Acta vet. scand. 1999, *40*, 299-305.

Response of Corriedale Ewes to the “Ram Effect” after Priming with Medroxyprogesterone, Fluorogestone, or Progesterone in the Non-Breeding Season

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Ungerfeld R, Pinczak A, Forsberg M, Rubianes E: Response of Corriedale ewes to the “ram effect” after priming with medroxyprogesterone, fluorogestone, or progesterone in the non-breeding season. Acta vet. scand. 1999, 40, 299-305. – One hundred eighty-nine Corriedale ewes were used during the non-breeding season to study the “ram effect“ stimulus after priming with progestogens. Intravaginal sponges containing either medroxyprogesterone acetate (MAP group, n = 49), fluorogestone acetate (FGA group, n = 49), or progesterone devices (CIDR group, n = 46) were inserted on Day -6 (Day 0 = introduction of the rams). Forty-five ewes were untreated and kept as a control group. On Day 0 the sponges were removed and rams provided with marking harnesses for oestrous detection were placed with the ewes. Onset of estrus was monitored until Day 25, and conception was determined by transrectal ultrasonography. Ewes came into heat during 4 periods: Days 0-3, 5-7, 17-20, and 21-23. The overall number of oestrus ewes were 29%, 53%, 35%, and 50% for the control, MAP, FGA, and CIDR groups, respectively (MAP and CIDR > control, $p < 0.05$). Control ewes presented oestrus only on Days 17-20 and 21-23. Oestrus in the progestogen-primed ewes was concentrated during Days 0-3 and 17-20, and some ewes came into oestrus on Days 5-7. There were no differences between different primings neither in oestrous response nor in conception rate. The conception rate from matings occurring on Days 0-3 was higher than on those occurring on Days 17-20. We conclude that MAP, FGA, and CIDR is equally effective in improving the response to the ram effect, and the pattern of oestrus in primed ewes was different than previously reported.

progestogen; oestrous induction.

Introduction

The reproductive response of isolated anoestrous ewes to the introduction of rams (the “ram effect”) has long been known (*Underwood et al.* 1944). If ewes are preconditioned by a period of isolation, the introduction of rams induces changes in their reproductive physiology, LH pulsatility is increased, and ovulation is induced in many of the ewes (for review, see *Martin et al.* 1986). This ovulation is not associated with heat. In some of the ewes, the first heat appears in conjunction with the second ovulation 17 to 20 days after ram introduction. In others, there is at first a short luteal phase (4-5 days), then a second ovulation without signs of oestrus, followed by a luteal phase of normal duration. Thereafter, another ovulation associated with heat occurs. This explains why oestrus occurs in a highly characteristic pattern after ram exposure, i.e. a bimodal response with 2 oestrous peaks: 17 to 20 and 21 to 25 days after rams are introduced (*Martin et al.* 1986).

Progestogen priming prevents the occurrence of short luteal phases (*Cognié et al.* 1982, *Pearce et al.* 1985). Although the mechanism is not clear, it is known that progestogens have a direct effect on the preovulatory follicle, modulating the effects of LH and the steroid secretion (*Hunter et al.* 1987). The delay of the LH peak observed after progestogen treatment may allow follicles to obtain a better synchrony with endocrinological events (*Pearce et al.* 1985). Progestogen priming has also been reported to induce heat at the first ovulation when provoked by the "ram effect" (*Lishman et al.* 1971), thus hampering the return of some ewes to anoestrus after the first ovulation without oestrous signs (*Lindsay et al.* 1984).

The objective of this experiment was to study the "ram effect" stimulus on the onset of oestrus and the conception rate in Corriedale ewes after intravaginal priming with medroxyprogesterone acetate (MAP), fluorogestone acetate (FGA), and progesterone in the non-breeding season.

Materials and methods

The experiment was conducted on a farm located in Artigas, Uruguay (latitude: 31° S), during the non-breeding season (November-December 1997) (natural light:dark ratio = 14L:10D). Ewes were grazing native pastures. During the experimental period, there were unusually heavy rains and storms. Between 1966 and 1996, from November to December, the local rainfall ranged from 30 to 610 mm, but during the experiment, precipitation was more than 1200 mm.

One hundred eighty-nine Corriedale ewes weighing 35.6 ± 0.5 kg (mean ± SEM) and with a body condition score of 1.9 ± 0.0 (1 extremely emaciated, 5 with excess fat) were used in the experiment. Ewes were isolated (by sight, sound, and smell) from rams (minimum distance = 4000 m) for more than 7 months. Ewes had lambed in July, and all lambs were withdrawn one month before the experiment started. Ewes were tagged and divided into 4 homogeneous groups according to body condition.

On Day -6 (Day 0 = introduction of the rams) MAP sponges (60 mg, SincrOvin, Lab Santa Elena, Montevideo, Uruguay) (MAP group, n = 49), FGA sponges (30 mg, Chronogest, Intervet, The Netherlands) (FGA group, n = 49), or devices containing progesterone (CIDR-G, 0.3 g, InterAg, Hamilton, New Zealand) (CIDR group, n = 46) were inserted. Forty-five ewes served as a control group. The sponges remained in situ for 6 days. At sponge withdrawal (Day 0) all ewes were placed with sexually experienced Corriedale rams fitted with marking harnesses for oestrous detection in a 1:13 ram:ewe ratio. All ewes were managed together until marked by rams. Marked ewes were removed from the flock with rams maintaining the ram:ewe ratio.

Corriedale anoestrous ewes submitted to the "ram effect" express maximum response if introduced to the rams together with oestrous ewes (*Rodriguez Iglesias et al.* 1991). Consequently, 50 additional ewes were brought into oestrus between Days -2 and +4 by an injection of 400 IU of eCG (Folligon, Intervet, The Netherlands) after a 6-12-day MAP priming period (60 mg, IVU, Montevideo, Uruguay).

Oestrous ewes were identified twice daily from Day 0 to 6 and 17 to 25, and once daily from Day 7 to 16. The onset of oestrus was considered to occur at a point half way between the last control where the ewe was not marked by a ram and first one in which it was. Five to 6 weeks after oestrus, pregnancy was determined by transrectal ultrasonography (Pie Medical

Table 1. Number of Corriedale ewes that came into estrus after exporure to rams. Ewes were primed during 6 days either with medroxyprogesterone acetate (MAP, n = 49) or fluorogestone acetate (FGA, n = 49) sponges or progesterone devices (CIDR, n = 46), or were unprimed (Control, n = 45).

Group	Days				Total
	0-3	5-7	17-20	21-23	
Control	0/13 (0) a	0/13 (0)	9/13 (69)	4/13 (31) a	13/45 (29) a
MAP	12/26 (46) b	2/26 (8)	12/26 (46)	0/26 (0) b	26/49 (53) b
FGA	6/17 (35) b	2/17 (12)	9/17 (53)	0/17 (0) b	17/49 (35)
CIDR	9/23 (39) b	0/23 (0)	12/23 (52)	2/23 (9)	23/46 (50) b
Total primed	27/66 (41) **	4/66 (6)	33/66 (50)	2/66 (3) ***	66/144 (46) *

For the same column: a vs b: $p < 0.05$; * vs Control: $p < 0.05$; ** vs Control: $p < 0.01$; *** vs Control: $p < 0.001$.

480, Maastricht, The Netherlands, with a dual linear probe 5/7.5 MHz).

Frequencies of ewes in oestrus and rates of conception were compared by a Chi square test. The interval from withdrawal of the intravaginal device to onset of oestrus was compared by ANOVA using SAS (1996). Data are expressed as mean ± SEM.

Results

Ewes came into oestrus in 4 periods: Days 0-3, 5-7, 17-20, 21-23 (Table 1). No differences were observed in the total number of ewes in oestrus and the number of ewes that came into oestrus in each period between MAP, FGA, and CIDR primings, so data from the 3 groups were pooled (Table 1, bottom row).

No control ewes were in oestrus before Day 17. All responding control ewes came into oestrus on Days 17-20 and 21-23. Progestogen-primed ewes came into oestrus in all periods, but most did so on Days 0-3 and Days 17-20. Overall, more primed (46%) than control (29%) ewes came into oestrus ($p < 0.05$).

The time (h) from sponge withdrawal to onset of oestrus on Days 0-3 tended to be higher for the MAP group (59.0 ± 13.0) than for FGA (48.0 ± 10.0, $p = 0.06$) and CIDR (50.0 ± 8.5, $p = 0.08$) groups. On Days 17-20 oestrous onset was earlier in control ewes than ewes of the MAP and CIDR groups (18.6 ± 0.9 vs. 20.0 ± 1.2 and 20.0 ± 0.9 days, respectively; $p < 0.05$) FGA being intermediate (19.1 ± 0.9).

The overall conception rate for control ewes was 15.4%. Conception rate for primed ewes mated during Days 0-3 and 5-7 was significantly higher (35.5%) than those that mated during Days 17-20 and 21-23 (8.6%; $p < 0.01$) (Fig. 1).

Discussion

The periods during which control ewes showed onset of oestrus in response to the "ram effect" were similar to that which has been previously reported (*Martin et al.* 1986). There are few reports of the "ram effect" on Corriedale ewes (*Louw et al.* 1974; *Rodriguez Iglesias et al.* 1997), but we consider the proportion of control ewes that showed oestrus to be relatively low. We observed that 29% of control and 53% of primed ewes came into oestrus, but previous authors using the same breed have reported oestrus as a response to the "ram effect" in approximately 90% of ewes regardless of whether they had been primed (*Rodriguez Iglesias et al.* 1997). The response to the "ram effect" is re-

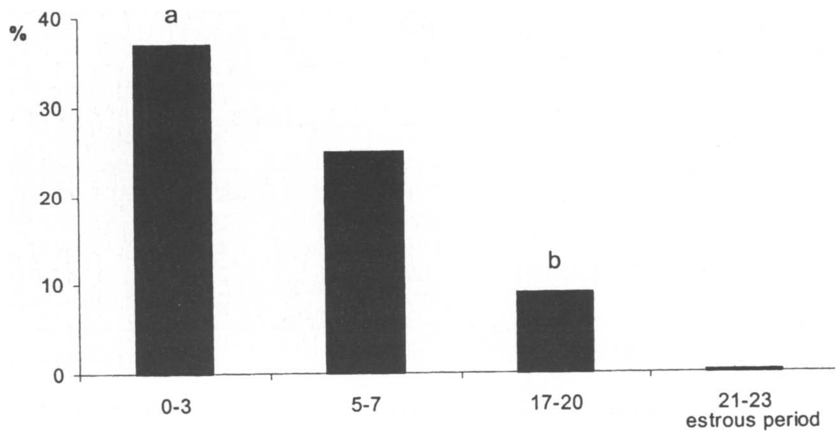

Figure 1. Conception rate of Corriedale ewes primed with progestogens during 6 days shown in relation to estrous period after ram introduction during the non-breeding season.

lated to the level of nutrition (*Khaldi* 1984, *Folch et al.* 1988), and the influence on reproductive system is mediated by changes in GnRH/LH secretion, which is directly affected by body condition (*Rhind et al.* 1989). Thus, the poor body condition of the ewes in this study could partly account for the low oestrous response.

Some progestogen-primed ewes in this study showed their first oestrus 17-20 days after ram introduction. *Rodriguez Iglesias et al.* (1997) reported that 90% of progestogen-treated ewes presented a corpus luteum 5 days after ram introduction, and this luteal phase was preceded by signs of heat. In a later study we observed a similar pattern: ewes primed with MAP had an increase in progesterone before the first heat on Days 17-20 (unpublished observations). Taken together, these findings indicate that primed ewes in this study had a luteal phase preceding the first oestrus on Days 17-20. This implies that the priming had an effect similar to a short luteal phase, being sufficient to allow ewes to have a normal luteal phase, but not sufficient to elicit heat. It is unclear why the ewes did not show signs of heat during the first follicular phase, as can be expected after progestogen priming. We can not relate the delayed response to body condition or to the short-term priming used, which were the main differences between our experimental design and previous experiments (*Rodriguez Iglesias et al.* 1997). In a recent experiment using Corriedale ewes in good condition (body condition score: ~3.5), we observed the same pattern of oestrous distribution in MAP-primed ewes (unpublished observations), and short-term priming (5 days) has been effective in inducing heat at first ovulation after ram exposure (*Reeve & Chamley* 1984). *Martin et al.* (1981) also observed that only 2 ewes from 12 primed anoestrous ewes that ovulated after ram introduction showed oestrus.

Two mechanisms can explain why more progestogen-primed ewes than control ewes came into oestrus: (1) priming caused more ewes to ovulate, and/or (2) as oestrus was delayed in unprimed ewes, some responding ewes may have returned to anoestrus. This differs from previous reports in which the oestrous responses were similar regardless of whether or not the ewes had been subjected to progestogen treatment (*Lindsay et al.* 1984, *Rodriguez Iglesias et al.* 1997). The oestrous responses observed in those experiments were probably too high to reveal any differences between treatments.

Intravaginal devices containing MAP, FGA, and progesterone have been used with equal success for oestrous synchronisation in cyclic ewes (*Walker et al.* 1989) and in combination with eCG to induce oestrus in the non-breeding season (*Crosby et al.* 1988). Our results confirm that these compounds can also be used successfully in combination with the "ram effect" to induce oestrus in anoestrous ewes. In addition, our study confirms the previously reported differences in time from cessation of treatment to the onset of oestrus, when MAP, FGA and progesterone are used for oestrous synchronisation. This is an important aspect that should be considered when artificial insemination programs are applied (*Robinson et al.* 1967, *Crosby et al.* 1988).

A small number of ewes came into oestrus between Days 5 and 7. Some authors have suggested that some ewes do not ovulate immediately after ram introduction during the non-breeding season: ovulation is delayed 5 to 9 days (*Hunter & Lishman* 1967, *Fulkerson et al.* 1981). This has also been confirmed by daily ultrasound examination in our laboratory (*Ungerfeld et al.* 2000).

The higher conception rate observed at earlier oestrous periods compared with that observed at later oestrous periods confirms previous observations (*Martin & Scaramuzzi* 1983). However, the overall conception rate was lower than expected (*Louw et al.* 1974, *Rodriguez Iglesias* 1997). Various factors could have affected conception rate, mainly the poor body condition and incidental stress experienced during the experimental period. Although pathways that couple energy balance with ovulation are not completely understood, it is known that spontaneous ovulation is suppressed or at least depressed during periods of negative energy balance (*Bronson* 1998). Fertility in anoestrous ewes, subjected to ram or eCG stimulation, is directly related to nutritional status (*Folch et al.* 1988). Low feeding levels decreases ovulation rate (*Khaldi* 1984) and increases egg loss (Gunn & Doney 1973). Moreover, the exposure to heavy rainfall, which occurred during our experiment, affects both ovulation rate and egg loss (*Gunn & Doney* 1973).

In summary: (1) more progestogen-primed ewes than control ewes came into oestrus throughout the experimental period, and no differences were detected between MAP, FGA, or CIDR priming; (2) some primed ewes showed their first oestrus during Days 5-7 and Days 17-20, a period not previously reported; and (3) conception rate from matings occurring 1-3 days after rams were introduced was higher than those occurring 17-20 days later.

Acknowledgements

The authors thank Julio Pirotto and all people working on Estancia San Lorenzo, the farm where this research was conducted; Ing Jorge Zoppolo (Allflex Latinoamericana) for supplying tags, and Labs. Santa Elena and IVU for donating the sponges. This work was supported by CIDEC, Veterinary Faculty, University of Uruguay, Montevideo, Uruguay.

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Sammanfattning

Inverkan av baggstimulering på äggstocksaktiviteten hos Corriedale tackor under icke parningssäsong efter behandling med medroxyprogesterone, fluorogestone eller progesterone.

Etthundraåttionio tackor behandlades under 6 dagar med tamponger som innehöll progestogener; medroxyprogesteronacetat (MAP, n = 49), flourogeston (FGA, n = 49) och progesterone (CIDR, n = 49). Fyrtoifem tackar lämnades utan behandling som kontrollgrupp. När tampongerna avlägsnades på dag -6 introducerades baggar i flocken av tackor. Brunst registrerades genom färgmärkning vid betäckning under 25 dagar. Dräktighet registrerades med ultraljud. Tackorna kom i brunst under fyra perioder: dag 0-3, 5-7, 17-20 och 21-23. Det total antalet tackor i brunst i de olika grupperna var 29% (kontrollgruppen), 53% (MAP), 35% (FGA) och 50% (CIDR)(MAP och CIDR > kontrollgruppen, P< 0.05). Kontrollgruppen visdae endast brunst dag 17-20 och 21-23. Brunsterna i de progestogenbehandlade grupperna var koncentrerade till dag 0-3 och 17-20 med vissa djur i brunst också mellan dag 5-7. Inga skillnader i brunst eller dräktighet registrerades mellan de progestogenbehandlade grupperna. Däremot blev fler djur dräktiga vid parningar som ägt rum dag 0-3 jämfört med dag 17-20. Slutsatsen av undersökningen är att alla 3 progestogenerna är effektiva och ökar antalet tackor i brunst vid baggstimulering utanför normal parningssäsong.

(Received March 22, 1999; accepted June 3, 1999).

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