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ORIGINAL ARTICLE

Comparative Survey of Testosterone Enanthate and Nandrolonedecanoate Administration on the Number and Function of Sperms in rat

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ABSTRACT
Anabolic-androgenic steroid compounds are one of the most widely abused drugs by athletes and muscle builders with the goal of improving performance/muscle mass. However, increasing concern has been expressed because these compounds not only offer unappreciable benefits to infertile and subfertile males, but also might have deleterious effects on both human and animal physiology including sperm quality. In this study, 21 male Wistar rats (220–250 g and 2-3 month age) were selected for the study and were purchased from Animal House, Islamic Azad University and randomly divided into 3 equal groups: group1; healthy control rats received standard diet without any drug; Group 2 received standard diet plus testosterone enanthate at a dose of 5mg/100gBW weekly for 8 weeks; Group 3, received standard diet plus nandrolonedecanoate at a dose of 10mg/kg weekly for 8 weeks. After 8 weeks, animals were anesthetized using ether and an incision was made on the epididymis and sperms was gathered then were counted using Neubauer'shemocytometer.Data showed that Testosterone enanthate and Nandrolonedecanoate decrease No. of sperms and Mobility but not viability.
Keywords:*Testosteroneenanthate, Nandrolonedecanoate, sperms quality, rats.*

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INTRODUCTION

Anabolic steroids, technically known as anabolic-androgenic steroids (AAS), are drugs that have similar effects to testosterone in the body. They increase protein within cells, especially in skeletal muscles. Anabolic steroids also have androgenic and virilizing properties, including the development and maintenance of masculinecharacteristics such as the growth of the vocal cords, testicles (primary sexual characteristics), and body hair (secondary sexual characteristics). Anabolic steroids were first made in the 1930s, and are now used therapeutically in medicine to stimulate bone growth and appetite, induce male puberty, and treat chronic wasting conditions, such as cancer and AIDS. The American College of Sports Medicine acknowledges that AAS, in the presence of adequate diet, can contribute to increases in body weight, often as lean mass increases, and that the gains in muscular strength achieved through high-intensity exercise and proper diet can be additionally increased by the use of AAS in some individuals.

Health risks can be produced by long-term use or excessive doses of anabolic steroids[1,2]. These effects include harmful changes in cholesterol levels (increased low-density lipoprotein and decreased high-density lipoprotein), acne, high blood pressure, liver damage (mainly with oral steroids), and dangerous changes in the structure of the left ventricle of the heart[3]. Conditions pertaining to hormonal imbalances such as gynecomastia and testicular atrophy may also be caused by anabolic steroids.

Ergogenic uses for anabolic steroids in sports, racing, and bodybuilding as performance-enhancing drugs are controversial because of their adverse effects and the potential to gain unfair advantage is considered cheating. Their use is referred to as doping and banned by all major sporting bodies. For many years, AAS have been by far the most detected doping substances in IOC-accredited laboratories[4]. In countries

where AAS are controlled substances, there is often a black market in which smuggled, clandestinely manufactured, or even counterfeit drugs are sold to users.

The pharmacodynamic of anabolic steroids are unlike peptide hormones. Water-soluble peptide hormones cannot penetrate the fatty cell membrane and only indirectly affect the nucleus of target cells through their interaction with the cell's surface receptors. However, as fat-soluble hormones, anabolic steroids are membrane-permeable and influence the nucleus of cells by direct action. The pharmacodynamic action of anabolic steroids begin when the exogenous hormone penetrates the membrane of the target cell and binds to an androgen receptor located in the cytoplasm of that cell. From there, the compound hormone-receptor diffuses into the nucleus, where it either alters the expression of genes[5]or activates processes that send signals to other parts of the cell[6]. Different types of anabolic steroids bind to the androgen receptor with different affinities, depending on their chemical structure[4]. Some anabolic steroids such as methandrostenolone bind weakly to this receptor in vitro, but still exhibit androgenic effects in vivo. The reason for this discrepancy is not known [7].

The effect of anabolic steroids on muscle mass is caused in at least two ways[8]:first, they increase the production of proteins; second, they reduce recovery time by blocking the effects of stress hormone cortisol on muscle tissue, so that catabolism of muscle is greatly reduced. It has been hypothesized that this reduction in muscle breakdown may occur through anabolic steroids inhibiting the action of other steroid hormones called glucocorticoids that promote the breakdown of muscles[9]. Anabolic steroids also affect the number of cells that develop into fat-storage cells, by favouringcellular differentiation into muscle cells instead[10]. Anabolic steroids can also decrease fat by increasing basal metabolic rate (BMR), since an increase in muscle mass increases BMR.The objective of present study was to comparative survey of Testosterone enanthate and Nandrolonedecanoate administration on the Number and Function of sperms in rats.

MATERIALS AND METHODS

Preparation the environment

Animal care and experiments confirmed with the Guide for the Care and Use of Laboratory Animals of China and approval of the ethics committee of Islamic Azad University was obtained before the commencement of the study. The animals were housed under standard environmental conditions (23±1°C, with 55±5% humidity and a 12 h light/12 h dark cycle) and maintained with free access to water and a standard laboratory diet *ad libitum*.

In this study, 21 male Wistar rats (220–250 g and 2-3 month age) were selected for the study and were purchased from Animal House, Islamic Azad University and randomly divided into 3 equal groups: group1; healthy control rats received standard diet without any drug; Group 2 received standard diet plus testosterone enanthate at a dose of 5mg/100gBW weekly for 8 weeks; Group 3, received standard diet plus nandrolonedecanoate at a dose of 10mg/kg weekly for 8 weeks.

After 8 weeks, animals were anesthetized using ether and an incision was made on the epididymis and sperms was gathered then were counted using Neubauer'shemocytometer.

Extracting the sperms

In this step, after epididymis was collected they were divided into the small parts then PBS was added onto them and was incubated at 37°C for 30 minutes. Spermatozoa were counted as per the method described in earlier studies [11,12]. Briefly, sperm suspension drops were placed on both sides of Neubauer'shemocytometer, allowed to settle by in a humid chamber (wet) for 1 h. The numbers of spermatozoa in the appropriate squares of the new improved neuberhaemocytometer were counted under the microscope at X100 magnification as previously described by Shittu[11,12].

Morphological assessment

For evaluation the sperms morphology, Eosin-nigrosin staining was carried out. In this method, the head of live and dead sperms appear white and red, respectively.

RESULTS

Data related to the number of sperms, mobility and viability is given in table 1.

Table 1: the comparison of Mean±SD in groups

Groups / Parameters	Group 1 (Control)	Group 2 (testosterone enanthate)	Group 3 (nandrolonedecanoate)
No. of sperms	14.37±3.6 [a]	9.3±3.2 [b]	10.2±4.1 [b]
Viability	62.93±2.04 [a]	54.76±4.3 [a]	57.13±3.8 [a]
Mobility	81.1±2.3 [a]	48.23±5.8 [b]	52.73±6.76 [b]

a,b; Dissimilar letters show significant difference in rows ($p<0.05$)

As shown in table, there is a significant difference among control and both treatment groups in term of number of sperms. Although there is a decrease in number of sperms in both treatment groups but there is no statistically difference among them.
Also, data showed that there is no significant difference among groups in term of sperms viability. At the end, the difference among control and both treatment groups was significant but it was not seen among treatment groups.

DISCUSSION AND CONCLUSION

We now know that combination of well-characterized animal models with stereological techniques always allow for proper quantitative study of any hormonal impact on male reproductive system [13,14]. In addition, rat appears to be a more suitable animal model in studying the roles of the androgenic hormones within the male reproductive system. Hence was used for this study because, it operates on a two-way androgen model (DHT and T) for its sexual differentiation. However, unlike mouse model that is dependent on testosterone action alone for the differentiation of its male urogenital tract [14,15].
Both sperm motility and percentage normal sperm morphology were significantly lowered in the treated groups compared to the control. These were similar to the findings in other studies where testosterone undecanoate and high dose of T-esters were used in rats and human models [16,17]. However, in another similar study, significant increase in sperm motility was observed without any significant increase in sperm morphology in proviron treated adult oligo-asthenozoospermic male human subjects [18].
The low level of testosterone found in the treated group in this present study must have been responsible for the low sperm density and motility obtained as reflected in Table. In addition, the significant reduction in sperm count in the treatment group was found to be well correlated to the significant reduction in spermatogonia count and decreased in spermatocyte count seen in testicular tissues histology.
Moreover, the above finding may be as a result of the reduction in proliferation of stem cells or spermiogenesis as large masses of seminiferous tubules epithelium appeared to be sloughing into the tubular lumen of the treated groups with associated evidence oftesticular atrophy. In addition, studies have shown that the administration of T-esters at high doses is associated with both morphological/structural and cytological changes in adult testes of rat and humans [19,20]. Such that, the degenerative features like increased luminal dilatation (up to 35%) and reduced epithelial height seen in other previous studies [14], were amongst the other factors responsible for the testicular atrophy.
Russell and co-workers had also speculated that both FSH and T might co-operate and thus have a common post receptor pathway of action[21]. Moreover, other studies have shown that both FSH and androgen act in a co-existing additive and synergistic manners in regulating spermatogenesis and Sertoli cell activity [22]. However, the Sertolicell still retains a significant capacity for activity that is independent of direct hormonal regulation [22].
Although, spermatogenesis in the adult male is a complex hormonal interplay that is FSH and androgen dependant[14]. However, ablation of either hormone has deleterious effects on sertoli cell functionand the progression of germ cells through spermatogenesis.Thus, a reduction of intratesticular androgen isan essential factorneeded for the inhibition of spermatogenesisas reflected with the low sperm quality associatedwith a significantly low T.
It is obvious that there is a complex hormonal interplay existing at the level of the hypothalamic testicular axis with negative feedback and rebound effects, which may be the cause of the significant low FSH. Moreover, we know that FSH has a role in facilitating the transport and localization of testosterone within Sertoli cells involved in spermatogenesis. The role of AAS in management of male infertility will need to be reviewed based on results of present study. In fact, AAS could probably serves as hormonal contraceptive in the light of these present findings.

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