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Increased number of large non-atretic follicles and co-dominance effects account for high litter sizes in Bonga sheep

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Abstract

To understand the ovarian basis for prolificacy of Bonga sheep, a total of 31 ewes were selected based on litter size (LS) records and divided into two groups: High Prolificacy (HP) (n = 20) with LS ≥ 2 and Low Prolificacy (LP) (n = 11) with LS = 1. At a synchronized estrus, follicular dynamics were determined using transrectal ultrasonography. Plasma estradiol concentrations were also monitored. In total 27 ewes were observed in estrus being 9/11 LP (82%) and 18/20 HP (90%). On the day of estrus (day 0), the mean number of large follicles was higher (p < .05) in HP (1.78 ± 0.19) than in LP (1.0 ± 0.28) ewes. Prior to estrus, more (p < .05) medium follicles were visible for HP compared to LP ewes. Plasma estradiol concentrations were higher in HP compared to LP ewes (18.91 ± 0.41 vs. 14.51 ± 0.65 pg/ml; p < .05) and similarly was ovulation number (2.3 ± 0.15 vs. 1.28 ± 0. 14; p < .05). Higher ovulation rates and litter size in Bonga sheep are evidenced by the previous presence of more large follicles and the existence of co-dominance effects as most likely medium follicles are selected to ovulate.

KEYWORDS

Bonga sheep, estradiol, follicles, litter size, ovulation rate

1 | INTRODUCTION

Sheep population in Ethiopia is among the largest in East Africa and sub-Saharan Africa and it increased from 25.5 million in 2012 to 31.8 million in 2017 (FAOSTAT, 2017). Small ruminants are mainly kept by smallholder farmers and the rural poor. Sheep contribute substantially as a source of income, food (meat and milk), and industrial raw materials (skin and wool). In addition, sheep production has a socioeconomic and cultural function and contributes to risk mitigation during crop failures, increase property security, and serve as a form of investment (Tibbo, 2006).

Ethiopia has a large and diverse sheep population which is divided to four groups based on morphological characteristics and geographic distribution: (a) Sub-Alpine short-fat-tailed, (b) Highland long-fat-tailed, (c) Lowland fat-rumped/tailed, and (d) Lowland thin-tailed groups (Gizaw, Komen, Hanotte, & Van Arendonk, 2008). Bonga sheep belong to the Highland long-fat-tailed group and have physical features characterized by long fat tail with straight tapering end (98.4%), hairy coat, large size, and predominantly plain brown in color (57.9%; Gizaw et al., 2011). Average adult body weights of female and male Bonga sheep are 32 and 48 kg, respectively (Edea, 2008), and the breed is considered among the large-sized sheep breeds in Ethiopia. Kaffa, Sheka, and Bench zones of Southern State are the home region of the Bonga sheep with 66% of the total population being reared in Kaffa zone (CSA, 2017).

The reproductive performance of sheep in Ethiopia varies among breeds, types, and locations. Major traits differences include age at first lambing, litter size, lambing interval, and lifetime productivity of the ewe (Abate, 2016).

In a study carried out in Adiyo Kaka (Southern Nations, Nationalities and Peoples' Regional State of Ethiopia) district, Edea et al. (2012) calculated an average age at first lambing of 14.9 ± 3.12 (±*SE*) months for Bonga sheep which is younger than the age reported for Menz sheep (523 ± 13 days) in the Ethiopian highlands (Mukasa-Mugenva et al., 1991). Litter size is directly related to ovulation rate, genotype, and environmental factors (Mukasa-Mugerwa & Lahlou-Kassi, 1995). A litter size of 1.36 was reported for Bonga sheep (Edea et al., 2012) which was higher than the 1.14 reported for Menz sheep under traditional village conditions (Agyemang, Negussie, Voorthuize, & Anderson, 1985). Average lambing interval for Bonga breed is 8.92 ± 2.13 months (Edea et al., 2012) being shorter than the 10 to 11 months figure for Menz sheep (Dibissa, 1990). On average, a Bonga ewe delivers 12.2 ± 1.80 lambs in her lifetime which is lower than the 15.3 ± 4.3 lambs reported for Horro sheep (Edea et al., 2012). Bonga sheep are naturally managed in accelerated lambing rhythm which is shorter than the conventional year-round lambing schedule for sheep. Much of the above data backed by the findings of Edea (2008) point out that, most likely, Bonga sheep have a non-seasonal reproductive rhythm. In a recent report related to the community-based breeding program (CBBP) of the breed (Aynalem Haile, personal communication), lambing interval for 2,900 Bonga ewes under village conditions was estimated at 258.1 ± 1.14 days (nearly, 8.6 months). The same report provides further evidence to the relatively prolific trend in Bonga sheep with an average litter size of 1.54 ± 0.006 for a total of 10,814 observations under village conditions. Large variability characterizes the dataset with records varying between 1 and 4; village, year, season, age, and parity significantly affected the performances.

Globally, Bonga sheep breed seems to reproductively perform better than most other indigenous breeds in Ethiopia, particularly for litter size. Recently, our research conducted a selection signature analysis using genome-wide genotype data from the prolific Bonga sheep in Ethiopia and identified 41 candidate regions associated with fertility and reproduction traits. The analyses confirmed the presence of selection signatures in genomic regions that span or lay adjacent two genes, *GDF5* and *BMP15*, that are known to be associated with prolificacy (Dolebo et al., 2019). Nevertheless, the physiological mechanisms behind this high and variable performance remain unknown. To better understand the physiological mechanisms underlying expression of litter size in Bonga sheep, current study aimed at characterizing and comparing follicular dynamics, ovulatory response, and some ovarian endocrine attributes during the follicular and the luteal phases in synchronized Bonga sheep with different retrospective average records for litter size.

2 | MATERIALS AND METHODS

2.1 | Study area

The study was conducted in Adiyo Kaka district of Kaffa zone. The district is located 509 km South West of Addis Ababa in Southern Nations, Nationalities and Peoples Regional State (SNNPRS). The study area is a wet, humid agro-ecology that lies at an altitude of 2,511 m above sea level, at 7°17′ N latitude and 36°24′ E longitude with temperature range 17.5–22.5°C. The area is characterized by large evergreen natural forest receiving rainfall almost year-round. The annual rainfall ranges from 1,700 to 2,000 mm with peak rainy season being mid-June to early October. The district has 35% highland, 55% midland, and 10% lowland agro-ecology. There are no ethical concerns to be reported and animals were handled in the presence of their owners by adhering to local animal practices and handling rules.

2.2 | Animals and experimental design

A total of 31 Bonga ewes aged between 4 and 5 years old and having a body condition score varying between 2.5 and 3.5 were selected from private farmers of Boka-Shuta CBBP cooperative, for the study. A subjective body condition scoring system was based on the scale of Russel, Doney, and Gunn (1969). The ewes were reared in small flock sizes of less than 10 ewes (Mamiru, Banerjee, & Haile, 2017) and were chosen based on existing litter size records for three consecutive lambing seasons with no history of reproductive disorders (extended lambing intervals, abortions, etc.) as per the database of the CBBP. Ewes were classified as high prolific (HP) (n = 20; average body condition score 3.1 ± 0.56) producing litter sizes ≥ 2 and low prolific (LP) (n = 11; average body condition score 3.0 ± 0.42) producing litter sizes equal to 1 during each of the three considered lambing seasons. All the ewes were confirmed to be non-pregnant using ultrasound-based pregnancy diagnosis.

Throughout the experiment, animals were assembled and kept in a community shed, grazed on natural grassland, had ad libitum access to clean water, and were exposed to natural daylight during the entire trial. Three sexually mature and experienced rams of the Bonga breed were used for estrus detection.

On November 5, 2017 estrous cycle was synchronized for the 31 selected ewes, using intravaginal progesterone sponges (Syncropart®; CEVA laboratories) inserted for 14 days. Determination of follicular dynamics was performed daily by transrectal ultrasonographic assessment of the number and size of all follicles ≥ 2 mm, from the day of sponge removal to the day following the onset of estrus (day of estrous being considered day 0 for uniformity of data presentation) or for 5 days following sponge withdrawal in those sheep that failed to exhibit estrus. Prior to echographic examination, all the ewes were subjected to 12-hr overnight fasting.

The presence and number of *corpora lutea* (CL) were also assessed by transrectal ultrasonography approximately 9 days following the onset of estrus.

2.3 | Assessment of preovulatory follicular development and the presence and number of corpora lutea

Ultrasonographic observations of the ovaries were performed by the same experienced operator using a 7.5-MHz transducer for

transrectal ultrasonography (Honda®, HS-2200V). After placing the sheep in the dorsal position as during laparoscopy, the probe was placed in the rectum with the transducer orientated perpendicularly to the abdomen wall using a hydrosoluble contact gel to enhance ultrasound transmission. Once the uterine horns were located, the probe was rotated laterally 90° clockwise and 180° counterclockwise to observe both ovaries and their structures after surpassing the urinary bladder (Gonzalez-Bulnes, Santiago-Moreno, Garcia-Lopez, Gomez-Brunet, & Lopez-Sebastian, 1994). Each ovary was scanned several times from different angles in order to image all follicles ≥ 2 mm. The largest diameter of each of these follicles was measured and its position was recorded on a paper-back diagram of each ovary. In order to ensure correct identification of all follicles at successive observations and follow their individual growth, both position and distance from the largest follicle and the ovarian medulla and hillus were recorded every day on the diagram of each ovary (Gonzalez-Bulnes, Souza, Campbell, & Baird, 2004).

Follicles recorded by ultrasonography from the left and right ovaries were classified as small [2–3 mm], medium [4–5 mm], and large [≥6 mm] and total follicles corresponding to all follicles larger than 2 mm. Follicles were further classified as atretic when being in a regressing phase (those that decreased in size between two successive ultrasound sessions) and new follicles (not previously detected; Cueto, Gibbons, Alberio, Taddeo, & Gonzalez-Bulnes, 2006).

Around day 9 following the onset of estrus, the left and right CLs were identified through their echogenic pattern and their numbers determined as described by Gonzalez-Bulnes, Santiago-Moreno, Gomez-Brunet, and Lopez Sebastián (2000).

2.4 | Estrus and mating

Sixteen hours following the removal of the intravaginal sponges, estrous behavior was detected at 8-hr intervals via direct observation of the ewes using three aproned teaser rams. Estrous detection continued for four consecutive days or until estrus was detected. Ewes standing to be mounted were considered to be in estrus and were mated with rams allocated at a mating ratio of 10 ewes to 1 ram. Each ewe was mated twice at 12 hr interval. Ewes not displaying estrus were also recorded.

2.5 | Assessment of estradiol and progesterone secretion

Blood sampling for estradiol determination took place every 8 hr, from 16 to 96 hr after sponge removal. This corresponded to the time period during which follicular dynamics and estrous behavior were being monitored. Blood was collected using vacutainer tubes infused with heparin (Vacutainer® Systems Europe, Becton Dickinson). For progesterone analysis, blood samples were collected at 48-hr intervals for 20 days following the removal of sponges. Samples were then immediately transported in a cooling box filled with ice to Bonga Research Center, Animal Health Laboratory, and centrifuged at 1,500 *g* for 15–20 min. Plasma, recovered from each centrifuged sample, was stored at −20°C for 3 weeks prior to undertaking progesterone and estradiol assays.

Plasma progesterone and estradiol concentrations were determined by enzyme-linked immunosorbent assay (ELISA) in duplicate using an ELISA assay kit (MyBioSource®) based on standard procedures following the manufacturer's instructions. The inter- and intra-assay variation coefficients were 8.6% and 11.2%, respectively, for progesterone and 6.2% and 10.3% for estradiol.

2.6 | Statistical analysis

For uniformity of variables, day 0 was equaled to be the day of onset of estrous. Day 0 in ewes that did not show estrus was assimilated to day 2 after the introduction of teaser rams on which more than 90% of the ewes displayed estrus. For changes in the frequency of follicular size, factorial ANOVA with two independent factors (time and prolificity) was used to test the difference between LP and HP ewes. The Students *t* test was used to compare differences in follicular numbers between the LP and HP ewes. One-way ANOVA was used to test differences in the number of CLs, atretic follicles, new follicles, and plasma concentration of estradiol and progesterone between the LP and HP ewes.

Mean number small, medium, large, total, atretic, and new follicles were expressed as mean ± *SEM*. Mean plasma estradiol concentrations and progesterone concentrations were expressed as mean ± *SD*. Statistical significance was set at $p < .05$ (Schwartz, 1993).

3 | RESULTS

3.1 | Estrous response

Following sponges' removal and the introduction of teaser rams, 27 of 31 ewes were detected to be in estrus in the following 3 days. Nine LP ewes (82%) were detected to be in estrus and 18 HP ewes (90%) were standing to estrus.

3.2 | Growth dynamics of preovulatory follicles

The changes in frequency of follicular size (mean number of small, medium, and large follicles) are presented in Figure 1.

The mean number of medium follicles tended to be higher in HP than in LP ewes ($p = .07$). In fact, in the HP group, the mean number of medium follicles increased from 0.89 ± 0.65 3 days prior to estrus to 4.89 ± 0.30 and 3.89 ± 0.30 at days −2 and −1 before estrus, respectively (Figure 1). Corresponding values in LP ewes remained lower (1.17 ± 0.70, 2.83 ± 0.37, and 1.83 ± 0.27 at days −3, −2, and −1 before estrus, respectively; Figure 1). Differences between LP and HP ewes were significant on days −2 and −1 preceding estrus

($p < .05$). However, for small and large follicles, no significant differences were observed between the two groups except on the day of estrus (day 0) when the mean number of large follicles was higher in HP compared to LP ewes (1.78 ± 0.19 and 1.0 ± 0.28 for HP and LP ewes, respectively; $p < .05$; Figure 1).

3.3 | Atretic and new follicles

Frequency distributions of small, medium, and large atretic and new follicles pooled together are reported. No differences were observed in the trend follicles were undergoing atresia. The mean number of new follicles was significantly higher in the ovaries of LP compared to those of their HP counterparts on the day of estrus and on the day prior to estrus ($p < .05$; Figure 2).

3.4 | Plasma Estradiol

For most of the sampling period, HP ewes had higher means of plasma estradiol but at some sampling points the difference failed to attain statistical significance because of high individual variations (Figure 3). Overall, the plasma estradiol concentrations were higher for HP than LP ewes (18.91 ± 0.41 vs. 14.51 ± 0.65 pg/ml; $p < .05$).

3.5 | Luteal function and plasma progesterone

The mean number of CL was significantly higher ($p < .05$) in HP (2.3 ± 0.15) than in LP (1.28 ± 0. 14) ewes. The mean plasma progesterone concentrations were also significantly higher ($p < .05$) in HP than in LP ewes (Figure 4) and these differences were more pronounced between days 10 and 15 following the removal of sponges. The largest difference was observed on day 12 when progesterone concentrations rose to an average value of 5.60 ± 0.71 ng/ml in HP ewes compared to 1.95 ± 1.63 ng/ml in LP ewes.

4 | DISCUSSION

The present study confirms that the LP and HP ewes of Bonga sheep exhibit different ovulation rates. The lambing data that were made available and consecutive to the following protocol is consistent with our hypothesis as LP ewes ($n = 7$) yielded an average litter size of 1.25 ± 0.433, while HP ewes ($n = 11$) produced 2.12 ± 0.499 lambs on average. Results of the current study suggest that differences in ovulation rate between the LP and HP ewes are caused by differences in the number of follicles growing to the large stage and the existence of co-dominance effects. Beyond the overall result confirming ovarian-related differences between LP and HP ewes, some features in the response of the two groups of sheep are very crucial.

It is important to highlight that the estrous response (87% of all experimental ewes) in this study was high. This was expected because the Bonga breed, like most tropical breeds of sheep, is not susceptible to changes in photoperiod and is commonly known to be non-seasonal. Although discrete periods of seasonality, resulting from other environmental and social cues, such as feed availability, ambient temperature, and disease incidences, can be reported for such breeds, it is anticipated that such effects would be negligible in the home region of Bonga sheep. Indeed, the area is characterized by mild temperatures and sufficient, well-distributed rainfall pattern over the year to maintain lush vegetation cover (Mamiru et al., 2017). With the exception of two individuals, all the sheep displayed estrus in a very synchronous way 2 days after the removal of sponges. The two exceptions, one LP and one HP had a delayed response on day

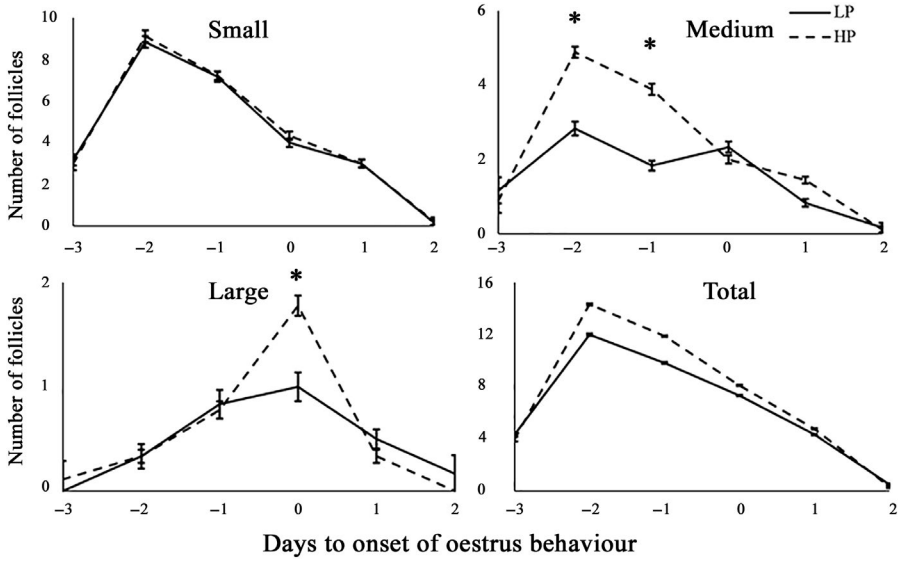

FIGURE 1 Mean number (±*SEM*) of small [2–3 mm], medium [4–5 mm], large ≥6 mm, and total follicles ≥2 mm during the follicular phase of Low (LP) and high prolific (HP) Bonga sheep. Statistically significant differences are indicated with * if $p < .05$

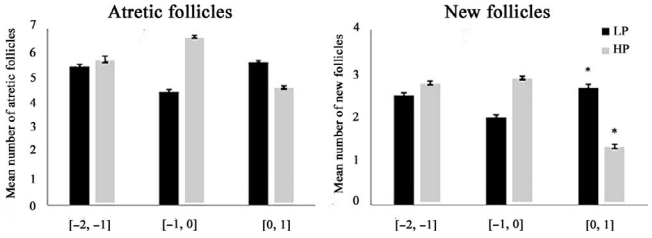

FIGURE 2 Mean number (±*SEM*) of atretic and new follicles for all follicular sizes during the follicular phase of Bonga ewes with single (LP) and multiple (HP) litter sizes. Day 0 corresponds to the day of onset of estrus. Statistically significant differences are indicated with * if $p < .05$

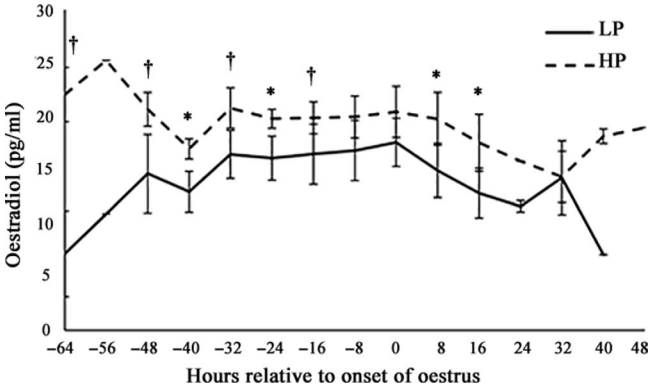

FIGURE 3 Plasma estradiol concentrations (mean ± *SD*) around estrus for Bonga ewes with single (LP) and multiple (HP) litter sizes. Statistically significant differences are indicated with * if $p < .05$ and † if $0.09 > p > .05$

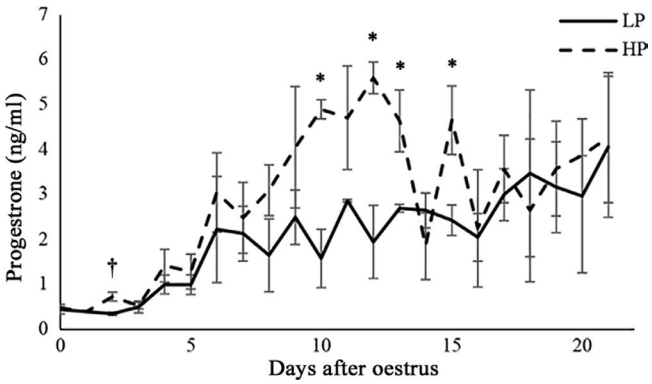

FIGURE 4 Plasma progesterone concentrations (mean ± *SD*) for Bonga ewes with single (LP) and multiple (HP) litter sizes every 2 days for 20 days after sponge removal. Statistically significant differences are indicated with * if $p < .05$ and † if $0.09 > p > .05$

3 after the removal of sponges. This observation is important as it discards, at least for HP Bonga ewes, the notion that increases in ovulation rate are related to an extended period of ovulatory follicle recruitment (Scaramuzzi & Radford, 1983). An extended period of follicle recruitment would allow a higher number of follicles growing to ovulatory stages as confirmed for various temperate sheep breeds (Bartlewski et al., 1999; Driancourt, 2001; Souza, Campbell, Webb, & Baird, 1997).

The number of small and medium follicles decreased during the follicular phase in both groups of ewes. This indicates that preovulatory follicles in both LP and HP ewes grew in a fast and continuous manner during the period of terminal growth, therefore exerting dominance over the remaining follicles. We also anticipate such a dominance effect of the preovulatory follicles to be higher in HP ewes due to the higher number of atretic follicles a day prior to estrus (although not significant) and the lower number of new follicles during the 24 hr following estrus. Such patterns of follicular growth are similar to those reported in other sheep breeds (Bartlewski et al., 1999; Ben Salem, Rekik, Gonzalez-Bulnes, Lassoued, & Kraïem, 2010; Evans, Duffy, Hynes, & Boland, 2000; Gonzalez-Bulnes et al., 2001; Lopez-Sebastian et al., 1997).

However, if we analyze the data obtained in the current study in chronological detail, it reveals some interesting trends. The mean number of CL in HP ewes is much higher than the average number of large follicles on the day of estrus. Furthermore, while the number of medium follicles declined slightly during the follicular phase in LP ewes, it rapidly declined in HP ewes over the 48 hr preceding estrus. Based on these two observations, it can be hypothesized that the medium follicles in HP ewes are able to be selected to ovulate, hence contributing to a higher ovulation rate. This agrees with the results of previous study that compared two strains of Tunisian Barbarine sheep (Lassoued, Rekik, Gonzalez-Bulnes, Ben Salem, & Tounsi, 2013) in which ewes of the prolific strain exhibited a codominance effect with several medium follicles ovulating. The existence of medium follicles contributing to the increase in ovulation rate supports the idea of codominance in sheep; i.e., the coexistence of two or more follicles, from the same or subsequent growth waves, reaching dominance and even ovulation (Bartlewski, Baby, & Giffin, 2011). Commonly, dominant follicles impair the development of smaller gonadotrophin-dependent follicles by suppressing FSH and inducing atresia (Campbell, Scaramuzzi, & Webb, 1995), but in sheep the dominance exerted by large follicles is weak (Gonzalez-Bulnes, Diaz-Delfa, et al., 2005a) and does not completely suppress ovulations from supplementary growing follicles of smaller sizes (Evans et al., 2000).

Throughout the follicular phase, estradiol secretion was consistently higher in HP ewes. This is probably due to the higher number of ovulatory follicles, but this does not preclude differences in functionality of the growing medium and large follicles. Such a hypothesis can only be verified with an appropriate experimental design to assess in vitro follicle competency, but it should be noted that estradiol is also a marker for follicular function (Souza, Campbell, & Baird, 1996) and, as a result, preovulatory (large and medium) follicles of the HP ewes have a higher responsiveness to gonadotrophin secretion, hence promoting their growth prior to ovulation. What should also be noted regarding estradiol pattern of secretion is the absence of the rise which should normally appear at estrus time in both HP and LP ewes. This can be argued by the negative effects of progestogens on the functionality of ovulatory follicles (Gonzalez-Bulnes, Veiga-Lopez, et al., 2005b). Indeed, they demonstrated that ovulatory follicles from sheep treated

with exogenous progestogens showed deficiencies in the secretion of estradiol during the preovulatory phase and no marked rise was detected at the time of estrus. This did not prevent estrus to be exhibited and ovulation to occur. However, in their study, subsequent corpora lutea secreted low progesterone.

None of the sheep had a short cycle on the basis of the echogenicity of the CL tissue and the screening of progesterone levels from day 2 to 14 following the withdrawal of sponges. The mean number of CL was significantly higher in HP than LP ewes. As the production of progesterone is done by the luteal cells, this could explain the significantly higher plasma progesterone concentrations in HP ewes during the mid-luteal phase (Mesen & Young, 2016). The overall reduction in plasma progesterone levels in HP ewes after day 14–16 could be explained by three ewes for which plasma progesterone levels declined to less than 0.4 ng/ml and remained undetectable until day 20. These three ewes most likely did not conceive to the synchronized estrus and underwent luteolysis.

In conclusion, this study provides evidence at the ovarian level of the phenotypic variability characterizing litter size in Bonga sheep found in Ethiopia. This is mainly reflected in the growth of larger preovulatory follicles and in the existence of co-dominance effects that could account for the difference in ovulation rates and litter sizes between LP and HP ewes.

ANIMAL WELFARE STATEMENT

The ethical polices of the journal have been adhered to, and that European Union (EU) standards on the protection of animal used for scientific purposes and/or feed legislation have been met.

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