Serial levels of PGF were quantitated by radioimmunoassay in the serum of 8 ewes during an induced estrous cycle. Daily samples were obtained via chronic catheterization of the inferior vena cava. Mean PGF levels increased throughout the cycle. In addition, a highly significant peak in the mean PGF level occurred on Day 13 following estrus (Day 12 following the serum LH peak) at a time when luteal progesterone levels remained stable. These observations tend to cast doubt on the physiologic role of PGF as the uterine luteolytic factor.
INTRODUCTION

The uterus plays an important role in regulating the normal ovarian cyclicity in several mammalian species (1,2), with the exception of primates (3,4). However, the mechanism by which the uterus causes luteal regression and brings about the onset of a new cycle remains speculative. It has been postulated that prostaglandin F₂α, PGF₂α, might be the substance responsible for luteolysis (5). However, PGs are ubiquitous in the body and have an amazingly wide range of pharmacological actions (6). In this context many experiments have shown that PGF₂α is luteolytic in the ewe when infused into the ovarian artery (7,8,9) or the uterine vein ipsilateral to the ovary bearing a corpus luteum (10). Also, PGF₂α has been identified by mass spectrometry in the uterine vein plasma of anesthetized ewes towards the end of the estrus cycle (11) and PGF have been quantitated by radioimmunoassay in the utero-ovarian venous plasma of two ewes during the estrus cycle (12). However, these isolated data have not been correlated with values for other hormones during the estrous cycle nor have the levels of PGF in the peripheral blood been measured.

As part of a study of the hormonal variations in the cycling ewe, changes in PGF concentration in the peripheral blood were measured and the results are reported herein.

MATERIALS AND METHODS

Animals: Eight mature cross-bred ewes were studied at the start of the breeding season (September-October, 1971). The animals were kept stantioned in a barn and fed hay and water ad libitum. The onset of estrus was synchronized in all animals by inserting intra-vaginal sponges impregnated with 40 mg of 6α-methyl, 17α-acetoxyprogesterone (Repromat, Tuco Products Co., Orangeville, Ontario). These sponges were left in place for 13 days. On the day of their removal, 750 I.U. of PMS (Gonadin, Haver-Lockart Co., Calgary, Alberta) were injected intramuscularly. Estrus would be expected to occur the following day (13) and for convenience this is termed Day 0.

Blood Collection: On the day of PMS injection, a polythene catheter (1.5 mm O.D.) was inserted into the inferior vena cava via an external saphenous vein so as to lie at or just distal to the junction of the utero-ovarian vein and its anastomosis near the bifurcation of the vena cava. The length of the catheter was precalibrated in an animal of average size by the following procedure. Silver clips were surgically placed at the aforementioned junction. A radio-opaque catheter was then introduced via the saphenous vein and localization of the tip near the clips was accomplished under radiographic control. All catheters were secured in the vein and the external portion was sutured onto the back of the animals. Starting with Day 0 (the day following PMS treatment), eighteen daily samples of 25 ml of blood were collected between
9:00 and 10:00 a.m. Blood samples were allowed to clot in the cold (40°C). Serum was separated and kept frozen (-20°C) until analyzed.

Detection of estrus: At the end of the 18-day period of blood collection, four ewes were saved for another investigator's purposes. They were run twice a day with a raddled vasectomized ram, but no further blood samples were obtained.

Prostaglandin assay: Prostaglandin concentrations were measured in duplicate in 0.5 ml serum samples using the radioimmunoassay method of Caldwell, Burstein, Brock and Speroff (14) with minor modifications. PGF2α-3H (14 Ci/mM, New England Nuclear) was used instead of PGF1α-3H. Since the antiserum used in this study exhibited about 50% cross-reactivity with PGF1α and the system of column chromatography does not separate these two prostaglandins, values are reported as PGF. As the standards were run through the same procedure as used for unknown samples, the values were not corrected for procedural losses (overall recovery = 75%).

Statistics: Data were subjected to a factorial analysis of variance, the two factors being subject and time, to the new Duncan's multiple range test (15) and to linear regression analysis using the University of Manitoba Health Sciences computer facilities.

RESULTS

Six of the eight ewes No. 96, 152, 114, 9200, 7068 and 8172 (Fig. 1 and 2) had a major peak of PGF on Day 13. Sheep No. 159 exhibited a single peak on Day 10 and a slight increase on Day 16-17. Sheep No. 8172 showed a complex series of peaks from day to day with the highest value occurring on Day 17. To a lesser extent, Sheep No. 9001 and 96 presented a similar pattern. From sheep No. 582, only 6 samples of blood were collected. The results have not been plotted in a separate graph. But signs of estrus were recorded for this sheep.

Differences among mean daily levels of PGF (Fig. 3) were highly significant (p<0.01). They appear to rise throughout the estrus cycle as the coefficient of regression was highly significant (r=0.695; p<0.01). A complex series of variations was observed from Day 0 to Day 11 (0.42 - 1.35 ng/ml) with a well defined peak on Day 13 (2.85 ng/ml) and a secondary peak on Day 16-17 (1.98 ng/ml). The new Duncan's multiple range test indicated that this peak on Day 13 was significantly different from the mean levels on Day 16 and 17 (p<0.05) and highly significant from all other means (p<0.01).

Among the 4 sheep run with a ram, ewe No. 7068 showed signs of estrus behaviour on Day 17, ewe No. 582 and ewe No. 152 on Day 18, and ewe No. 9200 on Day 19 (16).
Fig. 1  Daily levels of PGF in the estrous cycle of 4 ewes. Dotted lines stand for missing values.
Fig. 2 Daily levels of PGF in the estrous cycle of 4 ewes.
Fig. 3  Mean daily levels of PGF throughout the estrous cycle of 8 ewes.
DISCUSSION

This is the first report of the changes in PGF concentration in the peripheral blood of conscious ewes during the estrus cycle. By placing the tip of the catheter opposite to or slightly distal to the cavo-uterine venous junction, it was expected to sample a blood with a higher content of hormones than that taken, for instance, from the jugular vein. However, simultaneous measurements of progesterone, estradiol and LH (17) showed that the levels of these hormones are comparable to those already reported for jugular blood during the estrus cycle (18,19,20). It is therefore reasonable to assume that the PGF levels reported herein are close to the situation prevailing in the peripheral blood.

In fact, little information is available with which to compare the results of the present study. Frequent sampling of the utero-ovarian venous blood was made in 2 ewes only (12) and the levels of PGF reported were about 4-fold higher than in the present investigation. Also the same authors detected a complex series of peaks in the concentration of PGF between Day 13 and Day 17 of the cycle, and very low levels were measured at other times. The present data, gathered from a larger group of animals and statistically analyzed, make clear the existence of a major peak in PGF concentration in the peripheral blood on Day 13. Other data (17) indicate that the maximum serum LH concentration was observed on Day 1 of this estrus cycle. Thus, the PGF peak occurred 12 days following the LH peak. It was also observed that the mean progesterone concentration on this day was still on the plateau part of the luteal phase curve and did not decline until 3 days later. On the other hand, the antisera used in the above report (12) compete in an identical manner for PGF₁α and PGF₂α. Although it is uncertain which PGF was actually measured, those authors concluded that PGF₂α is the ovine luteolytic factor.

The existence of a major peak of PGF comparatively early in the luteal phase (Day 12 after the peak of LH) at a time when progesterone levels remain stable, along with preliminary data from 15 ewes showing that PGF₂α-ÖH does not cross from the utero-ovarian vein to the ovarian artery (21), leave unsettled the question of a direct luteolytic action of PGF₂α.

Addendum: In a very recent study (1), PGF levels in the jugular vein of two sheep were reported. The values were lower than those reported in the present study. Levels averaged 0.21 ng/ml on days 3-12, 0.43 ng/ml on day 13, and 0.77 ng/ml on day 14 of the estrous cycle. These lower values in jugular vs. caval venous blood may be due to catabolism of PGF by the lung and/or liver (2).

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