Reprod Nutr Dev (1997) 37, 559–571

Original article

Role of the uterus in early regression of corpora lutea induced by the ram effect in seasonally anoestrous Barbarine ewes

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(Received 31 August 1996; accepted 11 August 1997)

Summary – The involvement of the uterus in early regression of corpora lutea induced by the ram effect was studied in seasonally anoestrous Barbarine ewes. In experiment 1, group I was only submitted to the male effect (control, $n = 15$) while group II ($n = 14$) was injected every 12 h with flunixin meglumine, a PGF2α synthetase inhibitor (finadyne), from day 3 to day 6 (day 0: day of ram introduction). The preovulatory LH surge appeared at the same time (around 21 h after ram introduction) in both groups. Finadyne treatment significantly decreased 13-14-dihydro-15-keto-PGF2α (PGFM) pulses (1.3 ± 0.3 versus 0.4 ± 0.2; $P < 0.05$), the number of short cycles (50 versus 14%, $P < 0.05$), and provided a single peak of oestrus, 15–16 days after the introduction of the rams, instead of between day 14 and day 23 ($P < 0.01$). In experiment 2, 17 hysterectomized ewes were allocated into two groups: group III ($n = 8$) was injected with oil and group IV ($n = 9$) received an intramuscular injection of 20 mg of progesterone immediately before introduction of rams. An additional group of intact ewes was used as control (group V, $n = 9$). Hysterectomy did not affect the ovulation response to the ram effect, but completely suppressed short cycles (0 versus 78% , $P < 0.001$). The preovulatory LH surge was delayed in hysterectomized females (36.0 ± 14.1 versus 16.6 ± 11.4 h; $P < 0.004$). Treatment with progesterone significantly ($P < 0.01$) increased the interval between introduction of rams and the preovulatory LH surge. In conclusion, suppression of short cycles by hysterectomy and an inhibitor of PGF2α synthetase suggest that the uterus is essential for determining the lifespan of ram-induced corpora lutea and that premature release of PGF2α is the cause of early luteal regression. The hypothesis that lower secretion of progesterone before D5 could be the initial cause of the premature induction of the luteolytic signal is discussed.

ram effect / corpus luteum lifespan / hysterectomy / prostaglandin / ewe

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Résumé – Rôle de l'utérus dans la régression précoce des corps jaunes induits par effet bélier chez des brebis de race Barbarine en anœstrus saisonnier. L'implication de l'utérus dans la régression lutéale précoce des corps jaunes a été étudiée chez des brebis de race Barbarine soumises à l'effet bélier pendant l'anœstrus saisonnier. Une première expérience concerne deux lots de brebis : les femelles du lot I sont seulement soumises à l'effet mâle (lot témoin, $n = 15$) ; celles du lot II ($n = 14$) reçoivent, toutes les 12 h, un inhibiteur de la synthèse des PGF2α (finadyne), de J3 à J6 (J0 étant le jour d'introduction des béliers). Un pic préovulatoire de LH apparaît environ 21 h après introduction des béliers chez la quasi-totalité des femelles des deux lots. Le traitement à la finadyne diminue significativement le nombre de pulses de 13-14-dihydro-15-céto-PGF2α (PGFM : 1,3 ± 0,3 vs 0,4 ± 0,2 ; $p < 0,05$), diminue également la fréquence d'apparition des cycles courts (50 vs 14 %, $p < 0,01$) et permet ainsi une synchronisation des chaleurs à J15 et J16 au lieu d'un étalement entre J14 et J23 dans le lot témoin ($p = 0,01$). La deuxième expérience concerne 17 brebis hystérectomisées et réparties en deux groupes : les brebis du lot III ($n = 8$) sont injectées avec de l'huile et celles du lot IV ($n = 9$) reçoivent une injection intramusculaire de 20 mg de progestérone immédiatement avant l'introduction des béliers. Des femelles intactes servent de témoins (lot V, $n = 9$). L'hystérectomie n'affecte pas le pourcentage de femelles ovulant après l'effet bélier mais supprime totalement l'apparition des cycles courts (0 vs 78 %, $p < 0,001$). Le pic préovulatoire de LH est plus tardif ($p < 0,004$) chez les brebis hystérectomisées (36,0 ± 14,1 h) que chez les brebis témoins (16,6 ± 11,4 h). L'injection préalable de progestérone augmente encore l'intervalle introduction des béliers–pic préovulatoire de LH ($p < 0,01$). En conclusion, la suppression des cycles courts soit par un inhibiteur de la synthèse des prostaglandines F2α, soit par hystérectomie met en évidence le rôle essentiel que jouent l'utérus et particulièrement les prostaglandines dans la régression prématurée des corps jaunes induits par effet mâle chez des femelles en anœstrus saisonnier. L'hypothèse qu'une plus faible concentration de progestérone avant J5 pourrait être la cause initiale de la mise en place du signal lutéolytique est également discutée.

effet mâle / corps jaune / hystérectomie / prostaglandine / brebis

INTRODUCTION

Introduction of a male to a group of previously isolated anovulatory ewes or goats can induce oestrus and ovarian activity. The induced ovulation occurs within the first 4 days following exposure (ewe: Knight et al, 1978; goat: Chemineau, 1983). Such induced corpora lutea often have a short lifespan and exhibit early regression after transitory progesterone release (Knight et al, 1981; Chemineau, 1983). The proportion of short cycles varies and depends on factors such as nutrition, season and breed, all of which may affect the 'depth' of anoestrus (goat: Chemineau, 1983; ewe: Khaldi, 1984; Lassoued and Khaldi, 1993). In all cases, the length of these cycles is less than 7 days (Oldham, 1980; Knight et al, 1981; Chemineau, 1983; Southee et al, 1988; Lassoued and Khaldi, 1993; Lassoued et al, 1995).

The mechanism of short cycles induced by the male effect in anoestrous females is still unknown and little work has been carried out on the subject. Early regression could be attributed to the lack of maturity of preovulatory follicles (Cognié et al, 1982; Lindsay et al, 1982; Martin et al, 1983). However, this hypothesis does not explain why this regression occurs at a definite stage of the cycle, which corresponds to the beginning of the sensitivity of the corpus luteum to prostaglandin injections (Acritopoulou and Haresign, 1980). If it were only a matter of follicular maturity, the luteal phases should be of variable periods between 2 and 13 days.

Hypothesis of higher sensitivity of hypofunctional corpora lutea to PGF2α is less probable owing to the fact that the corpora lutea induced by GnRH are not more sensitive to the luteolytic action of prostaglandin F2α than those having normal lifespan

(Copelin et al, 1988). If such an effect is also valid for corpora lutea induced by the ram effect, then we have to suppose that their early regression is related to trophic factor insufficiency and/or to the early release of luteolytic factors.

Priming with a single injection of 20 mg of progesterone, or with a progestagen for several days, eliminates these abnormal ovarian cycles (Cognié et al, 1982; McLeod and Haresign, 1984). Progesterone may act on the hypothalamic–pituitary pathway (Martin et al, 1983), or at the level of the ovary (Hunter et al, 1986; Brown et al, 1988) or uterus. This latter suggestion has been widely studied (Vallet et al, 1990).

Corpus luteum regression depends on PGF2α as the uterine luteolysin, the surge of which increases rapidly following coupling of oxytocin to its receptor in the endometrium (McCracken et al, 1984). This release is correlated to oxytocin receptor concentrations in the endometrium (Flint et al, 1990). Maximum concentrations occur during oestrus, and are lower, relatively, 4 to 5 days earlier or later (Roberts et al, 1976; Sheldrick and Flint, 1985). During oestrus, plasma concentrations of progesterone are minimal whereas oestradiol concentrations are maximal, suggesting that oxytocin receptor synthesis is inhibited by progesterone (Hunter et al, 1989; Vallet et al, 1990) and increased by oestradiol (Hixon and Flint, 1987).

Various studies have highlighted the role of uterine PGF2α in the early regression of induced corpora lutea. In seasonally anovular ewes, hysterectomy prolongs the lifespan of the corpus luteum after GnRH-induced ovulation (Southee et al, 1988) or those induced by the male effect (Chemineau et al, 1993). Injected into the uterus as late as day 12 (ewe) or day 14 (heifer) of the oestrous cycle, indomethacin inhibition of PGF2α synthesis, prevented normal luteal regression (Lewis and Warren, 1977). In Barbarine ewes, when ovulation is induced by the male effect, treatment with indomethacin is less efficient, it reduces the frequency of short ovulatory cycles by 30%. The lifespan of hypofunctional corpora lutea is extented by 2 days and the concentration of progesterone in plasma is increased (Lassoued and Khaldi, 1989).

The objective of this study was to use a more efficient inhibitor of prostaglandin secretion (finadyne, Battye et al, 1988), and to assess the luteal activity in hysterectomized ewes treated or not with progesterone to evaluate the trophic action of this hormone on the induced corpora lutea.

MATERIAL AND METHODS

Animals and treatments

Two experiments were conducted in May during 1989–1991 at Inra-Tunisia using adult Barbarine ewes with fat tail; spring is the period of lowest sexual activity (Khaldi, 1984). For both experiments, females were isolated from males for more than 2 months. Adult Barbarine rams were introduced into the flock at a ratio of one male to ten females. Day 0 (D0) was defined as the first day of ram introduction. Beginning on D0 detection of oestrus was carried out twice daily, morning and afternoon, for 1 month.

The work of Khaldi (1984) showed that there is a close relationship between the proportion of short cycles and the ewes liveweight 2 months before mating. This proportion can reach 67% when the body weight is lower than 42 kg. To obtain similar live weight evolution, feeding restriction was initiated at the end of pregnancy and continued up to mating. The food quantity was controlled according to the weight progress measured every 10 days. Initially, animals were raised on summer stubbles supplemented with hay ad libitum plus 300 g of concentrate.

Experiment 1

Twenty-nine anovulatory ewes (observed by coelioscopy at D–1) were divided into two homogeneous groups (I and II) according to liveweight (respectively, 41.9 ± 3.7 and 42.1 ± 5.1 kg) and age (2–6 years): group I: untreated control females ($n = 15$); group II: females receiving an

intramuscular injection of Flunixin Meglumine, an inhibitor of PGF2α synthetase (finadyne, Rigaux Galéna, France) at a dose rate of 2.2 mg/kg/female at 12-h intervals. Treatment began at D3 and was continued for 4 days until D6 (n = 14).

Experiment 2

Seventeen ewes, hysterectomized for more than 1 year, and weighing 41.2 ± 2.7 kg were injected intramuscularly, on D–7, with a luteolytic dose of prostaglandin F2α analog (1.25 mg of Prostavet, Virbac, France), in order to synchronize the timing of luteal regression. The absence of a corpus luteum before the ram effect was assessed by plasma progesterone concentrations (Thimonier, 1978) on D–1 and D0.

The ewes were divided into two homogeneous groups (groups III and IV) according to liveweight (respectively, 40.9 ± 3.2 and 41.7 ± 2.3 kg) and age (2–6 years): group III (n = 8) were untreated control females; group IV (n = 9) females received an intramuscular injection of 20 mg progesterone (Sigma, France) immediately before ram introduction; group V (n = 9) were intact anovulatory ewes (coelioscopy on D–1) weighing 36.9 ± 3.3 kg.

After the analysis of progesterone concentrations, two females of group III were discarded because they showed spontaneous ovulatory activity before male contact.

Samples, hormonal assays and ovarian dynamics

Blood samples

To determine the occurrence of preovulatory LH surge, blood was collected by jugular venepuncture at 4-h intervals for 76 h following male introduction.

Blood samples for progesterone assays were taken daily from D1 until D17 (experiment 1: groups I and II; experiment 2: groups III and IV) and from D1 until D8 only for group V in experiment 2.

The 13-14-dihydro-15-keto-PGF2α (PGFM) concentrations were estimated in blood samples collected hourly on D4 and D5 between 0900 and 1700 hours. Under our conditions, pulse detection was carried out using the Munroe algorithm (Merriam and Wachter, 1982). Ewes with a late preovulatory surge were discarded.

The blood was centrifuged and the plasma stored at –20 °C until assayed.

Hormone radioimmunoassays

Concentrations of LH, progesterone and PGFM were determined by radioimmunoassay.

Plasma LH concentrations were determined using the method of Pelletier et al (1982) and modified by Montgomery et al (1985). The limit of sensitivity of the assay was 0.1 ng/mL and the inter- and intra-assay coefficients of variation were 10.1 and 11.3%, respectively.

The time of the initiation of the preovulatory surge of LH was considered as the point of the first LH concentration higher than 10 ng/mL, and the moment of ovulation 24 h later.

Progesterone assay was performed using kits supplied by IAEA (Vienna, Austria). The sensitivity of this method was 0.09 ng/mL and the inter- and intra-assay coefficients of variation were 9.2 and 11.0%, respectively.

The estimation of PGFM concentration in the blood is a direct reflection of PGF2α release by the uterus (Peterson et al, 1976; Zarco et al, 1984). PGFM was assayed according to the method of Dray et al (1980). The assay sensitivity was 0.4 pg/100 μL and intra- and inter-assay coefficients of variation were 10 and 11%, respectively.

Ovarian dynamics

Coeslioscopy was carried out on D4 (experiment 1: groups I and II), D9, and 8–12 days following first oestrus (experiment 1: groups I and II, experiment 2: group V) in order to define the day of ovulation onset according to the criteria proposed by Oldham and Lindsay (1980) and to determine the number of corpora lutea (ovulation rate). In the case of hysterectomized ewes (experiment 2: groups III and IV), ovary observation using coelioscopy is difficult and therefore plasma progesterone levels were used as an indicator of ovulation and luteal activity. Cycles were classified as short when the induced corpus luteum regressed within 5 days. Only ewes with nonfunctional or regressed corpora lutea on D–1 were considered anovulatory or non-cyclic.

Statistical analysis

Data were analysed using SAS software (Statistical Analysis System Institut). The results of the hormone assays (progesterone and PGFM) were analysed using a three-factor analysis of variance (ewe, treatment, prelevement). Comparison between groups were carried out using Duncan's multiple range test. Chi-square analysis was used to compare ovulation rate, short cycle frequencies, LH surge start point and day of the onset of first oestrus. Data are expressed as mean ± standard deviation (SD).

RESULTS

Inhibition of PGF secretion

Concentration and pulse frequency of PGFM on D4 and D5 in the peripheral circulation were lower in ewes with normal cycles than in those with short cycles within each group and between groups (table I, $P < 0.05$).

Figure 1 shows the variation in plasma PGFM concentration for ewes with short and normal cycles. Mean concentrations were higher in short cycles compared to normal cycles (table I, $P < 0.05$), but within each group the difference was not significant.

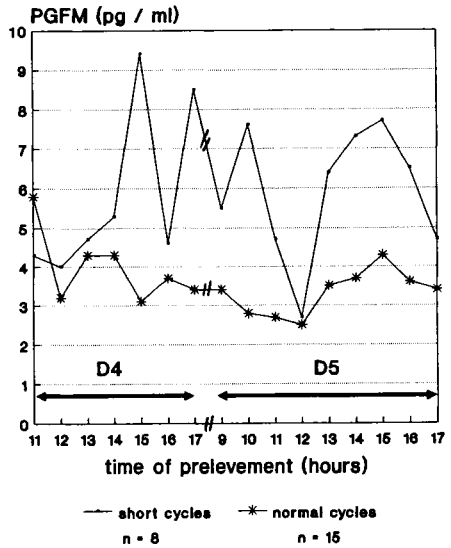

Fig 1. Plasma PGFM concentrations in short and normal cycles at D4 and D5 after ram introduction.

The number of PGFM peaks was significantly higher in short cycles (table I, $P < 0.05$) and in the control group than in the finadyne-treated group (1.3 + 0.3 versus 0.4 +0.2; $P < 0.05$). Peaks of PGFM were detected in all ewes (100%) that exhibited short cycles but only 20% of ewes with normal cycle showed one peak through the detection period.

Table I. Concentrations and pulse frequency of PGFM in both control and finadyne groups, and in females with short or normal induced cycles.

Groups	*Concentration (pg/mL)*		*Number of pulses*	
	Short cycles	*Normal cycles*	*Short cycles*	*Normal cycles*
I (control)	5.9 ± 3.2	2.9 ± 0.6	2.0 ± 0.6 a	0 b
	$n = 7$	$n = 4$	$n = 7$	$n = 4$
II (finadyne)	5.6	3.9 ± 1.3	2.0 a	0.3 ± 0.4 b
	$n = 1$	$n = 11$	$n = 1$	$n = 11$
Total	5.9 ± 1.1 a	3.6 ± 0.3 b	2.0 ± 0.2 a	0.2 ± 0.1 b
	$n = 8$	$n = 15$	$n = 8$	$n = 15$

a versus b: $P < 0.05$.

Preovulatory LH secretion

Experiment 1

A preovulatory LH peak was detected in all females that ovulated. The mean time of onset of this peak was about 20 h after male introduction in groups I and II (18.5 ± 10.8 and 20.0 ± 14.6 h, respectively). Four days after male introduction, one or more corpora lutea were observed by coelioscopy in each of the females that responded to the male effect. Ovulations occurred between D1 and D3.

Experiment 2

The preovulatory LH surge occurred within 11.6 ± 7.8 h in intact females (group V), and later in hysterectomized females which did not receive progesterone (group III: 33.6 ± 13.3 h; $P < 0.004$). For females of group IV (injected with 20 mg of progesterone immediately before ram introduction), no LH surge had been detected up to 76 h. The initiation of the LH surge probably occurred after 76 h. Hysterectomy significantly delayed the initiation of the preovulatory LH surge in comparison to intact females. This result was confirmed by subsequent analysis of progesterone profiles.

Ovarian activity

Ovulation rate

The ovulation rate accompanying the induced ovulation was higher when compared to the ovulation rate at first oestrus in groups I and II. However, the difference between the ovulation rates of the first and second ovulation was statistically significant only for the finadyne-treated females (table II; $P < 0.05$).

Progesterone levels

Experiment 1

Only one ewe from the control group did not ovulate after ram introduction. When early luteal regression did not occur plasma progesterone concentrations increased gradually beginning at day 3 following ram introduction (fig 2a, c), reaching maximum values of approximately 3 ng/mL between D9 and D11 (3.16 ± 0.77 and 2.93 ± 1.04 ng/mL, respectively, for groups I and II). This concentration returned to low levels at D15 (group I) and D16 (group II). In cases of early luteal regression, short cycles were characterized by a brief increase in progesterone concentrations. The maximum level reached was at day 2 after ovulation in control group and day 3 in the finadyne group (fig 2b, d). At days 2 and 3 after ovulation, levels were always lower in short cycles than in normal cycles. This short luteal phase

Table II. Ovulatory response and luteal regression in ewes exposed to the ram effect alone (group I) or treated with finadyne (group II), m ± SD.

Groups	*Number of ewes*	*Number of ovulated ewes*	*Number of short cycles (%)*	*Ovulation rate*	
				Induced	*At first oestrus*
I (control)	15	14	7 (50) a	1.5 ± 0.5	1.3 ± 0.5
II (finadyne)	14	14	2 (14) b	1.4 ± 0.5 c	1.1 ± 0.3 d

a versus b and c versus d: $P < 0.05$.

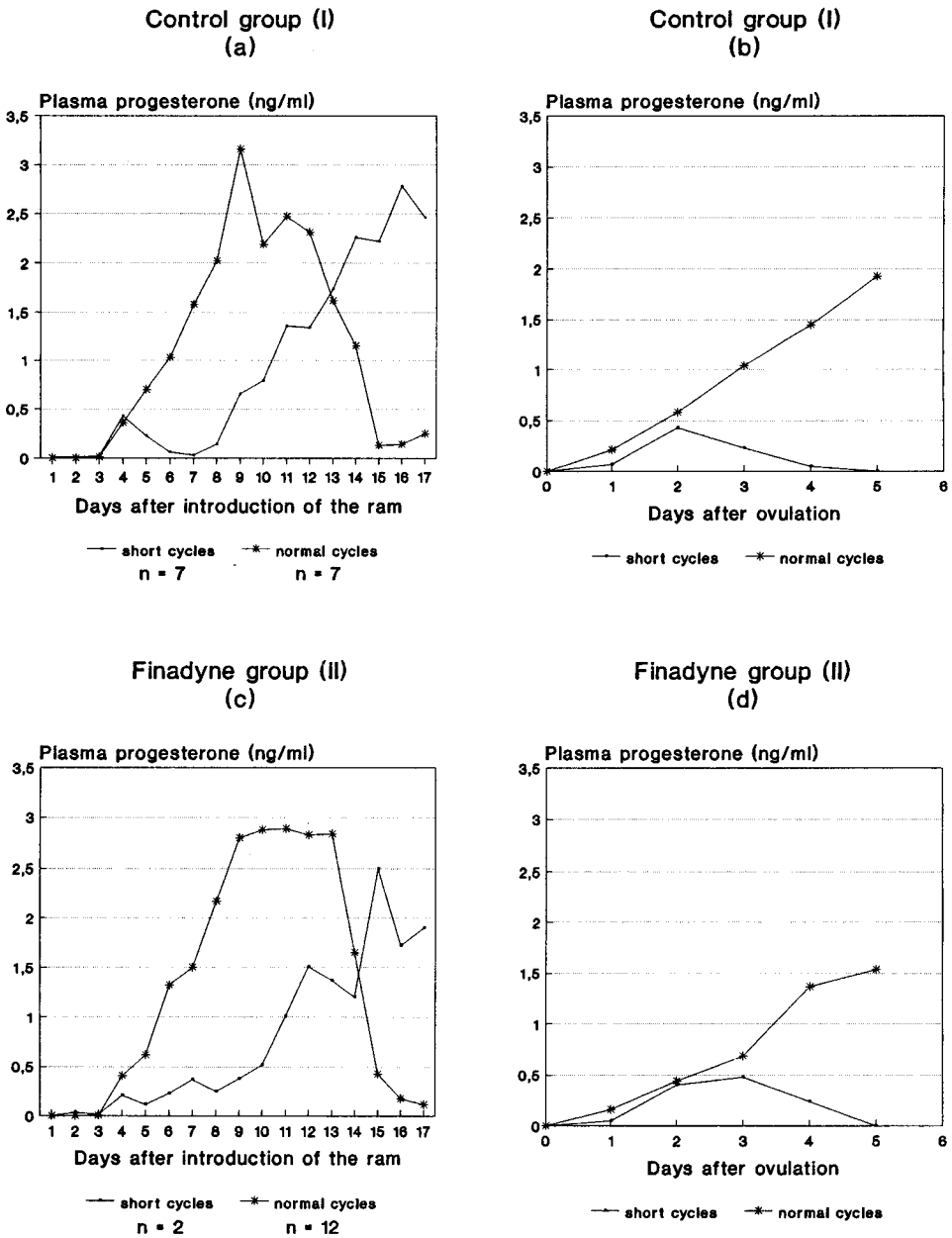

Fig 2. Plasma progesterone concentrations in control (group I) and finadyne-treated (group II) ewes after ram introduction (a, c) and after induced ovulation (b, d).

was followed in all females (except for the one that entered anoestrous after ovulation induction, fig 2b) by a new luteal phase similar to those of normal cycles.

Experiment 2

In hysterectomized ewes (group III), the increase in plasma progesterone was similar to that observed in intact females with normal cycles (group V). Nevertheless, levels of plasma progesterone were significantly lower in these females; mean levels between D1 and D8 after male introduction were 0.5 ± 0.6 (group III) and 0.9 ± 0.9 ng/mL (group V; $P < 0.001$, fig 3a, c).

In hysterectomized ewes injected with 20 mg progesterone (group IV), progesterone levels were over 1 ng/mL at D1 and decreased progressively. The minimum was reached on D5 with individual temporal variations. From that time, progesterone levels increased progressively until D11 and remained over 1.5 ng/mL (fig 3c). In all hysterectomized females (groups III and IV), the luteal activity (plasma progesterone > 1 ng/mL) was maintained at least up to D17.

Progesterone levels increased similarly in hysterectomized females (groups III and IV) from ovulation until day 8 (fig 3d).

Corpora lutea lifespan

Experiment 1

Regressed corpora lutea were found on the ovaries of 50% (7/14) of animals (table II) in the control group and only 14% (2/14) in the finadyne-treated group (group II versus group I: $P < 0.05$).

These short cycles were followed by new ovulations as evidenced by plasma progesterone profiles and coelioscopy performed on D9. The length of the short cycles estimated by coelioscopy was around 5 days (groups I and II: 4.9 ± 1.1 and 4.5 ± 0.7 days) while normal cycles lasted 15–16 days (15.0 ± 1.0 and 15.7 ± 0.8 for ewes of groups I and II, respectively).

The progesterone levels were over 0.1 ng/mL for approximately 12 days in normal cycles (11.8 ± 0.8 and 11.8 ± 1.3 days for females of groups I and II, respectively) and about 2–3 days in short cycles (2.0 ± 0, group I; 2.5 ± 0.7 days, group II).

Experiment 2

No short cycles were observed in hysterectomized ewes. In contrast, these types of cycles were observed in 78% of females (7/9) in the intact control group (group III or IV versus group V: $P < 0.001$). In these cases, progesterone levels were over 0.1 ng/mL for 3.1 ± 0.7 days.

Onset of oestrus

Experiment 1

All females in the control groups having ovulated after ram introduction, showed at least one oestrus. Only one female showed behavioural oestrus at induced ovulation (D1). All others showed first oestrus between D14 and D23. Oestrus behaviour was characterized by two peaks of activity in the flock, on days 14–16 and 20–23 post-joining (fig 4).

Only one treated female (group II) did not show oestrus. All ewes exhibiting normal induced cycles were in oestrus between D15 and D18, accompanying the second ovulation. The female that had a short induced cycle showed oestrus on D22, accompanying the third ovulation. The distribution of the first oestrus behaviour of the finadyne-treated females was significantly different ($P < 0.01$) from that of the control females.

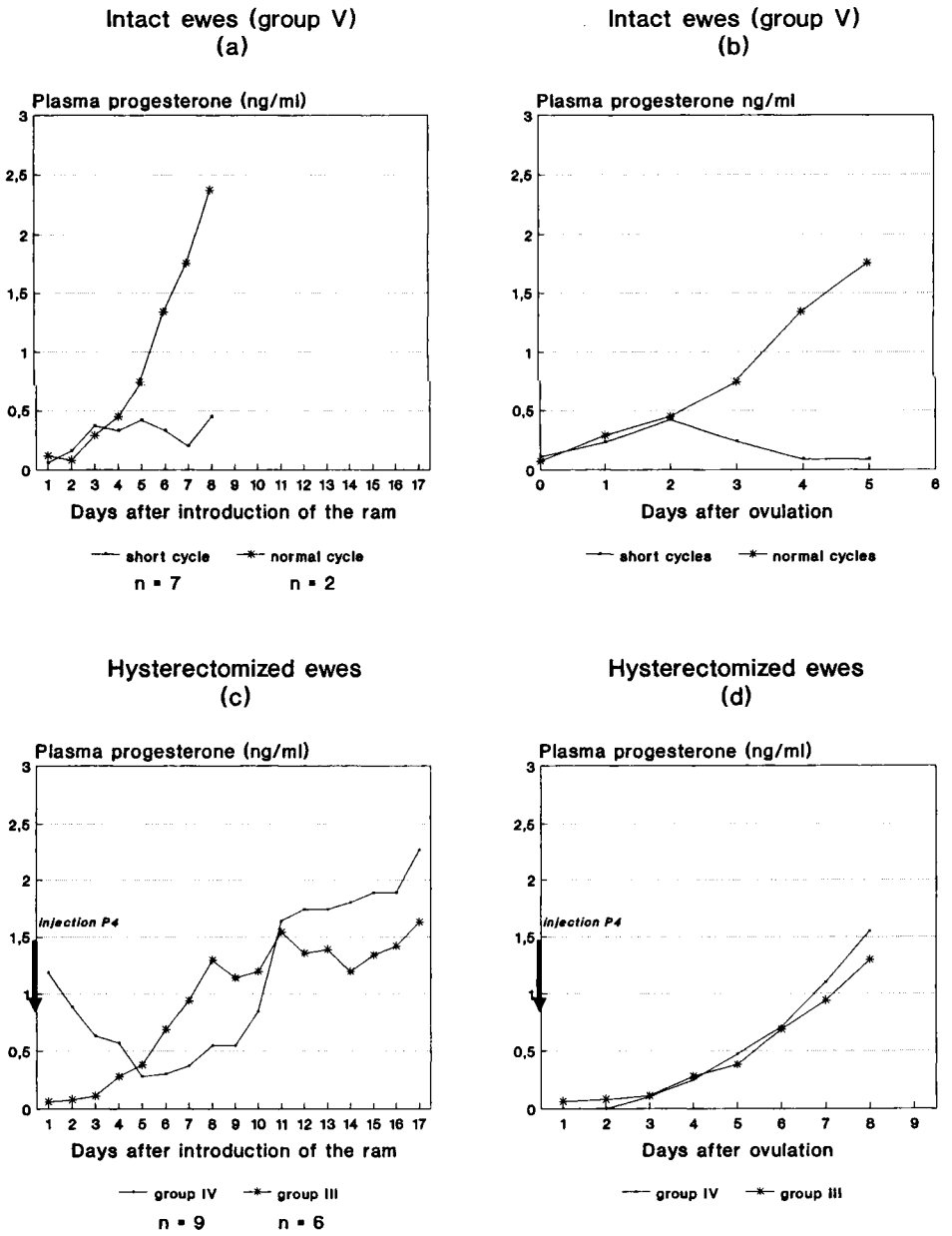

Fig 3. Plasma progesterone concentrations in intact (group V) and hysterectomized ewes, with (group IV) or without (group III) progesterone pretreatment, after ram introduction (a, c) and induced ovulation (b, d).

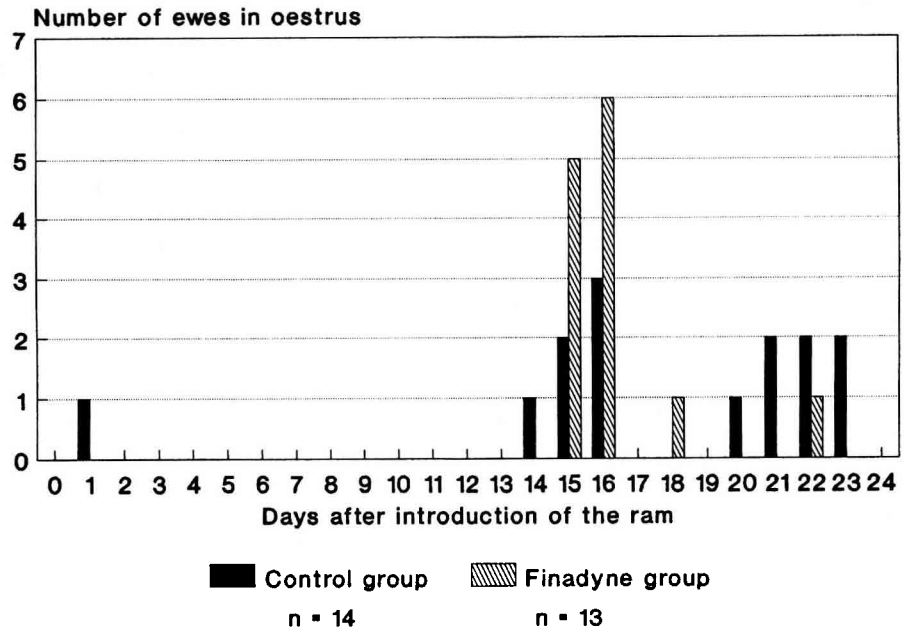

Fig 4. Distribution of first oestrus after ram introduction in control and finadyne-treated groups.

Experiment 2

All the intact females (group V) that reacted after ram introduction showed oestrus behaviour at least once. Just one came into oestrus at D6 with ovulation following a short cycle. Among the hysterectomized ewes, five showed oestrus. Two ewes, having received progesterone immediately before male introduction (group IV) showed oestrus behaviour with the induced ovulation.

DISCUSSION

The results of this study present evidence for the involvement of the uterus in early luteal failure associated with the ram effect in seasonal anovular Barbarine ewes.

Hysterectomy completely eliminated short luteal phases. These results confirm those of Chemineau et al (1993) and Southee et al (1988) obtained in Préalpes du Sud and Romney Marsh sheep, respectively.

The secretion of PGF2α is implicated in this process because both the concentration and the number of pulses were significantly higher in ewes with short cycles regardless of the treatment. Furthermore, the administration of finadyne resulted in a significant reduction in PGFM production (experiment 1). When injected from D3 to D6, finadyne prevented premature luteal regression in 86% of females. Its inhibitory action on PGFM pulsatility was significant compared to the controls at D4 and D5. PGFM concentrations were low compared to those found by Battye et al (1988) in the goat. This could be due to the fact that plasma

had been frozen without adding an inhibitor of prostaglandin degradation (Granström and Kindahl, 1982). However, as all samples (controls and treated) were treated in the same manner, and the values recorded were higher than the limit of sensibility of the assay, the effect of finadyne treatment can be considered as reliable. This treatment seemed to be more efficient than intra-uterine administration of indomethacin in Barbarine sheep for 3 days (Lassoued and Khaldi, 1989). However, in sheep and cattle, the intra-uterine administration of indomethacin prevents luteal regression (Lewis and Warren, 1977) and decreases PGFM in the plasma (Troxel and Kesler, 1984).

Removal of the uterus prior to ram introduction did not change the percentage of ewes ovulating but delayed by 1 day the moment of ovulation and the increase in plasma progesterone concentrations. Ovulation was even further delayed in progesterone-treated ewes. The reasons for such a difference between intact and long-term hysterectomized females are not known. One hypothesis may be that the modifications of the ovarian vascular system by surgery have consequences for hypothalamo-pituitary-ovarian feedback mechanisms.

It seems, in hysterectomized females, that progesterone secretion, as assessed by peripheral plasmatic levels, is lower than in intact ewes. Considering the very small number of intact females having a normal luteal lifespan, and the lack of knowledge about the induced ovulation rate, cellular composition and characteristics of the induced corpora lutea in hysterectomized ewes, we can not confirm that hysterectomy induced lower progesterone secretion. However, a luteotropic effect of the uterus has been demonstrated in hysterectomized ewes before ovulation induction (Southee et al, 1988) and at different moments after ovulation induction (Sankot and Murdoch, 1991). On the other hand, hysterectomized ewes were heavier than intact ones (41 versus 37 kg) and Parr et al (1987) observed that overfeeding reduces peripheral progesterone concentration in sheep. This effect could be due to a higher clearance rate of progesterone since blood flow to the liver of ewes increases with feeding (Bensadoun and Reid, 1962). However, this is unlikely as when one administers intramuscularly 20 mg progesterone to hysterectomized ewes, the elimination of the hormone is slower (4 days, fig 3c) than in intact ewes (1 day, Lassoued et al, 1995).

Progesterone injection in hysterectomized females delayed the LH surge and ovulation but had no effect on progesterone secretion by corpora lutea during the first week. This result indicates that the timing of the preovulatory LH surge is not the most important factor in eliminating short cycles. Furthermore, this result may suggest that progesterone action is not at the ovarian level but is probably mediated through the uterus.

Luteolysis depends on oxytocin receptor concentration in the uterus (McCraken et al, 1984), which increases while plasma concentrations of progesterone decrease and oestradiol increase. In the case of induced short luteal phases in anoestrous ewes, endometrial concentrations of oxytocin receptors are higher at D5 than those found in sheep with normal induced cycles following progesterone treatment (Hunter et al, 1989). Progestagen treatment inhibits the establishment of oxytocin receptors and the release of prostaglandins in response to oxytocin administration (Vallet et al, 1990).

The length of short cycles induced by the ram effect in seasonally anoestrus ewes is remarkably constant (5–6 days). The analysis of progesterone profiles at day 4 after ram introduction or day 3 after ovulation showed a lower concentration associated with hypofunctionnel corpora lutea despite their normal appearance. This difference was not significant owing to the small num-

ber of ewes involved in experiment 1, but using a larger sampling of monoovulatory Barbarine ewes after ram effect ($n = 68$), a statistical difference was obtained (0.61 ± 0.29 ng/mL versus 0.48 ± 0.32 ng/mL; $P < 0.05$; unpublished results). This could be attributed to immaturity of the preovulatory follicle induced to ovulate by the ram effect during seasonal anoestrus. It was shown in sheep that supplementation with progesterone soon after ovulation decreases follicular growth during the first follicular wave (Rubianes et al, 1996) and probably reduces the increase in E2 secretion observed by Mattner and Braden (1972).

Thus, the lack of progesterone and its inhibitory action on oestradiol secretion and establishment of endometrial oxytocin receptors at day 5 could explain the existence of short luteal phases. The mechanism by which the uterus starts to secrete PGF2α early in the cycle could then be similar to that causing corpus luteum regression at the end of a normal cycle. This phenomenon may exist in other situations such as puberty or the onset of breeding season.

ACKNOWLEDGEMENTS

This work was supported by the International Atomic Energy Agency (IAEA) and the Franco-Tunisian project supported by the French Foreign Minister. We would like to thank the following people for their contributions: M Latifa Abdennebi from the Institut National Agronomique Tunisie for her assistance in statistical analysis, G Charpigny from Inra (Jouy-en-Josas) for PGFM assay, A Locatelli, D André and P Lonergan from Prmd, Inra (Nouzilly), for, respectively, hysterectomy, LH assay and help with the English version of this manuscript.

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