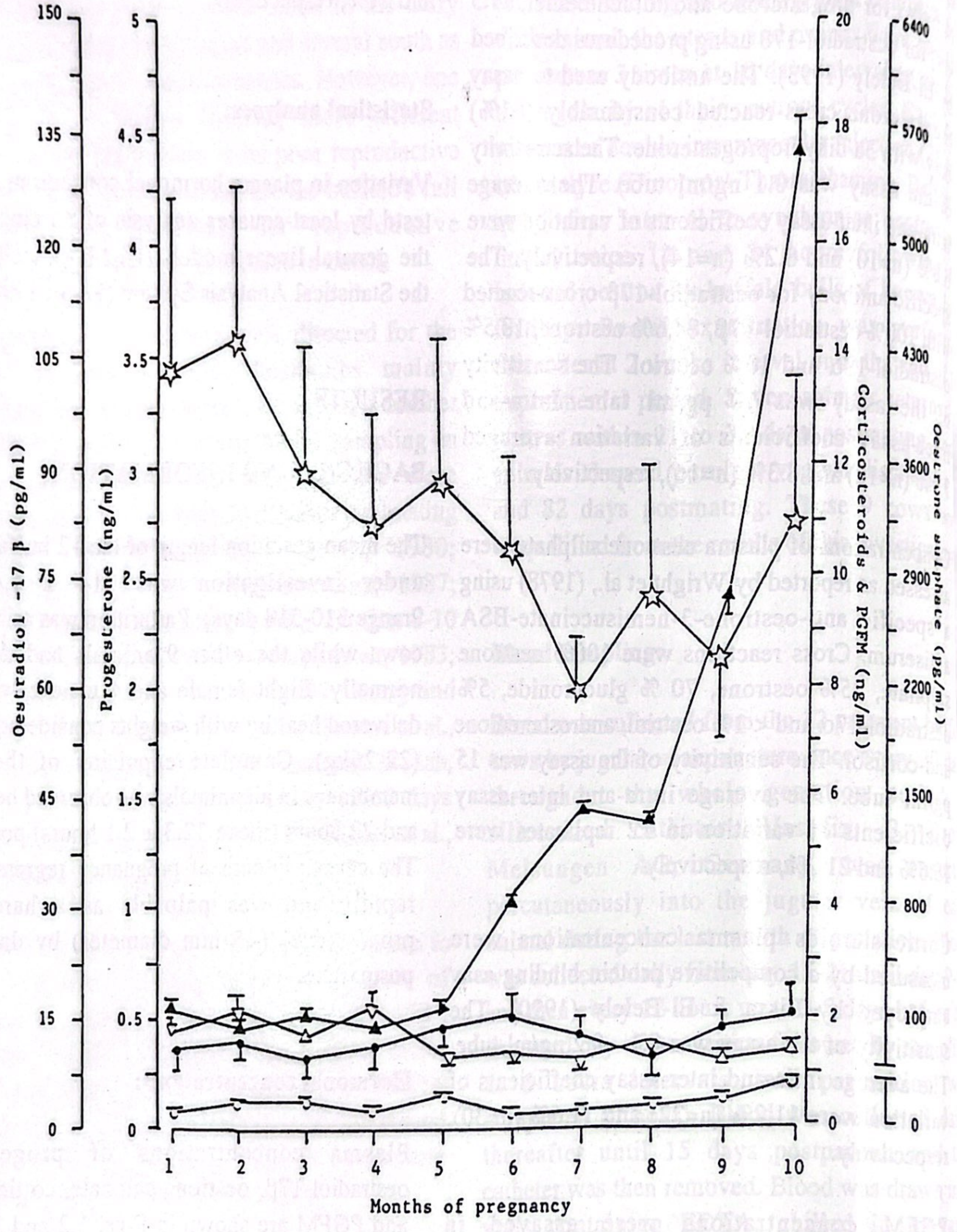

Fig. 1: Mean (± SE) plasma concentrations of progesterone (☆), oestradiol -17 β (△), oestrone sulphate (▲), corticosteroids (●) and PGFM (○) throughout the whole gestation period in buffalo cows (n = 12).

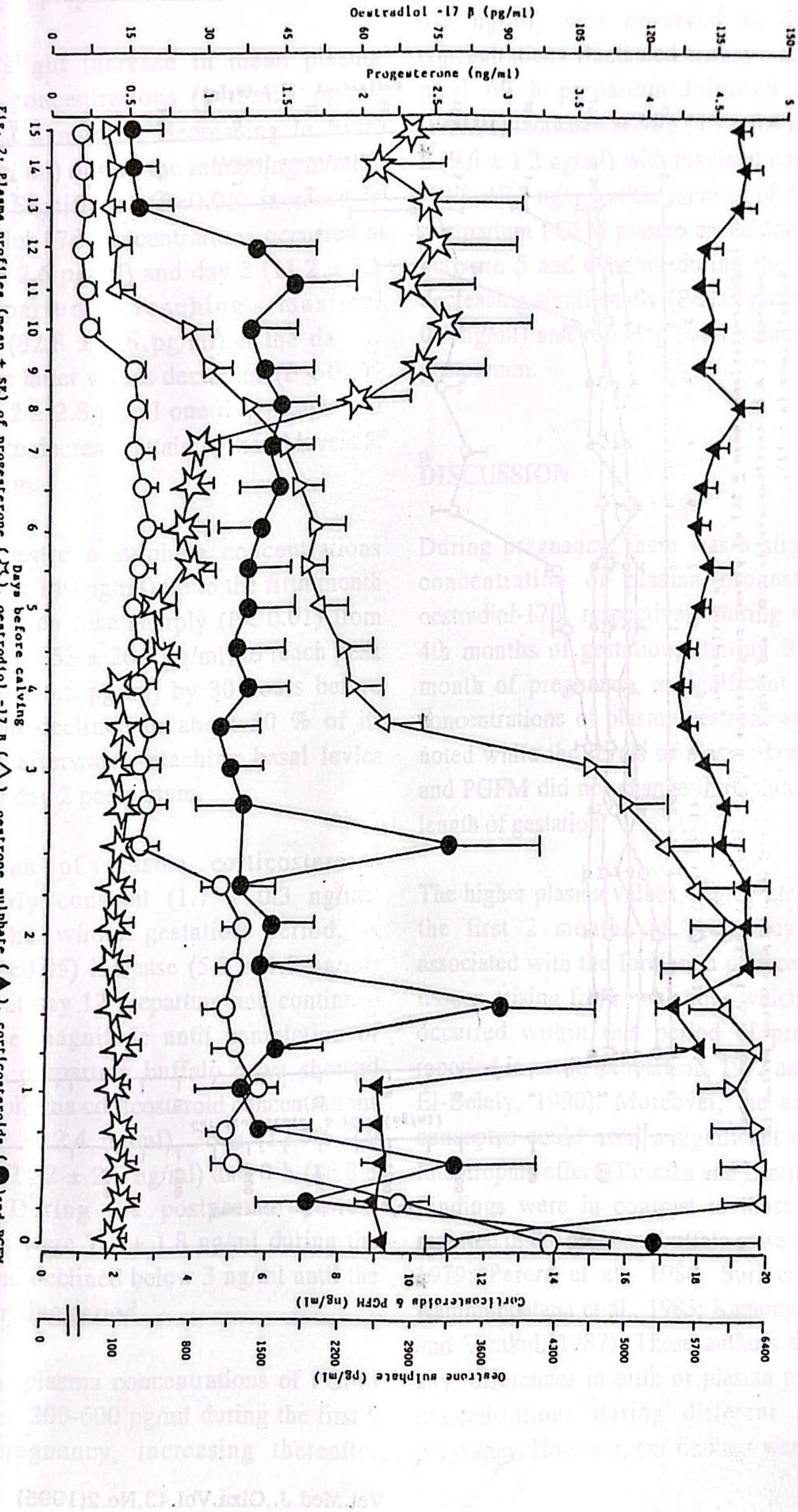

Fig. 2: Plasma profiles (mean ± SE) of progesterone (☆), oestradiol -17 β (△), oestrone sulphate (▲), corticosteroids (●) and PGFM (○) before and during parturition in buffalo cows (n = 12). The point designated 0 means completion of delivery.

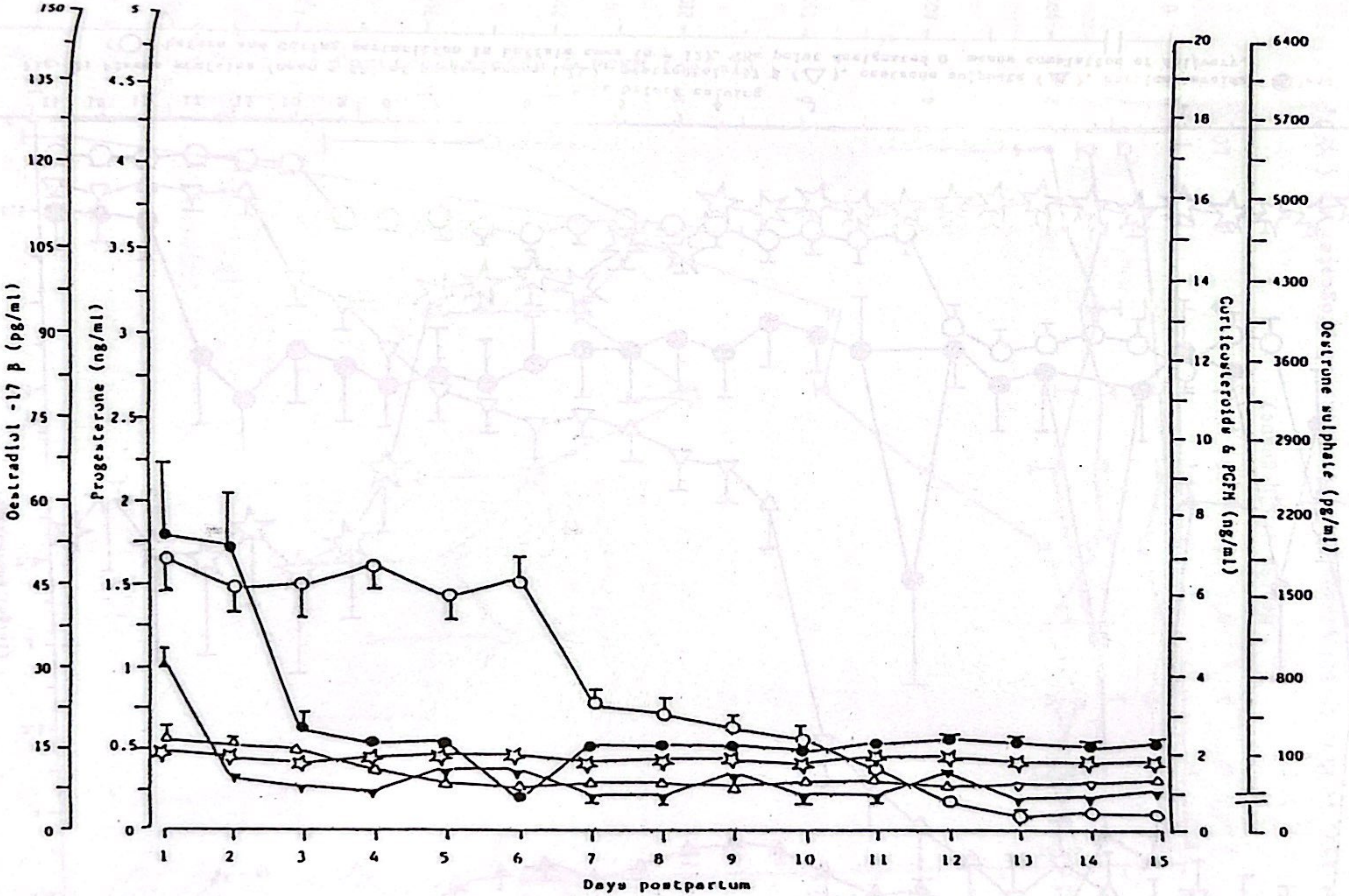

Fig. 3: Concentrations (mean ± SE) of plasma progesterone (☆), oestradiol -17 β (△), oestrone sulphate (▲), corticosteroids (●) and PGFM (○) during the early postpartum period in buffalo cows (n = 12).

(p<0.05) thereafter reaching baseline values (≤0.5 ng/ml) from day 4 prepartum onwards.

There was a slight increase in mean plasma oestradiol-17β concentrations (14.8±2.1 pg/ml) during the first 4 months, decreasing to basal values (< 12 pg/ ml) during the remaining months of pregnancy. Significant (P<0.01) increase in plasma oestradiol-17β concentrations occurred at day 10 (26.3 ± 2.6 pg/ml) and day 3 (11.2 ± 5.1 pg/ml) prepartum, reaching maximal concentration (82.8 ± 3.6 pg/ml)on the day of parturition. The latter values decreased (P < 0.01) abruptly to 14.2 ± 2.8 pg/ml one day postpartum and continued to decrease attaining basal levels at day 5 postpartum.

The plasma oestrone sulphate concentrations remained low (< 140 pg/ml) up to the fifth month of pregnancy, then rose sharply (P< 0.01) from the sixth month (853 ± 26.3 pg/ml) to reach peak levels (5920 ±116.5 pg/ml) by 30 hours before parturition and declined to about 50 % of its concentration afterwards reaching basal levles (<80 pg/ml) at day 2 postpartum.

Concentrations of plasma corticosteroids remained fairly constant (1.7 ± 0.3 ng/ml) throughout the whole gestation period. A significant (P<0.05) increase (5.3 ± 1.8 ng/ml) was detected at day 12 prepartum and continued with the same magnitude until completion of delivery. The prepartum buffalo cows showed four peaks of plasma corticosteroid concentrations at 60 h (10.8 ± 2.4 ng/ml), 36 h (12.5% 2.9 ng/ml), 12 h (11.2 ± 2.5 ng/ml) and 0 h (16.8 ± 3.2 ng/ml). During the postpartum period, concentrations were 7.1 ± 1.8 ng/ml during the first 2 days and declined below 3 ng/ml until the end of the sampling period.

The peripheral plasma concentrations of PGFM ranged between 200-600 pg/ml during the first 9 months of pregnancy, increasing thereafter between 700-1000 pg/ml until day 10 before parturition. A significant (P<0.01) increase (2.2 ± 0.2 ng/ml) was observed at day 9, and concentrations fluctnated within a narrow range until 60 h prepartum followed by marked (P<0.01) increases at 54 h (4.9 ± 0.4 ng/ml) and 6 h (9.6 ± 1.2 ng/ml) with maximal concentrations (13.8 ± 2.3 ng/ml) at the moment of delivery. The postpartum PGFM plasma concentrations ranged between 5 and 8 ng/ml during the first 6 days decreasing significantly (P<0.01) at day 7 (3.2 ± 0.3 ng/ml) and reaching basal values on day 12 postpartum.

DISCUSSION

During pregnancy, there was a slightly higher concentration of plasma progesterone and oestradiol-17β, respectively during the 2nd and 4th months of gestation; starting from the 6th month of pregnancy, a significant increase in concentrations of plasma oestrone sulphate was noted while the levels of plasma corticosteroids and PGFM did not change throughout the whole length of gestation.

The higher plasma values of progesterone during the first 2 months of pregnancy might be associated with the formation of accessory luteal tissues arising from ovulations which frequently occurred within this period of pregnancy as reported in cattle (Robertson, 1972 and Eissa and El-Belely, 1990). Moreover, the early bovine conceptus could exert a significant and positive luteotrophic effect (Bulman and Lamming, 1978). Findings were in contrast to those previously reported in the pregnant buffalo cows (Batra et al., 1979; Perera et al., 1980; Suri et al., 1980; Kammonpatana et al., 1983; Kamonpatana, 1984 and Virakul, 1987). These authors did not find any differences in milk or plasma progesterone concentrations during different months of pregnancy. However, our findings were supported by the higher plasma oestradiol- 17β concentrations during the first 4 months of pregnancy indicating that 3 or more follicular waves had occurred during this period of pregnancy producing 2 or more accessory luteal tissues under the influence of LH together with prolactin (Kammonpatana, 1984).

The pattern of change for plasma oestrone sulphate in the present investigation confirmed earlier reports for cattle (Gaiani et al., 1982). Kammongpatana et al., (1983) and Hung and Prakash (1990) recorded a progressive increase in oestrone sulphate concentrations in plasma of buffaloes after the 4th to 5th months of pregnancy which differed from our observation in which there is a sharp and distinct rise in oestrone sulphate levels beginning in the 6th month of pregnancy. The probable reason for increasing oestrone sulphate concentrations with advancing pregnancy in buffaloes is not known but the placenta in conjunction with a viable conceptus might be incorporated in the synthesis and release of this steroid as in bovine and swine (Robertson and King, 1974 and Wright et al., 1978).

The finding that plasma progesterone concentrations decreased profoundly 7 days before parturition differed from those reported in buffaloes. Progesterone concentrations were found to decrease significantly 60 days before parturition (Rao et al., 1978); 15 to 21 prepartum (Batra et al., 1982; Heshmat et al., 1985 and Prakash and Madan, 1986) or at the day of parturition (Kammonpatana, 1984 and El-Belely et al., 1988). This discrepancy of results could be due, in part, to few number of animals or the insufficient blood and milk samplings.

Several investigations in buffaloes reported that plasma oestrone concentrations increased substantially during the last 2 months of gestation with maximal concentrations 5 days prior to parturition (Perera et al., 1981; Kamonpatana, 1984; Virakul, 1987 and Hung and Prakash, 1990), while total oestrogens increased markedly during the last month of pregnancy peaking 1-2 days before delivery (Rao et al., 1978 and El-Belely et al., 1988). No data are available in buffaloes regarding plasma concentrations of oestradiol-17β during the periparturient period. The present findings agreed well with those reported in cattle that plasma oestradiol-17β concentrations showed a marked elevation on day 10 prepartum reaching maximal concentration at the moment of delivery (Henricks et al., 1972; Dobson and Dean, 1974; Hunter et al., 1977; Seren et al., 1977 and Hrada, 1980).

This may be the first report on oestrone sulphate status in buffaloes or cattle around parturition. The 50% decrease in plasma concentrations of this steroid during the last 24 hours prior to parturition is surprising in the presence of peak concentrations of oestradiol-17β, the main precursor of oestrone sulphate, during this period. To our opinion, the activity of the foetal adrencls concerning the release of sulphokinase enzyme (Gower, 1979) is decreased, due to unknown causes, resulting in a slow conversion of unconjugated oestrogens to oestrone sulphate.

A similar prepartum increase in plasma corticosteroid concentrations was reported in buffaloes by Kamonpatama (1984) who found that concentrations increased dramatically 15 to 20 days before parturition but their report is devoid of any peaks of this hormone (possibly due to the small number of the studied animals or the infrequent blood samplings).

PGFM plasma levels showed trifle changes until 10 days before calving then markedly increased until the last 54 hours where there was a dramatic rise, reaching peak levels during labour. These results were similar to those reported in bffaloes by Perera et al., (1981). Howerver, the present magnitude of PGFM elevations was greater than those reported by these authors.

On the bases of a comparative analysis of the hormonal profiles presented above, we may be able to formulate the endocrine mechanisms initiating parturition in buffalo-cows. A fundamental role is certainly played by the marked elevation of corticosteroids around day 12 prepartum. The mechanism of action of this hormone in inducing parturition is likely due to its luteolytic effect (Prakash and Madan, 1986) causing a sharp drop in plasma progesterone concentrations 7 days prepartum. Furthermore, the increased corticosteroid concentrations at this time stimulated increased secretion of placental oestradiol-17β at day 10 prepartum resulting in increased release of PGF2a within few hours. Higher levels of placental oestradiol-17β also created a large oestrogen:progesterone ratio which became critical for stimulating the development of oxytocin receptors (Wendorf e tal., 1983). In this way, the inhibiting action exerted by progesterone on uterine motility would cease allowing prostaglandins and oxytocin to act on the myometrium, so that birth may ensue.

The higher plasma concentrations of both corticosteroids and PGFM persisted for about 7 days postpartum resulting in expulsion of the foetal menbranes within about one day and complete regression of the corpus luteum within about 10 days postpartum. These findings are in agreement with those reported in buffaloes by Jainudeen at al., (1983) and El-Belely et al., (1988). The plasma concentrations of oestrone sulphate reached baseline values immediately following expulsion of the foetal membranes.

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