Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

SHORT COMMUNICATION

Changes in the Testicular Morphology of Chinchillas *(Chinchilla lanigera)* Implanted with Zeranol

Francisco R Carvallo[1], Carolina A Santibañez[2] and Tamara A Tadich[2]*

[1]California Animal Health and Food safety Laboratory, 105 W Central Av, San Bernardino, CA. 92408, USA
[2]Department of Animal Production, Faculty of Veterinary and Animal Sciences, University of Chile, Santa Rosa 11735, La Pintana, Santiago, Chile
*Corresponding author: tamaratadich@u.uchile.cl

ARTICLE HISTORY (14-160)

Received: March 30, 2014
Revised: June 07, 2014
Accepted: July 05, 2014

Key words:
Chinchilla
Estrogen
Testicular degeneration
Zeranol

A B S T R A C T

Zeranol is a semi-synthetic non-steroidal product used by chinchilla breeders to improve fur quality. The aim of this study was to assess the potential deleterious effects of zeranol in chinchilla at testicular level. For this purpose, 27 chinchillas received 6 mg of zeranol and 9 untreated chinchillas were used as control. Testicles were recovered after routine sacrifice, and the weight, length, width and testicular perimeter were recorded. Individual information regarding sex, age, weight at birth, at weaning and at sacrifice of chinchilla was obtained from the breeders records. The testes were processed for routine histological evaluation. Treated animals evidenced a significant reduction in testicular weight, length, width and perimeter. Histological findings included a reduction in the tubular lumen, loss of epithelium and disappearance of spermatozoids in the seminiferous tubules. Data suggests that zeranol produces toxic effects on the testis including degenerative damage in germ cells and Leydig cells, thus affecting spermatogenesis.

To Cite This Article: Carvallo FR, CA Santibañez and TA Tadich, 2015. Changes in the testicular morphology of chinchillas *(Chinchilla lanigera)* implanted with zeranol. Pak Vet J, 35(1): 103-105.

INTRODUCTION

The chinchilla (*Chinchilla lanígera*) is a histricomorphic rodent endemic from Chile (South America), and has one of the most valuable furs worldwide. Because of this it has been domesticated, selected and produced according to fur quality, colour and growth rate (Sanchez-Toranzo *et al.*, 2014).

Among fur characteristics, the length (up to 2.5cm), softness and high density (20-80 hairs per follicle) result in a very high price within the industry (Badillo *et al.,* 1999). The obtainment of a high quality product in a short period of time is the aim of producers, and also the main reason for incorporating anabolic drugs in the production system.

In Chile, as in other countries, the use of zeranol (Ralgro ®) has been incorporated empirically in the production system of chinchillas, aiming to stimulate the hair follicle and dermal cells, achieving shorter maturation periods and a more homogeneous finishing of the fur (Figueroa *et al.,* 2001). Zeranol implants also stimulate the growth rate of the animal, which in turn results in bigger size furs (Badillo *et al.,* 1999).

Studies in rats demonstrate permanent changes in development of sexual organs, mainly testis degeneration following treatment with zeranol. The damage of the seminiferous epithelium in rats has been associated with the suppressive action of zeranol over the transcription of enzymes that participate in the biosynthesis of testosterone by Leydig cells (Shidaifat *et al.,* 2013).

The effect of zeranol on the histologic structure of the uterus of chinchillas was described by Figueroa *et al.* (2001), but its effect on the macro and microscopical structure of Chinchillas testicle has not been studied. Therefore, the current study was conducted to describe the productive body weight variations and damage in the testicle of zeranol-treated Chinchillas in a quantitative manner.

MATERIALS AND METHODS

Animals: This study comprised 36 sexually mature male chinchillas (*Chinchilla lanígera*). The chinchillas were kept in individual cages under the same husbandry practices (12 hours light cycles, 55% humidity and 20ºC, alfalfa and special commercial concentrate for rodents, ad libitum water). Individual characteristics such as date of birth, date of implantation of Zeranol, date of sacrifice, age, weight at birth, at weaning and at sacrifice were recovered from the breeders' records. Two groups were

formed; Control group consisted of nine chinchillas that did not receive zeranol implants, and Treatment group of 27 chinchillas that received a dose of 6mg of zeranol, administered as a subcutaneous implant at the base of the tail. All treated chinchillas were implanted between 30 and 45 days before sacrifice, corresponding to ages between 10 and 34 months. This study was approved by the Bioethics committee of the Faculty of Veterinary and Animal Sciences of the University of Chile.

Collection of samples: All samples were obtained at sacrifice. Testicles were recovered, weighted, measured and kept in 10% buffered formalin for at least 24 hours before routine histological process.

Macroscopic variables: The weight, length, width and perimeter of each testicle were measured with a caliper. For the gonado-somatic index (GSI), the following equation was used:

GSI(%)= (Testicular weight (g)/Body weight (G)) x 100

Microscopic variables: A sagittal cut was done in each testicle, dividing the organ in two. The testicles were then processed through routine histological technique and stained with haematoxylin and eosin for evaluation of general morphology and different cell types. The microscopic variables registered were diameter (μm) of seminiferous tubules; number of elongated spermatids embedded in the apical edge of the tubules at stages 5, 6 & 7 of maturation development (Leal and França, 2009). The evaluation of the number of spermatids was done by a semi-quantitative method as described by Campion *et al.* (2013) and is presented in Table 1.

Statistical analysis: Descriptive statistics were used for semi quantitative data, and expressed as percentage of individuals in each score. Individual variables (age, weight) and testicular variables (weight, length, width, perimeter, volume, tubular diameter and GSI) were analysed with a two samples Students t-test or U-Mann Whitney test according to parametric or non-parametric characteristic's of data. For all analysis the Statistix 8.0® software was used.

RESULTS AND DISCUSSION

The age at sacrifice and body weight at birth, weaning and sacrifice for chinchilla of treated and control groups are given in Table 2. The weight at birth and at weaning did not differ between the two groups (P>0.05). These results were expected since at both ages none of the individuals had been implanted with the anabolic drug, but do represent the homogeneity of the animals of the two groups. However, the weight at sacrifice differed between groups, with implanted chinchillas being heavier (P<0.05). Both groups had weight at sacrifice higher than that described for chinchillas at full sexual maturity (552g±26g; Leal and França, 2009). The higher weight in the treated group can be explained by the anabolic effect of zeranol, increasing nitrogen synthesis favouring tissue development, effect that has been reported in other species such as bovines (Toso *et al.,* 1999), and rats (Yang *et al.,* 2007). This increase in body weight and size is a secondary effect for producers, benefiting fur production, since bigger animals produce furs of bigger size, which in turn gives a higher price.

Regarding the age at sacrifice, the treated group was significantly older (P<0.05), this difference can be explained by husbandry practices. Chinchillas that do not reach fur maturity during the first or second year are the ones usually implanted by the producer in order to stimulate hair growth. Although puberty is reached at 3 months of age, sexual maturity is achieved at around 17mo, at the same time it has been shown that the coefficient of efficiency of spermatogonial division, the meiotic index and the daily sperm production per gram of testicle in chinchillas does not present significant differences between 5 and 30 months of age (Leal and França, 2009), indicating that the probability of our findings being the result of degenerative changes would remain very low.

Macroscopic and Microscopic changes: All macroscopic testicular variables studied presented significant differences between groups (Table 3). Treated individuals presented smaller size testicles in relation to weight, length, width and gonado-somatic index. The microscopic effect of zeranol on testicular tissue was observed at tubular level. A significant (P<0.05) decrease in tubular diameter (average 124.78±73.7μm) was present in the treated group in comparison with an average diameter of 176.67±33.3μm for the control group (Table 3). These results are in agreement with the level of damage observed in the seminiferous epithelium (Table 4; Fig. 1), where most treated animals (62.9%) had a score of 0. The lower number of cells and a higher level of disorganization could result in the reported lower tubular diameter.

Recent studies gave zeranol similar features to natural and synthetic estrogens, showing a high affinity of zeranol to alpha and beta estrogen receptors (Shidaifat *et al.,* 2013), characteristics that explain its effect on testicles. zeranol could have a potential suppressor effect over the transcription of the enzymes involved in the biosynthesis of androgens by Leydig cells, specifically through a decrease in protein P450scc (Yang *et al.,* 2007; Warita *et al.,* 2010). The P450scc protein works by cutting the lateral chain of 6 carbons of cholesterol molecules, giving

Table 1: Semi-quantitative grading scheme for testis evaluation

Score	Description
0	Loss of greatest part of the seminiferous epithelium. Presence of vacuolated Sertoli cells; few spermatogonia without primary or secondary spermatocytes; no elongated spermatids. The lumen is empty or contains sparse cell debris.
1	Presence of Sertoli cells and primary spermatocytes; secondary spermatocytes are not always recognized; no elongated spermatids. The lumen contains small amounts of proteinaceous material.
2	Presence of Sertoli cells, spermatogonia, primary and secondary spermatocytes and low development of elongated spermatids at the apical portion of the epithelium. The lumen is narrower.
3	Normal structure of the seminiferous epithelium, with complete Sertoli cells, I and II spermatogonia and numerous elongated spermatids at the apex of the epithelium. The lumen is almost completely occupied by mature cells.

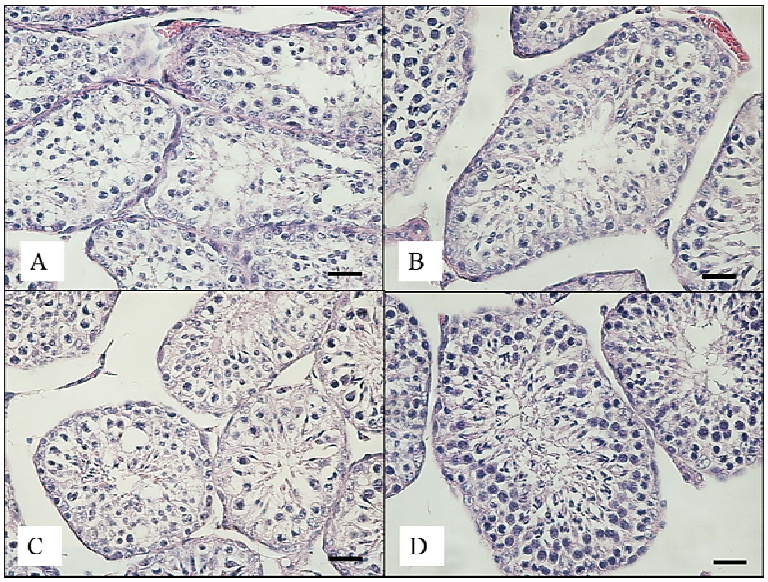

Fig. 1: Representative images of histological scoring of the seminiferous epithelium. For the description of each score, please refer to Table 1. A, score 0; B, score 1; C, score 2; D, score 3. Hematoxylin and eosin, 400X. Bar: 20μm

Table 2: Productive variables assessed for the control and treated chinchillas (mean±SD)

Variable	Control Group (n=9)	Treated Group (n=27)	P value	T/W
Age at sacrifice (days)	371.8±95[a]	538.0±140[b]	0.0009	75.5
Weight at birth (g)	47.9±10.8[a]	56.5±10.8[a]	0.340	-0.99
Weight at weaning (g)	261.0±59.5[a]	271.4±57.9[a]	0.339	0.99
Weight at sacrifice (g)	563.9±59.5[a]	631.9±72.2[b]	0.023	-2.58

Different superscripts indicate significant differences between groups (P<0.05). T/W=value of the t-test or U-Mann Whitney test.

Table 3: Macroscopic variables assessed in the testicles of control and treated chinchillas (mean±SD)

Variables	Control Group (n=9)	Treated Group (n=27)	P value	W/T
Testicular weight (g)	3.01±0.7[a]	1.75±0.8[b]	0.0005	263
Testicular length (cm)	2.03±0.2[a]	1.53±0.3[b]	0.0003	265
Testicular width (cm)	1.26±0.1[a]	1.07±0.3[b]	0.019	252
Testicular perimeter (cm)	4.07±0.4[a]	3.22±0.7[b]	0.001	5.10
Tubular diameter (μm)	176.7±33.3[a]	124.8±73.7[b]	0.0028	3.22
GSI	0.54±0.6[a]	0.28±0.1[b]	0.001	273

Different superscripts indicate significant differences between groups (P<0.05). W/T= value of the t-test or U-Mann Whitney test.

Table 4: Results of the semi-quantitative testis evaluation of treated and control chinchillas (n=36). Re-structure the table as indicated.

Score	Control Group (n=9)		Treated Group (n= 27)	
	Number	%	Number	%
0	0	0	17	62.9
1	1	11.1	7	25.9
2	1	11.1	3	11.1
3	7	77.7	0	0

origin to isocaproic acid and pregnenolone, playing an important role in the early stages of steroidogenesis. By blocking this pathway, Zeranol would decrease the production of testosterone, impeding normal gonadal development and function. Estrogenic endocrine disrupters are known to reduce reproductive success in laboratory animals and reduce variables such as weight of reproductive organs and sperm count among rodents (Warita *et al.,* 2010). The study of Figueroa *et al.* (2001) also adds important evidence of this estrogenic action of Zeranol when studying its effect on the uterine tissue in chinchillas, where intense vascular congestion of the endometrium, with changes in the histological organization of the uterus was evidenced. Studies in rats treated with Zeranol have also reported severely affected seminiferous tubules, with lumens deprived of spermatozoids and reduction in serum testosterone (Shidaifat *et al.,* 2013).

Conclusions: Our findings demonstrate that the use of Zeranol implants in Chinchillas results in similar changes at testicular level as those reported for other species. Zeranol is a potent destructor of the structural integrity of the testicle in Chinchillas.

REFERENCES

Badillo I, R Fernández and R Ulloa, 1999. Effect of zeranol over the maturation of the fur in *Chinchilla lanígera*. Vet Mex, 30: 63-66.

Campion S, F Carvallo, R Chapin, W Nowlan, D Beauchamp, R Jamon, R Koitz, T Winton, G Cappon and M Hurtt, 2013. Comparative assessment of the timing of sexual maturation in male Wistar Han and Sprague-Dawley rats. Reprod Toxicol, 38:16-24.

Figueroa S, R Fernández, S Anzaldua and M Pérez-Martínez, 2001. Histologic changes in the uterus of zearalenol subcutaneously implanted chinchillas (*Eryomis laniger)*. Vet Méx, 32: 7-12.

Leal MC and LR França, 2009. Slow increase of Sertoli cell efficiency and daily sperm production causes delayed establishment of full sexual maturity in the rodent *Chinchilla lanígera*. Theriogenology, 71: 509-518.

Sanchez-Toranzo G, A Torres-Luque, MC Gramajo-Büller and MI Büller, 2014. Histology of the ovary of *Chinchilla lanigera*in captivity. Anim Reprod Sci (In press). DOI: 10.1016/ j.anireprosci.2014.05.007.

Shidaifat F, S Kulp and Y Lin, 2013. Zeranol induces deleterious effects on the testes and the prostate gland of mature rats. Endocr Res, 38: 232-241.

Toso R, M Toribio, L Morini and H Magalhaes, 1999. Neonatal administration effects of zeranol on calve's testes and body weight. Ciencia Vet, 1: 15-19.

Warita K, T Mitsuhashi, T Sugawara, Y Tabuchi, T Tanida, Z Wang, Y Matsumoto, T Yokoyama, H Kitagawa, T Miki, Y Takeuchi and N Hoshi, 2010. Direct effects of diethylstilbestrol on the gene expression of the cholesterol side-chain cleavage enzyme (P450scc) in testicular Leydig cells. Life Sci, 87: 281-285.

Yang J, Y Zhang, Y Wang and S Cui, 2007. Toxic effects of zearalenone and α-zearalenol on the regulation of steroidogenesis and testosterone production in mouse Leydig cells. Toxicol in Vitro, 21: 558-565.