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·*Original Article*·

The reversibility of sperm quality after discontinuing nandrolone decanoate in adult male rats

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Abstract

Aim: To investigate the reversibility of the effect of nandrolone decanoate (ND) on sperm parameters after discontinuing the drug. **Methods:** Three groups of rats received peanut oil (control), low and high doses of ND for 14 weeks, respectively. Each group was divided into subgroups A and B, in which rats were killed 14 and 28 weeks after initiating the injection, respectively. **Results:** Sperm count and motile sperm fraction were decreased in the subgroups A and B that received low and high doses of ND in comparison with the controls A and B, respectively. The sperm count and motile sperm fraction increased in the subgroups B that received low and high doses of ND in comparison with their corresponding subgroups A. The number of normal morphology sperm was decreased significantly in subgroups A receiving low and high doses of ND in comparison with the control subgroup A. However, this parameter was not decreased in subgroups B receiving low and high doses in comparison with the control subgroup B. The normal sperm morphology did not show any significant differences in the subgroups B in comparison with their corresponding subgroups A. **Conclusion:** The 14-week injection of low and high doses of ND decreases sperm quality and quantity in rats. These parameters were improved after discontinuing ND, but not recovered completely even when they are left untreated for 14 weeks. *(Asian J Androl 2007 Mar; 9: 235–239)*

Keywords: nandrolone decanoate; sperm count; sperm morphology; sperm motility; anabolic androgenic steroid; rats

1 Introduction

Anabolic-androgenic steroid (AAS), when used to improve athletic ability or muscle mass compounds, ranks among the most widely abused drugs [1]. AAS compounds were developed as synthetic analogs of testosterone and are currently prescribed for the treatment of refractory anemia, hereditary angioedema, breast cancer and starvation states [1]. The doses and combinations of these compounds used by athletes are typically in excess of therapeutic doses (10- to 100-fold).

As derivatives of testosterone, anabolic steroids greatly affect the male pituitary-gonadal axis. Hypogonadism can be induced, characterized by decreased serum testosterone concentrations, testicular atrophy and impaired spermatogenesis. These effects result from negative feedback of androgens on the hypothalamic-pituitary axis and possibly from local suppression of excess androgens on the testis. Serum follicle stimulating

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hormone (FSH) and luteinizing hormone (LH) concentrations are also low in this steroid-induced state of hypogonadotropic hypogonadism, with impaired sperm production and decreases in semen quality, and often resultant infertility. The evidence suggests that at doses used by athletes, AAS compounds can lead to changes in human and animal sexual physiology and sperm quality [1–6]; however, little is known about how reversible these effects are on sperm quality. The present study was conducted to evaluate the effects of weekly injection of low and high doses of nandrolone decanoate (ND) for 14 weeks (which is one of the AAS compounds) on sperm quality in rats and quantity, and reversibility of these effects 14 weeks after stopping the drug injection.

2 Materials and methods

2.1 Ethics

All the procedures were carried out under the ethical guidelines of the Shiraz University of Medical Sciences and the studies received prior approval by the Ethics Committee of the Shiraz University of Medical Sciences.

2.2 Chemicals

ND (25 mg/mL) was purchased from Iran Hormone (Tehran, Iran).

2.3 Animals

Ninety Sprague-Dawley male rats, weighing between 250 g and 300 g, were randomly selected (simple random sampling procedure) from the Laboratory Animal Center of Shiraz University of Medical Sciences. After 1-week acclimatization, they were placed in polycarbonate cages under a 12 h :12 h Light:Dark cycle with room temperature of 22 ± 2ºC. The rats were given tap water and fed *ad libitum*.

2.4 Treatment

The rats were divided randomly into three different groups with 30 animals each. Group 1 (control): rats received 0.4 mL/(kg·wk) of the vehicle (peanut oil); group 2: received 3 mg/(kg·wk) (low doses) of ND [7–9]; and group 3: received 10 mg/(kg·wk) (high doses) of ND for 14 weeks [7–9]. The vehicle and ND were injected i.m. to the gluteal region once every week. Our previous experiments (unpublished data) showed that sperm analysis results of vehicle treated animals had no differences with the untreated control rats. So in the present study, a normal control group was not included. The rats in each group were divided into two subgroups (A and B). The rats in subgroups A were killed 14 weeks after the injection (just after stopping the injection) and those in subgroups B were left untreated for another 14 weeks; after this period of withdrawal from the hormone injection, the rats in subgroups B were killed. The selected doses were lower than therapeutic doses of ND. According to Parfitt [10], ND is usually given once every 3 or 4 weeks. Doses of 25–100 mg have been used in debilitating illness. Doses of 50 mg have been used in postmenopausal osteoporosis and doses of 25–100 mg in postmenopausal breast carcinoma; therefore, the given doses in the present study were lower than the therapeutic doses [7–9].

Injection and withdrawal periods were selected according to the spermatogenesis period in rats. The period of one spermatogenesis is approximately 48–56 days in rats [11]; therefore, 14-week ND injection (98 days) and then 14-week discontinuation of the injection seem to be a reasonable period for recovery of sperm quality.

2.5 Sperm quality

The procedure for obtaining and analyzing semen was the same as that of Seed *et al*. [12]. The sperm samples obtained from the distal region of the right vas deferens of the rats were used in the present study. This involves excision of a small piece (1.0 cm) of the vas deferens just distal to the cauda epididymis. They were placed in a Petri dish containing an aliquot of buffer (Hank's Balanced Salt Solution, Appendix I) and agitated gently at 37ºC for 15 min. Total sperm count, morphological defects and characteristics such as motility were determined through microscopic examination.

2.5.1 Sperm count

Specimens were spread on a hemocytometer and the heads were counted manually under an optical microscope. In each rat, 300–400 sperm were counted and the data were expressed as total number of sperm/mL [12].

2.5.2 Sperm motility

The spermatozoa were classified as motile or immotile. Aliquots of the sperm suspension prepared for analysis were placed on a slide. The slides were evaluated with phase contrast microscopy in 10 microscopic fields and 200–300 sperms per animal were analyzed at a final magnification of × 1 000 [12]. The assessment of

the motile sperm fraction was defined as the mean number of motile sperm × 100 /total number of sperm [12].

2.5.3 Sperm morphology

Spermatozoa were classified as normal or abnormal. Abnormality was classified into a variety of head and tail abnormalities, including blunt hook, banana-head, amorphous, pin-head, two-head, two-tail, small head and bent tail [12]. One aliquot of the sperm suspension prepared for analysis was placed on a slide and air dried. The sample was stained with Eosin Y. The slides were evaluated with phase contrast microscopy in 10 microscopic fields, and 200–300 sperm per animal were analyzed at a final magnification of × 400. Finally, this parameter (normal morphology sperm fraction) was defined as the mean number of normal sperms × 100 /total number of sperm [12].

2.6 Statistical analysis

Statistical analysis was performed by comparing sperm count, morphology and motility between the control group and experimental groups using analysis of variance. Significant results were examined by further Mann–Whitney test, and $P < 0.05$ was considered significant.

3 Results

Sperm count and motile sperm fraction were decreased significantly in the subgroups A (the rats were killed just after 14 weeks of ND injection) and the subgroups B (the rats were killed 14 weeks after ceasing ND injection) of animals that received low and high doses of ND in comparison with the control subgroups A and B, respectively (Table 1).

The sperm count and motile sperm fraction increased in the subgroups B of animals that received low and high doses of ND in comparison with the corresponding subgroups A (Table 1).

Sperms with normal morphology decreased significantly in the subgroups A in which animals received low and high doses of ND in comparison with the control subgroups A (Table 1). However, this parameter did not show any significant differences in subgroups B in comparison with control subgroup B.

The normal sperm morphology did not show any significant differences in the subgroups B of animals that received low and high doses of ND in comparison with the subgroups A.

4 Discussion

The present work describes the study on the reversibility of ND-induced changes in sperm quality and quantity after discontinuing ND injection in male rats. In the present study we first compared sperm quality and quantity of the control (peanut oil treated) and ND-treated groups that were killed 14 weeks after injection. The results showed that sperm count and motile sperm decreased and sperm with abnormal morphology increased in these rats. This finding is in coincidence with Schurmeyer *et al.* [4], Torres-Calleja [5], Holma [13], Turek [14] and Feinberg *et al.* [15], who all found that AAS can cause azoospermia and reduce normal morphology sperm in athletes.

Second, to show whether these effects are reversible or not, we studied these parameters in ND-treated rats left untreated for 14 weeks. In this group, sperm count and percentage of motile sperm improved in the low and high dose-treated animals in comparison with the controls but the parameters do not reach the normal values of the control rats even 14 weeks after discontinuing ND

Table 1. Mean ± SD of the sperm count, motile sperm fraction and normal morphology sperm fraction in subgroups A (killed after 14 weeks of nandrolone decanoate [ND] injection) and B (killed 14 weeks after ceasing nandrolone decanoate injection) in the control, low dose (LD) and high dose (HD) of ND treated groups. $^{\dagger}P < 0.01$, LD A or HD A *vs.* control A, LD A *vs.* LD B, HD A *v.s* HD B; $^{\square}P < 0.05$, LD B *vs.* control B; $^{\blacklozenge}P < 0.01$, HD B *vs.* control B; $^{\ddagger}P < 0.01$, LD A or HD A *vs.* control A, HD B *vs.* control B, LD A *vs.* LD B, HD A *vs.* HD B; $^{*}P < 0.05$, LD B *vs.* control B; $^{\blacksquare}P < 0.01$, LD A *vs.* control A; $^{**}P < 0.05$, HD A *vs.* control A.

Parameters	Groups					
	Control A	Control B	LD A	LD B	HD A	HD B
Sperm count ($\times 10^6$ /mL)	92.0 ± 2.9	86.4 ± 3.3	58.0 ± 6.5†	80.2 ± 3.6$^{\square}$	41.4 ± 8.9†	66.0 ± 3.8$^{\blacklozenge}$
Motile sperm (%)	69.4 ± 4.7	68.0 ± 6.4	40.0 ± 3.6‡	59.0 ± 3.6$^{\ddagger *}$	35.0 ± 3.6‡	55.0 ± 3.6‡
Normal morphology sperm (%)	95.0 ± 2.9	95.0 ± 1.9	84.0 ± 5.5$^{\blacksquare}$	90.0 ± 3.6	88.4 ± 5.1**	91.0 ± 7.3

injection. In the study of Boyadjiev *et al.* [16], the process of complete recovery took more than 10 months. In sperm count of Holma [13], motility and number of sperm with normal configuration completely recovered 3 months after AAS discontinuance in athletes. However, in the present study theses parameters did not recover completely. This difference might be because this is an animal study, or because of different drugs used and duration of injection. Long-term AAS abuse causes a decrease in testosterone concentration and impairment of spermatogegenesis, resulting in azoospermia [13, 17]. As for many of steroid side effects, the semen parameter deficit is thought to be reversible. Discontinuation of all steroids, therefore, seems to be appropriate as an initial therapy. Previous studies suggested the reversibility of the anabolic steroid-induced endocrine imbalance [13, 16], but some reports casted doubt on this theory [18, 19].

Our previous quantitative study of the testis showed that a significant decrease in seminiferous tubule length, testis volume and weight is associated with the use of ND [20]. Additionally, our stereological study on the prostate showed that prostate weight, volume, glandular tissue, epithelium, fluid, collagen content, luminal surface of glands and vessel length decrease in nandrolone-treated rats [21]. This finding might provide some reasons for the decrease in the quality of sperm count. The effect of anabolic steroids on testis results from negative feedback of androgens on the hypothalamic-pituitary axis and possibly from local suppressive effects of excess androgens on the testis [1–6, 14]. Zirkin *et al*. [22] showed that there is a dose-response relationship between the concentration of testosterone in seminiferous tubule fluid and the quantitative maintenance of advanced spermatogenic cells in rat testis. Therefore, reduction in sperm count, motile sperm and normal morphology sperm in the present study could be due to inappropriate concentration of testosterone in seminiferous tubule fluid. Dohle *et al*. [23] reported that exogenous administration of synthetic testosterone results in negative feedback on the hypothalamic-pituitary axis and, therefore, inhibits the secretion of both FSH and LH. Although normal-to-high serum androgen concentrations are achieved with anabolic steroid use, those concentrations might not produce the testicular concentration necessary to maintain spermatogenesis and many male users of anabolic steroids develop hypogonadotrophic hypogonadism with subsequent testicular atrophy. Therefore, it can be concluded that testicular concentrations of testosterone are necessary to maintain the normal length of the seminiferous tubule and the reduction in the tubule length might be one reason for reduction in testis weight and volume and sperm count and motility.

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Appendix I. Components of Hanks' balanced salt solution (HBSS).

Components	Quantity
Calcium chloride dehydrate (mg/L)	185.4
Potassium chloride (mg/L)	400
Potassium phosphate monobasic anhydrous (mg/L)	60
Magnesium sulfate heptahedra (mg/L)	200
Sodium chloride (g/L)	8
Sodium bicarbonate (mg/L)	350
Sodium phosphate dibasic heptahedra (mg/L)	90
Dextrose (g/L)	1
Distilled water→Made up to (mL)	1 000