



## BEHAVIORAL AND ENDOCRINE RESPONSES OF HAIR SHEEP EWES EXPOSED TO DIFFERENT MATING STIMULI AROUND ESTRUS

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### ABSTRACT

Hair sheep ewes were used to evaluate the influence of various levels of mating stimuli on the duration and timing of estrus and LH concentrations around estrus. Ewes were treated with PGF2 $\alpha$  (15 mg, im) 10 d apart. At the time of the second PGF2 $\alpha$  treatment (Day 0) ewes were placed in groups and exposed to different types of mating stimuli. One group of ewes (n = 16) was exposed to an epididymectomized ram (RAM), a second group of ewes (n = 16) was exposed to an epididymectomized ram wearing an apron to prevent intromission (APRON) and a third group of ewes (n = 17) was exposed to an androgenized ovariectomized ewe (T-EWE). Jugular blood samples were collected from ewes at 6-h intervals through Day 5. Plasma was harvested and LH concentration was determined by RIA. The ewes were observed at 6-h intervals to detect estrus. A ewe was considered to be out of estrus when she no longer stood to be mounted by the teaser animal. There was no difference (P > 0.10) in the proportion of ewes expressing estrus (79.6%) or having an LH surge (85.7%) among the treatments. Neither the time to estrus nor the duration of estrus were different (P > 0.10) among APRON, RAM or T-EWE groups (41.6  $\pm$  3.8 vs 43.6  $\pm$  3.6 vs 46.1  $\pm$  3.6 h, respectively, and 26.5  $\pm$  2.2 vs 24.8  $\pm$  2.3 vs 30.5  $\pm$  2.2 h, respectively). The time to LH surge was similar (P > 0.10) among APRON, RAM and T-EWE groups (51.2  $\pm$  4.5 vs 51.2  $\pm$  4.7 vs 52.7  $\pm$  4.5 h, respectively). The magnitude of the LH surge was similar (P > 0.10) in the T-EWE, APRON and RAM ewes (99.7  $\pm$  4.9 vs 87.2  $\pm$  4.9 vs 85.8  $\pm$  5.0 ng/mL, respectively). The time from estrus to the LH surge was not different (P > 0.10) among APRON, RAM or T-EWE ewes (10.1  $\pm$  2.2 vs 9.8  $\pm$  2.3 vs 11.6  $\pm$  2.3 h, respectively). These results show that the expression and duration of estrus are not influenced by different types of mating stimuli in hair sheep ewes. In addition, the timing and the magnitude of LH release does not appear to be influenced by mating stimuli around the time of estrus.

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Key words: sheep, estrus, LH, behavior, ram

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## INTRODUCTION

The ram effect is well documented in sheep and was shown to be useful for inducing estrous cyclicity in anestrus ewes (6, 16). The presence of the male around the time of estrus may also have some impact on the physiology of the female, although reports describing this effect in sheep are limited in number. The presence of a ram around estrus was shown to increase ovulation rate (8), to increase conception out of season (16) and to enhance P4 secretion (5). The presence of the boar at mating was shown to enhance LH levels in sows and mating with a sterile boar hastens ovulation (14). With the increasing use of estrus synchronization and artificial insemination (AI) in sheep the effect of the ram around the time of estrus is being investigated. When using synchronization and AI it may be beneficial, in some instances, to not have a ram with the ewes. The cost of producing and keeping a sterile ram for estrus detection may be prohibitive for some producers. The use of androgenized females for estrus detection is another option available. This project was designed to evaluate the effect of different types of mating stimuli around the time of a synchronized estrus on the behavior and endocrine response of hair sheep ewes in the tropics.

## MATERIALS AND METHODS

### Animals and Treatments

Multiparous St. Croix White hair sheep ewes ( $n = 49$ ) were given PGF $2\alpha$  (15 mg im, Lutalyse®, Pharmacia & Upjohn Co., Kalamazoo, MI, USA) 10 d apart to synchronize estrus. At the time of the second PGF $2\alpha$  treatment (Day 0) the ewes were placed in treatment groups. One group of ewes ( $n = 16$ ) was exposed to an epididymectomized ram equipped with a marking harness (RAM). The second group of ewes ( $n = 16$ ) was exposed to an epididymectomized ram wearing an apron to prevent intromission (APRON). The apron was constructed using denim material attached to a modified ram marking harness. The apron completely covered the abdomen of the ram and prevented intromission when mounting ewes. Both rams had been used previously to detect estrus and possessed adequate libido. The third group of ewes ( $n = 17$ ) was exposed to an ovariectomized androgenized ewe wearing a marking harness. The ewe was given 100 mg of testosterone propionate in corn oil (25 mg/mL im) at 3-d intervals for 2 wk, and then once weekly for the duration of the study. The libido of the androgenized ewe was evaluated before use in the study by exposing the ewe to a group of ewes, some of which were in estrus, and observing which ewes were mounted. A ram of known libido was then exposed to the same ewes and observed for mounting. The androgenized ewe was able to identify the ewes in estrus with the same degree of accuracy as the ram. The 3 groups of ewes were kept in separate pens (6.1 m x 6.1 m) with an empty pen between each group. This prevented physical contact among animals in different groups but did not prevent visual or possible olfactory stimuli. While in the pen, the sheep were fed a pelleted ration and had ad libitum access to guineagrass hay, water and mineralized salt.

Beginning on Day 0 each group of ewes was observed at 6-h intervals for mounting or crayon marks from the stimulus animal. The onset of estrus was determined as the time when a ewe was first observed to stand when mounted. The duration of estrus was determined as the time interval between the onset of estrus and when a ewe no longer stood to be mounted.

## Blood Sampling and Hormone Assays

Jugular vein blood samples were collected at 6-h intervals from all ewes beginning at the time of the second PGF2 $\alpha$  injection and ram introduction on Day 0 and continuing through 96 h. Plasma was harvested from all blood samples and stored at -20°C. Plasma LH was measured in all samples using a double-antibody RIA (2, 4). The intra- and inter-assay CV for the LH RIA were 4.4 and 11.2%, respectively. Sensitivity of the assay was 2.0 ng/mL.

## Statistical Analyses

Data were analyzed using General Linear Models (GLM) procedures of SAS (13). Independent variables were the three treatment groups. Dependent variables analyzed were time to estrus, duration of estrus, time to preovulatory LH surge (maximal LH concentration) and magnitude of the LH surge. Preovulatory LH surges were identified in individual ewes after plotting their LH concentrations over time. Cumulative percentages of ewes in estrus in each treatment were analyzed using the CATMOD procedure using treatment, time after treatment and their interaction in the model (13). Results are expressed as least squares means  $\pm$  SEM.

## RESULTS

The proportion of ewes exhibiting estrus was similar ( $P > 0.10$ ) among ewes in the RAM, APRON and T-EWE groups (Table 1). The time to estrus after the second PGF2 $\alpha$  treatment and the duration of estrus were similar ( $P > 0.10$ ) among the treatment groups. The cumulative percentage of ewes in estrus after the second PGF2 $\alpha$  was not different ( $P > 0.10$ ) among treatments (Figure 1).

Table 1. Time to onset of estrus, duration of estrus and magnitude of the LH surge in St. Croix White ewes exposed to various male stimuli

	Male Stimuli		
	Control ram	Apron ram	Androgenized ewe
Time to estrus (h) <sup>a</sup>	43.6 $\pm$ 3.6 <sup>b</sup>	41.6 $\pm$ 3.8	46.1 $\pm$ 3.6
Duration of estrus (h) <sup>a</sup>	24.8 $\pm$ 2.3	26.5 $\pm$ 2.2	30.5 $\pm$ 2.2
Time to LH surge (h) <sup>c</sup>	51.2 $\pm$ 4.7	51.2 $\pm$ 4.5	52.7 $\pm$ 4.5
Magnitude of LH surge (ng/mL) <sup>c</sup>	85.8 $\pm$ 5.0	87.2 $\pm$ 4.9	99.7 $\pm$ 4.9
Proportion (%) of ewes			
Exhibiting estrus	81.2	81.2	76.5
Having an LH surge	81.2	87.5	82.3
Number of ewes in group	16	16	17

<sup>a</sup>Checked at 6-h intervals after second PGF2 $\alpha$  injection and stimulus animal introduction.

<sup>b</sup>Least squares mean  $\pm$  SEM.

<sup>c</sup>Measured in blood samples collected every 6 h after second PGF2 $\alpha$  injection and stimulus animal introduction.

Neither the time to LH surge after introduction of mating stimulus animal (Day 0) nor the magnitude of the LH surge were different ( $P > 0.10$ ) among the treatment groups (Table 1). The time of the LH surge ranged from 24 to 66 h in ewes exposed to the control ram, 18 to 78 h in ewes exposed to the androgenized ewe and 12 to 96 h in ewes exposed to the apron ram (Figure 2). In ewes exposed to the apron ram, 78.6% of ewes having an LH surge had it within

1 SD of the mean. In ewes exposed to the control ram, 69.2% of ewes having an LH surge had it within 1 SD of the mean. In ewes exposed to the androgenized ewe, 64.3% of ewes having an LH surge had it within 1 SD of the mean.

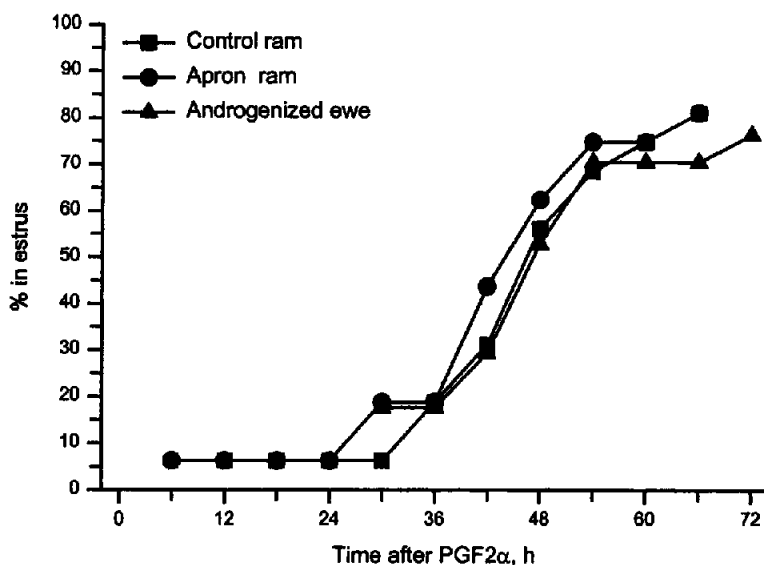


Figure 1. Cumulative percent of St. Croix White ewes exhibiting estrus after PGF $2\alpha$  treatment and exposure to either a sterile ram, a sterile ram equipped with an apron or an androgenized ovariectomized ewe.

Of the 39 ewes that exhibited estrus, 97.4% also had a detected LH surge, but only 30% of the 10 ewes that did not exhibit estrus had a detectable LH surge ( $P < 0.001$ ). There was no difference ( $P > 0.10$ ) among treatments in the proportion of ewes exhibiting estrus, having an LH surge, or exhibiting estrus and having an LH surge (Table 2). The proportion of ewes in the RAM, APRON and T-EWE groups that did not express estrus and had no detected LH surge was not different ( $P > 0.10$ ).

Table 2. Proportion of ewes in each treatment group that expressed estrus or had a detectable LH surge.

Treatment	Ewes that had:			
	Estrus and LH surge	Estrus only	LH surge only	Neither
Control ram	81.3 <sup>a</sup> (13)	0 (0)	18.7 (3)	0 (0)
Apron ram	81.3 (13)	0 (0)	12.5 (2)	6.2 (1)
Androgenized ewe	70.7 (12)	5.9 (1)	11.7 (2)	11.7 (2)

<sup>a</sup>Percentage of ewes in each group. The number in parenthesis is the actual number of ewes.

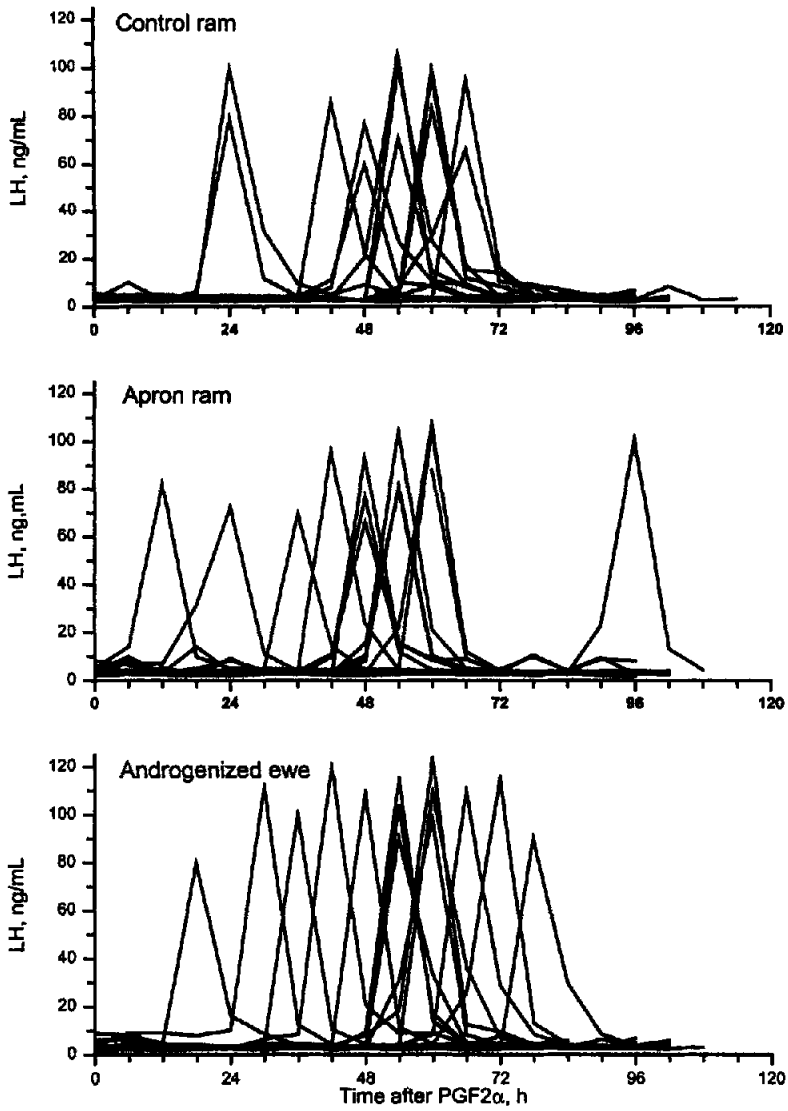


Figure 2. Concentration of LH in St. Croix White ewes after PGF2 $\alpha$  treatment and exposure to either a sterile ram, a sterile ram equipped with an apron or an androgenized ovariectomized ewe.

## DISCUSSION

In the present study the various mating stimuli did not influence the timing of behavioral or endocrine events around estrus in the ewes. In a study by Romano (9) it was reported that does had a longer period of estrus when they were mounted but not served by a buck in comparison to does that were serviced by the buck at least once. In another study, Romano et al. (12) reported that does that were only mounted, but not serviced, during the first 12 h of estrus had a shorter duration of estrus than does serviced during this time period. Both of these studies disagree with the data being presented in the present study where there was no difference in the duration of estrus in the treatment groups (Table 1). The ram with the apron and the androgenized ewe were able to mount the ewes but there was no vaginal penetration and this is similar to the groups in the previously mentioned studies (9, 12) where the buck was only permitted to mount the does but not to service them. Both of these studies (9, 12) were performed during the natural breeding season of the goats, and since hair sheep in the tropics do not exhibit seasonal patterns of estrous cycles (1, 15) it is unlikely that season is responsible for the difference in results. In a subsequent study (10) it was found that service by the buck or mechanical stimulation of the cervix shortened the duration of estrus in comparison to natural service by the buck or the placement of seminal fluid in the cervical os. This implies that there is some influence of the physical contact of the penis with the vagina and cervix on the duration of estrus in goats. This outcome was not observed in the present study, although the duration of estrus was numerically lower in the ewes exposed to the control ram when compared to the ewes exposed to the androgenized ewe. If there is an effect of penile stimulation of the cervix on the duration of estrus in sheep, as there appears to be in goats, it was not detected in the present study.

In the present study where there was no difference in the time to estrus after synchronization and "male" introduction among the treatment groups (Table 1). This is in contrast to the study of Mellado and Hernandez (7) where exposure of does to bucks resulted in a shorter time to estrus in comparison to does exposed to androgenized wethers or does during both the breeding and the nonbreeding seasons. In another study, it was reported that ewes synchronized during the breeding season had a shorter time to estrus after sponge removal when they were exposed to a ram at the time of sponge removal (11).

There was also no effect of the mating stimuli used in the present study on the timing or magnitude of the preovulatory LH surge in the ewes (Table 2). It was reported that in the sow natural mating may prolong LH surge in comparison to AI (17). The duration of the LH surge in the present study was not evaluated, in part because of the frequency of sampling (6-h intervals). In a study by Husein et al. (5) the effect of having a ram present at the time of pessary removal and estrus on fertility to AI was evaluated, but the conception rate after AI was too low (5%) to make any conclusions. The authors did report that P4 concentrations at 12 to 14 d after the synchronized estrus were higher in ewes exposed to teaser rams at the time of pessary removal than in ewes not exposed to rams. They suggested that the higher P4 concentrations were due to enhanced LH secretion during the preovulatory period in response to the presence of the ram (5). Fertility to natural service could not be evaluated in the present study because of the nature of the treatments, and AI was not used. In addition, it is not known if the treatments in the present study had an influence on either the timing or frequency of ovulation or subsequent luteal function in the ewes since these responses were not monitored and P4 concentrations after estrus were not determined.

The proportion of ewes in each group that exhibited estrus or had an LH surge was not different in this study (Table 2 and Figure 2). These results are similar to those reported by Mellado and Hernandez (7) in goats where exposure of does to bucks, or androgenized wethers or ewes had no effect on the induction of estrus. The response to synchronization in the present study (Figure 1) was similar to that of a previous trial conducted in our laboratory (3). In a subsequent study when ewes were synchronized using PGF2 $\alpha$ , 100% of ewes were in estrus by 42 h after treatment (2). When PGF2 $\alpha$  was used on our research farm as part of our routine breeding program, the response rate was variable, but comparable to what is being reported in this study.

Based on the results of this study, hair sheep ewes will express estrus and have a preovulatory LH surge when exposed to either natural mating stimuli (control ram) or mounting only (apron ram and androgenized ewe) after synchronization treatment with PGF2 $\alpha$ . There was also no detectable influence on the timing or magnitude of the preovulatory LH surge among the mating stimuli treatments. It appears that the use of an androgenized female would be suitable for detecting estrus with no negative influence on estrus response or LH secretion in hair sheep. The effect of the type of animal (intact male or androgenized female) used to detect estrus on subsequent fertility, after either natural mating or AI, still needs to be investigated.

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