Concentrations of LH, oestradiol-17 β and progesterone in the peripheral plasma of swamp buffalo cows (*Bubalus bubalis*) around the time of oestrus

J. A. Avenell*, Y. Saepudin and I. C. Fletcher

Balai Penelitian Ternak, P.O. Box 123, Bogor, Indonesia

Summary. Peripheral plasma concentrations of LH, oestradiol-17 β and progesterone were measured in 13 mature swamp buffalo cows at 4-h intervals from 36–40 h before until 36–40 h after the onset of oestrus. Mean LH concentrations increased sharply to a peak of 35 ng/ml and returned to basal levels of 5 ng/ml within a 12-h period beginning soon after the onset of oestrus. Mean oestradiol-17 β concentrations were within the range 9–13 pg/ml from 36–40 h before until 12–16 h after the onset of oestrus, and within the range 7–9 pg/ml thereafter. Progesterone concentrations remained around 0.1 ng/ml throughout the sampling period. There were no significant differences in hormone concentrations or changes between cows that conceived and those that did not conceive to artificial insemination 12–24 h after onset of oestrus.

Introduction

Hormonal changes associated with ovulation and oestrus in buffalo cows have not been clearly established. Broad changes in progesterone concentration during the oestrous cycle have been determined (Perera, Pathiraja, Buvanendran, Abeywardena & Piyasena, 1978; Kamonpatana, Kunawongkrit, Bodhipaksha & Luvira, 1979), but information on LH and oestradiol-17 β concentrations, particularly in relation to each other and to oestrus, is limited (Kaker, Razdan & Galhotra, 1980; Batra, Arora, Bachlaus, Pahwa & Pandey, 1980; Kanai & Shimizu, 1984). The primary purpose of this investigation was to establish relationships between oestrus and plasma concentrations of LH, oestradiol-17 β and progesterone in swamp buffalo cows. Because conception rates to artificial insemination in Indonesian village buffalo are commonly in the range 20–40% (Toelihere & Siregar, 1981), a secondary aim of the investigation was to determine whether the success or failure of conception after artificial insemination could be related to differences in hormone concentrations around the time of oestrus.

Materials and Methods

Sixteen mature non-lactating swamp buffalo cows (*Bubalus bubalis*) showing regular cyclic ovarian activity were available for the investigation. The cows were well nourished and in good health. They were maintained on an ad-libitum ration of freshly chopped elephant grass (*Pennisetum purpureum*) with a daily supplement of 3 kg commercial concentrate/cow. Body weight during the investigation, which covered a 3-week period during June 1981, was 405 ± 15 kg (mean $\pm$ s.e.m.).

The cows were run as two groups, each with a vasectomized bull fitted with chinball marking harness (Lang, Hight, Uljee & Young, 1968), and observed every 4 h for evidence of oestrus. At each observation, indications of oestrus from fresh paint marks on cows' backs were confirmed by

* Present address: CSIRO, Division of Animal Production, P.O. Box 239, Blacktown, New South Wales 2148, Australia.

direct observation of cows standing for mounting by the bull. The beginning and end of oestrus were considered to have occurred 2 h before and 2 h after the first and last observations respectively when cows were seen to stand for a bull. Cows were inseminated with a single straw of frozen semen (Avenell, 1982) 12–24 h after the onset of oestrus, using a schedule which could be applied under field conditions. Cows first detected in oestrus at any time during the night (before 06:00 h) were inseminated at 16:00 h in the afternoon, and those first detected in oestrus at any time during the day (before 18:00 h) were inseminated at 08:00 h the next morning. Sustained plasma progesterone concentrations above 1 ng/ml for 6 weeks after insemination were taken as evidence of conception (Batra, Arora, Bachlaus & Pandey, 1979).

Initially, 5 ml blood were collected by jugular venepuncture each day for immediate determinations of plasma progesterone concentrations. When individual cows showed a decline in progesterone concentration denoting the end of the luteal phase of an oestrous cycle, 10-ml blood samples were then taken every 4 h until 36–48 h after the onset of oestrus for determinations of plasma progesterone, oestradiol-17 β and LH concentrations. Further 5-ml blood samples were taken for progesterone assay, at first daily and then 3 times weekly, until 6 weeks after insemination. Blood was collected into heparinized tubes on ice, transferred to a cold room for plasma separation by centrifugation, and stored at -20°C until assayed.

Progesterone concentration was determined by a radioimmunoassay procedure similar to that described by Scaramuzzi, Corker, Young & Baird (1974). Progesterone was extracted from 0.2 ml plasma with 2 ml freshly distilled *n*-hexane (Merck). Antiserum (A.S. 334), raised in sheep against progesterone-11 α -hemisuccinate-BSA, was used at an initial dilution of 1 in 4000. Cross-reactions with other steroids were 2% with 17 α -hydroxyprogesterone, 3% with corticosterone, and <1% with pregnenolone and cortisol. Assay sensitivity, defined as twice the standard deviation of the zero standard, was 0.10 ± 0.03 ng/ml (mean $\pm$ s.e.m., $n = 15$). Solvent blanks and plasma from an ovariectomized buffalo cow contained <0.1 ng progesterone/ml, and the addition of 0.5, 2.5 and 5.0 ng progesterone gave recoveries of 98%, 98% and 99% respectively. Intra- and inter-assay coefficients of variation for concentrations of 0.3 and 1.0 ng progesterone/ml were 10% and 14%, and 10% and 15% respectively.

Oestradiol-17 β concentration in 4 ml plasma (or 2 ml for samples taken before and after the 4-h observation period, when plasma volume was limited) was determined by radioimmunoassay as described for cattle plasma by Dobson & Dean (1974), using an antiserum (A.S. 727) raised in rabbits against 17 β -oestradiol-6-(O-carbomethyl)oxime. Antiserum used at an initial dilution of 1 in 12 000 showed cross-reactions of 5% with oestrone, 3% with oestradiol-17 α and <1% with oestriol. Assay sensitivity was 0.02 ± 0.003 pg/ml (mean $\pm$ s.e.m., $n = 13$). Solvent blanks contained <0.01 pg oestradiol-17 β /tube, and the addition of 2.0, 5.0, 10.0 and 25.0 pg oestradiol-17 β /ml plasma from an ovariectomized buffalo cow gave recoveries of 86%, 98%, 92% and 91% respectively. Intra- and inter-assay coefficients of variation were 10% and 14% respectively.

Concentration of LH was determined from duplicate 0.2-ml plasma samples within a single assay, using a heterologous radioimmunoassay procedure as described for sheep plasma by Scaramuzzi, Caldwell & Moor (1970). The bovine pituitary LH preparation used for standards and for iodination was NIH-LH-B10. The antiserum (R 115), raised in rabbits against NIH-LH-S11, was used at an initial dilution of 1:100 000, and showed cross-reactions of 5% with NIH-FSH-B1, <1% with NIH-P-B1, and <1% NIH-GH-B17. No standard buffalo pituitary preparations were available for testing, but serial dilution of a buffalo plasma sample collected at the time of oestrus gave an inhibition curve parallel to that of NIH-LH-B10. Assay sensitivity was 0.04 ng/tube, and intra-assay coefficients of variation for levels of 1.6 and 5.2 ng LH/ml were 5% and 6% respectively.

Results

Fifteen cows were detected in oestrus and 14 were inseminated. Plasma progesterone concentrations recorded daily and then 3 times weekly are shown in relation to the day of oestrus for

Text-fig. 1. Mean $\pm$ s.e.m. plasma progesterone concentrations recorded daily or 3 times weekly in buffalo cows that conceived ($\bullet$ — $\bullet$, $N = 9$) or failed to conceive ($\circ$ -- $\circ$, $N = 5$).

the 14 inseminated cows in Text-fig. 1. The figure illustrates the decline in progesterone concentration recorded before the beginning of 4-h sampling, and shows the difference in progesterone profiles after insemination between cows that conceived and those that failed to conceive.

Full records of oestrus and 4-h samples of plasma from 44 h before until 32 h after the LH peak were collected from 8 of 9 cows that conceived and from all 5 cows that failed to conceive. Mean concentrations of LH, oestradiol-17 β and progesterone from these 13 animals are shown in relation to oestrus and the time of peak LH concentration in Text-fig. 2. Progesterone concentrations remained around 0.1 ng/ml (range 0–0.2) throughout the sampling period. LH concentration arose from a base level of 5 ng/ml (range 1–8) to a peak of 35 ng/ml (range 20–52) over a period of only 4 h, and returned to basal levels within a further 8 h. Peak LH concentration was recorded 6.5 ± 1.1 (mean $\pm$ s.e.m.) h after the onset of oestrus. Oestradiol-17 β concentrations fluctuated between consecutive samples and showed a less pronounced pattern of change, but there was a distinct decrease in concentration 12–16 h after the onset of oestrus from within the range 9–13 pg/ml to within the range 7–9 pg/ml. For every animal, maximum concentrations (range 11–19, mean 12.9 ± 0.6 pg/ml) were recorded before the end of oestrus, and minimum concentrations (range 5–9, mean 6.6 ± 0.4 pg/ml) after the end of oestrus. Mean oestradiol-17 β concentrations before and after the 4-h collection period, determined from the relatively few samples in which 2 ml plasma remained after progesterone assay, were 4.6 ± 0.4 pg/ml (14 samples taken from 7 cows) 3–5 days before the LH peak, and 5.4 ± 0.4 pg/ml (18 samples taken from 8 cows) 3–5 days after the LH peak.

It is evident from Text-fig. 2 that there were no substantial differences in hormone concentrations or changes between cows that conceived and those that failed to conceive, in duration of oestrus (19.8 ± 1.1 versus 19.6 ± 2.6 h), interval from onset of oestrus to insemination (18.2 ± 2.2 versus 16.4 ± 2.0 h), or interval from LH peak to insemination (12.8 ± 3.3 versus 8.8 ± 2.9 h).

Discussion

Peripheral plasma hormone concentrations recorded from 36–40 h before until 36–40 h after the onset of oestrus can be summarized as showing unchanging basal levels of progesterone, a sharp

Text-fig. 2. Mean $\pm$ s.e.m. concentrations of LH, oestradiol-17 β and progesterone recorded at 4-h intervals in buffalo cows that conceived ($\bullet$ — $\bullet$, N = 8) or failed to conceive ($\circ$ -- $\circ$, N = 5). Horizontal bars show mean durations of oestrus (■ conceived, □ failed to conceive), with times of insemination arrowed.

peak in LH concentration soon after the onset of oestrus, and a decrease in oestradiol-17 β concentration towards the end of oestrus.

Basal concentrations of progesterone for several days around the time of oestrus are a common feature of most farm animals (reviewed by Jöchle & Lamond, 1980). A transient rise in progesterone concentration just before oestrus, reported for cattle by Ayalon & Shemesh (1974), was not evident in the present study. Basal and peak concentrations of LH were similar to those previously reported for buffalo cows (Kaker *et al.*, 1980; Kanai & Shimizu, 1984) and for cattle (Lemon, Pelletier, Saumande & Signoret, 1975). As in cattle, the LH peak was of short duration and occurred soon after the onset of oestrus. Oestradiol-17 β concentrations were similar to or slightly lower than those previously recorded for buffalo (Batra *et al.*, 1980; Kanai & Shimizu, 1984) and cattle (Ayalon & Shemesh, 1974; Lemon *et al.*, 1975; Dobson, 1978). Although the pro-oestrous rise in oestradiol-17 β concentration occurred before the beginning of 4-h sampling and therefore was

not determined precisely, lower concentrations recorded from prior daily sampling suggest that it occurred during the 3rd day before onset of oestrus, at about the same time as progesterone concentrations were declining to basal levels. This observation, and the decrease in oestradiol-17 β concentration recorded towards the end of oestrus, are consistent with changes reported for other farm animals (Jöchle & Lamond, 1980).

There were no obvious differences in hormone profiles or in time relationships between oestrus, insemination or hormone changes between cows that conceived and those that failed to conceive. This contrasts with the report of Rosenberg *et al.* (1982) that conception rate was higher in dairy cows that showed oestrous behaviour before the LH surge reached its peak than in cows in which oestrus coincided with or occurred later than the LH surge, though this conclusion was based on an assumption that onset of oestrus coincided exactly with the 6-h observation at which cows were first seen to stand for mounting by other cows.

In view of the similarity between buffalo cows and cattle in their hormone profiles around the time of oestrus, it is not surprising that satisfactory conception rates (9 of 14 cows in this investigation, and similar results from other experiments at this laboratory: Avenell, 1982) can be achieved from artificial insemination of buffalo cows using similar time intervals between onset of oestrus and insemination as have proved successful in cattle (Roberts, 1971). Arora & Pandey (1982) showed that late insemination was one of the causes of conception failure in 'repeat breeder' buffalo cows, and cited reports for cattle for which failure of conception has been associated with high progesterone concentrations at the time of insemination. Such situations could arise from insemination at inappropriate times in relation to oestrus or inaccuracies in the detection of oestrus which, while not apparent under experimental conditions of intensive observation in the present study, might well be a problem under village conditions, particularly in view of the reputed weak expression of oestrus in buffalo cows (Mahadevan, 1978; Perera, 1979). While it is possible that poor nutrition, high ambient temperatures or draught use may cause hormone imbalances in village buffalo cows which were not evident under the conditions of this study, we suggest that other factors, and particularly a failure to inseminate cows at the appropriate time after an accurately determined onset of oestrus, are more likely to be responsible for the low conception rates recorded in artificial insemination programmes under village conditions.

We thank Suparman, Wayan, Tarso, Alit, Ida and Murtiyeni for care of the experimental animals and assistance with observations and assays, and Dr R. I. Cox and Dr R. J. Scaramuzzi for providing antisera A.S. 334, A.S. 727 and R115, prepared in the CSIRO Division of Animal Production laboratories at Prospect, New South Wales, Australia. The work was supported in part by the Australian Development Assistance Bureau.

References

- Arora, R.C. & Pandey, R.S. (1982) Plasma concentrations of progesterone, oestradiol-17 β and luteinizing hormone in relation to repeat breeding in buffalo (*Bubalus bubalis*). *Anim. Prod.* **34**, 139–144.
- Avenell, J.A. (1982) Freezing of swamp buffalo semen. *Anim. Reprod. Sci.* **5**, 141–145.
- Ayalon, N. & Shemesh, M. (1974) Pro-oestrous surge in plasma progesterone in the cow. *J. Reprod. Fert.* **36**, 239–243.
- Batra, S.K., Arora, R.C., Bachlaus, N.K. & Pandey, R.S. (1979) Blood and milk progesterone in pregnant and non-pregnant buffalo. *J. Dairy Sci.* **62**, 1390–1393.
- Batra, S.K., Arora, R.C., Bachlaus, N.K., Pahwa, G.S. & Pandey, R.S. (1980) Quantitative relationships between oestradiol-17 β in the milk and blood of lactating buffaloes. *J. Endocr.* **84**, 205–209.
- Dobson, H. (1978) Plasma gonadotrophins and oestradiol during oestrus in the cow. *J. Reprod. Fert.* **52**, 51–53.
- Dobson, H. & Dean, P.D.G. (1974) Radioimmunoassay of oestrone, oestradiol-17 α and -17 β in bovine plasma during the oestrous cycle and last stages of pregnancy. *J. Endocr.* **61**, 479–486.
- Jöchle, W. & Lamond, D.R. (1980) *Control of Reproductive Functions in Domestic Animals*. Martinus Nijhoff, The Hague.
- Kaker, M.L., Razdan, M.N. & Galhotra, M.M. (1980) Serum LH concentrations in cyclic buffalo (*Bubalus bubalis*). *J. Reprod. Fert.* **60**, 419–424.
- Kamonpatana, M., Kunawongkrit, A., Bodhipaksha, P. & Luvira, Y. (1979) Effect of PGF-2 α on serum progesterone levels in the swamp buffalo (*Bubalus bubalis*). *J. Reprod. Fert.* **56**, 445–449.

- Kanai, Y. & Shimizu, H. (1984) Plasma concentrations of LH, progesterone and oestradiol during the oestrous cycle in swamp buffaloes (*Bubalus bubalis*). *J. Reprod. Fert.* **70**, 507–510.
- Lang, D.R., Hight, G.K., Uljee, A.E. & Young, J. (1968) A marking device for detecting oestrous activity of cattle. *N.Z. J. Agric. Res.* **11**, 955–958.
- Lemon, M., Pelletier, J., Saumande, J. & Signoret, J.P. (1975) Peripheral plasma concentrations of progesterone, oestradiol-17 β and luteinizing hormone around oestrus in the cow. *J. Reprod. Fert.* **42**, 137–140.
- Mahadevan, P. (1978) Water buffalo research—possible future trends. *World Anim. Rev.* **25**, 2–7.
- Perera, B.M.A.O. (1979) Synchronization of oestrus and fertility in buffaloes of Sri Lanka. In *Buffalo Reproduction and Artificial Insemination*, FAO Animal Production and Health Paper No. 13, pp. 211–215. FAO, Rome.
- Perera, B.M.A.O., Pathiraja, N., Buwanendran, V., Abeywardena, S.A. & Piyasena, R.D. (1978) Plasma progesterone levels during natural and prostaglandin-synchronised oestrous cycles in buffaloes. *Ceylon Vet.* **26**, 29–34.
- Roberts, S.J. (1971) In *Veterinary Obstetrics and Genital Disease*, pp. 726–755. Ithaca, New York.
- Rosenberg, M., Folman, Y., Herz, Z., Flamenbaum, I., Berman, A. & Kaim, M. (1982) Effect of climatic conditions on peripheral concentrations of LH, progesterone and oestradiol-17 β in high milk-yielding cows. *J. Reprod. Fert.* **66**, 139–146.
- Scaramuzzi, R.J., Caldwell, B.V. & Moor, R.M. (1970) Radioimmunoassay of LH and estrogen during the estrous cycle of the ewe. *Biol. Reprod.* **3**, 110–119.
- Scaramuzzi, R.J., Corker, C.S., Young, G. & Baird, D.T. (1974) Production of antisera to steroid hormones in sheep. In *Steroid Immunoassay*, pp. 111–122. Eds E. M. D. Cameron, S. G. Hiller & K. Griffiths. Alpha Omega Publishers, Cardiff.
- Toelihere, M.R. & Siregar, A. (1981) Results of the second evaluation of artificial insemination in swamp buffalo cows in the district of Brebes, Central Java. Report from Fakultas Kedokteran Veterinar, Institute Pertanian Bogor, October 1981.

Received 16 August 1984