



## Ovarian activity in dairy Saanen goats subjected to a short-term ovulation induction protocol and a single injection of lecorelin (GnRH analog) given 28 h or 34 h after progestin pre-treatment

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### ABSTRACT

Fixed-time artificial insemination (FTAI) and hormonal superovulation (SOV) are instrumental in enhancing genetic gain and productivity of commercial goat herds. The goal of this study was to document changes in antral follicle populations and ovulation in dairy Saanen does. Fifty-six animals received MAP sponges that were left in place for 6 days as well as 200 IU of eCG and 120 µg of cloprostenol i.m. 24 h before sponge withdrawal. Does were randomly divided into three groups: G<sub>con</sub> (n = 18) received 1 mL of saline 28 h after, whereas G<sub>28h</sub> (n = 19) and G<sub>34h</sub> (n = 19) received 25 µg of lecorelin i.m. 28 h or 34 h after sponge removal. Estrus was detected with intact bucks every 12 h and transrectal ovarian ultrasonography was carried out from the time of sponge removal until ovulation detection (8/8 h – 60 h after sponge removal) and then until 156 h after sponge withdrawal (12/12 h). There were no differences ( $P > 0.05$ ) among GnRH-treated and control goats in estrus responses (G<sub>28h</sub>–57.9%; G<sub>34h</sub>–84.2%; G<sub>con</sub>–83.3%) and mean ovulation rates (G<sub>28h</sub>–100%; G<sub>34h</sub>–91.7%; G<sub>con</sub>–81.8%), but the intervals from MAP sponge withdrawal to ovulation and from the estrus onset to ovulation were less variable ( $P < 0.05$ ) in both GnRH-treated groups. The number of large antral follicles ( $\geq 6$  mm) decreased ( $P < 0.05$ ) from 52 h to 72 h after sponge removal and then rose ( $P < 0.05$ ) to 144 h and 122 h in G<sub>28h</sub> and G<sub>34h</sub>, respectively. Synchronous ovulation and distinctive pattern of antral follicle growth after short-term estrus synchronization with lecorelin injections at 28 h or 34 h after MAP sponge removal can pave the way for improving FTAI and SOV yields in goats.

### 1. Introduction

The application of assisted reproductive techniques in small ruminant farming has significantly improved animal genetic gains and production efficiency, especially in difficult natural and climatic conditions (Menchaca and Ungerfeld, 2017). Ultrasonography is a useful diagnostic and research tool, which paved the way for the development or improvement of assisted reproductive technologies. The use of transrectal ovarian ultrasonography in small ruminants has been instrumental in understanding the mechanisms governing antral follicular development and ovulation (Soboleva et al., 2000; Riesenber et al.,

2001; Esteves et al., 2013; Pietroski et al., 2013; Alvarado-Espino et al., 2016; Balara et al., 2016; Teixeira et al., 2016; Menchaca and Ungerfeld, 2017; Almeida et al., 2018; Nascimento-Penido et al., 2018; Hameed et al., 2020).

In the last decade, hormonal induction and synchronization of estrus, followed by natural mating or artificial insemination (AI), have been extensively tested in dairy goats (Abecia et al., 2012; Fonseca et al., 2019a,b; Gonzalez-Bulnes et al., 2020). In cattle, fixed-time artificial insemination (FTAI) has been widely used around the world (Bó et al., 2018; Fejoo et al., 2019; Thatcher and Santos, 2020), but due mainly to a lack of synchronous ovulations after estrus synchronization protocols

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currently used, FTAI is not commonly performed in goats (Maffili et al., 2006; Holtz et al., 2008; Esteves et al., 2013; Fonseca et al., 2017). Determining optimal time for insemination in small ruminants typically requires estrus detection, which is cumbersome and time-consuming, especially in large flocks, and not sufficiently accurate without appropriate personnel training (Fonseca et al., 2017). Therefore, several attempts have been made to improve the synchrony of ovulations in small ruminants undergoing estrus induction protocols, and the most promising results were obtained using GnRH-based protocols with (Husein and Kridli, 2003; Pierson et al., 2003; Luther et al., 2007; Titi et al., 2010) or without (Holtz et al., 2008; Al Yacoub et al., 2011; Martemucci and D'Alessandro, 2011; Nur et al., 2013) pre-treatment with progesterone-releasing intravaginal devices.

The Multiple Ovulation and Embryo Transfer (MOET) program is important for attaining genetic improvement in dairy goat operations. However, the outcome of MOET in small ruminants is highly variable due mainly to multiple intrinsic and extrinsic influencing factors (Candappa and Bartlewski, 2011; Ledda and González-Bulnes, 2018; Fonseca et al., 2019a,b). The best superovulatory results were obtained when superovulatory (SOV) treatments were initiated in presence of large numbers of small antral follicles and in the absence of a large/dominant follicle(s) (Guilbault et al., 1991; Huhtinen et al., 1992; Nasser et al., 1993; Menchaca et al., 2002, 2007b, 2009;). Considering that supposition, Menchaca et al. (2007b) devised the "Day 0 superovulatory protocol" for goats and Balaro et al. (2016) for ewes, wherein a GnRH agonist (buserelin or lecorelin respectively) is given to synchronize ovulation after a progestogen treatment and the SOV protocol commences 84 h or 80 h after progestin device withdrawal in goats and ewes, respectively; consequently, the superovulatory FSH regimen begins in the absence of dominant ovarian follicles.

Since the preovulatory LH peak heralding ovulation occurs in goats approximately 35 h after progestin pre-treatment (Pierson et al., 2003; Titi et al., 2010; Zarazaga et al., 2014), in the present study we decided to examine and compare the effects of lecorelin administered 28 h or 34 h after the end of progestin priming. Those times were chosen to induce a synchronous LH discharge prior to or around the time of the expected endogenous rise in LH secretion in most animals. The main objective of this study conducted in Saanen goats kept under tropical conditions was to employ transrectal ovarian ultrasonography to determine the time of ovulations and changes in numbers of ovarian antral follicles following lecorelin injections at 28 h or 34 h after progestogen sponge removal. We anticipate that the present results may pave the way for improving the synchrony of ovulation and hence conception rates during the FTAI procedure, and for maximizing ovarian responses and embryo yields in goats subjected to the "Day 0 protocol" in MOET programs.

## 2. Materials and methods

### 2.1. Location and experimental animals

All experimental procedures described in this section had been approved by the Ethical Committee for Animal Use at the Universidade Federal Fluminense (protocol 1021), and complied with the guidelines of the Brazilian Society of Animal Experimentation and of the Animal Research: Reporting of In vivo Experiments (Kilkenny et al., 2010). The present study was conducted in a dairy goat farm located in Rio de Janeiro state, Brazil (22°07'50.2"S), during the transition to the breeding season (January–February; Balaro et al., 2019). It used 56 Saanen goats [2.9 ± 0.5 years old, body condition score: 2.9 ± 0.3 (scale 1–5; Suiter, 1994) (mean ± SD)]. According to Köeppen (1948), the local climate is a tropical hot-humid type (Aw). None of the goats had any reproductive abnormalities detected by ultrasonography or clinical examination. Throughout the entire experiment, the does were kept in group pens and fed twice a day with corn silage and concentrate according to their maintenance requirement [16% crude protein; (National Research Council (NCR), 2007)]. Water and mineral salt licks

(Caprinofós, Tortuga, São Paulo, Brazil) were provided *ad libitum*.

### 2.2. Experimental procedures

All goats received intravaginal sponges containing 60 mg of medroxyprogesterone acetate (MAP; Progespon; Schering Plough, SP, Brazil) for 6 days. One day before sponge withdrawal, 200 IU of equine chorionic gonadotropin (eCG; Folligon, MSD, São Paulo, SP, Brazil) and 120 µg of cloprostenol sodium (Estron, Agner União, São Paulo, SP, Brazil) were administered i.m. After sponge withdrawal, the does were divided into three groups:  $G_{con}$  ( $n = 18$ ) received 1 mL of saline i.m. 28 h after sponge withdrawal;  $G_{28h}$  ( $n = 19$ ) received 25 µg of lecorelin i.m. 28 h after sponge withdrawal; and  $G_{34h}$  ( $n = 19$ ) received 25 µg of lecorelin i.m. 34 h after sponge withdrawal. Estrus was detected every 12 h with sexually mature, intact bucks for 96 h after sponge withdrawal.

Transrectal ovarian ultrasonography was performed using a portable scanner (Sonoscape S6, Shenzhen, China) equipped with a 7.5 MHz linear rectal transducer adapted for use in small ruminants. Animals were restrained and examined in a standing position. Eleven, 12 and 12 goats from  $G_{con}$ ,  $G_{28h}$  and  $G_{34h}$ , respectively, were used for ultrasonographic examinations carried out every 8 h from sponge withdrawal until ovulation detection (Day 0) and then every 12 h until Day 4 of the estrous cycle studied (96 h after ovulation). All detectable antral follicles were categorized into the following 3 size classes: small follicles ( $\leq 3.0$  mm), medium-sized follicles ( $< 3.0$  and  $< 6.0$  mm), and large follicles ( $\geq 6.0$  mm) (Ginther and Kot, 1994; De Castro et al., 1999; González-Bulnes et al., 1999). The preovulatory follicle diameter was defined as the last diameter recorded prior to ovulation detection. Ovulation time was defined as the mid-way time between the last ultrasonographic detection of the preovulatory follicle(s) and first time where such a follicle(s) was/were no longer recorded. Fig. 1 shows the experimental proceedings.

### 2.3. Statistical analyses

Data were analyzed using SAEG 9.0 statistical program (Universidade Federal de Viçosa, Minas Gerais, Brazil). The following variables were determined: (1) rate of estrus response, (2) duration of estrus, (3) interval from sponge withdrawal to the beginning of estrus, (4) interval from sponge withdrawal to ovulation, (5) interval from the beginning of estrus to ovulation, (6) number of ovulations, and (7) number of follicles in different size classes. Lilliefors's test was used to verify the normality of variables and Bartlett's test was used to see if the data were from populations with equal variances. The F variance test was used to examine the differences in variability among experimental groups. Parametric data (e.g., follicle growth–data presented in Fig. 2) were analyzed by one-way analysis of variance and Fisher's least significant difference (LSD) test for comparison on individual mean values. Non-parametric data (e.g., reproductive responses–data presented in Table 1) were analyzed using Kruskal-Wallis test and Dunn's test. For all tests,  $P$  value  $< 0.05$  was considered statistically significant.

## 3. Results

There were no significant differences among the three groups of goats for various intervals between MAP sponge removal, the onset of estrus and ovulation or for the duration of behavioral estrus, ovulation rates and preovulatory follicle diameter (Table 1). However, the standard deviation values for the mean interval from sponge withdrawal to ovulation and from the estrus onset to ovulation were lower ( $P < 0.05$ ) in both GnRH-treated groups ( $G_{28h}$  and  $G_{34h}$ ) than in the saline group ( $G_{con}$ ).

Mean numbers of small follicles did not differ ( $P > 0.05$ ) over time or among treatment groups (Fig. 2) In general, the number of medium-sized follicles increased ( $P < 0.05$ ) from 8 h before to 24 h after ovulation, and then began to decline with slight differences over time

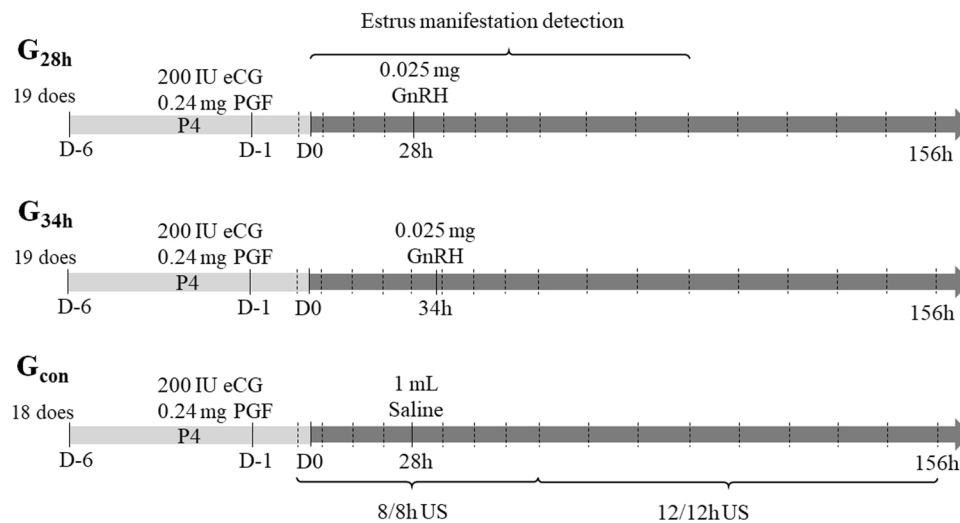


Fig. 1. Experimental proceedings timeline.

within each group (Fig. 2). The  $G_{28h}$  group exceeded ( $P < 0.05$ )  $G_{34h}$  and  $G_{con}$  in medium-sized follicle numbers at 52 h, 60 h, 72 h and 96 h, and  $G_{con}$  animals at 60 h after sponge withdrawal. In both GnRH-treated groups, large follicle numbers decreased ( $P < 0.05$ ) from 52 h to 72 h after sponge removal and then rose ( $P < 0.05$ ) to 144 h and 122 h in  $G_{28h}$  and  $G_{34h}$ , respectively. In  $G_{con}$ , a minimum number of large antral follicles was recorded at 96 h and then increased ( $P < 0.05$ ) to 144 h after sponge removal. The mean number of large follicles remained greater ( $P < 0.05$ ) in  $G_{con}$  compared with both GnRH treatment groups from 52 h to 72 h after sponge removal.

#### 4. Discussion

To the best of authors' knowledge, this is the first study of ovulatory responses and changes in antral follicular numbers after the progestin-based estrus synchronization protocol combined with the GnRH analog lecorelin treatment in goats. It is evident that lecorelin synchronized the occurrence of ovulation since both  $G_{28h}$  and  $G_{34h}$  groups exhibited less variable ovulation times than  $G_{con}$ . Similar results were obtained in cyclic ewes receiving lecorelin 24 h or 36 h after progestin-sponge withdrawal (Balara et al., 2016). Therefore, the lecorelin injections given at 28 h or 34 h after sponge withdrawal can potentially be used as a method to induce synchronous ovulation for FTAI in small ruminants.

In a previous study in cyclic Alpine goats subjected to the 5-day progesterone treatment and induced to ovulate with estradiol benzoate (EB) or eCG (Menchaca et al., 2007a) given at the time of eCG or 24 h after EB CIDR® withdrawal, ovulations occurred ~60 h and ~70 h after CIDR® removal in the treatment group and saline-injected controls, respectively. Variability in the mean ovulation times observed by Menchaca et al., 2007a was 50% less than in the present study (Table 1), but it can be attributed to synchronizing Alpine goats during the breeding season. However, the potential differences due to an application of either natural progesterone (CIDR®) or synthetic progestin (medroxyprogesterone acetate) and the duration of the treatment with progestogen may also play a role and remain to be elucidated.

In the present study, the number of does in estrus was lower for animals that received lecorelin injections 28 h after MAP sponge withdrawal than in goats injected 34 h after MAP treatment. These results are similar to those reported for ewes that received lecorelin injections 24 h or 36 h after MAP sponge removal (Balara et al., 2016). Diminished manifestations of estrous signs may be problematic if natural breeding is used, but a lack of behavioral estrus during application of the FTAI protocol is somewhat less important. The suitability of the presently

used GnRH treatments for FTAI and superovulatory protocols in goats has yet to be evaluated. In a recent trial conducted in our facility, the pregnancy rate in Saanen does subjected to a short-term estrus synchronization protocol and a single dose of lecorelin 34 h after sponge withdrawal was 56% (20/36; unpublished results); the experiment was carried out during the early anestrus period (August–October) and the does were inseminated 48 h after MAP sponge withdrawal. Those results were better compared with a trial by Nur et al. (2013) using buserelin before sponge insert at 48 h after sponge withdrawal in Saanen goats (24%–38%–breeding season at 40.19 °N), but similar to those obtained by Al Yacoub et al. (2011) (50%) also using buserelin at 48 h after prostaglandin treatment (does with at least 5 ng/mL of serum progesterone) in Boer goats (October–January, breeding season at 51°46'N). Therefore, lecorelin appears to be equally or more effective than currently used buserelin for synchronizing ovulation during AI protocols in goats.

Based on the number of large antral follicles detected using ultrasonography, the optimal time to begin the superovulatory regimen with exogenous FSH in lecorelin-treated goats (both 28 h and 34 h after sponge removal) would be within 12 h of ovulation or between 60–72 h

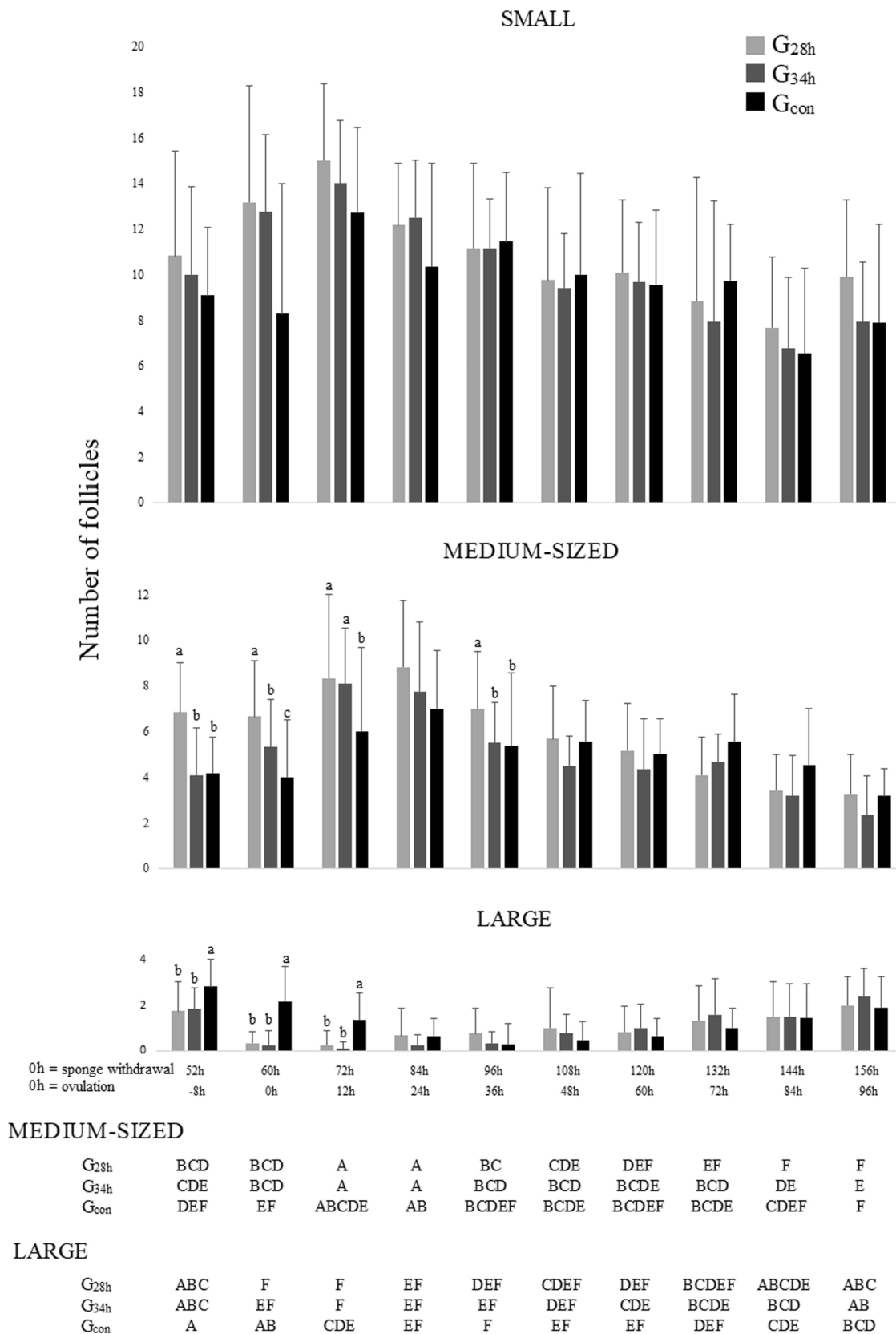
Table 1

Reproductive outcomes (mean  $\pm$  SD) recorded in Saanen does subjected to short-term estrus synchronization and receiving a saline solution ( $G_{con}$ ) or lecorelin treatment at 28 h ( $G_{28h}$ ) or 34 h ( $G_{34h}$ ) after sponge withdrawal.

Variables	$G_{28h}$	$G_{34h}$	$G_{con}$	Total
Rate of estrus manifestation (%) <sup>*</sup>	57.9 (11/19)	84.2 (16/19)	83.3 (15/18)	75.0 (42/56)
Duration of estrus (h) <sup>*</sup>	32.5 $\pm$ 17.1	26.8 $\pm$ 12.2	36.6 $\pm$ 19.6	31.8 $\pm$ 16.6
Sponge withdrawal to the beginning of estrus manifestation (h) <sup>*</sup>	26.4 $\pm$ 9.2	29.4 $\pm$ 9.5	28.7 $\pm$ 11.3	28.4 $\pm$ 9.9
Sponge withdrawal to ovulation (h) <sup>**</sup>	52.7 $\pm$ 4.2 <sup>B</sup>	53.8 $\pm$ 5.5 <sup>B</sup>	59.6 $\pm$ 13.6 <sup>A</sup>	55.0 $\pm$ 8.5
Beginning of estrus to ovulation (h) <sup>**</sup>	24.5 $\pm$ 10.4 <sup>AB</sup>	23.6 $\pm$ 10.2 <sup>B</sup>	35.3 $\pm$ 22.2 <sup>A</sup>	26.9 $\pm$ 14.5
Largest follicle diameter (mm) <sup>**</sup>	7.1 $\pm$ 0.9	7.9 $\pm$ 1.4	7.9 $\pm$ 1.3	7.6 $\pm$ 1.2
Number of ovulations <sup>**</sup>	2.0 $\pm$ 0.4	1.8 $\pm$ 0.7	2.3 $\pm$ 0.5	2.1 $\pm$ 0.6
Ovulatory response (%) <sup>**</sup>	100 (12/12)	91.7 (11/12)	81.8 (9/11)	91.4 (32/35)

<sup>A,B</sup> Different letters within rows denote a significant difference (F test,  $P < 0.05$ ).

<sup>\*</sup>  $G_{con}$  - n = 18;  $G_{28h}$  - n = 19;  $G_{34h}$  - n = 19 <sup>\*\*</sup>  $G_{con}$  - n = 11;  $G_{28h}$  - n = 12;  $G_{34h}$  - n = 12.



**Fig. 2.** Small (detectable follicles  $\leq 3.0$  mm), medium-sized ( $< 3.0$  and  $< 6.0$  mm) and large ( $\geq 6.0$  mm) antral follicle numbers determined ultrasonographically in Saanen does subjected to short-term estrus synchronization and receiving a saline solution (G<sub>con</sub>) or leirelin dose at 28 h (G<sub>28h</sub>) or 34 h (G<sub>34h</sub>) after sponge withdrawal from 52 to 156 h after sponge withdrawal (from 8 h before to 96 h after it).

Each column represents the mean and the error line presents the SD ( $\pm$ ).

<sup>a, b</sup> Different letters within the chart area represent a significant difference between treatments (Fisher LSD test,  $P < 0.05$ ).

<sup>A, B</sup> Different letters denote means with significant differences over time (for medium-sized and large antral follicles; Fisher LSD test,  $P < 0.05$ ).



after MAP sponge removal. Due mainly to highly synchronous ovulation times, detectable numbers of large antral follicles are consistently low during that time window. During that period, there is also a gradual (numerical) increase in small follicle numbers and a significant rise in the numbers of medium-sized follicles. The latter may indicate an increasing responsiveness of ovarian follicles to FSH stimulation. Bartlewski et al. suggested that the number of medium-sized antral follicles in superovulated ewes is a better predictor of ovulatory responses in SOV ewes than small follicle numbers (Bartlewski et al., 2016, 2008). This suggested time to commence the SOV treatment in goats differs from the original protocol developed by Menchaca et al. (2007b), wherein FSH administration begins 84 h after sponge withdrawal. According to our study, at 84 h after sponge withdrawal the numbers of large antral follicles are still relatively low, but the numbers of medium-sized antral follicles begin to decline in GnRH treatment groups. In control goats, however, the 84-h mark appears to be optimal due to the lowest number of large and peak number of medium-sized antral follicles. As mentioned earlier, more confirmatory studies are needed to corroborate the effectiveness of this treatment in superovulated goats.

## 5. Conclusion

The administration of 25 µg of GnRH analog leirelin, either 28 h or 34 h after the removal of MAP-soaked intravaginal sponges inserted for 6 days, improves the synchrony of ovulations in Saanen goats raised in a tropical climate. Therefore, this treatment can potentially be used for FTAI programs. In addition, ultrasonographic observations revealed that the highest numbers of small antral follicles and the absence of large, ostensibly dominant, follicles occurred between 60 h and 72 h after sponge withdrawal in GnRH-treated animals, suggesting that this would be an optimal period to start a SOV treatment (Day 0 protocol) in Saanen goats under tropical conditions.

## Authors' contributions

IOC, MFAB, FZB co-designed the present study and organized the experiment. IOC, MFAB, FSCL, ALCB, LFCB, FMG, PRCS, PVSP, FZB collected and analyzed the data as well as wrote and revised the manuscript. PMB helped with data interpretation and critical review of the present paper. All authors approved its final version.

## Declaration of Competing Interest

None of the authors has any conflict of interest to declare.

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