

Characterizing differences in the time to onset of puberty and reproductive performance between East Friesian and Corriedale ewe lambs

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ABSTRACT - The objective of the study was to characterize differences in the time to onset of puberty and reproductive variables between East Friesian and Corriedale ewe lambs in an extensive pastoral system. The study was carried out for two consecutive years, and 73 pure-breed ewe lambs were evaluated (35 East Friesian and 38 Corriedale). Plasma progesterone was determined weekly between 4.5±0.5 and 7.2±0.5 months old. The time to onset of puberty was assumed when the progesterone levels were ≥0.6 ng/mL for at least three consecutive blood collections. Body weight and body condition score were also determined. The reproductive variables calculated were fertility (ewe lambs' pregnancy/ewe lambs mated), prolificacy (lambs born/ewe lambs' pregnancy), and fecundity (lambs born/ewe lambs mated). We found that 97.1% of East Friesian lambs and 44.7% of Corriedale reached puberty during the blood-sampling period, and there was no significant age difference between breeds. Average body weight at puberty was significantly higher for East Friesian than for Corriedale ewe lambs (44.2±0.8 and 40.6±0.9 kg, respectively). Fertility significantly differed between breeds (85.7 vs 34.2% East Friesian and Corriedale, respectively) and was not affected by year. Of the pregnant ewe lambs, 88.4% carried a single lamb. Prolificacy did not differ with the breed, year, or dam parity but was significantly higher for those lambs born as twins. Fecundity was significantly higher for East Friesian than for Corriedale lambs. Breeding Corriedale and East Friesian ewe lambs in their first year results in different reproductive outcomes.

Keywords: fertility, progesterone, reproductive traits

1. Introduction

The reproduction rate is defined as lambs born/breeding ewes per year (Kremer et al., 2010; Washaya et al., 2023) and is the major indicator of the profitability of the flock. Several strategies can be implemented to improve flock productivity, such as increasing the reproduction rate and prolificacy (lamb-born/pregnant ewe) by genetic, nutritional, and hormonal tools. An additional option involves expanding the number of females available for breeding, with one alternative being the inclusion of ewe lambs in the breeding process (Kenyon and Corner-Thomas, 2022).

The productive and technical sectors are increasingly advocating for the identification of sexual precocity in sheep, as it serves as the primary catalyst for the initiation of selection and genetic enhancement initiatives. By identifying precocious animals, which present puberty earlier and anticipate the moment of the first lambing, the reproductive life of these females is increased in the flock (Ferra et al., 2010).

In extensive sheep breeding systems in Uruguay, half of the ewe hoggets are mated when they are 10-18 months old, while the remaining half are mated when they are 24-36 months old. It is not expected to mate ewe lambs in this context (Bianchi and Garibotto, 2007). Therefore, Corriedale, a dual-purpose breed, is the largest breed in the country (MGAP, 2016), while East Friesian is a dairy breed with an improved reproductive rate (Allison, 1995; Farid and Fahmy, 1996; Bell et al., 2021) and is currently being used as dams in sheep-meat-intensive systems.

Ewe lamb puberty refers to the physiological stage of female lambs' development at which they attain sexual maturity and become capable of reproductive activities, including estrous cycling and conception (Dýrmundsson, 1973; Alexander et al., 1978; Dýrmundsson, 1981; Martin, 1981). Several factors determine the time to onset of puberty: breed, live weight, age, nutritional status, and breeding season (photoperiod-dependent) (Dýrmundsson, 1983; Martin et al., 2004). Ewe lambs typically attain puberty at 40-70% of their mature live weight (Dýrmundsson, 1973; Jainudeen et al., 2000). For instance, Dickerson and Laster (1975) found that only 41.4% of Corriedale ewe lambs achieved puberty at a live weight of 43.5 ± 3.1 kg. Prolific breeds such as Finnsheep and Romanov have been observed to reach puberty at a comparatively lower live weight of 30-35 kg (Farid and Fahmy, 1996). Regarding age at puberty, studies conducted on pure East Friesian sheep in Great Britain revealed that 89% of ewe lambs evaluated reached puberty within 7-8.2 months with 26-48 kg (approximately 34.7-64% of adult body weight [BW]) (Ward and Williams, 1993). Furthermore, the onset of puberty of these lambs occurred later in the breeding season compared with older ewes. However, under pastoral conditions in Uruguay, only 21-25% of Corriedale ewe lambs reached puberty at eight months (Bianchi and Garibotto, 2007), while 90% of East Friesian ewes reached puberty at 8.2 months old (Banchero et al., 2014).

Advances in understanding the onset of reproduction activity of East Friesian and Corriedale ewe lambs, especially in extensive pastoral systems, may provide further knowledge to facilitate reproductive management and improve reproductive rate. Thus, the objective of the study was to characterize differences in the time to onset of puberty and reproductive variables (fertility, prolificacy, and fecundity) between East Friesian and Corriedale ewe lambs in an extensive pastoral system.

2. Material and Methods

2.1. Experimental location and animal management

The experiment was carried out for two consecutive years (summer to spring - January to September in the Southern Hemisphere) at Migue's Experimental Station (34.22° S, 55.36° W), Canelones, Uruguay. Research on animals was conducted according to the institutional committee on animal use (CEUA FVET- 451). In addition, the climatic conditions were registered; on average, daily ambient temperature ranged from 12°C in July (winter) to 24°C in January (summer). Accumulated rainfall from August (lamb birth) to December (lamb weaning) was 492 mm in the first year and 748.6 mm in the second. From weaning to breeding (April), rainfall was 305 and 311.9 mm first and second year, respectively.

Seventy-three ewe lambs of two pure breeds, East Friesian and Corriedale, from an experimental flock were used; 19 East Friesian and 22 Corriedale in the first year, and 16 East Friesian and 17 Corriedale in the second one. These ewe lambs were born in August-September, and data of date of birth, birth weight, birth rank, and dam parity (primiparous or multiparous) were registered. Weaning occurred on January 14th, when the ewe lambs were 135.3 ± 13.1 days old. From weaning to breeding, the ewe lambs of both breeds grazed together in natural pastures, with a forage allowance of at least 6 kg of DM/100 kg of BW. If the forage allowance decreased, they were supplemented with concentrates

(17% crude protein). A reproductive evaluation of the rams was conducted in February, followed by a seminal evaluation 15 days before their use. At the time of breeding (April 12th), the ewe lambs were 223.3 ± 13.1 days old, with a body condition score (BCS) of 2.8 and 2.9 for Corriedale and East Friesian ewe lambs, respectively, the first year and 3.0 and 2.9 the second year, without significant difference between breeds ($P > 0.05$). They were stocked separately by breed, and one ram was introduced into each group for 40 days. The animals were strategically drenched in pre-breeding, pre-lambing, and weaning with Derquantel 10 mg/mL - Abamectina 1 mg/mL (Startect®, Laboratorio Zoetis, Uruguay). The diseases were not assessed and, therefore, represent a limitation of the study.

2.2. Plasma progesterone determination

The blood-sampling period was from January 16th to April 10th, when the ewe lambs were 135.3 ± 13.1 and 221.3 ± 13.1 days old, respectively. The samples were collected weekly (total of 12 samples/ewe lamb) via jugular venipuncture with Vacutainer tubes while plasma was harvested by centrifugation at 2500 rpm for 10 min and stored at -20°C . Blood progesterone concentrations were determined using a commercial kit (MP Biomedicals LLC, Eschwege, Germany). The sensitivity of the assay was 0.02 ng/mL, and the intra-assay CV for the low (1 ng/mL) and high (5 ng/mL) controls were 9.06 and 9.15%, respectively. The inter-assay CV for the low (1 ng/mL) and high (5 ng/mL) controls were 14.55 and 12.74%, respectively. A value of ≥ 0.6 ng of progesterone per mL of plasma was used to indicate luteal activity. Consequently, the first values equal to or greater than 0.6 ng/mL for at least three consecutive blood collections were considered the beginning of puberty (Meikle and Forberg, 2001).

2.3. Determinations

From day 135.3 ± 13.1 to day 221.3 ± 13.1 of ewe lamb's life, BW was registered weekly, and the BCS was recorded fortnightly (scale 1-5 score, Russel et al., 1969). The BW measurement closest to the onset date of puberty was taken as BW at puberty. The daily gains per period were calculated as: final weight – initial weight/days.

To describe the proportion of ewe lambs that onset puberty, cumulative frequencies curves of age to onset of puberty by breed were constructed. At lambing of the ewe lambs, fertility (ewes lambs pregnancy/ewes lambs mated $\times 100$), prolificacy (lambs born/ewes lambs' pregnancy $\times 100$), and fecundity (lambs born/ewes lambs mated $\times 100$) were calculated.

2.4. Statistical analysis

Age of ewe lambs (days), BW, and BCS of ewes that reached puberty were analysed using a general linear model of the statistical package STATA (Stata Corp, 2014). The following statistical model was adjusted:

$$y_{ijklmn} = \mu + \text{breed}_i + \text{year}_j + \text{birth rank}_k + \text{dam parity}_l + \text{puberty}_m + e_{ijklmn},$$

in which y is the response variable, μ is the overall mean, breed is the effect of breed class (Corriedale, East Friesian), year is the effect of year class (1 = 2017, 2 = 2018), birth rank is the effect of birth rank (single, twin), dam parity is the effect of dam parity (primiparous, multiparous ewes), puberty is the effect of puberty (Yes, No), and e is the error. Body weight at the beginning and end of the sampling period and average daily gain (ADG) during the period mentioned before were also analysed using a general linear model, including the effects of breed, year, birth rank, and dam parity. Since the interactions of the effects were not significant for all traits ($P > 0.05$), the interactions were ignored. None of the tested interactions (breed by year, breed by birth rank, breed by dam parity, year by birth rank, year by dam parity, birth rank by dam parity, puberty by breed, puberty by year, puberty by birth rank, puberty by dam parity) were statistically significant and, therefore, were not included in subsequent models.

Accumulated frequency of age to onset of puberty, fertility, and fecundity were analysed by association test Chi-square. The prolificacy was analysed by the Brown test (Brown, 1988). Statistical significance was set at $P < 0.05$. No sample size calculations were performed which represents a limitation of the study as it may be underpowered for analysis conducted.

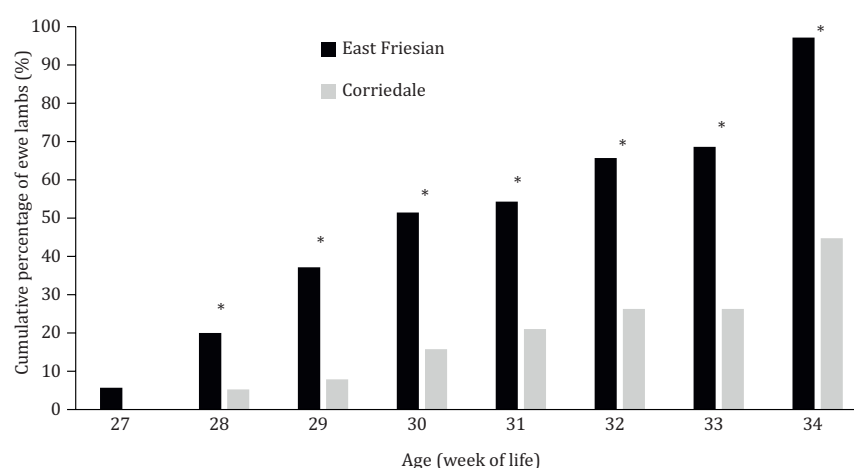
3. Results

3.1. Time to onset of puberty in ewe lambs

Of the 73 ewe lambs evaluated in the experiment, 69.8% reached puberty at the end of the blood-sampling period (in the first week of April). In both years, the accumulated frequency of the onset of puberty was higher for East Friesian than for Corriedale lambs (97.1 vs 44.7, respectively, $P < 0.01$) (Figure 1).

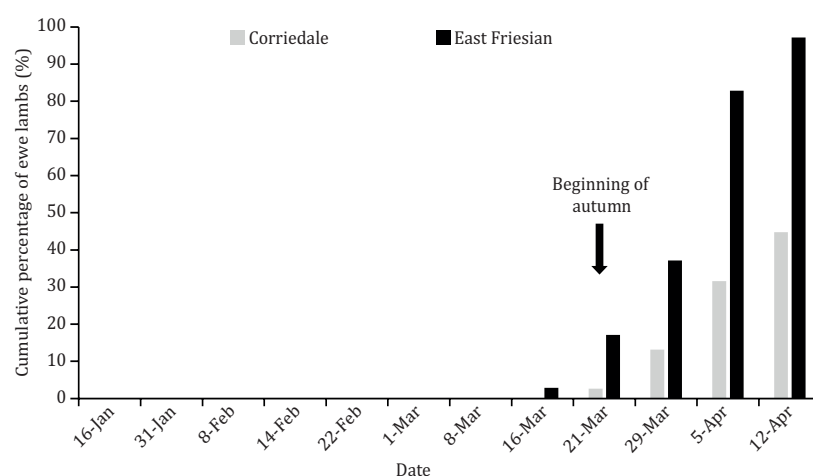
In year 1, 94.7% of East Friesian and 42.8% of Corriedale ewe lambs had reached puberty at the end of the blood-sampling period ($P < 0.01$), while in year 2, it was 100 and 47%, respectively; ($P < 0.01$) (Figure 1). There was no significant difference in age at puberty between both breeds: 214.9 ± 5 and 218.0 ± 4 days for East Friesian and Corriedale, respectively ($P > 0.05$).

In both breeds, the first lambs to enter puberty did so in the second half of March, almost at the beginning of autumn. After that, the percentage of East Friesian ewe lambs entering puberty was double that of Corriedale ewe lambs in each sample (Figure 2).



* $P < 0.05$.

Figure 1 - Cumulative percentages of East Friesian and Corriedale ewe lambs that reached puberty by age (expressed in weeks of life) in two years in an extensive production system.



Summer began on 22nd December and autumn on 21st March.

Figure 2 - Cumulative percentages of East Friesian and Corriedale ewe lambs that reached puberty during the blood-sampling period in both years.

3.2. Ewe lambs' body weight, body condition score, and average daily gain

Considering breed effect, no statistically significant differences were found between Corriedale and East Friesian ewe lambs' birth weight (4.5 ± 0.2 and 4.4 ± 0.2 kg, respectively). However, ADG from birth to the beginning of the blood-sampling period was higher for East Friesian than for Corriedale (224.7 ± 42.3 and 183.1 ± 27.0 g/d, respectively, $P < 0.05$). Consequently, their BW at the beginning of the blood-sampling period were also greater ($P < 0.01$) (Table 1). However, there were no significant differences concerning BCS.

During the blood-sampling period, ADG showed no differences between breeds, but BW at the end of it (beginning of breeding) was higher in East Friesian than in Corriedale ewe lambs ($P < 0.01$).

Concerning the year effect, ewe lamb BW was not affected. However, ADG from birth to the beginning of the blood-sampling period was higher in year 1 ($P < 0.01$) than in year 2, and as a result, BW at the beginning of the blood-sampling period was also greater ($P < 0.01$) in that year and even reached at a younger age. Furthermore, ADG during the blood-sampling period was higher in year 2 than in year 1 ($P < 0.05$); despite this, BW at the end of it was significantly higher ($P < 0.05$) in the first year.

No significant effects of birth rank on BW at the beginning or end of the study or on ADG were found. Concerning dam parity, BW at the beginning and end of the sampling period was significantly higher for ewe lambs born from multiparous dams ($P < 0.05$ and $P < 0.01$, respectively).

Table 1 - Means $\pm$ SEM for body weight at the beginning and end of sampling period and average daily gain during the experiment of the total ewe lambs; effects of breed, year, birth rank, and dam parity

	n	BW1 (kg)	BW2 (kg)	ADG (g/d)
Breed				
Corriedale	38	$28.5 \pm 4.3a$	$37.1 \pm 4.1a$	108.7 ± 31.5
East Friesian	35	$34.8 \pm 4.9b$	$43.7 \pm 5.3b$	114.1 ± 28.3
Year				
1	40	$32.7 \pm 5.6a$	$40.9 \pm 6.0c$	$103.4 \pm 31.6c$
2	33	$30.1 \pm 5.2b$	$39.6 \pm 5.5d$	$120.0 \pm 25.7d$
Birth rank				
Single	50	30.6 ± 5.3	39.2 ± 5.5	109.7 ± 30.9
Twin	11	34.4 ± 5.3	43.3 ± 5.7	113.8 ± 26.6
Dam parity				
Primiparous	26	$28.6 \pm 4.8a$	$36.6 \pm 4.7a$	102.9 ± 32.2
Multiparous	36	$33.3 \pm 5.0b$	$42.3 \pm 5.1b$	114.7 ± 28.1

BW1 - body weight at the beginning of sampling period (January 16th); BW2 - body weight at the end of sampling period (April 10th); ADG - average daily gain in the sampling period.

a,b - Means within a column and within a treatment group with different letters are statistically different ($P < 0.01$).

c,d - Means within a column and within a treatment group with different letters are statistically different ($P < 0.05$).

No differences were found between Corriedale and East Friesian for age at puberty (Table 2). However, BW was significantly higher ($P < 0.05$) for East Friesian than for Corriedale, whereas BCS was not affected by breed. The average BW of those ewe lambs that did not reach puberty were 35.2 ± 0.7 kg for Corriedale and 37.5 ± 3.5 kg for East Friesian and were significantly different ($P < 0.05$) from those ewe lambs that reached puberty in each breed.

Considering the year effect, ewe lambs reached puberty at an older age in the second year than in the first one ($P < 0.001$). No significant differences were found between years for BW or BCS at puberty.

Dam parity had a significant effect only on BW at puberty ($P < 0.05$); those ewe lambs born from multiparous dams showed a higher BW than those born from primiparous dams ($P < 0.05$). No significant interactions between effects were registered.

Table 2 - Means $\pm$ SEM for age, body weight (BW), and body condition score (BCS) of ewe lambs that reached puberty; effects of breed, year, birth rank, and dam parity

	n	BW1 (kg)	BW2 (kg)	ADG (g/d)
Breed				
Corriedale	17	215.0 $\pm$ 4.1	40.6 $\pm$ 0.9c	2.9 $\pm$ 0.1
East Friesian	34	212.8 $\pm$ 3.0	44.2 $\pm$ 0.8d	2.9 $\pm$ 0.0
Year				
1	27	203.4 $\pm$ 2.0a	44.3 $\pm$ 0.9	2.8 $\pm$ 0.1
2	24	223.5 $\pm$ 3.2b	42.0 $\pm$ 1.0	2.9 $\pm$ 0.0
Birth rank				
Single	31	214.8 $\pm$ 2.8	42.2 $\pm$ 0.8	2.8 $\pm$ 0.0c
Twin	12	215.1 $\pm$ 6.0	44.4 $\pm$ 1.4	3.0 $\pm$ 0.1d
Dam parity				
Primiparous	11	205.0 $\pm$ 2.5	40.6 $\pm$ 1.1c	2.7 $\pm$ 0.1
Multiparous	29	218.2 $\pm$ 3.1	43.5 $\pm$ 0.8d	2.9 $\pm$ 0.0

a,b - Means within a column and within a treatment group with different letters are statistically different (P<0.01).

c,d - Means within a column and within a treatment group with different letters are statistically different (P<0.05).

3.3. Fertility, prolificacy, and fecundity rate

Forty-three ewe lambs were pregnant in both years. The fertility was different between breeds (85.7 vs. 34.2%, East Friesian and Corriedale, respectively; P<0.05) and it was not affected by year (Table 3). Fertility was higher for ewe lambs born from multiparous dams than for those born from primiparous (69.4 and 46.1%, respectively; P<0.05). From total pregnant ewe lambs, 88.4% were carrying a single lamb and 11.6% were carrying twins. Prolificacy of ewe lambs that onset puberty did not differ among breeds, years, or dam parity, but it was affected by the birth rank (Table 3). Fecundity was significantly higher for East Friesian than for Corriedale ewe lambs (P<0.01) and it was not affected by year, birth rank, or dam parity. No interactions between the effects were found.

Table 3 - Fertility, prolificacy, and fecundity in ewe lambs that onset puberty; effects of breed and year

	Fertility (%)	Prolificacy (n)	Fecundity (%)
Breed			
Corriedale	34.2a	1.00	34.2a
East Friesian	85.7b	1.16	101.7b
Year			
1	65.0	1.11	72.1
2	51.5	1.11	57.2

a,b - Means within a treatment group with different letters are statistically different (P<0.01).

4. Discussion

Puberty was reached by 69.8% of ewe lambs, mostly (66.7%) East Friesian ewes. Similar results were obtained by Banchemo et al. (2014) in East Friesian breed, but they reported that none of their Corriedale ewe lambs reached puberty in their first year of life. In agreement with these authors, progesterone levels higher than 0.6 ng/dL were first detected when the ewe lambs were 27 weeks old, five weeks earlier than the age reported by Crescionini et al. (2021). These authors reported that 33% of Corriedale ewe lambs were cycling at 32 weeks old. These results agreed with those obtained by Dýrmundsson (1973) and Smith and Knight (1998), who reported that the timing of puberty in ewe lambs occurred later in the breeding season than the onset of seasonal breeding activity in mature ewes.

The timing of puberty and the success of ewe lamb breeding are influenced by several factors, including age, live weight, breed, nutrition, and breeding season (Kenyon et al., 2014). In our study, in which ewe lambs were of the same age and raised in identical conditions, the difference in weight observed at the beginning of the blood-sampling period could be attributed to a combination of high milk production and high daily gain of the East Friesian genotype (Kremer et al., 2010). This suggests that the year effect observed on ADG from birth to the time of onset of puberty and BW at that moment could be a result of annual variations in the availability of natural pastures or the possibility that the ration was consumed in greater quantity by East Friesian than by Corriedale lambs.

A high growth rate is associated with an increase in the proportion of ewe lambs that reach puberty in their first year of life (Moore and Smeaton, 1980). Despite this, during the trial period, ADG was low (111 g/d, average) and without significant differences between breeds but significant between years. Regarding BW at puberty, Corriedale ewe lambs attained puberty at 40.6 kg while East Friesian ewe lambs at 44.2 kg. These values were higher than those obtained by Bianchi and Garibotto (2007), who reported live weights of 30 and 33 kg for Corriedale and East Friesian Corriedale crossbreeds, respectively. Moreover, Ward and Williams (1993) reported that East Friesian's average BW at puberty was 48.9% of adult BW, ranging from 26 to 48 kg.

According to Fogarty et al. (2007), there is an evident variation between breeds for BW at puberty, the timing of puberty, and the proportions displaying oestrus at differing time points within the season. Different studies show poor performance when BW at breeding is below 35 kg; conversely, higher performance occurs above 40-45 kg (Dýrmundsson, 1973; Kenyon et al., 2010). Therefore, following Kenyon et al. (2014), there is some merit in using 40 kg as a minimum when deciding which ewe lambs will be presented for breeding. Although BW is a crucial factor in determining the likelihood of an ewe lamb attaining puberty in its first year, this is not the only contributing factor, and it is not responsible for all significant variability observed in the percentage of ewes attaining puberty from year to year (Edwards et al., 2015).

The age of ewe lambs is also important and, in the present study, was estimated at 215 and 212 days for Corriedale and East Friesian, respectively. Similar results were obtained with East Friesian by Ward and Williams (1993) (seven months). Moreover, Bianchi and Garibotto (2007) reported that 25% of Corriedale lambs reached puberty at eight months (240 days average), while 85% of East Friesian × Corriedale crossbred lambs reached puberty at 7.5 months (225 days average). These values were higher than those in this study but maintained the same difference between breeds. According to Kenyon et al. (2014), ewe lambs at 7-9 months (210-270 days) of age have the potential to be successfully bred.

Fertility rates have been reported to be higher for ewes than for ewe lambs (Annett and Carson, 2006). Fertility rates of 47 and 82% for ewe lambs compared with 85-97% in mature multiparous ewes have been reported (Donald et al., 1968; Forrest and Bichard, 1974; Annett and Carson, 2006; Mulvaney et al., 2013). In the present study, fertility differed between breeds, showing higher values for East Friesian than for Corriedale lambs. However, lower fertility values (58.82%) were reported for Friesian ewes by Simonetti et al. (2017). According to Allison et al. (1975), a lower fertility percentage may be due to ewe lambs needing to be served at least on three occasions by the ram to achieve 100% of ewe lambs having semen inside their reproductive tract.

Piaggio et al. (2015) stated that heavier Corriedale ewe lambs at the beginning of the breeding season (on average 38 kg) were the most successful in getting pregnant (35% of pregnancy for lambs over 35 kg and 13% for lambs under 35 kg, $P = 0.0044$). In the present study, Corriedale ewe lambs that did not achieve pregnancy showed an average BW at the end of the sampling period of 35.2 kg against 40.6 kg for those that lambed.

On the other hand, even though BCS has been identified to affect the proportion of ewe lambs successfully bred (Kenyon et al., 2010), in the present essay, there were no differences in BCS between pregnant and non-pregnant ewe lambs. However, Piaggio et al. (2015) reported that BCS did not affect the number of ewe lambs that got pregnant even though these had low body condition (2.15-2.3 units).

Not all pregnant and lambing ewe lambs presented detectable progesterone levels during the blood-sampling period. However, as ewe lambs usually onset puberty later in the breeding season (Foster et al., 1985), luteal activity likely occurred after the last sampling.

East Friesian is considered a twin-bearing breed, with birth rates in commercial conditions and experimental stations ranging from 106 to 195% (Farid and Fahmy, 1996). Despite this, the percentages reached by the ewe lambs of this trial were not high (11.6%). Similarly, for this breed and category, Simonetti et al. (2017) reported a percentage of multiple births of 10%. According to these authors, multiple pregnancies would increase as females' age rises in the flock.

Concerning fertility, introducing young animals in the flock increases the number of females in service without having more lambs, which agrees with Kenyon and Corner-Thomas (2022). In addition, the fact that a low percentage of Corriedale ewe lambs would come into oestrus until the beginning of April would make it necessary to carry out long breedings. One limitation of this study is that the data was collected from a limited number of animals within a single herd in Uruguay. Consequently, further studies are warranted to assess variations associated with different locations and their intrinsic factors.

5. Conclusions

Corriedale and East Friesian ewe lambs could be mated in their first year but with different reproductive results. East Friesian reach puberty earlier and are more fertile than Corriedale ewe lambs.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: Neimauro, K.; Perdígón, F. and Kremer, R. **Data curation:** Neimauro, K.; Perdígón, F.; Negrín, F.; Capucho, M. C. and Minteguiaga, M. **Formal analysis:** Neimauro, K.; Negrín, F.; Cal-Pereyra, L.; Capucho, M. C.; Minteguiaga, M. and Kremer, R. **Funding acquisition:** Neimauro, K. **Investigation:** Neimauro, K.; Perdígón, F. and Kremer, R. **Methodology:** Neimauro, K. and Kremer, R. **Project administration:** Neimauro, K. **Resources:** Neimauro, K.; Perdígón, F.; Cal-Pereyra, L. and Minteguiaga, M. **Supervision:** Neimauro, K. **Validation:** Neimauro, K. **Visualization:** Neimauro, K.; Perdígón, F.; Cal-Pereyra, L. and Kremer, R. **Writing – original draft:** Neimauro, K.; Negrín, F.; Cal-Pereyra, L.; Capucho, M. C. and Minteguiaga, M. **Writing – review & editing:** Neimauro, K.; Perdígón, F.; Negrín, F.; Cal-Pereyra, L.; Capucho, M. C.; Minteguiaga, M. and Kremer, R.

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