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Research in Veterinary Science 78 (2005) 93–97
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Electroejaculation in the Chinchilla (*Chinchilla lanigera*): effects of anesthesia on seminal characteristics

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Received 19 June 2003; accepted 15 June 2004

Abstract

Repetitive electroejaculation is probably extremely stressful in conscious animals and could adversely affect fertility. The present study was designed to (a) evaluate the effects of anesthesia (40 mg ketamine/kg body weight, i.m.) on a method of electroejaculation used previously in conscious chinchillas (*Chinchilla lanigera*), and (b) determine the quality of the electroejaculated semen obtained under anesthesia. In Experiment 1 (8 animals), a 4×4 Latin square design was used to study the effects of anesthesia and ejaculatory voltage on semen collection, ejaculate volume, sperm concentration, motility, viability, response to the hypoosmotic swelling test (HOST), and acrosomal status. In Experiment 2 (12 animals), the effects of differing voltages and the number of stimuli on ejaculation by conscious or anesthetized males was determined. In both experiments, all the conscious animals ejaculated, but only 60% ejaculated under anesthesia and they required more stimuli and higher voltages to achieve ejaculation. Ejaculate volume was significantly lower in anesthetized (<5 μl) than in conscious animals (>40 μl), but sperm concentration was unaffected. None of the indices of sperm quality were affected by anesthesia. The techniques we have developed in anesthetized domestic chinchillas should be applicable to endangered chinchillas in the wild, and though the number of sperm available is reduced, there are still sufficient for assisted reproduction.

1. Introduction

Members of the genus *Chinchilla* are either critically endangered or vulnerable in the wild owing to habitat loss and trapping (Jiménez, 1996; Amori and Gippoliti, 2001). Successful ''integrated'' conservation programmes often depend on a detailed understanding of the gamete biology of the species (Holt and Pickard, 1999; Wildt et al., 2001). Our knowledge of the semen of *Chinchilla* spp. is mostly based on samples obtained by natural ejaculation (Healey and Weir, 1967; Weir, 1970; Calderón Fernández, 1976; Barnabe et al., 1994; Ponce et al., 1998a) or from epididymal spermatozoa (Ponce et al., 1998b).

Electroejaculation has the advantage that it can be applied to chinchillas caught in the wild but the results have been varied. Dalziel and Phillips (1948) report that 67% of attempts to electroejaculate guinea pigs or chinchillas were successful. Healey and Weir (1967) achieved 92% success using 9.5 V and 5.5 mA. Later, Calderón Fernández (1976) employed 22 V and 100 mA with 80% success and Barnabe et al. (1994) applied 12 V and 250 mA with 75% success. Since then we have developed a method that is always effective in conscious chinchillas using 6.8 V (Ponce et al., 1998a). Differences in success rates probably reflect technical differences in the procedure. Our method, unlike others used on small rodents (Scott and Dziuk, 1959; Anderson et al., 1983;

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doi:10.1016/j.rvsc.2004.06.004

Tecirlioglu et al., 2002a,b), yielded spermatozoa that were suitable for use in assisted reproduction.

Repeated electroejaculation of conscious animals is probably extremely stressful and could also adversely affect fertility. Anesthesia could overcome this problem and would greatly facilitate electroejaculation of wild-caught chinchillas (Durrant, 1999). We therefore set out to study (a) the effects of anesthesia on the method of electroejaculation already applied to conscious chinchillas in our laboratory, and (b) determine the characteristics of the semen collected under anesthesia.

2. Materials and methods

2.1. Animals

Twenty sexually mature *Chinchilla lanigera* males (2.1 ± 0.3 years) weighing 555 ± 15 g were used. They were individually housed indoors under natural photoperiod (temperature range, 17–26 °C) with free access to food and water. The animals were returned to the breeders after the study, and neither reproductive nor behavioral changes were reported.

2.2. Spermatozoa collection

The method of electroejaculation has been described by Ponce et al. (1998a). Briefly, each chinchilla was placed in a box covering the cranial half of the body in the prone position. The caudal half rested on a metal grid. The tail was gently raised and, after cleaning the genital area, the prepuce was retracted, and the penis was introduced into a 2 ml Eppendorf plastic tube. 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). The electrical potential across the poles of the electrode was controlled with a rheostat and measured with a voltmeter. The circuit was calibrated in advance using a known resister. Four series of up to 5 pulses were applied to each animal. The ejaculate was collected and diluted in 150 μl modified Tyrode's medium. All the materials coming in contact with the semen were kept at 37 ± 1.0 °C throughout the analysis. Anesthesia was induced with ketamine (40 mg/kg body weight, i.m.; Morgan et al., 1981).

2.3. Experiment 1

Experiment 1 had a 4 × 4 Latin square design in which all animals were subjected successively to all treatments. The treatments were voltage (6–6.5 or 7.5–8) and anesthesia (present or absent). There were four groups of 2 animals giving a total of 8 animals and eight data points for each treatment. Treatments were performed once a week.

2.4. Experiment 2

Twelve animals were used to determine if there were differences in the voltage required to obtain semen from conscious or anesthetized chinchillas. Each animal was subjected to electroejaculation twice: first conscious, then 14 days later, anesthetized. In each case, the voltage was progressively increased in 4 steps until either ejaculation occurred or the highest voltage level was reached. Each step constituted a series of stimuli as before, at the following levels: (1) 6–6.5, (2) 7.5–8, (3) 9.5–10, and (4) 11.5–12 V.

2.5. Semen evaluation

All variables were measured as previously described by Ponce et al. (1998a,b): Semen volume was measured with automatic pipettes (0–500 μl). Sperm concentration and motility were assessed in a Makler counting chamber. Motility was expressed as the percentage of motile cells (progressive and non-progressive). 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 results are expressed as the percentage of viable cells.

The integrity of the sperm membrane was determined by the hypoosmotic swelling test (HOST). The procedure used was similar to the one described by Jeyendran et al. (1984) and adapted by Ruiz et al. (1996). The sperm suspension was mixed with the hypoosmotic solution (100 mOsm/l; sodium citrate plus fructose) at pH 7.4 for 45 min (37 ± 1.0 °C). 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 labeled with fluorescein isothiocyanate. Spermatozoa with an intense green-turquoise blue fluorescent acrosome were regarded as having an intact acrosome. No fewer than 200 spermatozoa were assessed and the results were expressed as the percentage of viable spermatozoa (H258 negative) with an intact acrosome.

2.6. Statistical analysis

Values are expressed as mean ± standard error of mean (SEM). The following tests were applied: analysis of variance for Latin square design (Sokal and Rohlf, 1997) with Fisher's LSD for testing the differences be-

tween means (Experiment 1), and Student's *t*-test for dependent samples (Experiment 2). A *p*-value of 0.05 was regarded as significant. All tests were carried out with Infostat 2000 (Infostat version 1.1, Grupo Infostat, FCA-UNC, Argentina).

3. Results

In Experiment 1, only 3 of 8 anesthetized chinchillas ejaculated with both treatments, while all 8 conscious animals did so. Nonetheless, those anesthetized animals that did not ejaculate showed penile erection and extension of the hind limbs. Only the 3 animals that ejaculated in response to all four treatments were included in the analysis. Seminal volume obtained in conscious animals was 93.3 ± 16.3 and 95.5 ± 13.2 μl for 6–6.5 and 7.5–8 V, respectively. Ketamine administration significantly diminished the volume of semen obtained to less than 5 μl for both applied voltages.

There were significant differences between conscious and anesthetized animals regarding the number of stimuli necessary to obtain semen. The conscious chinchillas needed 2.3 ± 0.9 and 3.0 ± 0.6 stimuli for 6–6.5 and 7.5–8 V, respectively, while the anesthetized animals needed 15.0 ± 0.0 and 11.7 ± 1.7 stimuli for 6–6.5 and 7.5–8 V, respectively (conscious vs. anesthetized, $p < 0.01$).

Sperm concentrations and functional characteristics are shown in Table 1. There were no significant differences either between treatments or between individual animals (not shown in the table). The ejaculates obtained from the 5 animals that responded only when conscious were similar in quality to the others.

In Experiment 2, when higher voltages were applied, 7 of 12 anesthetized animals ejaculated as did all the conscious ones. It is remarkable that 92% of conscious males ejaculated with only 1 series of stimuli; in contrast, in anesthetized animals, more stimuli ($p < 0.0001$) and higher voltages ($p < 0.0002$) were required to obtain semen samples (Fig. 1). Anesthesia significantly reduced

Table 1
Concentration and functional characteristics of electroejaculated spermatozoa obtained by applying 6–6.5 or 7.5–8 V in conscious or anesthetized chinchillas (mean ± SEM)

Variable	Conscious		Anesthetized	
	6–6.5 V	7.5–8 V	6–6.5 V	7.5–8 V
Sperm concentration (10^6/ml)	483.8 ± 4.4	137.7 ± 6.3	280.0 ± 0.7	156.6 ± 9.0
Motility (% motile cells)	98.0 ± 1.1	96.3 ± 1.7	93.7 ± 3.9	92.3 ± 1.9
Viability (% living cells)	91.7 ± 2.2	95.3 ± 1.3	91.7 ± 2.6	86.7 ± 5.4
HOST (% swollen cells)	90.0 ± 1.7	90.0 ± 2.0	85.0 ± 4.0	79.3 ± 2.8
Viable intact acrosome (%)	75.7 ± 9.9	89.0 ± 4.4	78.7 ± 0.9	86.3 ± 3.0

The data are for the three chinchillas that responded to all treatments in Section 2.3. There were no significant differences between groups.
HOST, hypoosmotic swelling test.

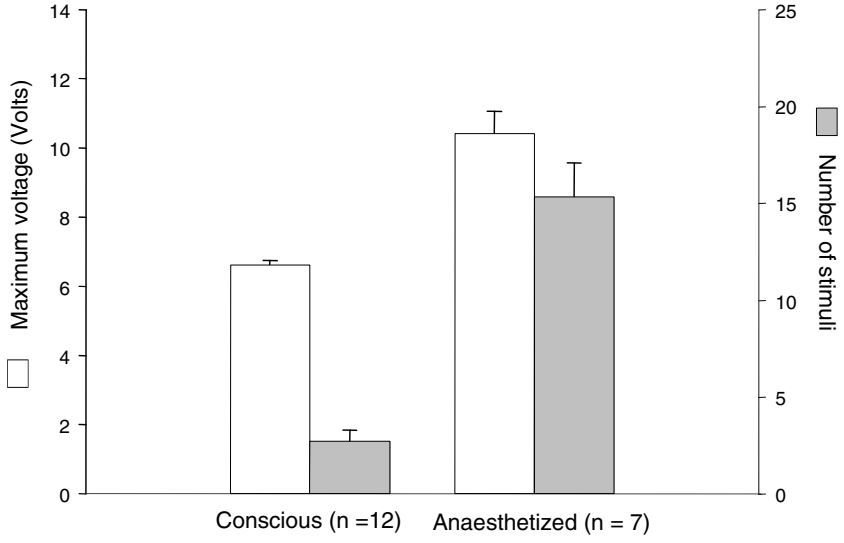

Fig. 1. Maximum voltage used (open columns) and number of stimuli required at that voltage (shaded columns) to collect semen from chinchillas. Both variables were significantly different in conscious and anesthetized animals.

Table 2
Semen volume, and concentration and functional characteristics of electroejaculated spermatozoa of conscious or anesthetized chinchillas (mean ± SEM)

Variables	Animal condition	
	Conscious $n = 12$	Anesthetized $n = 7$
Semen volume (μl)	45.0 ± 11.5	3.9 ± 0.4*
Sperm concentration (10^6/ml)	175.8 ± 76.2	204.1 ± 107.4
Motility (% motile cells)	97.8 ± 0.9	84.7 ± 9.4
Viability (% living cells)	95.8 ± 1.2	86.7 ± 5.1
HOST (% swollen cells)	82.3 ± 4.1	73.7 ± 6.6
Viable intact acrosome (%)	81.1 ± 4.2	66.0 ± 6.6

HOST, hypoosmotic swelling test.
* $p \leqslant 0.05$ (paired t-test).

semen volume but sperm concentration and quality were not significantly affected (Table 2).

4. Discussion and conclusions

The main objectives of this study were to improve electroejaculation in anesthetized chinchillas and to determine the effects of anesthesia and stimulatory voltage on semen collection and sperm quality. To our knowledge, there are no other reports of a standardized electroejaculation procedure for anesthetized chinchillas.

When electroejaculation was performed on conscious chinchillas the success rate (100%) was similar to that reported earlier (Ponce et al., 1998a) but anesthetized animals responded in only 60% of cases. Similar rates were reported in mice when ketamine was used as the anesthetic (Tecirlioglu et al., 2002a). Higher voltages and more prolonged stimulation were required to induce ejaculation in ketamine anesthetized chinchillas than in conscious animals. Similar diminished sensitivity has been reported in other small non-domestic species (Howard et al., 1986, Durrant, 1990).

Abnormal ejaculation has been reported before in anesthetized animals including retrograde ejaculation (Dooley and Pineda, 1986; Pineda and Dooley, 1994) and semen contamination with urine (Howard et al., 1986). In our experiments, these phenomena were not observed, but it is noteworthy that although in some animals (40%) ejaculation did not occur, penile erection was always evident. In rats, ketamine exerts a more powerful inhibitory effect on the sympathetic outflow than on the parasympathetic one (Clanachan and McGrath, 1976). In the chinchilla, similar actions of ketamine could explain our observations, because erection is a parasympathetic-mediated phenomenon, while ejaculation is achieved by activation of sympathetic nerves (Calderón Fernández, 1976).

In conscious animals, the seminal volume was similar to that reported previously for chinchillas (Barnabe et al., 1994; Ponce et al., 1998a) but the volume of semen was greatly diminished by anesthesia. It is possible that with higher voltages the volume would have been larger.

In our experiments, the concentration and quality of sperm (motility, viability and integrity of membranes) did not seem to be influenced by either anesthesia or the voltage used to induce ejaculation. However, because anesthesia reduced ejaculatory volume, the total number of sperm available was reduced. Nevertheless, sufficient sperm were ejaculated by a majority of anesthetized chinchilla males for use in assisted reproduction. Taken together, our data also suggest that male chinchillas can provide a high quality ejaculate at least once a week for a month.

Finally, we believe the methods described here are a practical prerequisite for the use of assisted reproduction in wild chinchillas and that they are likely to diminish stress in target animals. However, it could still be argued that electroejaculation using low voltages in conscious animals is a reasonable approach in domestic chinchillas.

Acknowledgements

This study was supported by Grants from Agencia Córdoba Ciencia, SECyt – UNC and FONCyT. The animals were kindly provided by Mr. D. Martins and by ACRICHI (Córdoba).

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