https://doi.org/10.15218/zjms.2017.021

Nandrolone effects on men's semen parameters in Erbil city

Received: 30/11/2015 Accepted: 19/6/2016

Abdalmuhaimn Y. Sharef * Govand Sh. Tawfeeq * Asos M. Rasoul **

Abstract

Background and objective: Anabolic androgenic steroids are synthetic compounds based on the structure of testosterone, and are used to treat various conditions such as reproductive system dysfunction. High doses of anabolic androgenic steroids and exercise influence the hypothalamic pituitary gonadal axis, which can, in turn, affect testicular apoptosis. This study aimed to investigate the influence of anabolic androgenic steroids on semen parameters in bodybuilders (heavy exercise) in Erbil city.

Methods: Semen specimens and serum were collected from 150 which divided into three groups; each consists of 50 men. The control group (A) didn't practice exercise so didn't receive nandrolone. The exercise group (B) who practice daily without taking nandrolone. The exercise and treated group (C) who practice exercise and had been using nandrolone (200 mg- wk^{-1}, intramuscularly) for at least three months. Smear prepared by methyline blue stain and assessment of semen volume, sperm morphology, sperm concentration ,motility were carried out .Serum levels of follicle-stimulating hormone, luteinizing hormone, and testosterone were also carried out.

Results: There was no difference in the semen volume within three groups. Sperm concentration and the percentage of sperm motility in the group C was significantly lower (P <0.001) than that in the other groups. A significantly increased percentage of sperm with the tapered head was found in the group C. Our results also demonstrated a significant decrease in testosterone and follicle-stimulating hormone in the group C compared to group A and B.

Conclusion: Users of anabolic-androgenic steroids have sperm with abnormal shape, especially tapered head, and low concentration of sperm with sluggish motility attributing to infertility.

Keywords: Nandrolone; Men; Semen parameters; Abnormal shape; Infertility

Introduction

The anabolic-androgenic steroids (AAS) are a family of hormones that includes the natural male hormone testosterone, together with its many synthetic relatives, all of which exhibit both *anabolic* ("muscle building") and *androgenic* ("masculinizing") properties.[1,2] Reports indicate that Anabolic androgenic steroids abuse impacts upon several hormone systems like the hypothalamic-pituitary-adrenal (HPA), hypothalamic pituitary- thyroid (HPT) and hypothalamic-pituitary gonadal (HPG) axes.[3] The administration of high doses of exogenous androgens in men has been reported to result in decreased levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) through negative impact on the hypothalamic-pituitary-gonadal (HPG) axis with reduced endogenous testosterone production leading to decreased spermatogenesis, reduced semen density, testicular atrophy and abnormal sperm morphology attributing to infertility.[4] Some authors have reported that AAS decreased density, motility and normal morphology of sperm,[5] even low doses of AASs decrease sperm

* Department of Clinical Analysis, College of Pharmacy, Hawler Medical University, Erbil, Iraq.
** Department of Pharmaceutics, College of Pharmacy, Hawler Medical University, Erbil, Iraq.

https://doi.org/10.15218/zjms.2017.021

quality and quantity.[6] In addition, numerous studies have shown that severe depletion of Leydig cells following treatment by AAS,[7] Leydig cells are known to have receptors for LH that stimulates these cells to produce testosterone.[8] On the other hand, exercise training frequently results in a decrease of serum testosterone, and may rarely be associated with reduced libido, sperm production, and fertility.[9] It appears, however, that a high volume of endurance running was associated with subclinical alterations in both the profile of sex hormones (decreased levels of total and free testosterone) and the quality of semen (particularly decreased motility and increased number of in mature cells).[10] It has been reported that both hypothalamic and testicular endocrine functions are suppressed during acute prolonged physical exercise. The exercise-induced suppression of serum testosterone is associated with suppressed endogenous GnRH stimulation of gonadotropin release during exercise.[11] Qualitatively and quantitatively normal spermatogenesis is critically dependent on an intact hypothalamus–pituitary–testis (HPT) axis.[12] Anabolic-androgenic steroids seem to be appealing to abusers because when taken in conjunction with exercise, they increase muscle mass and strength, and increase libido.[13] The combination of exercise training and nandrolone decanoate treatment produce a decrease in testicular somatic index and relative wet weights of accessory sex glands which also support the inhibition in testicular steroid genesis.[14] A number of abnormalities in the architecture of the seminiferous tubules of treated rats with AAS namely atrophy in the seminiferous tubules, abnormal organization of the germinal epithelium, and maturation arrest.[15] Sertoli cells support a finite number of germ cells, and thus their number determines the spermatogenic capacity of the adult.[16] It is indicated an AAS-induced reduction in Sertoli cell number which might be due to a structural response of Sertoli cells to the deprivation of testosterone.[17] The reduction in Sertoli cell number in treated rats could have resulted in a subsequent reduction in the number of spermatogonia leading eventually to a decrease in sperm count.[15] In this study, the effects of exercise-induced nandrolone on the gonadotropin releasing hormones levels from the pituitary gland (FSH and LH) that have growth promoting effects on testis development and the standard semen parameters is being tested. The second part of the study focused on assessing the effect of exercise alone, on the above named markers. There has not been any previous study regarding this topic in Erbil City. Therefore, this study aimed to determine the effect of nandrolone on sperm concentration, sperm motility percentage, sperm morphology of bodybuilders in Erbil City, and to improve the health (fertility) of our young bodybuilder for future planning.

Methods

Sample collection

This study was conducted in Erbil city during the period between Feb 2014 to June 2015 on 150 men aged between 20-40 years. Semen specimens and serum were collected from three groups. Group A, as a control group (n=50), included healthy volunteers, i.e. not having any disease or condition that affect the fertility, no previous past medical history, nonsmoking and normozoospermia. Group B, as an exercise group, included 50 men who practiced exercise and didn't receive any steroids or other drugs. Group (C): as an exercise and treated group included 50 bodybuilders who practiced exercise and received a weekly intramuscular injection containing 200 mg nandrolone for at least three months. Both groups B and C were selected from many fitness centers in Erbil city, Kurdistan region, Iraq. Men with systemic and endocrine diseases, male accessory gland infection, varicocele, microrchidism, cigarette smoke, alcohol or drug abuse were excluded. Seminal fluid

https://doi.org/10.15218/zjms.2017.021

and serum were collected from each group. Serum levels of reproductive hormones (FSH, LH and testosterone) were measured. Both samples were immediately brought to the laboratory in Rizgary Teaching Hospital, Erbil city, Kurdistan region.

Smears of semen and staining procedure

All semen samples were processed for staining (Berg's method for spermatozoa) within two hours after collection and after liquefaction. The following seminal characteristics were considered: sperm concentration, total progressive motile sperm count, and sperm morphology. All results were compared and contrasted with published normal values by World health organization.[18] Sperm progressive motility was assessed using a Motic microscope at 100X by placing semen sample on a pre-warmed (37°C) glass slide and covered with a coverslip.

Evaluation of sperm morphology

Spermatozoa were classified as normal or abnormal. The abnormality was classified into a variety of head and tail abnormalities, including tapered-head, double head, vaculated head, two-tail, small head and bent tail[19]. Two smears were thus prepared by using a single semen droplet. Slides were air-dried in a near vertical position then put into a fixative in a jar for two minutes, and then rinsed with tap and distilled water. Wash the smear with sodium bicarbonate formalin solution to remove any mucus which may be present .rinse the smear with several changes of water. Slides were put into jars containing the Fuchsin solution and allow so stain for 3 minutes at room temperature. Wash off the stain with water. Counterstain by covering the smear with dilute methylene blue for 2 minutes. (Slides were rinsed again with tap and distilled water for 2 minutes. Microscopic evaluation of sperm cells was performed at 40x and 100x oil immersion magnification. Based on staining characteristics of sperm cells we differentiated three categories: The acrosome stains pale blue. The postacrosomal chromatins stain dark blue. The middle piece and the tail stain read a total of 200 sperm cells were observed with a 1000x magnification (100x objective under oil immersion). The number of normal and morphological defective sperm were be registered, as well as the type of defect, and the cell region where it is detected (i.e., vaculated heads, tapered head, double headed sperm, etc.).

Statistical analysis:

Data were analyzed using the statistical package for the social sciences (version 13). One way ANOVA was used to compare means of different parameters among three groups. All data were expressed as mean ± standard deviation (SD). The results were considered statistically significant when probability was less than 0.05.

Results

The sperm concentration in 8 of the anabolic user was within normal range (15-120X10^6/mL), 8 with azoospermia. There was one case of oligozoospermia in the control (A) group and tow cases of oligozoospermia in exercise (B) group. Results of the study in sperm parameters showed the difference in sperm concentration, the percentage of sperm motility and normal morphology between group B and A were not statistically significant (P = 1.000). The difference in seminal fluid volume between the two groups also was not statistically significant (P = 1.000). In the Table 1a shows that the nandrolone-using group results are seen, sperm concentration was significantly reduced after three months of using the agent. Thus, sperm concentration was 26.16±14.53X10^6/mL (Mean ± SD) compared with group A at 72.16 ±19.71 X10^6/mL (P <0.001). Table 1b shows the decline of the percentage of sperm motility and normal morphology in group C compared with control group (P <0.001). Table 1b shows serum LH level was significantly lower in

https://doi.org/10.15218/zjms.2017.021

group B compared with control group (A) (*P* = 0.003). However, the levels of testosterone and FSH were not statistically different between the two groups A and B (*P* = 0.102 and *P* = 1.000, respectively). As can seen in Table 1b serum testosterone, FSH and LH levels were also significantly lower in group C compared with control group (*P* <0.05). While seminal fluid volume changes were not statistically significant (*P* = 1.000) between group C and A. Sperm concentration was also significantly reduced in group C compared with group B which had sperm concentration at 68.2 ±23.1 $X10^6$/mL (*P* <0.05).Normal sperm morphology and percentage of sperm motility were also significantly lower (*P* <0.05) in group C compared with group B (Table 1a). The follicular stimulating hormone was also significantly reduced in group C compared with group B (*P* = 0.007). Whereas seminal fluid volume, the level of LH were not statistically different between group C and B (*P* = 1.000 and *P* = 0.709, respectively) as shown in Table 1b.

Table 1a: Mean ± SD sperm parameter and hormone in 3 groups.

Parameters	Group (A) N=50	Group (B) N=50	Group (C) N=50
Ejaculate volume(ml)	4.171± 1.81	4.14± 1.66	4.181± 1.81
106/ml Sperm concentration $X10^6$/mL	72.16 ±19.71	68.20 ± 23.1	26.16± 14.53
Total motility%	70.70 ± 14.53	66.70 ± 12.51	38.44± 17.34
Normal morphology%	71.30 ± 11.50	59.80 ± 16.3 4	32.84± 22.92
ng/ml Testosterone	4.72 ±1.80	3.95 ± 2.03	3.50± 1.45
mIU/ml FSH	6.47 ± 3.82	6.03 ± 2.42	4.13± 1.62
LH mIU/ml	4.65 ± 2.13	4.05 ± 1.76	3.00± 1.36

Table 1b: Statistical analysis within groups A, B and C.

	P(ANOVA)		P(Bonferroni)
Volume	0.877	AxB	1.000
		AxC	1.000
		BxC	1.000
Concentration	< 0.001	AxB	1.000
		AxC	< 0.001
		BxC	< 0.001
Total motility	< 0.001	AxB	1.000
		AxC	< 0.001
		BxC	< 0.001
Normal morphology	< 0.001	AxB	0.270
		AxC	< 0.001
		BxC	< 0.001
Testosterone	0.003	AxB	0.102
		AxC	0.003
		BxC	0.005
FSH	< 0.001	AxB	1.000
		AxC	0.001
		BxC	0.007
LH	< 0.001	AxB	0.003
		AxC	0.002
		BxC	0.709

https://doi.org/10.15218/zjms.2017.021

The result in the present study showed Table 2, Figure 1 that the highest percentage of abnormal head sperm with tapered head in the group used nandrolone (15.88% of 50 cases) after three months. The present study, thus, highlights the abnormality in head sperm in group C compared with the two groups (control and exercise groups). As can seen in Table 2 there was no statistically significant difference between group C and group A in the percentage of double head sperm (*P* = 0.413) and the percentage of vacuolated head sperm (*P* = 0.393). There was a significant decrease in the percentage of double and vacuolated head sperm was found between group C and group B, *P* <0.001 for both (Table2). It has been calculated statistically as shown in Table (2) that the percentage of normal head sperm reduced in the group used nandrolone compared with control and exercise group (*P* <0.001).

Table 2: Mean of normal and abnormal head %sperm in 3 groups A, B and C.

Groups		N	Mean	SD	p (ANOVA)	LSD groups	*P* (LSD)
Tapered head%	A	50	5.94	3.63	< 0.001	A X B	0.143
	B	50	8.66	5.10		A X C	<0.001
	C	50	15.88	14.72		B X C	<0.001
	Total	150	10.16	10.09			
Double head%	A	50	6.58	3.51	< 0.001	A X B	0.001
	B	50	9.46	4.69		A X C	0.413
	C	50	5.90	4.14		B X C	<0.001
	Total	150	7.31	4.40			
Vacuolated head%	A	50	7.06	3.36	< 0.001	A X B	0.001
	B	50	9.76	4.61		A X C	0.393
	C	50	6.36	4.19		B X C	<0.001
	Total	150	7.73	4.32			
Normal head%	A	50	80.48	8.89	< 0.001	A X B	0.038
	B	50	72.94	10.96		A X C	<0.001
	C	50	57.56	27.80		B X C	<0.001
	Total	150	70.33	20.28			

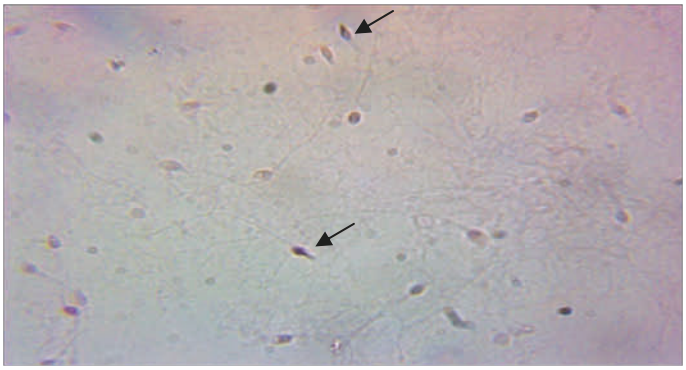

Figure 2: Semen smear stained with methylene blue. Shows tapered head a raw (100X magnification).

https://doi.org/10.15218/zjms.2017.021

Discussion

The Kurdistan region in Iraq has witnessed great social and economic changes since 1990. The area has since seen large increases in the availability of leisure and sport facilities. Many sport centers goers are turning to AAS use in an attempt to increase muscle bulk and improve their athletic abilities; Nandrolone being the drug of choice. The difference in ejaculating volumes between group C and the other two groups, A and B was not statistically significant. However; the difference in all of the other parameters investigated in between the group that used Nandrolone and the other two control groups were significant. The result of sperm morphology assessment revealed a highly significant increase in the number of tapered head sperm in the group that used Nandrolone. This may be due to the degenerated cells, shrinked pyknotic or pale degenerated nuclei, clumped chromosome degenerated cytoplasmic organelles. Our results agreement with Ahmed F Al domairy[20] as he showed the ultra structural changes included apoptotic and completely degenerated cells in the seminiferous tubules of testes of rats treated with therapeutic and high doses of nandrolone decanoate. In this study, vacuolated and double head sperm percentage were, however, not affected by exercise or by nandrolone. Interestingly, it is notable that heavy exercise does not appear to have significantly altered the percentage of the tapered head. Torres-Colleja et al. studied the effect of AAS in 30 adult male bodybuilders,[6] who reported that sperm count, percentage of normal sperm morphology and FSH level were significantly reduced, this results agree with our results, on the other hand they found that the LH level didn't vary in the same group. This finding disagrees with our result (LH level significantly reduced in exercise and treated group) may be due to the different doses of AAS taken by bodybuilders. Serum testosterone was statistically reduced in group C because nandrolone decanote caused decrease in the percentage of Leydig cells of the interstitial compartment, addition reduce its volume cytoplasmic and cellular.[21] It is well recognized that a long term use of nandrolone frequently results in male infertility as a predominant side effect[22]. Moreover, treatment with relatively high doses of nandrolone leads into decrease of sperm number, sperm motility and change of its morphology.[23] Our results showed significant decrease in sperm concentration which could be due to the exposure to AAS which have effects on the interruption in normal testosterone production which might cause disturbance in regulation of spermatogenesis and eventually leads to decrease in sperm count.[24,25] Other reasons for diminution of sperm concentration may be due to the apoptosis of germ cells, disarranged intratubular cells, destruction of the seminiferous tubules, these demonstrated by AlDomery[20] who examined a histological section of rat testes. Our results agree with studies which found that low and high doses of nandrrolone decrease sperm quality and quantity in rats in the form of low sperm count, decrease motile sperm fraction and decreased sperm with normal morphology.[26] The present study shows that the percentage of sperm motility decrease in group C compared with others this may be due to the mitochondrial content decrease with many swollen and disrupted mitochondria in rat soleus muscle after six weeks of treatment.[27] The result of S. Shokri et al[15] who studied the effect of exercise and supraphysiological doses of Nandrolone on sperm characteristics in male mature rats revealed that exercise and Nadrolone alone or in combination produced a significant decrease in sperm count and motility compared with the control group produced a significant decrease in sperm count and motility compared with the control group (sedentary rat without any injection or exercise). Both exercise alone and exercise plus Nandrolone had an

increase in the percentage of dead sperms and sperm abnormality. Exercise produced the least change followed by Nadrolone use with the highest change was caused by the exercise and Nandrolone use.

Conclusion

Data from the current study suggest that AAS has started its effects on the normal shape of spermatozoa, diminution of total motility percentage and concentration of sperm which lead to male infertility. The percentage of the tapered head (abnormal head) is higher in bodybuilder how received (AAS). This change of sperm parameters may be due to the diminution of follicular stimulating hormone and testosterone hormone. We believe that this is a vital issue that needs to be highlighted and addressed by health policy makers to avoid long term harmful effects of AAS use. Our study did not monitor the level of exercise performed by the body builders, how strenuous or how light. The severity of exercise could potentially, have a significant impact on the parameters monitored in our research. This is a factor to be considered in future research. The study highlights inadequate knowledge and wide spread ignorance regarding the serious impact of AAS use on the general health of young males in the region.

Conflicts of interest

The authors report no conflicts of interest.

References

1. Kopera H. The history of anabolic steroids and a review of clinical experience with anabolic steroids. Acta Endocrinologica 1985;271:11–8.
2. Sheffield-Moore M, Urban RJ. An overview of the endocrinology of skeletal muscle. Trends Endocrinol Metabol TEM 2004; 15: 110–5.
3. Deyssig R, Weissel M. Ingestion of androgenicanabolic steroids induces mild thyroidal impairment in male body builders. J Clin Endocrinol Metab 1993;76:1069-107.
4. Sravonee P, Rita M. Effect of Nandrolone Decanoate on Serum FSH, LH and Testosterone Concentration in Male Albino Mice. World Journal Life Science and Medical Research 2012; 2(3) : 123.
5. Torres-Calleja J, González-Unzaga M, DeCelis-Carrillo R, Calzada-Sánchez L, Pedrón N. Effect of androgenic anabolic steroids on sperm quality and serum hormone levels in adult male bodybuilders. Life Sciences 2001;68:1769–74.
6. Dohle GR , Smith M ,Weber RF. Androgen and male fertility.World J Urol 2003; 21:341 – 5.
7. Mesbah SF , Shokri S, Doust SK. Effects of nandrolone decanoate on ultrastructure of testis in male adult rats . Iran J Med Sci 2008 ; 33 (2): 94 – 100 .
8. Johnson LG, Fisher G, Silvester LJ, Hofhein CC. Anabolic steroid :Effects on strength, body Weight, oxygen uptake and spermatogenesis upon mature males. Med Sci Sports 1972; 4: 43– 5.
9. Eliakim A, Nemet D. Exercise and the male reproductive system. Harefuah 2006;145(9): 677-81.
10. De Souza MJ, Arce JC, Pescatello LS, Scherzer HS, Luciano AA. Gonadal hormones and semen quality in male runners. A volume threshold effect of endurance training. Int J Sports Med 1994; 15 (7):383-91.
11 Kujala UM, Alen M, Huhtaniemi IT. Gonadotrophin-releasing hormone and human chorionic gonadotrophin tests reveal that both hypothalamic and testicular endocrine functions are suppressed during acute prolonged physical exercise. Clinical Endocrinology 1990; 33: 219–25.
12. Safarinejad MR, Azma K, Kolahi AA. The effects of intensive, long-term treadmill running on reproductive hormones, hypothalamus–pituitary–testis axis, and semen quality: a randomized controlled study. Journal of Endocrinology 2009; 200: 259-71.
13. Feinberg MJ, Lumia AR, McGinnis MY. The effect of anabolicandrogenic steroids on sexual behavior and reproductive tissues in male rats. Physiol Behav 1997;62:23–30.
14. Shokri S, Aitken RJ, Abdolvahhabi M, Abolhasani F, Ghasemi FM, Kashani I, et al. Exercise and Supraphysiological Dose of Nandrolone Deconoate Increase Apoptosis in Spermatogenic cells. Basic & Clinical Pharmacology & Toxicology 2010; 106: 324–30.
15. Tahtamouni LH, Mustafa NH, Hassan IM, Ahmad IM, Yasin SR, Abdalla MY. Nandrolone Decanoate Administration to Male Rats Induces Oxidative Stress, Seminiferous Tubules Abnormalities, and Sperm DNA Fragmentation. Jordan Journal of Biological Sciences 2010; 3:165 –74.
16. McLachlan RI, Wreford NG, O'Donnell L De, Kretser DM , Robertson DM. The endocrine regulation of spermatogenesis independent roles for testosterone and FSH. J Endocrinol 1996;148: 1-9.

17. Watanabe N. Decreased number of sperms and Sertoli cells in mature rats exposed to diesel exhaust as fetuses. Toxicology Letters 2005;155: 51-8.
18. WHO laboratory manual for the examination and processing of human semen - 5th ed. Geneva: Switzerland World Health Organization; 2010.
19. Seed J, Chapin RE, Clegg ED, Dostal LA, Foote RH, Hurtt ME, et al. Methods for assessing sperm motility, morphology, and counts in the rat, rabbit, and dog: a consensus report. ILSI Risk Science Institute Expert Working Group on Sperm Evaluation. Reprod Toxicol 1996; 10: 237-44.
20. Ahmed F. AlDomairy MD. Histological and Ultrastructural Study of the Effect of Nandrolone on the Testis of Adult Male Albino Rat. J Am Sci 2013;9(5):104.
21. Ferrari F, de Paiva Foletto M, de Moraes SM, Peres SB, Segatelli TM, da Costa CE..Testis morphophysiology of rats treated with nandrolone decanoate and submitted to physical training. Acta Scientiarum Health Sciences 2013; 35: 164-66.
22. Lombardo F, Sgro P, Salacone P, Gilio B, Gandini L, Dondero F, et al . Androgens and fertility. Journal of Endocrinological Investigation 2004 ;28:51-5.
23. Takahashi M, Tatsugi Y, Kohno T. Endocrinological and pathological effects of anabolic-androgenic steroid in male rats. Endocrine Journal 2004; 51(4):425-34.
24. Pope Jr HG, Brown KJ. Treatment of anabolic androgenic steroid-related disorder. USA: American Psychiatric publishing Inc ;2008.
25. Farrell SF, Mc Ginnis MY. Long –term effect of pubertal anabolic androgenic steroid exposure on reproductive and aggressive behaviours in male rats. Horm Behav 2004;46:193-203. [PubMed].
26. Karbalay-Doust S, Noorafshan A, Ardekani FM, Mirkhani H . The reversibility of sperm quality after discontinuing nandrolone decanoate in adult male rats. Asian J Androl 2007; 9(2):235-9.
27. Soares JM, Duarte JA . Effects of training and an anabolic steroid on murine red skeletal muscle, a stereological analysis. Acta Anat1991; 142 (2):183-7.