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ORIGINAL ARTICLE

Studying the Effect of the Anabolic Androgen Methandrostenolone on Some Fertility Parameters in Local Male Rabbits

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ABSTRACT

The current study was conducted to investigate the effect of the anabolic androgen Methandrostenolone (Dianabol) on some parameters of fertility in male rabbits. In this experiment, (20) adult male rabbits were used, randomly divided into two groups, each group 10 animals. Control group: The animals were dosed with distilled water. The treatment group: The animals were orally dosed with (Dianabol) within a course that lasted (8) weeks in an ascending manner: (0.076, 0.152, 0.304, 0.38, 0.456, 0.532, 0.608, 0.684) mg/kg/d each dose for consecutive 7days. In the end of the experiment period the results showed a significant decrease (P≤ 0.05) in the ratio of testes and epididymis weight to the body weight of the animal in addition to tissue damage that included a significant decrease (P≤0.05) in the level of germinal epithelium height of the seminiferous tubules and a significant decrease (P≤0.05) in the thickness of the epididymis wall, also our results showed a significant decrease (P<0.05) in the total number of sperm, sperm motility and survival rate in the treatment group animals compared with control group animals.

We conclude from this study that anabolic androgen Methandrostenolone has a significant negative effect on fertility in male rabbits.

INTRODUCTION

Many young people are seeking to get the perfect body by using various drugs that help them get toned muscles **(1)** represented by anabolic steroids which also known as anabolic androgenic steroids (AAS), as manufactured substances that has an effect similar to the effect of testosterone in building muscles**(2)** Anabolic androgens were synthesized in the 1930s and used as a medical treatment in many conditions such as growth delay and bone marrow stimulation to treat anemias due to leukemia or kidney failure as well as to stimulate appetite and maintain muscle mass **(3)**. There are many types of anabolic androgens, the most prevalent and used by young people at the present time is Dianabol (methandrostenolone, methandienone) which is a derivative from testosterone and has been modified in a way that reduces its male characteristics but maintains its anabolic properties. It was first described as an activator in 1955 **(4)** and was introduced in the market of prescription drugs in the United States in 1958 under the trade name (Dianabol) and it was classified as an anabolic steroid used as a drug, then Dr. Ziegler and Ciba developed the drug **(5)**. Therefore, the main objective of this study was to find out the effect of this anabolic androgen on Fertility of young adults.

Experimental animals: In this experiment, 20 healthy adult male rabbits were used, their weights ranged between (1700-2000) grams and ages ranged between (15-18) months. The animals were placed in a room provided with a large opposite air window to ensure good ventilation. The experimental animals randomly placed in iron cages with dimensions of 90 cm x 65 cm x 60 cm. Tow cages for each group in each cage five animals only. The cages were placed in a room with a temperature of (23-27) C. The animals were left for two weeks before the start of the experiment for the purpose of Adaptation to the place and the nature of the food.

Dianabol Anabolic Androgen: The anabolic androgen tabs were purchased from a bodybuilding gym and It was crushed into a fine powder to prepare a suspension that facilitates the animals orally administration.

Experience design: The study included 20 male rabbits distributed randomly into two equal groups (each group has 10 animals) treated as follows:

The first group (C): represents the control group: Animals dosed with distilled water for the experimental period, which is (8) weeks.

Treatment group (T): In this group, the animals dosed with the anabolic androgen Dianabol by following the weekly ascending as follows: -

- Animals dosed Dianabol at a concentration of 0.076 mg/kg/d for 7 consecutive days.
- Animals dosed Dianabol at a concentration of 0.152 mg/kg/d for 7 consecutive days.
- Animals dosed Dianabol at a concentration of 0.304 mg/kg/d for 7 consecutive days - Animals dosed Dianabol at a concentration of 0.304 mg/kg/d for 7 consecutive days
- Animals dosed Dianabol at a concentration of 0.456 mg/kg/d for 7 consecutive days
- Animals dosed Dianabol at a concentration of 0.532 mg/kg/d for 7 consecutive days
- Animals dosed Dianabol at a concentration of 0.608 mg/kg/d for 7 consecutive days
- Animals dosed Dianabol at a concentration of 0.684 mg/kg/d for 7 consecutive days

Animal Sacrifice: After 24 hours from the last dose process, the animals were anesthetized by exposing them to inhalation of a quantity of chloroform and then the abdominal cavity was opened to remove the genitals (testes and epididymis) and isolate them separately.

Weight ratios and histological sections of the testes and epididymis: Histological sections of the testes and tail of the epididymis preserved in 10% formalin were prepared for the two groups, method of **(6)** was followed in preparing the histological sections.

Fertility parameters: The left caudal epididymis was isolated for sperm extraction by immersing them in 1 ml of warm physiological saline in a clean bottle. Using a sharp blade, it was cut into small pieces to prepare the sperm mixture. Then the following fertility tests were performed:

Sperms Mortality and Motility: To distinguish live sperm from dead sperm, a drop of the sperm mixture was taken and placed on a warm glass slide near one end. A drop of dye (eosin-necrosin) was added to it, after mixing for (30 seconds), a smear was prepared and left to dry in the air (7). Sperm motility, the mixture of sperms (from the caudal epididymis) was stirred well, then one drop was taken from it to placed on a dry and warm glass slide, covered it with the cover of the slide, then placed under the microscope to count at least 200 sperm along the slide using the magnification power 40x (8). After completing the sperm motility count the prepared smear is prepared. It was examined at (40x) power, then at least 200 sperms were counted along the smear and the percentage of live and dead sperms was calculated **(7)**.

Total number of sperm: By using a Pasteur pipette, a drop of the sperm mixture is taken after being stirred and placed on a glass slide (dry and warm) and then covered with a slide cover to count the number of sperm in ten selected zigzag microscopic fields at the power of (40x). For sperm calculated in the multiplier factor (1 million) **(7)**.

Statistical analysis: After data collection and classification, statistical analysis software SPSS V.25 was used. Where the data were statistically analyzed according to one-way ANOVA and the averages of the experimental groups were compared using the LSD test at the level of significance of 0.05 **(9)**.

RESULTS

Weight ratios and tissue section: Table (1) shows that there was a significant decrease (P≤0.5) in testes weight ratios of the animals that were given doses of anabolic androgen compared with the control group. As for the weight of the epididymis, the treated group recorded a significant decrease (P≤0.5) in the percentage of weight of the epididymis compared with the percentage of its weight in the control group. There was a significant decrease (P≤0.5) in seminal tubular endothelial elevation in the testes of the animal group that took doses of anabolic androgen compared to the control group. The results also showed a significant decrease (P≤0.5) in the wall thickness of the epididymal tubules of the animals that took doses of the anabolic androgen (Dianabol) compared to the control group.

Sperm parameters: Table (2) shows that animal treated with Dianabol had a clear effect in reducing sperm motility and survival rate in the treated group animals, significantly (P≤0.5) compared to the control group animals. Table (2) also showed a significant decrease (P≤0.5) in the total sperm count in the anabolic androgen-treated group, in ascending order, compared to the normal total sperm count in the control group.

Table 1: Effect of Dianabol on (testes and epididymis weight ratios epididymal wall thickness testicular lining height)

Standards The group	Testicular weight ratio (g)	Epididymis weight ratio (g)	Epididymal wall	thickness Lining height
C	1.96±0.17 a	0.428±0.02 a	9.83±0.10 a	13.53±0.22 a
T	0.78±0.15 b	0.1320±.01 b	5.49±0.30 b	7.43±0.34 b
LSD	0.361	0.0356	0.724	0.636

The values are mean ± standard error.
Different letters within the same column indicate significant differences (0.5 ≥p) between the two groups.

Table 2: Effect Dianabol on fertility parameters

Standards The group	Sperm count /million	Sperm movement %	Living %
C	9544000.±728934.83 a	86.40±0.97 a	88.80±0.73 a
T	3030000±214662.52 b	23.00±2.15 b	51.50±3.50 b
LSD	1224220	4.11	6.54

The values are mean ± standard error.
Different letters within the same column indicate significant differences (0.5 ≥p) between the two groups.

DISCUSSION

Weight ratio and tissue sections: current study showed that the testicles of animals of the group treated with the androgen Dianabol significantly decreased in weight and size compared to the animals of the control group. This negative change is maybe due to the androgen effect, as Dianabol can cause changes that affect the weight and size of the testicles, including the length and diameter of the seminiferous tubules, making them shorter in length and narrower in diameter, which in turn makes the size of the testicles smaller and lighter **(10).** This androgen may also affect the germinal epithelium lining the seminiferous tubules, causing a decrease its height affecting volume and weight as well as tissue aberrations that Dianabol may cause in testicular tissue and its contribution to preventing Leydig cell proliferation therefore, these anatomical and tissue damages can make the testicles smaller compared to their normal size and less weight, causing testicular atrophy **(11).**

The atrophy of the epididymis can also be explained by the cessation of its growth, as the decrease in the natural testosterone hormone inside the body as a result of taking this androgen will negatively affect the growth and atrophy of the epididymis significantly, as well as tissue damage that may occur to it as a result of taking Dianabol, as it can cause tissue changes that may be Cause of atrophy of the epididymis, such as necrotic degenerations and stenosis of the epididymal lining in the epididymis that's affecting its structure and weight **(12).**

The height of the lining of the seminiferous tubule and the thickness of the wall of the epididymal tubule: The results of the study showed significant decrease in the elevation of the lining of the seminiferous tubules in the testes of the treatment group animals compared to the control group, and this change can be attributed to the effect of the dianabol, The decrease in natural testosterone secretion by dianabol lead to thinner lining of the seminal tubule in addition this androgen affecting the basement membrane of the seminal tubules which may contribute to the decrease in the seminiferous epithelium height **(13).** Dianabol has negative effects on spermatogonia include lack mitochondria, decrease in the amount of cytoplasm and filling with vacuoles with lipid droplets causing its die and reducing their numbers leading to decrease the height of the seminal lining **(14).**

This androgen can cause Sertoli cells to gradually lose their cytoplasm, causing programmed death and decrease their numbers in the epithelium of the tubule, which in turn significantly reduce the numbers of germ cells because of the lack of nutrients needed for cell division this leads to a decrease in the thickness of the epithelium **(15).**

Thickness of the epididymis tubules walls, according to our histological study severed significant decrease in its thickness in the treatment group compared to control animals, That may be due to dianabol, because it negatively affects the interstitial tissues in the wall causing damage, and this leads to a decrease in the thickness of the wall of the epididymal tubule **(16)**. It can also cause degeneration and necrosis in the tubule wall cells leading to severe wall damage and decrease in its thickness than it was before the dose **(17)** .

Sperms number - motility – mortality: Our results showed a significant decrease in the total number of sperms for rabbits that were treated with anabolic androgen, To achieve the process of sperm production the natural testosterone concentration inside the testicles must be about 40 times higher than its concentration in the serum but when you take dianabol the level of synthetic testosterone increases inside the body, which negatively affects the secretion of natural testosterone from the Leydig cells inside the testicle **(18)** . That Leading to a significantly lower level than it was before the arrival of exogenous testosterone, and as a result, this may lead to inhibition of sperm production and a severe decrease in sperm count **(19)** .

Also, Dianabol can inhibit the secretion of the gonadotropin-stimulating hormones, (LH) and (FSH) from the pituitary gland **(16)** . This is done by affecting Hypothalamic-Pituitary-Gonadal (HPG) axis and inhibition the secretion of Gonadotropin Releasing Hormone (Gn-RH) which is responsible for the production of sperm **(11)** . The action of the hormone LH is to stimulate the Leydig cells to produce testosterone by contributing to the conversion of Acetate to Sequalen, which is the basis for the formation of Cholesterol. Also, the low level of FSH impedes the transformation of spermatids into mature sperm because it is the hormone responsible for stimulating Sertoli cells to produce spermatoblasts nutrients and thus the number of sperms can decrease **(20).**

Also, taking anabolic androgens may lead to a programmed death of spermatogonia cells and consequently lack of sperm formation, causing a decrease in their total number **(21) (22).**

Sperm motility is a prerequisite for ensuring male fertility and directly related to its active metabolism **(23).** Dianabol decrease the level of the FSH hormone by its effect on the hypothalamic-pituitary axis and that inhibit the Adenyl cyclase enzyme, which is responsible for stimulating Sertoli cells to secrete transferrin and

other proteins important in transporting nutrients necessary for sperm development and providing energy for sperm movement **(19).** This may explain the results of our current study, which showed a significant and clear decrease in sperm motility in animals that dosed with Dianabol, considering that this dosing process is the only difference from control animals.

Our study also showed that the mortality of sperm in the treated animals decreased significantly, the cause of sperm death may be due to the effect of the androgen Dianabol on the genetic expression of sperm, as this androgen causes a chromosomal imbalance (chromosomal aberration) that appears during the process of filling the genetic material in the sperm head, which leads to deformations in the head, including swelling of the head, loss of the apical hook, imbalance of the head and becomes in many forms, including hammer head, ribbon shape, pin shape, irregular head shape and other forms and these abnormalities may develop to loss of the head causing death of sperm **(15)**, Sperm death can be attributed to a genetic point mutation in the testicular DNA due to the effect of anabolic androgen, which may lead to sperm cell damage **(20).**

REFERENCES

1- Sagoe, D., Andreassen, C. S., & Pallesen, S. (2014). The aetiology and trajectory of anabolic-androgenic steroid use initiation: a systematic review and synthesis of qualitative research. Substance abuse treatment, prevention, and policy, 9(1), 1-14.

2- Roman, M., Roman, D. L., Ostafe, V., Ciorsac, A., & Isvoran, A. .(2018). Computational assessment of pharmacokinetics and biological effects of some anabolic and androgen steroids. Pharmaceutical Research, 35(2), 1-25.

3- Patanè, F. G., Liberto, A., Maria Maglitto, A. N., Malandrino, P., Esposito, M., Amico, F., Cocimano, G., Rosi, G. L., Condorelli, D., & Nunno, N. D. (2020). Nandrolone decanoate: use, abuse and side effects. Medicina, 56(11), 606.

4- Joukar, S., Vahidi, R., Farsinejad, A., Asadi-Shekaari, M., & Shahouzehi, B. (2017). Ameliorative effects of endurance exercise with two different intensities on nandrolone decanoate-induced neurodegeneration in rats: Involving redox and apoptotic systems. Neurotoxicity Research, 32(1), 41-49.

5- Karimooy, F. N., Bideskan, A. E., Pour, A. M., & Hoseini, S. M. (2019). Neurotoxic effects of stanozolol on male rats 'hippocampi: does stanozolol cause apoptosis? Biomolecular Concepts, 10(1), 73-8.

6- Luna, L. G. (1968). Manual of histologic staining methods of the Armed Forces Institute of Pathology.

7- Hinting, A. (1989). Methods of semen analysis in: assessment of human sperm fertilizing ability. Ph. D. thesis by Hinting, A., University of Michigan state,

8- Levine, R. J., Brown, M. H., Bell, M., Shue, F., Greenberg, G. N., Bordson, B. L. J. F., & sterility. (1992). Air-conditioned environments do not prevent deterioration of human semen quality during the summer. 57(5), 1075-1083..

9- Daniel, W. W., & Cross, C. L. (2018). Biostatistics: a foundation for analysis in the health sciences: Wiley.

10- Rolf, C., Nieschlag, E. J. B. s. c. e., & metabolism. (1998). Potential adverse effects of long-term testosterone therapy. 12(3), 521-534.

11- Baharan, O. (2018). Protective effects of curcumin on the structural parameters of seminiferous tubules and leydig cells in methandienone treated mice. Research Journal of Pharmacognosy, 5(4), 33-41.

12- Morewood, T. (2013). Fertility preservation in male cancer patients. In: Taylor & Francis

13- Falih, M. Y., Mohammed, A. A., & Sulaiman, G. M. J. B. B. R. A. (2018). The Morphological Effects of Methandienone on Sperm Head in Male Mice. 15(3), 643-648.

14- Albano, G., Amico, F., Cocimano, G., Liberto, A., Maglietta, F., Esposito, M., . . . Montana, A. (2021). Adverse Effects of Anabolic-Androgenic Steroids: A Literature Review. Healthcare 2021, 9, 97. In: s Note: MDPI stays neutral with regard to jurisdictional claims in published …

15- Reitzner, S. M., Hengevoss, J., Isenmann, E., Diel, P. J. T. J. o. S. B., & Biology, M. (2019). Modulation of exercise training related adaptation of body composition and regulatory pathways by anabolic steroids. 190, 44-53.

16- Zabiba, I. M. J. J. A.-Q. J. o. V. M. S. (2017). Effect of long-term administration of Deca-Durabolin on reproductive organs of male rats. 16(2), 120-125.

17- Jassim, A. M., Alzamely, H. A., Hamad, A. G. J. I. J. o. A., & Science, V. (2015). Study of the testicular damage induced by dianabol and its effect on morphological and histological changes in albino male rats. 8(8), 24-32.

18- Al-Janabi, A. (2017). The cytogenetic effect of Euphorbia tirucallistems methanolic extract on sperm head morphology in male albino mice. In: licensee Herbert Publications HOAJ Biology.

19- Linhares, B. L., Miranda, E. P., Cintra, A. R., Reges, R., & Torres, L. O. J. S. m. r. (2021). Use, Misuse and Abuse of Testosterone and Other Androgens

20- Hallak, J., Teixeira, T. A., & Souza, G. L. d. (2020). Effect of exogenous medications and anabolic steroids on male reproductive and sexual health. In Male Infertility (pp. 455-468): Springer.

21- Christou, M. A., Christou, P. A., Markozannes, G., Tsatsoulis, A., Mastorakos, G., & Tigas, S. J. S. M. (2017). Effects of anabolic androgenic steroids on the reproductive system of athletes and recreational users: a systematic review and meta-analysis. 47(9), 1869-1883.

22- Kanayama, G., Kaufman, M. J., Pope Jr, H. G. J. C. o. i. e., diabetes,, & obesity. (2018). Public health impact of androgens. 25(3), 218.

23- El Osta, R., Almont, T., Diligent, C., Hubert, N., Eschwège, P., Hubert, J. J. B., & andrology, c. (2016). Anabolic steroids abuse and male infertility. 26(1), 1-8.