The “female effect” positively affects the appetitive and consummatory sexual behaviour and testosterone concentrations of Alpine male goats under subtropical conditions


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ABSTRACT: The aim of this study was to evaluate the possible action of the “female effect” by evaluating if exposure to estrogenized females would affect sexual behaviour and testosterone concentrations while affecting the length of the reproductive season of Alpine bucks in northern Mexico (26°N). In January, two experimental groups were formed: (i) treated males (MH; n = 8) kept in a pen aside to another pen with four estrogenized females during four weeks; pens were separated by a metal mesh, and (ii) control males (GC; n = 8) which had no contact with any female during the same period. At the end of the study, an appetitive and consummatory sexual behaviour test was performed by exposing males from both groups to estrogenized females. Besides, serum concentrations of testosterone were quantified in each male on days 0 and 28. On day 0 the serum levels of testosterone were similar in both groups (217 ± 86 vs 320 ± 89 ng/dl in MH and GC respectively; P > 0.05). However, on day 28, serum testosterone levels favoured to the MH group (164 ± 56 vs 49 ± 18 ng/dl; P = 0.06). With respect to the consummatory (80%) and appetitive (62%) sexual behaviour, the best reproductive performance was depicted by the MH group with respect to the GC group (P < 0.05). Results obtained confirm a positive action of the “female effect” upon behavioural, reproductive, and endocrinological outcomes, while extending the breeding season of Alpine male goats. These findings should be relevant in the design of clean, green, and ethical reproductive management strategies in goat production systems and potentially important to the animal industry.

Keywords: seasonal reproduction; pheromonal communication; Alpine bucks; sexual performance

INTRODUCTION

The length of the reproductive season of male and female goats can be affected by several factors, such as breed, latitude, photoperiod, and nourishment among others (Ibrahim 1997; Zamiri and Haidari 2006; Meza-Herrera 2012; Meza-Herrera and Tena-Sempere 2012). The seasonal reproductive pattern of goats has been studied in different breeds and geographical locations (Chemineau et al. 1992; Ahmed et al. 1997; Al-Ghalban et al. 2004). Nevertheless, information available related to the length of the reproductive season either in females and males is scarce under subtropical latitudes, and in most cases only few variables are taken into consideration, predominantly breed. Certainly, just few studies have considered the effect of other environmental variables that may affect the yearly cycle of reproductive activity such as chemo-sexual cues, e.g. pheromone (Walkden-Brown et al. 1999; Carluccio et al. 2013).
Regarding chemo-sexual cues, the pheromonal signal reaches the brain via the accessory olfactory system, which projects into the medial amygdala and the bed nucleus of the stria terminalis, and into the hypothalamic subnuclei, including the medial preoptic area and the ventromedial hypothalamic nucleus. The activation of this neuronal circuitry controls the neuroendocrine system (Carluccio et al. 2013). The vomeronasal system shows sexual dimorphism at many stages of its projection pathways, and has been well established that male chemical signals can modify female physiology; e.g. increased luteinizing hormone and follicle-stimulating hormone secretion, augmented follicle number and size, accelerated onset of puberty while modifies oestrus and ovulation (Carluccio et al. 2013).

Actually, in goats, more reports have been generated in temperate latitudes (> 35°N or S), while under subtropical latitudes (25–35°N or S) such information is scarce (Walkden-Brown et al. 1999; Delgadillo 2005; Gonzalez-Bulnes et al. 2011). In addition, it is difficult to extrapolate results from temperate areas to subtropical conditions because physiological performance can be affected in a different fashion due to differences regarding photoperiod, temperature, climate, as well as other environmental factors such as feeding strategies and socio-sexual and chemo-sexual cues (Gonzalez-Bulnes et al. 2011).

In male goats, consummatory sexual behaviour (mounted attempt, mounting, and ejaculation) depends upon testosterone production, which decreases during spring and summer, pointing out to the paramount influence that photoperiod exerts upon testosterone release, even under subtropical conditions (Delgadillo 2005). Recently, Carrillo et al. (2010) demonstrated that Alpine male bucks raised in an intensive system with an accurate nutrition level and exposed to natural variations of photoperiod observed in northern Mexico (26°N) exhibited a decreased sexual activity from January to July, with significant decreases in spermatic quality, libido, and testicular weight.

On the other hand, the presence of oestrus females can influence the consummatory sexual activity of male goats and rams; a socio-sexual cue known as the “female effect” (Fahey et al. 2012; Carluccio et al. 2013). Certainly, under temperate conditions, either in Australian Cashmere buck (Walkden-Brown et al. 1994) or in Île-de-France rams (Schanbacher et al. 1987; Gonzalez et al. 1991a, b), exposure of males to oestrus females increased not only the serum levels of LH and testosterone but also the consummatory sexual behaviour during the non-breeding season. Nevertheless, the impact of this so-called “female effect” upon highly seasonal genotypes such as Alpine bucks and under the subtropical conditions in northern Mexico remains unknown. The purpose of this study was to determine the possible influence of the “female effect” upon sexual behaviour, either appetitive or consummatory, as well as testosterone concentrations in Alpine bucks exposed to estrogenized females in northern Mexico.

MATERIAL AND METHODS

Location, animals, and their management. The experiment was carried out in the Comarca Lagunera located in northern Mexico (26°23’N and 104°47’W, 1200 m a.s.l.), with an annual precipitation rate of 230 mm, distributed in an erratic fashion throughout the year, although more frequent during the summer and autumn seasons. The maximum average temperature is of 41°C during May and June, while the lowest (–3°C) occurs in December and January. Relative humidity varies between 26.1 and 60.6%, with a range in the photoperiod from 13 h 41 min during the summer solstice (June) to 10 h 19 min during the winter solstice (December). Alpine adult goat males (n = 16, 2–4 years old) were subjected to the natural variations of photoperiod in an intensive production system. All animals had free access to alfalfa hay (17% crude protein (CP), 1.95 Mcal metabolizable energy (ME)), and each of them received 200 g of a commercial concentrate per day (14% CP, 1.7 Mcal ME) while trace minerals were provided in a 25 kg block with no less than 17% P, 3% Mg, 5% Ca, 5% Na, and 75% NaCl, with free access to fresh water throughout the experimental period which lasted from January 11 to February 14. All methods used in this study were conducted in accordance with accepted international guidelines (ASAB, 2006).

On January 11, the male goats were distributed in two homogeneous groups regarding body weight and condition, age, horn odour intensity, and testicular weight. Thereafter, a group of males (MH; n = 8) was placed for four weeks in a 5 × 5 m open pen, aside to another pen (5 × 4 m) with four estrogenized females which were kept apart from
males with the use of chain link mesh, yet visual and olfactory contact was allowed. Furthermore, females where introduced into the males’ pen daily for one hour, and males where covered with an apron to prevent them from engaging intercourse. On day 3, females where treated with one dose (20 mg) of progesterone in order to promote an increased response to the estradiol treatment as well as to produce faster oestrus behaviour. This treatment regime allowed goats to be brought into a permanent oestrus during the three weeks of exposure to bucks (Carrillo et al. 2011). Females received 2 mg of estradiol cypionate intramuscularly beginning on day –2 and finishing by the end of the study. The male control group (GC; n = 8) was kept in a 5 × 5 m pen to avoid either olfactory or visual contact with females which were placed 200 m apart. According to previous studies, this separation allows the animals to be isolated from any pheromonal and acoustical input (Delgadillo et al. 2002; Veliz et al. 2002).

**Appetitive and consummatory sexual behaviour evaluation.** Three days after the end of the experimental period, on days 31–33, an appetitive and consummatory sexual behaviour test was conducted; males from both experimental groups were individually exposed to two estrogenized females for 15 min. The behavioural components recorded were (i) appetitive sexual behaviour (ASB) considering flehmen response (lip curled and head erected), as well as ano-genital sniffing (nasal investigation of anal-genital region), and (ii) consummatory sexual behaviour (CSB): mount attempts (mounts accompanied by pelvic oscillations with or without ejaculation) and male-female aggressions (when a male hitting a female with his head or horns) (Rivas-Munoz et al. 2010; Carrillo et al. 2011; Carluccio et al. 2013).

**Body condition and weight, scrotal circumference, and horn odour intensity index.** Body condition score and body weight were determined every two weeks throughout the experimental period. Regarding body condition score, the determination methodology was that outlined by Walkden-Brown et al. (1993). Briefly, the muscle mass in the animal was measured at the lumbar region, and the value was based in a 1–4 points scale (1 = very thin, 4 = very fat). Scrotal circumference was determined in the widest part of both testicles, using a measuring metric tape graduated in mm. The volatile emission in the area behind the base of the horns was determined through the method described by Walkden-Brown et al. (1993), and reported as horn odour intensity (HOL) index. The horn odour intensity evaluated at such a distance has a positive correlation (r = 0.71) with serum testosterone concentrations responsible for male sexual activity. This method considers a 0–3 point scale, where 0 = neutral odour of females or castrated males up to 3 = an intense sexual odour; 1 and 2 runs from light to moderate, respectively (Walkden-Brown et al. 1993). Blood samples (10 ml) were collected on days 0, 28, and 35 by jugular venipuncture into sterile vacuum tubes (Corvac Kendall Health Care, St. Louis, USA) and allowed to clot at room temperature during 30 min. This daily protocol allowed detecting any difference between males depicting sexual activity or reproductive arrest (Delgadillo et al. 2002).

**Blood sampling and testosterone quantification.** Blood samples were collected in the morning in both groups, and in the MH group also prior to full contact to estrogenized females. Serum was separated by centrifugation (1500 g, 15 min), decanted, and transferred to polypropylene micro tubes (Axygen Scientific, Union City, USA) and stored at −20°C until hormonal analysis. Peripheral serum testosterone concentrations were determined in duplicate in a single solid phase radioimmunoassay, using a commercial testosterone kit (Coat-A-Count; Diagnostic Products Co., Los Angeles, USA), following the manufacturer’s instructions. The value of the inter-assay coefficient of variation (CV) for testosterone quantification was of 8.9%, the intra-assay CV was of 11.6%, with a detection limit of 0.013 ng/dl, and control high, low, and medium were 22, 404, 1582 ng/dl, respectively.

**Statistical analyses.** The mean, standard deviation, and standard error were calculated and the tests for normality (Kolmogorov-Smirnov) and homogeneity of variances (Levene’s test) were performed for all variables. Differences between sexually inactive and active bucks in the frequencies of buck sexual behaviours were analyzed using a chi-square test for goodness of fit, with a null hypothesis of equal repartition of behavioural frequencies in the two groups. Data regarding scrotal circumference, body weight, body condition, odour score, and plasmatic testosterone were submitted to a split-plot analysis of variance. The model included treatment in the main plot, which was tested using animal within treatment as the error term. Time and the time × treatment interac-
tion were included in the subplot, and were tested by using the residual mean square. In addition, odour intensity data was analyzed using the Mann-Whitney U test. All the statistical analyses were performed with the statistical package SYSTAT (Version 10, 2006). Differences were considered to be statistically significant at \( P < 0.05 \). Reported values are defined as Least Squares Means ± standard error of the mean (SEM).

RESULTS

Analyses of Variance did not reveal any effect of group, time or group × time interaction upon body condition \( (P > 0.05) \). Average body condition for MH and GC was 1.6 ± 0.1 and 1.7 ± 0.1, respectively. Overall scrotal circumference averaged 23.1 ± 0.8 cm and was only affected by time \( (P < 0.01) \). Horn odour intensity score was affected by experimental group \( (P < 0.05; \text{Figure 1}) \), time \( (P < 0.001) \), and the group × time interaction \( (P < 0.01) \). Certainly, odour score was higher in the MH on days 21 and 28 of the experiment \( (P < 0.05; \text{Figure 1}) \). With respect to the consummatory (80%) and appetitive (62%) sexual behaviour, the best reproductive performance exhibited the MH group with respect to the GC group \( (P < 0.05; \text{Figure 2}) \). On the other hand, while serum levels of testosterone were not affected by experimental group, an effect of time and the group × time interaction affected this variable \( (P < 0.05) \). Certainly, the MH showed higher serum testosterone values on day 28 \( (P = 0.06; \text{Figure 3}) \). Nevertheless, after the sexual behaviour test (day 35), serum testosterone concentration decreased in the MH group, with a corresponding increase in the GC males \((43 ± 14 \text{ vs } 252 ± 63 \text{ ng/dl}, P < 0.05; \text{Figure 3})\).

DISCUSSION

Results of this study suggest a positive pheromonal effect exerted by the oestrus females, the so-called “female effect”, demonstrating a positive influence upon reproductive and behavioural outcomes of Alpine male goats which extended their reproductive season in northern Mexico. Certainly, the contact with estrogenized females delayed the end of the reproductive season of Alpine bucks under subtropical conditions. In addition, an increased appetitive and consummatory sexual behaviour was observed in males exposed to the female effect. This positive effect could be exerted because of the pheromonal stimulation throughout the two olfactory

![Figure 1. Horn odour intensity index of Alpine goat males exposed to estrogenized females (MH) or of control Alpine goat males without contact with females (GC) at the end of sexual activity season in northern Mexico (26°N) (* \( P < 0.05 \), ** \( P < 0.01 \)). Horn odour intensity index was affected by experimental group \( (P < 0.05) \), time \( (P < 0.001) \), and the group × time interaction \( (P < 0.01) \).](image1)

![Figure 2. Sexual behaviour of Alpine goat males exposed to estrogenized females (MH) or control Alpine goat males without contact with females (GC) at the end of sexual activity season in northern Mexico (26°N) (* \( P < 0.05 \), ** \( P < 0.01 \)).](image2)
pathways previously demonstrated in sheep: (i) the main olfactory system receiving sensorial stimuli throughout the olfactory mucosa connected to the central nervous system by means of the olfactory bulb, and (ii) the accessory olfactory system which receives information from the vomeronasal organ which is in turn connected to other brain centres throughout the accessory olfactory bulb (Alvarez and Zarco 2001). Both systems have connection to the hypothalamus where the neuronal centres controlling LH secretion through activation of GnRH neurons reside (Alvarez and Zarco 2001; Carluccio et al. 2013). Also, serum testosterone concentrations on day 28 of the experimental period as well as the male-female aggression pattern increased in the female-treated bucks. Remarkably, such reproductive, endocrinological, and sexual-behavioural responses were promoted in the Alpine breed, a genotype which typically displays a high reproductive seasonality or a significant reproductive arrest period even under subtropical conditions (Carrillo et al. 2010). Certainly, such results were obtained in Alpine male goats during a season of the year commonly defined as photo-inhibitory of reproductive function because of the observed photoperiod at this latitude (26°N) (Delgadillo et al. 2002; Carrillo et al. 2010).

In this study, the increased sexual activity shown by the males exposed to the “female effect” was probably promoted because of an increase in the secretion of testosterone, which in turn generated an increase in the reproductive and sexual performance observed in the treated Alpine males (Meza-Herrera and Tena-Sempere 2012). Similarly, in Australian Cashmere male goats briefly exposed to oestrus females during the natural sexual resting period, an increased pattern in the secretion of both LH and testosterone was observed (Walkden-Brown et al. 1994). Nevertheless, in our study the serum testosterone levels of the treated males decreased by half on day 28, reaching basal levels on day 35, time at which the sexual behavioural tests were performed. The reason could be that the males became refractory to the stimuli generated by oestrus females, and/or the males were facing a deep sexual resting period, and under such conditions, the inhibitory reproductive effect generated by the increased photoperiod seems to be enough to abolish any rise in the libido of males, suppressing, in this way, any possible influence of the “female effect” (Delgadillo et al. 2008). However, males from the control group increased their horn odour index as well as the serum testosterone levels once the sexual behavioural tests were performed. The last could be promoted because these males were not in contact with females prior to the behavioural tests and may be they were not refractory to the stimulatory “female effect” depicting a definitive sexual behaviour. Certainly, males with no previous contact to estrogenized or oestrus females by at least three weeks, and then suddenly exposed to oestrus females, display an increase in both LH and testosterone, as well as in semen volume while a perfectly defined sexual behaviour, by increasing the HOI even during the natural period of sexual arrest (Walkden-Brown et al. 1994, 1999; Carluccio et al. 2013).

In this respect, the contact of males with oestrus females halfway through the sexual resting period, when the effect of the photoperiod is significantly inhibitory of sexual activity, generated no increases either in sexual behaviour or in serum testosterone levels, even in adequately fed males subjected to such inhibitory photoperiod (Walkden-Brown et al. 1994; Veliz et al. 2002). On the contrary, the exposure of oestrus females to males during the resting season increased the frequency of LH pulses, observing that both visual and audiovisual signals were less effective than the stimuli of the animal’s presence per se, suggesting that both visual and audiovisual cues are possibly less important regarding olfactory signals (Walkden-Brown et al. 1994; Hawken et al. 2009). The hypothalamic gene KiSS1 encodes a 54 amino acid precursor that is cleaved to a family of peptides also known as kisspeptins. Kisspetin and its GPR54 receptor linked to G-proteins have emerged as key elements in the regulation of GnRH secretion (Meza-Herrera
et al. 2010; Meza-Herrera 2012; Meza-Herrera and Tena-Sempere 2012). In sheep, over 90% of GnRH cells express the GPR54 receptor (Clarke 2011). Nonetheless, despite the huge body of evidence linking kisspeptin to GnRH secretion, no studies have yet directly correlated activation of kisspeptin neurons with the increase in LH secretion characteristic of the male effect (Hawken and Marin 2013). The same scenario is true regarding the increases in testosterone release generated by the “female effect”; further studies in this respect are therefore warranted.

Under temperate conditions, photoperiod is the most important environmental regulator of seasonal reproduction. However, in small ruminants from northern latitudes transferred to subtropical conditions, as is the case of our study, it seems that besides photoperiod, other environmental signals such as nutrition, socio-sexual or chemo-sexual communication may also play a paramount role as modulators of reproductive activity (Walkden-Brown et al. 1993, 1994; Meza-Herrera et al. 2011; Meza-Herrera et al. 2013a, b). Certainly, under subtropical environmental conditions, animals may be less sensitive to the prevailing photoperiod and could be more influenced by socio-sexual or chemo-sexual signals, particularly by the “female effect” during the transition stage from the reproductive season to the sexual resting season. The last scenario might have probably generated lengthening regarding the function of the hypothalamic-hypophysial-gonadal axis in those Alpine male goats exposed to the “female effect”, generating a positive influence not only in the sexual behaviour but also in the reproductive outcomes observed in those males exposed to the “female effect”.

CONCLUSION

The female socio-sexual stimuli and chemo-sexual communication upon the hypothalamic-pituitary-gonadal axis of male goat are complex and multi-factorial both in terms of the nature of the female stimuli as well as regarding the response of the male. Results of this study confirm that exposure of estrogenized females to Alpine male goats during the normal resting reproductive season invoked key physiological responses, not only at behavioural level but also regarding reproductive and endocrinological outcomes. Consequently, the “female effect” emerges as an important socio-sexual/chemo-sexual cue modulating the reproductive function in Alpine male goats under subtropical conditions. Such findings are therefore important in the design of clean, green, and ethical reproductive management strategies and potentially attractive to the animal industry.

REFERENCES


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