Relative concentration of 13,14-dihydro-15 keto-prostaglandin F-2 α in blood and milk of buffaloes during the oestrous cycle and early pregnancy

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Summary. Concentrations of PGFM in blood and milk of 28 Murrah buffaloes increased significantly ($P < 0.001$) in non-pregnant animals before oestrus but not in pregnant animals. The PGFM level in milk was similar to that in blood plasma and was positively correlated in non-pregnant ($r = 0.981$) and pregnant ($r = 0.521$) animals. Mean blood plasma PGFM concentration was not significantly ($P < 0.05$) different from that of milk.

Introduction

Prostaglandin (PG) F-2 α is known to play an important role in ovarian function of dairy cows (Louis, Hafs & Morrow, 1972; Rowson, Tervit & Brand, 1972; Lamond, Tomilson, Drost, Henricks & Jochle, 1973) and buffaloes (Bachlaus, Arora, Prasad & Pandey, 1979; Kamonpatana, Kunawongkrit, Bodhipaksha & Luvira, 1979). However, the measurement of PGF-2 α released from the uterus is difficult because of the need to cannulate the utero-ovarian blood vessels. To avoid these difficulties, attempts have been made to measure concentrations of PGF-2 α in peripheral blood plasma (Caldwell, Tillson, Brock & Speroff, 1972) but direct measurement is hindered because of the rapid metabolism of PGs (Granstrom, 1972). Furthermore, the estimations of PGF-2 α are complicated by the possibility of platelet synthesis of PGF-2 α during collection and handling of blood samples (Samuelsson, 1972).

The major metabolite of PGF-2 α in the blood of cows is 13,14-dihydro-15 keto-prostaglandin F-2 α (PGFM) (Peterson, Fairclough, Payne & Smith, 1975; Kindahl, Edqvist, Bane & Granstrom, 1976a). The concentrations of progesterone in milk have been shown to reflect those in the blood in cows (Dobson, Midmer & Fitzpatrick, 1975) and buffaloes (Batra, Arora, Bachlaus & Pandey (1979) and we have therefore studied the concentrations of PGFM in blood and milk during the oestrous cycle and early pregnancy in buffaloes.

Materials and Methods

Animals and sampling. Twenty-eight Murrah buffaloes (11 primiparous and 17 multiparous) were selected from the Institute herd. They were maintained under general herd management conditions of feeding and loose housing. Oestrus was detected by behavioural symptoms of the cow and the response of the teaser bull. Blood samples were taken by jugular venepuncture from Day 0 (day of insemination) to Day 5 daily, followed by collection on alternate days until Day 15. Daily samples

were again taken until Day 25 or return to oestrus, whichever occurred earlier. Blood samples were taken on alternate days until Day 40 of pregnancy for animals that did not return to oestrus. In another group, 6 cyclic buffaloes with known oestrous cycle lengths (not inseminated at the previous oestrus) were sampled at 6-h intervals from 4 days before the expected oestrus. Blood samples were collected rapidly into pre-cooled tubes and centrifuged immediately at 4°C. The plasma was stored at -20°C until analysis.

Pooled milk samples from the bulk yield of individual animals were collected at the evening milking (18:00–20:00 h) on the same schedule as blood was collected. The samples were preserved with 0.01% formalin and stored at 4°C until analysed.

Radioimmunoassay. PGFM concentration was determined by a modification of radioimmunoassay technique of Manns (1975). The antiserum was raised in rabbits against a PGFM-BSA conjugate. Duplicate milk samples (200 µl) were extracted with 2 ml petroleum ether (b.p. 40–60°C) for 30 sec. The lower aqueous phase was frozen and the upper organic phase was discarded. The aqueous phase was thawed and acidified with 1 N-HCl to pH 3–4. The samples were again extracted for PGFM with distilled diethyl ether (2 ml) and the extract was evaporated to dryness under nitrogen. The residue was dissolved in 80% methanol (2 ml) and incubated at 40°C for 1 h. The excess fat precipitated by this step was separated by centrifugation at 2600 g for 20 min at -10°C. The supernatant was decanted and evaporated to dryness. The residue was dissolved in 100 µl phosphate-buffered saline (PBS; 0.1 M-phosphate buffer pH 7.0 containing 0.15 M-sodium chloride, 0.015 M-sodium azide and 0.1% gelatin in distilled water) by maintenance at 45°C for 15 min and vortexing for 30 sec to ensure complete solubility. The resulting mixture was combined with 100 µl PGFM antiserum (1:5000 dilution) and 100 µl [5,6,8,9,11,12,14(n)-³H]PGFM (4000 c.p.m./tube; sp. act. 80 Ci/mmol; Radiochemical Centre, Amersham, U.K.). The mixture was vortexed and incubated at 4°C overnight. The bound and unbound fractions were separated by the addition of 500 µl dextran-coated charcoal (1% activated charcoal Norit A, Sigma Chemical Company, St Louis, U.S.A.; 0.25% dextran T-70, Pharmacia Company, Uppsala, Sweden). The contents of the tubes were shaken and allowed to equilibrate for 10 min and centrifuged at 2600 g for 10 min at 4°C. The supernatant containing antiserum bound to PGFM was decanted into scintillation vials containing 10 ml Triton-toluene scintillation fluid (7 g PPO, 0.3 g POPOP, 60 g naphthalene, 333 ml Triton X-100 and 666 ml toluene). The radioactivity was counted after 8 h equilibration between liquid and organic phase. A series of PGFM standards (Ono Pharmaceutical Company, Osaka, Japan) ranging from 2.5 to 1280 pg in 100 µl PBS were concurrently reacted to prepare the standard curve.

PGFM in blood plasma was estimated by extracting a 100 µl sample (acidified to pH 3–4 with 1 N-HCl) with 2 ml diethyl ether. The ether was evaporated to dryness under nitrogen and the residue dissolved in 100 µl PBS. The remaining procedure was similar to the milk PGFM assay as described above.

The recovery of PGFM added to plasma and milk was calculated after allowance for procedural losses and subtraction of the endogenous level (Table 1). The average recovery of [³H]PGFM (4000

Table 1. Recovery of unlabelled PGFM added to blood plasma and milk (8 replicates)

Amount added (pg)	Amount recovered (pg)		% Recovery		Coefficient of variation	
	Blood plasma	Milk	Blood plasma	Milk	Blood plasma	Milk
50	50.5 ± 1.7	49.1 ± 1.5	108.1 ± 3.3	98.2 ± 3.1	8.7	8.9
100	105.5 ± 3.3	99.9 ± 2.8	105.6 ± 3.3	99.9 ± 2.8	8.9	7.9
200	206.2 ± 3.0	196.6 ± 5.1	103.1 ± 1.5	98.4 ± 2.5	4.1	7.3
400	410.5 ± 4.6	397.3 ± 5.0	102.6 ± 1.2	99.3 ± 1.2	3.2	3.6

Blood plasma: $r = 0.998$ ($y = 1.017x + 3.266$).

Milk: $r = 0.999$ ($y = 0.993x - 0.5$).

c.p.m./tube) was $87.1 \pm 0.6\%$ (80.8–91.8%) for plasma and $65.2 \pm 0.9\%$ (57.0–71.3%) for milk. The least amount detected from zero dose was 2.83 pg/tube which was calculated as twice the standard deviation of zero dose tubes. Cross-reaction of the antiserum was obtained by comparing the relative amounts of PGFM and the compounds that were required to reduce the initial binding of [^3H]PGFM by 50%: cross-reactions were 0.79% for 13,14-dihydro-PGF-2 α , 0.27% for 15-keto PGF-2 α , 0.05% for PGF-1 α and <0.04% for PGA-2, PGB-2, PGE-2 and PGF-2 α . The intra-assay coefficient of variation was evaluated by 8 replicate measurements of the same sample in the same assay and was 9.27 and 10.11% for plasma and milk, respectively. The interassay variation was evaluated by the measurement of the 8 replicates of the same sample in 5 different assays and was 14.4 and 16.2% for plasma and milk, respectively.

Results

Since the variation in oestrous cycle length was 22–31 days, the results were calculated for the first 17 days after insemination and 4 days before the next oestrus (Tables 2 and 3). Out of 28 animals, 18 animals returned to oestrus and the rest were diagnosed pregnant at 50–60 days after insemination.

Table 2. Mean $\pm$ s.e.m. PGFM concentrations (no. of samples in parentheses) in blood plasma and milk during the oestrous cycle in buffaloes (Day 0 = day of oestrus)

Day of cycle	PGFM conc. (pg/ml)		Day of cycle	PGFM conc. (pg/ml)	
	Blood	Milk		Blood	Milk
0	233 $\pm$ 15 (18)	235 $\pm$ 16 (18)	15	248 $\pm$ 19 (17)	254 $\pm$ 17 (17)
1	245 $\pm$ 16 (17)	229 $\pm$ 15 (16)	17	258 $\pm$ 13 (17)	262 $\pm$ 17 (17)
3	319 $\pm$ 30 (17)	257 $\pm$ 18 (17)	–4	372 $\pm$ 30 (18)	429 $\pm$ 16 (17)
5	245 $\pm$ 22 (15)	256 $\pm$ 16 (16)	–3	843 $\pm$ 34 (17)	692 $\pm$ 35 (17)
7	260 $\pm$ 19 (16)	274 $\pm$ 16 (16)	–2	913 $\pm$ 27 (16)	792 $\pm$ 32 (16)
9	231 $\pm$ 15 (17)	240 $\pm$ 11 (17)	–1	428 $\pm$ 46 (15)	445 $\pm$ 42 (15)
11	297 $\pm$ 35 (17)	254 $\pm$ 13 (18)	0	221 $\pm$ 09 (18)	248 $\pm$ 12 (18)
13	234 $\pm$ 18 (17)	238 $\pm$ 11 (17)			

Table 3. Mean $\pm$ s.e.m. PGFM concentrations (no. of samples in parentheses) in blood plasma and milk during early pregnancy in buffaloes (Day 0 = day of insemination)

Day of pregnancy	PGFM conc. (pg/ml)		Day of pregnancy	PGFM conc. (pg/ml)	
	Blood	Milk		Blood	Milk
0	244 $\pm$ 23 (10)	240 $\pm$ 20 (10)	21	290 $\pm$ 16 (10)	275 $\pm$ 13 (10)
1	242 $\pm$ 28 (9)	239 $\pm$ 22 (9)	22	326 $\pm$ 39 (7)	266 $\pm$ 22 (9)
3	326 $\pm$ 26 (9)	246 $\pm$ 26 (9)	23	295 $\pm$ 26 (10)	275 $\pm$ 19 (10)
5	271 $\pm$ 22 (10)	261 $\pm$ 22 (10)	24	261 $\pm$ 21 (10)	252 $\pm$ 18 (10)
7	267 $\pm$ 29 (10)	274 $\pm$ 21 (10)	25	253 $\pm$ 14 (10)	262 $\pm$ 14 (10)
9	259 $\pm$ 18 (9)	269 $\pm$ 25 (9)	27	265 $\pm$ 14 (10)	261 $\pm$ 13 (9)
11	278 $\pm$ 26 (9)	267 $\pm$ 19 (10)	29	242 $\pm$ 27 (9)	260 $\pm$ 16 (10)
13	240 $\pm$ 17 (10)	244 $\pm$ 16 (10)	31	361 $\pm$ 39 (10)	290 $\pm$ 14 (10)
15	250 $\pm$ 15 (10)	253 $\pm$ 13 (10)	33	255 $\pm$ 23 (10)	262 $\pm$ 16 (9)
17	249 $\pm$ 23 (10)	263 $\pm$ 23 (10)	35	252 $\pm$ 15 (10)	251 $\pm$ 11 (10)
18	277 $\pm$ 18 (8)	270 $\pm$ 22 (9)	37	258 $\pm$ 20 (10)	263 $\pm$ 15 (10)
19	273 $\pm$ 21 (10)	264 $\pm$ 25 (10)	40	259 $\pm$ 19 (10)	274 $\pm$ 20 (9)
20	272 $\pm$ 17 (10)	273 $\pm$ 21 (10)			

The PGFM concentration fluctuated around the basal level of 200–250 pg/ml during the oestrous cycle and started increasing 4 days before oestrus in non-pregnant animals. The temporal changes of PGFM concentration at the time of luteolysis revealed that PGFM concentration fluctuated dramatically, with a major peak occurring 43.83 ± 1.30 h before the onset of oestrus (Text-fig. 1).

Text-fig. 1. Changes in PGFM concentrations in the blood of buffaloes at the time of oestrus (0 h).

Discussion

The basal level of PGFM concentration in peripheral plasma of buffaloes was similar to that reported by Peterson *et al.* (1975) for cows although Kindahl *et al.* (1976a) obtained a lower basal level (50–100 pg/ml). The increase in PGFM concentration before oestrus coincided with the decline of progesterone concentration that is known to occur at this time (Batra *et al.*, 1979; Suri, Batra, Pahwa & Pandey, 1981; Pahwa & Pandey, 1982). Since administration of PGF-2 α to buffaloes resulted in the occurrence of oestrus 2–4 days after injection (Prasad, Bachlaus, Arora & Pandey, 1979), the peak PGFM concentration observed in the present study before oestrus is presumably a reflection of the elevation of endogenous PGF-2 α responsible for regression of the corpus luteum. The temporal changes of PGFM concentration showed a number of peaks before the oestrus, but these peaks were less pronounced than those reported by Kindahl, Edqvist, Granstrom & Bane (1976b) for cows.

The PGFM concentration did not show any variation during early pregnancy, which agrees with the report of Kindahl *et al.* (1976b) for cows, and Peterson, Tervit, Fairclough, Havik & Smith (1976) for ewes. There was no significant difference ($P > 0.05$) in PGFM concentrations in primiparous and multiparous buffaloes.

The level of PGFM in buffalo milk was very similar to that of blood plasma and was positively correlated with it during the oestrous cycle ($r = 0.981$; $P < 0.001$) and early pregnancy ($r = 0.521$; $P < 0.01$). However, there was no significant difference in the concentration of PGFM ($P > 0.05$) between milk and blood plasma. In the present study, PGFM concentration in milk increased simultaneously with the increase in blood plasma, suggesting the transfer of PGFM into milk down the concentration gradient, but PGFM in milk may also be due to metabolism of PGF-2 α in the mammary gland as reported by Maule Walker & Peaker (1980) for goats.

We conclude that because the PGFM level in milk reflects the uterine PGF-2 α concentration, milk PGFM measurements would be a more convenient technique for assessment of some endocrine changes during clinical disorders of buffaloes.

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