Quest Journals
Journal of Research in Agriculture and Animal Science
Volume 10 ~ Issue 12 (2023) pp: 01-06
ISSN(Online) : 2321-9459
www.questjournals.org

Research Paper

Analysis of sexual behavior in dairy genotype sheep according to litter size

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ABSTRACT: *The objective was to evaluate some variables of sexual behavior in dairy genotype ewes according to litter size. We worked* with *East Friesian ewes, which were synchronized in their estrus during the autumn and mated in a paddock (1 h in the morning and 1 h in the afternoon) until the end of estrus. From field observations and real-time recording during mating, the following variables were determined: onset of estrus (ONSET), duration of estrus (DURA), duration of courtship received by ewe (COURT), proceptivity (PROC; degree of search for close contact with the male), receptivity (REC; degree of immobilization at courtship/mounting), effective mounts (EJAC; number of ejaculations received during the matting period) and shifts with ejaculation (SHIFT; number of shifts with at least one effective mount). During the lambing period, the litter size was recorded for each lambing ewe (1=single lamb and 2=twins). The variables were analyzed by PROC GLIMMIX according to litter size. None of the measured variables of sexual behavior was related to litter size, since no differences were recorded at the time of mating between ewes that gave birth to single lambs or twins (P>0.05). Only in the case of EJAC, a trend near to P=0.10 for more ejaculations could be noted in those ewes that finally lambed twins.*

KEYWORDS: *Ewes, Estrus, Sexual behavior, Litter size.*

R*eceived 02 Dec., 2023;* ***R****evised 10 Dec., 2023;* ***A****ccepted 12 Dec., 2023 © The author(s) 2023.*
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I. INTRODUCTION

In Argentina, the Pampas region, which encompasses the province of Buenos Aires and parts of La Pampa, Córdoba and Santa Fe provinces, is highly suitable for the development of agricultural activities (Arzubi et al., 2009). However, in recent years, due to the loss of profitability in traditional agricultural productions and/or land fragmentation, many farms have ceased to be economically viable (Arzubi et al., 2009). Sheep production emerges as an interesting option, particularly dairy activity. While milk is its main product, lamb production significantly contributes to income, followed by wool (Simonetti et al., 2014). This means that sheep dairying allows for the use of this species in its "multipurpose" form. For this purpose, the dairy genotype Ost-Friesisches Milchschaf (also known as East-Friesian) is often used, characterized by its high prolificacy, i.e., its tendency for multiple births.

Estrus, or heat, is defined as the period of sexual behavior in females. Its average duration in sheep is 24 to 36 hours (Fernández Abella et al., 1997), but with significant variations from 6 to 72 hours (Fernández Abella et al., 1997; Das et al., 2000; Hafez and Hafez, 2020; Simonetti et al., 2021; Ngcobo et al., 2022). During estrus, ewes, like other species, exhibit characteristic sexual behavior. It involves three components: proceptivity, receptivity and attractiveness (Beach, 1976). Proceptivity refers to the positive sexual behavior of the ewe in estrus, directing itself towards the ram, involving physical stimulation (Beach, 1976). Receptivity indicates the attitude towards the consummation of the sexual act (Beach, 1976), primarily involving the stillness reflex. Attractiveness refers to the female's ability to stimulate the male's interest and depends on the stimuli passively emitted by her (Beach, 1976), measurable from the number of Flehmen reflexes produced by the male (Chenoweth et al., 2022). Through courtship, the ram seeks to confirm the receptivity of ewes in estrus and, at the same time, prepares for mating (Silva Mena, 2008). Thus, estrus has been described not only in terms of its duration but also its intensity (Chenoweth et al., 2022).

Ovarian steroids are important endocrine and behavioral regulators during the estrus cycle (Kara et al., 2010; Pinto-Santini and Ungerfeld, 2019). The increase in estradiol concentration that occurs in the mid-follicular phase is responsible for behavioral changes during estrus (Ben Said et al., 2007). The concentration of estradiol is related to the number of growing follicles and its maximum value, preceding the LH peak, is higher in more prolific breeds (Cahill et al., 1981). Thus, working with ovariectomized ewes, Ben Said et al. (2007) found different estradiol requirements to induce estrus behavior and LH increase in two breeds varying in prolificacy.

The objective of this study was to analyze some variables of sexual behavior in dairy genotype sheep, under paddock mating, according to litter size.

II. MATERIALS AND METHODS

The present study was performed following the ethic requirements of the Institutional Comittee for Care and Use of Experimental Animals (CICUAE), belonging to the Faculty of Agricultural Sciences, National University of Lomas de Zamora.

2.1 Location

The study was carried out in a dairy sheep farm located in Uribelarrea (35º 06' S and 58º 53' W), Buenos Aires province, Argentina. The area is characterized by an annual rainfall of about 1100 mm and an average daily temperature ranging from 12ºC in July to 24ºC in January. The activity in the farm is carried out under a semi-intensive production system based on pasture with strategic supplementation.

2.2 Animals

Dairy genotype East Friesian ewes weighing approximately 60 kg, with previous sexual experience, were used in this study. The ewes had been weaned from their lambs 5 months prior to the assay and their milking, finished 3 months before.

Rams of the same breed were destined for mating. To confirm estrus, teasers (entire rams equipped with a protective apron to prevent mating) were also used.

Figure 1: Experimental flock.

2.3. Mating

During the autumn reproductive season, the ewes were synchronized with intravaginal sponges impregnated with progestagen (60 mg medroxyprogesterone acetate). These devices were inserted deep into the vagina and removed after 14 days.

After sponge removal, partial paddock mating was performed twice daily at 12-hour intervals (2 daily mating shifts, at 6 a.m. and 6 p.m., each lasting one hour) until the end of estrus. Rams were individually introduced into the paddock (surface of approximately 60 m2) for each mating shift. Each group of ewes was mated with only one ram, always the same one per group. Ewes that were effectively mounted were not removed from the paddock, thus always working with the same group of ewes.

During this period, animal behavior was recorded in real-time, from distance and without noise or interruptions. Immediately after each mating shift, ewes not courted were individually exposed to teasers to confirm if they were in estrus or not.

Figure 2: Paddock mating.

Figure 3: Lambing period.

2.4. Records

From field observations and detailed recordings, the following variables were measured:

Onset of estrus (ONSET; h): Time elapsed between the end of synchronization treatment and the onset of estrus.

Estrus duration (DURA; h): Time elapsed between the start (acceptance of the first mounting) and the end of estrus (last accepted mount), according to Romano et al. (2001)

Courtship (COURT; min): Duration of courtship received by ewe throughout estrus.

Receptivity (REC; n): Immobility during courtship/mating, classified into three categories: 0 "non-receptive" (fleeing from courtship/mating), 1 "moderately receptive" or 2 "highly receptive" (showing stillness or immobility during courtship/mating), adapted from Edey et al., (1978)

Proceptivity (PROC; n): Seeking close contact with the male, classified into three categories: 0 "non-proceptive" (not seeking the ram), 1 "moderately proceptive" or 2 "highly proceptive" (constantly seeking the ram), classification developed similarly to REC.

Ejaculations (EJAC; n): Number of effective mounts received throughout estrus; the effective mount was characterized by the insertion of the penis accompanied by pelvic contraction and backward movement of the head, with or without visualization of semen dripping after dismount; it was further confirmed by the loss of sexual interest immediately after dismount.

Shifts (SHIFT; n): Number of mating shifts in which each ewe received an effective mount.

During the lambing period, daily paddock observations were conducted, and litter size was recorded for each ewe that gave birth (1 = single lamb and 2 = twins)

2.5. Statistical Analysis

Descriptive statistics (mean, standard error (SE), minimum, maximum) were calculated. Each sexual behavior variable was analyzed using mixed generalized linear models (PROC GLIMMIX) according to litter size (1 vs. 2). All analyses were performed using the statistical package SAS (2023)

III. RESULTS AND DISCUSSION

The results regarding the analysis of sexual behavior according to litter size in dairy genotype sheep are presented in Table 1. The incidence of single births was 47%, while twin births were 53%.

As observed, none of the sexual behavior variables (ONSET, DURA, COURT, REC, PROC and SHIFT) were related to litter size, as no significant differences were recorded at the time of mating between ewes that finally gave birth to single lambs or twins ($p>0.05$). In the case of the number of ejaculations received during mating (EJAC), there was only a trend ($p=0.1063$) to be higher in ewes that gave birth to twins, with an average difference of 1.37 ejaculations. This result indicates that it would be necessary to increase the sample size to confirm whether this trend is maintained or not.

Table 1. Sexual behavior variables in East Friesian ewes according to litter size (1=single y 2=twins) (Mean ± standard error (minimum – maximum))

Variable	Litter size*		p-Value
	1 (n=16)	2 (n=18)	
ONSET (h)	37.50 ± 1.86 (24 – 48)	36.67 ± 2.05 (24 – 48)	0.7865
DURA (h)	43.50 ± 3.07 (24 – 72)	45.33 ± 3.30 (24 – 72)	0.8982
COURT (min)	13.22 ± 1.37 (6.02 – 20.55)	14.64 ± 1.05 (7.70 – 24.35)	0.2765
PROC (n)	1.56 ± 0.10 (1 – 2)	1.49 ± 0.10 (0.67 – 2)	0.5302
REC (n)	1.61 ± 0.12 (0.67 – 2)	1.64 ± 0.07 (1 – 2)	0.6182
EJAC (n)	5.07 ± 0.51 (2 – 9)	6.44 ± 0.66 (3 – 15)	0.1063
SHIFT (n)	2.50 ± 0.20 (1 – 4)	2.89 ± 0.31 (1 – 6)	0.3643

ONSET: estrus onset; DURA: estrus duration; COURT: duration of courtship received; PROC: proceptivity; REC: receptivity; EJAC: ejaculations or effective mounts received; SHIFT: mating shifts in which each ewe received an effective mount.

Regarding the literature, Ben Said et al. (2007) reported that, although the onset of estrus did not vary, more prolific Romanov ewes had longer estrus periods (mean of 42 h) compared to less prolific Ile de France ones (mean of 24 h). According to these authors, the longer estrus duration could be related to a longer time required for follicular recruitment and mating in sheep of high prolific breeds (Ben Said et al., 2007)

Kaldhi (1989), studying the Barbary breed, noted extended estrus periods especially when the ovulation rate was higher; they reported durations of 27, 30.6 and 42 h in ewes and 26, 29.5 and 36 h in hoggets, for ovulation rates of 1, 2 and 3, respectively. The difference between females with ovulation rates of 1 and 2 corpus luteums was only 3.5 h, while in those with 2 and 3 corpus luteums, their estrus differed by 6.5 to 11.4 h. In our case, ewes with triplets were not included due to their low frequency.

However, Dally and Hohenboken (1980) reported that the variation in the number of lambs born per ewe was not adequately described in either of the two age categories at mating (18 and 30 months of age) of 25% Finnsheep, 25% Rambouillet and 50% Blackface crosses. These authors found an adjustment with the best statistical model corresponding to an R^2 of only 0.16 and 0.33, respectively, concluding that it might not be sufficient to predict litter size based on estrus duration.

Land (1970) found that, according to the ovulation rate and estrus duration, Finnish Landrace x Blackface, Blackface and Merino x Blackface breeds were ranked in the same order, meaning that a longer estrus was associated with higher ovulation rate: 52.45, 42.90 and 28.16 h with 1.86, 1.33 and 1.14 corpus luteums, respectively. This same author found 10% more lambs for every extra 10 h of estrus in Finnish Landrace females, characterized by their high prolificacy (average of 2.65, with variations between 1 and 5 lambs). Hanrahan and Quirke (1975), working with ewes of different ovulation rates (Texel: ovulation rate of 1.75; Finnish Landrace, of 4.03; Galway, of 1.53 and Fingalway (Finnish Landrace x Galway), of 2.53), found that the ranking of such breeds according to this parameter differed from the ranking according to estrus duration. However, excluding the Texel breed, they could confirm Land's (1970) results. Furthermore, Hanrahan and Quirke (1975) found that the correlation of ovulation rate with estrus onset and duration depended on the breed. For instance, while its relationship with estrus duration was positive for almost all breeds (and significant at 5% only in Finnish Landrace), in Texel, the relationship was negative and significant at 10%, probably due to the small sample size. In contrast, the correlation between ovulation rate and estrus onset was positive and significant at 1% in Texel, but tended to be negative in Finnish Landrace. It was concluded that, in view of the breed differences, generalizations should not be made.

Maurya et al. (2008) noted not only earlier and longer estrus but also increased sexual behavior intensity, determined from various patterns (tail wagging, head turning, seeking the ram, stillness during courtship), in Garole breed sheep carrying a gene for high ovulation rate (3.7 ovulations) compared to monovulatory Malpura breed sheep.

The potential association between the number of mounts received during estrus and litter size, although not statistically significant in the present study, was suggested by Land (1970). This author emphasizes that the number of mounts could be associated with longer estruses, resulting in the ovulation of more follicles or the fertilization of a higher proportion of eggs. Our previous work (Simonetti et al., 2021) observed that as ewes had longer estrus, they received more ejaculatory mounts and were mated for more shifts during the paddock mating period, however these three variables did not affect litter size in the present assay.

Some other factors, not measured herein, could impact on ovulation rate/litter size through sexual behavior.

Gelez et al. (2003) observed that temperament also affects sexual behavior, i.e. ewes of 'calm' temperament were more proceptive and tended to be more receptive than those classified as 'nervous'. Furthermore, according to van Lier et al. (2007), differences in reproductive outcomes between 'calm' and 'nervous' ewes could be explained by the higher ovulation rate of the former. In our study, measures of sexual behavior were registered but not temperament, as well as litter size instead of ovulation rate.

The nutritional status of sheep influences ovulation rate, as reported in numerous studies and reviewed by Blache (2011), among others, conditioning litter size. However, a recent study (Alhamada et al., 2017) found that while attractiveness would be related to nutritional status – meaning that rams prefer to mount ewes in better body condition – proceptivity and receptivity would be independent. A more recent study (Veliz-Deras et al., 2023) in goats located in a semi-arid area of Mexico demonstrated that an improvement in body condition not only leads to higher ovulation rates and litter size but also improves sexual behavior.

The recent work by Woods and Adcock (2023) highlights greater attractiveness and receptivity in ewes without tail docking compared to those docked; however, this improvement in reproductive behavior had no impact on pregnancy or litter size. In our case, tails were not docked as East Friesian is characterized by their "rat tail" because it is thin and free of wool.

Litter size is an important economic indicator in multiparous species (Smith and Akinbamijo 2000; Mc Cormick et al., 2020). The three most important variables contributing to litter size are ovulation rate, embryo survival and fetal survival (Martin et al., 2004). A study (Karea et al., 2006) conducted on crossbred females (wool and meat breeds x East Friesian) mated with males of prolific breeds (Finn and Romanov) described that the average difference between the number of corpus luteums in the ovaries and the number of lambs born was 0.35, but variable depending on maternal age, birth type, and the percentage of prolific breeds in the genotype. Although litter size depends not only on ovulation rate, Hulet and Foote (1967) reported a high positive relationship between the number of corpus luteum and lambs born. In our case, however, it is possible that these events contributed, at least partially, to the results. Similar to what happened in the present study, our previous work (Simonetti et al., 2021) determined that fertility did not depend, among other variables, on the proceptive behavior of ewes under paddock mating.

IV. CONCLUSION

Litter size was not significantly related with any of the measured sexual behavior variables of dairy genotype ewes under paddock mating. Only a trend close to P=0.10 was found towards more effective mounts in ewes that finally lambed twins.

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