EFFECT OF PROSTAGLANDIN $F_{2\alpha}$ ON THE ULTRASTRUCTURE AND FUNCTION OF SHEEP CORPORA LUTEA

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Summary. Early morphological changes in the ultrastructure of CL of ewes treated with prostaglandin $F_{2\alpha}$ were examined in relation to luteal function as judged by plasma progesterone concentration. The luteolytic effect of prostaglandin $F_{2\alpha}$ was confirmed, but there was little synchrony between morphological and functional luteolysis. Significant changes included a decrease in the amount of smooth endoplasmic reticulum, a change in the shape of mitochondria and a decrease in the number of membrane-bound granules. There was also an accumulation of lipids.

INTRODUCTION

There is good agreement between steroidogenic function and cytological and biochemical changes in sheep CL during the late luteal phase of the oestrous cycle (Deane, Hay, Moor, Rowson & Short, 1966; Bjersing, Hay, Moor, Short & Deane, 1970). During normal luteal regression, there is an abrupt decline in plasma progesterone concentration (Stabenfeldt, Holt & Ewing, 1969; Sarda, Robertson & Smeaton, 1973). A similar cessation of luteal function has been demonstrated following the infusion of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in the ewe (McCracken, Glew & Scaramuzzi, 1970; Thorburn & Nicol, 1971). Clinical evidence of luteolysis following intramuscular injection or intrauterine deposition of $PGF_{2\alpha}$ has been presented by Rowson, Tervit & Brand (1972) in the cow and Douglas & Ginther (1973) in the ewe. The mechanism by which $PGF_{2\alpha}$ brings about this luteolysis is still unknown.

The purpose of this study was to assess early changes in CL structure following a single intramuscular injection of a luteolytic dose of $PGF_{2\alpha}$ to cyclic ewes and to correlate any regressive changes with luteal function.

MATERIALS AND METHODS

In January and February 1974, ten Clun Forest ewes were housed with a rad-dled vasectomized ram and were examined twice daily for oestrus. The oestrous cycle length in this group of ewes was found to be between 15.5 and 16.5 days.

The day the ewes were marked was designated Day 1 of the oestrous cycle. On Day 10 of the cycle, six animals were given 10 mg PGF_{2α}-tromethamine salt (Upjohn) intramuscularly in 5 ml saline. This dose of PGF_{2α} has been shown to be luteolytic (Douglas & Ginther, 1973). Two control animals received 5 ml of vehicle alone. Anaesthesia was induced by an intravenous injection of thio-pentone sodium 12 hr after treatment, and was maintained with fluothane and oxygen. The ovaries were exposed through a ventral mid-line incision. The CL were carefully dissected out and immediately placed on a small quantity of fixative on a pad of dental wax. Other CL were obtained from two ewes on Day 15 of the cycle. A portion of the CL was finely chopped (0.5 to 1 mm³ pieces) and put in fixative for subsequent processing for electron microscopy.

Blood collection

A cannula was inserted into a saphenous vein (Coudert, Phillips, Palmer & Faiman, 1972) on Day 9 of the oestrous cycle. Blood samples were obtained twice daily before treatment with PGF_{2α}, four times after treatment at 3-hr intervals and once on the day after removal of the CL. Plasma was separated and stored at -20°C for progesterone assays. The radioimmunoassay method of Abraham, Swerdloff, Tulchinsky & Odell (1971) was used without prior chromatography or the use of an internal tracer. Plasma samples from anoestrous ewes, to which known amounts of progesterone were added, were included in each assay. The mean recovery of progesterone ($n = 8$) was $75.3 \pm 2.3\%$. The progesterone data was analysed using Student's *t* test.

Electron microscopy

Tissue collected as above was fixed in 2.3% glutaraldehyde in 0.1 M-cacodylate buffer for 2 hr and 2% osmium tetroxide for a further 2 hr at 4°C. Both fixatives were adjusted to pH 7.2. Blocks fixed in osmium tetroxide were washed with cacodylate buffer, dehydrated and embedded in Epon (Taab). Sections (1 μm) were cut and stained with toluidine blue to verify the type of tissue. Thin (gold or silver) sections were cut with a Reichert OMU2 ultratome and placed on coated grids. Dried sections were stained in uranyl acetate for 5 min, and counterstained in lead citrate for a further 5 min. Examination and photography were carried out with an AEI EM6B electron microscope at magnifications of 3000 to 15,000 and photographically enlarged 2.4 times.

RESULTS

Progesterone concentration

The plasma progesterone concentration in blood collected from the inferior vena cava of treated and control ewes is presented in Table 1. The mean peripheral plasma progesterone concentration dropped significantly in all animals by 6 hr after PGF_{2α} treatment. By 12 hr after treatment, a near minimum detectable concentration of 0.5 ng/ml was reached in all animals. The peripheral plasma progesterone concentration in the untreated group was unchanged during the experimental period.

FIG. 1. Portions of two luteal cells from an untreated ewe on Day 10 after oestrus, showing polymorphic mitochondria (M), nucleus (N), Golgi lamellae (G), numerous membrane-bound granules (double arrows) and collagen fibres (C). The plasma membrane shows folds and microvilli (mv). $\times 7200$.

(Facing p. 288)

FIG. 2. Portions of luteal cells from an untreated ewe on Day 15 after oestrus, showing indistinct cell outlines, nucleus (N), mitochondria (M) with a rarefied matrix, and masses of electron-dense lipid droplets (L). Collagen fibres (C) are abundant. $\times 7200$.

FIG. 3 A portion of a luteal cell from a ewe treated with $\text{PGF}_{2\alpha}$ on Day 10 after oestrus, showing the nucleus (N), spherical mitochondria (M) and the vesicular appearance (V) of the smooth endoplasmic reticulum. Note absence of membrane-bound granules. $\times 12,000$.

FIG. 4. A portion of another luteal cell from a ewe treated with $\text{PGF}_{2\alpha}$ on Day 10 after oestrus, showing the nucleus (N), mitochondria (M), vesicular appearance (V) of the smooth endoplasmic reticulum, and the accumulation of lipid (L). $\times 24,000$.

Ultrastructure

Two types of cells could be identified on the basis of size in CL from untreated ewes on Day 10. Large angular cells (20 to 25 μm) had several Golgi lamellae and an abundance of smooth endoplasmic reticulum (Pl. 1, Fig. 1). The plasma membrane of the large angular cell was often thrown into many thin microvillous projections and folds. The second cell type was smaller (4.5 to 10 μm) and more elongated; it had fewer polymorphic mitochondria but showed numerous vesicles which may represent a poorly defined agranular reticulum. Both cell types had a fairly large nucleus (2 to 10 μm), with a smooth nuclear membrane. Surface specializations of the plasma membrane such as invaginations and pinocytotic vesicles were found occasionally. Small membrane-bound electron-dense granules (0.15 to 0.45 μm) were numerous and appeared to be unevenly distributed in the cytoplasm (Pl. 1, Fig. 1). A few spherical lipid droplets (0.4 to 0.65 μm) were also found. Collagen fibres were present in the intercellular spaces often in association with blood vessels. A few fibroblast cells could be seen in the same areas. A small number of eosinophilic leucocytes were present.

Table 1. The plasma progesterone concentration in blood from the inferior vena cava of control ewes and ewes treated with 10 mg PGF_{2α} on Day 10 after oestrus

Ewes	Plasma progesterone conc. (ng/ml)					
	Day before treatment	Time after treatment with PGF _{2α} (hr)				Day after removal of CL
		3	6	9	12	
Treated (N = 6)	4.8 ± 0.2	4.6 ± 0.9	1.3 ± 0.4*	0.9 ± 0.5*	0.5 ± 0.4*	0.4 ± 0.2
Controls (N = 2)	5.2 ± 0.2	4.6 ± 0.2	4.9 ± 0.2	5.0 ± 0.1	4.9 ± 0.1	—

The results are expressed as Mean ± S.D.

* Significantly different ($P < 0.001$) from the pre-treatment concentration, or 3 hr after treatment and the controls.

In CL from untreated ewes on Day 15, there was evidence of cellular disorganization. This was marked by a general diminution in cell size, massive invasion by collagen and a poorly defined plasma membrane (Pl. 2, Fig. 2). The smooth endoplasmic reticulum appeared vesicular and the mitochondria showed higher electron density and an indistinct inner structure. Blood vessels were less abundant than in the CL from control ewes on Day 10. There was a marked increase in the size (0.5 to 2 μm), number and electron density of lipid droplets (Pl. 2, Fig. 2). These appeared coarse and tended to coalesce, forming large lipid masses which obscured the cell outlines. In a few specimens, cytoplasmic debris and myelin-like figures (2 to 4 μm) were present. A few small electron-dense bodies lacking the membrane and uniformity of the granules found in the CL of untreated ewes on Day 10 were observed.

The majority of cells showed various degrees of minor alterations following treatment with PGF_{2α}. There was an obvious reduction in cell size compared to

the CL from Day 10 untreated ewes, and this gave the intercellular spaces a wider appearance, but the smooth endoplasmic reticulum was more vesicular and vacuolar. Mitochondria appeared more uniformly spherical (0.2 to 0.3 μm) and their matrix, though rarefied, showed an increase in electron density (Pl. 3, Fig. 3). The small membrane-bound granules were either scarce or completely absent. There was a marked increase in the number of lipid droplets (Pl. 3, Fig. 4), as in the regressing CL from the Day-15 untreated ewe though, in this case, their form was fairly regular. In some specimens, karyolysis of the endothelial cells of the blood vessels could be observed.

DISCUSSION

The results of the present study showed that the precipitous decline in plasma progesterone concentration following treatment with $\text{PGF}_{2\alpha}$ was accompanied by limited evidence of cellular disorganization. This may be explained by the short period between treatment and removal of CL for the present study. Giannina, Butler, Sawyer & Steinetz (1973) showed that administration of $\text{PGF}_{2\alpha}$ to 5-day pregnant hamsters caused a dramatic decrease in progesterone concentration with apparently no damage to the CL and Koering, Kirton & Thor (1973) also showed that following the administration of $\text{PGF}_{2\alpha}$ to pregnant rabbits progesterone concentration decreased before any significant changes in cellular structure were observed.

The present observations support those of Deane *et al.* (1966) and Bjersing *et al.* (1970), who showed conclusively that significant fine cytological changes in sheep CL were not evident before the 15th or 16th day of the cycle, even though involutionary changes were detectable histochemically as early as Day 12 or 13 of the cycle.

The mitochondrial changes observed 12 hr after treatment of ewes with $\text{PGF}_{2\alpha}$ are interesting. The apparent spherical form and reduced size of these mitochondria may indicate a reduction in cellular activity and are consistent with the view that these organelles are involved in the biosynthesis of progesterone.

The relative paucity of the electron-dense granules in the CL of ewes treated with $\text{PGF}_{2\alpha}$ suggests that these granules may be associated with progesterone secretion, especially since specimens which showed larger numbers of these granules were from sheep with higher plasma progesterone concentrations at the time the CL were removed. Dingle, Hay & Moor (1968) have suggested, however, that an increase in 'lysosomal fragility' at the time of normal luteal regression brought about the demise of the CL of the cycle in sheep. Though it is not possible to say whether the electron-dense granules seen in the CL of ewes on Day 10 of the cycle represent the 'lysosomes' described by Dingle *et al.* (1968), in our study there was no evidence of the rapid lysis of luteal cells that such an hypothesis would suggest.

Changes in the form and arrangement of the smooth endoplasmic reticulum resembled those seen by Bjersing *et al.* (1970). As in our study, these authors noted a decline in the amount of endoplasmic reticulum and also pointed out that it became more vesicular as luteolysis progressed. Deane *et al.* (1966)

were unable to demonstrate any clear changes in this organelle before the luteal cells became grossly involuted.

A few lipid droplets were evident in the CL from untreated ewes on the 10th day of the cycle. This is at variance with the observations of Bjersing *et al.* (1970) who found "no, or almost no, lipid droplets" in the CL of sheep on Days 10 to 13 of the cycle. The obvious accumulation of lipids in the CL of ewes treated with PGF_{2α} is an involutionary change and is consistent with the accepted view that, during the decline in the secretory activity of a steroid secretory cell, the ability to synthesize lipids is retained but the capacity to convert these lipids to steroid hormones is lost (Deane, 1958; Enders & Lyons, 1964).

The increase in the number of eosinophilic leucocytes coupled with focal karyolysis observed in a few endothelial cells of blood vessels following PGF_{2α} treatment indicates the involvement of a vascular factor in luteolysis induced by PGF_{2α} and perhaps in normal luteal regression as suggested by Bjersing *et al.* (1970).

In conclusion, the administration of PGF_{2α} to cyclic ewes on Day 10 of an oestrous cycle resulted in functional luteolysis and limited ultrastructural changes similar to those seen during the early stages of natural luteolysis. This may reflect the fact that the first stage of PGF_{2α}-induced luteolysis, and also of natural luteolysis, is biochemical in nature and that marked cellular disorganization is a late consequence of the biochemical change. More work will be needed to resolve these points.

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REFERENCES

- ABRAHAM, G. E., SWERDLOFF, R., TULCHINSKY, D. & ODELL, W. D. (1971) Radioimmunoassay of plasma progesterone. *J. clin. Endocr. Metab.* **32**, 619-624.
- BJERSING, L., HAY, M. F., MOOR, R. M., SHORT, R. V. & DEANE, H. W. (1970) Endocrine activity, histochemistry and ultra-structure of ovine corpora lutea. 1. Further observations on regression at the end of the oestrous cycle. *Z. Zellforsch. mikrosk. Anat.* **111**, 437-457.
- COUDERT, S. P., PHILLIPS, G. D., PALMER, M. & FAIMAN, G. (1972) Prostaglandin F concentration in the peripheral blood of the ewe during the oestrous cycle. *Prostaglandins*, **2**, 501-509.
- DEANE, H. W. (1958) Intracellular lipids: their detection and significance. In *Frontiers in Cytology*, pp. 227-250. Yale University Press, New Haven.
- DEANE, H. W., HAY, M. F., MOOR, R. M., ROWSON, L. E. A. & SHORT, R. V. (1966) The corpus luteum of the sheep: relationship between morphology and function during the oestrous cycle. *Acta endocr., Copenh.* **51**, 245-263.
- DINGLE, J. T., HAY, M. F. & MOOR, R. M. (1968) Lysosomal function in the corpus luteum of the sheep. *J. Endocr.* **40**, 325-336.
- DOUGLAS, R. H. & GINTHER, O. J. (1973) Luteolysis following a single injection of prostaglandin F_{2α} in sheep. *J. Anim. Sci.* **37**, 990-993.

- ENDERS, A. C. & LYONS, W. R. (1964) Observations on the fine structure of lutein cells: the effects of hypophysectomy and mammotrophic hormone in the rat. *J. Cell Biol.* **22**, 127-141.
- GIANNINA, T., BUTLER, M., SAWYER, W. & STEINETZ, B. (1973) On the mechanism of prostaglandin $F_{2\alpha}$ -induced abortion in hamsters. *Biol. Reprod.* **9**, 104-105.
- KOERING, M. J., KIRTON, K. T. & THOR, M. J. (1973) The effects of prostaglandin $F_{2\alpha}$ on the structure and function of the rabbit ovary. *Biol. Reprod.* **9**, 226-245.
- MCCRACKEN, J. A., GLEW, M. E. & SCARAMUZZI, R. J. (1970) Corpus luteum regression induced by prostaglandin $F_{2\alpha}$. *J. clin. Endocr. Metab.* **30**, 544-546.
- ROWSON, L. E. A., TERVIT, R. & BRAND, A. (1972) The use of prostaglandins for synchronization of oestrus in cattle. *J. Reprod. Fert.* **29**, 145, Abstr.
- SARDA, I. R., ROBERTSON, H. A. & SMEATON, T. C. (1973) Sequential changes in plasma progesterone levels in the ewe during the oestrous cycle and during pregnancy in intact and ovariectomised sheep. *Can. J. Anim. Sci.* **53**, 25-34.
- STABENFELDT, G. H., HOLT, J. A. & EWING, L. L. (1969) Peripheral plasma progesterone levels during the ovine estrous cycle. *Endocrinology*, **85**, 11-15.
- THORBURN, G. D. & NICOL, D. H. (1971) Regression of ovine corpus luteum after infusion of prostaglandin $F_{2\alpha}$ into ovarian artery and vein. *J. Endocr.* **51**, 751-752.