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Seasonal evaluations of urinary androgen metabolites and semen quality in domestic long-tailed chinchilla (*Chinchilla lanigera*) under natural photoperiod

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ABSTRACT

Chinchilla spp. is a South American hystricomorph rodent genus currently considered almost extinct in the wild. The high quality of chinchilla fur motivated the harvesting of chinchillas for the fur market. Reproductive biology advances come from studies on commercially exploited animals, especially *Chinchilla lanigera*. We studied seasonal variation of urinary androgen metabolites, sperm concentration and sperm functional activity in males of domestic *Chinchilla lanigera* under natural photoperiod. In Córdoba city (31° S–64° W; Argentina), within the same latitudes as those of the historic Andean distribution (tropical deserts; 15°–34° S), domestic males ($n = 7$) were studied in May (autumn), August (winter), November (spring), and February (summer). Urine was seasonally collected (over 24 h; once for season, 4 in total) to measure urinary androgen metabolites (RIA), before semen collection by electroejaculation. The results indicated that although testicular volume (relative to body weight) and values of sperm functional activity did not show seasonal changes, a seasonal variation in androgen excretion was detected, with the highest values occurring during "short" light/dark cycles (autumn–winter). In addition, viable spermatozoa with intact acrosome mean values during winter–spring were higher than in autumn or summer. This study provides information that might contribute to the assessment of testicular activity in male chinchilla subjected to genetic selection in the fur industry. In addition, since domestic chinchilla still share some genomic characteristics with their counterparts in the wild, results presented may alsocontribute to ex situ breeding program of endangered chinchilla. In conclusion, natural photoperiod cycle affects testicular activity in domestic chinchilla.

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1. Introduction

Photoperiodism is the ability of organisms to "employ" day length as an anticipatory cue to time seasonal events in their life histories. Photoperiodism is especially important in initiating physiological and developmental processes that are typically irrevocable and that culminate at a future time or at a distant place (Bradshaw and Holzpfel, 2007). Particularly in reproductive physiology, whether a species reaches full reproductive maturity under "long" or "short" days depends, among other factors, upon its length of gestation (Short, 1985). Reproduction in rodents with long gestation (i.e. 3–4 months) is triggered by "short" days, whereas in rodents with short gestation (i.e. 21 days), reproduction is initiated by "long" days (Prendergast et al., 2001). Nevertheless, a few studies in desert rodents indicate that reproductive functions are less affected by photoperiod (El-Bakry et al., 1998). In chinchilla, there are still no studies about the effect of photoperiod on gonadal activity.

Chinchilla spp. lives in colonies in extensive burrow systems in the Andes mountains (above 1700 m asl, tropical deserts) (Redford and Wisenberg, 1992). The short-tailed chinchilla (*Chinchilla brevicaudata*) and the long-tailed chinchilla (*Chinchilla lanigera*) were once widely distributed along the Andes. The high quality of their fur motivated the harvesting of chinchillas for the fur market. Therefore, free-living *Chinchilla* spp. is under threat (IUCN Red List of Threatened Species; http://www.iucnredlist.org) because of a drastic past and ongoing population decline (Chebez, 2008; D'elia and Teta, 2008) in South America. We found few studies available about the presence of free-living chinchillas in Chile (Cortés et al., 2003, 2002). By contrast, domestic chinchilla is now widespread on breeding farms around the world (Amori and Gippoliti, 2003). Most, if not all, present domestic chinchillas derive from few free-living long-tailed chinchillas captured and taken to California in 1923 (Jiménez, 1996; Spotorno et al., 2004a, 2004b). Results of cytochrome b sequence analyses show that domestic chinchillas still share some genomic characteristics with their free-living counterparts (Spotorno et al., 2004b).

Studies of reproductive physiology were extensively revised (Busso et al., 2012), and scientific information comes from studies on commercially exploited animals, especially domestic *Chinchilla lanigera*. We studied different aspects about reproductive gonadal activity in the laboratory under similar environmental conditions to those of breeding farms (Busso et al., 2012, 2007, 2005a, 2005b). On breeding farms, births occur throughout the year, with two annual activity peaks in spring and summer, usually producing two litters a year. Chinchillas show long gestation (3.5–4 months length). Particularly, we bi-weekly detected that sperm functional activity reaches values that might indicate an adequate fertilizing capability over the annual cycle. In addition, the peaks of testicular and seminal volumes and number of spermatozoa/ejaculate were detected in winter and spring (Busso et al., 2005a). Up to the present, the effects of environmental factors on gonadal activity have not been individually studied; consequently, natural reproductive seasonality is still poorly understood (Busso et al., 2012). We hypothesized that testicular activity in domestic chinchilla (*Chinchilla lanigera)* males are affected by photoperiod. The aim of this study was to measure urinary androgen metabolites and semen quality of chinchillas under natural photoperiod on a seasonal basis.

2. Materials and methods

2.1. Animals and husbandry

This research was conducted using sexually mature males (between 1 and 3 years old) of domestic *Chinchilla lanigera*. Chinchillas ($n = 7$) were housed individually and maintained indoors under controlled temperature ($22.2 \pm 1.0\,^{\circ}C$) and near females but without access to them. Pelleted chinchilla food (GEPSA Feeds, Córdoba, Argentina) and water were provided ad libitum, and a cube of compressed alfalfa was fed once weekly. Chinchillas were randomly chosen from our laboratory facilities with all individuals being subjected to this experiment over one year, starting at the autumn equinox. They were exposed to natural photoperiods in Córdoba, Argentina (31° S–64° W). The photoperiod length (winter solstice 10.1 h light/day and summer solstice 14.2 h light/day) was calculated from data provided by the Servicio de Hidrografía Naval, Armada Argentina (http://www.hidro.gov.ar). The experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH, publication 85–23, revised 1996).

2.2. Urine and semen collection

We collected urine and semen from male chinchillas in autumn (May), winter (August), spring (November) and summer (February), covering the four seasons (four samples per individual throughout the study). Urine samples were collected over a 24 h period in tubes containing 0.5 mL ethanol as preservative, according to Busso et al. (2007, 2005a), and centrifuged for 15 min (400G); the supernatant was frozen at $-20\,^{\circ}C$ until processing. The supernatant of urine was further diluted in PBS before radioimmunoassay (see below). Semen was collected by electroejaculation on conscious animals. Briefly, a bronze bipolar electrode (40 mm length, 4.2 mm diameter) was lubricated with glycerin, inserted into the rectum to a depth of 20–30 mm and held in place by a technician. Alternating current (sinusoidal wave, 50 cycles/s) was applied for the first 5 s of a 15-s period (one pulse; 6–6.5 V). Four series of up to five pulses (of 5 s), with a 3–5 min interval between series, were applied to each conscious animal until either ejaculation occurred or the last series was reached. The ejaculate was collected into a 2 mL Eppendorf plastic tube containing 150 μL Tyrode modified medium to prevent coagulation (Busso et al., 2005a; Ponce et al., 1998). After the electroejaculation trials, animals did not show any sign of discomfort, alteration or pain and returned immediately to their normal behavior, which is consistent with our previous findings in the laboratory (Ponzio et al., 2011).

2.3. Testicular volume, urinary androgen metabolite and semen quality measurements

The day before urine and semen collection, testicular volume (see results) and body weight were recorded. Body weight (grams = g) records showed no seasonal differences: 600.2 ± 27.3; 531.6 ± 37.4; 525.7 ± 31.5 and 552.3 ± 45.1 g, respectively. To measure testicular volume, length and width of the right and left testes were measured using calipers and these values were converted into paired testicular volume (cm^3), according to Busso et al. (2005a).

Urinary androgen metabolites were measured (assay dilution 1:4 in PBS) using an I-^{125}-testosterone RIA (Total Testosterone, DPC, Coat-A-Count). Briefly, the antiserum for testosterone has less than 5% cross-reactivity to other steroids, except 19-nortestosterone (20%) in serum, plasma or urine (data provided by the company). The kit, equipped with human serum-based calibrators, was employed to measure non-invasive urinary androgen metabolites, according to Busso et al. (2005b). The intra- and inter-assay coefficients of variation (CV) for the urine control samples and the internal control of the kit were 5.0 ± 2.8% and 13%, and 5.4 ± 1.6% and 8.5%, respectively. Creatinine was also quantified in all unprocessed urine samples and hormonal data were expressed on a per mg creatinine basis to account for day-to-day fluctuations in fluid balance.

Semen volume was quantified with automatic pipettes (10–100 μL); spermatozoa concentration and motility were assessed in a Makler counting chamber. Sperm viability was assessed by Hoechst 33258 (H258) supravital staining; sperm membrane integrity was evaluated using the hypoosmotic swelling test (HOST, 100 mOsm/L; sodium citrate plus fructose at pH 7.4 for 45 min), and acrosomal integrity was determined by staining using *Pisum sativum* agglutinin labeled with fluorescein isothiocyanate (Busso et al., 2005a; Ponce et al., 1998; Ruiz et al., 1996).

Motility was expressed as the percentage of motile cells (progressive and non-progressive), with sperm with brightly fluorescent nuclei being scored as "dead" and those that excluded the H258 being scored as "viable". In total, 200 cells were assessed and results were expressed as the percentage of viable cells. Sperm membrane integrity was evaluated via phase-contrast microscopy at a magnification of 400 and the percentage of spermatozoa showing swelling was reported (a total of 200 cells were assessed). Finally, spermatozoa with an intense green-turquoise blue fluorescent acrosome were regarded as having an intact acrosome and these results were expressed as the percentage of viable spermatozoa (H258 negative) with this characteristic (a total of 200 cells were assessed).

2.4. Statistical analysis

A one-way repeated measures ANOVA was applied and a multiple comparison method was used to assess the differences between seasonal means (Di Rienzo et al., 2002). Data were modeled using a mixed linear model to evaluate different structures of serial correlation. In this model, seasons (May, August, November and February samples) were the fixed part, whereas the animals were the random factor. The model was fitted using the "lme" routine of the R's nlme library, using the REML (Restricted Maximum Likelihood) estimation method. All the data were checked for normal distribution and equal homogeneity variances. Significance level was determined at 0.05 and all tests were conducted using Infostat 2000 (Infostat version 1.1, Grupo Infostat, Facultad de Ciencias Agropecuarias-UNC, Argentina). Pearson correlation coefficients were performed for: testicular volume and testicular volume/body weight; testicular and seminal volumes; and seminal volume and total number of spermatozoa. Results were expressed as mean ±standard error of mean (SEM).

3. Results

The statistical analysis revealed no seasonal changes in testicular volume/body weight, even when variables were analyzed separately (19.63 ± 2.18; 20.25 ± 2.23; 20.73 ± 0.82; and 16.20 ± 0.82 cm^3 for autumn – May, winter – August, spring – November and summer – February, respectively). Urinary androgen metabolite response to natural photoperiod changes in chinchilla is depicted in Fig. 1. Samples collected in autumn and winter exhibited higher values than in spring or summer ($p = 0.04$). The statistical analysis did not reveal any significant differences between the total number of ejaculated spermatozoa and seminal volume (Table 1). In addition, none of the sperm samples exhibited seasonal variations in sperm functional activity variables, such as motility, viability and swollen cells (Table 1). Nevertheless, viable gametes with intact acrosome peaked in winter and spring ($p = 0.02$), with all seasonal mean values being higher than 60% (Fig. 1). Finally, the following correlations were determined: testicular volume and testicular volume/body weight ($p = 0.001$; $r = 0.83$); testicular and seminal volumes

Table 1
Concentration and functional characteristics of electroejaculated chinchilla spermatozoa obtained seasonally for 1 year[a]

Variable	Autumn	Winter	Spring	Summer
Seminal volume (μL)	66.2 ± 16.6	75.0 ± 24.0	57.1 ± 16.6	44.3 ± 14.1
Total number of spermatozoa (×10^6)	34.3 ± 10.7	162.2 ± 77.6	67.7 ± 32.6	10.6 ± 3.5
Motility (% motile cells)	98.5 ± 0.6	98.3 ± 1.1	96.8 ± 1.3	97.7 ± 0.5
Viability (% viable cells)	94.5 ± 2.5	92.5 ± 2.5	95.1 ± 0.9	94.7 ± 1.5
HOST (% swollen cells)	89.5 ± 1.9	91.0 ± 2.2	85.1 ± 5.4	84.7 ± 3.4

[a] The data are from seven domestic chinchillas under natural photoperiod changes. In Córdoba (31° S–64° W; Argentina) during May (Autumn), August (Winter), November (Spring), and February (Summer); sperm functional activity was immediately evaluated in fresh semen (obtained by electroejaculation).

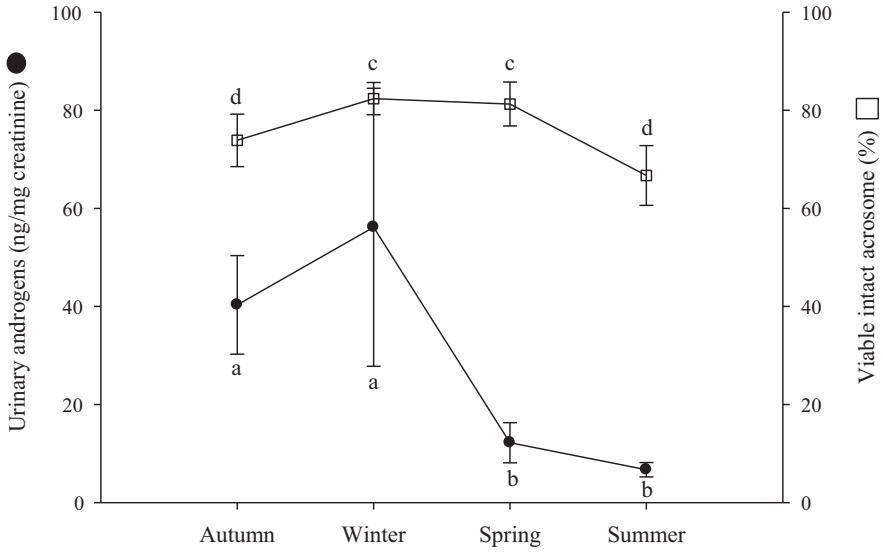

Fig. 1. Seasonal variations in urinary androgen excretion and viable intact acrosome (VIA) of spermatozoa during Autumn (May), Winter (August), Spring (November), and Summer (February). Chinchillas ($n = 7$) were maintained under natural photoperiod in Córdoba, Argentina (31° S–64° W) and urine (collected over 24 h-period) was obtained by electroejaculation before semen collection. Different letters indicate statistically significant differences for urinary androgen or VIA, respectively, a vs. b or c vs. d, $p < 0.05$.

($p = 0.001$; $r = 0.40$); and seminal volume and total number of spermatozoa ($p = 0.002$; $r = 0.37$).

4. Discussion

We recently revised information about reproductive biology of domestic chinchillas on breeding farms (Busso et al., 2012). Births can take place at any time, with peaks occurring in spring and summer. Chinchilla breeding facilities are normally exposed to significant macro- and microclimatic variations. In mammals, the environmental factors of major importance for reproductive physiology are food availability, ambient temperature, rainfall, day/night cycle (photoperiod), and a variety of social cues (Bronson, 1985). In rodents, annual changes in day length are largely involved in the generation of seasonal rhythms in reproduction (Prendergast et al., 2001). Nevertheless, few studies in chinchilla have investigated photoperiod as the source of variation in the experimental design (Busso et al., 2012). A study that measured monthly values of testicular volume found the lowest values in the middle of the summer, indicating that photoperiod has a significant influence on this variable (Busso et al., 2005a).

Domestic chinchilla males are fertile throughout the year (Weir, 1970). Accordingly, we detected that males exhibited spermatozoa with high quality functional activity bi-weekly (Busso et al., 2005a). Hence, we hypothesized that the male chinchilla can reproduce continuously, exhibiting constant sperm functional activity throughout the annual cycle. In addition, we considered that testicular volume, seminal volume and spermatozoa production might be controlled by environmental factors, such as photoperiod. Thus, male chinchillas synchronize their reproductive performance to winter–spring peaks in the occurrence of oestrus in females (Busso et al., 2012). In our study, we demonstrated that natural photoperiod affected testicular activity of domestic chinchilla. Under laboratory conditions similar to those in breeding farms, the highest values of testicular activity were detected in winter, anticipating the highest peaks of births on breeding farms over spring and summer seasons. Thus, natural photoperiod might synchronize some reproductive variables in indoor-housed domestic chinchillas, considering that "short" days were associated with high levels of endocrine testicular activity and percentage of viable sperm with intact acrosome; the opposite was true for "long" days, when lowest values of testicular activity were detected. Sperm production showed a similar tendency, and it was significantly positive correlated with testicular volume and seminal volume. We think that perhaps some individuals fail to adopt a photoperiodic breeding performance in the laboratory, and they may be classified as "photoperiod non-responders" (see below further discussion). Sperm functional activity showed no seasonal changes throughout the study, similarly to data collected previously in our laboratory (Busso et al., 2005a).

It is well known that endocrine activity of gonads usually increases immediately before and during the breeding season. In our experiments, under natural photoperiod changes, the urinary androgen profile was highest during autumn and winter. In a study employing male domestic chinchillas under a natural changing photoperiod in Chile, a similar plasma testosterone profile was collected during the annual reproductive cycle (Cepeda et al., 2006). Therefore, we consider that endocrine testicular function is affected by photoperiod. We speculate that the increase in urinary androgen excretion in autumn could be associated with the early preparation of the reproductive system and with intra-sexual reproductive behavior. In fact, a male's reproductive success is generally determined not only by the ability to produce semen (with physical dominance over other males) but also by the ability to attract females (Bronson, 1985).

The percentage of viable spermatozoa with intact acrosome was highest during winter–spring, which is consistent with peaks of estrus (Spotorno et al., 2004a), as well as with the seasonal profile of female urinary progestagens detected non-invasively under similar environmental laboratory conditions (Busso et al., 2007). Nevertheless, viable gametes with intact acrosome exhibit mean values above than 60% during four seasons. Considering that revisions of reproductive biology of this species pointed out that chinchilla males are fertile throughout the year (Busso et al., 2012; Weir, 1970), we speculate firstly that this seasonal pattern is consistent with births that occur year round in breeding farms (Spotorno et al., 2004a; García et al., 1989). Thus, natural changes in photoperiod also affected this variable, although its seasonal variation was associated with the daily increment in day light, which is typically observed during winter and spring.

Although there were no significant differences in other reproductive variables, there were important correlations between testicular and seminal volumes and seminal volume and total number of spermatozoa. Our finding shows the presence of winter peaks in seminal volume and in the total number of ejaculated sperm. In addition, as expected, we detected the lowest values of sperm production in summer, after endocrine testicular activity declined during spring. At present, our results do not support that testicular volume, seminal volume and spermatozoa production might be controlled by photoperiod. We think that a possible explanation may be individual differences in photoperiodic responses. In fact, a great variation in these variables has been observed in several experiments in our laboratory (Busso et al., 2012). However, to assess whether these animals can be classified as photoresponsive or nonphotoresponsive, a thorough study is required, as previously proposed for other rodents (Prendergast et al., 2002, 2001).

From an ecological perspective, based on our data obtained in the laboratory, we detected that photoperiod shapes male reproductive profiles in order to achieve the highest activity during winter. This phenomenon has already been demonstrated in other small and medium-sized male desert rodents housed in laboratories and inhabiting the same latitudes as the chinchilla (Wube et al., 2008; El-Bakry et al., 1998; Jackson and Bernard, 1999). Thus, reproductive functions in desert rodents could be less affected by photoperiod because they live in habitats where opportunistic behaviors are advantageous to face energetic challenges and nutrient availability. A similar strategy for feeding behavior was proposed for chinchilla in the wild (Cortés et al., 2002). Perhaps the use of plant predictors and/or other environmental factor may be a common strategy to modulate reproduction in free- living chinchilla, because of chinchilla births have been reported to only occur in spring and summer in the southern hemisphere.

In conclusion, natural photoperiod partially affected reproductive testicular activity in domestic chinchillas. Endocrine testicular activity was driven by photoperiod, whereas sperm production was not affected by this primary environmental factor. Viable sperm with intact acrosome peaked in winter and spring (with increasing photoperiod), with sperm functional activity showing no seasonal changes across the annual photoperiodic cycle.

Finally, we suggest the following lines of action:

a) Studying the effect of artificial photoperiod (long and short days) on testicular activity, based on descriptions of typical photoperiodic responses in mammalian species: long- or short-day breeding species (Goldman, 2001). This approach would be useful to explore possible polyphenisms in this species, as pointed out for other rodents (Prendergast et al., 2001). This fundamental research line may increase our understanding of chinchilla reproductive biology, and may contribute manipulation of reproduction in breeding farms.
b) Considering that we detected sperm functional activity and high values of gametes with intact acrosome in all seasons, semen samples could be useful for assisted reproduction techniques, such as artificial insemination. Nevertheless, it is essential to evaluate whether the sperm concentrations reported here are sufficient for successful artificial insemination.

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