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Original Article

Effects of Nandrolone Decanoate on Ultrastructure of Testis in Male Adult Rats

S.F. Mesbah, S. Shokri,
S. Karbalay-Doust, H. Mirkhani[1]

Abstract

Background: To improve the athletic ability and muscle mass, anabolic androgenic steroids are abused by athletes. Little is known about how these compounds affect the fine structures of the testis. This study aimed to identify the changes in the fine structure of testis following administration of nandrolone decanoate.

Methods: Twenty five Sprague-Dawley rats were randomly divided into two experimental, two vehicle, and one control groups. Experimental groups were treated by 3 or 10 mg/kg/wk intramuscular injection of nandrolone decanoate. The vehicle groups were treated by the same amount of peanut oil, for 14 weeks. One week after the last injection, the rats were sacrificed and their testes were prepared for transmission electron microscopy study.

Results: The cells in the interstitial space of the experimental rats were considerably degenerated. The basement membrane was thick and the diameter of seminiferous tubule was reduced. Several degenerated Sertoli cells and apoptotic germ cells were considerably observed in the experimental rats.

Conclusion: The results of this study show that fine structure of the testis is affected by nandrolone decanoate.
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Keywords ● Nandrolone decanoate ● ultrastructure ● testis ● rat

Introduction

Sport plays important roles in our lives and thus becomes a subject of various discussions.[1] Historically, athletes have used plants and natural and synthetic agents to increase their performance. The most commonly abused drugs are anabolic androgenic steroids (AASs), which are used by some athletes.[2] AASs are synthetic derivatives of testosterone, which are pharmacologically important in the treatment of growth deficiency, some blood disorders, and osteoprosis.[3,4] Many abusers believe that the side-effects of AASs are neither serious nor permanent; however, several studies have reported that AASs have negative health consequences including endocrine, hepatic, cardiovascular, and behavioral disturbances.[2,5] While many undesirable side-effects have been reported for the use of AASs, little is known about how AASs may affect the cells and tissues of the reproductive system.[4]

AAS compounds alter the function of the hypothalamic–pituitary–gonadal (HPG) axis, and as a result, affect the target

Departments of Anatomical Sciences,
[1]Pharmacology,
Shiraz University of Medical Sciences,
Shiraz, Iran.

Correspondence:
Seyed Fakhroddin Mesbah PhD,
Department of Anatomical Sciences,
Shiraz University of Medical Sciences,
Shiraz, Iran.
Tel/Fax: +98 711 2304372
Email: mesbahf@sums.ac.ir
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reproductive tissues. Under normal hormonal condition, luteinizing hormone (LH) is regulated by gonadotropin releasing hormone (GnRh) synthesized by the hypothalamus. LH interacts with receptors on the Leydig cells within the testis to produce testosterone. Testosterone is then transported to the target tissues such as testis, seminal vesicle, and prostate glands to regulate the growth and maintenance of these tissues. Testosterone also regulates the released GnRH by acting on the hypothalamus.[6] High level of exogenous androgen causes a decrease in LH release from the pituitary, resulting in suppression of endogenous testosterone production.[4]

Histopathological changes in reproductive organs of AASs abusers are equivocal. Some authors have reported that spermatogenesis still continues after AASs treatment,[7,8] whereas others showed that AASs decreased density, motility, and normal morphology of the sperm in the human.[9,10] Ludwig's findings represented that the spermatogenesis in rat continued normally following AASs treatment.[11] In contrast to this report, some investigations have indicated a severe depletion of Leydig cells in the interstitial tissue and also the development of the advanced steps of spermatids were arrested.[4,12] Based on the Takahashi's findings, severe pathological damage of the testis has been observed following AASs administration.[13] We have previously found that reduction of the testis volume and length of seminiferous tubule still remained 14 weeks after the last injection of high dose (10 mg/kg/wk) nandrolone decanoate.[14] Also, our findings showed that the weight of the testis and epididymis and sperm parameters decreased in rats following nandrolone decanoate treatment.[15]

To our knowledge, the effects of AASs on fine structure of the testis have not been described. The aim of the present study was to define the effects of nandrolone decanoate on the fine structure of the testis.

Materials and Methods

Twenty-five adult Sprague-Dawley male rats (weight = 180 – 210 g) were used and housed at 22-25 ºC (12-h light/dark cycle). The animals were equally randomized into two experimental, two vehicle, and one control groups. The experimental groups were treated by 3 and 10 mg/kg/wk intramuscular injection nandrolone decanoate, and the vehicle groups received the same volume of peanut oil for 14 weeks.[4,5] Control rats were housed under the same condition but did not receive any treatment. The animals had access to water and normal rat chow *ad libitum*. The volume of injection was 1 ml/200 g body weight. For descriptive purposes, the experimental groups were named "treatment low dose" (TLD) and "treatment high dose" (THD). The administered doses were selected based on the studies in which the effect of nandrolone decanoate on the muscle mass and physical performance of rats had been considered.[16-20] Moreover, we used sperm parameters to ensure adequacy of selected doses and bioavailability of nandrolone decanoate after intramuscular injections to rats.[15]

The rats were sacrificed one week after the last injection. The testes were removed and sliced into one cubic millimeter segments. The segments were fixed in 2.5% glutaraldehyde and post-fixed in 1% osmium tetroxide, dehydrated in graded series of ethanol, infiltrated with propylene oxide-resin and embedded in resin (agar 100). Following polymerization, five semi-thin sections of each animal were stained with 1% toluidine blue for light microscopy and ultrathin sections with uranyl acetate and lead citrate. The measurement of seminiferous tubules diameter was performed using ocular micrometer calibrated with micro stage and ultrastructural analysis under Philips CM10 transmission electron microscopy (TEM). One-way ANOVA followed by Duncan and LSD tests were used to compare the mean diameter of seminiferous tubules among the studied groups. A P value < 0.05 was considered statistically significant.

Results

Testicular interstitium

Light microscopy of the interstitial tissue in the control and vehicle rats showed normal appearance and contained Leydig cells, blood vessels, and fibroblasts. The Leydig cells had spherical and oval nuclei. In both TLD and THD groups less Leydig cells, blood vessels, and fibroblasts were observed and the interstitial spaces were wider.

Study of interstitium using electron microscope revealed a great abundance of lipid droplets in Leydig cells in the vehicle and less in control animals, as well as a great number of mitochondria with well-developed cristae. Tubular and vesicular smooth endoplasmic reticulum were distributed through cytoplasm in both the control and vehicle groups. Figure 1 shows the interstitium in control group. The size and number of Leydig cells were reduced in the treated animals. Contrary to control and vehicle animals, more pyknotic and apoptotic Leydig cells associated with vacuolated cytoplasm were seen in the treated animals especially in the THD group (figure 2).

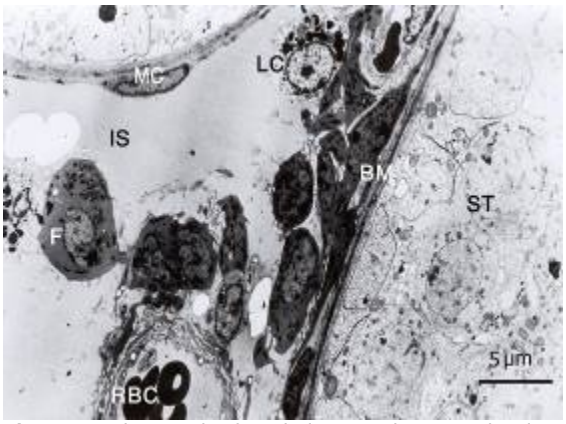

Figure 1: Micrograph of testis in control group, showing: interstitial space (IS), seminiferous tubule (ST), fibroblast (F), Leydig cell (LC), basement membrane (BM), myoid cell (MC), and red blood cell (RBC).

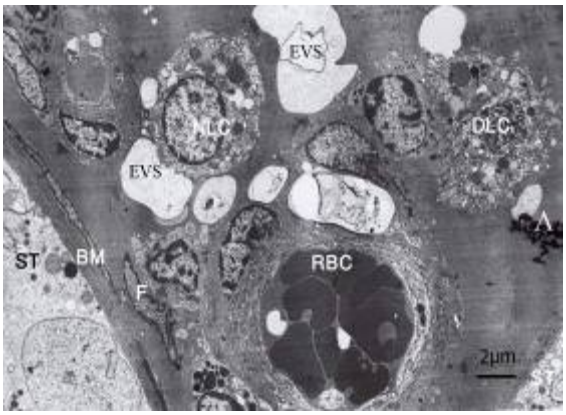

Figure 2: Micrograph of interstitial space of THD group, showing: degenerated Leydig cell (DLC), normal Leydig cell (NLC), seminiferous tubule (ST), basement membrane (BM), fibroblast (F), red blood cell (RBC), artifact (A), and empty vacuolar spaces(EVS).

Testicular epithelium

At the light microscopic level, the seminiferous tubules showed a normal arrangement and population of cellular components of germ cells and Sertoli cells in the control and vehicle animals (figure 3). Compared to the control and vehicle groups, the seminiferous epithelium of the treated animals was disrupted with broad spaces between the cellular components showing the presence of copious vacuoles frequently associated with degenerating germ cells (figure 4). The mean ± SD of seminiferous tubule diameter was 319.89±6.14 µm in the control, 319.82±6.14 µm in the vehicle, 285.70±4.26 µm in the TLD, and 285.71±5.56 µm in the THD rats (figure 5).

Ultrastructural analysis of the seminiferous epithelium revealed that the thickness of the basement membrane in THD and TLD rats were increased with irregular wavy multilaminar appearance, compared with the control and vehicle groups (figure 6). Collagen fibers were increased in both THD and TLD groups mostly in the vertical and oblique direction.

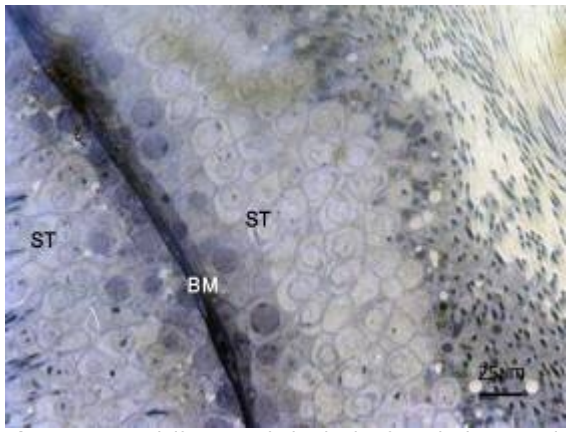

Figure 3: Seminiferous tubule (ST) of testis in control group at light microscope level, stained with toluidine blue. BM: basement membrane

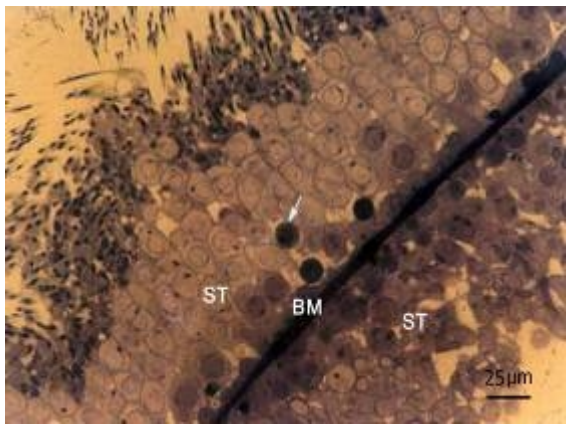

Figure 4: Two seminiferous tubules in TLD group at light microscope level, stained with toluidine blue. The arrow shows degenerated germ cells. BM: basement membrane

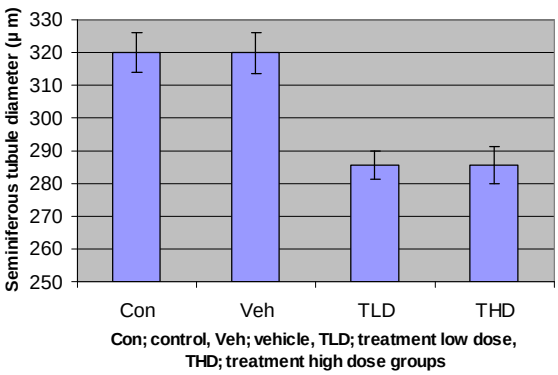

Figure 5: Seminiferous tubule diameter in various study groups (vehicle low dose not shown).

Well developed myoid cells covered the basement membrane of seminiferous tubule in all groups; however, pyknotic nucleus was observed in few myoid cells in the TLD rats.

Sertoli cells with triangular nucleus and apical invaginations were observed in all the animals, and basilateral invaginations in treated rats. In contrast to the control and vehicle animals, lysosome and phagolysosome and more abundant vacuoles were present in the cyto-

plasm of Sertoli cells in the treated groups (Figure 7). The mitochondria were distinct and possessed vesicular cristae. Fine structural features of seminiferous epithelium revealed no morphological alterations in spermatogonia, spermatocytes and spermatids in the control and vehicle animals. Also spermatogonia were in contact with basement membrane.

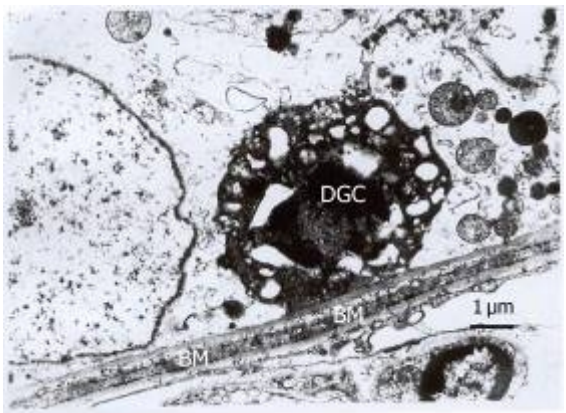

Figure 6: Micrograph of seminiferous tubule in THD group to show: basement membrane (BM) and degenerated germ cell (DGC).

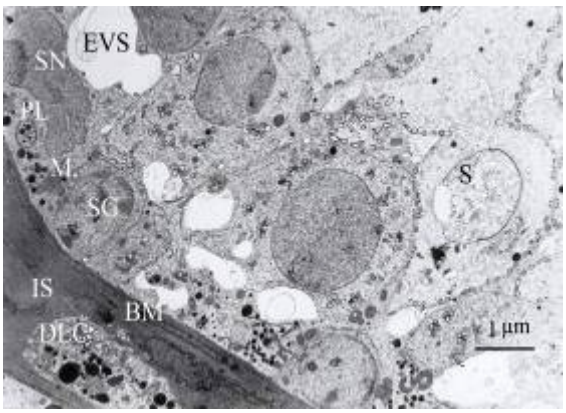

Figure 7: A micrograph in THD group to show: interstitial space (IS), degenerated Leydig cell (DLC), basement membrane (BM), spermatogonia (SG), empty vacuolar spaces (EVS), Sertoli cell nucleus(SN), phagolysosome (PL), lysosome (L), mitochondria (M), and spermatid (S).

Compared with control and vehicle animals, apoptotic and small flat spermatogonia were seen in the treated animals, especially in the THD group, in which nucleus had more prominent clumps of chromatin. The cytoplasm of spermatogonia contained numerous vacuoles, large lipid droplets and mitochondria with vesicular cristae (figures 6 & 7). In some treated animals in THD group, spermatocytes were close to the basement membrane and pyknotic spermatocytes associated with a great numbers of cytoplasmic vacuoles, lipid droplets and vacuolated mitochondria were observed. The spermatids contained more vesicles and vacuoles. The presence of detached and degenerated germ cells was identified and some of them were in close contact with the basement membrane. Residual body and fewer numbers of spermatozoa were seen in the lumen of both TLD and THD groups. Figure 7 shows a simiiniferous tubule and interstitial space in the THD group.

Discussion

The results of the present study show a remarkable decrease in the number and size of the Leydig cells and depletion of intact cells in treated, especially THD, animals. These results were consistent with Michele,[4] Ludwig,[11] and Grokett,[12] who found pyknotic and severe depletion of Leydig cells following treatment by AASs.

Close relationship between Leydig cells and blood vessels suggests that these cells are at high risk of exogenous toxicants and multivacuolated Leydig cells are probably a form of cell involution. Leydig cells are known to have receptors for LH that stimulates these cells to produce testosterone.[8] Both LH and testosterone are responsible for normal spermatogenesis in male rats.[21,22] Therefore, depletion of LH receptors and decrease in peripheral LH by exogenous testosterone administration result in the reduction of testosterone secretion.[6,8,23] Decreasing in testosterone level could be involved in the involution of seminiferous epithelium.[24]

The basement membrane plays an important role in maintaining the structural and functional integrity of tissues.[25] It provides structural stability of organs and sends signals to cells through cell surface receptors.[26] Altered basement membrane structure has been associated with severe functional impairment of the testis.[25] The basement membrane contains several proteins including laminin, type IV collagen, various heparin sulfate proteoglycans, and ectatin/nidogen.[27] Type IV collagen is a major constituent of mammalian basement membrane that has been localized in both the inner and the outer extracellular matrix (ECM) layers of the basement membrane of seminiferous tubules.[28] This collagen is secreted by myofibroblasts and Sertoli cells.[29,30]

In the present study, the increased thickness and irregular wavy multilaminar appearance of basement membrane in the treated, especially THD, animals are associated with significant decrease in the diameter of seminiferous tubules and well developed myoid cells. This is in accordance with other reports presented negative correlation between basement membrane thickness and seminiferous tubule diameter.[31,32] Mausle *et al*., have shown a considerable decrease in the mean diameter of seminiferous tubules of rat following oestradiol benzoate treatment.[33]

Some reports have demonstrated that exogenous stimulants, such as AASs, affect myoid cells to produce more type IV collagen, fibronectin, and extracellular matrix that are responsible for basal lamina thickness.[31,32,34]

Interactions between Sertoli cells, peritubular myoid cells, Leydig cells, and germ cells are thought to be essential for spermatogenesis. Each of these interactions must be communicated through the ECM of the basement membrane.[35]

Many reports have demonstrated that overexpression of the subtypes of type IV collagen correlates with abnormally thickened basement membrane and it is related to spermatogenic dysfunction in human and other mammals.[27,36,37] *In vitro* studies point toward a role for type IV collagen and laminins in spermatogenesis.[38,39]

Alternating with the spermatogonia are the highly polarized Sertoli cells, which act as nursery units for the developing sperm.[40] Sertoli cells foster the development and maintain the viability of germ cells by secreting hormonal and nutritive factors into a specialized compartment, formed by tight junctions between the adjacent Sertoli cells (blood-testis barrier), that surrounds the germ cells. Sertoli cells also form the sites of attachment to germ cells that serve both to maintain a close association between these two cell types and to provide physical support to the germ cells.[41] During spermatogenesis, spermatogonia differentiate into spermatocytes that cross through the blood-testis barrier as they mature and traverse the tubular lumen.[42,43]

Among the testicular epithelium observed, two major changes in the Sertoli cells and in their vicinity were noticed. One was the presence of pyknotic Sertoli cells associated with cytoplasm vacuolization, vesicular-like crista of the mitochondria, numerous lipid droplets and lysosome and phagolysosome in the THD rats. These are in agreement with the reports showing that exogenous stimulants may cause progressive apoptosis of the Sertoli cells, which affect spermatogenesis and sperm parameters.[9,44,45]

The second major change was the empty vacuolar spaces between Sertoli cells that are regarded to be the place where spermatogonia and spermatocytes should be located. In addition, the results of the present study showed that apoptosis occurred in all germline cells especially in spermatogonia and spermatocyte. The presence of apoptotic germ cells in this study is supported by Blanco's finding that has described apoptosis in hamster testis following treatment with AASs.[46] However, Michele has shown apoptosis only in the Leydig cells following testosterone propionate treatment in rats.[4]

Spermatogenesis is a complex and dynamic process that results in the continual production of spermatozoa in mammals. The Sertoli cells are largely responsible for orchestrating the germ cells through sequential phases of mitosis, meiosis, and differentiation. The Sertoli cells accomplish this task by providing hormonal, nutritional, and physical support. Apoptosis of germ cells that occurs in the testicular epithelium serves as a mechanism to reduce the germ cell population to the level that the Sertoli cells can support. Drugs such as AASs that injure or disrupt the function of Sertoli cells can effectively reduce their supportive roles and result in an increase in the elimination of the germ cell numbers via apoptosis[41]. Also it has been described that apoptosis in the germ cells is related to the Fas-signaling system that is activated by exogenous toxicants.[41]

Observation of detached germ cells, amorphous head sperm, and missed location of spermatid and spermatozoa that are closely related to the basement membrane in this study may be due to the rapid disruption of the Sertoli-germ cells interaction. This physical interaction ultimately leads to the sloughing of the germ cells from seminiferous epithelium.[41] It seems that the spermatogenesis cycle is reduced due to the presence of high level of androgens.

Conclusion

Administration of nandrolone decanoate exerts a clear effect on testicular structure including degenerated changes of germ cells, Sertoli cells, and Leydig cells. These are accompanied by changes in semen parameters and testis atrophy.[15] Our findings suggest major adverse effects on male reproductive organs of athletes and those who abuse AAS compounds.

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Conflict of Interest: None declared

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