Correlation between estrus onset and ovarian parameters in dairy goats

Initio do estrou e sua correlação com os parâmetros ovarianos em cabras leiteiras

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Abstract
This study assessed the effects of social dominance on the behavioral estrus and ovarian parameters of dairy goats subjected to synchronous estrus induction during the non-breeding season. Synchronous estrus was induced in 23 dairy goats allocated to collective pens. Ovarian ultrasonography was performed at 12-hour intervals from device removal to ovulation. Upon estrus onset (EO) detection, the goats were immediately removed from the pens. Dominance was scored according to EO in relation to device removal in each pen, with HD – high dominants (<36 h; n = 8), MD – medium dominants (36 to 48 h; n = 10) and LD – low dominants (>48 h; n = 5). Goats in estrus underwent flexible time artificial insemination (FxTAI) according to EO. The estrus response was 100.0%. The interval to estrus was longer (P<0.05) in LD (53.7 ± 6.5 h) than in MD (37.9 ± 5.5 h) and HD goats (32.3 ± 6.6 h), while the interval from device removal to ovulation was similar (P>0.05) among all groups. The interval from EO to FxTAI was longer in HD goats compared to the other groups (P<0.05). In conclusion, although social hierarchy influenced the time of EO, the ovarian parameters, including ovulation, were not affected.

Keywords: goats; estrus behavior; follicular dynamics; social hierarchy; ovulation.

Introduction
In tropical weather conditions, Saanen goats show reproductive seasonality as they are in the anestrus condition in spring (1). Specialized breeds, including Saanen, require reproductive management to ensure sufficient milk production throughout the year. The implementation of a flexible time artificial insemination (FxTAI) program is key to overcoming this challenge (2). The use of reproductive biotechniques, such as the application of exogenous hormones, is hereby essential to achieving higher rates of estrus response and synchrony to guarantee the efficiency of a FxTAI program (2). However, even with the use of hormonal protocols, small ruminants present a high degree of estrus onset dispersion (3).

Sequential transrectal ultrasound examinations have been used to identify ovarian follicular dynamics in dairy goats, using the associated ovulation parameters to conduct comparisons of how each animal responds to synchronous estrus induction protocols (4, 5, 6). The combination of ovarian parameters with estrus behavioral characteristics has been used to define the most appropriate time to apply artificial insemination and
achieve high pregnancy rates (7, 5, 8). The final benchmark of a synchronous estrus induction protocol is the time of device withdrawal. This parameter was taken into account in the development of the FxTAI strategy in dairy goats (9). It is known that the onset of estrus after device removal can be susceptible to the effect of social hierarchy, which determines the efficiency and fertility of goats (10). However, no information on ovulatory parameters in relation to social hierarchy has been recorded for synchronous estrus-induced dairy goats.

The social hierarchy effect on productivity can be substantial, depending on the species and the production system (10). Dominance is determined by a variety of features (11) and has been defined as the relationship between individuals in situations of dispute, wherein one is dominant and the other is subordinate (12). This hierarchy can be observed in access to resources such as food, resting places, shade, and mates (10). In a natural environment, the dominant females are normally the most actively reproductive (13) and partially or completely suppress the reproductive behavior and breeding of their subordinate counterparts. In studies on domestic animals, although dominance is recognized, (10, 13) its influence on reproduction remains unclear. Nonetheless, it is well known that small ruminants express social hierarchy behavior in the herd (10), and social dominance may play an essential role in reproductive management (14).

We hypothesize that in synchronous estrus-induced dairy goats, the hierarchical order influences the onset of estrus, but not the ovarian follicular dynamics and ovulation parameters. Specifically, this study aims to compare the reproductive parameters of dairy goats submitted to synchronous estrus induction and classified in order of dominance according to the onset of estrus.

**Material and Methods**

This study was conducted under the principles of the Brazilian Society of Laboratory Animal Science and was approved by the Ethics Committee for the Use of Animals of Universidade Federal Fluminense under protocol #6405230719. It was carried out at Embrapa, in Coronel Pacheco (21° 35’S and 43° 15’W), Minas Gerais state, Brazil, in the non-breeding season. All goats were equally kept in collective pens and managed in an intensive system under natural photoperiod at room temperature. The animals were fed with corn silage and received a balanced concentrated supplement. Mineralized salt (Caprinofós® Tortuga, São Paulo, Brazil) and water were available ad libitum.

A total of 23 clinically healthy multiparous dairy goats were used, with body condition scores of 3.0 ± 0.4 (on a scale from 1 to 5, where 1 = very thin and 5 = very fat) and a body weight of 58.2 ±13.2 kg. Goats were subjected to short-term progestogen synchronous estrus induction (9). After device removal, estrus detection was performed twice daily over 120 min (30 min per pen) for three days. According to the estrus onset recorded in each pen, the animals were categorized into three groups: HD (high dominants; <36 h; n = 8), MD (medium dominants; 36 to 48 h; n = 10) and LD (low dominants; >48 h; n = 5) (13). Females in estrus were removed from their pens at estrus onset and underwent FxTAI (9).

Ovarian follicular dynamics were assessed every 24 h from the day of device insertion to the day of removal and every 12 h from device removal to ovulation. All ultrasound exams were done by the same experienced operator using ultrasound equipment (Mindray® MS5Vet, Shenzhen, China) coupled with a 7.5 MHz linear transducer. The following ovarian parameters were calculated: follicular wave emergence time after device insertion or removal (the day a follicle reached 3 mm in diameter followed by an increase to 4 mm the next day) (h); estrus response (%); interval from device insertion and removal to estrus onset (h); interval from device insertion and removal to ovulation (h); interval from estrus onset to ovulation (h); animals ovulating (%); interval from follicular wave emergence to ovulation (h); diameter of the largest and second-largest ovulatory follicle (mm); average diameter of ovulatory follicle (mm); number of ovulations (total number of ovulations/animals that ovulated × 100); number of corpora lutea (CL) on Day 7 after estrus onset; interval from device removal to FxTAI (h); and interval from estrus onset to FxTAI (h); and pregnancy (%).

Statistical analysis was performed, whereby P < 0.05 considered as significant. Parametric variables were subjected to one-way ANOVA, followed by a Tukey test, and non-parametric variables were subjected to a Kruskal–Wallis test, using a Dunn test to determine differences. The results are described as mean ± S.E.M.

**Results and Discussion**

This study characterized for the first time the ovarian follicular parameters preceding and after device removal in synchronous estrus-induced dairy goats in relation to their social hierarchy. The ovarian follicular wave pattern was associated before and after device removal with ovulation. For the first time, this association was compared in goats presenting estrus early or late in relation to sponge removal in each pen. Some studies applying estrus synchronization (13) and synchronous estrus induction (14) have reported that the interval to estrus in relation to the end of the protocol (i.e., second prostaglandin administration or progestogen device removal) was negatively associated with estrus duration (14). Conversely, the interval to ovulation appears to not be significantly affected by estrus onset in goats presenting either earlier or later (16). The reproductive parameters are shown in Table 1.
Table 1. Reproductive parameters of different hierarchical categories of dairy goats after short-term progestogen synchronous estrus induction during the non-breeding season

| Parameters                                      | HD            | Groups | LD            | P-values |
|------------------------------------------------|---------------|--------|---------------|----------|
| Animal (n)                                      | 8             | 10     | 5             | -        |
| Follicular wave emergence time after device insertion (h) | 78.0 ± 11.1  | 74.4 ± 21 | 72.0 ± 24 | 0.847    |
| Follicular wave emergence time after device removal (h) | 64.4 ± 10    | 70.4 ± 20.7 | 78.0 ± 7.8 | 0.322    |
| Estrus response (%)                             | 100.0 (8/8)   | 100.0 (10/10) | 100.0 (5/5) | -        |
| Interval from device insertion to estrus onset (h) | 94.1 ± 5.7   | 101.9 ± 3.4 | 114.7 ± 6.1 | <0.001   |
| Interval from device removal to estrus onset (h) | 32.3 ± 6.6   | 37.9 ± 5.5 | 53.7 ± 6.5   | 0.004    |
| Interval from device insertion to ovulation (h)  | 129.8 ± 10.6 | 139.5 ± 20.3 | 140.8 ± 6.0 | 0.188    |
| Interval from follicular wave emergence to ovulation (h) | 132.3 ± 15.6 | 145.9 ± 40.5 | 157.8 ± 10.5 | 0.082    |
| Interval from estrus onset to ovulation (h)      | 67.9 ± 11.0  | 75.5 ± 20.4 | 79.8 ± 7.1  | 0.380    |
| Interval from estrus onset to ovulation (h)      | 35.6 ± 11.1  | 37.6 ± 20.7 | 26.1 ± 10.6 | 0.258    |
| Diameter of the largest ovulatory follicle (mm)   | 8.2 ± 1.1     | 8.3 ± 1.4 | 8.1 ± 1.7    | 0.974    |
| Diameter of the second-largest ovulatory follicle (mm) | 7.8 ± 1.1 | 7.7 ± 1.2 | 8.1 ± 1.7 | 0.877 |
| Average ovulatory follicle diameter (mm)          | 8.0 ± 1.0     | 8.0 ± 1.3 | 8.1 ± 1.7    | 0.991    |
| Animals ovulating (%)                            | 100.0 (8/8)   | 100.0 (10/10) | 100.0 (5/5) | -        |
| Number of ovulations                             | 1.9 ± 0.6     | 1.9 ± 0.6 | 1.5 ± 0.6    | 0.483    |
| Number corpora lutea on Day 7 after estrus onset | 1.4 ± 0.5     | 1.4 ± 0.5 | 1.5 ± 0.6    | 0.970    |
| Interval from device removal to artificial insemination (h) | 60.7 ± 7.3   | 55.2 ± 5.5 | 63.6 ± 5.7 | 0.025    |
| Interval from estrus onset to artificial insemination (h) | 28.4 ± 6.4   | 17.4 ± 0.1 | 9.9 ± 7.0 | <0.001   |
| Pregnancy (%)                                    | 71.4 (5/8)    | 20.0 (2/10) | 60.0 (3/5) | -        |

(n) Number of animals; (h) hours; (mm) millimeters; * values with different superscripts differed among the groups (P < 0.05). HD (high dominant goats), MD (medium dominant goats) and LD (low dominant goats).

The present study showed that all females started estrus between 24 and 60 h after device removal; the consideration of the time of estrus onset facilitated their categorization. As hypothesized, the goats displaying estrus earlier in the HD group did not necessarily have the different ovarian follicular profiles able to affect the ovulation time in relation to device removal when compared to the low hierarchical position (LD). The ovarian follicular wave emergence before and after device removal was similar among the categories studied. In fact, shorter intervals for the onset of estrus are mainly associated with longer intervals from the onset of estrus to ovulation, as the duration of estrus is therefore longer (14).

Because of the negative correlation between the interval to estrus and estrus duration (17) and ovulation occurring near the end of estrus (18), a longer estrus duration was expected for HD goats. Consequently, dominant goats in a given herd are likely to engage in more natural matings (shorter estrous interval and longer estrus duration), while subordinate goats are likely to engage in fewer or no matings. This occurrence can be circumvented with the use of preferential natural improvement (9). On the other hand, adjusting the FxTAI (8) may allow the FxTAI-like strategy of goats being inseminated at a fixed time in a short period of time and before the expected mean time of ovulation (Figure 1).

The social rank is not related to the conception rate or the number of kids born after estrus synchronization treatment and FxTAI in dairy goats (15). However, high-dominant goats have a higher level of progesterone than medium- and low-dominant goats (19). If, like progesterone, the estrogen profile during the follicular phase of the estrous cycle is higher in dominant goats, this category could reach the threshold estrogen level earlier for estrus presentation, which would explain the earlier onset of estrus observed in the present study.

The FxTAI strategy resulted in an acceptable pregnancy rate (43.5%), similar to that reported earlier for...
goats (47.8% \cite{19}) subjected to fixed time artificial insemination. However, it should be remembered that the animals in the present study were continuously monitored with various interventions for ultrasound assessments. Animals in similar conditions have been reported to achieve lower pregnancy rates than those simply subjected to the protocol of synchronous estrus induction, estrus monitoring, and artificial insemination \cite{5,7}.

Finally, in the present study, at each check, goats that were identified as being in estrus were promptly removed from their pens, allowing the remaining goats to be checked by males in their absence. This could be an interesting strategy to increase the estrus response, allowing subordinate goats to externalize behavioral estrus that could not be observed if the dominant goats had remained in the pens. Moreover, strategic breeding schemes must be employed to prevent dominant goats from receiving successive natural matings, which compromises the amount of matings and semen received by subordinates, as previously proposed \cite{19}.

**Conclusion**

Hierarchical order is an important parameter that requires consideration when estrus is induced in dairy goats during the non-breeding season. Females classified as having a higher level of dominance are the first to allow artificial insemination to be performed for all categories at an almost fixed time.

**Conflict of interest**

The authors declare no conflicts of interest.

**Author contributions**

*Conceptualization: P. R. Cortat, M. B. Dias, M. E. F. Oliveira, J. M. G. de S. Fabjan, J. F. da Fonseca; Methodology: P. R. Cortat, M. B. Dias, M. E. F. Oliveira, J. M. G. de S. Fabjan, J. F. da Fonseca; Investigation: C. J. C. de Paula, J. M. G. de S. Fabjan, J. F. da Fonseca; Data curation: J. F. da Fonseca, C. J. C. de Paula; Supervision: J. M. G. de S. Fabjan, J. F. da Fonseca; Funding acquisition: J. M. G. de S. Fabjan, J. F. da Fonseca; Project administration: J. M. G. de S. Fabjan, J. F. da Fonseca; Formal analysis: P. S. C. Rangel, J. F. da Fonseca; Writing - original draft: P. R. Cortat, M. B. Dias; Writing - review & editing: P. R. Cortat, M. B. Dias, P. S. C. Rangel, M. E. F. Oliveira, J. M. G. de S. Fabjan, J. F. da Fonseca; Resources: J. M. G. de S. Fabjan, J. F. da Fonseca. Validation: P. R. Cortat, J. F. da Fonseca.*

**Acknowledgements**

The authors wish to thank EMBRAPA staff for providing and housing the animals employed in this study. This study was supported by the National Council for Scientific and Technological Development (CNPq; Project: 403909/2021-0), Fundação de Amparo à Pesquisa de Minas Gerais (Fapemig: Project 002017-1), and EMBRAPA (Project 20.19.01.004.00.03.001). The authors also thank the support by the Carlos Chagas Filho Foundation for Research Support in Rio de Janeiro (FAPERJ). MEFO, JFF and JMGS-F are CNPq fellows and JMGS-F is a fellow of Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ).

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