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Response of the 1–5 day-aged ovine corpus luteum to prostaglandin F2 α

E. Rubianes^{a,b,*}, A. Menchaca^b, B. Carbajal^b

^a Department of Animal Production, Faculty of Agriculture, Lasplacas 1550, Montevideo, Uruguay

^b Laboratory of Reproductive Physiology, Faculty of Veterinary Sciences, PEDECIBA, Lasplacas 1550, Montevideo, Uruguay

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Abstract

The hypothesis that, in the ewe, prostaglandin (PG) F2 α administration on day 3 after ovulation is followed by luteolysis and ovulation was tested using 24 animals. The ewes were treated with a dose of a PGF2 α analogue (delprostenate, 160 μ g) on days 1 ($n = 8$), 3 ($n = 8$) or 5 ($n = 8$) after ovulation, was established by transrectal ultrasonography. Daily scanning and blood sampling were performed to determine ovarian changes and progesterone serum concentrations by radioimmunoassay. The treatment induced a sharp decrease of progesterone concentrations followed by oestrus and ovulation in all ewes treated on days 3 and 5 and in one ewe treated on day 1 (8/8, 8/8, 1/8; $P < 0.05$). Seven ewes treated on day 1 did not respond to PGF2 α treatment and had an inter-ovulatory cycle of normal length (17.4 ± 0.5 days). However, the profile of progesterone concentrations during the cycle of these ewes was delayed 1 day ($P < 0.05$) compared with a control cycle. The overall interval between PGF2 α and oestrus for the 17 responding ewes was 42.4 ± 2.3 h. In 15 of these ewes the ovulatory follicle was originated from the first follicular wave and the ovulation occurred at 60.8 ± 1.8 h after PGF2 α treatment. The other two responding ewes ovulated an ovulatory follicle originated from the second follicular wave between 72 and 96 h after treatment. These results support the hypothesis and suggest that refractoriness to PGF2 α of the recently formed corpus luteum (CL) may be restricted to the first 1–2 days post-ovulation.

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1. Introduction

Once prostaglandin (PG) F2 α was identified as a uterine luteolytic factor in the oestrous cycle of the ewe (McCracken et al., 1972), synthetic forms were developed which can be used

* Corresponding author. Fax: +598-2-6280130.

E-mail address: rubianes@adinet.com.uy (E. Rubianes).

to induce premature luteolysis. The injection of an analogue of PGF2 α to a flock of randomly cyclic ewes is effective to induce luteal regression in most ewes with a consequent return to oestrus (Acritopoulou and Haresign, 1980). However, the responding ewes present a greatly variable interval to oestrus and some of the ewes do not respond, which precludes a more extensive use of the hormone, particularly if oestrous synchronisation is used associated with timed artificial insemination. The variability of the response was attributed to differences in ovarian status among the ewes at the time of the treatment. The day of the cycle on which PGF2 α was given influences the interval to onset of oestrus (Houghton et al., 1995). Early treatment was followed by shorter intervals to oestrus. This was attributed to the different time span necessary to reduce progesterone concentrations to basal level as the luteal phase progresses and corpus luteum (CL) acquires its full endocrine functionality (Houghton et al., 1995). This was consistent with the observation that the interval to oestrus after attaining basal levels ($<0.2 \text{ ng ml}^{-1}$) is constant (Wiley et al., 1997). The variation in the interval between PGF2 α administration and ovulation could also be attributed to the wave giving origin to the ovulatory follicle (Viñoles and Rubianes, 1998).

The existence of non-responding ewes after a PGF2 α injection has been related to the percentage of ewes in early luteal phase at time of the treatment. The recently formed ovine CL has been considered to be refractory to PGF2 α (Acritopoulou and Haresign, 1980; Wiltbank and Niswender, 1992). However, in two experiments where the ovarian response was studied by transrectal ultrasonography, the administrations of PGF2 α 3 days after ovulation was effective to induce a rapid onset in decrease of progesterone concentrations (Rubianes et al., 1997a,b). In these experiments either FSH or GnRH were administered in association with PGF2 α treatment. In ewes in which ovarian follicular development was stimulated with a schedule of multiple doses of FSH starting soon after ovulation, the injection of PGF2 α on day 3 after ovulation induced luteolysis and oestrus (Rubianes et al., 1997a). In addition, when a dose of PGF2 α was administered together with a dose of GnRH around day 3 after ovulation luteolysis occurred in 85% of the ewes, and this was followed by ovulation without oestrus (Rubianes et al., 1997b). However, the use of ultrasonography to characterise the responsiveness of a 3 day-aged CL to a treatment with PGF2 α alone was not evaluated.

In the present work, we tested the hypothesis that in spontaneous oestrous cycling ewes the administration of PGF2 α on day 3 after ovulation is followed by luteolysis and ovulation.

2. Materials and methods

2.1. Animals and treatments

Multiparous Corriedale ewes ($n = 24$) were used during the middle of the breeding season (April) at a farm located in Flores, Uruguay (34° SL). The ewes weighed $41.2 \pm 0.7 \text{ kg}$ (mean \pm S.E.), and had a mean body condition score of 2.9 ± 0.1 (scale: 1–5). During the experiment the animals grazed nature pastures. The daily ultrasonic examinations were performed in inside box stalls of a building (3 m \times 3 m).

Stage of oestrous cycles were synchronised with an injection of a PGF2 α analogue (delprostenate, 160 μg , i.m., Glandinex, Universal Lab, Montevideo, Uruguay). The ewes remained with marking vasectomised rams and were observed twice a day for oestrous

behaviour during 45 min. Marked ewes by the rams were also recorded. Oestrus was defined as the moment when the ewe stood to be mounted by the ram. Seven days prior to the next expected oestrus ultrasonic examinations were performed daily in order to establish ovulation and to monitor follicular development. Ovulation was defined as the disappearance of a large follicle (usually ≥ 5 mm in diameter) from one examination to the next (Rubianes et al., 1997a) and this day was designated as day 0. Ewes were allocated randomly in one of three groups that received a dose of the PGF2 α analogue (delprostenate, 160 μg) on days 1 ($n = 8$, group D1), 3 ($n = 8$, group D3) or 5 ($n = 8$, group D5) after ovulation, respectively.

2.2. Ultrasonic evaluations and follicle data analysis

Ovarian images were obtained with a B-mode machine (Aloka 500, Tokyo, Japan) equipped with a 5 MHz linear array transducer. A slightly arched plastic open tube (diameter, 25 mm; length, 40 cm) was fixed to the transducer with tape so that the probe could be manipulated externally into the rectum (Rubianes et al., 1996). During examination the ewes were restrained in a standing position in a wooden chute designed for that purpose. Faecal pellets were removed by hand and carboxymethylcellulose gel (50 ml) was introduced with a syringe into the rectum. Ovaries were located as described previously (Rubianes et al., 1996). The diameter, positions and characteristics of the antral follicles ≥ 3 mm in diameter and the CL were recorded. Examinations were also recorded on videotape for further analysis of data. The day of emergence of a follicle was the day on which a follicle was 3 mm in diameter followed by an increase in diameter to ≥ 4 on the following day. The term wave was defined as a group of follicles that gave origin to one or more follicles ≥ 5 mm in diameter. The day of emergence of the largest follicle of a wave was considered the day of emergence of that wave. As ewes were scanned once a day, to determine the interval between PGF2 α treatment and ovulation the time of ovulation was considered to be half the time between the last scanning when ovulatory follicle was recorded and the following when it had disappeared.

2.3. Hormone analysis

Blood samples for progesterone determination were collected daily at 08.00 h by jugular venipuncture. Samples were allowed to clot at room temperature and were centrifuged within 2 h after collection. Serum was stored at -20°C until hormone determination. Concentrations of progesterone were determined in duplicate by a direct solid-phase RIA (DPC, Diagnostic Products Co., Los Angeles, CA, USA). The detection limit of the assay was 0.02 ng ml^{-1} . The intra-assay coefficient of variation was 12 and 6% for control samples of 0.8 and 4.2 ng ml^{-1} , respectively.

2.4. Statistical analysis

The intervals from PGF2 α treatment to oestrus and from PGF2 α treatment to ovulation (the duration of the period with progesterone concentrations over 1 ng ml^{-1} , and the mean days of progesterone increase up to 1 and below of 1 ng ml^{-1} , respectively) were compared

by ANOVA and non paired *t*-test was used. Frequencies of ewes in oestrus were compared by Fisher's exact test. The data are presented as Mean \pm S.E.M., and differences were considered to be significant when $P < 0.05$.

3. Results

All ewes treated with PGF2 α on days 3 and 5 post-ovulation came into oestrus whereas in only one ewe treated on day 1 ($P < 0.05$, Table 1) the treatment was followed by oestrus. The ultrasonic study showed that all ewes showing oestrus ovulated and no ovulations without associated oestrus behaviour were observed. The ovulatory follicle of the ewe responding (day 1), of the eight ewes responding from the day 3 group and of six ewes responding of the day 5 group originated from the first wave of follicular development during the oestrous cycle. In the other two ewes from the day 5 group, the ovulatory follicle originated from the second wave of follicular development during the oestrous cycle.

As depicted in Fig. 1, the interval between PGF2 α and ovulation for the 15 ewes that ovulated from first follicular wave was very homogenous (in 14 ewes it occurred at 60 h). The interval for the two ewes that ovulated from second follicular wave was 84 ± 0.0 h.

The mean serum progesterone concentration at the time of PGF2 α treatment were 0.4 ± 0.1 , 1.8 ± 0.1 and 3.2 ± 0.2 ng ml $^{-1}$ for day 1, 3 and 5 groups, respectively ($P < 0.05$). In all ewes from the day 3 and 5 groups, administration of PGF2 α induced a decrease of serum concentrations of progesterone to basal within 24 h (0.4 ± 0.3 and 0.4 ± 0.1 ng ml $^{-1}$, respectively) (Fig. 2). In one ewe from the day 1 group, progesterone remained low (mean: 0.4 ± 0.1 ng ml $^{-1}$) during the following 6 days (Fig. 2). This ewe came into oestrus 48 h after PGF2 α treatment. In the other seven ewes of the day 1 group, progesterone serum concentrations did not decrease after PGF2 α treatment and the inter-ovulatory cycle had a normal length (17.4 ± 0.5 days). However, mean daily progesterone concentrations on days 2 (0.4 ± 0.1 ng ml $^{-1}$) and 3 (0.8 ± 0.1 ng ml $^{-1}$) post-ovulation were significantly lower compared with ewes of the day 3 group (1.0 ± 0.1 and 1.8 ± 0.1 ng ml $^{-1}$, respectively, $P \leq 0.05$) and ewes of the day 5 group (0.8 ± 0.1 and 1.4 ± 0.1 ng ml $^{-1}$, respectively, $P \leq 0.05$).

The progesterone serum concentrations during the entire inter-ovulatory interval of the seven non-responding ewes of the day 1 group were compared with those of the next inter-ovulatory interval (spontaneous) of the eight ewes treated on day 3 that was used as a control luteal phase. A delayed increase up to 1.0 ng ml $^{-1}$ in progesterone concentrations

Table 1

Oestrous response, treatment to oestrus and treatment to ovulation intervals of ewes treated with PGF2 α on days 1 ($n = 8$), 3 ($n = 8$) or 5 ($n = 8$) after ovulation^a

	Day 1	Day 3	Day 5
Ewes in oestrus	1/8	8/8	8/8
Interval, PGF2 α –oestrus (h)	48	39 ± 3.8	48.0 ± 2.3
Interval, PGF2 α –ovulation (h)	60	60 ± 0.0	67.5 ± 5.0

^a Mean \pm S.E.M.

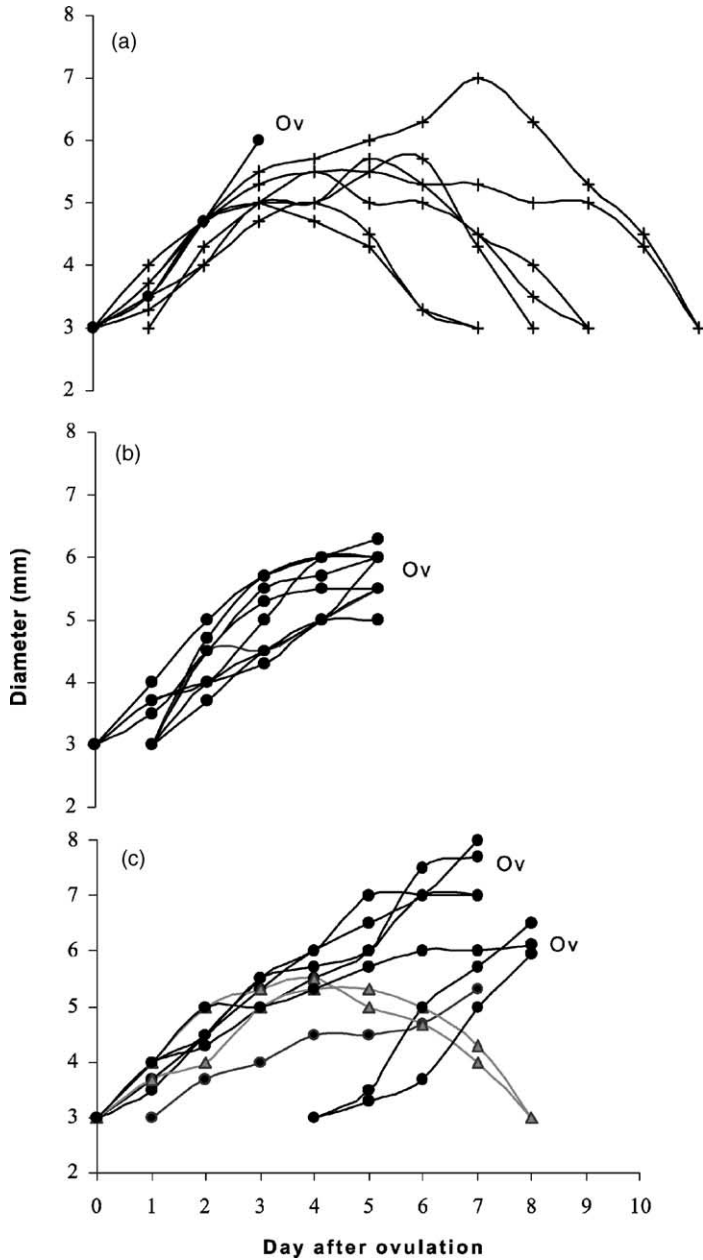


Fig. 1. Growth profile of the largest ovarian follicles of ewes treated with a PGF2 α analogue on (a) day 1, ($n = 8$), (b) day 3 ($n = 8$) or (c) day 5 ($n = 8$) after ovulation. In all ewes of the day 3 group and 6 of the ewes of the day 5 group, the ovulatory follicle originated from the first wave of follicular development. The other two ewes of the day 5 group had ovulatory follicles that originated from the second follicular wave. In only one ewe of the day 1 group, the treatment was followed by ovulation.

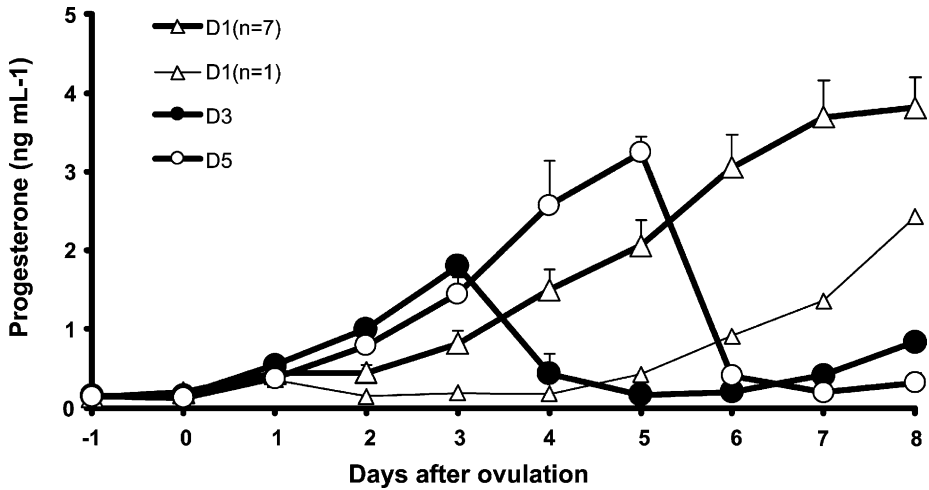


Fig. 2. Mean (\pm S.E.M.) daily progesterone concentrations in ewes treated with a PGF $_{2\alpha}$ analogue on days 1 (D1, $n = 8$); 3 (D3, $n = 8$) or 5 (D5, $n = 8$) after ovulation. The profile of a ewe from the day 1 group that responded to the treatment is depicted separately. Mean progesterone concentrations on days 2 and 3 after ovulation of the other seven ewes of the day 1 group were significantly different ($P < 0.05$) than those of ewes of the days 3 and 5 groups.

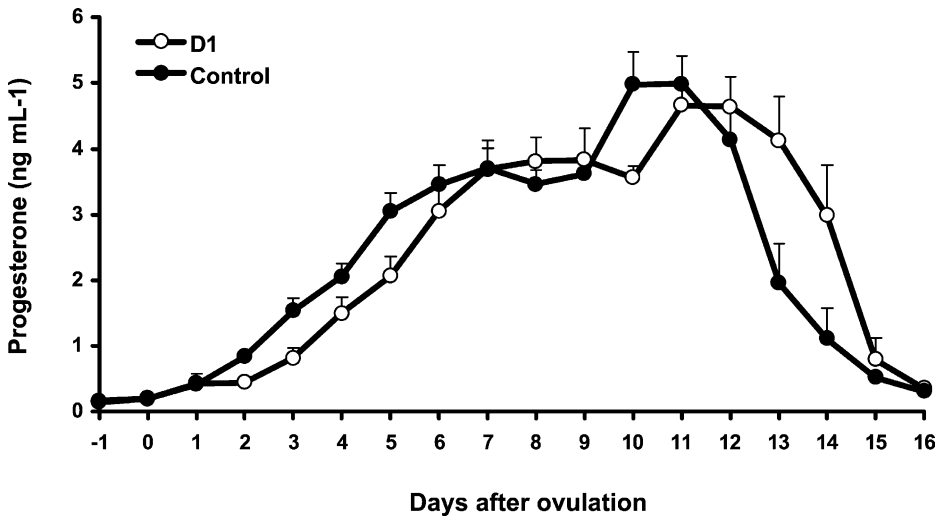


Fig. 3. Mean (\pm S.E.M.) daily progesterone concentrations during the inter-ovulatory cycle of the seven non-responding ewes treated with a PGF $_{2\alpha}$ analogue on day 1 (D1) compared with a control cycle (following spontaneous cycle of the eight ewes of the day 3 group). Mean progesterone concentrations differ between groups on days 2, 3, 5 and 10 after ovulation ($P < 0.05$).

(day 4.1 ± 0.3 versus day 3.0 ± 0.2 ; for ewes of the day 1 group and control ewes, respectively, $P < 0.05$) and a delayed decrease to below 1.0 ng ml^{-1} progesterone concentrations (day 15.0 ± 0.3 versus day 13.9 ± 0.3 , respectively, $P < 0.05$) were observed. However, the length of the total period of progesterone over 1.0 ng ml^{-1} was similar (10.9 ± 0.4 versus 10.8 ± 0.3 , respectively, $P > 0.05$). Overall comparison showed that PGF2 α administered on day 1 post-ovulation induced a 1 day delayed progesterone profile in these seven ewes (Fig. 3).

4. Discussion

The results of the present study show that the 3 day-aged CL of oestrous cycling ewes is sensitive to the luteolytic effect of PGF2 α . All ewes treated on day 3 after ovulation responded with a sharp decrease of progesterone concentrations and ovulated after a typical interval after PGF2 α administration (between 48 and 72 h) from treatment. These findings expand previous results observed in FSH-stimulated and GnRH-challenged ewes (Rubianes et al., 1997a,b).

Early experiments showed that some ewes treated with PGF2 α during the first days of the oestrous cycle did not respond to the treatment (Acritopoulou and Haresign, 1980); similar to what has previously been described in cattle (Rowson et al., 1972). Thereafter, the general concept that PGF2 α is not effective when given before day 5–6 of the oestrous cycle (day 4–5 after ovulation) in sheep was widely accepted (Wiltbank and Niswender, 1992). Although, there are several *in vitro* studies on the response of bovine and ovine CL to PGF2 α , few new *in vivo* studies regarding this issue in ewes have been conducted. Strong evidence derived from *in vitro* studies confirms the refractoriness of the recently formed CL (≤ 4 days) to PGF2 α (see reviews: McCracken et al., 1999; Niswender et al., 2000), the mechanism involved, however, remains unknown. Nevertheless, it has been demonstrated that the concentrations and/or affinity of PGF2 α receptors present in the large luteal cells do not change during the oestrous cycle (Wiltbank et al., 1995).

Recently, Silva et al. (2000) showed that the CL obtained from ewes on day 4 of the oestrous cycle had a greater capacity to catabolize PGF2 α than those obtained on day 13, a finding which could explain the resistance of the young ovine CL to the luteolytic effects of PGF2 α . In this regard, the success of inducing luteolysis of 3 day-aged CL in this work might be due to particular pharmacological characteristics of the PGF2 α analogue (delprostenate) used. However, in a previous work where PGF2 α was administered to FSH-stimulated ewes (Rubianes et al., 1997a) another analogue (cloprostenol) was also used successfully. In the present study, in seven out of the eight ewes treated on day 1 the PGF2 α analogue did not induce luteolysis, however, a short-term effect was observed. In effect, although the length of the period with progesterone concentrations above 1 ng ml^{-1} was not affected the pattern of progesterone concentrations was delayed 1 day. A transient luteolytic effect (progesterone concentrations fall $< 24 \text{ h}$) was also observed after the administration of a subluteolytic dose of PGF2 α (1/3 of the effective dose) during the late luteal phase in the ewe (Juengel et al., 2000).

In all responding ewes (one D1, eight D3 and eight D5 ewes, respectively), luteolysis was followed by oestrus. This was an unexpected observation as it is considered that before the

preovulatory oestrogen increase a priming period of greater progesterone concentrations is necessary for the ewe to completely express oestrous behaviour (Goodman, 1994). In a previous study (Rubianes et al., 1997b), ewes treated simultaneously with GnRH and PGF2 α on day 3 after ovulation ovulated without associated oestrus. This could be explained by the shorter period of oestrogen production from the ovulatory follicle as it was also stimulated by an induced LH surge at time of luteolysis. In the present study, full development of the ovulatory follicle was allowed and, therefore, oestrogen production might have been greater.

All ewes treated on day 3 and the responding ewe treated on day 1 ovulated a follicle from the first wave of ovarian follicular development, which emerged around day 0 of the inter-ovulatory cycle. The fact that six out eight treated ewes of the day 5 group in the present study also ovulated from the first follicular wave agrees with previous observations (Viñoles and Rubianes, 1998). In ewes treated on day 5, the dominant follicle of the first follicular wave became the ovulatory follicle whenever luteolysis was induced during its growing or plateau phases. However, if luteolysis occurred after regression had started, a newly developed ovarian follicle of the second follicular wave became the ovulatory follicle (Viñoles and Rubianes, 1998). In this case the interval to ovulation, as previously shown in heifers (Kastelic and Ginther, 1991), may be delayed. In the present study, most of the ewes ovulating from first follicular wave did so between 48 and 72 h after treatment but the two ewes of the day 5 group that ovulated from the second follicular wave did so after this period. The highly synchronised ovulation from first follicular wave observed in responding ewes treated early in the luteal phase, may have practical implications for timed artificial insemination protocols and needs to be evaluated.

In conclusion, the administration of PGF2 α on day 3 after ovulation was followed by luteolysis and ovulation showing that, in the ewe, a 3 day-aged CL is not refractory to the hormone. The fact that one ewe treated on day 1 ovulated after the treatment and in the other seven ewes of this group the recently formed CL was affected and its progesterone secretion pattern was retarded in 1 day suggests that the administration of PGF2 α on day 2 after ovulation could be—at least partly—effective. The present study strongly suggests that the precise period of refractoriness to PGF2 α of a recently formed ovine CL is most likely restricted to 2 days or less after ovulation.

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