BUCK-INDUCED ESTRUS IN GRAZING GOATS DURING INCREASING PHOTOPERIOD AND UNDER COLD STRESS AT 25° N

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Abstract. This study characterizes the goat response to the buck stimulus during the transition from anestrus to complete sexual function in mixed-breed goat herds maintained on an arid rangeland (25°N). In a first experiment, seasonal incidence of behavioural estrus was recorded by exposure of does to bucks (buck to doe ratio 1:18-1:24; n= 60-96) in February, April, May and June. Signs of estrus were observed in 89, 1, 100 and 88% of goats exposed to bucks during these months, respectively, within a mean \pm SD of 5.6 \pm 3.8, 8.0 \pm 0.0, 9.4 \pm 4.9 and 4.7 \pm 3.7 days from the initiation of teasing to first standing estrus. In a second experiment, two groups of goats were joined to bucks in January, one group (n= 44) undergoing cold stress (constant drizzling and 8°C mean daily temperature), the other without rain and mild temperatures (13.6°C; n= 44). A lower proportion of goats under cold stress, manifested by higher (p<0.01) plasma cortisol levels, showed estrus compared with goats under mild weather (27 vs. 80%; p<0.01). From a production standpoint, a high estrus response is expected in winter and early June in mixed-breed grazing goats in agropastoral production systems at 25°N, although wet cold weather in winter hampers the responsiveness of does to the buck stimulus.

Keywords: anestrous, estrus, sexual seasonality, male effect, short ovarian cycle.

Introduction

Several studies with dairy mixed-breed goats at 26°N under conditions of natural photoperiod and confinement (no interaction between bucks and does) indicate that female and male goats display a long reproductive seasonality independently of food availability. The breeding season of females lasts from September to February-March (Duarte *et al.*, 2008; Rivera-Lozano *et al.*, 2011), whereas bucks exhibit an intense sexual activity between May and December (Delgadillo *et al.*, 1999). Thus, coincidence of full natural sexual activity in both sexes occurs during a four-month period (September to December).

However, some non-dairy goats reared under pastoral/extensive goat farming systems in arid regions of northern Mexico are mated throughout the year, due to their reduced seasonal pattern of sexual inactivity (Mellado *et al.*, 1996). Breeding of goats in winter is particularly important for goat producers in northern Mexico, because kiddings coincide with the beginning of the rainy season (June), which is vital for dairy goat producers on rangeland, due to the higher availability of biomass during summer and fall. When does are bred in winter, cold weather could interfere with the response of does to the buck stimulus. However, to our knowledge, no experiments have been carried out in goats to determine the role played by low temperature conditions in the breeding process.

Breeding activity of goats under commercial conditions is at odds with controlled studies where breeding seasons of male and female goats have been characterized separately. This apparent contradiction is

because seasonal sexual activity of goats varies primarily with breed (Rivera-Lozano *et al.*, 2011; Amoah *et al.*, 1996; du Preez *et al.*, 2001), intensity of buck stimulus (Rivas-Muñoz *et al.*, 2007), level of nutrition (Zarazaga *et al.*, 2005; Estrada-Cortés *et al.*, 2009), body energy reserves (De Santiago-Miramontes *et al.*, 2009) and the interaction among these variables.

Based on this knowledge, we hypothesized that mixed-breed goats maintained under communal arid rangelands in agro-pastoral systems of northern Mexico (25° N) are not strictly a short-day breeders and exhibit a short circannual pattern of variation in reproductive activity, and that cold stress hampers the response of goats to the buck stimulus.

Materials and Methods

The experiment was conducted from January to June in a semi-desert area of northern Mexico (25° 07' N, 101° 40' W; altitude 2150 m) dominated by a microphyll desert scrub. Mean annual temperature is 16° C and average annual precipitation is 299 mm, with 75% falling from June to October. Historically, this communal pasture has been heavily stocked and continuously grazed by large herds of goats, bovines and equines, therefore goats grazed on a deteriorated rangeland showing a low forage production potential.

In a first study, four commercial flocks of goats typical of the farming systems of the arid zones of northern Mexico ranging from 60 to 96 adult animals were used. Breed of goats was undefined (mixture of breed of goats that originated in the Alps, as well as Nubian and native goats); all flocks were non-lactating

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and non-pregnant (goats had been isolated from bucks since last breeding period), from the same community and shared the same pasture.

Goats of all herds grazed, driven by herdsmen, during 7 h daily (11:00 to 18:00 h) and were penned from 18:00 to 11:00 h, with no extra food or salt supplementation throughout the year. Goats spent the night in unroofed corral and had access to water only once a day. Goats were not subjected to an anthelmintic drenching program and were not immunized against endemic diseases.

Goats ranging in body weight from 35 to 44 kg had been isolated from mature bucks since the previous winter breeding. Mean body condition score (1= extremely thin; 5= extremely fat; palpation over lumbar vertebrae, ribs and sternum) was 2.25.

Herds were randomly assigned to four breeding periods: February, April, May and June. Goats were exposed to well-fed adult mixed-breed bucks at a buck to doe ratio of approximately 1:20 during two weeks. Bucks remained with does permanently during the breeding period, following the flock during the daily grazing route. Small differences in buck to doe ratios in the goat groups were considered not important, as these ratios induce the same estrous response in goats (Carrillo *et al.*, 2007).

Different bucks not treated with artificial supplemental light or exposed to does in estrus were used in each herd of goats. To detect behavioural signs of estrus in does, observations were made by trained staff twice per day from 08:00 to 09:00 and from 16:00 to 17:00. Estrus was confirmed when bucks courted and copulated with the estrous doe.

In a second experiment, a flock of crossbred (native x dairy) goats kept on rangeland was randomly divided into two groups, each consisting of 44 does. Goats were placed in unroofed open pens. One group was exposed to three adult intact bucks in January under mild climatic conditions (daily mean temperature of 13.6°C, with no rainfall); the other group was also exposed in January (starting eight days later) to three different intact mature bucks under wet and cold climatic conditions (average daily air temperature of 8.0°C; continuous drizzling during 5 days). The does were checked visually for standing estrus and copulation, twice daily, during one hour, at 09:00 and 17:00 h.

Blood samples were collected after the morning meal (0800) three days after buck exposure via jugular venipuncture. Blood was collected in 10 mL heparinized tubes, packed in ice and centrifuged at 2000 × g for 10 min at 4°C within 1 h of collection, and plasma was stored in plastic vials at -20°C until assayed for cortisol. This hormone present a circadian periodicity with the highest plasma concentrations in the morning (Mesbah and Brudieux, 1982). Thus, plasma cortisol levels reported in this study correspond to peak cortisol concentrations. Some alteration in circulating cortisol levels due to restraint and vein puncture of animals during blood collection is not discarded.

Heart rate was measured in all animals using a battery operated HR monitor (Polar S610TM), which was mounted

comfortably on the animals. Respiration rate was recorded by counting the rate of flank movements for 1 minute. Rectal temperature was recorded with electronic thermometers having an accuracy of 0.1°C. Goats kept in pen were tame and tolerant to the presence of humans; therefore human interference during recordings was of minor concern. All physiological variables were recorded twice daily at 08:00 and 15:00 h and the averages of these measurements were used for statistical analyses.

Proportions of does exhibiting estrus behavior in different breeding seasons or during the cold wet and mild weather were analyzed as binomial data with the LOGIT function of the PROC GENMOD procedure of SAS (SAS Inst. Inc., Cary, NC, USA). The model statement contained either the effects of weather conditions (cold or mild) or month of breeding. If significant differences were found among treatments, the LSMEAN/DIFF procedure of SAS was used to compare the means.

Analysis of variance (PROC GLM; SAS Inst. Inc., Cary, NC) was used to compare the number of days between estrus in repeated breeders does, hearth rate, respiration rate and plasma cortisol levels.

Results

In experiment 1 most does exposed to bucks in February May and June exhibited estrus during the first 15 days of buck stimulus, with no difference between this time points for these months (Table 1). Only 12% of goats exposed to bucks in May were detected in estrus after 4 days of joining, but estrus response was over 36% (p<0.05) within the first 4 days post-joining in goats stimulated by bucks in February and June. On the other hand, only 1% of the goats exposed to bucks in April manifested standing estrus. The effect of buck exposure on estrus response of goats during the first four days was more pronounced (p<0.01) in June than in February. However, by day 11 of joining, over 80% of goats in contact with bucks either in February or June had shown estrus (Table 1).

The mean number of days from joining to first standing estrus was similar for goats exposed to bucks in February and June (Table 1). On the other hand, the interval from the introduction of bucks to the onset of estrus was much longer (p<0.01) in goats stimulated in April and May than goats in contact with bucks in February and June. Likewise, the proportion of does showing two estrus during the first 15 days following the introduction of bucks did not differ in goats bred in February compared to goats bred in May and June.

For experiment 2, average ambient temperature and humidity for two groups of goats joined to bucks in January are depicted in Table 2. Low temperature combined with constant drizzling conditions during four days (wet hair coats greatly reduces insulating properties and make animals more susceptible to cold stress; Young, 1981), caused the animals to show discomfort (immobility, reluctance to graze and lethargy) during the breeding period.

Table 1. Percentage of goats in estrus, interval to first estrus, percentage of repeat breeders and interval between estrus in mixed-breed goats maintained under range conditions and exposed to bucks in February, April, May and June in northern Mexico (25° N)

Item	February	April	May	June
Number of goats	70	96	70	60
Buck to doe ratio	1:18	1:24	1:18	1:15
Goats in estrus (cumulative)				
4 days (%)	36 (25/70) ^a	0 (0/96) ^b	$12(8/70)^{c}$	57 (34/60) ^d
8 days (%)	74 (52/70) ^a	1 (1/96) ^b	$12(8/70)^{c}$	$78 (47/60)^a$
11 days (%)	84 (59/70) ^a	1 (1/96) ^b	$76 (53/70)^{a}$	$82 (49/60)^a$
15 days (%)	89 (62/70) ^a	1 (1/96) ^b	$100 (70/70)^{c}$	$88 (53/60)^a$
Days to estrus after joining $(\bar{x} \pm SD)$	5.6 ± 3.8^{a}	8 ± 0^{a}	9.4 ± 4.9^{a}	4.7 ± 3.7^{a}
Repeat breeders in 15-d period (%)	44 (27/70) ^a	0 (0/96) ^b	17 (3/18) ^c	29 (17/60) ^a
Days between estrus $(\bar{x} \pm SD)$	6.1 ± 0.3^{a}	$0 \pm 0.0^{\rm b}$	4.5 ± 0.05^{a}	7.2 ± 0.7^{a}
^{a,b,c} Values that share different superscripts letters are different at p<0.01				

Table 2. Climatic and physiological variables, plasma cortisol levels and percentage of mixed-breed goats in estrus when exposed to bucks under a short period of cold stress or no stress at 25° N

Item	Cold stress* No stress [†]	
Number of goats	44	44
Percentage of bucks	6	6
Mean daily ambient temperature (°C)	8.0 ± 5.9	13.6 ± 3.4
Humidity (%)	80.1 ± 14.6	64.4 ± 22.5
Total rainfall during joining (mm)	17.7	0.0
Rectal temperatura (°C)	39.2 ± 0.4	37.9 ±0.4**
Resting hearth rate (beats per minute)	95.6 ± 16.2	82.4 ± 14.6**
Respiratory rate (breaths/minute)	26.8 ± 9.6	19.1 ± 6.4**
Cortisol (ng/mL)	28 ± 12	12 ± 4.6**
Goats in estrus (%) in a 15-d period	27 (12/44)	80 (35/44)**
Estrus after teasing (days; $x \pm SD$)	9.3 ± 4.7	$4.5 \pm 3.2^{**}$

^{*}Average daily air temperature 8.0°C and continuous drizzling during 5 days; [†]Daily mean temperature of 13.6°C with no rainfall; **p<0.01

The rectal temperature of the does under cold stress averaged 1.3° C higher (p<0.01; Table 2) than those under mild temperatures. Does exposed to a cold wet environment showed a greater (p<0.01) rise in plasma cortisol levels than goats under mild weather, which seems to explain the lower (p<0.01) estrus response of goats subjected to cold stress compared to goats exposed to bucks under a mild weather (Table 2).

Discussion

The null response of does to bucks in April indicates that bucks were sexually inactive, and therefore incapable to induce estrus in does (Vielma *et al.*, 2009). Mixedbreed bucks at this latitude present no sexual drive at this time of the year (Carrillo *et al.*, 2011; Luna-Orozco *et al.*, 2012), which apparently impeded the male-induced ovulation in goats.

Depth of anestrus at joining is an important factor in determining the response to the buck effect. Mixed-breed goats at this latitude display ovulation cycles in spring without exposure to bucks (Urrutia-Morales *et al.*, 2009) or anestrus goats display full estrus activity from April to May, provided that sexually active bucks are used (Veliz *et al.*, 2006; 2009). Even with the use of bucks under

natural photoperiod, mixed-breed goats at this latitude respond to the buck stimulus in spring, although with lower intensity (Mellado *et al.*, 1996; De Santiago-Miramontes *et al.*, 2011). In the present study, the lack of response of goats to the buck stimulus in April could be explained by the displayed sexual inactivity of bucks. Bucks probably ceased breeding, not because they were inhibited by increasing day lengths, but because they became photorefractory (Delgadillo *et al.*, 2011).

The discrepancy of these results with previous reports in this zone seems to stem from the genotypes of goats used: in the present study goats had high proportion of Criollo genes, whereas data from others researchers derived from mixed-breed dairy goats. Moreover, there is considerable variation between herds and animals within herds in the responsiveness of does to the buck stimulus (Chemineau *et al.*, 2004; Delgadillo *et al.*, 2009).

The fact that estrous response at 15 d after joining began was high during most of the non-breeding season, indicated that bucks were sexually active during this period, and therefore they stimulated does to become sexually receptive. This is contrary to what has been observed by several researchers (Vielma *et al.*, 2009; Flores *et al.*, 2000; Rivas-Muñoz *et al.*, 2010), who

observed that none of the goats joined with bucks nonstimulated with extra light in spring at this latitude showed sexual activity.

Goats exposed to bucks in May showed a slower response to the buck stimulus compared to goats bred in February and June. The delayed response of does to buck stimulation in May can be due to a diminished sexual activity of bucks during the transitional period, which possibly resulted in a weaker stimulus of bucks, which in turn, could have resulted in slower estrus response of does. In goats, the most sexually active males are more effective at stimulating ovulation than those that are less sexually active (Walkden-Brown et al., 1999), and the intensity of sexual stimulus in bucks has been shown to be markedly influenced by season (Zarazaga et al., 2009). These results suggest that lengthening the breeding period in May in crossbred goat herds at 25°N could be beneficial, because of the delay in sexual response of does during this month.

This study does not support observations by other researchers (Delgadillo et al., 1999) indicating that sexual activity of bucks is suppressed during the winter months at this latitude, because under the conditions of the present study bucks were fully capable of inducing estrous behaviour of does in February. Does in the present study had a strong perception of male sexual cues in February, May and June, which indicates that for these mixed-breed goats (animals with high percentage of Criollo genes) at this latitude photoperiod is a "weak" signal for the reproduction rhythm and apparently other environmental clues, particularly body energy reserves (Urrutia-Morales *et al.*, 2009) are used as complements to regulate the length of the reproductive cycle.

Crossbred goats at this latitude cannot be considered short-day breeders (does showed complete sexual response to the buck stimulus before the summer solstice), which is in line with data of others (Urrutia-Morales *et al.*, 2009) who observed, at this latitude, ovulation cycles in 60 to 80% of Criollo goats in spring, but these data are contrary to what has been stated by other researchers in this zone with mixed-breed dairy goats (Duarte et al., 2008; Delgadillo *et al.*, 1999).

In the present study, it was unknown if does were cycling at the time of buck exposure in winter and spring months. This is relevant because it has been postulated that the buck effect is ineffective in cycling goats, as the reproductive axis of does is inhibited by progesterone secreted during the luteal phase (Delgadillo *et al.*, 2009). However, this situation appears not to be entirely true, because in cyclic goats, the presence of bucks can stimulate LH secretion (Hawken *et al.*, 2009) and a high estrus response (Chemineau *et al.*, 1983; Mellado *et al.*, 1996).

The interval from joining to first standing estrus and accumulated percentage of does showing two estrus during the first 15 days following the introduction of bucks either in February or June was similar. Several researchers have documented that immediately after introduction of bucks, LH pulsatility increases, which leads to an increase in plasma estradiol pulsatility and the

preovulatory surge of LH, resulting in an induced ovulation 2–4 days after buck stimulus (Chemineau *et al.*, 2006). However, most goats have a short ovarian cycle of 5–7 days of duration, followed by a second ovulation, which is associated with estrous behaviour and a normal luteal phase (Flores *et al.*, 2000; Chemineau *et al.*, 1987). The short ovarian cycle after buck exposure seems to be due to low secretion of progesterone from corpus luteum with low granulosa cell quality derived from poor quality follicles forced to ovulate (Chemineau *et al.*, 2006).

The estrus that accompanies the second ovulation after teasing and is followed by a normal luteal phase suggests that the hypothalamo-pituitary system requires priming by progesterone secreted after the first ovulation (Lassoued *et al.*, 1995; Gonzalez-Bulnes *et al.*, 2006). The proportion of repeat breeders during the short mating period in the present study is comparable to that found by others in crossbred goats of this zone (Vielma *et al.*, 2009; De Santiago-Miramontes *et al.*, 2011).

Physiological responses observed in the present study in cold-stressed goats have been observed by other authors (Marques *et al.*, 1981), and this response is a sign of cold acclimatization, which involves elevated peak metabolic capability and a temporary elevation in resting metabolism. Higher respiration rate observed in the cold stressed group is an attempt by does to maintain normal body temperature by pumping blood faster to keep normothermia. As pulse increases, the need for oxygen also increases since lungs transfer oxygen to the blood and this explains the higher respiratory rate.

Short-term stress exposure in goats is associated with activation of the hypothalamic-pituitary-adrenal axis and a consequent rise in blood glucocorticoids and catecholamines (Marques *et al.*, 1981; Zimmer *et al.*, 2009), as it was observed in goats in the present study.

The data from the present study indicate that wet and cold ambient conditions prevented most does to become in estrus when stimulated by bucks. Whether the effect of the low environmental temperature is due to decreased pituitary activity or to some other mechanism cannot be determined from the present study. If light is responsible for triggering the physiological process or mechanism responsible for sexual activity in goats, it is clear that climatic conditions also have some effect on the expression of this phenomenon.

Conclusions

These results indicate that under rangeland conditions at 25° N, herds of mixed-breed goats show a reduced period of sexual inactivity, with absence of sexual function in the early spring, but with full sexual competence in the winter and late spring. These experiments also provide evidence that mixed-breed goats under cold stress are less responsive to the buck stimulus.

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