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Plasma progesterone, oestradiol-17β, oestrone sulphate, corticosteroids and a metabolite of PGF$_{2α}$: evolution throughout pregnancy, before, during and after parturition in buffalo cows

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Summary — The concentration of plasma progesterone, oestradiol-17β, oestrone sulphate, corticosteroids and 13,14-dihydro-15-ketoprostaglandin F$_{2α}$ (PGFM) was measured in 12 buffalo cows during the whole period of gestation, around parturition and for 15 d postpartum. The concentration of progesterone and oestradiol-17β increased slightly during the first 2 months (3.5 ± 0.9 ng/ml) and 4 months (14.8 ± 2.1 pg/ml) of pregnancy, respectively. Their values remained consistently below these levels until near the end of the pregnancy period when progesterone concentrations decreased significantly (P < 0.05) at d 7 prepartum (0.9 ± 0.1 ng/ml) and oestradiol-17β increased markedly (P < 0.01) at d 10 prepartum (26.3 ± 2.6 pg/ml). Progesterone showed basal values (< 0.5 ng/ml) from d 4 prepartum to d 15 postpartum. Oestradiol-17β concentrations were maximal (82.8 ± 3.6 pg/ml) during labour and returned to their basal values (< 12 pg/ml) at d 5 postpartum. The concentrations of oestrone sulphate remained low (< 140 pg/ml) during the first half of gestation period. It increased sharply (P < 0.01) thereafter to 5 620 ± 116.5 pg/ml by 30 d prepartum and afterwards declined to about 50% of this value before calving reaching basal level (< 80 pg/ml) at d 2 postpartum. The concentration of corticosteroids fluctuated narrowly (1.7 ± 0.3 ng/ml) throughout gestation, increasing significantly (P < 0.05) at d 12 prepartum (5.3 ± 1.8 ng/ml) and peaking to 16.8 ± 3.2 ng/ml at the moment of delivery. Its value declined below 3 ng/ml from d 3 onwards postpartum. The concentration of PGFM was at basal level (200–600 pg/ml) throughout the first 9 months of pregnancy. It began to increase progressively 9 d prepartum (2.2 ± 0.2 ng/ml) and reached a maximum concentration (13.8 ± 2.3 ng/ml) during delivery. The postpartum concentration of PGFM remained in excess of 5 ng/ml during the first week and returned to a basal value at d 12 postpartum. We concluded that progesterone, in particular 3α-dihydroprogesterone, is essential for maintaining pregnancy in buffaloes and the maturational events leading to parturition are linked with both the luteolytic effect of corticosteroids and the oestrogen-stimulated increased synthesis and release of PGFM$^{2α}$.

steroid hormone / PGFM / pregnancy / parturition / buffalo

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Résumé — Concentrations plasmatiques en progestérone, œstradiol-17\(\beta\), sulfate d'oestrone, corticostéroïdes et en un métabolite de PGF\(_{2\alpha}\) : évolution pendant la gestation, avant et après la mise bas chez la femelle buffle. Les concentrations plasmatiques en progestérone, œstradiol-17\(\beta\), sulfate d'oestrone, corticostéroïdes et en 13,14-dihydro-15-kéto-prostaglandine F\(_{2\alpha}\) (PGFM) ont été mesurées chez 12 femelles buffle pendant toute la durée de la gestation, autour de la mise bas, et jusqu'à 15 j après la mise bas. Les concentrations en progestérone et en œstradiol-17\(\beta\) ont augmenté légèrement durant les 2 (3,5 ± 0,9 ng/ml) et 4 (14,8 ± 2,1 pg/ml) premiers mois de gestation, respectivement. Puis leurs valeurs sont demeurées constamment en-dessous de ces niveaux, jusqu'à pratiquement la fin de la gestation. La concentration en progestérone a alors diminué significativement (\(P < 0,05\)) 7 j avant la mise bas (0,9 ± 0,1 ng/ml), et celle en œstradiol-17\(\beta\) a augmenté significativement (\(P < 0,01\)) 10 j avant la mise bas (26,3 ± 2,6 pg/ml). La concentration en progestérone était à son niveau de base (< 0,5 ng/ml) de 4 j avant jusqu'à 15 j après la mise bas. La concentration en œstradiol-17\(\beta\) a été maximale (82,8 ± 3,6 pg/ml) pendant la mise bas, revenant à sa valeur de base (< 12 pg/ml) 5 j après la mise bas. La concentration en sulfate d'oestrone est restée faible (< 140 pg/ml) pendant la première moitié de la gestation. Elle a ensuite augmenté brusquement (\(P < 0,01\)) pour atteindre un pic de 5 620 ± 116,5 pg/ml 30 j avant la mise bas, a ensuite diminué jusqu'à 50% de sa valeur, puis est revenue à sa valeur de base 2 j après la mise bas. La concentration en corticostéroïdes a peu varié (1,7± 0,3 ng/ml) durant toute la gestation ; elle a augmenté significativement (5,3 ± 1,8 ng/ml, \(P < 0,05\)) 12 j avant la mise bas, avec un pic (16,8 ± 3,2 ng/ml) au moment de la mise bas ; elle a ensuite diminué en-dessous de 3 ng/ml à partir de 3 j après la mise bas. La concentration en PGFM était à son niveau de base (200-600 pg/ml) pendant les 9 premiers mois de gestation. Elle a commencé à augmenter progressivement 9 j avant la mise bas (2,2 ± 0,2 ng/ml) pour atteindre un maximum (13,8 ± 2,3 ng/ml) pendant la mise bas ; elle est ensuite restée élevée (5 ng/ml) pendant 1 sem, et est revenue à sa valeur de base 12 j après la mise bas. Nous concluons que la progestérone, et plus particulièrement la 3\(\alpha\)-dihydroprogestérone, est essentielle au maintien de la gestation chez la femelle buffle, et que la maturation entraînant la mise bas est liée à la fois à l'effet lutéolytique des corticostéroïdes, et à l'augmentation de la synthèse et de la libération de PGF\(_{2\alpha}\) due au œstrogènes.

hormone stéroïde / PGFM / gestation / parturition / buffle

INTRODUCTION

The buffalo constitutes a major portion of the dairy and meat industries of Egypt and several south and south-eastern 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.

Many of the investigations concerning buffaloes have studied only 2 or 3 hormones, in particular progesterone and the oestrogens, and have used only small numbers of animals often with insufficient blood sampling. Moreover, these studies were confined to the first 30–50 d postmating (Batra et al, 1979; Suri et al, 1980; Chan-taraprateep, 1987; Kamonpatana, 1987; 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 d postpartum (Perera et al, 1981; Jainudeen et al, 1983; El-Belely et al, 1988).

The present study was, therefore, undertaken to give detailed information on the pattern of changes in several reproductive hormones (plasma progesterone, œstradiol-17\(\beta\), oestrone sulphate, corticosteroids and the major circulating prostaglandin F\(_{2\alpha}\) metabolite (PGFM)) throughout the whole gestation period, continuing through parturition and into the early postpartum period.
MATERIALS AND METHODS

Animals

Twenty-one multiparous and non-pregnant buffalo cows were chosen from a governmental buffalo farm near Cairo. They were between 6–9 years of age and 4–6 months had passed since their last calving. All the animals were kept under identical feeding and management conditions. For each animal, the uterus and ovaries were palpated per rectum 3 times with a 10 d interval between palpations in order to confirm the regularity of their oestrous cycles. Each cow received a double intramuscular injection (25 mg) of Lutalyse (Dinoprost Thromethamine, Belgium) with an 11 d interval between injections in order to synchronize their oestrous cycles. They were mated twice, 12 and 24 h following the induced oestrus, by buffalo bulls that were known to be of high fertility. Rectal examinations were performed thereafter at 2 week intervals until the end of the experimental period. Seven animals returned to oestrus within 21–33 d postmating and 2 animals showed purulent vaginal discharges 64 and 82 d 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 d 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. The blood was collected daily from d 15–21 prepartum until d 7–10 prepartum and then every 12 h up to 3–5 d before the expected day of parturition followed by sampling at 6 h intervals until parturition was completed (0 h). The blood was collected daily thereafter until 15 d postpartum and the catheter was then removed. The blood was drawn into vials containing EDTA, chilled in ice and centrifuged at 2 000 g for 15 min. The plasma was stored frozen at -20°C until assay.

Hormonal assays

The plasma steroid hormone concentrations were assessed in duplicate on toluene/petroleum ether (4:5 v/v) for progesterone, and toluene/ether (4:5 v/v) for oestradiol-17β, using the procedures described by El-Belely (1993). The antibody used to assay progesterone only cross-reacted (61%) with 3α-dihydroprogesterone. The detection limit of the assay was 0.1 ng/ml. The average intra- and interassay 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% oestradiol. The detection limit of the assay was 10 pg/ml. Intra- and interassay coefficients of variation averaged 7.9% (n = 10) and 8.3% (n = 16), respectively.

The concentration of plasma oestrone sulphate was assessed using the method of Wright et al (1978) using a specific anti-oestrone-3-hemisuccinate-BSA antiserum. The cross-reactions were 100% oestrone sulphate, 25% oestrone, 70% glucuronide, 5% oestradiol-17α and -17β, < 1% oestradiol, androstenedione and cortisol. The detection limit of the assay was 15 pg/ml. The average intra- and interassay coefficients of variation in 12 replicates were 18.6 and 21.2%, respectively.

The corticosteroid plasma concentration was measured by a competitive protein binding assay employed by Eissa and El-Belely (1990). The detection limit of the assay was 0.1–0.2 ng/ml. The average intra- and interassay coefficients of variation were 11.2% (n = 22) and 11.6% (n = 30), respectively.

The PGFM concentration was assayed in unextracted plasma by radioimmunoassay according to Kindahl et al (1976). The antiserum cross-reacted approximately 16% against 13,14-dihydro-15-keto-PGF2α, 4% against 13,14-dihydro-PGF2α, and 0.4% against PGF2α. A cross-reaction of < 0.1% was detected against other prostaglandins. The detection limit of the assay was 40 pg/ml. The intra- and interassay coefficients of variation averaged 16 and 21%, respectively.

Statistical analyses

Variation in plasma hormonal concentrations was tested 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 in the study was 314 ± 1.6 d (range: 310–318 d). Parturition was unassisted in 9 cows while the other 3 animals had malpresentation dystocia. Correction and delivery of the foetuses in these 3 cows occurred within 1–2 h. Eight female and 4 male calves were delivered. They were healthy and had normal birth weights (22–26 kg). Expulsion of the foetal membranes in all animals was observed between 6 and 22 h (mean 12.3 ± 2.1 h) postpartum. The corpus luteum of pregnancy regressed very rapidly following parturition and by d 10–15 postpartum; it was palpable as a hard small protuberance (< 5 mm diameter).

Hormonal concentrations

The plasma concentration of progesterone, oestradiol-17β, oestrone sulphate, corticosteroids and PGFM are shown in figures 1, 2 and 3, plotted as means ± SE. The plasma concentration 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 stayed below the latter value until d 8 prepartum when it significantly decreased (0.9 ± 0.1 ng/ml)

Fig 1. Mean (± SE) plasma concentrations of progesterone (■), oestradiol-17β (△), oestrone sulphate (▲), corticosteroids (○) and PGFM (○), throughout the whole gestation period in buffalo cows (n = 12).
(P < 0.05), thereafter reaching baseline values (< 0.5 ng/ml) from d 4 prepartum onwards.

The 12 buffalo cows showed slight increases in mean plasma oestradiol-17β concentration (14.8 ± 2.1 ng/ml) during the first 4 months decreasing to basal values (< 12 pg/ml) during the remaining months of pregnancy. Significant (P < 0.01) increases in plasma oestradiol-17β concentrations occurred at d 10 (26.3 ± 2.6 pg/ml) and d 3 (11.2 ± 5.1 pg/ml) prepar-
um reaching maximum concentrations (82.8 ± 3.6 pg/ml) at the day of parturition. The concentration then decreased ($P < 0.01$) abruptly to 14.2 ± 2.8 pg/ml 1 d postpartum and continued to decrease until they attained basal levels at d 5 postpartum.

The plasma oestrone sulphate concentration remained low (< 140 pg/ml) up to the 5th month of pregnancy, and then rose sharply ($P < 0.01$) from the 6th month (853 ± 26.3 pg/ml) to reach peak levels (5 620 ± 116.5 pg/ml) by 30 d before parturition when it declined by about 50% of this maximum value and reached basal levels (< 80 pg/ml) at d 2 postpartum.

The concentration of plasma corticosteroids remained fairly constant (1.7 ± 0.3 pg/ml) throughout the whole gestation period. A significant ($P < 0.05$) increase (5.3 ± 1.8 ng/ml) was detected at d 12 prepartum and continued at this level until completion of the delivery. The prepartum buffalo cows showed 4 peaks of plasma corticosteroid concentration 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, concentration was 7.1 ± 1.8 ng/ml during the first 2 d and dropped below 3 ng/ml until the end of the sampling period.

The peripheral plasma concentration of PGFM ranged between 200–600 pg/ml during the first 9 months of pregnancy and increased thereafter between 700–1 000 pg/ml until d 10 before parturition. A significant ($P < 0.01$) increase (2.2 ± 0.2 ng/ml) was observed at d 9 and the concentration fluctuated thereafter 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 concentration (13.8 ± 2.3 ng/ml) at the moment of delivery. The postpartum PGFM plasma concentration ranged between 5 and 8 ng/ml during the first 6 d postpartum decreasing significantly ($P < 0.01$) at d 7 (3.2 ± 0.3 ng/ml) and reaching a basal value at d 12 postpartum.

DISCUSSION

The data concerning the endocrine profile during pregnancy could be summarized as follows. There were slightly higher concentrations of plasma progesterone and oestra
diol-17β during the first 2 and 4 months of gestation. A significant increase in the concentration of plasma oestrone sulphate started in the 6th month of pregnancy. Finally, the levels of plasma corticosteroids and PGFM remained unchanged throughout the whole gestation period.

The higher plasma concentration of progesterone during the first 2 months of pregnancy might be associated with formation of accessory luteal tissues that are the result of ovulations. This is known to occur frequently within this pregnancy period in cattle (Robertson, 1972; Eissa and El-Belely, 1990). Moreover, the early bovine conception could exert a significant and positive luteotrophic effect (Bulman and Lamming, 1978). These findings are in contrast to those previously reported in the pregnant buffalo cows (Batra et al, 1979; Perera et al, 1980; Suri et al, 1980; Kamonpatana et al, 1983; Kamonpatana, 1984; Virakul, 1987). These authors did not find any differences in milk or plasma progesterone concentrations during the 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 (luteotrophic hormone) together with prolactin (Kamonpatana, 1984).

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

The finding that the plasma progesterone concentrations decreased sharply 7 d before parturition differed from those previously reported in buffaloes. Progesterone concentrations were found to decrease significantly 60 d before parturition (Rao et al, 1978); 15–21 d prepartum (Batra et al, 1982; Heshmat et al, 1985; Prakash and Madan, 1986) or at the day of parturition (Kamonpatana, 1984; El-Belely et al, 1988). The discrepancy between the results of the present work and those of the previously mentioned authors could partly be due to the small numbers of animals or to insufficient blood milk samplings in these previous studies.

Several investigations in buffaloes reported that plasma oestrone concentrations increased substantially during the last 2 months of gestation with maximal concentrations 5 d prior to parturition (Perera et al, 1981; Kamonpatana, 1984; Virakul, 1987; Hung and Prakash, 1990), while total oestrogens increased markedly during the last month of pregnancy peaking 1–2 d before delivery (Rao et al, 1978; 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 d 10 prepartum reaching a maximal concentration at the moment of delivery (Henricks et al, 1972; Dobson and Dean, 1974; Hunter et al, 1977; Seren et al, 1977; Harada, 1980).

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

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

PGFM plasma levels showed little change until 10 d before calving when they increased markedly until the last 54 h, where there was a dramatic rise, reaching peak levels during labour. These results were typically similar to those reported in buffaloes by Perera et al (1981). However, the present magnitude of PGFM elevations was greater than that reported by those authors.

On the basis of a comparative analysis of the hormonal profiles presented above, we have formulated an endocrine mechanism initiating parturition in the buffalo animal. A fundamental role is certainly played by the marked elevation of corticosteroids around d 12 prepartum. The mechanism of the action of this hormone in inducing parturi-
tion is probably due to its luteolytic effect (Prakash and Madan, 1986) causing a sharp drop in plasma progesterone concentrations 7 d prepartum. Furthermore, the increased corticosteroid concentration at this time stimulated increased secretion of placental oestradiol-17β at d 10 prepartum resulting in increased release of PGF2α a few hours later. Rising levels of placental oestradiol-17β also created a large oestrogen/progesterone ratio which would be critical for stimulating the development of oxytocin receptors (Wendorf et al, 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 d postpartum resulting in expulsion of the foetal membranes within about 1 d and complete regression of the corpus luteum within about 10 d postpartum. These findings were in agreement with those reported in buffaloes by Jainudeen et al (1983) and El-Belely et al (1988). The plasma concentration of oestrone sulphate reached baseline values immediately following expulsion of the foetal membranes.

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