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Luteal Function Following the Infusion of Prostaglandin $F_{2\alpha}$ into the Uterine Vein of the Ewe

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Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or saline was infused into sheep on Day 7 or 8 after estrus. Infusions were made into the uterine vein, ipsilateral to the ovary bearing a corpus luteum (CL), or into a jugular vein at dose rates of 1000–20 μg/h for 3–9 h. Plasma progesterone levels were suppressed to low levels in those ewes which received $PGF_{2\alpha}$ into the uterine vein but not in ewes which received saline into the uterine vein. In three ewes which received $PGF_{2\alpha}$ by the systemic route (200 μg/h or 500 μg/h for 3 h), some reduction in plasma progesterone concentration occurred in the one animal infused at the higher dose.

These findings give support to the hypothesis that a countercurrent mechanism, by means of which a uterine luteolysin could be transferred from the uterine vein into the ovarian artery, may exist in the sheep. Histological studies in one animal suggest that $PGF_{2\alpha}$ may cause luteolysis as a result of interference with the arteriolar blood supply of the CL.

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has been shown to be luteolytic when infused into the ovarian arterial circulation of ewes with ovarian transplants (McCracken, Glew, and Scaramuzzi, 1970; Barrett *et al.*, 1971; Chamley *et al.*, 1972) as well as into the arterial circulation of the intact ewe (Thorburn and Nicol, 1972).

Evidence was put forward which suggested that some form of countercurrent transfer may be operative, by which prostaglandins, when secreted into the uterine vein, could gain access to the ovarian artery without passing through the systemic circulation. This evidence was based upon the results of surgical separation of the relevant vessels (Barrett *et al.*, 1971) and also of infusing tritiated $PGF_{2\alpha}$ into the uterine vein, with its subsequent recovery from the ovarian artery (McCracken, Baird, and Goding, 1971; McCracken *et al.*, 1972).

This paper attempts to demonstrate the physiological significance of the transfer mechanism by showing that circulating progesterone levels are suppressed to low levels following infusion of unlabeled $PGF_{2\alpha}$ into the uterine vein of intact ewes. A preliminary report of some of this data has been given by Goding *et al.* (1971), while similar studies have been described by Thorburn and Nicol (1972).

Further information concerning the quantitative aspects of this mechanism is presented, and concomitant histological studies are presented which offer one possible explanation of the mode of action of $PGF_{2\alpha}$ on the corpus luteum.

MATERIALS AND METHODS

In all experiments, mature cyclic Merino ewes were used. The animals were run continuously with a vasectomized ram fitted with a Sire Sine

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TABLE 1

DETAILS OF TREATMENT, NUMBER OF CORPORA LUTEA, AND PROGESTERONE RESPONSE IN EWES INFUSED WITH $PGF_{2\alpha}$ OR SALINE INTO THE UTERINE VEIN OR SYSTEMIC CIRCULATION

Sheep no.	Solution—infusion rate	Length of infusion (h)	Reduction in plasma progesterone	No. of corpora lutea
1	$PGF_{2\alpha}$—1000 μg/h[a]	3	+	1
2	$PGF_{2\alpha}$— 200 μg/h[a]	3	+	1
4	$PGF_{2\alpha}$— 20 μg/h[a]	9	+	3
5, 6, 7	$PGF_{2\alpha}$— 20 μg/h[a]	7	+, +, −	1, 1, 1
37	$PGF_{2\alpha}$— 500 μg/h[b]	3	+	1
3, 53	$PGF_{2\alpha}$— 200 μg/h[b]	3	−, −	1, 1
8, 9	NaCl (0.154 M)—4.2 ml/h[a]	7	−, −	1, 1

[a] Infusion into the uterine vein on the same side as the ovary bearing a CL.
[b] Infusion into the jugular vein.

harness (Radford, Watson, and Wood, 1960), and daily checks for mating were made during the course of the experiments. Infusions were made in all animals on Day 7 or 8 after mating.

A total of 11 animals were included in the study. Cannulation of the uterine vein on the same side as the ovary bearing a corpus luteum (CL) was carried out under general anesthesia (Fluothane-ICI). In the first two studies only, a second cannula was placed in the uteroovarian vein and the entire experiment was carried out under general anesthesia.

Infusions were made into the uterine vein of six other animals on the day after surgical insertion of the cannulae. Ewes 3, 37, and 53 were used as controls, the infusion in this case being made into the jugular vein. $PGF_{2\alpha}$ solutions were made up in NaCl solution (0.154 M) and all infusions were given at the rate of 4.2 ml/h.

Experimental procedure and response to treatment are shown in Table 1. Blood samples were collected before the start of each infusion, during the course of the infusion and for up to 50 h thereafter. In all animals except ewe 1, peripheral blood samples were taken: in ewes 1 and 2 timed collections were made of uteroovarian vein blood. Plasma progesterone was determined by the method of Cain *et al.*, (1972). The treatment of blood samples and the calculation of progesterone secretion rates was as described previously (Chamley *et al.*, 1972).

An effect upon luteal function was established on the basis of a fall in progesterone secretion rate and/or peripheral plasma progesterone concentration, on the results of laparotomy and, in some cases, also on histological evidence.

Laparotomy was performed upon all animals 7 days after treatment. At this time the ovaries from ewes 2, 3, and 4 were fixed in Bouin's solution for histological examination. Paraffin sections were stained with hematoxylin and eosin (H and E) and van Gieson's stain.

RESULTS

At the time at which these experiments were conducted, there was no information available which would be of assistance in choosing a physiological dose rate for the infusion of $PGF_{2\alpha}$ into the uterine vein. Accordingly, the first two experiments (on ewes 1 and 2) were carried out at 1 mg/h and at 200 μg/h, respectively, to determine whether luteolysis could be achieved by this route.

The secretion rates of progesterone for ewes 1 and 2 (Fig. 1) during the control period were 219.8 ± 80.1 and 99.7 ± 14.4 μg/h (mean ± SD: $n = 4$), respectively. By the end of the infusion period, these rates had fallen to 70.0 and 78.4 μg/h and continued to fall to 14.0 and 42.7 μg/h approximately 6 h from the start of infusion. In ewe 1, the uteroovarian blood flow was somewhat lower during the early part of the PG infusion than subsequently. However, a lower blood flow was also noted for the sample taken immediately before the PG infusion was begun, and is therefore unlikely to be due to the infusion. In ewe 2, the uteroovarian blood flow remained between 310 and 460 ml/h for the duration of the experiment.

The changes in peripheral plasma progesterone concentration resulting from the

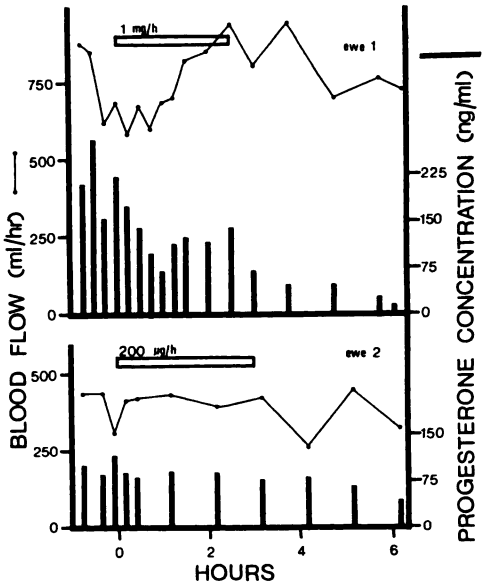

FIG. 1. Changes in progesterone secretion rate and ovarian blood flow in ewes 1 and 2 after infusion of $PGF_{2\alpha}$ into the uterine vein *in situ*.

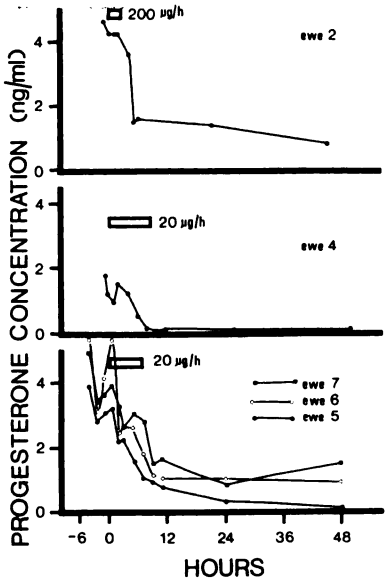

FIG. 2. Changes in peripheral plasma progesterone concentration in conscious ewes infused with $PGF_{2\alpha}$ into the uterine vein *in situ*.

infusion of $PGF_{2\alpha}$ into the uterine vein of ewes 2, 4, 5, 6, and 7 are shown in Fig. 2. Progesterone levels decreased when $PGF_{2\alpha}$ was given at a dose rate as low as 20 µg/h for 7 h. However, there was no suppression of progesterone in ewes which received saline. In the three ewes which received $PGF_{2\alpha}$ by the systemic route (200 µg/h or 500 µg/h for 3 h), some reduction in plasma progesterone concentration occurred in the one animal (ewe 37) infused at the higher dose.

Laparotomy

On visual appraisal, one ovary of ewe 1 contained a degenerate CL. In all the other animals, the CL were of normal size and color.

Histology

The ovary of ewe 2 (which had been given 200 µg/h $PGF_{2\alpha}$ into the uterine vein) contained a single corpus luteum, 5 mm in diameter and which showed advanced regressive changes (Plate 1, no. 1). Two 5-mm follicles were present, and showed histological evidence of active growth.

In ewe 3 (control, 200 µg/h $PGF_{2\alpha}$ into

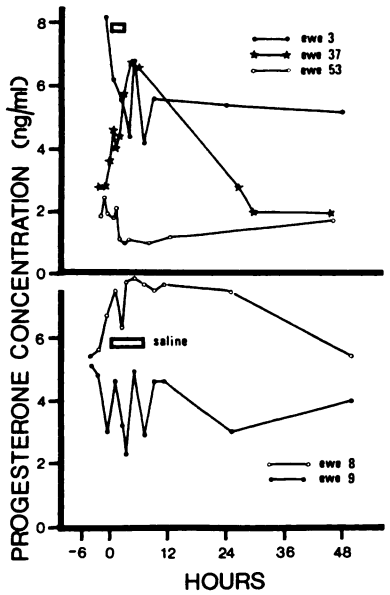

FIG. 3. Peripheral plasma progesterone levels in control sheep receiving $PGF_{2\alpha}$ (ewes 3 and 37—200 µg/h; ewe 53–500 µg/h) into the jugular vein or saline (ewes 8 and 9) into the uterine vein *in situ*.

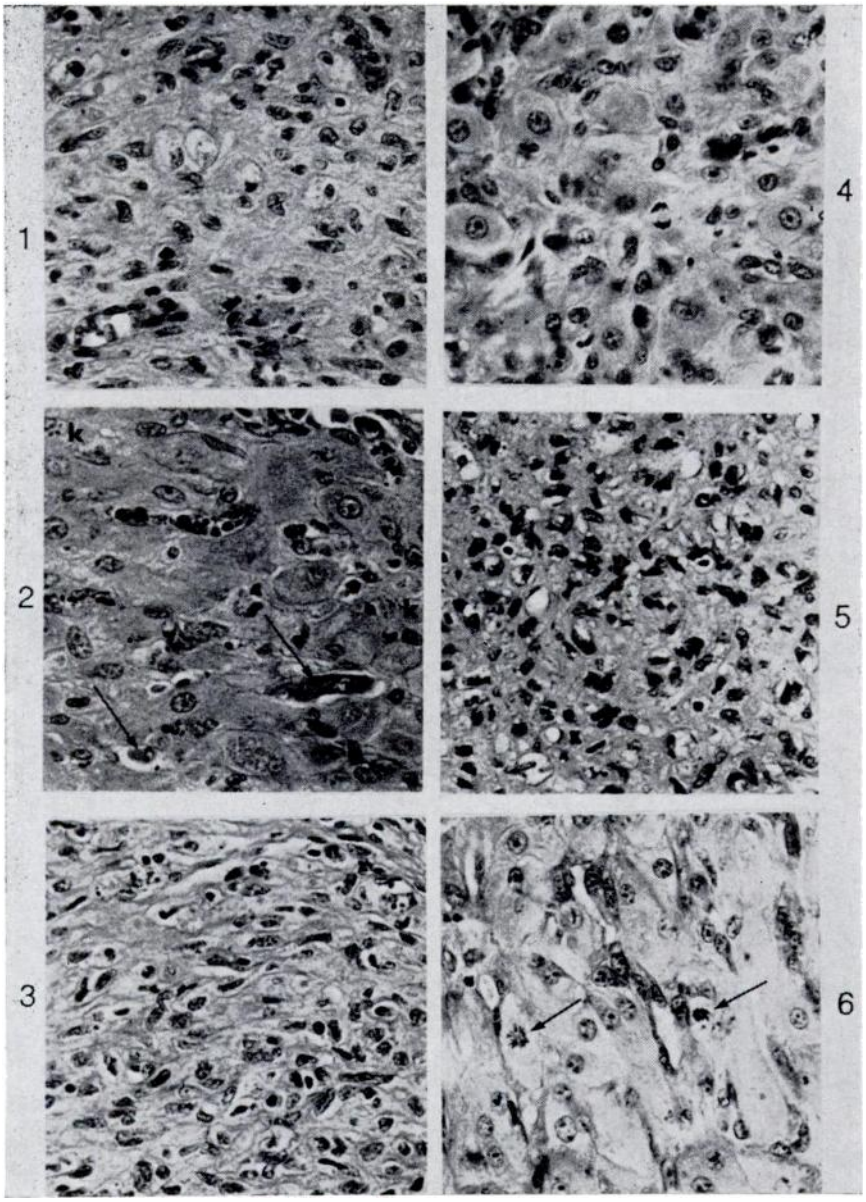

PLATE 1. Histological appearance of corpora lutea taken from ewes infused with $PGF_{2\alpha}$ into the jugular vein or uterine vein *in situ*. 1—Corpus luteum of ewe 2, showing advanced regressive changes. H and E, ×400. 2—Corpus luteum of ewe 3, showing early signs of regression. Karyorrhectic nuclei (k) are present, and shrunken, degenerate cells with hyperchromatic nuclei (arrowed) are seen among typical large cells. H and E, ×400. 3—Corpus luteum from ewe 4, showing advanced regression. H and E, ×400. 4—First active corpus luteum from ewe 4. A mitotic figure is present in the center of the field. H and E, ×400. 5—Same corpus luteum as no. 4, showing portion of a wedge-shaped area of apparent regression. H and E, ×400. 6—Second active corpus luteum from ewe 4, showing luteal cells and mitotic figures (arrowed). H and E, ×400.

the jugular vein), the ovary contained a single CL, 9 mm in diameter, with a large, fluid-filled central cavity. No large follicles were present. Histologically, the CL showed signs of early regression (Plate 1, no. 2).

The ovary of ewe 4 (20 μg/h $PGF_{2\alpha}$ into the uterine vein) contained one 5-mm CL which showed advanced regression (Plate 1, no. 3) as well as two apparently active CL. There was also one 7-mm antral follicle whose wall showed focal areas of luteinization. The first active CL, 7 mm in diameter, consisted mainly of mature luteal tissue containing many mitotic figures (Plate 1, no. 4). However, in one large wedge-shaped area extending toward the ovarian surface, the structure was suggestive of regression. This area was composed of loose, relatively cellular, fibrous connective tissue containing many large vacuolated cells (Plate 1, no. 5), and was almost devoid of recognizable lutein cells. The second active corpus luteum, 8.5 mm in diameter, appeared to be of more recent origin. Its wall was thrown into many folds projecting into the antrum, and mitotic figures were numerous (Plate 1, no. 6). The antrum was large and contained a coagulum rich in fibrin, at whose borders were numerous proliferating fibroblasts. Since no ovulation point was detected on the surface, the possibility that this was an atretic CL could not be excluded.

DISCUSSION

The ability of $PGF_{2\alpha}$ to cause a decrease in plasma progesterone levels when infused into the uterine vein, but not when infused systemically, gives further support to the hypothesis that a countercurrent transfer mechanism by which a uterine luteolysin could be transferred from the uterine vein into the ovarian artery, may exist in the sheep (Barrett *et al.*, 1971; McCracken *et al.*, 1971, 1972). This hypothesis has not gained widespread support in the literature. Thorburn and Mattner (1971) described a surgical preparation in which the uteroovarian vein was separated from the ovarian artery and then anastomosed onto the anterior mammary vein. Most of the sheep prepared in this way continued to cycle, although the occurrence of persistent CL was noted in some. Restall *et al.* (1973) reported that $PGF_{2\alpha}$ was effective in interrupting luteal function in the ewe, when infused into the uteroovarian vein after it had been separated from the ovarian artery.

Both of these observations could be explained by the work of Baird and Land (1973). They have described a tubal venous arcade which anastomoses with both the uterine and ovarian veins and offers another route for drainage of the uterus. Thus, in the experiments of Thorburn and Mattner (1971) and Restall *et al.* (1973), a luteolytic substance could have entered the ovarian vein via this tubal arcade, thereby coming into close contact with the ovarian artery once again.

In the experiments reported in this paper, the lowest dose rate found to cause a reduction in peripheral plasma progesterone in two out of three ewes was 20 μg/h. This is lower than the dose rate (40 μg/h) used by Thorburn and Nicol (1972). Assuming a uteroovarian plasma flow in one uterine horn as 1.8 l/h (Thorburn and Mattner, 1971), an infusion rate of 20 μg/h of $PGF_{2\alpha}$ into the uterine vein would result in a plasma concentration of approximately 11 ng/ml. Concentrations of $PGF_{2\alpha}$ in uterine venous plasma of this order have been reported by Thorburn *et al.* (1972) and McCracken *et al.* (1972).

Although $PGF_{2\alpha}$ has been shown to be luteolytic in several species (Goding *et al.*, 1971/72), there is relatively little evidence to explain the means by which this compound exerts its luteolytic effect. Behrman, McDonald and Greep (1971) have described a mechanism whereby $PGF_{2\alpha}$ interferes with the biosynthetic pathway for progesterone in the PMS-treated prepubertal rat, and such a mechanism may operate in other species. However, the histologi-

cal evidence on ewe 4 in the present study leaves open the possibility that $PGF_{2\alpha}$ may cause luteolysis by vasoconstriction (Pharriss, 1971). It seems likely, however, that the action of $PGF_{2\alpha}$ in causing reduction in ovarian blood flow in the rat and rabbit represents a pharmacological rather than a physiological effect of this compound. Similar comments also apply to the situation when an intraarterial dose of 100 μg/h is given to a sheep with an ovarian autotransplant (McCracken *et al.*, 1970). In the case of ewe 4 in this present study, the dose of $PGF_{2\alpha}$, given into the uterine vein, was only 20 μg/h. Yet, the histological picture of the CL of this animal was one in which areas of healthy activity were seen together with areas undergoing regression. It is possible that localized regression could have arisen as a result of locally reduced luteal blood supply. Such an effect could be brought about by constriction of afferent arterioles of the CL: the incompleteness of the effect probably being the result of the low dosage use. Hence, an action of $PGF_{2\alpha}$ may be to cause a selective shunting of blood from the CL by acting upon afferent arterioles. Such an effect could result in interference with luteal function without there being any observable change in ovarian blood flow. Further studies on this subject appear warranted in the light of the foregoing evidence.

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RECOMMENDED REVIEWS

GODING, J. R., CUMMING, I. A., CHAMLEY, W. A., BROWN, J. M., CAIN, M. D., CERINI, J. C., CERINI, M. E. D., FINDLAY, J. K., O'SHEA, J. D., AND PEMBERTON, D. H. (1972). Prostaglandin $F_{2\alpha}$ the lutcolysin in the mammal? *Gynecol. Invest.* **2**, 73.

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