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Animal Reproduction Science 90 (2005) 127–134

ANIMAL REPRODUCTION SCIENCE

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Year-round testicular volume and semen quality evaluations in captive *Chinchilla lanigera*

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Received 15 December 2004; received in revised form 18 January 2005; accepted 3 February 2005
Available online 3 March 2005

Abstract

In mammals, reproductive performance is usually associated with seasons. *Chinchilla lanigera*, an endemic South American rodent, reproduces throughout the year in captivity but its seasonal breeding pattern is not fully understood. The present study was designed to evaluate (bi-weekly) over 1 year: (1) testicular volume variations and (2) seminal volume, sperm concentration and functional activity changes. Five animals were studied; they were individually housed indoors ($22.2 \pm 1.0\,^{\circ}C$) under natural photoperiod in Argentina (Córdoba, 31°S–64°W). Semen was obtained by electroejaculation; a total of 116 ejaculates was evaluated. Monthly values for paired testicular volume were less in the middle of the summer than in other seasons ($p < 0.006$), while those for seminal volume and total spermatozoa/ejaculate were not significantly different; these variables ranged between 7.2–30.9 cm^3, 10–130 μL and $0.9–432.6 \times 10^6$, respectively. Spermatozoa concentration was ($\times 10^6$/mL) 2145.9 ± 365.3 and the pH of semen was 7.3 ± 0.0. Spermatozoa functional activity showed no significant differences between monthly evaluations; confidence intervals were calculated for the means of: motility, 92.2–95.8%; viability, 92.2–96.1%; swollen cells (hypo-osmotic swelling test), 81.2–87.7% and viable intact acrosome, 83.5–89.0%. The present study represents the first lon-

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0378-4320/$ – see front matter
doi:10.1016/j.anireprosci.2005.02.001

gitudinal reproductive assessment in the chinchilla male. In conclusion, males produce spermatozoa continuously that exhibit high quality functional activity.

Keywords: *Chinchilla*; Sperm functional activity; Electroejaculation; Neotropical rodents; Testicular volume; Photoperiod

1. Introduction

An important factor regulating reproductive seasonality in several small mammals is photoperiodicity (Reiter, 1980), the most 'noise-free' annual environmental predictor (Turek and Campbell, 1979). The effects of photoperiod on the reproductive system may be dichotomous, depending on what time of the year the sexual activity takes place. In spring mating mammals, short photoperiods induce gonadal regression, whereas long photoperiods stimulate development; the opposite is true in fall- or winter-mating mammals (Turek and Campbell, 1979; Steinlechner and Niklowitz, 1992).

In South America there are endemic species from the *Chinchillidae* family living in colonies in extensive burrow systems (Redford and Wisenberg, 1992). The male vizcacha (*Lagostomus* spp.) exhibits a short hibernal period of sexual quiescence under natural conditions (Fuentes et al., 1991, 1993); however, this species reproduces continuously in captivity (Bronson, 1999). Chinchilla (*Chinchilla* spp.) also reproduce throughout the year in captivity with two breeding peaks: spring and summer (Weir, 1970; Gromadzka-Osrttowska et al., 1985). On this basis we postulate that chinchilla may produce good quality spermatozoa continuously.

The objective of the present study is to provide further knowledge on the male chinchilla reproductive biology; this study was designed to evaluate throughout the year in male *Chinchilla lanigera* variations in: (1) testicular volume and (2) seminal volume, spermatozoa concentration and spermatozoa functional activity (motility, viability, spontaneous acrosome reaction and membrane functional integrity).

2. Materials and methods

2.1. Animals

Five sexually mature *C. lanigera* males (2.6 ± 0.9 years old) weighing 502.8 ± 5.6 g were studied. They were individually housed indoors with controlled temperature ($22.2 \pm 1.0\,^{\circ}C$) with water and food ad libitum; and under natural short (11.4 ± 0.0 and 11.8 ± 0.0 h, autumn and winter, respectively) and long (14.2 ± 0.0 and 14.0 ± 0.0 h, spring and summer, respectively) photoperiod from birth (Córdoba, 31°S–64°W, Argentina). Photoperiod duration was calculated from data provided by 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. Measurement of testicular volume

The length and width of the right and left testis were measured using callipers. Values for length and width were converted to volumes by the formula for a prolate spheroid (Wildt et al., 1982; Fuentes et al., 1993). Paired testicular volume (cm^3) was calculated by adding the volume of both testes.

2.3. Spermatozoa collection

All animals were subjected bi-weekly to electroejaculation for 12 months, using the method described by Ponce et al. (1998). Briefly, the bronze bipolar electrode (length 40 mm, diameter 4.2 mm) was lubricated with glycerin, inserted into the rectum to a depth of 20–30 mm and held in place by a technician. An alternating current (sinusoidal wave, 50 cycles/s) was applied for 5 of every 10 s (one pulse; 6–6.5 V). Four series (interval between series was 3–5 min) of up to five pulses were applied to each conscious animal until either ejaculation occurred, or the last series was reached (Ponce et al., 1998). The ejaculate was collected and diluted in 150 μL modified Tyrode's medium into a 2 mL Eppedndorf plastic tube. As previously reported, neither reproductive nor behavioral changes were observed (Busso et al., 2005).

2.4. Semen evaluation

All variables were measured as previously described by Busso et al. (2005): Semen volume was measured with automatic pipettes (0–500 μL); indicator strips were used to determine the pH of semen. Spermatozoa concentration and motility were assessed in a Makler counting chamber. Motility was expressed as the percentage of motile (progressive plus non-progressive cells) spermatozoa, and concentration as number of spermatozoa $\times 10^6$ mL. Spermatozoa viability was assessed by Hoechst 33258 (H258) supravital staining; sperm having brightly fluorescent nuclei were scored as 'dead' and those that excluded the H258 were scored as 'viable' (200 cells were assessed and the results were expressed as the percentage of viable cells).

The integrity of the spermatozoa membrane was evaluated by the hypo-osmotic swelling test (HOST). The procedure employed was similar to the one described by Jeyendran et al. (1984) and adapted by Ruiz et al. (1996). The spermatozoa suspension was mixed with the hypo-osmotic solution (100 mOsm/L; sodium citrate plus fructose) at pH 7.4 for 45 min; evaluations were made by phase-contrast microscopy at a magnification of 400× (200 cells were observed, and the percentage of spermatozoa showing swelling was reported). Acrosomal integrity was determined by staining with *Pisum sativum* agglutinin labelled with fluorescein isothiocyanate. Spermatozoa with an intense green–turquoise blue fluorescent acrosome were regarded as having an intact acrosome (200 cells were observed and the results were expressed as the percentage of viable spermatozoa with intact acrosome).

Table 1
Concentration and functional characteristics of electroejaculated chinchilla spermatozoa obtained bi-weekly for 1 year

Variable	Mean ± S.E.M.	Range
Spermatozoa concentration (10^6/mL)	2145.9 ± 365.3	20–11712
Motility (% motile cells)	93.9 ± 0.7	69–100
Viability (% viable cells)	94.4 ± 0.7	63–100
HOST (% swollen cells)	83.9 ± 1.3	55–98
Viable intact acrosome (%)	85.7 ± 1.1	62–100

The data are from five chinchillas number of samples 116. HOST: hypo-osmotic swelling test.

2.5. Statistical analysis

Values are expressed as mean ± standard error of mean (S.E.M.). Monthly means were calculated from the data obtained in the two corresponding bi-weekly evaluations of semen. A randomized complete blocks design was applied, in which animals of two-way ANOVA represent blocks and factor treatment (months) at 12 levels. Influence of months on all variables was assessed by repeated measures ANOVA and DGC (Di Rienzo et al., 2002) for testing the differences between monthly means; significance at least for the 0.05 level was considered. Confidence intervals were calculated with $\alpha = 0.05$. All tests were conducted with Infostat 2000 (Infostat version 1.1, Grupo Infostat, FCA-UNC, Argentina).

3. Results

During 12 months, a total of 116 ejaculates was collected and 88.3% of the males needed only one series of stimuli. As depicted in Fig. 1, during January and February values for monthly-paired testicular volume were significantly less than the rest of the year ($p < 0.006$), while those for seminal volume and total spermatozoa/ejaculate were not significantly different. Values for these variables ranged between 7.2–30.9 cm^3, 10–130 μL, and 0.9–432.6 × 10^6, respectively. The pH of semen was 7.3 ± 0.0 and ranged between 7.0 and 7.6. Semen was typically homogeneous and cream or white in appearance.

Data for concentration and functional characteristics of electroejaculated spermatozoa are shown in Table 1; there were no significant differences between monthly means. Finally, confidence intervals were calculated for the means of spermatozoa functional activity; estimations were made for motility: 92.2–95.8%; viability: 92.2–96.1%; HOST: 81.2–87.7% and viable intact acrosome: 83.5–89.0%.

4. Discussion

The present study represents the first longitudinal assessment of testicular volume and seminal quality in *C. lanigera* males. Annual variation of concentration and functional characteristics of electroejaculated spermatozoa (motility, viability and integrity of membranes) are provided as a result of the present study. It was confirmed that chinchilla males produce

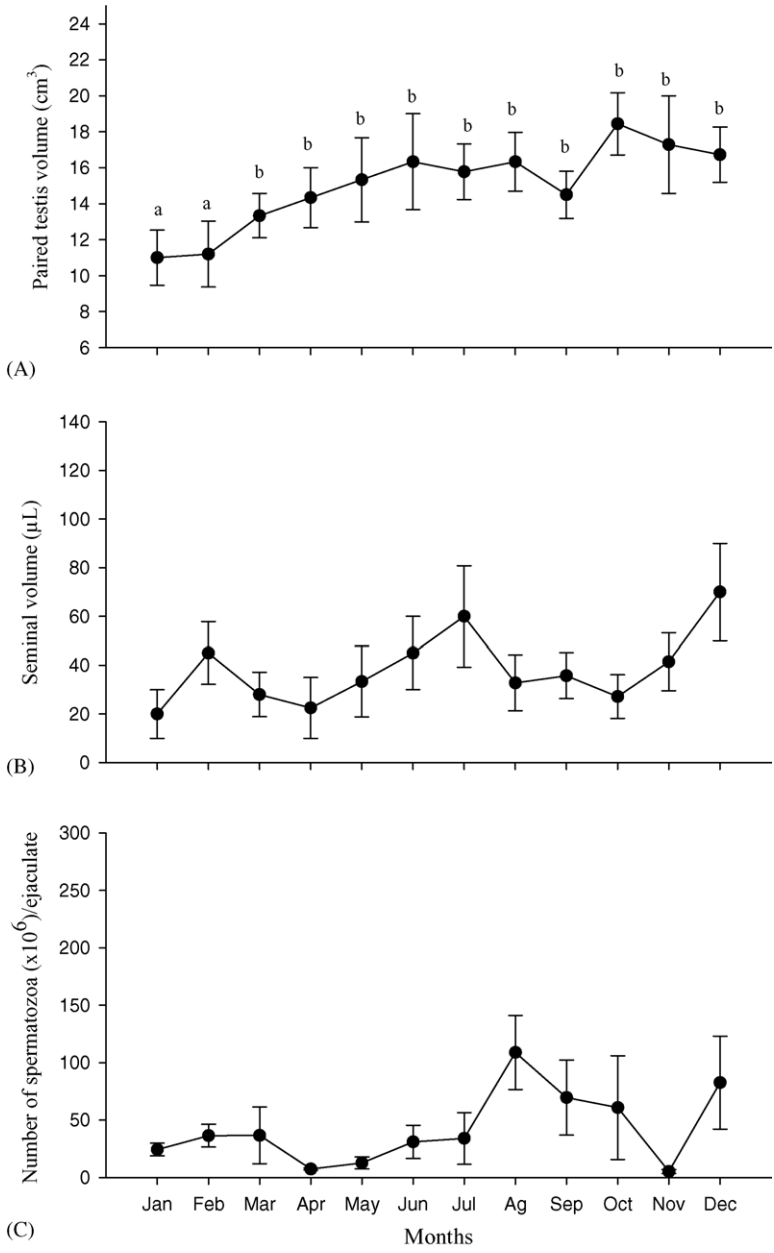

Fig. 1. Monthly means (±S.E.M.) for reproductive variables of chinchilla males ($n = 5$) in captivity: paired testis volume measured with callipers (A); semen was collected by electroejaculation for analysis of seminal volume (B) and total spermatozoa/ejaculate (C). Number of ejaculates: 116. Panel A: a as compared with b: $p < 0.006$.

spermatozoa continuously throughout the year of similar quality in captivity under natural photoperiod. Based on the present study, it would be possible to establish a normative data-basis on testicular volume, sperm quantity and semen quality in *C. lanigera*.

To our knowledge, there are no other reports on annual changes of testicular volume for chinchilla. Data obtained from five males in captivity demonstrated that there are significant season-associated changes, i.e. testicular volume was less in the middle of the summer than in the rest of the year. This finding corresponds to earlier findings that only a few females may come into estrus during this season (Weir, 1970). Some wild-trapped desert rodent species are responsive to photoperiod, but changes in only day length are not enough to induce robust variation in reproductive responses (El-Baryk et al., 1998). Furthermore, other species such as vizcachas reproduce continuously in captivity, but have seasonal reproductive patterns in the wild. This may be due to a photoperiodic regulation or to a negative energy balance caused by lesser temperatures and food shortage (Bronson, 1999). Accordingly, gonadal regression was reported in this rodent in its natural habitat during winter (Fuentes et al., 1993).

In the present study, a reliable collection of semen was possible for 12 months (bi-weekly) employing the modified electroejaculation method (Ponce et al., 1998). Results that reflect spermatozoa functional variables from April to October (autumn and winter in South America) were similar to those from our longitudinal assessment.

When results of spermatozoa concentration are compared, there were no significant differences. The mean calculated for this variable was five times greater than those reported in previous investigations from our laboratory (Ponce et al., 1998; Busso et al., 2005) or by other investigators of chinchilla reproduction (Barnabe et al., 1994). Previous results (Barnabe et al., 1994; Ponce et al., 1998; Busso et al., 2005) fall within the range for results obtained in the present study. Likewise, variations of spermatozoa density up to 10^9 have already been reported by Weir (1970). Seminal volumes in the present study were similar to those previously reported (Barnabe et al., 1994; Ponce et al., 1998; Busso et al., 2005).

In the present study, it was established that there are minimal changes in spermatozoa concentration and functional activity associated with month of the year. There were some trends that might be meaningful such as: mean values of seminal volume and total spermatozoa/ejaculate peaked twice during the year; these changes parallel the occurrences of estrus in this species with peaks during winter and spring (Weir, 1970). Possibly due to the limited number of animals in the present study, a significant influence of the photoperiod was only present for testicular volume. In this respect, Fuentes et al. (1993) considered photoperiod to be one of the most important factors in synchronizing the reproductive cycle in vizcacha, rodents belonging to the *Chinchillidae* family.

The typical rodent lives in a climate with little thermoregulatory challenge, therefore these species likely employ an opportunistic reproductive strategy (Bronson, 1985). Recently, a similar strategy for feeding behavior was proposed for chinchilla in the wild (Cortés et al., 2002). Therefore, taking into account that chinchilla males are fertile throughout the year (Weir, 1970), and on the basis of the present study, we hypothesize that the *C. lanigera* male is competent to reproduce continuously and is, therefore, capable of using an opportunistic reproductive strategy. Hence, it would need constant spermatozoa functional activity during the annual cycle, and testicular volume, seminal volume and spermatozoa production could vary according to variation in environmental factors (pho-

toperiod, temperature, feeding, social relationships). In this manner, the males could coordinate their reproductive performance with the peaks of the occurrence of estrus in the females.

From previous results (Busso et al., 2005), we know that the male can provide a high quality ejaculate once a week at least for a month. In the present study, we found that sperm functional activity and density throughout the year have no relevant changes. Considering both studies, semen could be collected monthly by electroejaculation for use with assisted reproductive techniques in this species.

Further experiments relative to the impact of environmental factors on testicular activity of this species under natural or captive conditions are in progress. At present, information on wild (Jiménez, 1996; Amori and Gippoliti, 2001) or domesticated chinchillas is scarce. Additional studies about reproductive physiology in the latter animals may help to develop new management strategies that would contribute to ensuring the survival of the wild populations, as well as to obtaining tools for a more sustainable use by the fur industry (Busso et al., 2004).

Acknowledgements

The present study was supported by grants from Agencia Córdoba Ciencia, SeCyT – UNC and FONCyT. We thank the valuable assistance of Mr. D. Martins and of ACRICHI (Córdoba).

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