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Follicular dynamics in dairy sheep within and out of the breeding season employing high or low progesterone concentration in estrous cycle synchronization

Dinâmica folicular em ovelhas leiteiras dentro e fora da estação reprodutiva, empregando alta ou baixa concentração de progesterona na sincronização do clico estral

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Abstract: Follicular dynamics in dairy sheep is poorly understood. This study assessed the follicular dynamics of dairy sheep within and out of the breeding season by observing the effect of two progesterone concentrations (60 and 120 mg) used in vaginal pessaries in pre-synchronization. The experiment was conducted in April/May and October/November using 40 healthy multiparous dairy ewes predominantly of the Lacaune breed, which underwent daily transrectal ultrasound examinations. The number of ovulations (1.35 vs 1.05), ovulatory follicle diameter (mm) (5.97 vs 5.05), length of the luteal phase (11 vs 9.14 days), and length of the estrous cycle (16.83 vs 16.6 days) were assessed within and out of the breeding season, respectively. The sheep out of the breeding season did not present a corpus luteum at the time of insertion of the vaginal pessary, characterizing anestrus. The ovulatory follicle had a larger diameter in the breeding season even with the shorter length of the follicular phase, suggesting higher viability and quality of the ovulated oocyte. The application of exogenous progesterone extends the luteal phase in the breeding season (11 vs 9.14 days). The anestrus observed out of season suggests the need for a higher progesterone concentration in reproductive protocols. Therefore, breeding protocols can be adapted for different seasons, suggesting the use of vaginal pessaries with a higher progesterone concentration (120 mg) out of the breeding season. The data allowed the characterization of follicular dynamics in dairy sheep, demonstrating their differences in each season based on the use of different progesterone doses in pre-synchronization.

Keywords: follicular development; dairy sheep; progesterone implant; ultrasound; pre-synchronization

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Resumo: A dinâmica folicular em ovelhas leiteiras é pouco compreendida. Neste trabalho foi avaliado a dinâmica folicular de ovelhas leiteiras, dentro e fora da estação reprodutiva observando o efeito de duas concentrações de progesterona (60 e 120mg) utilizadas em pessários vaginais na pré-sincronização. O experimento foi realizado nos meses de abril/maio e outubro/novembro, utilizando 40 ovelhas multíparas leiteiras hígidas, predominantemente da raça Lacaune, que foram submetidas a exames ultrassonográficos diários por via transretal. Na estação reprodutiva foram avaliados o número de ovulações (1,35 vs 1,05), o diâmetro(mm) do folículo ovulatório (5,97 vs 5,05), a duração da fase lútea (11 vs 9,14 dias) e a duração do ciclo estral (16,83 vs 16,6 dias) dentro da estação em comparação com fora da estação reprodutiva respectivamente. Fora da estação as ovelhas não apresentaram corpo lúteo no momento da inserção do pessário vaginal, caracterizando anestro. Mesmo com menor duração da fase folicular, o folículo ovulatório apresentou maior diâmetro na estação reprodutiva, sugerindo maior viabilidade e qualidade do oócito ovulado. A aplicação de progesterona exógena amplia a fase lútea na estação reprodutiva (11 vs 9,14 dias). O anestro observado fora de época sugere a necessidade de maior concentração de progesterona nos protocolos reprodutivos. Desta forma, é possível adaptar protocolos reprodutivos para diferentes épocas, sugerindo o uso de pessários vaginais com maior concentração de progesterona (120 mg) fora da estação reprodutiva. Os dados obtidos permitiram caracterizar a dinâmica folicular em ovelhas leiteiras, demonstrando suas diferenças em cada estação a partir do uso de diferentes doses de progesterona na pré-sincronização.

Palavras-chave: desenvolvimento folicular; ovelha leiteira; implante de progesterona; ultrassonografia; pré-sincronização

1. Introduction

Follicular dynamics in sheep has been studied, although initial research in this area encountered controversy regarding the described patterns of follicular development in the species. Ovarian ultrasound in sheep has demonstrated that a follicular wave emerges approximately every five days[8] and the corpus luteum is already sensitive to PGF2α at three days after ovulation[19], whereas follicular dominance presents varied growth patterns. Follicles larger than 5 mm have growth patterns in waves, that is, they appear less dispersed and more concentrated during the estrous cycle[10].

Follicular dominance in sheep is different, presenting varied growth patterns. Follicles smaller than 4 mm grow randomly and may appear in varying numbers and various ways during waves of follicular growth in an estrous cycle. Follicles larger than 5 mm have modulated growth patterns[5], that is, they appear less dispersed during an estrous cycle and more concentrated.

Pre-ovulatory follicles of each wave secrete high amounts of inhibin A and estradiol, causing a reduction in FSH concentration and atresia of smaller follicles[3]. The mechanism of follicular dominance in sheep and goats is different from that observed in cows[16], in which the dominant follicle is known to inhibit the growth of other follicles in the ovary. The dominant follicle in sheep prevents its own regression by switching its dependence from FSH to LH[7]. The pre-ovulatory follicle inhibits the growth of other follicles present in the ovaries at the time of emergence. However, the emergence of new follicles is reduced but not inhibited, although their subsequent growth is suppressed[9]. Different patterns are found in sheep ovaries in the luteal phase of follicular growth. Follicles become critically dependent on FSH, without dominance being established when LH concentrations remain low. This decrease in LH explains the suppressive effects of corpus luteum progesterone in the dominant follicles in sheep[1]. There is a lack of dominance during the luteal phase of the ovine estrous cycle until luteolysis when LH secretion increases due to an increase in estradiol secretion by pre-ovulatory follicles[3], determining that dominance becomes evident[14]. Low levels of serum progesterone increase the frequency of LH pulses, which in turn increases the size of the larger follicle and carries out positive feedback of estradiol with the GnRH and LH axis. Sub-luteal concentrations of progesterone increase LH pulses but not enough for the peak to occur, determining the persistence of the follicle and prolonging dominance[14].

The luteal phase interferes with follicular growth. There is a lack of dominance during the luteal phase until luteolysis when LH secretion increases due to an increase in estradiol secretion by pre-ovulatory follicles[3], determining that dominance becomes evident[14]. The persistence of large follicles is shortened when a progesterone implant is applied in the presence of a corpus luteum and is increased in the absence of a corpus luteum[20]. The persistence of these dominant follicles causes them to age, reducing fertility. The alternative to prevent ovulation in these old follicles is to use high progesterone doses, which would cause their atresia, allowing only follicles from the last wave to ovulate[5].

Therefore, this study aimed to characterize the follicular dynamics of dairy sheep within and out of the breeding season after the use of vaginal pessaries containing high (120 mg) or low (60 mg) progesterone concentrations in pre-synchronization of the estrous cycle.

2. Material and methods

2.1 Location

The experiment was conducted within the breeding season in April/May and out of the season in October/November to determine follicular dynamics at each time point. The experiment was conducted in Lages - SC, Brazil, at a latitude of 27°48′58″ S and a longitude of 50°19′34″ W, next to the Agroveterinary Sciences Center – CAV/UDESC.

2.2 Selection of animals

Forty multiparous, healthy, adult, female dairy sheep at reproductive age predominantly of the Lacaune breed and excellent body score, classified as 3 on a scale from 1 to 5[21], were used. The animals received concentrate based on corn, soybean, and a mineral mixture at 0.5% of live weight plus mineral salt for sheep *ad libitum*, in addition to grazing on *Pennisetum clandestinum* Hochst (kikuyugrass) and *Cynodon* spp. (Tifton-85) and water *ad libitum*.

2.3 Ultrasound evaluation

The females were handled in a quadrupedal or elevated position with the abdominal wall compressed [6]. The ultrasound equipment (Mindray/DP50, Shenzhen) was used in real-time B-mode, with a rectal linear transducer with frequencies varying between 6 and 8.5 MHz coupled to a slightly curved plastic tube (measuring 30 cm in length), allowing external manipulation. Initially, feces were removed from the rectum, followed by the deposition of 5 to 10 mL of gel in the rectal canal and on the transducer.

Figure 1. Transrectal ultrasound in a sheep in the season.

The ewes underwent daily ultrasound monitoring during the period of one estrous cycle, approximately 17 days. Ultrasounds were performed from the beginning of the estrous cycle. Ultrasound evaluation began 24 h after removal of the implants, continuing until the next ovulation, defined by the absence of the dominant follicle of the last follicular wave observed during the ultrasound examination.

The beginning of the estrous cycle was determined by pre-synchronizing the animals with vaginal pessaries based on medroxyprogesterone[9] (Merck, Darmstadt, TUDA, Germany) at two concentrations (60 or 120 mg), which was maintained for six days. Prostaglandin (75 µg of sodium cloprostenol, Sincrocio, Ouro Fino) and equine chorionic gonadotropin eCG (400 IU, Novormon, Zoetis) were applied at the time of pessary removal and, subsequently, ultrasounds were performed daily. Assessments were conducted every 8 h with the increase in follicular diameter and the proximity of ovulation, allowing the detection of the moment of ovulation. The occurrence of ovulation was defined by the absence of the dominant follicle detected in previous ultrasound examinations, thus characterizing the beginning of the estrous cycle. Each ultrasound assessment of each ovary was recorded on individual files and the images were recorded, allowing subsequent checking of the dimensions and location of each structure.

Some analysis parameters were adopted to establish follicular dynamics. Follicular wave was identified as a group of follicles that grow from 2 or 3 mm, with one or more reaching their maximum diameter. The day of emergence was considered when the largest follicle, still measuring 2 or 3 mm, was identified for the first time. Maximum follicular diameter was obtained from the follicle that exceeded the size of the others during the same wave. The largest-diameter follicle of the wave was the one that first reached this measurement when more than one follicle reached the same maximum diameter. The day of obtaining the maximum diameter was the one on which the follicle reached its largest diameter. Follicular growth phase was defined as the time required for the largest-diameter follicle to grow from its smallest observed diameter. Regression phase was defined as the time required for the largest-diameter follicle to reduce its size to its smallest diameter. Static phase was considered to be the time elapsed from the follicle reaching its largest diameter, at the end of the growth phase, until the beginning of the regression phase. Growth rate was considered as the difference (mm/day) between the maximum and minimum diameters of the largest follicle, divided by the length of its growth. Regression rate (mm/day) was calculated as the difference between the maximum and minimum diameters of the largest follicle, divided by the length of its regression period (days). The following variables were computed for the ovulatory wave, in addition to those already described: ovulatory diameter, which is the measurement obtained in the last measurement of the follicle before ovulation; number of ovulations, assessed as single, double, or triple, per cycle; analysis of the follicular population, in which the follicles were grouped by size, as follows: small (2-3.4 mm), medium (3.5-4.9 mm), and large (≥5 mm); length of the follicular phase (days), calculated from the moment CL disappeared until the appearance of the next CL; and length of the luteal phase, calculated from the CL appearance until the day of its disappearance.

2.4 Statistical analysis

The data were subjected to analysis of variance, using the MIXED procedure of the SAS® statistical package. The data were previously tested for normality of residuals using the Kolmogorov-Swirnov test. Means were compared using the Tukey test at a 5% significance level. The statistical model included the effect of season, progesterone concentration, and the interaction between them as explanatory variables.

3. Results

Table 1 shows a higher number of ovulations within season (1.35) compared to ovulations out of season (1.05) when considering only the period of treatments. A larger average diameter of follicles was also observed within season (5.97 mm) than out of season (5.05 mm). A variation was also observed in the length of the luteal phase, which was longer within season (11 days) than the 9.14 days out of season. As a consequence, the length of the follicular phase was shorter within season (6.25 days) than the 7.37 days out of season.

Finally, the length of the estrous cycle was longer within season (16.83 days) than the period out of season (16.60 days). Moreover, the length of the estrous cycle within season with the use of 60 mg progesterone (16.75 days) was similar to the length of the cycle out of season, regardless of the dose (Table 1). Assessments conducted out of season showed no corpus luteum in the sheep at the time of insertion of the vaginal pessary during pre-synchronization, characterizing an anestrus condition. The correlation between the two periods (within season x out of season) and progesterone doses (120 mg x 60 mg) showed an effect of doses on the number of ovulations, ovulatory follicle diameter, length of the luteal phase, length of the follicular phase, and total length of the estrous cycle (Table 1).

The 60 mg and 120 mg groups out of season showed lower values for the number of ovulations, ovulatory follicle diameter, length of the luteal phase, and total length of the estrous cycle when compared with the same variables and doses in the breeding season. Table 2 shows an influence on a series of aspects due to the treatments being performed within or out of the breeding season. Corpus luteum was observed earlier within season (4.1 days) than out of the breeding season (5.75 days).

The largest CL diameter was observed at 12.47 days within season compared to 10.55 days out of the breeding season (Table 2). There was also variation relative to the last day of CL observation, which occurred at 14.31 days within season versus 12.40 days out of the breeding season.

The correlation of the evaluation periods (within season x out of season) and progesterone doses (60 mg x 120 mg) showed differences for these variables, in which CL was initially observed at 5.85 days with 60 mg of progesterone and 5.65 days with 120 mg of progesterone out of season. However, it occurred earlier within the breeding season, with CL initially found at 4.30 days with 60 mg of progesterone and 4.40 days with 120 mg of progesterone (Table 2). The largest CL diameter was also influenced by the groups (within season x out of season) and progesterone doses (60 mg x 120 mg). The maximum CL diameter within season occurred at 12.50 days with 60 mg and at 12.44 days with 120 mg. In contrast, the maximum CL diameter occurred earlier out of season, i.e., 10.57 days with 60 mg and 10.53 days with 120 mg (Table 2). Finally, the last day of CL observation was later within season, occurring at 14.30 days of the cycle with 60 mg of progesterone and at 14.33 days with 120 mg of progesterone compared to 12.38 days with 60 mg of progesterone and 12.42 days with 120 mg of progesterone out of season (Table 2).

The number of follicles smaller than 3.4 mm was higher within season compared to the period out of season in the first (4.42 versus 2.16), second (3.57 versus 2.08), third (4.05 versus 1.19), and fourth waves (4.16 versus 2), within and out of season, respectively. Furthermore, the fourth wave showed a higher number of medium follicles (3.5–4.9 mm) within season (two) when compared to the period out of season (only one). Differences were found between some parameters of the different waves and evaluation periods (Table 3). In the first wave, the dominant follicle reached its maximum size at 5.51 days within season and 4.60 days out of season. The same was observed for the dominant follicle in the fourth wave, which reached its maximum size with 16.85 days within season against 16.25 days out of season.

The day of emergence of the largest-diameter follicle was not different in the first, second, or third waves within or out of season. However, the fourth wave showed a difference, with the emergence of the ovulatory follicle at 13.16 days within season and 12 days out of season. A difference was also observed in the maximum ovulatory follicle diameter in the fourth wave, which was larger within season (5.88 mm) and smaller out of the breeding season (5.05 mm).

The length of the static phase showed a difference in the first wave, lasting 0.98 days within season and 1.21 days out of season. The same was observed in the fourth wave, in which the length of the static phase was shorter within season (0.80 days) than out of season (1.16 days). The growth phase, which determines the growth time of the dominant follicle of each wave, was higher in the third wave, with a growth period of 4.06 days within season and 3.45 days out of season. A longer period of the regression phase was observed in the first and second waves within season (3.18 and 3.43 days, respectively) compared to the period out of season (2.61 and 2. 65 days, respectively).

The growth rate, which relates the daily growth time of the dominant follicle of each wave, showed differences between the first and second waves within season, with lower values (0.63 and 0.68 mm/day, respectively) than the period out of season (1.01 and 0.78 mm/day, respectively). The opposite was observed for the fourth wave, that is, higher values were observed within season (1.25 mm/day) than out of season (0.87 mm/day). The variable interval between waves showed no difference between periods in the different waves.

Table 1. Mean values ± SD for number of follicular waves per estrous cycle, ovulation rate, ovulatory follicle diameter, length of the luteal phase in days, length of the follicular phase in days, and length of the estrous cycle in days, according to the season of year (within and out of season) and two progesterone concentrations (60 and 120 mg) in dairy sheep. Complete cycle after implant removal

Group	Waves per cycle ± SD (No.)	Number of ovulations (N) ± SD	OF diameter (mm) ± SD	Luteal phase days ± SD	Follicular phase days ± SD	Estrous cycle days ± SD
WITHIN	3.35±0.15	1.35±0.36^{A}	5.97±0.058^{A}	11.00±0.17^{A}	6.25±0.17^{B}	16.83±0.05^{A}
OUT OF	3.16±0.19	1.05±0.52^{B}	5.05±0.078^{B}	9.14±0.21^{B}	7.37±0.21^{A}	16.60±0.06^{B}
WITHIN 60	3.20±0.21	1.25±0.33^{A}	5.98±0.079^{A}	10.95±0.23^{A}	6.30±0.23^{B}	16.75±0.07A^{B}
WITHIN 120	3.50±0.23	1.44±0.26^{A}	5.95±0.084^{A}	11.05±0.24^{A}	6.20±0.25^{B}	16.92±0.07^{A}
OUT OF 60	3.16±0.19	1.00±0.43^{B}	5.03±0.072^{B}	9.12±0.21^{B}	7.35±0.21^{A}	16.62±0.06^{B}
OUT OF 120	3.18±0.19	1.10±0.48^{B}	5.09±0.075^{B}	9.16±0.25^{B}	7.39±0.24^{A}	16.58±0.08^{B}

^{AB} *Different letters indicate statistical differences when compared between columns of the same grouping (P<0.05).*

Table 2. Mean values ± SD for maximum corpus luteum size (mm), ovulation in days after observation of ovulatory follicle (OF) emergence, day of CL observation, last day of CL observation, and day of maximum CL diameter, according to the progesterone dose (60 and 120 mg) and season of the year (within and out of season) in dairy sheep. Complete cycle after implant removal

GROUP	Maximum CL size (mm) ± SD	Ovulation in days after emergence of OF ± SD	Beginning of CL observation days ± SD	End of CL observation days ± SD	Day of maximum CL size ± SD
WITHIN	11.30 ±0.12	4.06±0.15	4.10±0.058^{B}	14.31±0.11^{A}	12.47±0.15^{A}
OUT OF	11.20 ±0.15	4.42±0.19	5.75±0.078^{A}	12.40±0.14^{B}	10.55±0.19^{B}
WITHIN 60	11.15 ±0.17	3.95±0.21	4.30±0.079^{B}	14.30±0.15^{A}	12.50±0.22^{A}
WITHIN 120	11.45 ±0.18	4.17±0.23	4.40±0.084^{B}	14.33±0.16^{A}	12.44±0.21^{A}
OUT OF 60	11.22 ±0.15	4.44±0.19	5.85±0.078^{A}	12.38±0.14^{B}	10.57±0.19^{B}
OUT OF 120	11.18 ±0.17	4.42±0.17	5.65±0.076^{A}	12.42±0.15^{B}	10.53±0.17^{B}

^{AB} *Different letters indicate statistical differences when compared between columns of the same grouping (P<0.05).*

Table 3. Mean values ± SD for the day of emergence of the largest-diameter follicle, largest diameter (mm) of the dominant follicle (DF), day of maximum DF diameter, DF static phase in days, DF growth phase in days, FD regression phase in days, FD growth rate in mm/day, FD regression rate in mm/day, and interval between waves for each wave of follicular development within and out of the breeding season

Variable	Wave 1		Wave 2		Wave 3		Wave 4	
	Within	Out of	Within	Out of	Within	Out of	Within	Out of
Emergence of the largest-diameter follicle (days)	0.88±0.09^{A}	0.87±0.09^{A}	6.26±0.42^{A}	6.56±0.42^{A}	11.00±1.03^{A}	11.68±1.10^{A}	13.16±0.81^{A}	12.00±0.90^{B}
Largest DF diameter (mm)	4.36±0.16^{A}	4.40±0.15^{A}	4.19±0.16^{A}	4.35±0.16^{A}	5.43±0.53^{A}	5.01±0.60^{A}	5.88±0.15^{A}	5.05±0.15^{B}
Maximum DF diameter (days)	5.15±0.51^{A}	4.60±0.52^{B}	10.68±0.82^{A}	10.26±0.82^{A}	15.40±1.54^{A}	15.89±1.59^{A}	16.85±0.41^{A}	16.25±0.44^{B}
DF static phase (days)	0.98±0.21^{A}	1.21±0.23^{B}	1.00±0.09^{A}	1.04±0.09^{A}	0.91±0.11^{A}	0.96±0.15^{A}	0.80±0.31^{B}	1.16±0.38^{A}
DF growth phase (days)	4.15±0.43^{A}	3.90±0.43^{A}	4.21±0.63^{A}	3.87±0.63^{A}	4.06±0.55^{A}	3.45±0.60^{B}	2.96±0.15^{A}	2.85±0.17^{A}
FD regression phase (days)	3.18±0.32^{A}	2.61±0.32^{B}	3.43±0.50^{A}	2.65±0.50^{B}	2.83±1.04^{A}	2.50±1.10^{A}	________	________
FD growth rate (mm/day)	0.63±0.18^{B}	1.01±0.18^{A}	0.68±0.1^{B}	0.78±0.15^{A}	0.78±0.18^{A}	0.88±0.15^{A}	1.25±0.15^{A}	0.87±0.13^{B}
FD regression rate (mm/day)	1.30±0.27^{A}	1.40±0.27^{A}	1.06±0.24^{A}	1.09±0.26^{A}	1.16±0.54^{A}	1.10±0.52^{A}	________	________
Interval between waves (days)	5.56±0.58^{A}	5.46±0.58^{A}	5.03±0.78^{A}	5.51±0.85^{A}	5.10±0.59^{A}	5.25±0.60^{A}	4.18±1.15^{A}	3.80±1.18^{A}

^{AB} *Different letters indicate statistical differences when compared between rows of columns of the same grouping (P<0.05).*

4. Discussion

This is the first report on the behavior of ovarian follicular development in dairy sheep. The period of a complete estrous cycle, which lasts an average of 17 ± 1 days[6,11] was also observed in this study, in which the average length of the estrous cycle was 16.83 ± 0.05 days within the breeding season. The season of the year influences the length of the estrous cycle, with a reduction in its duration out of the breeding season from 16.60 ± 0.06 days to 16.83 ± 0.05 days in the breeding season ($P < 0.05$), regardless of the progesterone concentrations used in synchronization (Table 1).

The number of waves of follicular development during the estrous cycle in each group was similar even though a variation was observed in the length of the follicular phase, according to the breeding season. This occurs because the regression rate between waves is similar in the different groups and the interval between waves is similar. Other studies have also identified differences in the interval between the emergence of these waves, mainly when evaluating sheep with three or four waves in their estrous cycle[22]. In this study, sheep with four follicular waves had shorter emergence intervals than animals that had three follicular waves. Oliveira[18] observed that the number of follicular waves can interfere with the period of their emergence, which is in line with the data found in this study. Natural estrous cycles have an interval between the emergence of follicular waves ranging from 3 to 7 days [15]. The results obtained in this study varied between 3.80 and 5.56 days, with no effect on the period in which the measurements were performed (within season x out of season).

Dairy breeds, especially Lacaune, tend to have a high incidence of twin births, indicating a high number of ovulations. Breed and season can influence the incidence of ovulation in sheep[13]. The study showed a variation in the number of ovulations, with a higher ovulation rate (1.35) within the breeding season compared to the period out of season (1.05). This demonstrates higher awareness of the synchronization protocol during the period of natural reproductive activity although dairy breed sheep can be induced to have sexual activity throughout the year, which provides a higher number of double ovulations[2].

The ovulatory wave showed a larger diameter of the dominant follicle within the breeding season, with an average of 5.97 mm. However, the maximum diameter out of season was 5.05 mm even with a longer length of the follicular phase during this period (7.37 days), which would provide more time for growth. This smaller diameter is probably due to dominance and feedback mechanisms, still little known in sheep[2], especially those with dairy aptitude[23], as well as the fact that these females are anestrous, that is, out of season. In contrast, the dominant follicle reached almost 6 mm even with a shorter length of the follicular phase (6.25 days) in the breeding period, which is smaller than in hair and non-dairy sheep. This may be due to the effect of lactation in these specialized sheep, which may interfere with the endocrine regulation of follicular dynamics[2]. The length of the luteal phase also varied depending on the seasons, i.e., the length was 11 days within the breeding season and 9.14 days out of season. It shows that the life span of the corpus luteum is shorter and the tested progesterone doses do not have a direct effect out of season. The longer CL length within the breeding season can be explained by the delay in luteolysis due to the high concentration of circulating progesterone present during this period[4]. Both 60 and 120 mg of progesterone within the breeding season maintained the corpus luteum for a similar time, but very differently with the same doses when used out of season (Table 2).

Despite this difference in the length of time CL remains, no difference was observed in CL size within and out of season, showing that its functionality was not affected by the period of activity in the estrous cycle[2]. An interesting fact was the early CL observation after ovulation, with CL in the breeding season occurring at 4.10 days, contrasting with 5.75 days out of season. This precocity of more than 1.5 days may explain the longer CL length in the estrous cycle of sheep within the breeding season. The data also suggest the possibility of synchronizing the sheep with PGF doses, starting four days after the last ovulation, allowing the anticipation of mating by a few days based on the idea that this corpus luteum would already have a response to PGF. Moreover, although CL emerged on different days depending on the evaluated seasons, its presence lasts until the 14th day of the estrous cycle both within and out of the breeding season. However, the maximum CL diameter occurred on different days depending on the season, with the maximum diameter being reached at 12.74 days within the breeding season and 10.55 days out of season. The moment of ovulation after the emergence of the ovulatory follicle was not statistically different, occurring on average four days within season and 4.5 days out of season. A population of follicles of various sizes with a variety of combinations was observed, suggesting that their growth does not follow the logic known for other species, such as cattle[2]. Higher numbers of small follicles, with a diameter lower than 3.4 mm, were found in the first, second, and third follicular waves during the breeding season when compared to the same period out of season, demonstrating higher follicular recruitment in the breeding season of dairy sheep. This is related to the higher activity of circulating FSH during the period of reproductive activity[2]. In the fourth wave, medium follicles, with a size between 3.5 and 4.9 mm, were influenced by the reproductive period, with an average of two follicles being found within the breeding season compared to one follicle out of season. The largest follicle[1] during the beginning of the luteal phase and the follicular phase of the estrous cycle can partially suppress the growth of smaller follicles, but the emergence of a new wave begins before the beginning of the static phase of the previous wave. This codominance situation can occur in multiple ovulating sheep[8]. Table 3 shows some variations in follicular development between the first, second, and third waves within and out of season, without respecting a standard behavior between them, which suggests that these waves with follicles smaller than 4 mm grow randomly and may appear in varying numbers and various ways during waves of follicular growth. The fourth wave, responsible for the ovulatory follicle of that cycle, showed that the day of emergence of the ovulatory follicle occurred closer to the end of the estrous cycle within the breeding season. This allows this oocyte to display a better quality, as it is not aged[5,21]. This follicle reaches a larger diameter due to its higher daily growth rate and shorter length of the static phase, even though it emerged later, compared to the same evaluations that occurred out of season. It determines that ovulatory follicles in the breeding season have higher viability than those ovulated out of season (Table 3). The elucidation of the existing variation in follicular dynamics in dairy sheep allows the formulation of fixed-time insemination/mating protocols, suitable to the moment of their use, increasing the chances of constant lambing throughout the year and ultimately leading to homogeneous milk production over time.

5. Conclusions

The data allowed us to characterize follicular dynamics in dairy sheep, demonstrating differences in their development depending on whether they occur within or out of the breeding season. Out-of-season ewes are in anestrus, and ovulatory follicle diameter and the number of ovulations are lower after being subjected to the protocol, but females respond well to the stimulation of estrous cycle induction and synchronization protocols, enabling cyclicity throughout the year.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

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