Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/repbio

Original Research Article

Reproductive performance and weaning success in fur-chewing chinchillas (*Chinchilla lanigera*)

María G. Galeano, Verónica I. Cantarelli, Rubén D. Ruiz, Marta Fiol de Cuneo, Marina F. Ponzio *

Cátedra de Fisiología Humana, INICSA-CONICET, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Santa Rosa 1085, X5000ESU Córdoba, Argentina

ARTICLE INFO

Article history:
Received 18 May 2013
Received in revised form
8 May 2014
Accepted 17 June 2014

Keywords:
Abnormal repetitive behavior
Fur-chewing
Stress
Reproduction
Seminal quality

ABSTRACT

In captive chinchillas, one of the most challenging behavioral problems is the development of a stress-related abnormal repetitive behavior (ARB) known as "fur-chewing". We investigated whether there is a relationship between the severity of fur-chewing behavior and reproductive function in male and female chinchillas. Regardless of the severity of abnormal behavior, fur-chewing males did not show significant differences in seminal quality (sperm concentration, motility and viability; integrity of sperm membrane and acrosome) and the response to the process of semen collection (the number of stimuli needed to achieve ejaculation) when compared to those with normal behavior. Also, females showing normal or fur-chewing behavior presented similar reproductive performance in terms of number of litters per female per year and litter size. However, pup survival rate was lower ($p = 0.05$) in fur-chewing females than in normal females. These results seem to be consistent with data suggesting non-significant effects of ARBs on reproductive performance.

1. Introduction

Although fur-chewing behavior was initially described 40 years ago [1], it was only quite recently recognized as a stress-related behavior triggered by a variety of environmental or management factors in the captive environment. For example, such behavior has been associated with an increased adrenocortical activity (increased plasma corticosterone and adrenocortical hyperplasia) [2,3]. Recently, we described a number of factors that may contribute to the development of the abnormal behavior in domestic chinchillas (i.e., crowding, number of wood shaving changes per week, dustbathing, etc.) and hypothesized that fur-chewing behavior in the chinchilla is caused by management/environmental stress factors and/or lack of natural stimuli in the caging conditions [4]. We provided additional evidence to support this concept, and suggested that a clear female sex bias exists in the expression

* *Corresponding author*. Tel.: +54 351 4332019; fax: +54 351 4332019.
E-mail addresses: ponziomarina@hotmail.com, mponzio@mater.fcm.unc.edu.ar (M.F. Ponzio).
http://dx.doi.org/10.1016/j.repbio.2014.06.002

of this behavior: females that exhibited the most severe form of fur-chewing excreted elevated concentrations of urinary cortisol, which suggested that the expression of this behavior was mediated, at least in part, by physiological stress. Furthermore, those females demonstrated increased anxiety-like behavior associated with the elevated plus-maze test (e.g., decrease in the percentage of entries and time spent in open arms, increase in freezing behavior) [5].

In addition to influencing the expression of abnormal behaviors, stress and a corresponding increase in glucocorticoid production can also be associated with compromised reproductive function. Although a wealth of literature exists about the effects of glucocorticoids on reproduction [6–10], few studies have focused on the inter-relationships between abnormal repetitive behaviors and reproduction. Whether or not reproductive function is altered in fur-chewing chinchillas is still a matter of debate. Although precise information on the underlying basis of chinchilla fur-chewing is currently ambiguous, the development of this behavior in commercial farms and the related economic loss, the possible reduced welfare of affected animals and the increasing popularity of this species as pet, have all increased the public demands for more information on this pathology. Therefore, the objective of this study was to examine the reproductive function in the affected male and female chinchillas.

2. Materials and methods

2.1. *Animals, housing and management*

Sexually mature domestic chinchillas (*Chinchilla lanigera*) with proven fertility were used in this study (age range: 2–4 years). The selected animals exhibited either normal behavior or fur-chewing behavior of different intensity, categorized as follows: (1) slight – only a few tufts of hair are chewed; (2) moderate – one of the sides or hips is extensively chewed; (3) severe – both sides of the body or hips are chewed; and (4) very severe – all the fur in regions of the body that the animal can reach are chewed [4].

The fur-chewing animals were obtained from local commercial breeding farms where they are usually sacrificed by breeders because while some individuals may stop the behavior, the fur recovery is generally uncompleted, the pelt therefore has no economic value and the affected animals finally have to be eliminated [1,6]. Therefore, the affected animals used in the current study were collected from different farms and taken to our chinchilla breeding facility. After transportation, the animals were observed for at least one month before the study onset, and were assessed by an experienced researcher to determine the fur-chewing intensity. The animals had access to pelleted chinchilla food (Chinworld, Escobar, Buenos Aires, Argentina) and water ad libitum, as well as they received a cube of compressed alfalfa weekly. The chinchilla were exposed to ambient photoperiod and controlled temperature (20–25 °C) and were housed in individual stainless steel cages (width: 0.32 m; height: 0.30 m; length: 0.50 m) with wood shavings as substrate. Females were maintained in a polygamous breeding system, in which individual female cages have a corridor in the back allowing the male to enter any of the family females' cages when the corridor gate is open by the researcher/breeder. Males were housed individually in cages of the same size as females. A tablespoon of marble powder was added to the substrate of each cage on a regular basis so that animals could perform a "dust bath" to keep the fur dry and uncompressed. The housing, environmental and management conditions were the same as those used in commercial breeding farms. All experiments were conducted in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals.

2.2. *Evaluation of reproductive function in males*

Semen quality was assessed weekly in normal ($n = 6$) and fur-chewing ($n = 18$) chinchilla males. A total of three semen samples were collected and assessed for each animal. Weekly values were then averaged to derive baseline seminal traits for each male. Semen was obtained by electroejaculation, and selected sperm parameters were assessed immediately and after 4 h of in vitro incubation to account for the overtime quality of the sample [11–16]. The examined sperm parameters included semen volume, sperm concentration, motility and viability, as well as the integrity of sperm membrane and acrosome (swollen spermatozoa and viable acrosome intact sperm, respectively) [11–16]. Due to the fact that particularly nervous animals often need more stimuli to achieve ejaculation (sometimes they do not ejaculate at all), the number of stimuli needed to achieve ejaculation and the effectiveness of the electroejaculation (number of electroejaculations per week resulted in an ejaculation/total number of electroejaculations × 100) was also recorded.

2.3. *Examination of reproductive function in females*

Fifteen chinchilla females exhibiting severe or very severe fur-chewing behavior were studied in this experiment. The females were maintained in a polygamous reproductive system and one behaviorally normal male was used for every five females. Reproductive performance for each female was evaluated over one year, allowing the male to mate with the females for the entire period (estrus and post-partum estrus), as it is usually on commercial farms. The examined reproductive parameters included: number of litters produced per female per year, litter size and weaning success (percentage of pups surviving through weaning; 60 days of age). Comparative data from behaviorally normal females were obtained from six local commercial breeding farms, which provided full reproductive databases for the preceding 5-year interval. Only females that delivered a litter during the study period were included in this comparison ($n = 1452$ females).

2.4. *Statistical analysis*

All values were expressed as mean ± SEM. Data analysis was performed using the Infostat statistical software package (Di Rienzo J.A., Casanoves F., Balzarini M.G., Gonzalez L., Tablada M., Robledo C.W. InfoStat versión 2012. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Córdoba, Argentina; URL http://www.infostat.com.ar). Modified Shapiro–Wilks

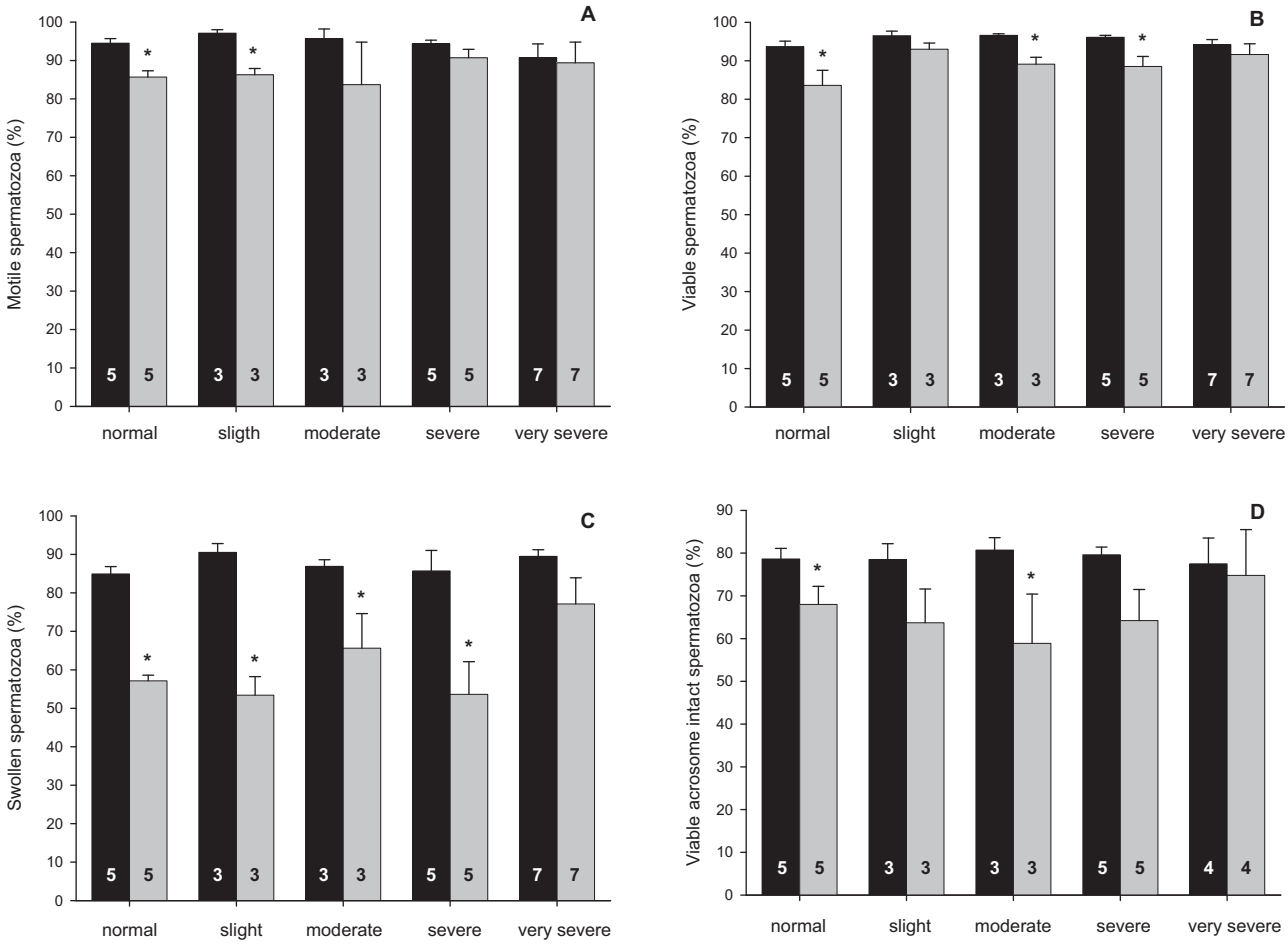

Fig. 1 – Selected sperm parameters (mean ± SEM) of *Chinchilla lanigera* males showing normal or fur-chewing behavior of different intensity. Semen samples were evaluated immediately after ejaculation (0 h; ■) and after 4 h of incubation at 37 °C (□). The number of animals is indicated at the bottom of each column. There were no differences in the examined parameters among the groups displaying different fur-chewing behavior; * shows difference between 0 and 4 h incubation ($p < 0.05$).

was applied to test normal distribution of data. Homogeneity of variances was evaluated, and nonconforming data were ln transformed before further analysis. Male data were analyzed using two-way ANOVA (incubation time and severity of the behavior being the factors) followed by the post hoc DGC test (DiRienzo, Guzmán and Casanoves test) [17]. Female data were analyzed by the Student's *t* test. The accepted level of significance for all statistical tests was $p \leq 0.05$.

3. Results

A total of 18 ejaculates from normal males and 54 ejaculates from fur-chewing males were collected. Both, in fresh semen (0 h) and after 4 h of incubation, sperm motility and viability as well as the integrity of sperm membrane and acrosome did not differ among animals with different levels of the fur-chewing behavior (Fig. 1). Similarly, no differences were detected in semen volume, sperm concentration, and the number of stimuli needed to achieve ejaculation and the effectiveness of the procedure (Table 1). However, the examined sperm parameters after 4 h incubation were lower compared to 0 h ($p < 0.05$; Fig. 1). The two-way analysis of variance showed that there was no interaction between the fur-chewing behavior severity and time of incubation ($p > 0.05$).

Females showing normal or fur-chewing behavior presented similar reproductive performance in terms of litter size and the number of litters per female and year. However, weaning success (pups survival determined at two months of age) was lower ($p = 0.05$) in fur-chewing females (Table 2).

4. Discussion

In the current study, fur-chewing chinchillas showed reproductive parameters similar to those of chinchillas with no fur-chewing behavior; nevertheless pups survival rate in ARB females were lower than that of control chinchillas. Regardless of the abnormal behavior severity, the examined sperm parameters and the response to the process of semen collection in fur-chewing males did not differ from those displaying normal behavior. Furthermore, the sperm parameters observed in normal animals were also similar to those previously described [11–16]. The present data are consistent

Table 1 – Selected characteristics (mean ± SEM) of electroejaculation procedure and semen of domestic *Chinchilla lanigera* showing different intensity of fur-chewing behavior.

Characteristics	Fur-chewing behavior				
	Normal ($n = 6$)	Slight ($n = 3$)	Moderate ($n = 3$)	Severe ($n = 5$)	Very severe ($n = 7$)
Semen volume (μL)	42.9 ± 5.9	53.3 ± 15	68.3 ± 31.8	40.0 ± 12.7	36.1 ± 10.2
Sperm concentration ($\times 10^6$ mL^{-1})	429 ± 118	1116 ± 296	1770 ± 1628	1050 ± 554	2193 ± 686
Number of stimuli to achieve ejaculation	4.0 ± 0.9	4.7 ± 1.2	5.0 ± 1.3	4.4 ± 1.0	6.4 ± 2.1
Electroejaculation efectiveness (%)	96.7 ± 3.3	91.7 ± 8.3	75.0 ± 14.4	88.3 ± 7.3	84.4 ± 7.4

Table 2 – Reproductive performance of normal and fur-chewing domestic *Chinchilla lanigera* females.

Parameter	Normal females	Fur-chewing females
Number of litters/female/year	1.4 ± 0.05	2.2 ± 0.5
Litter size at birth	1.9 ± 0.08	2.2 ± 0.2
Weaning success	88.9 ± 4.1[a]	59.0 ± 13.4[b]

Data are expressed as mean ± SEM; normal females: 1452 females from six breeding facilities; fur-chewing females: $n = 15$ animals. Weaning success is reported as the percentage of pups surviving through weaning (60 days of age); a vs. b: $p = 0.05$.

with previous results demonstrating the absence of elevated concentrations of cortisol metabolites and anxiety-related responses to the elevated plus-maze test in fur-chewing males [5]. Similarly, the number of litters produced per female per year and litter size was not affected by fur-chewing behavior. It should be emphasized that the housing, as well as environmental and management conditions provided in our laboratory were the same as in commercial breeding farms. Moreover, the reproductive performance found in fur-chewing females housed under laboratory conditions was similar to that reported previously for the species [18–20].

Several reports that addressed other forms of abnormal repetitive behavior (ARB; i.e., stereotypic behavior) revealed similar findings. Svendsen et al. [21] found no differences in fertility between high and low stereotypic lines of female mink. A recent retrospective analysis of Schonecker [22] yielded comparable results in litter size, number of weanlings and proportion of barren females between stereotypic and non-stereotypic bank vole dams. The results reported in the present study seem to be in line with the aforementioned evidence, suggesting non-significant effects of abnormal behaviors on reproduction. Furthermore, although the development of abnormal behavior is generally perceived as an indicator of sub-optimal environments, highly stereotypic individuals could contradictorily show better welfare and reproductive success than less stereotypic animals housed under the same conditions [23–25].

In the present study, the weaning success of normal females was consistent with previous reports [18–20], yet it was lower in fur-chewers. Some studies in voles suggested that pups born to dams that exhibit stereotypic behavior, experience maternal deprivation that is related to a reduced quality of maternal care [26]. Sorensen and Randrup [27] reported that stereotypes might interfere with the normal nurturing by the mother. Although pup mortality of the first litter did not differ between stereotypers and non-stereotypers, an increased mortality was demonstrated for the second litters of stereotypers [28]. It is likely that chinchilla fur-chewing mothers also exhibit inappropriate maternal behavior, and as a result, poor weaning success was observed. Indeed, some pups that were found dead in cages showed signs of maternal aggression.

In summary, the development of fur-chewing behavior in farmed chinchillas did not have a significant impact on reproductive functions of either males or females. The reduced survival of offspring from fur-chewing mothers may be related to deficient maternal care, and this aspect should be considered by breeders when selecting animals for the production stock. Future research should address the underlying motivational basis of the fur-chewing behavior as well as establish ways to prevent such behavior.

Authors' contribution

MGG, and MP performed all the experiments. MP, MFC and RR were involved in study design and data analysis. MP conceived the project and wrote the paper. All authors discussed the results and commented on the manuscript.

Conflict of interest

None declared.

Acknowledgments

Financial support was provided by Ministerio de Ciencia y Tecnología de la Provincia de Córdoba (PID 113/2011), FONCyT (PICT 2006-1974), CONICET (PIP 11420080100237) and SECyT-UNC. RDR, MFC and MFP are established investigators from the Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Argentina. The animals were kindly provided by ACRICHI (Córdoba, Argentina), and chinchilla pelleted food by ChinWorld S.A. (Escobar, Buenos Aires, Argentina).

REFERENCES

[1] Empress Chinchilla Breeders Cooperative Inc.. Rancher's handbook. New York: Empress Chinchilla Breeders Cooperative Inc.; 1987.

[2] Tisljar M, Janic D, Grabarevic Z, Marinculic A, Pinter L, Janicki Z, et al. Stress-induced Cushing's syndrome in fur-chewing chinchillas. Acta Vet Hung 2002;50:133–42.
[3] Vanjonack WJ, Johnson HD. Relationship of thyroid and adrenal function to fur-chewing in the chinchilla. Comp Biochem Physiol 1973;45:115–20.
[4] Ponzio MF, Busso JM, Ruiz RD, Fiol de Cuneo M. A survey assessment of the incidence of fur-chewing in commercial chinchilla (*Chinchilla lanigera*) farms. Anim Welf 2007;16:471–9.
[5] Ponzio MF, Monfort SL, Busso JM, Carlini VP, Ruiz RD, Fiol de Cuneo M. Adrenal activity and anxiety-like behaviour in fur-chewing chinchillas (*Chinchilla lanigera*). Horm Behav 2012;61:758–62.
[6] Breen KM, Karsch FJ. New insights regarding glucocorticoids, stress and gonadotropin suppression. Front Neuroendocrinol 2006;27:233–45.
[7] Dobson H, Smith RF. Stress and reproduction in farm animals. J Reprod Fertil 1995;49:451–61.
[8] Liptrap RM. Stress and reproduction in domestic animals. Ann N Y Acad Sci 1993;697:275–84.
[9] Moberg GP. How behavioral stress disrupts the endocrine control of reproduction in domestic animals. J Dairy Sci 1991;74:304–11.
[10] Rivier C, Rivest S. Effect of stress on the hypotalamic–pituitary–gonadal axis: peripheral and central mechanisms. Biol Reprod 1991;45:523–32.
[11] Busso JM, Ponzio MF, Chiaraviglio M, Fiol de Cuneo M, Ruiz RD. Electroejaculation in the chinchilla (*Chinchilla lanigera*): effects of anesthesia on seminal characteristics. Res Vet Sci 2005;78:93–7.
[12] Busso JM, Ponzio MF, de Cuneo MF, Ruiz RD. Year-round testicular volume and semen quality evaluations in captive *Chinchilla lanigera*. Anim Reprod Sci 2005;90:127–34.
[13] Carrascosa RE, Martini AC, Ponzio MF, Busso JM, Ponce AA, Lacuara JL. Storage of *Chinchilla lanigera* semen at 4 °C for 24 or 72 h with two different cryoprotectants. Cryobiology 2001;42:301–6.
[14] Ponce AA, Carrascosa RE, Aires VA, Fiol de Cuneo M, Ruiz RD, Ponzio MF, et al. Activity of *Chinchilla laniger* spermatozoa collected by electroejaculation and cryopreserved. Theriogenology 1998;50:1239–49.
[15] Ponzio MF, Busso JM, Fiol de Cuneo M, Ruiz RD, Ponce AA. Functional activity of frozen thawed *Chinchilla lanigera* spermatozoa cryopreserved with glycerol or ethylene glycol. Reprod Domest Anim 2008;43:228–33.
[16] Ponzio MF, Busso JM, Ruiz RD, de Cuneo MF. Time-related changes in functional activity and capacitation of chinchilla (*Chinchilla lanigera*) spermatozoa during in vitro incubation. Anim Reprod Sci 2007;102:343–9.
[17] Di Rienzo JA, Guzmán W, Casanoves F. A multiple comparison methods based on the distribution of the root node distance of a binary tree. J Agric Biol Environ Stat 2002;7:1–14.
[18] Felska L, Brzozowski M. Litter size, weaning success, and nursing mortality in chinchillas (*Chinchilla lanigera*) in relation to cage illumination. Scientifur 2004;28:234–7.
[19] Lanszki J. The effect of litter size and individual weight at birth on the growth and mortality of chinchillas. Scientifur 1996;1:42–6.
[20] Lanszki J, Jauk E, Bognár Z. Examination of traits related to prolificacy and suckling ability in chinchillas (*Chinchilla laniger*). Scientifur 1998;22:219–23.
[21] Svendsen B, Hansen J, Malmkvist S, Palme R, Jeppesen L. Selection against stereotypic behaviour may have contradictory consequences for the welfare of farm mink (*Mustela vison*). Appl Anim Behav Sci 2007;107:110–9.
[22] Schonecker B. Increased survival and reproductive success associated with stereotypical behaviours in laboratory-bred bank voles (*Clethrionomys glareolus*). Appl Anim Behav Sci 2009;121:55–62.
[23] Mason GJ, Latham NR. Can't stop, won't stop: is stereotypy a reliable animal welfare indicator? Anim Welf 2004;13: 57–69.
[24] Jones MA, Van Lierop M, Mason G, Pillay N. Increased reproductive output in stereotypic captive *Rhabdomys* females: potential implications for captive breeding. Appl Anim Behav Sci 2010;123:63–9.
[25] Jeppesen LL, Heller KE, Bildsoe M. Stereotypies in female farm mink (*Mustela vison*) may be genetically transmitted and associated with higher fertility due to the effects on body weight. Appl Anim Behav Sci 2004;86:137–43.
[26] Latham NR, Mason GJ. Maternal deprivation and the development of stereotypic behaviour. Appl Anim Behav Sci 2008;110:84–108.
[27] Sorensen G, Randrup A. Possible protective value of severe psychopathology against lethal effects of an unfavourable milieu. Stress Med 1986;2:103–5.
[28] Odberg FO. The influence of cage size and environmental enrichment on the development of stereotypies in bank voles (*Clethrionomys glareolus*). Behav Process 1987;14: 155–73.