Follicular and endocrine studies to characterize litter size variability in Bonga sheep

Bonga ewes with high litter sizes are characterized by a high number of large follicles and reduced atresia

High litter sizes in Bonga ewes are associated with high frequency of large, non-atretic follicles, secreting more oestradiol during the follicular phase

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Abstract

A total of thirty-one ewes belonging to the Bonga breed were selected based on existing records for at least the 3 last lambing seasons and divided into two groups for high and low prolificacy (HP, n = 20 and LP, n = 11, respectively). Oestrous cycle was synchronized, by using intravaginal progesterone pessaries. From the day of vaginal sponges' removal to the day of oestrus onset and for 96 consecutive hours, determination of follicular dynamics was performed using transrectal ultrasonography. Plasma oestradiol concentrations were determined every 8 hours between sponge removal and onset of oestrus. Luteal function was determined by assessing plasma progesterone every 2 days for a period of 20 days after onset of oestrus.

Following sponges' removal and introduction of rams, 27 out of 31 ewes were detected in oestrus. Nine out of the 11 ewes (82 %) belonging to the group LP were detected in oestrus and 18 out of the 20 high prolific ewes (90%) were also in oestrus. The number of total follicles was significantly higher in HP group than in LP ewes. This observation was accentuated two days before oestrus (129 ± 0.15 and 72 ± 0.18 for HP and LP animals, respectively) ($P < 0.001$). Mean number of large follicles was higher in HP animals compared to LP animals (16 ± 0.19 and 6 ± 0.28 for HP and LP group, respectively. However, a higher number of atretic follicles appeared in LP ewes between the day of oestrus and 1 day prior to oestrus. Overall the follicular phase, plasma oestradiol concentrations were higher for HP group in comparison to LP ewes (18.91 ± 0.41 vs. 14.51 ± 0.65 pg/ml; $P < 0.05$). During mid-luteal phase, mean number of CL was significantly higher in HP group compared to the LP ewes (2.3 ± 0.15 and 1.28 ± 0. 14 for HP and LP, respectively) ($P = 0.033$). It is concluded that prolific ewes of the Bonga breed have increased ovulation rate as a result of a higher number of large follicles appearing on the ovaries before ovulation and a reduced frequency of follicles undergoing atresia in the last 24 hours preceding onset of oestrus.

Keywords: Sheep – Bonga breed – Ethiopia – Ovulation rate – Follicles - Oestradiol

1. Introduction

Sheep population in Ethiopia is among the largest in sub-Saharan Africa and the largest in East Africa and the sheep population 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 and sheep contribute substantially as a source of income, food (meat and milk) and industrial raw materials (skins and wool). In addition, sheep production has a socioeconomic and cultural function and contribute to risk mitigation during crop failures, increase property security, and serve as a form of investment (Tibbo, 2006).

Ethiopia includes a huge and diverse sheep population and according to the morphological characteristics and geographic distribution it was divided to 4 groups (i) Sub-Alpine short-fat-tailed group (ii) Highland long-fat-tailed group (iii) Lowland fat-rumped/tailed group and (iv) Lowland thin-tailed group (Gizaw, 2008). Bonga sheep belongs to the second group (Highland long-fat-tailed group) and have physical feature and performance levels of long fat tail with straight tapering end (98.4%), hair sheep, large size and predominantly plain brown (57.9%) (Gizaw et al., 2011). Kaffa, Sheka and Bench zones of Southern State are the centre of distribution for Bonga and 66% of the total population of Bonga sheep are reared in Kaffa zone (CSA, 2017).

The reproductive performance of sheep in Ethiopia varies among breeds / types and locations. It depends on various factors including age at first lambing, litter size, lambing interval and the life time productivity of the ewe (Abate, 2016).

In a study carried out in Adiyo Kaka (Southern Nations, Nationalities and Peoples' Regional State of Ethiopia) and Horro (Oromia Regional State of Ethiopia) districts, Edea et al, (2012) calculated an average age at first lambing of 14.9 ± 3.12 months for Bonga sheep which is shorter than the age reported for Menz sheep (Gautsch, 1987 ; Mekoya, 1999). Litter size is directly related to ovulation rate, genotype and environmental factors (Mukasa-Mugerwa and

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 village conditions (Agyemang et al., 1985). Edea et al, (2012) calculated an average lambing interval of 8.92 ± 2.13 months. This value is shorter than what had been found for Menz sheep (Dibissa, 1990). On average a Bonga ewe delivers 12.2 ± 1.80 lambs in her life time which is lower than the 15.3 ± 4.3 lambs for Horro sheep (Edea et al., 2012). In a recent study, the lambing interval was calculated in 2017 for 453 Bonga sheep and was estimated to 259.0±2.26 days. Litter size was 1.44±0.013 for 1681 animals (unpublished data).

Although Bonga sheep breed perform better than most indigenous breeds showing higher prolificacy rates, litter sizes recorded in the field are very variable (from 1 to 4). The physiological mechanisms behind this variability is not characterized till today.

Therefore, the objective of the current study was to characterize and compare follicular dynamics, pituitary function, ovulatory response and luteal activity during the follicular phase and the luteal phase in synchronized Bonga sheep with different 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 kms South West of Addis Ababa in Southern Nations, Nationalities and Peoples Regional State (SNNPRS). The study area is wet humid agro ecology that lies at an altitude of 2511m above sea level at 7017' N latitude and 36024' E longitude with temperature range of 17.5-22.50C. The area is characterized by large ever green natural forest receiving rain fall almost all year. The annual rainfall range is between 1700-2000 mm with peak rainy season being mid-June to early October. The district has 35% highland, 55% midland and 10% lowland agro-ecology (Figure 1).

Figure 1. Map showing the project area

2.2. Animals and experimental design

A total of thirty one ewes belonging to private farmers of Boka-Shuta community based breeding program cooperative of the Bonga breed, aged between 4 and 5 years and weighing between .. and .. kg, were used. For this trial, selected females were randomly chosen of similar ages, body condition, management lines, to be non-pregnant and cycling, as assessed by ultrasonographic determination, and based on their birth history. Ewes were selected based on existing records for at least the 3 last lambing seasons as per the records of the breeding program. In fact, they were selected according to whether they consistently produced single, twin or triplet lambs with no history of reproductive disorders.

Throughout the experiment, animals were assembled and kept into the community shed, grazed natural grassland, had free access to clean water and exposed to natural daylight during the entire trial. Three adult, experienced rams of Bonga breed were kept in for oestrus detection.

On the 5th of November 2017 and for the 31 selected ewes, oestrous cycle was synchronized, by using intravaginal progesterone pessaries (Syncro-part®; CEVA laboratories, Libourne, France) inserted for 14 days. From the day of vaginal sponges' removal to the day of oestrus onset (considered day 0 for experimental purposes) and for 5 continuous days determination of follicular dynamics was performed using transrectal ultrasonography. Before the echographic examination, all experimental ewes were subjected to a 12-hour fasting overnight.

Plasma oestradiol concentrations were determined and luteal function was determined by assessing plasma progesterone. The presence and number of CL were also assessed by transrectal ultrasonography approximately 9 days after onset of oestrus.

2.3. Assessment of preovulatory follicular development and presence and number of corpora lutea

Ultrasonographic observations of the ovaries were performed by the same experienced operator using a 7.5MHz transducer for transrectal ultrasonography (reference). 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 an hydrosoluble contact gel into the rectum to enhance the ultrasound transmission. When 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 et al., 1994).

Follicles recorded by ultrasonography were classified as small]3-3 mm], medium [4-5 mm] and large follicles ≥ 6 mm from left and right ovaries.

Left and right corpus luteum (CLs) were identified through their echogenic pattern and their presence and number around day 9 after oestrus occurrence were undertaken as described by Gonzalez de Bulnes et al. (2000).

2.4. Oestrus and mating

Starting 16 hours after sponge removal, the oestrus behaviour was detected every 8 hours by direct observation of the ewes and three teaser rams. Oestrus checking continued for four consecutive days or until oestrus is detected. Ewes standing to be mounted from each group were considered as in estrus. These ewes were also mated with rams allocated at ewe to ram ratio of 10:1. If ewes do not display oestrus or ovulated, the information was recorded.

2.5. Assessment of oestrogen and progesterone secretion

Blood sampling for oestradiol determination was collected every 8 hours starting 8 hours after sponge removal. Blood was removed using vacuum blood evacuation tubes with heparin

(Vacutainer Systems Europe, Becton Dickinson, Meylan Cedex, France). Blood sampling continued for 96 hours corresponding to the period during which follicular dynamic and oestrus behavior were monitored. For progesterone analysis, blood samples were collected every 48 hours for the 20 days following removal of sponges. Blood samples collected at field were immediately transported to Bonga Research center Animal health laboratory and centrifuged at 1500 × g for 15-20 min. Plasma recovered from each tube was brought to ILRI laboratory and kept at -20°C until progesterone and estrogen assay.

The plasma progesterone and estradiol concentrations of experimental ewes were determined by enzyme-linked immunosorbant assay (ELISA) in duplicate using an ELISA® assay kit (MyBioSource, San Diego, USA), according to the manufacturer's instructions; of standard procedure. The inter- and intra-assay variation coefficients were 8.6 % and 11.2 %, respectively.

2.6. Statistical analysis

Animals were retrospectively classified, according to their birth history into two groups. The first group, low prolific group (LP) was composed of ewes that had a single type of birth (n = 11); the group high prolific (HP) comprised ewes that had twins and triplet (n = 20). For uniformity of variables, day 0 was equaled to the day of oestrus onset in the two groups.

Factorial ANOVA with two independent factors was used to test the difference between low and high prolific Bonga ewe. One-way ANOVA was used to test the difference of the number of CL and plasma concentrations of oestradiol and progesterone between LP and HP group.

A chi square Mantel–Haenszel test was used to test the difference between percentages of ewes having 0, 1, 2, 3 or 4 CL and percentages of atretic and new follicles according to prolificity. Results were expressed as mean ± SEM and statistical significance was set at $P < 0.05$ (Schwartz 1993).

3. Results

3.1. Oestrus response

Following sponges' removal and introduction of rams, 27 out of 31 ewes were detected in oestrus. Nine out of the 11 ewes (82 %) belonging to the group LP were detected in oestrus and 18 out of the 20 high prolific ewes (90%) were also in oestrus.

3.2. Growth dynamics of preovulatory follicles

Changes in frequency of follicular size (mean number of small, medium and large follicles) are represented in figure 2. Day 0 was equaled to the day of oestrus for uniformity of results.

The mean number of medium follicles tend to be higher in the HP group than the LP group ($p = 0.07$). In fact, mean number of medium follicles increased from 8 ± 0.65 three days before oestrus to 44 ± 0.30 and 35 ± 0.30 at days -2 and -1 before oestrus, respectively (figure 2). Corresponding values in LP animals remained roughly lower (7 ± 0.70, 17 ± 0.37 and 11 ± 0.27 at days -3, -2 and -1 before oestrus, respectively) (figure 2). However, for small and medium follicles, no significant difference was observed between the two groups of ewes (HP an LP) ($p > 0.05$) even if at day of oestrus, mean number of large follicles was higher in HP animals compared to LP animals (16 ± 0.19 and 6 ± 0.28 for HP and LP group, respectively) (figure 2). The number of total follicles was significantly higher in HP group than in LP ewes. This observation was accentuated two days before oestrus (129 ± 0, 15 and 72 ± 0, 18 for HP and LP animals, respectively) ($P < 0.001$) (figure 2).

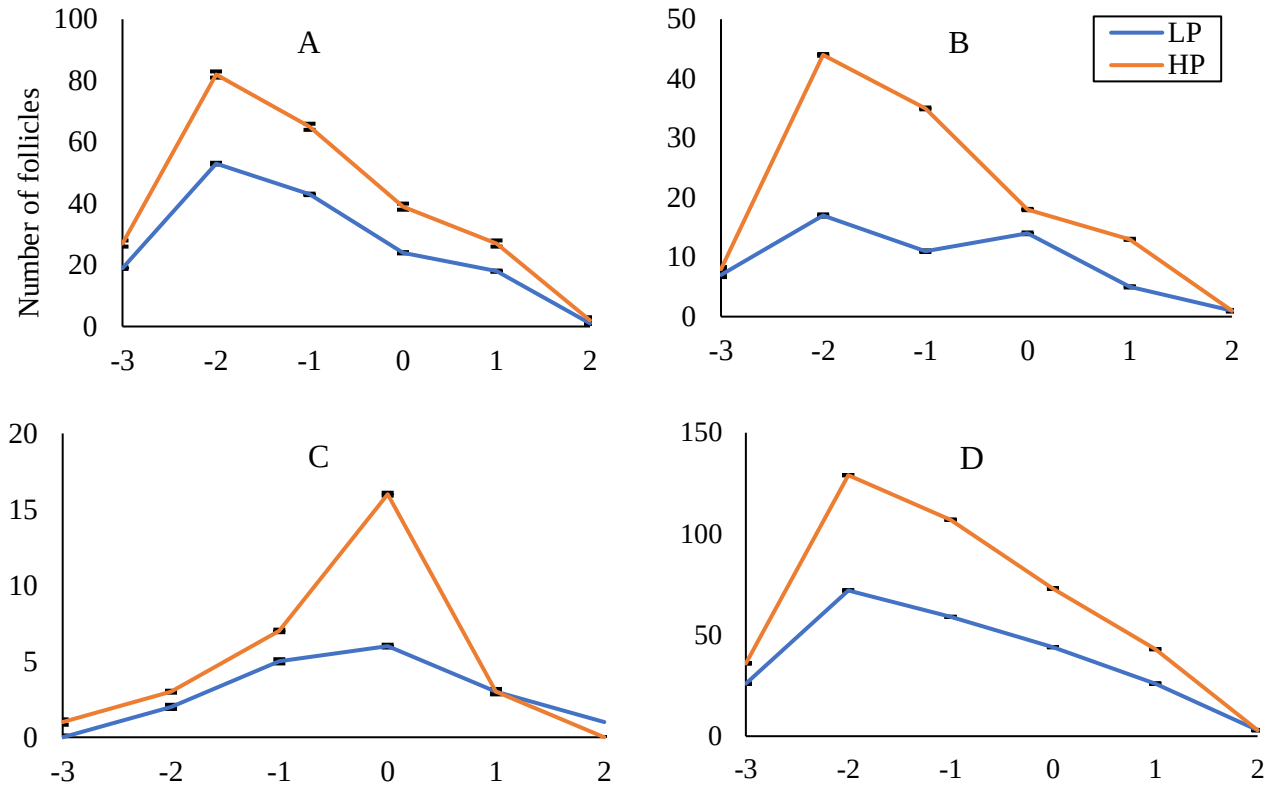


Figure 2. Number (± SEM) of (A) small]3-3mm], *(B) medium* [4-5mm], *(C) large* ≥6mm and total *(D) ovulatory follicle during the follicular phase of Low (LP) and high prolific (HP) Bonga sheep.*

3.3. Plasma Oestradiol

Mean plasma oestradiol for 3 days and with 8 hours interval were reported to the day of oestrus (considered as day 0) and plotted in the figure 3. Overall, plasma oestradiol concentrations were higher for HP group in comparison to LP ewes (18.91 ± 0.41 vs. 14.51 ± 0.65 pg/ml; $p < 0.05$). Throughout most of the sampling period, HP ewes had higher means of plasma oestradiol (figure 3).

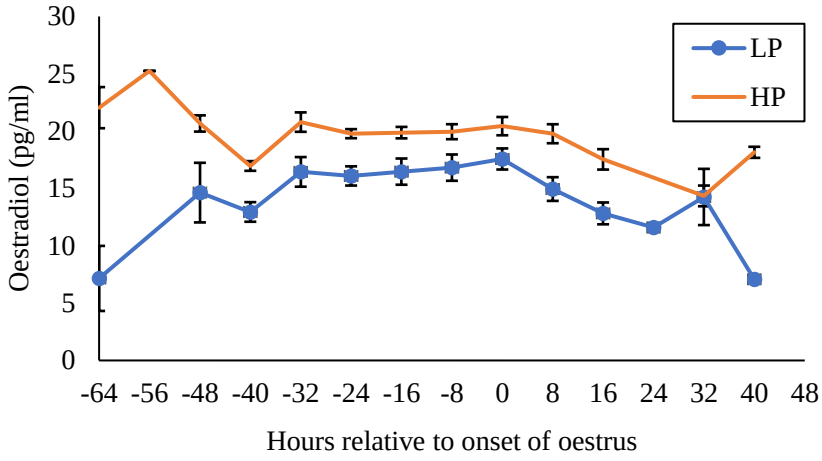
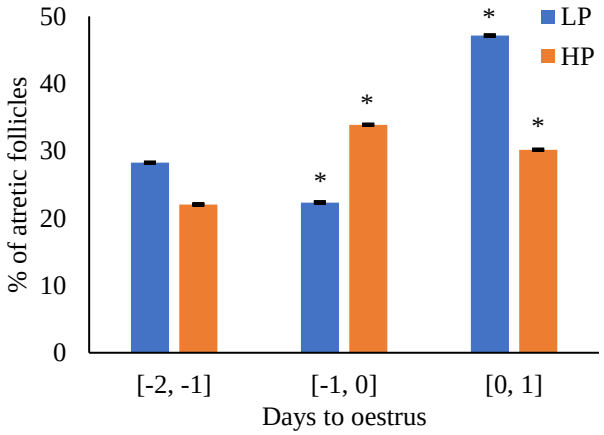


Figure 3. Plasma oestradiol concentrations (mean ± SEM) around oestrus for Bonga ewes that have single litter size (LP) and double or triple litter size (HP).

3.4. Atretic and new follicles

Frequency distributions of atretic and new follicles for small, medium, and large follicles pooled together were reported. More follicles did undergo atresia between the day of oestrus and 24h before oestrus in HP ewes than in LP ones ($p < 0.05$). However, a higher number of atretic follicles appeared on the ovaries of LP ewes between the day of oestrus and 1 day prior to oestrus ($p < 0.05$). For new follicles, a higher number appeared on the ovaries of LP ewes between the day of oestrus and 1 day prior to oestrus compared to the HP ewes ($p < 0.05$)



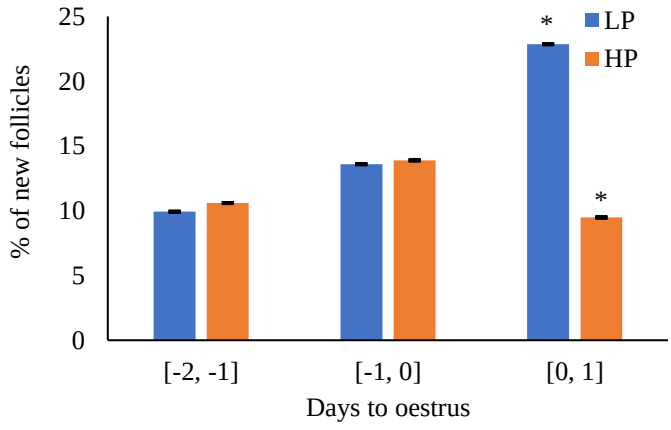


*Figure 4. Number (±SEM) of atretic and new follicles for all follicular sizes during the follicular phase of Bonga ewes that have single litter size (LP) and double or triple litter size (HP). Statistically significant differences are indicated with * ($p < 0.05$).*

3.5. Luteal function and plasma progesterone

Mean number of CL was significantly higher in HP group compared to the LP ewes (2.3 ± 0.15 and 1.28 ± 0. 14 for HP and LP, respectively) ($p = 0.033$). Most of the animals belonging to the LP group had 0, 1 and 2 CL while animals belonging to the high prolific group had 1, 2, 3 and 4 CL and the difference between the two groups of ewes was statistically significant ($p < 0.001$) (figure 5).

Mean plasma progesterone concentrations were significantly higher in HP than LP ewes (figure 6) ($p < 0.05$). At day 12 after oestrus, progesterone concentrations rose to a mean level of 5.60 ± 0.71 ng/ml in HP ewes which was higher ($P < 0.01$) than the concentration found in LP group (1.95 ± 1.63 ng/ml).

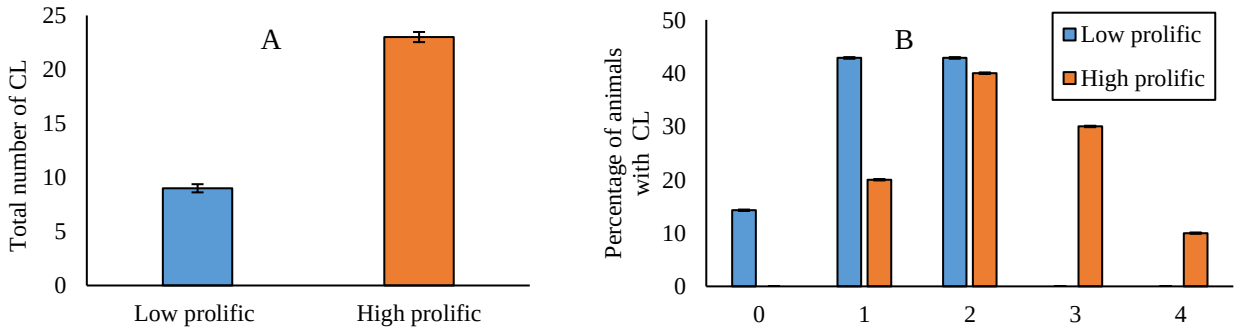


Figure 5. Number of CL (± SEM) (A) and distribution of the percentages (± SEM) of animals having 0, 1, 2, 3 or 4 CL (B) for low and high prolific Bonga ewes

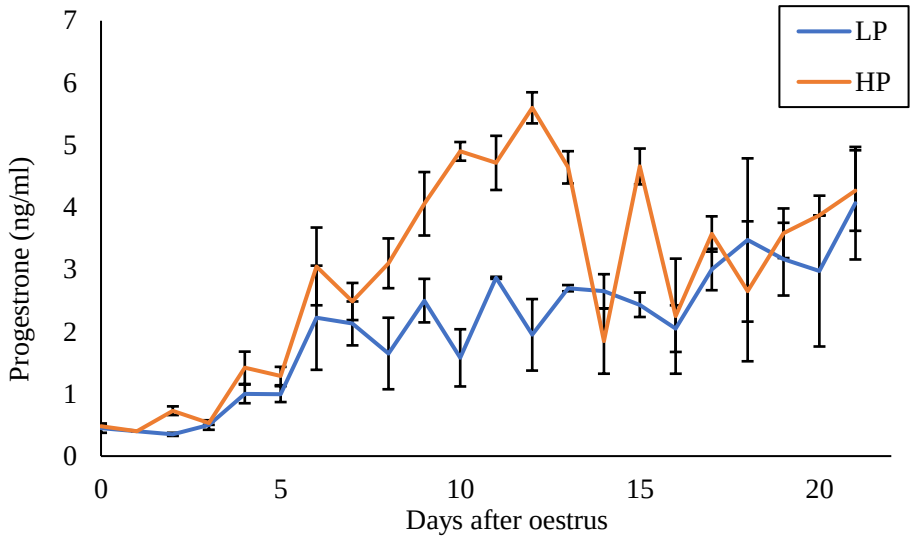


Figure 6. Plasma progesterone concentrations (±S.E.M.) for Bonga ewes that have single litter size (LP) and double or triple litter size (HP) after sponge removal every two days for 20 days

4. Discussion

Following sponges' removal and introduction of rams, 6/7 (85.71%) and 9/10 (90%) of ewes belonging to the low and high prolific group, respectively, were detected in oestrus. Therefore, the result of the current study indicates that manifestation of estrus was the same in the two groups of animals. This was not in concordance with what was obtained in other studies. In fact, Lassoued et al. 2013 found that 14 and 20 ewes of respectively prolific and non-prolific strains of the Barbarine breed were detected in oestrus following progestagen removal and introduction of rams. This difference may be due to the used breed and the period in which the trial was carried out.

The number of total follicles was significantly higher in HP group than in LP ewes. This observation was accentuated two days before oestrus. Furthermore, the mean number of medium follicles tend to be higher in the HP group than the LP group and this difference is especially observed 2 days prior to oestrus. Such result was observed by Lassoued et al. (2013) who found more total follicles for prolific ewes in comparison to non-prolific ones two days before oestrus. In this same study, a significantly higher numbers of medium follicles was

observed in prolific ewes two days before oestrus. Prior studies concerning follicular dynamics in prolific and non-prolific breeds of sheep showed that follicles attained maturity at a smaller diameter in prolific animals (Driancourt et al., 1986; Scaramuzzi and Radford, 1983 ; Souza et al., 1997). Zieba et al. (2001) indicated that in prolific sheep follicles achieve smaller maximum sizes and all phases of follicular development may occur with smaller follicles than they do in non-prolific breeds of sheep.

Plasma oestradiol concentrations and mean plasma progesterone concentrations were significantly higher in HP than LP ewes. When comparing prolific and non-prolific ewes, Lassoued et al. (2013) found also that plasma oestradiol concentrations were higher for prolific ewes. Authors think that oestradiol secretion was increased in prolific ewes because they have probably a higher number of ovulatory follicles (Lassoued et al., 2013).

In the present study, more follicles did undergo atresia between the day of oestrus and 24h before oestrus in HP ewes however a higher number of new follicles appeared on the ovaries of LP ewes between the day of oestrus and 1 day prior to oestrus. In the same way, Lassoued et al. (2013) found more atretic follicle during the last 24 h before oestrus in prolific ewes and a higher number of new follicles in non-prolific ewes between days 2 and 1 prior to oestrus.

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 significantly higher plasma progesterone concentrations in HP animals (Mesen and Young, 2016).

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Conflict of interest

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.