Effect of stage of the estrous cycle at the time of initial exposure to rams on the ovarian activity of Pelibuey ewes

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ABSTRACT: Forty non-lactating, cyclic adult Pelibuey ewes were randomly divided into six groups. Estrus was synchronized within each group using intravaginal sponges and prostaglandin F2α injection at the time of the sponge removal. The sponges were inserted and removed on different dates in each group, but all the groups except the control one were first exposed to rams on the same date (July 17th), so that at the time of the first exposure the ewes were either on day 0 (group D0; n = 7), 3 (group D3; n = 7), 8 (group D8; n = 7), 12 (group D12; n = 7) or 14 (group D14; n = 7) of their synchronized estrous cycle. Thereafter the ewes of these groups remained continuously exposed to the males until all the females showed estrus. The ewes in the control group (CG; n = 5) remained isolated from all the males, except for 5-minute periods at the time of estrus detection, which was carried out three times a day. Progesterone concentrations were determined in plasma samples taken daily from two days before the initial exposure to the males until the onset of the next estrus. There were no differences in estrous cycle length between the groups exposed to rams and the control group ($P > 0.05$). The interval from the assumed onset of the estrous cycle (48 h after sponge removal) until the occurrence of luteolysis was not different between the control group and any of the groups exposed to the males. The interval from luteolysis to estrus was not modified by exposure to the males ($P > 0.05$). Estrus duration was shorter ($P < 0.06$) in the control group than in group D3. It is concluded that the exposure of cyclic Pelibuey ewes to males does not advance the time of luteolysis and does not affect the length of the estrous cycle. Therefore, the male effect does not synchronize the next estrus of cyclic Pelibuey ewes.

Keywords: male effect; cyclic ewes; breeding season; African sheep

INTRODUCTION

The ram effect is a "clean, green, and ethical method of controlling reproduction" (Martin et al. 2004; Martin and Kadokawa 2006) and a useful and suitable tool to improve production in small ruminants. The effects of exposing anestrous ewes to males have been reported by several authors (Martin et al. 1986; Alvarez and Zarco 2001; Rosa et al. 2002; Ungerfeld et al. 2004; Delgadillo et al. 2009), but information regarding the role of the male effect on cyclic ewes is scarce and not conclusive.

In ewes of tropical origin, Ngere and Dzakuma (1975) reported that up to a quarter of presumably cyclic females were bred on the first day after exposure to males. They suggested that the presence of rams can alter and synchronize the estrous cycle of ewes from non-seasonal breeds. However, because in that study no estrus detection was carried out before male exposure and no progesterone determinations were made be-

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fore or after such exposure, it is not known if the animals that showed estrus immediately after the introduction of the males were really cyclic, or at which stage of the cycle were they at the time of exposure. Thus, it has been stated that lack of this sort of information prevents the development of management strategies based on the male effect for estrus synchronization during the breeding season in tropical sheep (Delgadillo et al. 2009).

The objective of this study was to evaluate the effects of male introduction at different stages of the estrous cycle on the ovarian activity of Pelibuey ewes, a low-seasonality breed of African sheep.

MATERIAL AND METHODS

The experiment was approved by the Institutional Committee for the Care and Use of Experimental Animals, and was carried out at a university farm located in Central Mexico (19°N) during the months of June, July, and August, when most Pelibuey ewes are cyclic at this latitude (Arroyo et al. 2007).

Animals. Forty adult, non-lactating Pelibuey ewes and two adult Pelibuey rams were used in the trial. All the ewes were cyclic before the onset of the experiment as confirmed by plasma progesterone concentrations higher than 1 ng/ml in at least one of two samples taken at weekly intervals. The rams were sexually experienced and they were stimulated by the presence of two estrous ewes during the two days preceding the onset of the experiment (Rosa et al. 2000). All the animals were kept in outdoor pens and were fed alfalfa hay, oat hay, corn silage, and a commercial concentrate according to their requirements.

General design of the experiment. All the ewes were kept completely isolated from any male for at least three months before the onset of the experiment, and all of them (except those in the control group) were first exposed to the males on the same date (July 17th). Previously, the ewes had been assigned to groups that were synchronized in such a way that at the time of the first exposure to the males they were either on day 0 (group D0; n = 7), 3 (group D3; n = 7), 8 (group D8; n = 7), 12 (group D12; n = 7) or 14 (group D14; n = 7) of the estrous cycle. The ewes in the control group (CG; n = 5) were not exposed to the males, but they had been synchronized to be on day 0 of the estrous cycle on July 17th, when the experimental groups were first exposed to the males.

The synchronization protocol consisted of the insertion of intravaginal sponges containing 20 mg of fluorogestone acetate (Chronogest®; Intervet, Mexico D.F., Mexico) lasting for 12 days. At the time of sponge removal the ewes were injected with 3.7 mg of the PGF2α analog, luprostiol (Prosolvin®; Intervet). Differential synchronization between groups was achieved by starting and finishing the 12-day synchronization protocol on different dates for each group (Figure 1). In all groups it was assumed that the onset of the synchronized estrous cycle (day 0) occurred 48h after sponge removal and PGF2α injection (Wildeus 2000).

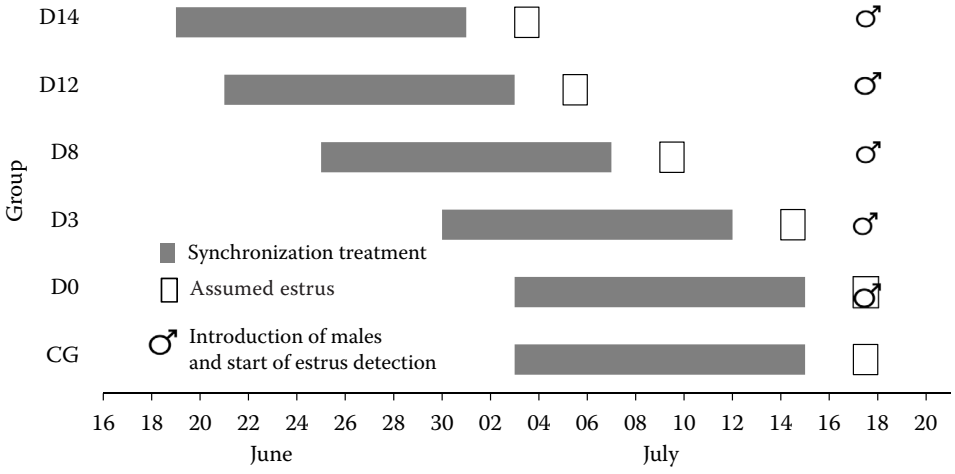

Figure 1. Timetable of activites carried out in Pelibuey ewes not exposed to males (control group, CG) and in the groups of ewes first exposed to males on July 17th, when they were on day 0 (D0), 3 (D3), 8 (D8), 12 (D12) or 14 (D14) of the estrous cycle. The males remained with the ewes from the time of introduction until all the ewes showed their following estrus. Synchronization treatment consisted in a 12-day insertion of a vaginal sponge containing FGA and a PGF2α injection at the time of the sponge removal. Synchronized estrus was assumed to occur 48 h after the sponge removal

Groups D0, D3, D8, D12, and D14 were maintained together in a single pen. The females in these groups were respectively on day 0, 3, 8, 12 or 14 on July 17th, when two adult Pelibuey rams were introduced to the pen and left continuously there until all the ewes had shown their following estrus. During the experiment each of the rams remained in a 4 × 2 m mesh-fence pen located within the pen where the ewes of the experimental groups were kept, since it has been reported that total contact through a clear mesh fence is sufficient to induce ovulation in a high proportion of anestrous ewes (Pearce and Oldham 1988). Estrus detection was carried out three times a day for periods of 30 min at 8:00, 14:00, and 20:00 h starting on the day of the first exposure to the males. At those times the males that had been kept within the mesh-fence pens were fitted with an apron to prevent mating and were allowed to interact directly with the females. In this way the males, that were prevented to directly interact with the ewes at other times, were very active detecting estrous females during the periods when direct contact was allowed, thus facilitating clear identification of estrous ewes.

The ewes in the control group were kept in a separate pen, where they remained isolated from the males at all times except at 8:00, 14:00, and 20:00 h, when a male was introduced to the pen for only 5 min in order to detect estrus with minimal stimulation to the females.

In all groups, a female was considered to be in estrus when she accepted to be mounted by a ram. Duration of estrus was defined as the interval between the first and the last time that the female accepted to be mounted by the males. Estrous cycle length was defined as the interval between the assumed onset of a synchronized cycle and the first detection of estrus by the males during the following cycle.

Sampling and hormone determinations. Heparinized blood samples were obtained daily from two days before the introduction of the males until all the ewes showed their following estrus. The samples were immediately centrifuged to separate the plasma, which was kept frozen at –20°C until assayed for progesterone by a solid-phase radioimmunoassay that has been validated for sheep (Padmanabhan et al. 1995). Sensitivity of the assay was 0.02 ng/ml; intra- and inter-assay variation coefficients for low concentrations were 5.4 and 10.85% respectively, and those for high concentrations were 3.1 and 7.1%. Luteolysis was considered to have occurred when progesterone concentration first decreased below 1 ng/ml.

Variables and statistical analysis. In order to avoid premature stimulation of the ewes by the rams, estrus detection was not carried out during the synchronized estrus that had occurred prior to the onset of exposure to the males. Instead, the onset of the synchronized cycle (day 0) was assumed to occur 48 h after the sponge withdrawal and PGF2α injection (Wildeus 2000). The length of the estrous cycle was therefore calculated as the interval between the assumed day 0 and the time when the onset of the next estrus was detected with the aid of the males. The interval from the onset of the estrous cycle to luteolysis was calculated as the time elapsed from day 0 until progesterone

Table 1. Estrous cycle length, interval from the estrous cycle onset till luteolysis, interval from luteolysis to estrus, and length of estrus in Pelibuey ewes first exposed to males at different stages of the estrous cycle (mean ± SEM)

Group	Estrous cycle length (days)	Estrus–luteolysis[1] (days)	Luteolysis–estrus[2] (h)	Estrus length (h)
Control	18.55 ± 0.88^{ab}	17.80 ± 0.86^{ab}	18.00 ± 5.37^{a}	25.20 ± 1.20^{c}
D0	18.05 ± 0.28^{ab}	17.20 ± 0.20^{a}	20.40 ± 3.60^{a}	31.20 ± 2.25^{cd}
D3	18.71 ± 0.62^{ab}	18.00 ± 0.63^{ab}	17.00 ± 5.24^{a}	35.00 ± 2.41^{d}
D8	21.18 ± 1.31^{b}	20.57 ± 1.27^{b}	14.57 ± 3.43^{a}	30.86 ± 2.42^{cd}
D12	17.54 ± 0.35^{a}	16.71 ± 0.36^{a}	19.71 ± 3.64^{a}	29.14 ± 1.57^{cd}
D14	18.64 ± 0.30^{ab}	17.86 ± 0.34^{ab}	18.86 ± 2.42^{a}	28.29 ± 1.71^{cd}

D0, D3, D8, D12, D14 = ewes first exposed to males respectively on days 0, 3, 8, 12, and 14 after the assumed onset of the estrous cycle, control = ewes not exposed to males

[1]interval between the assumed onset of the estrous cycle (48 h after sponge removal) and occurrence of luteolysis

[2]interval between the time of occurrence of luteolysis and the onset of estrus

[a,b]within a column, different superscripts indicate significant ($P < 0.05$) differences between groups

[c,d]within a column, different superscripts indicate significant ($P < 0.06$) differences between groups

concentrations first decreased below 1 ng/ml. The interval from luteolysis to estrus was calculated as the time elapsed from this moment until the onset of detected estrus. Estrus length was defined as the interval between the first and the last acceptance of the male.

The estrous cycle length, the interval from the cycle onset to luteolysis, the interval from luteolysis to estrus, and the duration of estrus were compared between groups by the analysis of variance followed by Tukey's multiple comparisons tests. A two-way analysis of variance followed by Tukey's multiple comparisons was used to compare the concentrations of progesterone between groups on different days of the estrous cycle.

RESULTS

Three ewes (two in group D0 and one in group D3) were removed from the study because of health problems unrelated to the experiment.

Table 1 shows that there were no differences in the length of the estrous cycle between the control group and any of the experimental groups ($P > 0.05$). The only significant difference ($P < 0.05$) in the length of the estrous cycle was between group D8 (21.18 ± 1.31 days) and group D12 (17.54 ± 0.35 days). The interval from the assumed onset of the estrous cycle to luteolysis was not different between the control group and any of the experimental groups ($P > 0.05$). However, this interval was longer ($P < 0.05$) in group D8 than in groups D0 or D12. The interval from luteolysis to estrus was not modified by the exposure to the males at any stage of the cycle ($P > 0.05$). The duration of estrus was longer in group D3 than in the control group ($P < 0.06$). No differences in estrus length were found between any other groups (Table 1).

Figure 2 shows the progesterone profiles for each group. Progesterone concentrations were higher ($P < 0.05$) in group D0 than in the control group on days 7, 8, 9, and 12 of the estrous cycle.

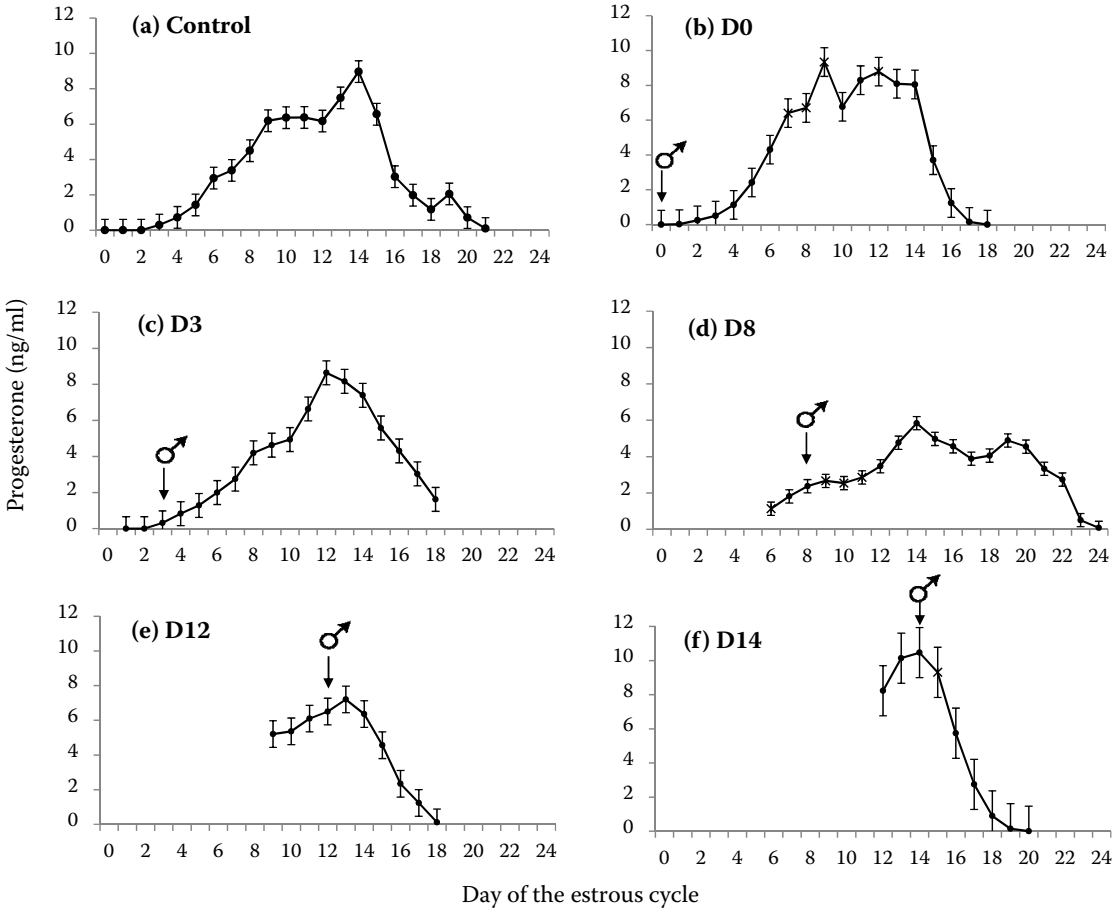

Figure 2. Progesterone concentrations in Pelibuey ewes not exposed to males (**a**) and in ewes first exposed to rams when they were on day 0 (**b**), 3 (**c**), 8 (**d**), 12 (**e**) or 14 (**f**) of the estrous cycle. The male sign indicates the time when the males were introduced to the females' pen (July 17th). The rams remained with the ewes until their next estrus

There were no significant differences between the control group and groups D3 or D12. Progesterone concentrations were lower ($P < 0.05$) in group D8 than in the control group on days 6, 9, 10, and 11. In group D14 the concentrations of progesterone were significantly ($P < 0.05$) higher than in the control group on day 15.

DISCUSSION

Exposure of cyclic Pelibuey ewes to males during the breeding season did not affect their estrous cycle length because no differences were found between any of the exposed groups and the control group. In all groups the average cycle length was close to the longest individual cycle length reported in other breeds (Zarco et al. 1988). However, it should be taken into account that in the present study it was assumed that the onset of estrus (and thus the onset of the estrous cycle) occurred in all animals 48 h after sponge removal (Wildeus 2000). Thus, the duration of the cycle measured from this assumed onset, useful as it was to compare the cycle length between groups, may not reflect the actual estrous cycle length. It cannot be ruled out that in some ewes, especially in those of group D8, the actual onset of estrus might have occurred more than 48 h after sponge removal, thus resulting in a longer apparent cycle length in this group.

Progestogen treatments to synchronize estrus are usually accompanied by the administration of equine chorionic gonadotropin (eCG) at the time of sponge removal in order to induce a more precise synchronization of estrus and ovulation (Haresign 1978). It is known that inclusion of eCG in a synchronization protocol can advance estrus (O'Doherty and Crosby 1990) and for this reason it was decided not to use eCG in this study, since it could interact with the male effect. However, in four ewes of group D8 there was a delay of 4–5 days in the increase of progesterone after the assumed estrus (Figure 2), probably indicating that ovulation occurred several days after the normal time for ewes synchronized with progestogen plus eCG (Wildeus 2000), and this could have resulted in apparent cycle lengths of 23–24 days when counting from the assumed estrus.

Chemineau (1983), working with goats, found that the distribution of estrus during the eight days that followed the introduction of males was significantly different from the expected uniform distribution, suggesting a possible luteolytic effect of exposure to males. Hawken et al. (2007) also suggested that a luteolytic effect of exposure to males could account for the advanced presentation of estrus found after such an exposure. However, in the present study shortening of the estrous cycle consistent with the luteolytic effect of the presence of rams was not observed. This finding agrees with the recent report that introduction of a buck to a group of cyclic goats does not exert a luteolytic effect and consequently does not advance the next estrus, irrespective of the stage of the luteal phase at the time of the initial exposure to the male (Valencia et al. 2010). Our results also agree with observations made by Ungerfeld (2011), which synchronized estrus in ewes with PGF2α and found that the introduction of rams 13 days after a single PGF2α injection did not substitute for a second administration of this hormone. They concluded that the presence of the male does not induce luteolysis in an estrous synchronization protocol. However, the results of the present study do not agree with previous observations by Ngere and Dzakuma (1975), which found that sudden exposure to rams altered the normal estrous cycle of some African ewes that showed behavioural estrus soon after being exposed to rams, resulting in 25% of the flock being bred on the first day of mating. We used ewes of the Pelibuey breed, which is also a tropical hair breed of African origin with poor reproductive seasonality (Valencia et al. 2006), and exposure to the male did not advance estrus behaviour in any of the ewes exposed.

In the present study the interval between luteolysis and the onset of estrus was not modified by exposure to the male, therefore, the hypothesis that proestrus could be shortened by the presence of males (Ungerfeld and Rubianes 1999; Evans et al. 2004; Hawken et al. 2007) is not supported by our results. Zarco et al. (1988) found that the timing of events after the onset of luteolysis is remarkably constant in sheep, and not related to the duration of the estrous cycle, making alterations in the duration of proestrus an unlikely cause of differences in the estrous cycle length.

It has been reported that continuous presence of rams reduces the duration of estrous behaviour (Parson and Hunter 1967; Lindsay et al. 1975). However, in our study the continuous presence of the male tended to increase the length of estrus, a finding difficult to explain, even though the duration of estrus in all groups was within the normal range for this species (24–36 h) (Jainudeen

and Hafez 1993). The brief exposure to the male during estrus detection (5 min) in the control group apparently did not affect the duration of estrus, since it was similar (25.20 ± 2.68 h) to the duration reported in ewes of this breed that were subjected to normal teasing schedules (27.18 ± 9.68 h) (Segura et al. 1991). Also, the 5 min that were available to the males to detect estrus among the five control ewes was equivalent, on a per ewe basis, to the 30 min available to the rams to detect estrus among the 32 experimental ewes that were kept together in a single pen.

Finally, in our study it was decided to include a group of females that were assumed to be in estrus at the time of male introduction (group D0) because during the breeding season some ewes in a flock could be randomly in estrus at the time of male introduction. Those females could interact with the male and potentiate the stimulus on the other females, a phenomenon known as "social facilitation" or "female effect" (Knight 1985; Zarco et al. 1995). Our results suggest that the presence of estrous females at the time of initial exposure to the males exerts only a slight effect, if any, on the estrous cycle of other ewes, even though the proportion of estrous females at exposure in our study was higher than the expected random distribution for cyclic ewes (normal distribution: 8.8% at a given time; in our study: 20%). Thus, the effect of "social facilitation" is probably only important in anestrous ewes (Zarco et al. 1995).

CONCLUSION

The results of the present study show that sudden introduction of males has no synchronizing effect in cyclic Pelibuey ewes and therefore cannot be used as a natural method for estrous synchronization during the breeding season.

REFERENCES

Alvarez L., Zarco Q.L. (2001): Sexual biostimulation phenomena in ewes and goats. Veterinaria México, 32, 117–129. (in Spanish)

Arroyo L.J., Gallegos S.J., Villa G.A., Berruecos J.M., Perera G., Valencia J. (2007): Reproductive activity of Pelibuey and Suffolk ewes at 19° north latitude. Animal Reproduction Science, 102, 24–30.

Chemineau P. (1983): Effect on oestrus and ovulation of exposing Creole goats to the male at three times of the year. Journal of Reproduction and Fertility, 67, 65–72.

Delgadillo J.A., Gelez H., Ungerfeld R., Hawken P.A., Martin G.B. (2009): The "male effect" in sheep and goats – revisiting the dogmas. Behavioural Brain Research, 200, 304–314.

Evans A.C., Duffy P., Crosby T.F., Hawken P.A., Boland M.P., Beard A.P. (2004): Effect of ram exposure at the end of progestagen treatment on estrus synchronisation and fertility during the breeding season in ewes. Animal Reproduction Science, 84, 349–358.

Haresign W. (1978): Ovulation control in sheep. In: Crighton D.B., Haynes N.B., Foxcroft G.R., Lamming G.E. (eds): Control of Ovulation. Butterworths, London, UK, 435–451.

Hawken P.A.R., Beard A.P., Esmaili T., Kadokawa H., Evans A.C.O., Blache D., Martin G.B. (2007): The introduction of rams induces an increase in pulsatile LH secretion in cyclic ewes during the breeding season. Theriogenology, 68, 56–66.

Jainudeen M.R., Hafez E.S.E. (1993): Sheep and goats. In: Hafez E.S.E. (ed.): Reproduction in Farm Animals. Lea and Febiger, Philadelphia, USA, 330–342.

Knight T.W. (1985): Are rams necessary for stimulation of anoestrous ewes with oestrous ewes? Proceedings of the New Zealand Society of Animal Production, 45, 49–50.

Lindsay D.R., Cognie Y., Pelletier J., Signoret J.P. (1975): Influence of the presence of rams on the timing of ovulation and discharge of LH in ewes. Physiology and Behavior, 15, 423–426.

Martin G.B., Kadokawa H. (2006): Clean, green and ethical animal production. Case study: Reproductive efficiency in small ruminants. Journal of Reproduction and Development, 52, 145–152.

Martin G.B., Oldham C.M., Cognie Y., Pearce D.T. (1986): The physiological responses of anovulatory ewes to the introduction of ram – a review. Livestock Production Science, 15, 219–247.

Martin G.B., Milton J.T.B., Davidson R.H, Banchero H.G.E., Lindsay D.R., Blache D. (2004): Natural methods for increasing reproductive efficiency in small ruminants. Animal Reproduction Science, 82, 231–245.

Ngere L.O., Dzakuma J.M. (1975): The effect of sudden introduction of rams on oestrus pattern of tropical ewes. The Journal of Agricultural Science, 84, 263–264.

O'Doherty J.V., Crosby T.F. (1990): The effect of progestagen type, PMSG dosage and time of ram introduction on reproductive performance in ewe lambs. Theriogenology, 33, 1279–1286.

Padmanabhan V., Evans N.P., Dahl G.E., McFadden K.L., Mauger D.T., Karsch F.J. (1995): Evidence for short or ultrashort loop negative feedback of gonadotropin-releasing hormone secretion. Neuroendocrinology, 62, 248–258.

Parson S.D., Hunter G.L. (1967): Effect of the ram on duration of oestrus in the ewe. Journal of Reproduction and Fertility, 14, 61–70.

Pearce G.P., Oldham C.M. (1988): Importance of non-olfactory ram stimuli in mediating ram-induced ovulation in the ewe. Journal of Reproduction and Fertility, 84, 333–339.

Rosa H.J.D., Bryant M.J. (2002): The "ram effect" as a way of modifying the reproductive activity in the ewe. Small Ruminant Research, 45, 1–16.

Rosa H.J.D., Juniper D.T., Bryant M.J. (2000): The effect of exposure to oestrous ewes on rams sexual behaviour, plasma testosterone concentration and ability to stimulate ovulation in seasonally anoestrous ewes. Applied Animal Behaviour Science, 67, 293–305.

Segura V.M.C., Quintal J.A.F., Sarmiento L.F. (1991): Estrus duration and timing of ovulation in Pelibuey ewes. In: Proc. 28th National Animal Research Meeting, Ciudad Victoria, Mexico, 94. (in Spanish)

Ungerfeld R. (2011): Combination of the ram effect with PGF2α estrous synchronization treatments in ewes during the breeding season. Animal Reproduction Science, 124, 65–68.

Ungerfeld R., Rubianes E. (1999): Estrus response to the ram effect in Corriedale ewes primed with medroxyprogesterone during the breeding season. Small Ruminant Research, 32, 89–91.

Ungerfeld R., Forsberg M., Rubianes E. (2004): Overview of the response of anoestrous ewes to the ram effect. Reproduction, Fertility and Development, 16, 479–490.

Valencia J., Porras A., Mejia O., Berruecos J.M., Trujillo J., Zarco L. (2006): Reproductive activity of Pelibuey sheep during the anestrous season: influence of the presence of the male. Revista Científica, 16, 136–141. (in Spanish)

Valencia J., Arroyo J., Trujillo J., Magana-Sevilla H., Zarco L. (2010): Exposure to the male does not exert a luteolytic effect in cyclic goats. Journal of Applied Animal Research, 38, 181–184.

Wildeus S. (2000): Current concepts in synchronization of estrus: sheep and goats. Journal of Animal Science, 77, 1–14.

Zarco Q.L., Stabenfeldt G.H., Quirke J.F., Kindahl H., Bradford G.E. (1988): Release of prostaglandin F-2 alpha and the timing of events associated with luteolysis in ewes with oestrous cycles of different lengths. Journal of Reproduction and Fertility, 83, 517–526.

Zarco Q.L., Rodriguez E.F., Angulo M.R.B., Valencia M.J. (1995): Female to female stimulation of ovarian activity in the ewe. Animal Reproduction Science, 39, 251–258.

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