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# Continuous presence of male on estrus onset, estrus duration, and ovulation in estrus-synchronized Boer goats



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## ABSTRACT

The objectives of the present study were to assess the effect of permanent contact of teasers without copulation on the interval from controlled internal drug release (CIDR) removal to estrus onset, estrus duration, ovulation time, number of ovulations, and interval from CIDR removal to ovulation time on estrus-synchronized Boer goats. During the fall season, a controlled randomized design experiment with two groups, control (CON;  $n = 18$ ) and treatment (TRE;  $n = 18$ ), was performed. The TRE group was maintained permanently in a pen with an aproned buck immediately after CIDR removal. The CON group was maintained in a different pen without permanent exposure to the male. All females were estrus synchronized with CIDR maintained in the vagina for 7 days and received 50  $\mu\text{g}$  of GnRH im at device insertion and 5 mg of natural prostaglandin F-2 $\alpha$  at device removal. Females were considered to be in estrus when they accepted mounting by the aproned bucks. Estrus was detected four times a day after CIDR removal (at 6 AM, 12 noon, 6 PM, and 12 midnight) using bucks with canvas apron as teasers. The ovulation time and number of ovulations were assessed by transrectal ultrasonography starting 24 hours after estrus onset and repeated every 6 hours until complete ovulation was detected. The estrus onset for the CON group was  $44.0 \pm 8.3$  hours and for the TRE group, it was  $37.0 \pm 7.7$  hours ( $P = 0.01$ ). Estrus duration from the CON group was  $43.7 \pm 9.2$  hours and for the TRE group, it was  $38.3 \pm 6.6$  hours ( $P = 0.05$ ). The first, last, and mean ovulation times for the CON group were  $32.4 \pm 5.3$ ,  $38.4 \pm 3.4$ , and  $35.4 \pm 3.9$  hours, and for the TRE group, the times were  $31.8 \pm 2.8$ ,  $36.7 \pm 3.0$ , and  $35.8 \pm 3.6$  hours, respectively ( $P = 0.85$ ,  $P = 0.23$ , and  $P = 0.82$ , respectively). The number of ovulations for the CON and TRE groups was  $2.6 \pm 0.7$  and  $2.6 \pm 0.6$  ovulations, respectively ( $P = 0.96$ ). The interval time for CIDR removal to ovulation for the CON group was  $79.2 \pm 8.2$  hours and for the TRE group, the interval time was  $73.2 \pm 6.2$  hours ( $P = 0.05$ ). It was concluded that the permanent presence of male without copulation with estrus-synchronized does hastened estrus onset, reduced estrus duration, and decreased the interval time from CIDR removal to ovulation without modification of ovulation time and number of ovulations in Boer goats.

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## 1. Introduction

Caprine estrus length exhibits great variations [1–4]. Estrus duration is essential to artificial insemination (AI) technology [5,6]. It has been traditionally recommended that goats be inseminated 12 hours after the onset of estrus

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and inseminated again the following day if they are still in estrus [6] or immediately after estrus detection and 12 hours later [7]. In previous investigations, a decrease in estrus duration from 9.3 to 19.2 hours (from 26% to 44% reduction compared with the CON group) was found after copulations during the first 12 hours of estrus [4,8–12]. This effect was independent of the number of copulas [8], and it was because of the mechanical effect of the penis against the vaginal fornix and not to the accessory sexual fluid [9]. The mechanical effect of the penis was eliminated by local and regional anesthesia [10]. In a further study, no differences between the CON and TRE groups, either in the number of ovulations or ovulation times when assessed at 8-hour intervals by laparoscopy, were detected [11]. However, in a recent study, it was shown that copulation at the beginning of estrus not only reduced estrus duration but also hastened the ovulation time when assessed at 4-hour intervals by transrectal ultrasonography [13]. Interestingly, in this last study, a small but significant difference of estrus duration between the CON and TRE groups was detected. The reason was the short estrus length of the CON group compared with earlier studies [4,8–12]. The close proximity of males during all period of investigation could be the potential factor of this short estrus length in the CON group. In this study, all the bucks were not only used more frequently for teasing (every 4 hours rather than every 6 hours) as in previous investigations but also were kept together after estrus detection in an adjacent pen in close proximity to the females' pens, therefore, permitting continual visual, olfactory, and auditory communication with the females [4,8–12]. In previous research, the permanent presence of bucks with female estrus synchronized using either progestogens or luteolytic hormones that hastened the estrus onset compared with females not exposed continuously to male; however, neither estrus duration nor ovulation was studied [14,15]. Consequently, the possible influence of permanent contact without copulation on estrus duration and ovulation warranted further investigation.

The objectives of the present study were to evaluate the effect of permanent contact of bucks without copulation on estrus-synchronized does on the interval period from controlled internal drug release (CIDR) removal to estrus, estrus duration, ovulation time, number of ovulations, and interval period from CIDR removal to ovulation during the fall season in Boer goats.

## 2. Material and methods

### 2.1. Animals

In this experiment, all procedures used were in compliance with the Guide for the Care and Use of Agriculture Animals in Research and Teaching and were approved by the Texas A&M University Institutional Animal Care and Use Committee; 36 Boer females (22 pluriparous and 14 nulliparous) and 5 bucks from the Teaching and Research Goat Herd at the Large Animal Clinical Sciences Department at the College of Veterinary Medicine and Biomedical Sciences of Texas A&M University were randomly selected. The animals were clinically healthy and

submitted to breeding soundness examination 4 weeks before the investigation according to the Society for Theriogenology criteria and were declared satisfactory potential breeders [16]. The females and males were maintained in different pastures with cover sheds before being moved to the building for this investigation. The males were separated from females by only one fence at the pastures. Does were housed in groups of no more than four does per cover pen, with free coastal hay available and supplemented with a commercial mixed concentration (16% crude protein, 3.0% crude fat, 16% crude fiber, 0.9% Ca, 0.55% P) and fed individually twice daily with 450 g per doe per meal. All does had free access to water and trace mineral salt. The age, weight, and body condition score were evaluated at the time of CIDR removal [17]. The female mean age was ( $\pm$ SD)  $2.9 \pm 1.6$  years (range 1–5), mean weight was  $52.3 \pm 13.1$  kg (17.6–75.0), and mean body condition score was  $3.2 \pm 0.3$  (2.5–3.75). All animals were vaccinated, had their hooves trimmed, and had their feces analyzed for gastrointestinal parasites according to the pre-established standard operating procedures. All the animals were free of caseous lymphadenitis and caprine arthritis encephalitis.

### 2.2. Experimental design

During the fall season, a controlled randomized design experiment with two groups, control (CON;  $n = 18$ ) and treatment (TRE;  $n = 18$ ), was performed. Each group was formed by 11 pluriparous and seven nulliparous does. All females were estrus synchronized with CIDR (progesterone 300 mg) maintained in the vagina for 7 days and received 50  $\mu$ g of GnRH im at device insertion and 5 mg of natural prostaglandin F-2 $\alpha$  im at device removal. CIDRs were inserted in the morning at unknown stages of the estrous cycles. The TRE groups were maintained permanently in pens with an aproned buck immediately after CIDR removal and were maintained during all investigation period. The number of does per buck and per pen was between 3:1 and 4:1. The CON group was maintained in different pens without permanent exposure to the male. All the animals were housed in the same barn, but the TRE group was separated from the CON groups by 10 m. The experiment was performed in three replicates of eight, 14, and 14 does each. At each replicate, the females were randomly divided equally in the TRE and CON groups. The intervals among replicates were 2 weeks. The CON and TRE groups did not receive any copula during estrus; only mounts were permitted. Females were considered to be in estrus when they accepted mounting by the bucks. Estrus was detected four times a day after CIDR removal (at 0600, 1200, 1800, and 2400 hours) using five bucks from 1 to 4 years old with high serving capacity as teasers with canvas apron conducted by leash as in previous investigations [4,7–11]. Estrus response was defined as the proportion of females in estrus within the 5 days of CIDR removal from the total estrus synchronized. Estrus onset was defined as the time elapsed from CIDR removal to the middle of the time between the last unaccepted mount and the first accepted mount. Estrus duration was the interval from the first to the last accepted mount. The ovulation time and number of ovulations were assessed by transrectal ultrasonography

starting 24 hours after estrus onset and repeated every 6 hours until complete ovulation was detected using a 7.5 MHz linear prostatic probe [18]. The time of first ovulation was the time from estrus onset to the middle time between when the first preovulatory follicles were seen and the next time when they disappeared. The time of last ovulation was the time from estrus onset to the middle time between the last preovulatory follicles seen and the next time when they disappeared. The mean ovulation time was the mean time from estrus onset to the average time between the first and last ovulations. The ovulation time was also related to behavioral estrus (inside or outside estrus). The numbers of ovulations per doe were recorded. The interval time from CIDR removal to mean ovulation time was also calculated.

2.3. Statistical analyses

The continuous variables (estrus onset, estrus duration, interval of estrus onset to ovulation, and interval CIDR removal to ovulation) were analyzed by Student *t* test for independent samples. The categorical variables (estrus response, number of ovulations, and ovulations inside or outside behavioral estrus) were analyzed by the chi-square or Fisher exact test as appropriate [19]. In addition, a multiple regression analysis including for estrus onset, estrus duration, and interval from CIDR removal to ovulation that included replicates was performed. All data were expressed as mean ± 1 SD. Differences were considered statistically significant when  $P \leq 0.05$ . Statistical software was used to perform all statistical analyses [20].

3. Results

A summary of all the results from this investigation can be seen in Table 1. No differences were detected among replicates for estrus onset ( $P = 0.66$ ), estrus duration ( $P = 0.10$ ), and for interval from CIDR removal to ovulation ( $P = 0.42$ ); therefore, all the data of the three replicates for each parameter were presented together. Estrus response between the TRE and CON groups was 100% in all the replicates. The estrus onset for the CON group was  $44.0 \pm 8.3$  hours and for the TRE group, it was  $37.0 \pm 7.7$  hours ( $P = 0.01$ ). The cumulative estrus onset between the CON and TRE groups at the different intervals is shown in Figure 1. Estrus duration from the CON group was  $43.7 \pm 9.2$  hours and for the TRE group was  $38.3 \pm 6.6$  hours ( $P = 0.05$ ). The first, last, and mean

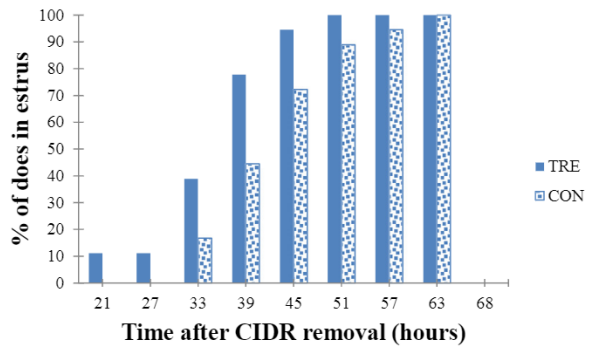


Fig. 1. Cumulative percentage of does in estrus from continual presence of the teaser (TRE group) and intermittent presence of the teaser (CON group).

ovulation times for the CON group were  $32.4 \pm 5.3$ ,  $38.4 \pm 3.4$ , and  $35.4 \pm 3.9$  hours and for the TRE group, the times were  $31.8 \pm 2.8$ ,  $36.7 \pm 3.0$ , and  $35.8 \pm 3.6$  hours, respectively ( $P = 0.85$ ,  $P = 0.23$ , and  $P = 0.82$ , respectively). The number of ovulations for the CON and TRE groups were  $2.6 \pm 0.7$  and  $2.6 \pm 0.6$  ovulations, respectively ( $P = 0.96$ ). The interval time from CIDR removal to ovulation for the CON group was  $79.2 \pm 8.2$  hours and for the TRE group, it was  $73.2 \pm 6.2$  hours ( $P = 0.05$ ). Most of the ovulations occurred inside behavioral estrus (31/37; 86%) without differences between treatments ( $P = 0.70$ ).

4. Discussion

The present results reported that the immediate and continual presence of a male after CIDR removal on estrus-synchronized females hastened estrus onset. This outcome agrees with the previous investigations that used different kinds of progestogens or luteolytic drugs for estrus synchronization in goats [14,15]. During the entire investigation period, the male had the opportunity to exhibit all its sexual behavior: vocalization, foreleg striking, mount, and close contact except copulation. This male effect on hastening onset of estrus in estrus-synchronized females during the breeding season was also reported in sheep [21,22]. Exposure of ewes to rams after progestogen sponge removal increased the number of females that showed luteinizing hormone (LH) surges between 24 and 48 hours [23]. One additional factor that could influence this male effect is the female effect. In an earlier study, it was shown that females in proestrus–estrus also hasten estrus onset in

Table 1

Comparison between does in permanent presence of male (TRE) and intermittent presence of male (CON) after CIDR removal on age, weight, body condition score, estrus response, estrus onset, estrus duration, ovulation times, ovulation rates, and interval between CIDR removal and ovulation in Boer goats.

Group	Number of does	Age (y)	Weight (kg)	Body condition score	Estrus response	Estrus onset (h)	Estrus duration (h)	First ovulation (h)	Last ovulation (h)	Ovulation rate	Interval from CIDR removal to ovulation (h)
TRE	18	$2.8 \pm 1.6^a$	$50.9 \pm 13.6^a$	$3.2 \pm 0.3^a$	18	$37.0 \pm 7.7^c$	$38.3 \pm 6.6^b$	$31.8 \pm 2.8^a$	$36.7 \pm 3.0^a$	$2.6 \pm 0.6^a$	$73.2 \pm 6.2^b$
CON	18	$2.9 \pm 1.7^a$	$54.0 \pm 12.7^a$	$3.2 \pm 0.4^a$	18	$44.0 \pm 8.3^a$	$43.7 \pm 9.2^a$	$32.4 \pm 5.3^a$	$38.4 \pm 3.4^a$	$2.6 \pm 0.7^a$	$79.2 \pm 8.2^a$

Columns with different letters are significant different (a, b:  $P = 0.05$ ; a, c:  $P = 0.01$ ).

Abbreviations: CIDR, controlled internal drug release; CON, control; TRE, treatment.

females estrus synchronized during the breeding season [24]. Therefore, the presence of females in proestrus–estrus could be considered a complementary stimulus to the male effect in the present study, especially because these females were kept in the same pen and not removed throughout the experimental period. Furthermore, these females in estrus were also persistent strong sexual stimuli to the male.

A novel finding of the present investigation was that the permanent presence of the male without copulation reduced the estrus duration compared with the group of females in intermittent presence with the male without copulation. This confirms the suspected influence from a previous study that the continual presence of male could decrease estrus duration [13]. Estrus duration in both studies was similar. In goats, copulation significantly reduced estrus duration because of the mechanical action of the penis against the vaginal fornix and not to the seminal plasma [9,10], and this effect was independent of the number of copulations for the same estrus [8]. Based on the findings from multiple independent studies, it seems that the degree of association between male and female during estrus could influence the estrus duration, from a weak stimulation when the male was in intermittent presence without copulation, a medium stimulation when the male was in permanent contact without copulation, to the maximum stimulus when the male was permitted copulation.

The ovulation time between the TRE and CON groups was not different, indicating that although the presence of the male was capable of reducing estrus duration, it was not able to hasten ovulation time. All the ovulations occurred inside the behavioral estrus. In a previous investigation, the copula at estrus onset not only reduced estrus duration but also established that ovulations were outside the behavioral estrus compared with a control group of noncopulated does assessed by laparoscopy [11]. In a recent investigation, the copulation at the beginning of estrus was not only able to reduce estrus duration but also to hasten the first ovulation and last ovulation when assessed by transrectal ultrasonography at 4-hour intervals [13].

The interval time from CIDR removal to ovulation was shorter in TRE group compared with the CON group because the male hastened estrus onset without changing the ovulation time; therefore, the final time of ovulation was earlier in the TRE group compared with the CON group. Successful fertilization depends on the time of insemination relative to ovulation, and this is related to the estrus onset [25]. Presently, it is recommended that fixed-time AI be performed between 48 and 60 hours after sponge removal [26,27]. If those recommendations had been followed in the present study, between 95% and 100% of the does from the TRE group would have been inseminated during estrus and between 72% and 95% of the does in the CON group (see Fig. 1). Females artificially inseminated between 42 and 52 hours after sponge removal that were not in estrus did not become pregnant [28]. Moreover, this situation is even more important when frozen semen is used. Frozen semen has reduced fertilizing capacity in comparison with fresh or refrigerated semen [29,30]. Therefore, the presence of the male could be one of the

factors responsible for the variation found among different experiments using the same drugs in estrus synchronization protocols [31,32]. Also, with adequate management of the teaser in females estrus synchronized, it could be possible to obtain more females in the optimal time for AI that could permit obtain satisfactory results in fertility.

The mechanism by which the buck hastens estrus and reduces estrus length in does is unknown. In small ruminants, the smell is an important source of sense stimulation that increases the LH pulses, producing an LH surge and ovulation in acyclic females when exposed to male odor [33,34]. Pheromones from the sebaceous glands of bucks, from the lipid and nonlipid fractions, are able to stimulate ovulatory cycles in noncycling female during the nonbreeding season [34]. An interesting point about the present study was that the bucks waiting to be used in the CON group, between teasing periods, were located 10 m away in adjacent pens. Therefore, the females of the CON group had the opportunity to smell and hear the bucks continuously during all the experimental period, including the time that they were introduced into the pen for estrus detection. In former goat experiments, the anosmia produced by destruction of the main olfactory bulb and/or accessory olfactory bulb did not annulated the male effect in anovulatory females [35,36]. Therefore, other senses seem to participate and complement the effect of smell. The buck teasing behavior was probably an important factor. In the TRE group, the male had the opportunity to exhibit all its sexual behavior: vocalization, foreleg striking, mount, and close contact except copulation during all the experimental period. Shelton [37], working with non-synchronized goats during the transitional breeding period, found that the male effect was completely effective to induce estrus when the male was in physical presence with the females, but the response was reduced when the male was far away from the females. In addition, in the present study, the influence of does in proestrus and in estrus (female effect) could be considered as an extra stimulus to the male effect as previously discussed and revealed in a former study some time ago [23].

It is concluded that the permanent presence of male without copulation on estrus-synchronized does hastened estrus onset, reduce estrus duration, and decreased the interval from CIDR removal to ovulation without modification of ovulation time and number of ovulations in Boer goats during the breeding season.

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## Competing Interests

All authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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