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# Continuous contact with females in estrus throughout the year enhances testicular activity and improves seminal traits of male goats

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## ABSTRACT

The “female effect” consists in the stimulation of males’ reproductive activity by different signals emitted by females. This stimulation leads to endocrine and behavioral changes that may modify the seasonal pattern of male ruminants. The aims of this experiment were (1) to describe the local reproductive seasonal pattern of Gabon bucks and (2) to determine if continuous chemical, auditory, and visual contact with does in estrus enhances bucks’ testicular activity and improves seminal traits throughout the year and modify their seasonal pattern. We used 16 adult Gabon bucks assigned to two experimental groups: nine bucks remained continuously isolated from females (isolated bucks, group IB) and seven bucks were in continuous chemical, auditory, and visual contact through a fence line with does in estrus (stimulated bucks, group SB). During 13 months, scrotal circumference and testosterone concentration were measured weekly and testicular echogenicity was measured every 2 weeks. Also, sperm motility mass and percentage of abnormal spermatozoa were determined, and sperm concentration and total number of motile spermatozoa were calculated every 2 weeks. Testicular echogenicity was greater in IB than that in SB bucks ( $P < 0.0001$ ), but there were no differences in scrotal circumference. Overall, testosterone concentration was greater in IB than that in SB bucks ( $P = 0.04$ ), but from late winter to mid-summer, when testosterone concentration presented basal concentrations, SB bucks had greater values than IB bucks ( $P = 0.004$ ). Sperm concentration ( $P = 0.05$ ) and sperm mass motility ( $P = 0.01$ ) were greater in SB than that in IB bucks, and the total number of progressive motile spermatozoa tended to be greater in SB than in that IB bucks ( $P = 0.1$ ). The percentage of abnormal spermatozoa was lower in SB than in IB bucks in several time points ( $P < 0.0001$ ). Testicular and seminal traits were better from the end of the spring until mid-autumn. We concluded that does in estrus stimulated bucks’ testicular activity, including better seminal quality and a greater increase of testicular fluid content than bucks isolated from females. However, the general seasonal pattern was not modified by stimulation with does in estrus.

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## 1. Introduction

In small ruminant species that evolved in temperate zones, the reproductive activity is mainly regulated by the annual cycle of photoperiod [1,2]. In males, the greatest

reproductive activity is achieved during autumn, when light time length is decreasing. At the beginning of this period, gonadotropin and testosterone concentration reach maximum concentrations, and semen quality is better than during the rest of the year [3]. The seasonal pattern of reproductive activity of Gabon bucks, a breed from tropical areas, has never been studied in detail. In Uruguay, using data from 8 years, we determined that

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births occur most frequently in May and September (unpublished data), and as goats gestation lasts 5 months, females have fertile estrous mainly in autumn (April) and summer (December).

The basic seasonal reproductive pattern can be modified by sociosexual signals produced by individuals from the same or the other gender [4]. Briefly, exposure to females induces a rapid increase in LH [5,6] and testosterone secretion [7] in rams, accompanied by an increase in testicular fluid content [7]. Also, exposing rams to ewes having estrous activity before semen collection increases volume and sperm concentration of the ejaculate [8]. The endocrine responses may be maintained during several days if there are still females in estrus [9]. Moreover, when rams are exposed to females for longer periods, testicular size [10] and testosterone concentration increases and enhances their sexual activity [10,11] and aggressive behavior [10]. Similarly, permanent contact with females increases testosterone fecal concentrations and improves semen quality in deer [12] and increase scrotal circumference and testosterone concentration in kid goats before their first breeding season [13].

Our hypothesis was that continuous contact throughout the year with does in estrus through a fence line could stimulate bucks' testicular activity. Therefore, the aims of the experiment were (1) to describe the local reproductive seasonal pattern of Gabon bucks and (2) to determine if chemical, auditory, and visual contact with does in estrus through a fence line could enhance bucks' testicular activity and improve seminal traits and modify their reproductive seasonal pattern.

## 2. Materials and methods

The experimental procedures were approved by the Comisión Honoraria de Experimentación Animal of the Facultad de Veterinaria.

### 2.1. Animal management and experimental treatments

The experiment was performed in Uruguay (35°S), from April 2012 to May 2013. Sixteen adult Gabon bucks (3–5 year old,  $29.2 \pm 1.5$  kg; mean  $\pm$  standard error of the mean) were assigned to two experimental groups. Although nine bucks remained continuously isolated from females (minimum distance = 5000 m) (isolated bucks, group IB), other seven bucks were continuously stimulated by females in estrus (stimulated bucks, group SB). Both groups of bucks were housed in similar pens (10 m  $\times$  17 m). The SB bucks' pen was separated by a fence line from three adult does allowing chemical, auditory, and visual communication. Bucks could have physical contact with does only through the small openings of the fence line (4  $\times$  4 cm). Each week one different doe was induced into estrus by hormonal treatment, remaining receptive at least during three consecutive days. Estrus was induced using intravaginal sponges impregnated with medroxyprogesterone during 5 to 7 days, followed by administration of estradiol benzoate (1.5 mg, Benzadiol 100; Laboratorio Universal, Montevideo, Uruguay) every 12 hours during 3 days.

During the experiment, both groups received equal amounts of lucerne hay per buck adjusted according to their nutritional requirements for maintenance and had free access to water.

### 2.2. Testicular characteristics

Scrotal circumference was measured weekly and testicular ultrasound scannings were performed every 2 weeks to determine testicular echogenicity according to Ungerfeld and Fila [14] using a B-mode ultrasound scanner (Wed9618V; Welld, Guangdong, China) equipped with a 7.5-MHz linear array transducer. Focus, gains, brightness, and contrast were standardized and used equally in all images. Echogenicity of the testicular ultrasound images may be useful to study the function and structure of tissues, providing information about the relationship between parenchyma density and fluid content [15]. The pixels' color intensity was measured (gray scale ranging from 0 to 255; 255 = white, 0 = black) using an appropriate software (Image Proplus 3.01, Media Cybernetics, Los Angeles, USA). This allowed to determine if there were changes in the amount of testicular fluid content (darker pictures indicate greater amounts of testicular fluid) [14]. In August, testicular ultrasound scans were not obtained due to technical problems.

### 2.3. Blood collection and testosterone measurement

Blood samples (5 mL) were collected weekly by jugular venipuncture, always in the morning and at the same time in both groups. Samples were allowed to clot for 60 minutes at room temperature before being centrifuged for 20 minutes, and serum was stored at  $-20$  °C until hormonal measurement. Testosterone serum concentration was determined at the Laboratorio de Técnicas Nucleares (Facultad de Veterinaria) with a Coat-A-Count solid-phase kit (Diagnostic Products Corporation, Siemens, Los Angeles, CA, USA). The detection limit of the assay was 0.1 nmol/L, and the intra-assay coefficient of variation for low and high control was 8.7% and 5.2%, respectively.

### 2.4. Semen evaluation

Semen samples were collected every 2 weeks by electroejaculation. The penis was grasped and held at the end of a glass vessel previously warmed up to 37 °C. Electrical stimulation (8 V) was applied for intervals of 3 seconds and alternated with rest periods of similar duration until semen was obtained. Sperm concentration and total number of progressive motile spermatozoa (individual motility  $\times$  total number of spermatozoa) in the ejaculate were calculated. Sperm motility mass (scale 0–5) was determined according to Evans and Maxwell [16], and the percentage of abnormal spermatozoa was determined.

### 2.5. Statistical analysis

Testosterone concentration was analyzed as the mean of the serum concentration of the samples measured every

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