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Endocrine and Ovarian Changes in Response to the Ram Effect in Medroxyprogesterone Acetate-primed Corriedale Ewes During the Breeding and Nonbreeding Season

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Ungerfeld R, Carbajal B, Rubianes E, Forsberg M: Endocrine and ovarian changes in response to the ram effect in medroxyprogesterone acetate-primed corriedale ewes during the breeding and nonbreeding-season. Acta vet. scand. 2005, 46, 33-44. – Two experiments were performed to determine the endocrine and ovarian changes in medroxyprogesterone acetate (MAP)-primed ewes after ram introduction. Experiment 1 was performed during the mid-breeding season with 71 ewes primed with an intravaginal MAP sponge for 12 days. While the control (C) ewes (n = 35) were in permanent contact with rams, the ram effect (RE) ewes (n = 36) were isolated for 34 days prior to contact with rams. At sponge withdrawal, all ewes were joined with eight sexually experienced marking Corriedale rams and estrus was recorded over the next 4 days. The ovaries were observed by laparoscopy 4-6 days after estrus. Four weeks later, pregnancy was determined by transrectal ultrasonography. In eight ewes from each group, ovaries were ultrasonographically scanned; FSH, LH, and estradiol-17ß were measured every 12 hours until ovulation or 96 hours after estrus. The response to the rams was not affected by the fact that ewes had been kept or not in close contact with males before teasing. No differences were found in FSH, LH, estradiol-17ß concentrations, growth of the ovulatory follicle, onset of estrus, ovulation rate, or pregnancy rate. Experiment 2 was performed with 14 ewes during the nonbreeding season. Ewes were isolated from rams for 1 month, and received a 6-day MAP priming. Ovaries were ultrasonographically scanned every 12 hours, and FSH, LH, estradiol-17ß, and progesterone were measured. Ewes that ovulated and came into estrus had higher FSH and estradiol-17ß levels before introduction of the rams than did ewes that had a silent ovulation. The endocrine pattern of the induced follicular phase of ewes that came into estrus was more similar to a normal follicular phase, than in ewes that had a silent ovulation. The follicle that finally ovulated tended to emerge earlier and in a more synchronized fashion in those ewes that did come into estrus. All ewes that ovulated had an LH surge and reached higher maximum FSH levels than ewes that did not ovulate, none of which had an LH surge. We conclude that (a) the effect of ram introduction in cyclic ewes treated with MAP may vary depending on the time of the breeding season at which teasing is performed; (b) patterns of FSH, and estradiol-17ß concentrations, as indicators of activity of the reproductive axis, may be used to classify depth of anestrus; and (c) the endocrine pattern of the induced follicular phase, which is related to the depth of anestrus, may be reflected in the behavioral responses to MAP priming and the ram effect.

anestrous depth, gonadotrophin, ram stimulus, teaser rams, ewe.

Introduction

Ovarian and hormonal changes resulting in ovulation following introduction of males to previously isolated females have been described for several species, including small ruminants (for review see *Walkden-Brown et al.* 1999). In ewes, the ram effect has been extensively used to induce out-of-season estrus in order to obtain births during autumn (*Martin et al.* 1986). Introducing rams to previously isolated ewes induces ovulation in some animals, and these may eventually become pregnant. The effect is mediated by a rapid increase in luteinizing hormone (LH) pulse frequency, followed by a surge in LH similar to that observed during the follicular phase of the estrous cycle (*Oldham et al.* 1978/1979). Ovarian responses to the ram effect were recently described using transrectal ultrasonography (*Ungerfeld et al.* 2002). The ovarian response may be related to anestrous depth: we observed a higher LH pulse frequency and higher FSH levels in ewes that responded to the ram effect with a luteal phase than in ewes that did not respond (*Ungerfeld et al.* 2000).

The ram effect has also been used to advance puberty (*Oldham & Gray* 1984) and shorten lactational anestrus (*Geytenbeek et al.* 1984). However, little is known about the effect of ram introduction on the estrous response of cyclic ewes. *Pearce & Oldham* (1983), using ovariectomized, progesterone-treated ewes during the breeding season, observed an increase in LH pulse frequency after ram introduction that was not affected by progesterone treatment. Cyclic ewes with an intravaginal sponge containing medroxyprogesterone acetate (MAP) also respond to ram introduction with an increase in LH secretion (A.C.O. Evans, personal communication), which is followed by a concentration of ovulation and an increase in pregnancy rate (*Lucidi et al.* 2001). When compared with ewes that have been near the pen of the rams, previously isolated ewes primed with MAP respond with a shorter latency to onset of estrus and an improved synchronization of heat (*Ungerfeld & Rubianes* 1999). Similarly, an earlier lambing period was observed in unprimed cyclic ewes stimulated with rams (*Ann Lai* 1988). An advancement in the onset of estrus was also observed, as well as an earlier LH surge and earlier ovulation (*Evans et al*. 2002).

In anestrous ewes, the ram effect stimulates ovulation; however, the first ovulation is not accompanied by heat. Heat has been reported to occur concurrently with the first ovulation when progestogen treatment is used before introduction of the rams (*Hunter et al.* 1971), even over 6 days (*Ungerfeld et al.* 2003). However, in previous experiments with Corriedale ewes, we observed that approximately 30-50% of animals primed with progestins showed their first estrus 17-20 days later (*Ungerfeld et al.* 1999, 2003), which was preceded by luteal progesterone levels, indicating a previous ovulation (*Ungerfeld et al.* 2003). Similar results have been reported by *Martin et al.* (1981), although in their study, sponges were withdrawn 48 hours before introduction of the ram.

The first objective of this study was to determine if the introduction of rams to MAP-primed cyclic ewes determines changes in endocrine and follicular profiles, estrous onset, ovulation and pregnancy rates. A second objective was to characterize the ovarian response and the endocrine profiles in MAP-primed ewes stimulated during the non-breeding season, and to determine if the endocrine stage at ram introduction may affect estrous expression and first ovulation.

Materials and methods

Experiment 1

The experiment was carried out on a commercial farm near Trinidad, Uruguay (33° SL), during the mid-breeding season (April-May). Alto-

gether 71 multiparous Corriedale ewes with a mean weight of 42.3 ± 4.7 kg and a body condition (BC) score of 2.7 ± 0.4 were used. Body condition was ranked on a scale of 1-5, where 1 = extremely emaciated and 5 = excessively fat; values are given as means ± standard error of the mean (SEM).

During the experimental period, ewes grazed on native pastures. On day -34 (day 0 = ram introduction), the experimental ewes were tagged and divided into two homogeneous groups with respect to BC: the ram effect group (n = 36) and the control group (n = 35). Ewes in the ram effect group were isolated from rams so that they could not see, hear, or smell them (minimum distance: 1,000 m). Ewes in the control group remained close to the pen where the rams were kept. Intravaginal sponges containing 60 mg MAP (Syntex SA) were inserted in ewes of both groups on day –12. At sponge withdrawal, all the ewes were mixed and placed in the same paddock with eight adult, sexually experienced Corriedale rams fitted with markers. Ewes in estrus were identified at 12-h intervals, from 12 h to 96 h after introduction of the rams. At 4-6 days after estrus, ovulation and ovulation rate were assessed by mid-ventral laparoscopy performed under local anesthesia. To determine pregnancy status, transrectal ultrasonography using a dual (5/7.5 MHz) linear probe (Pie Medical 480, Maastricht, The Netherlands) was performed 4 weeks after estrus.

A detailed study of the ovarian and endocrine patterns of the follicular phase was conducted in eight ewes from each group. Daily ultrasonographic observations of ovaries were performed by the same operator on all ewes from –72 h (0 h = introduction of the rams) to 0 h, and at 12-h intervals either until ovulation had occurred or until 96 h. Before each ultrasonographic examination, blood samples were collected by jugular venipuncture and allowed to clot for 1 h at room temperature before being centrifuged for 10-20 min, and stored at -20 °C until assayed for FSH, LH, and estradiol-17ß.

Experiment 2

Experiment 2 was conducted on a commercial farm located near Colonia, Uruguay (35° SL), in November (mid-seasonal anestrus). Fourteen adult multiparous Corriedale ewes with a mean weight of 52.7 ± 1.7 kg and a BC score of 3.2 ± 0.1 were used. Ewes had lambed in April-May, and lambs were withdrawn 2 months before the experiment started. During the experimental period, ewes grazed on improved pastures. From day -30 (day 0 = day on which rams were introduced), ewes were isolated from rams in terms of sight, sound, and smell (minimum distance: 1,000 m) .

On day -6, intravaginal sponges containing 60 mg of MAP were inserted in all ewes. At sponge withdrawal, ewes were placed together with three adult, sexually experienced marking Corriedale rams. Since anestrous Corriedale ewes submitted to the "ram effect" express maximum reproductive response when ewes in estrus are introduced together with the rams (*Rodríguez Iglesias et al.* 1991), 10 ewes were brought into estrus with a 6-day MAP priming plus 400 IU of eCG (Novormón, Syntex SA, Buenos Aires, Argentina). Ewes were checked twice daily from day 0 to day 5 for onset of estrus.

Transrectal ultrasonographic examinations of ovaries were performed every 12 hours, from –96 h until ovulation occurred or until 120 h. Blood was collected from the jugular vein of all animals on days –12 and –8. On day –8, all animals were fitted with indwelling jugular vein catheters, which were used until day 7 to collect blood samples. From day –4 to day 0, samples were obtained every 12 h and from day 0 until 120 h, samples were obtained every 4 h. A single sample was obtained on days 8, 11, and 14. Samples were allowed to clot for 1 h at room

temperature before being centrifuged for 10-20 min, and the serum was stored at –20 °C until assayed. Samples taken until day 5 were used for measurement of FSH, LH, and estradiol-17ß; samples taken from day 5 to day 14 were measured for progesterone.

Ultrasonographic observations

Ovaries were scanned with a B-mode ultrasound scanner (Pie Medical 480, Maastricht, The Netherlands) equipped with a dual (5/7.5 MHz) linear-array probe. A slightly arched plastic tube (25 cm long) was fastened to the transducer cable so that the intrarectally placed probe could be manipulated externally. During each examination, a sketch of both ovaries was made to record the diameter and position of follicles >2 mm in diameter. The observations were also recorded on video using individual videocassettes to verify and correct real-time data. After locations had been recorded, the sketch was compared with that of the previous day.

Hormonal measurements and definitions

Progesterone concentration was determined using a direct solid-phase ^{125}I RIA method (Count-A-Count TKPG, Diagnostic Products Corporation, Los Angeles, CA, USA) with a sensitivity of 0.3 nmol/L. LH concentrations were measured in all samples with a liquid-phase RIA previously validated for ovine serum (*Forsberg et al.* 1993); the detection limit was 0.4 µg/L. Concentrations of FSH and estradiol-17ß were measured in all samples, except the intensive bleeding period (day -6), from which only the first and the last sample were included. Concentrations of FSH were measured with a liquid-phase RIA previously validated for ovine serum (*Meikle* 2001); the detection limit was 0.4 µg/L. Estradiol-17ß was measured using a direct solid-phase ^{125}I RIA method (Count-A-Count TKPG, Diagnostic Products Corporation, Los Angeles, CA, USA) previously validated for ovine serum (*Meikle* 2001); the sensitivity of the assay was 5.5 pmol/L. The intraassay and interassay coefficients of variation were <10% for all assays.

Luteal activity was defined as the presence of progesterone concentrations >1.6 nmol/L (0.5 ng/ml) in three or more consecutive samples. An LH surge was defined as being at least 6 times the value of mean levels. Basal LH concentrations before the introduction of the rams were defined as the mean values of LH.

Statistical analysis

All results are presented as means ± standard error of the mean (SEM), with a significance level of $\alpha = 5\%$. Mean intervals from sponge withdrawal to estrus were compared by ANOVA; frequencies of ewes in heat were compared by Fisher's exact probability test. Ovulation rate (Experiment 1) and follicular popula-

Table 1. Percentages of ewes that showed estrus, length of interval to estrus onset, and ovulation and conception rates in cyclic ewes primed with intravaginal sponges containing 60 mg of MAP for 12 days. While Control ewes remained near rams during all the period before joining, ram effect ewes remained isolated from rams during that period (Experiment 1).

Group	Estrous ewes no. (%)	Interval to estrus onset (h)	Ovulation rate	Conception rate no. (%)
Control	34/35 (97.1)	51.0 ± 5.4	1.1 ± 0.3	26/34 (76.5)
Ram effect	32/36 (88.9)	52.5 ± 3.9	1.1 ± 0.3	28/32 (87.5)

C = control group; RE = ram effect group.

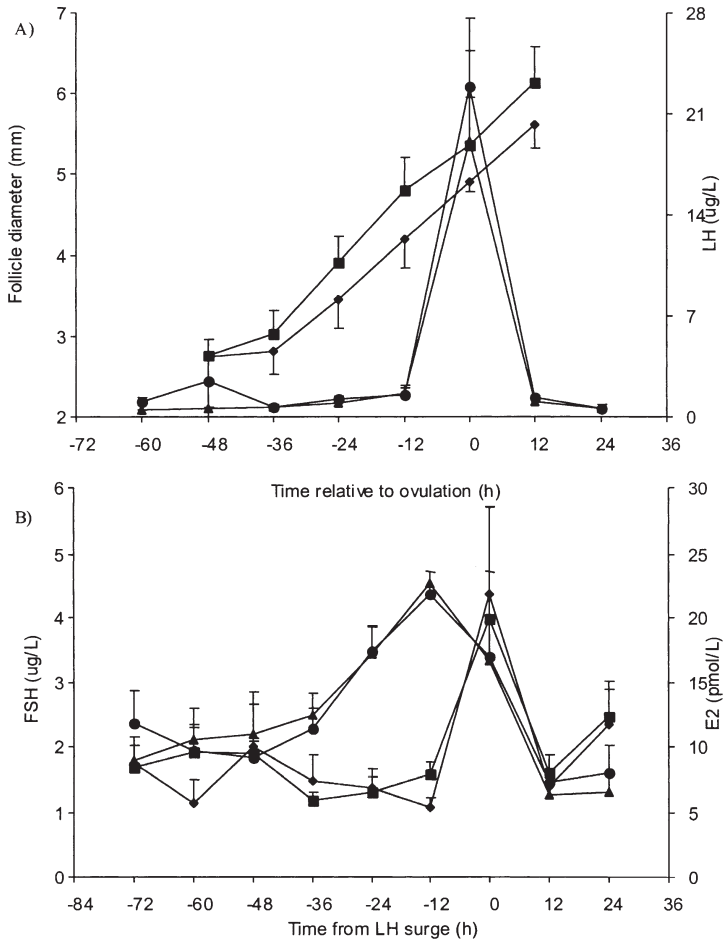

Fig. 1. A) Diameter of the ovulatory follicle (RE: -■-; C: -◆-), and LH levels (RE: -●-; C: -▲-) normalized to ovulation. B) FSH (RE: -■-; C: -◆-) and estradiol-17ß (RE: -●-; C: -▲-) normalized to the LH surge. Ewes were primed for 12 days during the breeding season with an intravaginal sponge containing 60 mg of MAP. Unlike control (C) ewes (n = 5), ram effect (RE) ewes (n = 6) were isolated from contact with rams 30 days before sponge withdrawal.

tions (Experimetn 2) were compared with the Kruskal-Wallis test The diameter of the largest follicle was compared with ANOVA, and LH surge values were compared with ANOVA (Experiment 2). Changes of hormonal concentrations over time for each group of ewes were compared by ANOVA; hormonal profiles and the growth profiles of follicles were analyzed with the general linear model procedure of the Statistical Analysis System (SAS 1996) using repeated-measures ANOVA. Hormonal data were analyzed after normalization by log transformation.

Results

Experiment 1

There were no significant differences between the ram effect and control groups regarding percentage of estrous ewes, time from sponge withdrawal to onset of estrus, ovulation rate, or pregnancy rate. Data are presented in Table 1.

Surges in LH, which were detected in five out of eight control ewes and in six out of eight ram effect ewes, occurred at 48.0 ± 6.0 and 48.0 ± 4.2 h, respectively, after sponge withdrawal. All ewes ovulated, and there was no difference in LH and growth of the ovulatory follicle (Fig. 1A), or FSH and estradiol-17ß (Fig. 1B) levels between control and ram effect ewes (P >0.05).

Experiment 2

No ewe showed luteal activity before introduction of the rams (days -12, -8, and -6).

Ten out of 14 ewes showed an LH surge, that reached maximum concentrations at 63.6 ± 6.8 h after introduction of the rams. After FSH levels were normalized with respect to the LH peak, we observed a significant increase in FSH, which began 6 h before the LH surge and reached maximum levels concurrently with the maximum LH concentrations (P <0.05; Fig. 2A).

Five ewes came into estrus, ovulated, and developed normal luteal phases. Of the remaining nine ewes, five ovulated and had normal luteal phases, but did not display heat. The remaining four ewes did not ovulate or display estrous behavior. Ewes were grouped according to their response: those that came into estrus and ovulated (E-O), those that did not display estrous behavior but ovulated (NE-O), and those that did not ovulate and did not show estrus (NE-NO).

The insertion of the intravaginal sponges (day -6) did not provoke significant changes in concentrations of FSH, LH, and estradiol-17ß (P >0.1). Thus, concentrations for this period are presented pooled in Table 2. Concentrations of FSH and estradiol-17ß before introduction of the rams differed according to the response pattern. While FSH (Fig. 2B) and estradiol-17ß concentrations were higher in E-O ewes than in NE-O and NE-NO ewes in samples obtained before introduction of the rams, there were no significant differences in LH basal concentrations.

The mean values for the number of large follicles and the maximum diameter of the largest follicle during the period before ram introduction are presented in Table 2. After introduction of the rams, the diameter of the largest follicle and the number of follicles >4 mm was similar between E-O, NE-O, and NE-NO ewes (P >0.05).The diameter of the largest follicle in-

Table 2. Concentrations and characteristics of FSH, LH and estradiol-17b, and number of large follicles (>4mm) and diameter of the largest follicles before introduction of rams (mean values until the introduction of the rams) to anestrous ewes primed for 6 days with intravaginal sponges containing 60 mg of MAP. Ewes were classified as E-O (those that came into estrus and ovulated), NE-O (those with a silent ovulation), and NE-NO (those that did not come into estrus or ovulate) (Experiment 2).

	E-O	NE-O	NE-NO	P
FSH levels (µg/L)	3.3 ± 0.1[a]	2.8 ± 0.1[b]	2.6 ± 0.3[b]	<0.01
LH basal levels (µg/L)	0.86 ± 0.05	0.84 ± 0.04	0.75 ± 0.05	>0.1
Estradiol-17ß levels (pmol/L)	12.6 ± 0.6[a]	10.0 ± 0.6[b]	9.7 ± 5.3[b]	<0.001
Number of follicles > 4mm	0.3 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	>0.1
Diameter of the largest follicle (mm)	3.8 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	>0.1

Different letters within the same row indicate statistically significant differences.

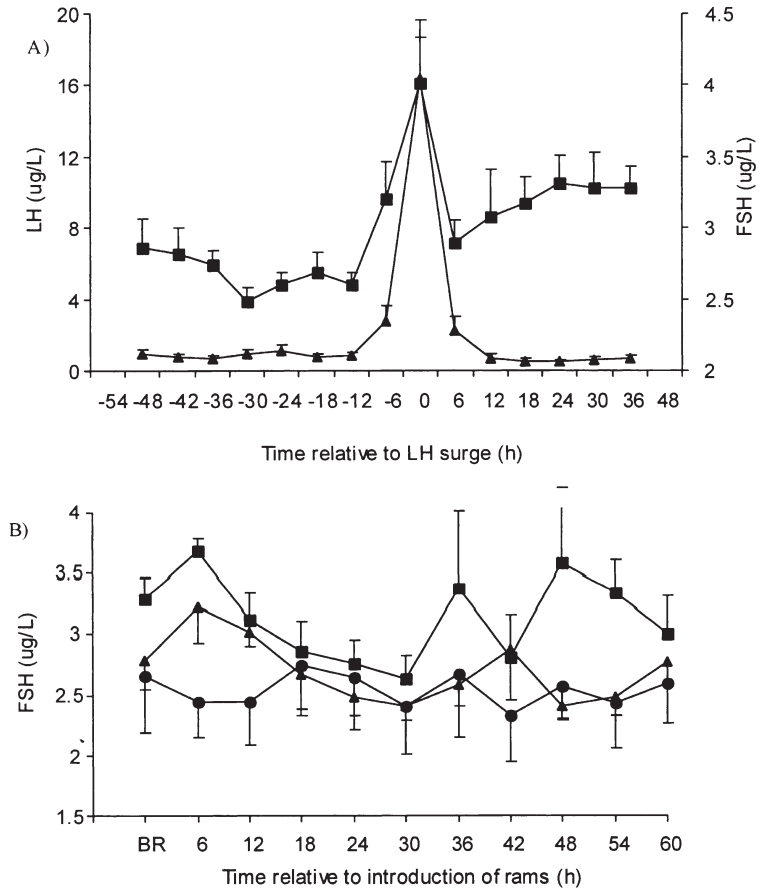

Fig. 2. Fourteen ewes were isolated from contact with rams for 30 days and primed for 6 days with an intravaginal sponge containing 60 mg of MAP during the nonbreeding season; rams were introduced at sponge withdrawal. A) FSH (-■-) and LH levels (-▲-) normalized to the LH surge (n = 10). B) FSH levels in E-O (-■-, n = 5), NE-O (-▲-, n = 5), and NE-NO (-◆-, n = 4) ewes. (BR = data pooled from the period before the introduction of the rams.) (Time with respect to the sponge withdrawal is equal to the time for ram's introduction.) E-O ewes displayed estrous behavior and ovulated after introduction of the rams, NE-O ewes ovulated but did not come into estrus, and NE-NO ewes neither ovulated nor came into estrus.

creased significantly after introduction of the rams (P <0.001), reaching the maximum value at 36 h (3.9 ± 0.1, 3.8 ± 0.1, 4.3 ± 0.1, 4.8 ± 0.2, 4.8 ± 0.2, and 4.7 ± 0.3 mm, for the period before rams, at 12, 24, 36, 48, and 60 h, respectively). The number of follicles >4 mm increased significantly (P <0.05) from 24 to 48 h compared with values before introduction of the rams (0.4 ± 0.1, 1.0 ± 0.2, and 1.25 ± 0.3 before introduction of the rams, at 24 and 48 h, respectively).

There were no differences in growth profiles of the largest follicle between ewes from different groups after introduction of the rams (P >0.1).

However, while in four E-O ewes the follicle emerged before introduction of the rams, in four NE-O ewes it emerged after introduction of the rams ($P = 0.06$). Moreover, the follicle that finally ovulated tended to emerge earlier in E-O (–7.2 ± 7.2 h) than in NE-O (19.2 ± 12.3 h; $P = 0.1$) ewes.

While in E-O ewes, there was a significant increase of estradiol-17ß concentration after introduction of the rams ($P < 0.05$), in NE-O and NE-NO ewes, changes in estradiol-17ß during this period did not reach statistical significance. Concentrations from ram introduction until 36 h later (the period where significant changes occurred) are presented in Fig. 3A. Maximum estradiol-17ß levels tended to be higher in E-O than in NE-O and NE-NO ewes (Table 3). During the same period, FSH concentrations decreased significantly ($P < 0.05$) in the E-O and NE-O ewes, but no significant differences were observed in FSH levels in NE-NO ewes (Fig. 3B). When normalized to the LH surge, estradiol-17ß concentrations tended to be higher in E-O than in NE-O, from 30 (17.3 ± 1.8 vs. 11.2 ± 1.8 pmol/L; $P = 0.06$), 24 (16.0 ± 2.4 vs. 10.0 ± 1.6 pmol/L; $P = 0.1$), and 18 h (20.7 ± 3.7 vs. 13.0 ± 1.1 pmol/L; $P = 0.08$) before the LH surge, and were significantly higher at the time of the surge (18.8 ± 3.1 vs. 10.8 ± 1.2 pmol/L; $P < 0.05$) in E-O than in NE-O ewes, respectively.

All ewes in the E-O and NE-O groups, but none in the NE-NO group, showed an LH surge. The surge tended to be earlier and attained higher concentrations in E-O than in NE-O ewes, and ovulation tended to be earlier in E-O than in NE-O ewes (Table 3). Maximum FSH levels tended to be higher in E-O and NE-O ewes than in NE-NO ewes (Table 3).

Discussion

In the breeding season (Experiment 1), the endocrine or ovarian changes found after introduction of the ram stimulus were similar in ewes that had been totally isolated from males before the stimulation and in ewes that had been kept in close contact with them. The fact that the ovulation was observed in all ewes, while the LH surge in only 11 ewes may be explained by the sampling regime (each 12 h), missing the remaining 5 surges between two consecutive bleedings. The lack of differences between stimulated and unstimulated ewes is in contrast to recently reported results (*Ann Lai* 1988, *Ungerfeld & Rubianes* 1999, *Evans et al.* 2002). However, in other studies it was observed that the introduction of rams induces an increase of LH pulsatility in cyclic ewes with an

Table 3. Concentrations and characteristics of FSH, LH, and estradiol-17b after introduction of rams to anestrous ewes primed for 6 days with intravaginal sponges containing 60 mg of MAP. (Abbreviations as in Table 2.) (Experiment 2).

	E-O	NE-O	NE-NO	*P*
LH surge	5/5	5/5	0/4	<0.001
LH surge concentration (µg/L)	21.3 ± 5.6	11.5 ± 1.9		0.07
Time to LH surge (h)	51.6 ± 8.4	75.6 ± 7.9		0.07
Time to ovulation (h)	67.2 ± 6.1	94.0 ± 8.9		<0.1
Mean FSH concentration (µg/L)	3.1 ± 0.1	2.7 ± 0.1	2.5 ± 0.4	<0.1
Maximum FSH concentration (µg/L)	5.1 ± 0.4[a]	4.3 ± 0.1[a]	3.4 ± 0.5[b]	<0.1
Mean estradiol-17ß levels (pmol/L)	13.4 ± 1.2	13.3 ± 2.1	10.5 ± 1.3	<0.1
Maximum estradiol-17ß levels (pmol/L)	23.8 ± 3.3	15.8 ± 1.6	17.5 ± 1.9	0.09

Different letters within the same row indicate statistically significant differences.

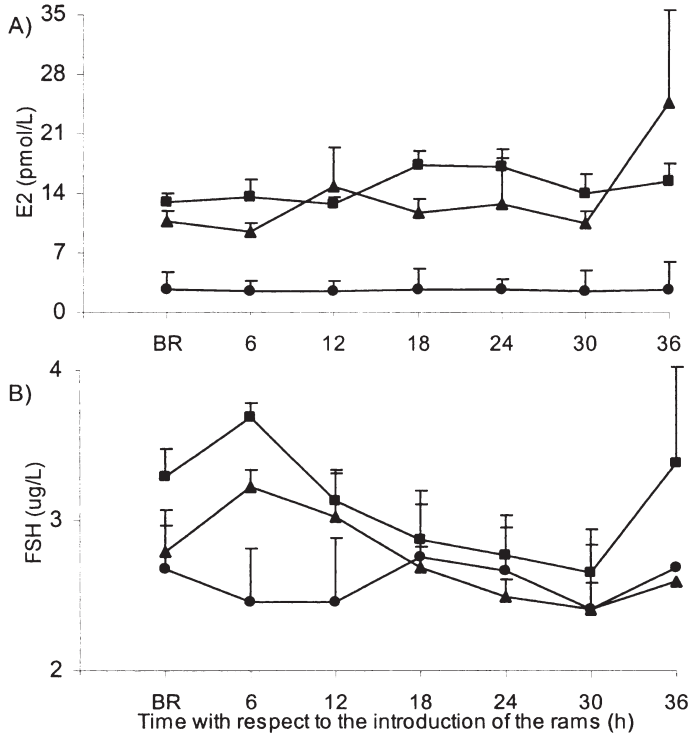

Fig. 3. Estradiol-17ß (A) and FSH (B) concentrations in 14 ewes that were isolated from contact with rams for 30 days, and primed for 6 days with an intravaginal sponge containing 60 mg of MAP during the nonbreeding season; rams were introduced at sponge withdrawal. Time with respect to the sponge withdrawal is equal to the time for ram's introduction. Ewes ovulated and came into estrus (n = 5; -■-), ovulated without estrus (n = 5; -▲-), or neither ovulated nor came into estrus (n = 4; -●-). BR = pooled values from the period before rams were introduced.

intravaginal MAP sponge, but it was followed by a lower pregnancy rate in subsequent estrus (*Evans et al.* 2004). The diverging results could be attributed to the physiological state of ewes in relation to when in the breeding season the experiment was performed. While previous observations in Corriedale ewes (*Ungerfeld & Rubianes* 1999) were made at the onset of the breeding season, the present experiment was performed in the mid-breeding season, when ewes spontaneously display their maximum reproductive activity. As in our experiment one aim was to determine possible effects of ram introduction in estrous onset, ovulation rate and pregnancy rate, we did not include a permanent isolated group. Thus, it remains to be elucidated if the introduction of rams may induce changes in the endocrine patterns or on the follicular development compared with those values in permanent isolated ewes.

In the non-breeding season (Experiment 2), we observed that concentrations of FSH and estradiol-17ß were higher before ram introduction in ewes that finally came into heat coincident with ovulation (E-O ewes). Although there are no clear parameters to characterize "deep" or

"shallow" anestrus, our results let us to suggest that the endocrine patterns of estradiol-17b and FSH, as well as LH pulsatility (*Martin et al.* 1985, *Ungerfeld et al.* 2000) may be useful tools to characterize anestrous depth. The use of arbitrary percentages, such as percentage of animals that are cyclic to differentiate deep from shallow or transitional anestrus (*Signoret et al.* 1982), is useful for flock studies, but such estimates do not take into account the physiological differences existing within noncyclic ewes. *Martin & Scaramuzzi* (1983) proposed that the "responsiveness" to the ram effect could be used to differentiate deep from shallow anestrus, while *Restall* (1992) suggested that use of the ovulation rate, LH pulse frequency, or basal LH levels would differentiate these "states", because these parameters better reflect variation in hypothalamic activity. However, specific values from any hormone that may be used to characterize the depth of anoestrous of an individual female should be considered only against data from a specific flock, because basal hormone concentrations may differ with factors such as breed, the stage of the anoestrous season, or the nutritional status of the animals.

Ewes that came into estrus also showed a preovulatory increase in estradiol-17ß concomitant with a fall in FSH concentrations (*Goodman et al.* 1981), which is similar to what occurrs before ovulation in a normal estrous cycle (*Baird et al.* 1976). In the same ewes, the emergence of the follicle that finally ovulated, the LH surge, and ovulation all occurred or tended to occur earlier. We can speculate that this difference in the endocrine response may have been a consequence of a high sensitivity of the hypothalamus-pituitary axis during shallow anestrus that determine that in more ewes a follicle that was present when rams were introduced finally ovulate. In ewes that were in deeper anestrus, the increase in estradiol-17ß did not reached significant differences and the LH surge and ovulation were delayed until a new follicle grew, suggesting the need for a more sustained stimulus before their reproductive system could respond, and determining a more widespread ovulation.

In agreement with reports from *Poindron et al.* (1980) and *Ungerfeld et al.* (2002), ewes that responded with an LH peak showed an increase in FSH levels (Experiment 2). As those experiments were performed with unprimed ewes, our results extends the information to the response of anestrous progestogen-primed ewes stimulated with rams.

We conclude that the effect of ram introduction in cyclic ewes treated with MAP may vary depending on the time of the breeding season at which teasing is performed. While anestrous ewes with higher spontaneous activity of the hypothalamus-pituitary-ovarian axis will respond to MAP primings and the ram effect with a follicular phase similar to that observed during a normal estrous cycle, and will come in estrus concurrently with ovulation, those in a deeper anestrus would show a less intensive endocrine response , would ovulate in a more dispersed way, and would not come into estrus.

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References

Ann Lai SC: Behavioral enhancement of ewe lamb reproduction through use of the "ram effect". Master in Science Thesis. Faculty of the Graduate School of Cornell University, USA, 1988, 108 p.

Baird DT, Land RB, Scaramuzzi RJ, Wheeler AG: Endocrine changes associated with luteal regression in the ewe: the secretion of ovarian oestradiol, progesterone, and androstenedione and uterine prostaglandin F2a throughout the oestrous cycle. J. Endocrinol. 1976, *69,* 275-286.

Evans ACO, Duffy P, Crosby TF, Hawken PA, Boland MP, Beard AP: Effect of ram exposure at the end of progesterone treatment on estrus synchronisation and fertility during the breeding season in ewes. Anim. Reprod. Sci. 2004, *84,* 349-358.

Evans ACO, Duffy P, Quinn KM, Beard AP, Crosby TF, Boland MP: Introduction of a ram three days before progestagen sponge withdrawal decreases fertility and alters the timing of oestrus, the LH surge and ovulation in ewes. Proc. Sixth International Symposium on Reproduction in Domestic Ruminants, Crieff, Scotland, August 14-17, 2002, p A22.

Forsberg M, Tagle R, Madej A, Molina JR, Carlsson M-A: Radioimmunoassay of bovine, ovine and porcine LH with a monoclonal antibody and a human tracer. Acta Vet. Scand. 1993, *34,* 255-262.

Geytenbeek PE, Oldham CM, Gray SJ: The induction of ovulation in the postpartum ewe. Proc. Aust. Soc. Anim. Prod. 1984, *15,* 353-356.

Goodman RL, Pickover SM, Karsch FJ: Ovarian feedback control of follicle-stimulating hormone in the ewe: evidence for selective suppression. Endocrinology 1981, *108,* 772-777.

Hunter GL, Belonje PC, Van Niekerk CH: Synchronized mating and lambing in spring-bred Merino sheep: the use of progestogen-impregnated intravaginal sponges and teaser rams. Agroanimalia 1971, *3,* 133-140.

Lucidi P, Barboni B, Mattioli M: Ram-induced ovulation to improve artificial insemination efficiency with frozen semen in sheep. Theriogenology 2001, *55,* 1797-1805.

Martin, G.B., Cognié, Y., Schirar, A., Nunes-Ribeiro, A., Fabre-Nys, C. & Thiéry, J.-C: Diurnal variation in the response of anoestrous ewes to the ram effect. J. Reprod. Fertil. 1985, *75,* 275-284.

Martin GB, Oldham CM, Cognie Y, Pearce D: The physiological responses of anovulatory ewes to the introduction of rams - a review. Livest. Prod. Sci. 1986, *15,* 219-247.

Martin GB, Scaramuzzi RJ: The induction of oestrus and ovulation in seasonally anovular ewes by exposure to rams. J. Steroid. Biochem. 1983, *19,* 869-875.

Martin GB, Scaramuzzi RJ, Lindsay DR: Induction of ovulation in seasonally anovular ewes by the introduction of rams: effects of progesterone and active immunization against androstenedione. Aust. J. Biol. Sci. 1981, *34,* 569-575.

Meikle A: Reproductive endocrinology of prepubertal and anestrous ewes. Regulation of uterine sex steroid receptors by ovarian hormones and effects of estradiol on gonadotropin secretion and follicular growth. Ph.D. Thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden, 2001, 51 p.

Oldham CM, Gray SJ: The 'ram effect' will advance puberty in 9-10 month old Merino ewes independent of their season of birth. Proc. Aust. Soc. Anim. Prod. 1984, *15,* 727.

Oldham CM, Martin GB, Knight TW: Stimulation of seasonally anovular Merino ewes by rams. I. Time from introduction of the rams to the preovulatory LH surge and ovulation. Anim. Reprod. Sci. 1978/1979, *1,* 283-290.

Oussaid B, Cognie Y, Mariana JC: Ovarian stimulation following repeated injections of LH or LH + FSH in Ile-de-France sheep in early and mid-seasonal anoestrus. Anim. Reprod. Sci. 1993, *31,* 83-98.

Pearce DT, Oldham CM: Ram effect in the breeding season. Proc. Aust. Soc. Rep. Biol., Canberra, Australia, September 4-7, 1983, p 49.

Poindron P, Cognie Y, Gayerie F, Orgeur P, Oldham CM, Ravault JP: Changes in gonadotrophins and prolactin levels in isolated (seasonally or lactationally) anovular ewes associated with ovulation caused by introduction of rams. Phys. Behav. 1980, *25,* 227-236.

Restall BJ: Seasonal variation in reproductive activity in Australian goats. Anim. Reprod. Sci. 1992, *27,* 305-318.

Rodríguez Iglesias RM, Ciccioli N, Irazoqui H, Rodriguez BT: Importance of behavioural stimuli in ram-induced ovulation in seasonally anovular Corriedale ewes. Appl. Anim. Behav. Sci. 1991, *30,* 323-332.

SAS: 1996. Statistical Analysis Systems, Version 6.12.

Signoret JP, Fulkerson WJ, Lindsay DR: Effectiveness of testosterone-treated wethers and ewes as teasers. Appl. Anim. Ethol. 1982, *9,* 37-45.

Ungerfeld R, Pinczak A, Forsberg M, Rubianes E: Response of Corriedale ewes to the "ram effect" after primings with medroxyprogesterone, fluorogestone, or progesterone in the non-breeding season. Acta Vet. Scand. 1999, *40,* 299-305.

Ungerfeld R, Pinczak A, Forsberg M, Rubianes E: Parámetros para determinar la profundidad del anestro estacional en la oveja. [Parameters to determine anestrus depth in sheep.] Quinta Reunión Latinoamericana de Cátedras de Fisiología Veterinaria, La Plata, Argentina, 14-15 de diciembre, 2000. [Fifth Latin American Veterinary Physiology Departments Meeting, La Plata, Argentina, December 14-15, 2000.]

Ungerfeld R, Pinczak A, Forsberg M, Rubianes E: Ovarian and endocrine responses of Corriedale ewes to ram effect in the non-breeding season. Can. J. Anim. Sci. 2002, *82,* 599-602.

Ungerfeld R, Rubianes E: Estrous response to ram effect in Corriedale ewes primed with medroxyprogesterone during the breeding season. Small Rum. Res. 1999, *32,* 89-91.

Ungerfeld R, Suárez G, Carbajal B, Silva L, Laca M, Forsberg M, Rubianes E: Medroxyprogesterone primings and response to the ram effect in Corriedale ewes during the non-breeding season. Theriogenology 2003, *60,* 35-45.

Walkden-Brown SW, Martin GB, Restall BJ: Role of male-female interaction in regulating reproduction in sheep and goats. J. Reprod. Fertil. (Suppl.) 1999, *52,* 243-257.

Sammanfattning

Baggeffektens inverkan på könshormonnivåer och follikeldynamik hos Corriedale tackor som behandlats med medroxyprogesteronacetat (MAP) under och utanför parningssäsongen.

Experiment 1 genomfördes under parningssäsongen med 71 tackor som behandlats intravaginalt med MAP under 12 dagar. De tackor som fungerade som kontroller (n=35) var i ständig kontakt med baggar medan tackorna i försöksgruppen (n=36) hölls isolerade från baggar i 34 dagar. Efter avslutad behandling med MAP sammanfördes kontroll- och försöksgrupperna och 8 sexuellt erfarna baggar. Tecken på brunst övervakades under de 4 följande dagarna och hos de tackor som visade brunst undersöktes äggstockarna med laparaskopi 4-6 dagar efter visad brunst. Fyra veckor senare gjordes en dräktighetsundersökning med ultraljud. Hos 8 tackor från kontroll- och försöksgrupp som visat brunst undersöktes äggstockarna med ultraljud var 12 timme samtidigt som blodprov togs för bestämning av FSH, LH och östradiol 17ß. Detta pågick fram till ägglossning eller till 96 timmar efter visad brunst. Av resultaten kunde man inte utläsa någon skillnad i om tackorna varit isolerade från baggkontakt eller inte. Det framkom inte heller några skillnader mellan grupperna i hormonkoncentrationer, tillväxt av den ovulatoriska follikeln, när brunsten startade, ovulationsfrekvens eller dräktighetsresultat.

Experiment 2 genomfördes med 14 tackor utanför parningssäsongen. Tackorna hölls isolerade från baggar under 1 månad. De sista 6 dagarna innan baggar återfördes till gruppen behandlades tackorna under 6 dagar intravaginalt med MAP. Innan baggarna återförts till gruppen, påbörjades undersökning av äggstockarna med ultraljud var 12 timme och blodprov togs för bestämning av FSH, LH, östradiol17ß och progesteron. Resultaten visade att de tackor som kom i brunst hade högre koncentrationer av FSH och östradiol17ß innan baggarna återfördes till gruppen. Det indikerar att dessa båda hormoner skulle kunna användas för att bedöma hur djupt anöstral en tacka är utanför parningssäsongen. Den follikelfas som inducerades av baggeffekten liknade hormonellt en normal follikelfas under parningssäsong. Hos alla tackor som ovulerade föregicks ägglossningen av höga halter av LH och FSH.

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