

The effect of oxytocin treatment on the levels of prostaglandin F in the blood of heifers

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Summary. Six heifers with normal oestrous cycles were treated i.m. with 100 i.u. oxytocin on 3 consecutive days, commencing on Days 1–6 after oestrus, and the levels of prostaglandin (PG) F in posterior vena cava plasma were compared with pretreatment values. An increase of PGF in response to oxytocin was significantly influenced by day, with the greatest response occurring on Day 3 after oestrus. In an ovariectomized heifer the levels of PGF in posterior vena cava plasma increased 24 h after priming with oestradiol, but no further increase occurred after oxytocin injection. Peak levels of PGF were higher in the plasma of the posterior vena cava than in the jugular vein. Various storage conditions of the blood before centrifugation and freezing (-20°C) produced significant differences in plasma levels of endogenous PGF, but storage experiments with added labelled PGF-2 α indicated that the PG was stable in plasma and whole blood.

Introduction

Daily administration of oxytocin to heifers during the first week after oestrus markedly shortens the ensuing dioestrus, and the subsequent oestrus occurs 8–12 days, instead of the usual 21 days, after the preceding oestrus (Armstrong & Hansel, 1959; Anderson & Bowerman, 1963; Anderson, Bowerman & Melampy, 1965; Black & Doby, 1965). Premature oestrus is also induced when oxytocin is administered on Days 3 to 6 (Hansel & Wagner, 1960; Black & Doby, 1965; Auletta, Currie & Black, 1972) or Days 3 to 7 after oestrus (Harms & Malven, 1969). Armstrong & Hansel (1959) found that oxytocin was ineffective in hysterectomized heifers, but induced precocious oestrus in sham-operated animals. In heifers receiving oxytocin for 7 days, beginning on Day 1, and hysterectomized on Day 2, the corpus luteum was maintained and oestrus was not induced (Anderson *et al.*, 1965). A uterine luteolysin therefore appears to be involved in mediating the effects of exogenous oxytocin.

Sharma & Fitzpatrick (1974) have demonstrated a release of prostaglandin (PG) F α in response to oxytocin treatment in anoestrous ewes primed with oestradiol, and endometrial PGF-2 α in sheep is affected by oestrogens (Liggins, Grieves, Kendall & Knox, 1972) and progesterone (Wilson, Cenedella, Butcher & Inskip, 1972). In the cow, during the week following oestrus, when there is a change from oestrogen to progesterone dominance, the success of nonsurgical transfer of eggs (Lawson, Rowson, Moor & Tervit, 1975) as well as the retention of radioactive 'imitation eggs' (Tervit, 1973) progressively increases with time after oestrus.

As a preliminary to investigating the role of PGF in the rejection of eggs from the bovine uterus, it was considered useful to examine at which stages during the early luteal phase PGF can be released in response to treatment with oxytocin.

Materials and Methods

Animals

The posterior vena cava and/or the jugular vein were cannulated in each of 12 predominantly Hereford cross-bred heifers weighing approximately 384 kg.

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Cannulae of polythene tubing (i.d. 0.86 mm) were inserted into the jugular vein and the posterior vena cava. Jugular cannulae were introduced through a 14-gauge needle and inserted 45 cm into the jugular vein. The vena cava cannula was inserted surgically through the external pudic vein. After fasting of the animals for 24 h, general anaesthesia was induced with thiopentone sodium and continued with a halothane/nitrous oxide/oxygen mixture. A skin incision 12 cm in length was made parallel and 5 cm lateral to the teats on the side to be cannulated. Blunt dissection was extended towards the inguinal canal and lateral to the mammary gland. An incision into the lateral ligament of the mammary gland enabled the external pudic vein to be located and cannulated as it passed through the inguinal canal. The cannula was inserted some 75 cm into the external pudic vein, ligated in position and the free end passed under the skin to the flank using a trochar and cannula. The incision was surgically repaired using braided silk. In 3 cows cannulated in this manner and subsequently slaughtered, the end of the cannula in each case was located within the posterior vena cava.

Cannula potency was maintained by flushing before and after every blood collection with 5 ml heparinized saline (100 i.u./ml). The heparinized saline present in the cannula also served to heparinize the blood sample collected. Blood samples were centrifuged (533 g at 20°C for 15 min) and the plasma samples frozen (-20°C) within 3 h of collection.

Experiment 1. Posterior vena cava cannulae were inserted into 6 heifers, $\frac{1}{2}$ -2 days before the start of the experiment. Pretreatment blood samples were collected at intervals of 20 min for 2 h. Immediately after collection of the last sample, 100 i.u. oxytocin was administered i.m. in the gluteal region and blood samples were then taken every 10 min for 2 h and every 20 min for a further hour. Each heifer was treated in this way on 3 consecutive days, but the day of initial bleeding varied between Days 1 and 6 after oestrus. It was anticipated that problems experienced in pilot studies of maintaining cannula patency for long periods would not arise, and that any possible release of PGF induced by stress associated with surgery or the experimental procedure itself would not influence the results.

Experiment 2. An ovariectomized heifer was subjected to the same bleeding and oxytocin treatment schedule as in Exp. 1. After 24 h an i.m. injection of 5 mg oestradiol benzoate (in arachis oil) was given, and 48 h later a similar oxytocin treatment and the blood sampling routine were followed.

Experiment 3. Plasma was obtained from 2 heifers with jugular cannulae, one at 3 days, and the other at 6 days, after oestrus. The treatment schedule was identical to that in Exps 1 and 2 but the oxytocin dose was 150 i.u.

Experiment 4. To examine the effect of different conditions of blood storage on the plasma PGF levels, jugular cannulae were inserted in 3 heifers. Blood samples were collected before and after oxytocin treatment (100 i.u. intravenously), at 20 min intervals over a 3 h period, centrifuged (533 g at 20°C for 15 min) and the plasma frozen (a) at -20°C immediately, (b) at -20°C after standing at bench temperature (20°C) for 3 h after the last blood sample was obtained, or (c) at -20°C after treatment (b) plus 18 h storage at 4°C. All samples from each animal were subsequently assessed for PGF within a single assay.

Radioimmunoassay of PGF

PGF was determined by a method similar to that described by Hennem *et al.* (1974). Plasma samples (1 ml) in duplicate were adjusted to pH 4.0 with 25% acetic acid (50 μ l). The samples were then extracted with freshly redistilled diethyl ether (10 ml) using an automatic shaker for 10 min, and subsequently frozen at -80°C to separate the ether and aqueous phases. The ether phases were decanted into all-glass assay tubes, and the solvent removed under a current of air over a waterbath maintained at 45°C. The antiserum for the assay had been prepared in sheep to PGF-2 α , and was kindly supplied by Dr M. T. Withnall (May & Baker Ltd, Dagenham, Essex, U.K.). The antiserum was diluted 1:16,000 with 0.05 M-phosphate-buffered saline (PBS) containing gelatin (1% w/v) and sodium azide (1% w/v); 0.2 ml was added to each assay tube and the tubes were then incubated for 30 min at 4°C. Then, 0.02 μ Ci [5,6,8,11,12,14,15(n)-³H]prostaglandin F-2 α , (sp. act.

120–170 Ci/mmol: Radiochemical Centre, Amersham, Bucks, U.K.) in 0.1 ml PBS was added to each tube and incubation was continued for 30 min at 4°C. The antibody-bound and free prostaglandin were separated from each other by the addition of a suspension (1 ml) of calcium sulphate in ammonium sulphate (see Hennan *et al.*, 1974). The tubes were vortexed, centrifuged and the supernatants aspirated and discarded. The precipitates were resuspended by vortexing in 1 ml deionized water and decanted into a scintillation vial. A Packard TRI-CARB liquid scintillation spectrometer (Model 3380) was used for the determination of radioactivity after adding 11 ml standard toluene-based scintillation fluid containing Triton-X100 detergent (370 ml/litre toluene) to each scintillation vial. Amounts of authentic PGF-2 α between 25 and 2000 pg (in duplicate), kindly supplied by Dr K. Crowshaw (May & Baker Ltd), and appropriate blanks were each taken up in 1 ml PBS and subjected to the extraction and radioimmunoassay procedure used for the plasma samples. Losses due to the ether extraction were assessed in a number of assays on the basis of radioactive PGF-2 α (0.05 μ Ci) recovered.

The results were computed and 95% confidence limits were calculated. The minimum concentration of PGF that could be detected 97.5% of the time was 15–20 pg. The intra-assay coefficient of variation on duplicate determinations was 8.2% ($n = 20$), and the inter-assay coefficient of variation on 1 ml aliquots taken from a pool of plasma from an oestrous cow was 9.6% ($n = 15$). The cross-reactions of various prostaglandins to the antiserum prepared against PGF-2 α when PGF-2 α is 100% at 50% binding were as follows: PGF-1 α 18.5%, PGE-1 0.2% and PGE-2 0.24%; metabolites such as 13,14-dihydro-15 keto PGF-2 α and the prostaglandins F-3 α and F- β group were not tested. Because the E group prostaglandins had a very low cross-reaction, the assay was considered specific for F compounds. Parallelism was found between standard curves for authentic PGF-2 α prepared in PBS or cow plasma which had been stripped with charcoal and validates the use of PBS instead of plasma for the standard curve and recovery samples. The recovery of labelled PGF-2 α after ether extraction was 96.0 \pm 0.8% (S.E.M.) ($n = 21$).

Radioimmunoassay of steroids

The methods adopted for measurement of total unconjugated oestrogen and progesterone were based on those described by Henricks, Guthrie & Handlin (1972) and Guthrie, Henricks & Handlin (1972) for pig plasma. The use of the method for the determination of oestrogen in cow plasma has been reported (Booth *et al.*, 1975). The radioimmunoassay for progesterone was similar to that for oestrogen, except that 20 μ l plasma were used after dilution to 100 μ l with PBS, before addition of the antiserum and tracer steroid, because the levels of progesterone are much higher than those of oestrogen in cow plasma. The antisera to oestrogen and progesterone were gifts from Dr B. J. A. Furr (I.C.I. Ltd, Mereside, Alderley Park, Macclesfield, Cheshire, U.K.) and were used at an initial dilution of 1:75,000 in 0.2 ml PBS for oestrogen and 1:4500 in 0.1 ml PBS for progesterone. The tracer steroids were [2,4,6,7(n)-³H]oestradiol-17 β and [1,2,6,7(n)-³H]progesterone (sp. act. 80–110 Ci/mmol: Radiochemical Centre, Amersham). The amount of labelled oestrogen and progesterone added to each assay tube was 0.01 μ Ci in 0.2 ml and 0.1 ml PBS, respectively. Incubation of the antisera and labelled steroids in the presence of the steroid extracts (blood plasma and standard curve samples) was carried out overnight at 4°C. The separation of bound and free steroid fractions was effected using a dextran-coated charcoal suspension, and the bound counts were finally extracted into 5 ml toluene-based scintillation fluid by shaking the scintillation vials for 10 min on an automatic shaker.

A series of initial investigations was carried out to test the effect of different volumes of plasma on the direct radioimmunoassay of progesterone. The results showed that amounts of charcoal-stripped plasma in excess of 20 μ l, from the cow or pig, caused a progressive deviation from parallelism when compared with steroid samples prepared in PBS. The values suggested that the loss of parallelism was due to non-specific binding factors in plasma. However, when 10 or 20 μ l samples of stripped plasma were added to steroid samples and diluted to 100 μ l with PBS for radioimmunoassay, the results obtained were similar to those with buffer alone.

The cross-reactions of various steroids to the antiserum prepared against oestradiol-17 β were as

Table 1. The mean \pm S.E.M. levels of PGF (pg/ml) in the posterior vena cava of heifers before and after i.m. treatment with 100 i.u. oxytocin

Day of cycle	No. of animals	Pretreatment levels	Post-treatment levels						
			Time from oxytocin injection (min)						
			0-20	20-40	40-60	60-80	80-100	100-120	120-180
2	2	59.7 \pm 10.4	106.5 \pm 34.3	107.8 \pm 31.5	121.7 \pm 29.6	173.0 \pm 35.3	159.5 \pm 65.9	58.2 \pm 23.5	43.7 \pm 14.5
3	3	55.4 \pm 7.4	152.0 \pm 41.9	144.6 \pm 39.1	135.4 \pm 40.1	211.6 \pm 75.1	273.1 \pm 41.5	252.6 \pm 56.5	138.1 \pm 36.7
4	3	26.1 \pm 3.6	31.2 \pm 16.5	24.3 \pm 1.9	31.6 \pm 7.0	45.5 \pm 7.2	31.1 \pm 10.6	59.0 \pm 17.8	45.3 \pm 15.2
5	3	16.3 \pm 2.1	12.8 \pm 3.7	24.4 \pm 2.7	18.6 \pm 4.8	26.5 \pm 7.3	33.6 \pm 11.1	36.5 \pm 7.6	28.9 \pm 6.5
6	3	25.6 \pm 3.5	42.4 \pm 6.1	71.6 \pm 20.3	74.9 \pm 25.0	65.2 \pm 21.2	44.3 \pm 15.4	44.8 \pm 10.3	33.0 \pm 4.8
7	2	8.6 \pm 2.3	11.1 \pm 4.4	13.0 \pm 3.2	25.0 \pm 14.1	18.2 \pm 12.5	18.8 \pm 2.5	26.3 \pm 2.7	44.5 \pm 8.8

Table 2. The mean \pm S.E.M. levels of PGF (pg/ml) in the jugular vein of heifers before and after i.m. treatment with 150 i.u. oxytocin

Day of cycle	Cow	Day of Pretreatment	Post-treatment levels						
			Time from oxytocin injection (min)						
			0-20	20-40	40-60	60-80	80-100	100-120	120-180
R	3	37.1 \pm 2.9	43.2 \pm 6.3	64.5 \pm 13.1	75.1 \pm 3.9	63.4 \pm 1.9	57.8 \pm 3.8	64.6 \pm 13.4	49.5 \pm 0.9
S	6	49.0 \pm 4.3	58.1 \pm 5.0	57.3 \pm 9.1	56.9 \pm 4.1	52.9 \pm 1.5	56.1 \pm 3.6	60.1 \pm 8.1	38.0 \pm 3.1

follows when oestradiol-17 β is 100% at 50% binding: oestrone 55%, oestriol 5.8%, oestradiol-17 α 4.7%, oestrone-3-sulphate 3.9% and oestradiol-3-sulphate 0.5%; androgens and progestins had no significant cross-reaction. For the antiserum prepared against progesterone the cross-reactions when progesterone is 100% at 50% binding were: pregnenolone 3.4%, 17 α -hydroxypregnenolone 1.3%, 17 α -hydroxyprogesterone 1.0%, and 20 α /20 β -dihydroprogesterone, cortisol, testosterone, androstenedione and oestrogens were all <1%.

The accuracy of the steroid determination was similar to that for the prostaglandin assay. The minimum amounts of oestrogen and progesterone that could be determined were around 3 pg and 20–30 pg, respectively.

The precision of the oestrogen assay has been assessed previously (Booth *et al.*, 1975). For the progesterone assay the intra-assay coefficient of variation was 6.7% ($n = 20$) and the inter-assay coefficient of variation was 12.0% ($n = 23$).

Results

Experiment 1

The effect on PGF levels in the posterior vena cava of oxytocin injections on various days after oestrus are shown in Table 1. An analysis of covariance on the post-treatment mean values, with day after oestrus (1–8), and number of cows as factors and with the pretreatment mean values as the concomitant variable, suggested that the post-treatment level of PGF depended upon the day of the oestrous cycle ($P < 0.02$) with some indication that Day 3 after oestrus gave the higher level. Basal levels of PGF did not differ greatly between posterior vena cava and jugular plasma, but post-treatment peak levels were lower in the jugular plasma.

Table 3. Comparison between mean \pm S.E.M. PGF levels (pg/ml) in plasma samples, of 3 heifers, taken before and after oxytocin treatment, and (a) immediately centrifuged at 20°C and stored at –20°C, or (b) kept for 3–6 h at 20°C before storage at –20°C, or (c) treated as for (b) but kept for 18 h at 4°C before storage at –20°C

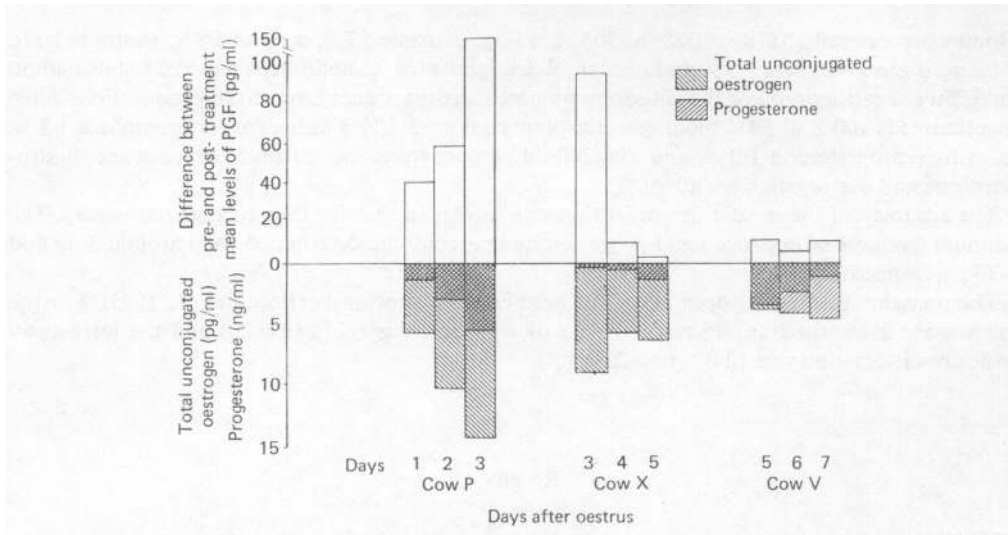
Storage treatment			Significance of storage	Significance of oxytocin treatment
a	b	c		
20.7 \pm 2.0	11.1 \pm 2.0	21.7 \pm 2.0	$P < 0.01$	N.S.
30.1 \pm 2.6	37.4 \pm 2.6	41.3 \pm 2.6	$P < 0.02$	$P < 0.06$
19.3 \pm 3.9	10.1 \pm 3.9	14.6 \pm 3.9	N.S.	N.S.

The relationship between PGF release, represented by the difference between mean pre-treatment and mean post-treatment levels of PGF, and the concomitant steroid levels in 3 heifers are shown in Text-fig. 1.

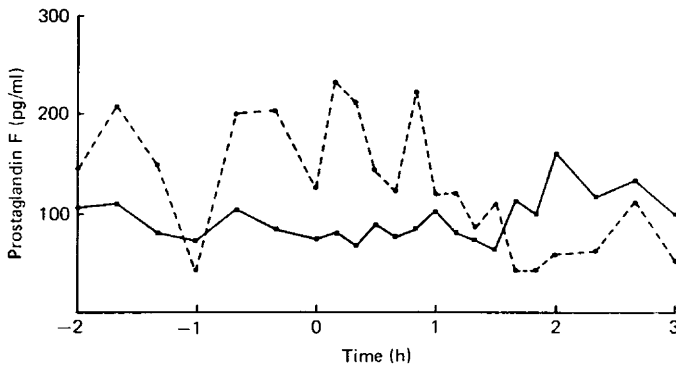
In this and other preliminary experiments in which 100 i.u. oxytocin was administered from Day 3 for 3 or 4 consecutive days, the cycle was shortened to 17 and 8 days in 2 out of 3 heifers. When injections were started before or after Day 3, only 1 heifer (treated on Days 4, 5, 6 and 7) out of 8 had a shorter cycle (17 days).

Experiment 2

The ovariectomized heifer (Heifer M) did not show the same pattern of release of PGF after oxytocin treatment (Text-fig. 2) as did the normal cyclic heifers (Exp. 1). The posterior vena cava levels of plasma PGF were elevated after oestradiol treatment,



Text-fig. 1. The difference between the mean pre- and post-treatment levels of PGF in three heifers on different days after oestrus in relation to steroid levels.



Text-fig. 2. Comparison between the levels of PGF in posterior vena cava plasma of an ovariectomized heifer in response to an i.m. injection of 100 i.u. oxytocin (time 0) 24 h before (—) and 24 h after (----) treatment with 5 mg oestradiol benzoate.

Experiment 3

The administration of a higher dose of oxytocin (150 i.u.) did not lead to large increases in the level of PGF in jugular plasma (Table 2).

Experiment 4

The various storage treatments resulted in significant differences in PGF levels in 2 out of 3 cows, but the pattern was not consistent between cows (Table 3).

Discussion

The release of PGF after oxytocin treatment had been anticipated, but although in Exp. 1 an initial rise was apparent in posterior vena cava plasma 20 min after the i.m. injection of oxytocin, a larger and more sustained rise occurred 1–2 h after treatment on Days 2 and 3. This finding had not been foreseen, since Sharma & Fitzpatrick (1974) found that in oestrogen-primed anoestrous sheep an i.v. injection of oxytocin caused a very rapid elevation of PGF α which returned to basal levels within

25 min. They concluded that this was in keeping with the known duration of the myometrial response to oxytocin in ruminants (Fitzpatrick, 1960). Mitchell, Flint & Turnbull (1975) suggested that there was no simple physical explanation (such as myometrial contractions) for the release of PGF after oxytocin treatment, but that it might be of metabolic origin. Such an explanation would also explain the prolonged release of PGF reported here. However, Rowson (1955) found that during the luteal phase of the cycle the bovine uterus is relatively unresponsive to oxytocin; and epinephrine treatment, which is known to inhibit the uterine contractions caused by oxytocin (VanDemark & Hays, 1951), when superimposed on oxytocin treatment inhibits its cycle-shortening action (Black & Duby, 1965). It is of interest that significant quantitative differences in the levels of PGF occurred ($P < 0.02$) when the same dose of oxytocin was administered on different days after oestrus.

The greater release of PGF on Day 3 coincides both with the stage when radioactive 'imitation eggs' are rapidly ejected after nonsurgical transfer to the uterus (Tervit, 1973), and with the low conception rate after surgical transfer of eggs (Newcomb & Rowson, 1975). While it is tempting to assume a relationship between these phenomena, such a conclusion is probably not justified because pharmacological doses of oxytocin were administered (a physiological release is of the order of 1 i.u. oxytocin; J. Linzell, personal communication).

The dosage of oxytocin has been shown to influence the extent of shortening of the oestrous cycle (Donaldson & Takken, 1968; Donaldson, 1969), although in anoestrous ewes Sharma & Fitzpatrick (1974) found that the amount of PGF α released appeared to depend more on the priming dose of oestrogen than on the challenging dose of oxytocin. It is somewhat surprising that cycle shortening was observed in so few animals, even with doses equivalent to those used by other workers (Black & Duby, 1965). A reduction in cycle length was most marked when the first of the 3 or 4 injections of oxytocin was given on Day 3. In ewes, the reduction in the length of the oestrous cycle after the introduction of intrauterine devices has been shown to be prostaglandin mediated (Spilman & Duby, 1972; Pexton, Ford, Wilson, Butcher & Inskeep, 1975) and does not occur unless the device is inserted on or before Day 3 (Tomaszewska, Hecker & Bray, 1974). In cattle, despite the presence of a corpus luteum which is apparently refractory before Day 4 to the effects of exogenous PGF-2 α (Rowson, Tervit & Brand, 1972), the large release of PGF demonstrable at Day 3 may be involved in the shortening of the cycle after oxytocin treatment.

In the ovariectomized cow, Hays & VanDemark (1953) showed that oestrogens caused a marked increase in response to oxytocin, whereas progesterone greatly reduced the response. Higher levels of PGF were present in posterior vena cava plasma after oestrogen priming in an ovariectomized animal (Exp. 2), but the response to oxytocin differed from that occurring in normal cyclic animals, and indicates that the interrelationship between ovarian steroids is important. This suggestion is further supported by the results shown in Text-fig. 1.

The significant alteration in the levels of endogenous PGF in blood samples, stored under different conditions, of 2 out of 3 animals shows that a standard sampling routine should be adopted. However, the variations that occurred were small and not of the same order of magnitude as those reported in sheep (Umo, 1975). In-vitro experiments were carried out with labelled PGF-2 α to investigate the stability of the prostaglandin in blood or plasma. Tritium-labelled PGF-2 α was added to plasma and stored at -80°C for various periods during 7 months, or added to plasma and whole blood and stored for periods up to 24 h at room temperature. The blood and plasma samples were subsequently extracted with ether and recovered on thin-layer chromatography plates (developed in benzene:dioxan:acetic acid, 20:20:1 by vol., solvent front 15 cm from the origin). In all instances a single peak coincident with authentic unlabelled PGF-2 α was found when the plates were scanned for radioactivity using a thin-layer chromatogram scanner Model RTLS-1S (Panax Equipment Ltd, Mitcham, Surrey, U.K.). These findings indicated that PGF-2 α is relatively stable in bovine plasma. Manns (1975) also found that PGF-2 α was quite stable when incubated in cow's milk for 6 h. However, when the levels of PGF increased slightly during storage of blood samples, this may have been due to synthesis from precursors such as arachidonic acid.

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