Journal of Animal Production 29 (3), 36-42, 2017

Influence of Penile Spicules of Covies (*Cavia porcellus*) on their Sexual Behavior, Fertility and Sperm Quality

Luis E. Ayala Guanga, Ramiro Rodas Carpio*, Andrés Almeida**, Carlos S. Torres Inga*, Pedro E. Nieto Escandón**

*** School of Veterinary Science, Faculty of Agricultural Sciences, University of Cuenca, Ecuador**

**** Ministry of Agriculture, Livestock, Aquaculture, and Fishing, Azuay, Ecuador**

luis.ayala@ucuenca.edu.ec

Abstract

This research took place in canton Cuenca, province of Azuay, Ecuador, located on UTM 717386 x 9675751, 2 714 meters above sea level, with a mean temperature of 14°C. The study determined the influence of penile spicules on sexual behavior, fertility and sperm quality parameters in cavies (*Cavia porcellus*), raised on the Ecuadoran highlands. A number of 5 whole males (with penile spicules), and other 5 males with their spicules removed were included in the study, along with 40 nulliparous females, type A, pelage type 1. The pregnancy percentage of females that copulated with extirpated males decreased 65% in relation to the control group ($P<0.05$). However, the sexual behavior pattern and sperm quality of the two groups in the study were similar ($P>0.05$). Therefore, the study concluded that extirpation of the penile spicules has effects on fertility, but not on sexual behavior and sperm quality.

Key words: *cavy, spicules, fertility*

INTRODUCTION

Surgical (or chemical) castration or sterilization is an alternative to reduce animal aggressiveness (Apráez, *et al*., 2011; Vega *et al*., 2012). Every castration technique has advantages and disadvantages, depending on factors like species, individuals, physiological states, etc. (Hernández and Fernández , 2012).

In rural areas of Ecuador, the traditional ancient castration technique used is the removal of penile spicules to sterilize covies, and control their aggressiveness. However, the literature shows no published papers on the function these structures have, and their influence on Covy´s reproductive behavior.

Anatomically, hystricomorph rodents have two invaginated structures or keratinized spicules below the urethra (Stan, 2015). When the penis is erected, they project from the gland´s end as two thin spines of approximately 4-5 mm long. But their function remains partially unknown, though it has been speculated that they can adapt to fitting cavities into the female genitals (Sachs *et al*., 1984).

Since no information is available on the function of Covy´s penile spicules, it has been sought for in other rodents. Accordingly, Abedayo *et al*. (2011) claimed that the penile spicules of rodents may act as accessory sensitive organs that contribute to genital sensitivity in the male, or they might confer an additional stimulus to the female genital tract.

Upon ejaculation, most rodent males deposit a stopper in the female´s vaginal tract to prevent further mating (Hartung and Dewsbury, 1978; Voss, 1979). Cena *et al.* (1995), determined that the penile spicules of covies contributed to the removal of the vaginal stopper deposited on the first mating, thus allowing new fertilization. Adebayo *et al.* (2011), claimed that the stimulation given by rodent spicules when mating may trigger the neuroendocrine process that leads to ovulation.

The aim of this research was to determine the presence of penile spicules on sexual behavior, fertility, and sperm quality parameters in covies (*Cavia porcellus*) that grow in the Ecuadoran highlands.

MATERIALS AND METHODS

The study was developed on a farm owned by a beneficiary of the Ministry of Agriculture, Livestock, Aquaculture, and Fishing (MAGAP), located on Panamericana road, km 1, Baños parish, Cuenca canto, province of Azuay, Ecuador. It is located on coordinates UTM 717 386 x 967 5751, 2 714 meters above sea level, average temperature 14 ºC, relative humidity 80%, and annual rainfall between 800 and 2 000 mm. This research followed the sanitary regulation standards for ground animals, chapter 7.8 "Use of Animals for Re-

search and Education", World Organization for Animal Health (OIE, 2016).

Animals in the study

The study included ten five-month old male covies, with an average weight of 988.3 ± 11.40 g, after fertility check. Also, 40 four-month old nulliparous females, weighing 815.3 ± 11.80, on average were included. The animals classified as type A, and type I pelage, according to (Solorzano and Sarria, 2014). The covies lodged in the same facility from June to September 2015, with the same management and care conditions, receiving associated *Pennisetum clandestinum*, *Lolium multiflorum*, *Trifolium pratense*, *Trifolium repens*, supplemented with mineral salts.

Protocol for removal of penile spicules

The determinations were made after surgical removal of the penile spicules. The males used for breeding (5) received a mixture of zolazepam and tiletamine anesthetics (Zoletil 50®) in a 50 mg/kg dose, subcutaneously, according to the protocol suggested by Álvarez (2010). When the animal was anesthetized, the area was shaved and embrocated, and the organ was exposed by penis protrusion through the urethral bag, by finger pressing. Then, the spicules were removed, according to the criteria for genital experimental surgery proposed by Gay and Heavner (1986). When the bone formations (spicules) were spotted, they were held with tweezers and cut using scissors, then the organ was returned to normal position. After that, the covies were kept in individual pits for monitoring and recovering, during 15 days.

Experiments

First experiment (Assessment of sexual behavior)

T1= 4 male covies with penile spicules (whole); each was placed in separate cages with 5 females.

T2= 4 covies (males) with removed spicules (extirpated), each was placed in separate cages with 5 females.

The number of genital grooming, sniffing, biting, and mounts made by the male to their cage mates, were evaluated.

Second experiment (pregnancy percent)

Pregnancy was evaluated in 20 T1 females, and 20 T2 females from the first experiment.

Third experiment (determination of sperm quality)

T1= 5 covies (males) with penile spicules (whole)

T2= 5 covies (males) without the spicules (extirpated)

Sperm quality was analyzed in the eight studied males.

Sexual behavior

This research evaluated the sexual behavior of whole and extirpated covies. A male was placed in a cage with five females (four replicas). The number of genital grooming, sniffing, biting, and mounts made by the male to the females, was assessed. Daily observations were performed, four hours in the morning and four in the afternoon, during the first 8 post-lodging days of the males and females in the cages. The cages were arranged strategically so all the animals could be observed at the same time, the covies were filmed along that period.

Fertility (pregnancy and proliferation percent)

Male fertility was determined by the number of inmate females fertilized. The males stayed with the females for 30 days, then they were withdrawn and put in separate cages. Pregnancy determination was made according to Kaufman and Davidoff (2012); however, real evidence was given by the number of females that delivered until 60 days after male withdrawal. The number of litters per delivery was set up according to the number of litters born per cage, then it was divided only by the number of females that gave birth to estimate the final value.

Parameters of sperm assessment

The spermatozoa were collected by diffusion from the epididyme (Scott et al., 1991). To achieve that, the testicles from every experimental individual was surgically removed. The epididyme was removed by dissection. The tail of the epididyme was placed in a 15 ml sterile Falcon tube (SSI, México), containing 2 ml of Holding Plus medium (BIONICHE, Pullman, WA, USA®). It was dipped in lukewarm water bath, at 37 ºC, for 10 min, to allow spermatozoa to spread in the medium; 1 ml of the supernatant was removed, and was placed into another sterile Falcon tube, containing 1 ml of Cushion fluid® solution. Then it was centrifuged at 100 g, for 20 min, until a fraction with spermatozoa could be collected for analysis.

To assess sperm quality, concentration, mass motility, single motility, vitality, and morphology

were determined, according to Boersma *et al.*, (2015). A photometer (SDM 1, Minitube; DE; USA®) was used to assess sperm concentration. Mass motility was established by direct microscope observation of wave formation (scale: 1:5); 1, no wave; 5, waves forming vortex. Evaluation of individual motility consisted of progressive rectilinear motion of spermatozoa. Eosine-nigrosine staining was used to determine sperm vitality. Saline solution with glutaraldehide (2%) was used to assess morphology.

Statistical analysis

The study was based on a completely randomized design (CRD).

SPSS 22, for Windows, was used for statistical analysis. The sexual behavior and sperm quality data were analyzed by the Shapiro-Wilk and Levine test ($P < 0.05$). The Student T test for single samples was used for analysis of normality and homogeneity of variances (scale variables). Proportion: comparison Z ($P < 0.05$) was applied for variable gestation percent (nominal).

RESULTS AND DISCUSSION

Assessment of sexual behavior

The sexual behavior of males in the two treatments was similar ($P > 0.05$) for sniffing, biting, and mounts (Table 1); however, the average number of grooming was higher in the whole male group, in comparison to the extirpated males ($P < 0.05$).

When analyzing the daily number of grooming actions during the assessment period (8 days), T1 was observed to show higher values than T2, on days 1; 2; and 3 ($P < 0.05$), with means of 17.5 ± 2.02(T1); 6.0 ± 0.41(T2)/8.8 ± 1.25(T1); 3.3 ± 0.48(T2)/4.8 ± 0.85(T1);1.0 ± 0.40(T2), respectively (Fig. 1). On the other days, the behavior of the variable was similar for the two treatments ($P > 0.05$).

Fertility assessment (pregnancy percent)

The group of female covies copulated by whole male covies were fertilized 65% or more, in comparison to the females copulated by the extirpated males ($P < 0.05$) (Fig. 2).

When comparing the number of litters obtained in the two treatments, a value of 1.28 ± 0.26 offspring/litters for females that copulated with whole males; and 1.18 ± 0.64 offspring/litters (extirpated), with ($P > 0.05$); hence, they did not show any statistically significant differences.

Assessment of sperm quality

The behavior pattern of variables sperm concentration, mass motility, individual motility, vitality, morphology, were all used to determine sperm quality in the treatments, were similar ($P > 0.05$); however, they were within the acceptable ranges for this species of rodents (Table 2).

The results achieved showed a highly significant influence ($P < 0.05$), of the covy´s spicules on fertility of females copulated by the whole males. What is more, group 1 (females copulated by whole males) had greater fertility (95%), compared to group 2 (females copulated by removed males). Solorzano and Sarria (2014); and Fernández (2010) analyzed the pregnancy percent of female covies, and set up average values of 90%, and 84.27%, respectively, which led to the assumption that the whole males (T1) were fertile.

Regarding the offspring average per mother, it was 1.28 ± 0.26 (whole), and 1.18 ± 0.64 (extirpated), within the range established by Solorzano and Sarria (2014), and Schöpper *et al.* (2011), who determined a range of 1-5 offspring/mother, when the mating density is 1 male with 5 females.

This research established that penile spicules do not have an effect on the sexual behavior of covies, except for the number of grooming actions, with significant differences between the groups ($P < 0.05$). It could be explained by the theory of Manteca (2009), who stated in his book Etología Veterinaria, that sexual behavior in male rodents is originated in the process of sexual differentiation of the central nervous system, influenced by the action of sexual hormones. This process occurs in two phases: first, the androgens act in an early stage of development, organizing neural pathways that will be responsible for the sexual behavior. Second (activation), takes place during puberty; the androgens act on previously differentiated neural pathways, allowing the occurrence of male sexual traits. Therefore, when sexual maturity is reached, the behavioral patterns of sexual conduct are already established. In this research only sexually mature males were used (five months old). Besides, Hull and Domínguez (2007) determined that the chemo-sensory entrance and the vomeronasal system are probably the two ways that regulate sexual behavior of mature male rodents during mating, but these remained intact in this study.

Quality analysis of spermatozoa collected from the tails of covies´ epididymes determined that no statistical differences were produced between the two compared treatments ($P > 0.05$). However, it is important to note that the values obtained for individual motility in the two groups (whole 58%, extirpated 55%) were close to the reports made by Freund (1969), who achieved 66% sperm motility. Besides, he also established 95% spermatozoa with a normal morphology as high, when compared to the results in this paper (63%).

The specific role of penile spicules in covy breeding could not be determined based on the results of this study; however, Stoddart (1979), established that spicules act as sensitive accessory organs, causing sensitivity in the males, or they might produce additional stimulus in the genitals of the females that can induce ovulation. Spotorno (1979) noted that in species of rodents with spontaneous ovulation, the physiological mechanisms for pre ovulating LH discharge had not been properly considered. So he suggested the existence of a mechanical way of arousing that could act in the vagina and cervix. Silva, *et al.* (2013) claimed that the penile spicules have two functions: stimulating the vagina during copulation and promote neuroendocrine processes that generate ovulation; and removing the vaginal mucous stoppers to succeed in fertilizing the female with a subsequent ejaculation. Moreover, these structures follow a peri puberal development pattern, which is androgen dependent. It was demonstrated through castration, which generated a regression of the structures. But when testosterone was applied exogenously, they were recovered. Although this research was left in the experimental field, it opens new possibilities for further research.

CONCLUSIONS

Pregnancy percent decreased in female covies copulated by males without penile spicules. However, the sexual behavior and sperm quality of male covies did not differ between the experimental groups.

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Received: 7-12-2017
Accepted: 7-20-2017

Table 1. Mean and standard error of grooming, sniffing, biting, and mounts, made by covies in the two treatments during the monitoring period (8days)

Variables	Treatments						P
	Wholes			Extirpated			
	$\bar{X}$ ± SE	CI 95 %		$\bar{X}$ ± SE	CI 95 %		
		inferior	superior		inferior	superior	
Genital grooming	4.67±1.05	2.61	6.73	1.59±0.36	0.88	2.30	0.01
Sniffing	11.53±1.29	9.00	14.06	9.87±1.30	7.32	12.42	0.37
Biting	17.97±1.24	15.54	20.40	20.28±1.53	17.28	23.28	0.25
Mounts	1.09±0.16	0.78	1.40	0.63±0.24	0.16	1.10	0.11

$\bar{X}$ = mean; SE=standard error; CI=confidence interval 95 %; P=significance value to 0.05; Student T test

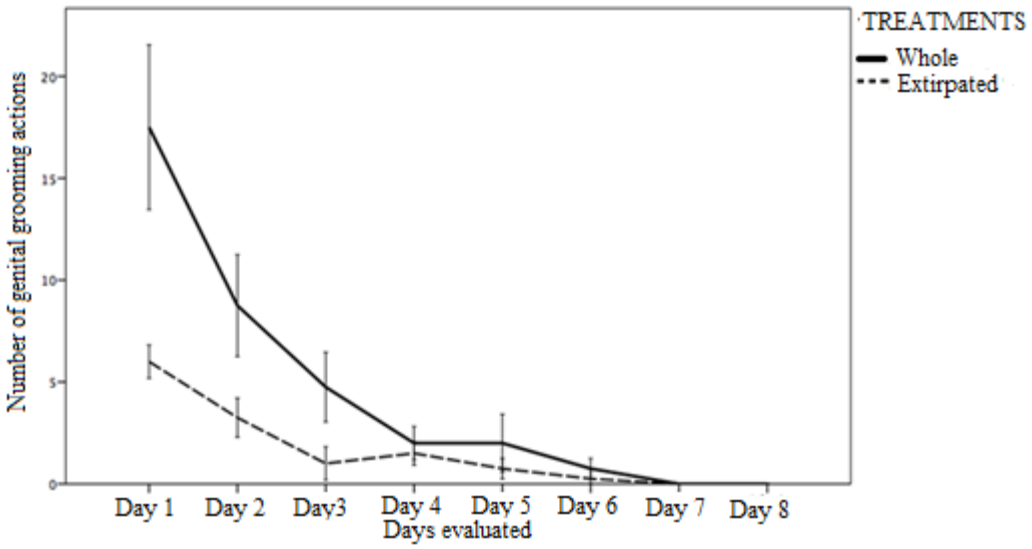

Fig. 1. Mean of grooming actions made by the males to their pertners in the cages, in the two treatments, assessed in different days. On days 1, 2 and 3, there was statistically significant differences between the treatments (P < 0.05) Student T test

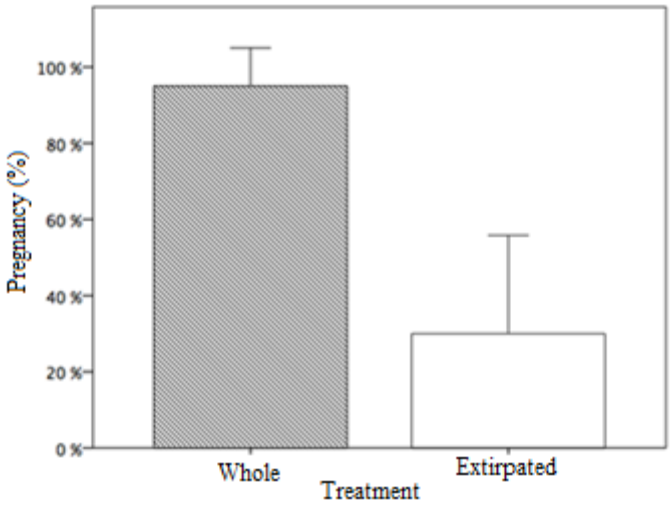

Fig. 2. Pregnancy percent in females copulated by males in the two treatments (Z test)

Table 2. Mean and standard error of sperm assessment parameters

Variables	Treatments						P
	Wholes			Extirpated			
	$\overline{X}$ ± SE	CI 95 % inferior	superior	$\overline{X}$ ± SE	CI 95 % inferior	superior	
Concentration (x10^6)	418±57	306.28	529.72	342±32	279.28	404.72	0.29

Mass motility (1-5)	3.4±0.24	2.93	3.87	2.8±0.2	2.41	3.19	0.09
Individual motility (%)	58±5.39	47.44	68.56	55±2.24	50.61	59.39	0.62
Vitality (%)	60.2±4.02	52.32	68.08	52.2±8.09	38.34	70.06	0.53
Morphology (%)	62.8±2.89	57.14	68.46	63.4±2.66	58.19	68.61	0.88

$\overline{X}$: = mean; SE=standard error; CI=confidence interval 95 <t3/>%; P=significance value to 0.05; Student T test