Ciência Animal Brasileira
Brazilian Animal Science

DOI: 10.1590/1809-6891v27e-83388E

e-ISSN 1809-6891
Animal science | Research article

Previous evaluation of ovarian follicular dynamics as an indicator to initiate superovulation protocol in acyclic Toggenburg goats

[Avaliação prévia da dinâmica folicular ovariana como indicador para iniciar protocolo de superovulação em cabras Toggenburg acíclicas]

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Received: Jul 14, 2025. Accepted: Feb 24, 2026. Published: Ap 02, 2026. Editor: Rondineli P. Barbero and José Felipe W. Spricigo

Abstract: This study evaluated ovarian follicular dynamics during estrus induction to determine the optimal time to initiate superovulation (SOV) in acyclic Toggenburg goats. In Experiment 1, goats (n = 6) underwent estrus induction using intravaginal sponges containing 60 mg medroxyprogesterone acetate for six days, combined with 37.5 µg d-cloprostenol administered via the laterovulvar (l.v.) route on Day 0 (D0). Ovarian ultrasonography was performed daily from D0 until ovulation (D9). The population of Class 1 follicles (≤ 3.9 mm) progressively increased, reaching a maximum on D4. On this day, significantly fewer Class 3 (5.0 - 5.9 mm) and Class 4 (≥ 6.0 mm) follicles were observed compared with Class 1 follicles ($P < 0.0001$). Based on the follicular profile identified in Experiment 1, SOV in Experiment 2 was initiated on D4, the first day characterized by the highest number of follicles ≤3.9 mm and a minimal number of follicles ≥6 mm. Goats were allocated into two groups: superovulated (G_{SOV}, n = 10) and non-superovulated ($G_{non-SOV}$, n = 10), followed by nonsurgical embryo recovery (NSER). No difference in estrus response was observed between $G_{non-SOV}$ (100 %) and GSOV (90 %) ($P > 0.05$). However, G_{SOV} exhibited a greater mean corpora lutea count and a superior mean number of recovered structures (10.8 ± 0.2 and 6.3 ± 0.8, respectively) compared with $G_{non-SOV}$ (1.6 ± 0.3 and 1.0 ± 0.2, respectively). Conversely, the proportion of viable structures was superior in $G_{non-SOV}$ (60 %) than in G_{SOV} (10.5 %) ($P < 0.05$). These findings suggest that ovarian follicular profiling may be a suitable approach for guiding SOV in acyclic goats. However, further investigation is warranted, particularly regarding the optimal timing of follicle-stimulating hormone (FSH) administration within the follicular wave.

Keywords: caprine; FSH; NSER; progesterone.

Resumo: Este estudo avaliou a dinâmica folicular ovariana durante a indução do estro para identificar o melhor momento para iniciar a superovulação (SOV) em cabras Toggenburg acíclicas. No Experimento 1, cabras (n = 6) foram submetidas à indução do estro com esponjas (60 mg de acetato de medroxiprogesterona) por seis dias, associada a 37,5 µg de d-cloprostenol via laterovulvar

(I.v.) no D0. A ultrassonografia ovariana foi realizada do D0 até a ovulação (D9). A população folicular (Classe 1, ≤ 3,9 mm) aumentou gradualmente, atingindo seu pico máximo no D4, no qual foram observados menos folículos de Classe 3 (5.0 a 5,9 mm) e Classe 4 (≥ 6 mm) em comparação à Classe 1 (P < 0,0001). Considerando o perfil folicular ovariano no Exp. 1, a SOV no Exp. 2 foi iniciada no primeiro dia que apresentou o maior número de folículos ≤ 3,9 mm, com apenas alguns folículos ≥ 6 mm (D4). As cabras foram divididas em dois grupos, superovuladas (G_{SOV}, n = 10) ou não ($G_{não\text{-}SOV}$, n = 10), posteriormente, submetidas à coleta não cirúrgica de embriões (CNCE). Não houve diferença na resposta ao estro entre Gnão-SOV (100 %) e G_{SOV} (90 %) (P > 0,05). G_{SOV} teve maior contagem média de corpos lúteos e maior média de estruturas recuperadas (10,8 ± 0,2 e 6,3 ± 0,8) em comparação com $G_{não\text{-}SOV}$ (1,6 ± 0,3 e 1,0 ± 0,2), respectivamente (P < 0,05). A taxa de estruturas viáveis foi maior em $G_{não\text{-}SOV}$ (60 %) em comparação com G_{SOV} (10,5 %) (P < 0,05). O uso do perfil ovariano para superovulação em cabras acíclicas parece ser apropriado, no entanto, requer mais investigação, particularmente em relação ao momento da administração de hormônio folículo estimulante (FSH) dentro da onda folicular.

Palavras-chave: caprino; FSH; CNCE; progesterona.

1. Introduction

In vivo embryo production efficiency in goats has been associated with several intrinsic and extrinsic factors [1]. Breed, nutrition, gonadotropin dose and type, progestogen/progesterone priming duration, and ovarian follicular status at the onset of superovulation (SOV) have been extensively investigated to improve embryo yield [2]. Despite efforts to control these variables, embryo production efficiency remains limited by variability in superovulatory response and reproductive seasonality [3].

Dairy goats managed under tropical conditions, such as in southeastern Brazil, exhibit marked reproductive seasonality characterized by the absence of estrous behavior and ovulation [4]. This phenomenon is particularly evident in specialized dairy breeds such as Toggenburg goats. In this context, the efficiency of SOV programs remains challenging, largely due to high variability in response to superovulatory treatments[2]. Consequently, variations in follicular populations must be considered when designing SOV protocols for embryo collection. It is well established that during the anestrus season, ovulation does not occur; however, follicular dynamics persist, with follicles progressing from the antral to the dominant stage under the influence of follicle-stimulating hormone (FSH) [5]. Therefore, implementing SOV protocols based on specific phases of the follicular wave may improve embryo collection outcomes in acyclic females.

In cattle, Kim et al. [6] demonstrated that removal of the dominant follicle 48 h prior to superstimulation in Holstein cows accelerated follicular growth and increased the number of medium and large follicles, corpora lutea, and progesterone concentrations. In goats, Menchaca et al. [7] initiated FSH superstimulation in the absence of a dominant follicle compared with a conventional protocol, resulting in a greater number of corpora lutea and improved embryo recovery. These findings support strategies aimed at preventing follicular dominance to promote more homogeneous follicular recruitment and enhance ovulatory and embryonic responses.

Superovulation studies rarely include simultaneous evaluation of unstimulated donors, possibly due to the cost and logistical constraints associated with surgical embryo recovery. In this regard, nonsurgical embryo recovery (NSER) may facilitate comparison of embryo production efficiency between superovulated and unstimulated animals under equivalent conditions [8]. Thus, considering B-mode transrectal ultrasonography performed to assess ovarian follicular dynamics,

this study aimed to determine whether prior characterization of follicular dynamics during estrus induction could identify the optimal timing for initiation of SOV treatment within the follicular growth wave in acyclic Toggenburg goats. Additionally, embryo production outcomes were compared between SOV and non-SOV protocols.

2. Material and methods

2.1. Ethics, location, period, animals and experimental design

This research was approved by the Animal Care Committee of Fluminense Federal University (0116-2011). The study was conducted on a farm in Piau, Zona da Mata region of Minas Gerais, Brazil (21º 35' S, 43º 15' W, 435 m altitude), during the anestrus period (September to December). According to Balaro et al.[4], seasonal anestrus in dairy goats in a semi-humid tropical region occurs between the end of August and mid-December, with progesterone concentrations approaching zero. During the experiment, animals were housed in collective pens and fed with balanced concentrate, corn silage, chopped elephant grass (*Pennisetum purpureum*), and/or sugar cane, in addition to water and mineral salt *ad libitum.*

Animals were pre-selected based on the absence of a corpus luteum in two consecutive ultrasonographic evaluations performed seven days before initiation of the hormonal protocols. The study consisted of two sequential experiments (Exp. 1 and 2). In Exp. 1, pluriparous goats (n = 6, 2.8 ± 0.1 Body Condition Score - BCS; 53.1 ± 4.3 kg) underwent estrus induction using intravaginal sponges containing 60 mg of medroxyprogesterone acetate (MAP; Progespon®, SD Saúde Animal, São Paulo, Brazil) for six days, plus 37.5 µg d-cloprostenol administered via the laterovulvar (l.v.) route at sponge insertion. The number and diameter of antral follicles, as well as the day of follicular wave emergence and ovulation, were evaluated by ultrasonography. Estrus was monitored twice daily (08:00 and 18:00) using mature males. Based on the ovarian follicular population profile observed in Exp.1, the probable day of follicular wave emergence was identified as the first day presenting the highest number of follicles ≤ 3.9 mm and a minimal number of follicles ≥ 6 mm.

Exp. 2 was conducted based on the ovarian follicular profile identified in Exp. 1. Goats were allocated to either a superovulated group (G_{SOV}; n = 10; 3.4 ± 0.2 BCS; 61.8 ± 2.9 kg) or a non-superovulated group ($G_{non\text{-}SOV}$; n = 10; 3.2 ± 0.3 BCS; 61.8 ± 5.2 kg). Estrus was induced using CIDR® (0.33 g progesterone; Zoetis, São Paulo, Brazil) for six days, with 37.5 µg d-cloprostenol administered l.v. on D0. $G_{non\text{-}SOV}$ received 37.5 µg d-cloprostenol l.v. plus 200 IU eCG (Novormon 5000®, Zoetis, São Paulo, Brazil) administered i.m. on D6. G_{SOV} received 200 mg FSH (Folltropin-V®, Vetoquinol, Ontario, Canada) administered i.m. in six decreasing doses (25–25–15–15–10–10 %) starting on D4 (as defined in Exp. 1), in addition to two doses of 37.5 µg d-cloprostenol l.v. on D6 and D6.5. Both groups received three doses of 2.2 mg/kg flunixin meglumine (Banamine®, Intervet, Rio de Janeiro, Brazil) administered i.m. on D9.5, D10.5, and D11.5. Additionally, 37.5 µg d-cloprostenol was administered i.m. at NSER on D14.

After removal of the intravaginal device, estrus behavior was monitored, and the females were mated every 12 h until the end of estrus. Adult bucks were used under controlled management, respecting a maximum mating ratio of 1:4 (buck:goat). Bucks underwent andrological examination to assess semen quality prior to mating. The NSER was performed according to Fonseca et al. [8]. Subsequently, recovered oocytes/embryos were identified and classified according to Mapletoft et al. [9].

2.2 Ovarian dynamics

In Exp. 1, ultrasonographic (US) examinations were performed at 24-h intervals from D0 to D6 and subsequently at 12-h intervals from D6 to D9 or until ovulation. In Exp. 2, the ovaries were identified, and the number and location of ovarian follicles were recorded every 12 h, beginning with the first dose of FSH and continuing until ovulation. The first ovulation was defined as the disappearance of at least one large follicle visualized during the previous examination, as described by Arashiro et al. [10]. The number of corpora lutea (CL) was determined by US 24 h prior to NSER. Monitoring of follicular wave patterns (Exp. 1) and assessment of superovulatory response (CL count; Exp. 2) were performed using B-mode US (Mindray M5 Vet, Shenzhen, China) equipped with a 7.5 MHz multifrequency linear transducer adapted for small ruminants. Follicles were classified according to diameter into four categories: small follicles (Class 1, ≤ 3.9 mm), medium follicles (Class 2, 4.0-4.9 mm), large follicles (Class 3, 5.0-5.9 mm) and preovulatory follicles (Class 4, ≥ 6.0 mm). The follicular wave was defined as a cohort of follicles emerging and growing from 2.5 to 3.9 mm [11]. The day selected for initiation of SOV treatment was determined based on identification of the ovulatory follicular wave in Exp. 1. Primary consideration was given to the day exhibiting a low number of Class 4 follicles, and secondarily to the day with the highest number of Class 1 follicles. If more than one day presented the same number of follicles ≥ 6.0 mm, SOV treatment was initiated on the day with the lowest combined number of Class 3 and Class 4 follicles (5.0 - 5.9 and ≥ 6.0 mm). The use of these follicular size classes (≤ 3.9 mm; 4.0 - 4.9 mm; 5.0 - 5.9 mm; ≥ 6.0 mm) was intended to provide a more precise distribution of intermediate follicular growth stages, thereby enabling a more detailed evaluation of ovarian response to the hormonal protocol employed.

2.3 Blood sampling and hormonal analysis

Blood samples were collected via jugular venipuncture into anticoagulant-containing tubes to determine plasma progesterone (P_4) concentrations from D0 to D14 and estradiol (E_2) concentrations at three time points: the day of ovulation, 24 h after ovulation, and the day of NSER. Plasma P_4 concentrations were measured using a solid-phase radioimmunoassay (RIA) (sensitivity: 0.05 ng/mL; intra-assay coefficients of variation [CV]: 10 %), whereas E_2 concentrations were determined by liquid-phase RIA (sensitivity: 5 pg/mL; [CV]: 7 %) using commercial kits (MP Biomedicals, LLC, Diagnostics Division, Orangeburg, NY, USA).

2.4 Statistical analysis

Statistical analyses for Exp. 1 and 2 were performed using the software SIRVAR 5.3. Parametric data were analyzed by analysis of variance (ANOVA), and means were compared using Student's *t* test or the Student–Newman–Keuls (SNK) post hoc test, as appropriate. Dichotomous qualitative variables were evaluated using contingency tables and analyzed by the chi-square (χ^2) test. Statistical significance was set at $P < 0.05$.

3. Results and discussion

To the best of the authors' knowledge, few studies in goats have utilized prior characterization of ovarian follicular wave patterns to determine the optimal timing for initiation of superovulation, with the objective of avoiding dominant follicles and prioritizing recruitment of younger follicles.

Although the administration of d-cloprostenol in anestrous females lacking a CL detectable by ultrasonography may appear contradictory, its use is supported by strategic considerations. Anestrus may vary among individuals, and sporadic ovulations can occur, thus, d-cloprostenol ensures luteolysis of any functional luteal tissue, thereby standardizing the hormonal status of the group. Moreover, the study was conducted on a commercial farm under field conditions, where complete isolation from males was not feasible. This may have induced an unintended "male effect," potentially triggering silent ovulations and formation of CLs not detected during the initial US examination. Additionally, d-cloprostenol may exert a beneficial uterine effect by promoting uterine clearance, thereby optimizing the uterine environment for implantation following mating or artificial insemination. Finally, at latitudes near the Equator, reproductive seasonality is attenuated due to minimal photoperiod variation. Under adequate nutritional management, breeds such as Toggenburg may exhibit near-continuous polyestrous activity, supporting the rationale for the protocol employed.

In Exp. 1, the predominant follicular population throughout the observation period consisted of small and medium follicles (Classes 1 and 2) compared with large and preovulatory follicles (Classes 3 and 4, respectively) ($P < 0.0001$) (Fig. 1).

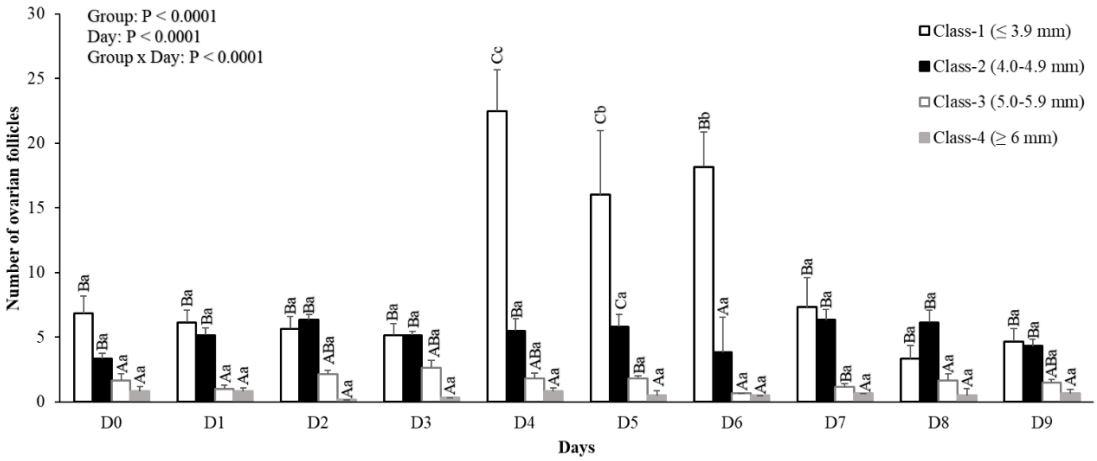

Figure 1. Average number of follicles identified and classified by size (Class 1, ≤ 3.9 mm; Class 2, from 4.0 - 4.9 mm; Class 3, from 5.0 - 5.9 mm; and Class 4, ≥ 6.0 mm) of Toggenburg goats subjected to estrus induction (Exp. 1). ^{A,B} Means compare the follicular size classes on each day. ^{a,b} Means compare the days in each follicular size class (Student-Newman-Keuls test; $P < 0.05$).

The population of Class 1 follicles varied across days, with a significant peak in the number of follicles ≤ 3.9 mm observed on D4 compared with other time points ($P < 0.0001$), indicating intense recruitment of small follicles and emergence of a new follicular wave. Accordingly, D4 was selected as the day to initiate SOV treatment, corresponding to 48 h before device removal. On D4, the greater number of small follicles (Class 1) suggested that the dominant follicle present since D0 was undergoing atresia. This physiological state favored recruitment of a new follicular cohort, as evidenced by the increase in small follicles. Thus, six days of progestogen treatment appeared to promote functional regression of the dominant follicle, enabling synchronized recruitment of a new follicular wave and defining a more appropriate time for initiation of superovulation. Characterization of follicular populations to guide estrus induction and embryo production protocols in acyclic goats during the non-breeding season is essential to optimize year-round reproductive efficiency. However, data supporting this approach remains limited.

Despite considerable variability, Exp. 1 demonstrated an increased number of follicles on D4, D5, and D6. The superovulatory strategy was therefore designed to minimize the deleterious effects of dominant follicles, which can suppress FSH secretion and compromise growth of subordinate follicles, or alternatively to initiate FSH supplementation when the majority of follicles are responsive to gonadotropin stimulation. Four days after insertion of the intravaginal P_4 device corresponded to the highest number of small follicles and a reduced number of large follicles. Nevertheless, the presence of dominant follicles may impair the development of gonadotropin-dependent follicles (3 – 5 mm), suppressing FSH and inducing atresia [12]. In the present study, although D4 showed a high number of Class 1 follicles, Class 4 follicles were still present and may have influenced the developmental potential of smaller follicles. Veiga-Lopez et al.[5] reported that the ideal follicular population for initiation of SOV consists of the absence of large follicles and a high number of gonadotropin-responsive follicles. It is noteworthy that Exp. 1 used intravaginal sponges containing 60 mg medroxyprogesterone acetate, whereas Exp. 2 employed a CIDR containing 0.33 g progesterone. Differences in P_4 source and release profile may have influenced follicular dynamics between protocols, potentially affecting both embryo quantity and quality.

In the present study, the CL count in goats subjected only to synchronized estrus induction (1.6 CLs) was considered within the physiological range and comparable to cyclic goats mated during the breeding season (1.7 prolificacy) [13] or to goats subjected to synchronized estrus induction (1.8 ovulations) [14]. The SOV strategy adopted resulted in an approximately sevenfold increase in ovarian response in superovulated goats (10.8 CLs; G_{SOV}) compared with non-superovulated goats (1.6 CLs; $G_{non\text{-}SOV}$), as expected. Furthermore, the CL count observed in the G_{SOV} (10.8 ± 0.2 CLs) exceeded values reported by Camacho et al. [15] (8.5 ± 0.5 CLs) in superovulated Boer goats, by Maia et al. [16] (8.5 ± 1.3 CLs) in dairy goats subjected to CIDR-based protocols, and by Bruno-Galarraga et al. [3] (9.9 ± 1.3 CLs) in superovulated Criolla-Neuquina goats using a MAP-based protocol.

Regarding plasma P_4 concentrations (Fig 2A), a significant interaction between treatment and day of evaluation was observed (P = 0.02). During the estrus induction phase (D0 - D6), concentrations were similar in G_{SOV} (3.6 ± 0.5 to 2.7 ± 0.9 ng/mL) and $G_{non\text{-}SOV}$ (2.0 ± 0.5 to 1.4 ± 0.4 ng/mL) respectively. Following device removal, P_4 concentrations decreased on D7 in G_{SOV} (0.3 ± 0.0 ng/mL) compared with preceding days. Thereafter, concentrations progressively increased in both groups, reaching peak values on D11 in $G_{non\text{-}SOV}$ (14.7 ± 3.3 ng/mL) and D12 in G_{SOV} (15.0 ± 2.9 ng/mL), followed by a marked decline on D13 and D14. No differences between groups were detected on individual assessment days (P > 0.05).

Both groups exhibited a decline in P_4 following CIDR removal; however, the pattern of decrease differed. In $G_{non\text{-}SOV}$, progesterone concentrations declined abruptly, suggesting that circulating P_4 primarily originated from the intravaginal device rather than endogenous luteal secretion. In contrast, G_{SOV} showed a more gradual reduction, consistent with prostaglandin-induced luteolysis of active C L combined with device withdrawal.

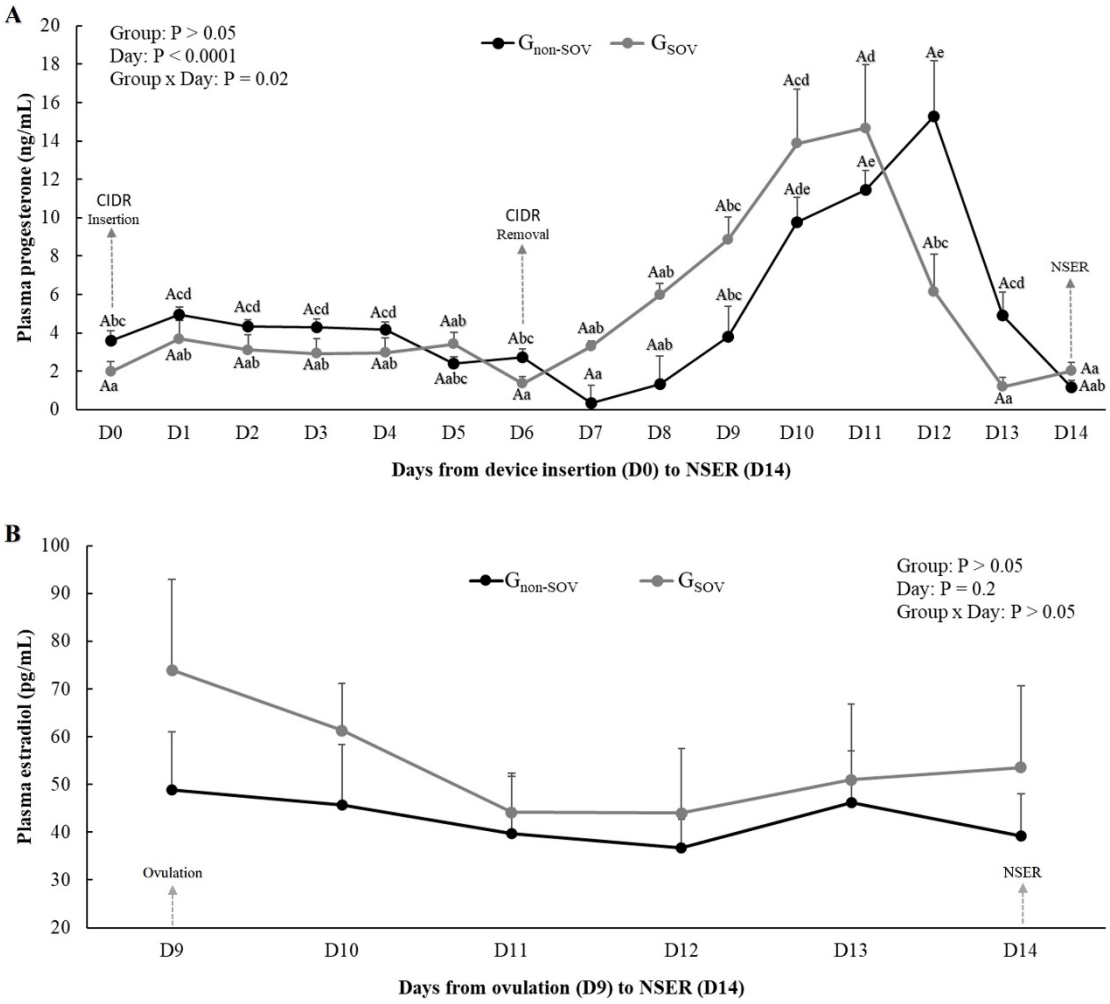

Figure 2. (A) Plasma progesterone concentration (ng/mL) and (B) estradiol concentration (pg/mL) of acyclic Toggenburg goats superovulated (G_{SOV}) non-superovulated ($G_{non\text{-}SOV}$) followed by nonsurgical embryo recovery (NSER; D14). [a,b] Means compare the days in each treatment (Student-Newman-Keuls test; $P < 0.05$).

In the present study, differences in fertilization rate were observed between non-superovulated and superovulated animals. However, the results obtained in G_{SOV} were comparable to those reported by Fonseca et al. [8], who observed unfertilized structures (4.2 ± 3.7 and 6.0 ± 4.2) in superovulated Saanen goats with or without recombinant bovine somatotropin (rbST) administration. An abrupt decline in P_4 concentrations was observed on D13 and D14 in both groups, despite the expectation of sustained high luteal P_4 levels at this stage. This decrease may be associated with premature luteal regression (PRCL), resulting in reduced P_4 concentrations and a suboptimal uterine environment for embryo implantation. Abnormal P_4 concentrations are known to negatively affect superovulatory response and embryo quality [17]. In the present study, the mean P_4 concentration on the day of NSER was 2.02 ng/mL in $G_{non\text{-}SOV}$ and 1.15 ng/mL in G_{SOV}. These relatively low concentrations may have adversely affected embryo quality. At the beginning of Exp. 2, plasma P_4 concentrations ranged between 2 and 4 ng/mL, suggesting that some goats may have retained functional CL. This finding may be related to a male effect induced during estrus detection in the previous experiment. Regarding plasma E_2 concentrations (Fig. 2B), no

differences were observed between groups (P > 0.05), across days (P = 0.2), or in the interaction between treatment and day (*P* > 0.05). Nevertheless, a progressive increase in E_2 concentrations was observed from D12 until the day of NSER, which was not anticipated.

Data related to superovulatory and estrus induction responses, as well as NSER efficiency (Exp. 2), are presented in Table 1. Considerable rates of unfertilized oocytes were observed. This may be attributed to estrogenic overstimulation in superovulated animals, which can impair sperm transport, induce asynchrony in oocyte maturation, and promote premature ovulation [18]. Repeated FSH administration may also disrupt the E_2-to- P_4 ratio following SOV, potentially inducing inadequate follicular luteinization[1], thereby compromising CL quality and function and ultimately affecting NSER recovery rates. The recovery rates observed in the present study (G_{SOV} 64.1 a $G_{não\text{-}SOV}$ 74.1 %) were superior than those reported by Camacho et al. [15], who obtained recovery rate of 39 % and 41 % in goats synchronized with intravaginal sponge and CIDR, respectively. However, they were lower than those described by Maia et al. [16], who reported an 81.7 % recovery rate in healthy superovulated goats.

It is also important to consider the potential impact of anestrous period on oocyte quality. Souza-Fabjan et al. [19] demonstrated that reproductive seasonality influences in *in vitro* embryo production in adult goats, reporting reduced cleavage and blastocyst formation rates during anestrus. These findings suggest reduced oocyte competence outside the breeding season, which may have contributed to the increased incidence of degenerated and unfertilized oocytes observed in the present study.

The present study did not include eCG in the hormonal protocols. Although P4 combined with eCG is widely used for estrus induction and synchronization in anestrous females, its role in superovulatory protocols remains uncertain. Due to its prolonged half-life and combined FSH and LH-like activity, eCG may interfere with exogenous FSH action, particularly depending on the timing of administration. The concomitant use of eCG and FSH may result in excessive follicular growth, heterogeneity in follicular quality, and premature luteinization, potentially compromising oocyte and embryo quality [20]. Therefore, the decision to use a P_4-and FSH-based protocol exclusively aimed to provide greater control over follicular dynamics while minimizing hormonal interactions that could negatively affect superovulatory results. Thus, the SOV protocol used was based on other studies[18, 21].

Table 1. Reproductive parameters of Toggenburg goats superovulated (G_{SOV}) and non-superovulated ($G_{non-SOV}$) followed by nonsurgical embryo recovery (NSER) during the anestrus season (data expressed as % or mean ± SEM).

Parameters	Experimental groups	
	$G_{non-SOV}$	G_{SOV}
Estrus response (%)	100.0 (10/10)	90.0 (9/10)
Interval to estrus (h)	32.0 ± 2.5[a] (10)	19.8 ± 0.9[b] (9)
Goats ovulating (%)	90.0 (9/10)	90.0 (9/10)
Interval from estrus onset to ovulation (h)	26.2 ± 2.4 (9)	34.3 ± 3.2 (9)
Interval from device removal to ovulation (h)	58.2 ± 3.3 (9)	53.8 ± 3.3 (9)
CL count	1.6 ± 0.3[b] (16)	10.8 ± 0.2[a] (97)
NSER success (%)	100.0 (9/9)	100.0 (9/9)
NSER duration (min)	21.4 ± 2.7	26.8 ± 1.8
Flushing recovery rate (%)	98.2 ± 0.6	96.6 ± 1.0
Recovery rate (%)	74.1 ± 25.7 (10)	64.1 ± 22.0 (57)
Structures recovered	1.0 ± 0.2[b] (10)	6.3 ± 0.8[a] (57)
Viable structures	0.6 ± 0.3 (6)	0.7 ± 0.8 (6)
Degenerated structures	0.2 ± 0.3 (2)	0.3 ± 0.6 (3)
Unfertilized eggs	0.2 ± 0.3[b] (2)	5.3 ± 0.8[a] (48)
Donors with structures recovered (%)	88.8 (8/9)	88.9 (8/9)

Interval to estrus - time in hours from device removal to the onset of estrus. ( ) Number of animals or structures counted. [a,b] Means with different superscripts within rows differed (Tukey or Kruskal-Wallis-Dunn; $P < 0.05$).

Although different progestogen sources were used across experiments, existing evidence indicates that stabilization of plasma P_4 concentrations and suppression of follicular dominance occur effectively regardless of the device used [(22)]. Furthermore, the frequency of handling and restraint of donors under commercial farm conditions was intentionally minimized to reduce stress and potential interference with reproductive performance. Nevertheless, the absence of a detailed evaluation of follicular dynamics in donors during Exp. 2 represents a limitation of the present study. Future investigations should incorporate systematic monitoring of follicular dynamics prior to initiation of SOV to enhance protocol standardization and improve reproducibility of results.

4. Conclusion

The use of the ovarian follicular profile as a criterion to initiate SOV protocols in goats during the non-breeding season warrants further investigation. In particular, future studies should evaluate the effects of initiating FSH administration at different stages of the follicular wave to determine the optimal timing for superovulatory response.

Conflict of interest statement

The authors declare no conflicts of interest.

Data availability statement

The complete dataset supporting the results of this study is available upon request from the corresponding author.

Author contributions

Methodology: Gonçalves, J. D., Maia, A. L. R. S., Esteves, L. V., Fonseca, J. F.; Validation: Gonçalves, J. D., Silva, M. R.; Investigation: Gonçalves, J. D., Maia, A. L. R. S., Esteves, L. V., Silva, M. R., Fonseca, J. F.; Formal analysis: Gonçalves, J. D., Silva, M. R., Souza-Fabjan, J. M. G., Brandão, F. Z.; Resources: Fonseca, J. F.; Writing – original draft:Maia, A. L. R. S., Esteves, L. V.; Writing – review & editing: Gonçalves, J. D., Souza-Fabjan, J. M. G., Oliveira, M. E. F., Brandão, F. Z., Fonseca, J. F.; Supervision: Fonseca, J. F.; Project administration: Fonseca, J. F.; Funding acquisition: Fonseca, J. F.

Generative AI use statement

The authors did not use generative artificial intelligence tools or technologies in the creation or editing of any part of this manuscript.

Acknowledgments

Os autores agradecem ao Dr. José Henrique Bruschi (*in memoriam*) e à Dra. Marlene Bruschi, da Agropecuária Água Limpa, pelo fornecimento dos animais, alojamento e alimentação. Agradecem também à Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA; Projeto 20.19.01.004.00.03), à Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG; Projetos CVZ-APQ 01367/09 e CVZ PPM 00119-11) e à Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) pelo apoio financeiro a este estudo. ALRSM, FZB e JMGSF são bolsistas da FAPERJ. JDG, FZB, JFF, JMGSF e MEFO são bolsistas do CNPq.

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