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The effect of ram exposure previous to progestagen oestrus synchronization on corpus luteum function and fertility in crossbred ewes

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Abstract

Merino and crossbred ewes were used to evaluate whether previously ram exposure in late winter could improve the response to further progestagen oestrus synchronization treatment in terms of luteal function and fertilization rate.

We used 56 adult Merino and crossbred ewes randomly allocated in two groups, control (C; n= 27) and Male Effect (ME; n= 29), which had been isolated from rams for at least 2 months. On the 20th of February and for 5 days (D0-D5), 15 males were introduced into ME group to induce male effect. Oestrous synchronization of animals from both groups began on D20 by the introduction of vaginal sponges containing 45 mg of FGA for 12 days and the administration of 500 IU of eCG on the day of sponge withdrawal (D32). Cervical AI with refrigerated semen (400 x 10^6 spz) was performed 55 h (D34) after sponge withdrawal. Progesterone levels were measured by RIA on blood samples collected twice a week for two weeks before D0, and on days 0, 3, 5, 12, 20, 27, 32, 34, 42 and 52 for ovarian activity evaluation. Blood samples were also collected each 4 hours, during 24 hours starting 44 hours after sponge withdrawal to identify LH preovulatory surge in 5 animals of each group.

On ME group the number of cyclic ewes on D12 and D20 was significantly higher than on D0. On D12, the number of cyclic ewes on ME group was significantly higher than in C group. There were no differences between both groups for the number of ewes lambing and not lambing, as well as for the number of ewes lambing as result of AI or natural service.

Introduction of rams enhanced the number of ewes cycling but this advantage relatively to the control group did not last until the synchronisation treatment. In spite of this stimulatory effect of rams on ovarian activity, it was not enough to improve the lambing rate achieved at the end of the essay probably because the number of acyclic animals at synchronisation treatment was higher than cyclic ewes. We conclude that when in deep anoestrus, ewes in this region do not respond favourably to the ram effect and no improvements on fertility are expected to occur.

Keywords: sheep, ram effect, fertility,

Introduction

Animals living in temperate latitudes have to face seasonal climatic changes in temperature and food availability. This cyclic variation in natural resources leaded to the development of seasonal reproduction of species, in order to give birth at the optimal time of year, usually spring, allowing the new-born to grow under favourable temperature and food availability conditions before the next winter (Thiéry et al., 2002). Domestication have attenuated or suppressed some of the physiological expressions of seasonality, but domesticated small ruminants still have seasonal reproduction (Rosa & Bryant, 2003). The seasonality of

reproduction in sheep breeds in temperate latitudes is controlled mainly by photoperiod, with ewes responding to shortening days in autumn by initiating breeding activity, and inhibiting breeding activity during lengthening days in spring. However, the dates of onset and cessation of anoestrous vary widely with breed, location and management (Notter, 2002). Breeds from intermediate latitudes, such as Mediterranean breeds, have a short anoestrous during which a proportion of ewes ovulate spontaneously.

The duration of seasonal anoestrous in the ewe may be modified by introduction of males to the flock, a process usually known as male effect, which is a suitable tool for out-of-season oestrus induction mainly because of its negligible cost. In anoestrous ewes that have been isolated from rams, introduction of males commonly induces a rise in LH pulse frequency within few minutes. This pattern of LH release causes ovulation, usually within 1 to 2 days of ram introduction. Two periods of oestrous activity, at about 19 and 24 days after ram introduction, will be detected, depending on the kind of corpus luteum formed (Martin et al., 1986; Rosa & Bryant, 2002). Induction of sexual activity during anoestrous can also be achieved by hormonal means. The association of intravaginal progestagen sponges with eCG in oestrous cycle synchronization treatments is extensively applied in reproductive management of sheep flocks during both breeding and non-breeding season.

The majority of research investigating the interaction between male effect and controlled breeding programmes has focused on the influence of the ram during or after removal of the artificial progestagen. Exposure of ewes to rams on the last three days of a progestagen synchronization programme induced an increase in LH concentrations in response of pre-mating ram exposure as well as a more rapid onset of oestrous, shorter oestrous period and earlier LH surge and ovulation, compared to ewes isolated from rams prior to breeding, although a decrease in fertility was observed (Evans et al., 2004; Hawken et al., 2005). Exposure of ewes to rams post sponge removal can reduce variation and hasten LH surge, ovulation and the onset of oestrous (Lucidi et al., 2001).

This work aimed to study whether previously ram exposure in late winter could improve the response to further progestagen oestrus synchronization treatment in terms of luteal function and fertilization rate.

Material and Methods

During late winter, 56 Merino and crossbred ewes (which had been isolated from rams for at least 2 months) were randomly allocated in 2 groups, control (C, n= 27) and ME group (ME, n=29). On the 20th of February and for 5 days (D0-D5), 15 males were introduced into ME group to induce male effect. Oestrous synchronization of animals from both groups began on D20 by the introduction of vaginal sponges containing 45 mg of FGA for 12 days and the administration of 500 IU of eCG on the day of sponge withdrawal (D32). In the two weeks before male introduction, ovarian activity was assessed by plasma progesterone (P4) quantification on blood samples collected twice a week. During the assay, corpus luteum function was assessed by measuring plasma P4 levels on samples collected on days 0, 3, 5, 12, 20, 27 32, 34, 42 and 52. Blood samples were also collected each 4 hours, during 24 hours starting 44 hours after sponge withdrawal to identify LH preovulatory surge in 5 animals of each group. Animals were submitted to cervical artificial insemination 55 hours after sponge withdrawal (D34) using refrigerated semen (400 x 10^6 spz). Rams were introduced to both flocks 4 weeks later allowing all ewes to become pregnant. Progesterone concentrations were determined by RIA using a commercial kit (Count-a-Count Progesterone, DPC, Los Angeles, USA). Plasma LH was measured by ELISA using a commercial kit (LH Detect®, INRA, France).

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Mean concentrations of progesterone were compared between treatments by ANOVA. Other values were compared between groups by chi-square test (Statistica 6.0, Statsoft Inc.)

Results

There were no differences between groups C and ME for the number of cyclic and acyclic ewes on D0 and D20 (table 1). On D12, seven days after finishing male effect, the number of cyclic ewes on ME group was significantly higher than in C group (table 1). In ME group the number of cyclic ewes on D12 and D20 was significantly higher than on D0 (table 1).

Table 1 – Number of cyclic and acyclic ewes on D0, D12 and D20

		Groups		
		C (n=27)	**ME (n=29)**	
D0	**CYCLIC (%)**	7 (25,93)	4 (13,79)	p>0,05
	ACYCLIC (%)	20 (74,07)	25 (86,21)	
D12	**CYCLIC (%)**	3 (11,11)	11 (37,93)	**p<0,05**
	ACYCLIC (%)	24 (88,89)	18 (62,04)	
D20	**CYCLIC (%)**	6 (22,22)	12 (41,38)	p>0,05
	ACYCLIC (%)	21 (77,78)	17 (58,62)	
		p>0,05	**p<0,05 (D0 vs D12 and D20)**	

Plasma progesterone concentrations on D12 were higher (P=0.07) on animals from group ME than those observed on group C (figure 1). On AI day (D34), all animals but one from group C, presented plasma progesterone concentration below 0.5 ng / mL.

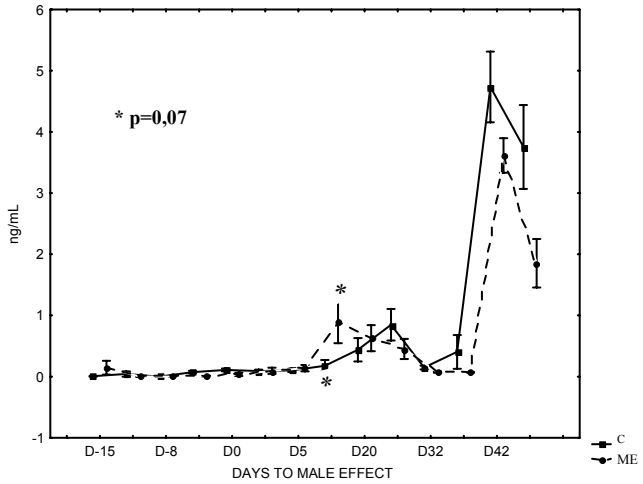

Figure 1 – Plasma progesterone concentrations on animals from groups C and ME during assay (mean ± standard error)

There were no differences between both groups for the number of ewes lambing and not lambing, as well as for the number of ewes lambing as result of AI or natural service (table 2).

Table 2 – Number of ewes lambing (from AI and natural service) and not lambing

		EWES LAMBING		
GROUPS	**TOTAL (n)**	**AI (n)**	**Natural service (n)**	**EWES NOT LAMBING (n)**
C	27	15	3	9
ME	29	11	8	10

The LH preovulatory surge was only detected in one ewe of each group. In the animal from C group it was detected 48 hours after sponge withdrawal (figure 2), while in the animal from ME group it was detected just before 44 hours (figure 3).

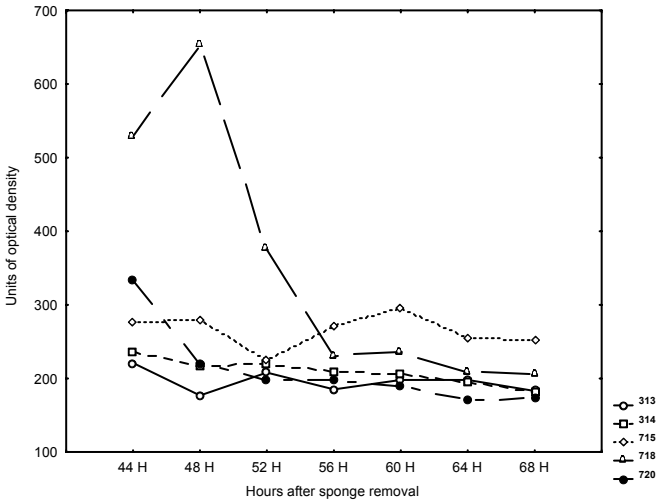

Figure 2 – LH plasma levels recorded in 5 ewes belonging to C group

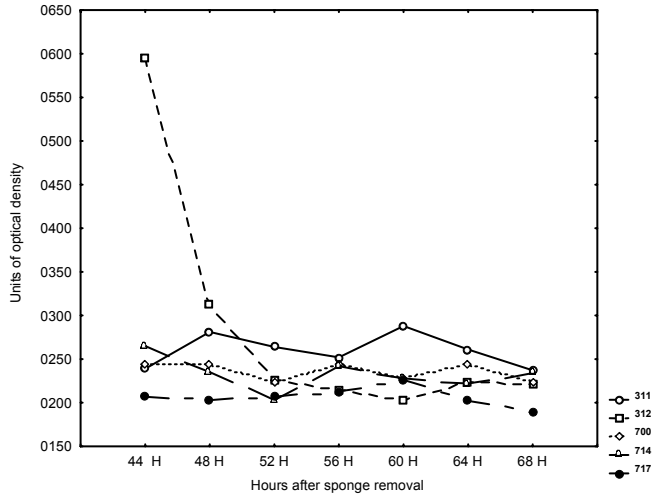

Figure 3 – LH plasma levels recorded in 5 ewes belonging to ME group

Discussion

Plasmatic levels of progesterone on D12 on animals from group ME confirm the occurrence of ovulations induced by the presence of males. LH preovulatory surge was detected only in one ewe from each group, and even in those cases it was observed only the descendent part of the surges. This means that preovulatory surge of LH occurred before the expected time which, according to several authors (Barbas, 1999, Menegatos et al., 2003) is observed 48,3 ± 2,7 or 56,5 ± 3,6 hours after sponge removal.

Introduction of rams to Merino and crossbred ewes enhanced the number of ewes cycling but this advantage relatively to the control group did not last until the synchronisation treatment. In spite of this stimulatory effect of rams on ovarian activity, it was not enough to improve the lambing rate achieved at the end of the essay probably because the number of acyclic animals at synchronisation treatment was higher than cyclic ewes.

In fact, in a previous work by Horta et al. (2004) using a similar methodology but with a greater percentage of cyclic animals at the beginning of the assay, it was obtained a higher fertility rate in animals from ME group. Plasma progesterone concentrations 7 days after the synchronized ovulations were also significantly higher on animals belonging to ME group, which might result from male effect inducing more competent ovulations.

We conclude that when in deep anoestrus, ewes in this region do not respond favourably to the ram effect and no improvements on fertility are expected to occur.

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