

## MORPHOLOGIC AND HISTOPATHOLOGIC CHANGES ASSOCIATED WITH NANDROLONE DECAONATE (NANDURABOLIN) ON THE SEMINAL VESICLE OF RATS

ZEINAB, S.DESOUKY<sup>1</sup>; SHAKKER, A.S.<sup>1</sup>; THABET IBRAHIM<sup>2</sup>; SALAH H. AFIFI<sup>3</sup> and WAFAA A. MUBARAK<sup>4</sup>

<sup>1</sup> Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, South Valley University, Egypt

<sup>2</sup> Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt

<sup>3</sup> Department of Pathology and Clinical Pathology, Assiut University, Assiut, Egypt

<sup>4</sup> Department of Anatomy, Faculty of Medicine, Assiut University, Assiut, Egypt

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### ABSTRACT

Twenty adult male rats three month old, weighing 80-190 gm were obtained from the Animal House Assiut University and divided into one control group and three treated groups (5 rats each group). Treated groups were injected intramuscular with Nandrolone Decaonate at doses of 2.5, 5, and 10mg/kg body weekly for three months. At the end of exp. the whole body weight and the seminal vesicle weight of both treated and control group were recorded. The relation wight of the seminal vesicle to the body weight was increased significantly in treated group compared to the control. The thickness of the smooth muscle fiber layer also was increased in treated groups compared to the control group. Histopathologically, shortening of the mucosal folds, abundant seminal secretions, fragmentation of the smooth muscle fiber layer and hemorrhage and hyperemia below the muscular layer were observed. Desquamation of the epithelium lining the folds of seminal vesicle was evident at a dose of 10mg/kg body weight of Nandrolone Decaonate. The study concluded that Nandrolone Decaonate induced alterations in the seminal vesicle.

**Key words:** Nandrolone Decaonate, seminal vesicle, intramuscular injection, histopathology.

### INTRODUCTION

The Anabolic-Androgenic Steroid (AASs) is a group of synthetic derivatives of testosterone with both skeletal muscle-building (anabolic) and masculinizing (androgenic) effects. Anabolic-Androgenic Steroids (AAS) are used in high doses by athletes to improve athletic ability, physical appearance, and muscle mass. The abuse of Anabolic Androgenic Steroids (AAS) is under constant debate world-wide. A large number of young adolescents abuse AAS to improve their physical fitness and appearance (Barceloux *et al.*, 2013). Soma *et al.* (2007) stated that anabolic steroids are used in equine medicine for building weight and muscle mass, and to alleviate anemia. These drugs used for growth in food producing animals like beef, cattle and sheep.

Most of the adverse effects following the use of AASs result from the enhancement of normal physiologic response to testosterone by

either direct receptor agonist activity or suppression of steroid biosynthesis. In general, toxic effects associated with AAS abuse involve the following: (1) anabolic side effects, (2) enhanced androgenic effects, (3) estrogenic side effects, (4) antiandrogenic effects from the suppression of the hypothalamus-pituitary-adrenal/ gonadal axes, (5) hepatotoxicity, and (6) neuropsychiatric effects (Buttner, and Thieme 2010).

Pathologic abnormalities from AAS abuse are best-documented in the cardiovascular system, reproductive system, liver, and serum lipids. Animals studies suggest that AAS can cause dysplasia of collagen fibrils and decreased tensile strength, and potentially the use of these drugs could cause disruption of connective tissue (Laseter and Russell, 1991).

Shokri *et al.* (2014) stated that the prostates and seminal vesicles weight were significantly different between control and group injected intramuscular with nandrolone at a dose of 10mg/kg bodyweight for 8 weeks (respectively,  $P < 0.05$  and  $P < 0.001$ ). The use of anabolic androgenic steroids in rats promotes structural changes in the prostate manifested by changes in

Corresponding author: Dr. SALAH H. AFIFI

E-mail address: [Afifi\\_s\\_4@hotmail.com](mailto:Afifi_s_4@hotmail.com).

Present address: Department of Pathology and Clinical Pathology, Assiut University, Assiut, Egypt

the weight, volume and epithelium height of the prostate ventral lobe and a predominance of collagen fibers in rats injected intramuscular with 10mg/kg-body weight using morphometric assessment. Ferrari *et al.* (2013) studied testis and seminal vesicle morpho-physiology of rats treated with nandrolone decanoate (injected with 0.5mg/kg body weight) and submitted to physical training in relation to the weight of the seminal vesicles. The anabolic caused significant increases in both sedentary and trained animals.

Little is known about the alterations might occur on the seminal vesicle due to nandrolone decaonate. The objective of the present study is to identify the effect of different doses of nandrolone decanoate (2.5, 5 and 10 mg/kg body weight) on the seminal vesicle morphologically and histopathologically.

## MATERIALS AND METHODS

Nandurabolin 25 mg mg/ml) as (Nandrolone Decanoate) was obtained from El-Nile Company for Pharmaceutical and Chemical Industries, Egypt.

### Animals

Twenty adult male Sprague-Dawley rats 3 month old, weighting 80-190gm were obtained from the Laboratory Animal House, Faculty of Medicine, Assiut University. The animals were housed in cages, fed a standard laboratory diet and water *ad libitum*.

### Experimental design

Rats were divided randomly into 4 groups each of 5 rats, one control (G1) and three treated groups (G2,G3,G4). The treated groups were injected intramuscularly with Nandrolone Decaonate at a dose level of 2.5, 5 and 10 mg/kg/weekly. The control group received the same volume of normal saline.

### Sampling

The whole body and seminal vesicle weights of both treated and control animal were taken and recorded. Specimen from the seminal vesicle was taken from each rat after three months post-injection and fixed in 10% formalin, dehydrated, embedded in paraffin, sectioned at 4-5 $\mu$ , stained with H&E and examined by light microscopy. The thickness of the smooth muscle fiber layer of the seminal vesicle was chosen for

morphometric measurements using Fiji Software. The data were expressed by the mean $\pm$  SEM.

### Statistical analyses

ANOVA test used for statistical comparison of data ( $b < 0.05$ ) was considered statistically significant. All statistical calculations were made with the SPSS computer program, version 16.0 (SPSS Inc., Chicago, IL, USA). Data are presented as the means  $\pm$  standard error (SE).

## RESULTS

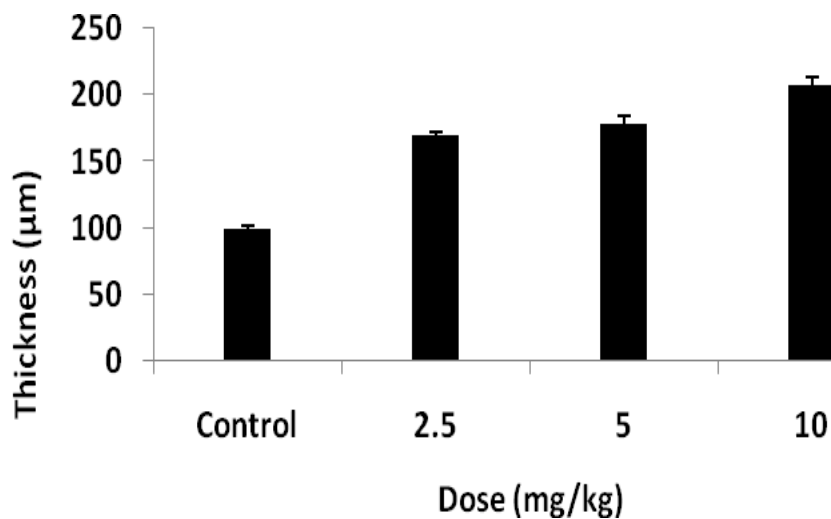
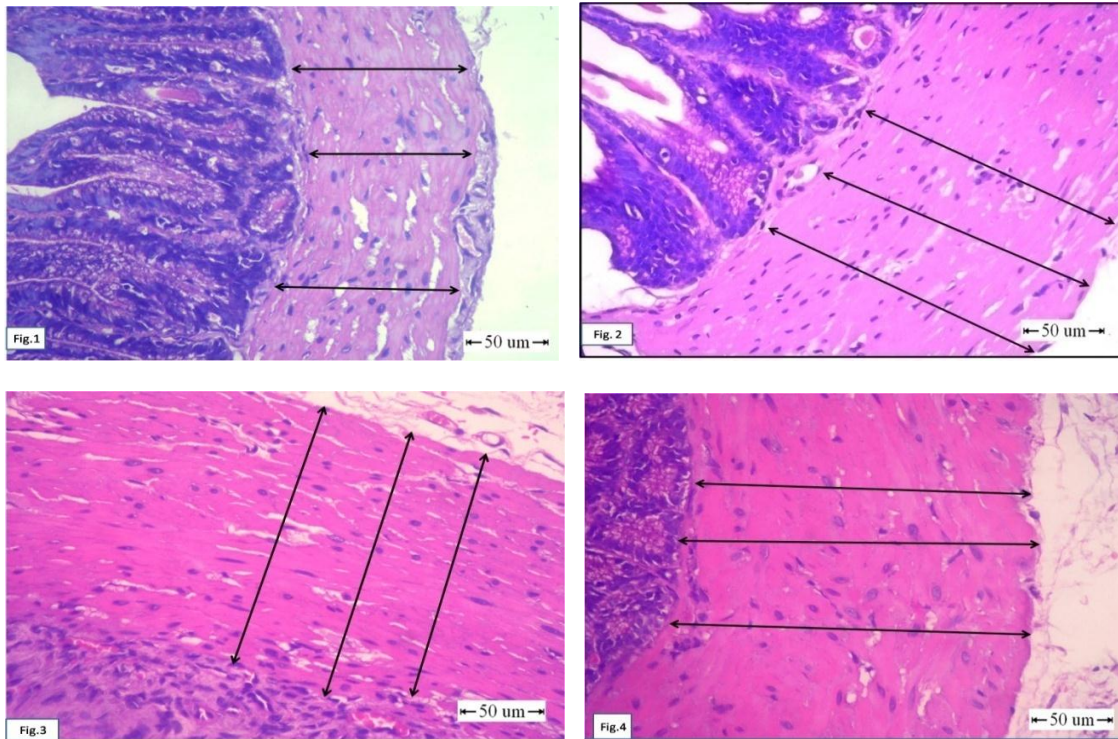
The seminal vesicle weight in relation to the body weight of rats injected with 2.5(Group1), 5(Group2), and 10mgkg/ body weight(Group3) and collected after three months post-injection was significant increased in weight compared to the control groups as shown in Table (1).

The thickness of the smooth muscle fiber layer was  $99.01 \pm 3.15 \mu\text{m}$  in the control group (Fig.1). While, rats injected with 2.5 mg/kg body weight was  $169.50 \pm 3.09 \mu\text{m}$  (Fig.2). In group 2 (5mg/kg body weight) the thickness was  $178.45 \pm 5.32 \mu\text{m}$ (Fig.3). Rats injected with 10mg/kg body weight showed thickness of  $207.56 \pm 5.31 \mu\text{m}$  as shown in Fig.4. Variation in the thickness among different groups and control was illustrated in Fig.5.

Histopathologically, the seminal vesicle from control group had the normal appearance and consisted of mucosal folds filled with red homogenous secretion and muscular layer made of smooth muscle fibers (Fig6). The seminal vesicle of rats injected with 2.5 mg/kg BW Nandrolone Decaonate and collected after three months post-injection showed shortening of the mucosal folds, abundant secretion and fragmentation of the smooth muscle fibers (Figs.7&8). The seminal vesicle from rats injected with 5 mg/kg BW Nandrolone Decaonate after three months post-injection had or also abundant secretion, shorten folds and fragmentation of smooth muscle fibers in addition to sub-muscular hemorrhage and hyperemia of blood vessel (Figs 9,10&11). Desquamation of the epithelium lining the mucosal folds was observed in the seminal vesicle of rats injected with 10 mg/kg BW Nandrolone Decaonate after three months post-injection (Fig.12).

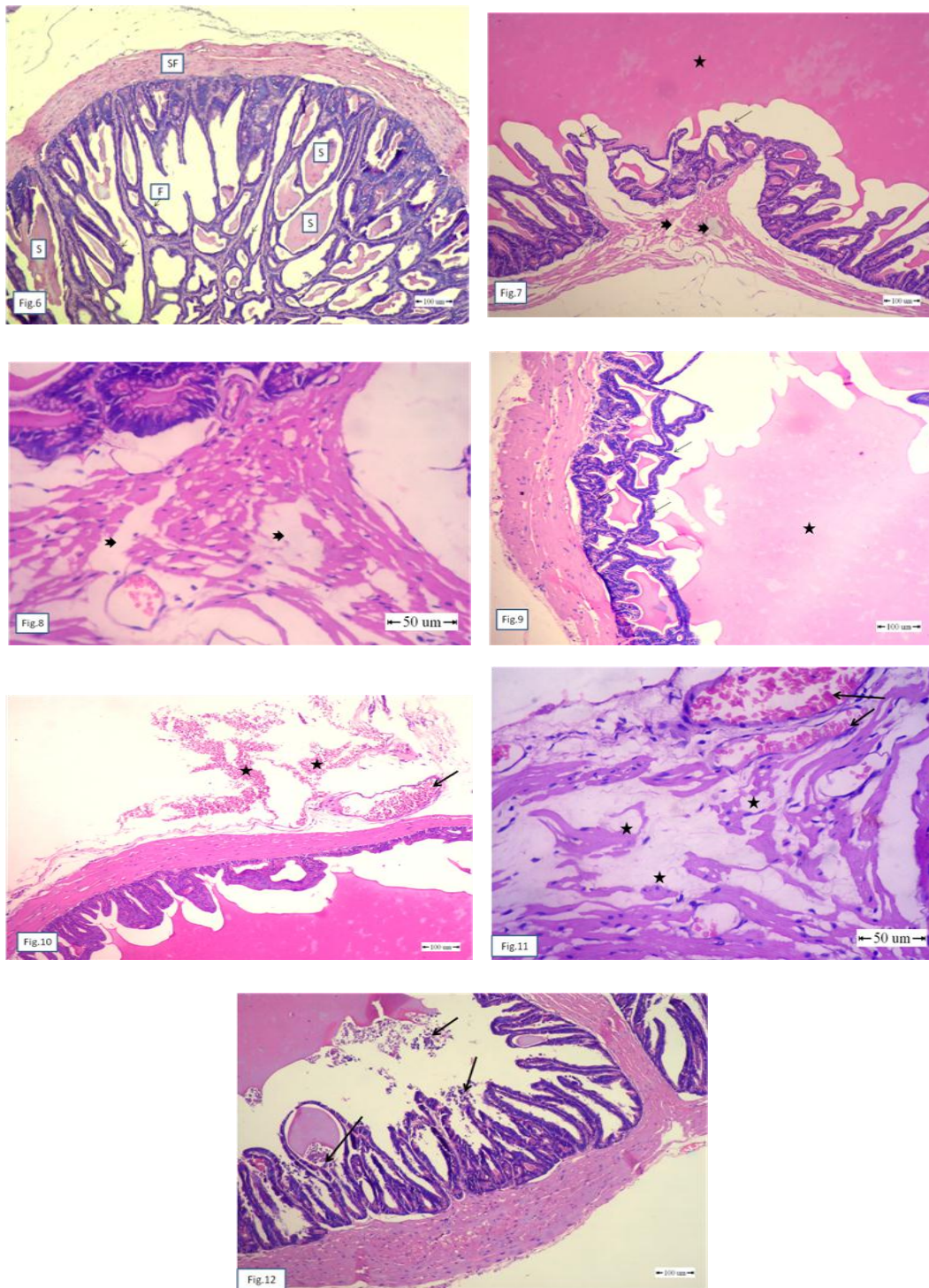
**Table 1:** The ratio ( $\pm$  SEM) of the seminal vesicle weight/gm in different doses of Nandrolene Decaonate in rats to the whole body weight.

	Control	Group1	Group2	Group3
Seminal vesicle weight(gm)	1.3 $\pm$ .061	2.2 $\pm$ .152	2.6 $\pm$ .096	2.2 $\pm$ .239
BODY WEIGHT (gm)	197.6 $\pm$ 2.088	188 $\pm$ 1.761	194.2 $\pm$ 11.074	200 $\pm$ 8.246
Relative seminal vesicle/Body weight ratio (%)	.68 $\pm$ .03	1.17 $\pm$ .08***a	1.38 $\pm$ .10***a	1.12 $\pm$ .13***a



**Fig. 5:** Variation in the thickness of the smooth muscle fibers of the seminal vesicle.





**Figure Legends**

- Fig.1:** seminal vesicle from rat of the control group showing the smooth muscle fibers (SMF) layer with a thickness of  $99.44 \pm 3.15\mu\text{m}$ . H&E. Bar=50um.
- Fig.2:** Seminal vesicle of rats IM injected with 2.5 mg/kg BW Nandrolene Decaonate weekly. Note increase in the thickness of SMF ( $169.50 \pm 3.09 \mu\text{m}$ , arrow) compared

- with the control and altered nuclei morphology H&E. Bar= 50um.
- Fig.3:** Seminal Vesicle of rats IM injected with 5 mg/kg BW Nandrolene Decaonate weekly showing thickness of SMF layer ( $178.45 \pm 5.32 \mu\text{m}$ (arrow). H&E. bar=100um.
- Fig.4:** Seminal Vesicle of rats IM injected with 10 mg/kg BW Nandrolene Decaonate weekly showing thickness of the SMF =  $207.56 \pm 5.31\mu\text{m}$ , arrow. H&E. bar=50um.

**Fig.5:** Variation in the thickness of the SMF of the seminal vesicle.

**Fig.6:** Control rat seminal vesicle IM injected with physiologic saline showing the normal appearance of seminal vesicle. Long folds of mucosa (F), SMF and homogenous red secretion (S).H&E. Bar=100um.

**Fig.7:** Seminal vesicle of rats IM injected with 2.5 mg/kg BW Nandrolene Decaonate weekly for three months showing abundant secretion (\*), shortening of folds (arrow) and fragmentation of SMF (➔) H&E. bar=100um.

**Fig.8:** Higher magnification of Fig.7 showing the SMF fragmentation (➔). H&E. bar=50um.

**Fig.9:** Seminal vesicle of rats IM injected with 5 mg/kg BW Nandrolene Decaonate weekly for three months showing abundant secretion(\*), shortening of folds (arrow) and fragmentation of SMF layer similar to 2.5mg/kg BW. H&E. bar=100um.

**Fig.10:** Seminal vesicle of rats IM injected with 5 mg/kg BW Nandrolene Decaonate weekly for three months showing submuscular hemorrhage (star) and hyperemia of blood vessel (arrow). H&E. bar=100um.

**Fig.11:** Seminal vesicle of rats IM injected with 5 mg/kg BW Nandrolene Decaonate weekly for three months showing pronounced fragmentation of the SMF (star) and hyperemia of blood vessel (arrow). H&E. bar=100um.

**Fig.12:** Seminal vesicle of rats IM injected with 10 mg/kg BW Nandrolene Decaonate weekly for three months showing pronounced desquamation of the epithelium lining the folds (arrows) H&E. bar=100um.

## DISCUSSION

In the present study, there was an increase in the body weight by three months post-injection. The increase in body weight was also associated with the seminal vesicle weight. The increase in the body weight in animals and human due to the use of Nandrolene Decaonate was also reported by many authors (Bhasin *et al.*, 1996, Beatriz *et al.*, 2000). Some authors however, believed that the gain in the body weight is apparently due to the muscular exercise rather than an anabolic effect (Schürmeyer *et al.*, 1984). Others reported that increase in body weight may be attributed to the accumulation of fluids and sodium in the body (Forbes, 1985). There is an acceptable consequence that supra-physiological doses of AASs can inhibit body growth and weight gain (Carson *et al.*, 2002).

The seminal vesicle weight in the present study had significant increase in the weight in all treated groups (2.5, 5 and 10mg/kg body weight) compared to the control group. These results were correlated to the increase in thickness of the smooth muscle fibers layer of the seminal vesicle ( $169.50 \pm 3.09$ ,  $178.45 \pm 5.32$  and  $207.56 \pm 5.31 \mu\text{m}$ ) in treated groups compared to the control group ( $99.01 \pm 3.15 \mu\text{m}$ ). These results may suggest that the increased weight and thickness are due to the direct effect of Nandrolene Decaonate. Ferrari *et al.* (2013) investigated the effect of intramuscular injections of Nandrolone Decanoate (0.5mg kg<sup>-1</sup> body weight) on male rats sedentary control, sedentary treated, trained control and trained treated for eight weeks. The study revealed that the weight of the seminal vesicles was significantly increased in both sedentary and trained animals and demonstrating the high sensitivity of the reproductive structure to Nandrolone Decanoate. Shokri *et al.* (2014) reported that the relative weights of the seminal vesicle in the Nandrolone Decanoate exercise groups significantly decreased compared with the control group.

Little is known about the effect of Nandrolone Decanoate on the histopathological alterations of the seminal vesicle. In the present study, there was an increase in the secretion of the seminal vesicle at the expense of the length of the mucosal folds at a dose of 2.5 and 5mg/kg body weight as well as fragmentation of the smooth muscle fibers. Vascular changes expressed by hyperemia of blood vessels at a dose of 5mg/kg bodyweight. Nandrolone Decanoate at 10mg/kg body weight induced sloughing of the epithelial lining the mucosal folds into its lumen. These results suggested that Nandrolone Decanoate induced both degenerative and vascular changes of the seminal vesicle.

In conclusion, the present study showed that Nandrolone Decanoate affects both the weight and morphology of the seminal vesicle and suggested that the seminal vesicle can be a sensitive organ to the anabolic steroids drug.

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### التغيرات المورفولوجية والنسجية المصاحبة لعقار الناندرولين ديكونات على الحويصلة المنوية للفئران

زينب دسوقي ، عبد اللطيف شاكر ، ثابت عبد المنعم ، صلاح عفيفي ، وفاء مبارك

Email: Afifi\_s\_4@hotmail.com Assiut University web-site: [www.aun.edu.eg](http://www.aun.edu.eg)

استخدم في هذه الدراسة (٢٠) عشرون فأر عمر ثلاثة أشهر وتتراوح اوزانهم ما بين ٨٠-١٢٠ جم قسمت هذه الفئران الى اربع مجاميع (٥ فئران لكل مجموعة). تم حقن الفئران بعقار الناندرولين ديكونات بتركيزات ٢.٥، ١٠، ٥٠ ملجم لكل كيلوجرام وزن اسبوعيا عن طريق العضل في الثلاث مجاميع المعالجة بالترتيب، أما في فئران مجموعة الضابطة فانه تم حقنها بمحلول الفسيولوجي المنظم عن طريق العضل وتم اخذ القياسات والعينات بعد ثلاث اشهر من بداية التجربة. كان هناك زيادة معنوية في الوزن النسبي للحويصلة المنوية بالنسبة الى الوزن الكلي في الفئران في المجاميع المعالجة بالعقار تتناسب طرديا مع الجرعات المستخدمة مقارنة بالمجموعه الضابطة. وايضا كان هناك زيادة في سمك طبقة العضلات للحويصلة المنوية في المجاميع المعالجة بالعقار مقارنة بالمجموعه الضابطة. اظهر الفحص الهستوباثولوجي تغيرات في الحويصلات المنوية للفئران تمثلت في زيادة افرازات الحويصلة وقصر حجم الثنايا المبطنه لطبقة الغشاء المخاطي ، كان هناك تكسير وعدم انتظام في الياف طبقة العضلات للحويصلة، تغيرات دموية تمثلت في احتقان الاوعية الدموية ونزيف تحت طبقة العضلات عند جرعات ٢.٥ ، ٥ ملجم. بينما أدت جرعة ١٠ ملجم الى تساقط الخلايا المبطنه لطبقة الغشاء المخاطي. أظهرت هذه الدراسة حدوث تغيرات مورفولوجية وباثولوجية للحويصلة المنوية للفئران نتيجة لاستخدام الناندرولين ديكونات وظهرت ايضا حساسية الحويصلات المنوية لهذا العقار.