Effect of Nandrolone decanoate induced-oxidative stress on rat testes, prostate, and seminal vesicle: Biochemical, morphometric and histopathological studies

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A B S T R A C T

Nandrolone decanoate (Nd) is a highly abused androgenic-anabolic steroid among body builders. Even though it has weak androgenic effects, its prolonged use may have harmful impact on male reproductive system which needs to be evaluated. This study aimed to reinvestigate its possible oxidative stress induced alteration on male rat reproductive system. Twenty-eight male rats were divided into two groups. Nd treated group (n = 18) injected intramuscular with 10 mg/kg body weight once a week for four weeks. While, the control group (n = 10) was injected with physiologic saline by the same route for four weeks. Body weight was recorded for all rats and after animal dissection weight of testes, prostate and seminal vesicles were also recorded. The results showed that the average testicular weight was decreased in treated group compared to the control. The average weights of the prostate and seminal vesicles were increased compared to the control. Morphometric study revealed that in Nd treated group, there was a decrease in the width of seminiferous tubules and the height of spermatogenic cell layer compared to the control. Testicular degeneration was expressed by presence of spermatid giant cells, vacuolation, and degenerated spermatozoa. Tunnel technique showed scattered positive reaction among the spermatogenic cell layers and interstitial cells. Severe alterations of the prostate were expressed by benign prostate hyperplasia and retained secretions. Lipid peroxidation products (malonaldehyde concentration as ng/g of testicular tissue) were increased in treated group compared to the control and suggested the occurrence of oxidative damage. Nd induced severe alterations in the male genital organs were resulted from oxidative stress. It is concluded that the male genital organs are highly sensitive to the anabolic steroids and there is a high extent of reproductive risk associated with the use of AASs.

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1. Introduction

Anabolic-androgenic steroids (AASs) are testosterone analogs with functional and structural hormonal elements. They are utilized to treat different and significant pharmacological circumstances including renal functional impairment, blood disorders, delayed puberty in men, hereditary edema, osteoporosis, and growth deficiency (Tan and Scally, 2009; Ergun-Longmire and Wajnrajch, 2018). The combinations and doses of such compounds are excessively utilized and consumed by athletes (10–100 folds) (Naraghi et al., 2010). It has been suggested that these elements can generate modifications in animal and human hormonal physiology and behavior, and quality of sperm when taken in high doses (Torres-Calleja et al., 2001).

Several studies have explained the adverse influences of nandrolone on the testis (Noorafshan et al., 2005; Tofighi et al., 2017; Min & Lee, 2018). Uncontrolled and prolonged utilization of nandrolone causes different morphological and histological irregularities in the testes, which includes seminiferous tubules length, mitigation in testicular volume, sloughing in sertoli cells and apoptosis in germ cells (Takahashi et al., 2004; Noorafshan et al., 2005). Male infertility had been resulted as a major impairment due to the frequent use of nandrolone. Furthermore, reduction in size of the testes, sperm number and motility, and epididymal weights have been reported due to treatment with comparatively high nandrolone doses (Takahashi et al., 2004; Noorafshan et al., 2005).
The impacts of AAS compounds are equivocal on male reproductive tissues (Mesbah et al., 2007). Some researchers posit that even after the management of AAS, the process of spermatogenesis continues (El Osta et al., 2016; McBride and Coward, 2016). While other reported that density, normal morphology, and motility of sperm in men is decreased through AASs (Durairajanayagam, 2018; Józków and Rossato, 2017). It was observed that spermatogenesis in rats was sustained normally after treatment with AASs (Soufir, 2017). However, some examiners have reported reduction of Leydig cells and arrest of developed phases of spermatogenesis (Smith and Walker, 2014). Previously, it was revealed that atrophy of reproductive accessory organs and testes is caused by AASs. Sperm quality and sperm count have a broader effect on male fertility (Gabrielsen and Tanrikut, 2016), and one of the frequent reasons of male infertility is the abuse of AASs.

Shokri et al. (2014) studied the effect of 10 mg/kg body weight of Nandrolone decanoate (Nd) injection per week for eight weeks on rats. The study found a significant difference between seminal vesicles and prostate weight among experimental and control groups. In the experimental group, left and right testes weights were considerably lower than the control group. Moreover, immunohistochemistry using Tunnel technique revealed that treatment with Nd caused an elevation in apoptosis of the germ cell layers of the testes. Saddick (2018) evaluated the morphometric and histopathological effects of Nd over the ovaries and uterus of female rats as it was administered and withdrawn. The study showed marked increase in the uterus and ovaries weight when compared to the control group, which decreased after withdrawal of administration. Moreover, uterine changes were observed after two weeks of post-injection and were expressed by papillary hyperplasia and increased thickness of myometrium, which decreased on drug withdrawal. Lipid peroxidation was substantially improved by Nd as evaluated with the monitoring of MDA production in experimental and control groups, which might be because of the production of free radicals (Mohamed and Mohamed, 2015).

This study is novel from the preceding study as it will see the difference after 4 weeks of intervention, instead of 8 weeks (Shokri et al., 2014) which will help in postulating the changes at earlier phase of intervention. Also, the study is not limited to morphometric analysis only. The objectives of the present study are to assess the biochemical, morphometric and histopathological changes of the testes, prostate, and seminal vesicles and elucidate possible oxidative damage and mechanism of cell death in rats injected with Nd.

2. Materials and methods

2.1. Chemicals

The purchase of Nandrolone decanoate (17β-hydroxy-19-nor-4-androstene-3-one) was made from a local store in the form of oily substance consisting of 25 mg of androgen in 1 ml injection. The generic name of the dug was Deca-Duraboli, company was Belugas pharmaceuticals, Brussels Belgium and was manufactured in the year 2017.

2.2. Animals

Male adult rats (n = 28) were obtained from the King Fahad Medical Research Center (KFMRC) animal house and maintained for one week under laboratory conditions and kept at 12:12 dark/light conditions and fed on commercial pellets. This was done after approval from the local ethical committee of the university on the study protocol.

2.3. Experimental design

Male adult rats were divided into control group (n = 10) and Nd treated group (n = 18). Small sample size (and smaller control group) was to ensure 3R principles of animal testing. (Törnqvist et al., 2014). Nd was injected intramuscularly to the treatment group (10 mg/kg body weight) every week for 4 weeks, while the control group was injected with physiologic saline.

Samples were taken at the end of 4 weeks post-injection, embedded in paraffin, sectioned at 4–5 μm, 10% neutral buffered formalin, and examined by light microscopy. For morphometric analysis, random 5–10 rounded seminiferous tubules were selected. The width of each tubule and height of spermatogenic cell layers were measured using Fiji ImageJ 2012 Software (Suva, Fiji Island). Tunnel technique for cell apoptosis was prepared on testes paraffin sections by following the guidelines proposed by Sun et al. (2011).

For lipid peroxidation products, one gram of testes was taken at the first (n = 3) and forth (n = 3) week post-injection for the control group (n = 6) based on malondialdehyde (MDA) using the strategy proposed by Ohkawa et al. (1979). This method relies on the response of TBA with MDA at temperature of 95 °C for 30 min in acidic medium in order to produce TBA reactive product. A spectrophotometric measurement was conducted for the optical density of the pink product at 534 nm. Findings were presented in the form of nmol/g testicular tissue.

2.4. Statistical analysis

SPSS version 25.0 software was used to analyze the data collected using both descriptive and inferential statistics. Mean ± SE were used for presenting continuous variables. Analysis of Variance (ANOVA) and Duncan test were used for analysis at levels of P < 0.01 and 0.05, respectively.

3. Results

The gross appearance of the seminal vesicles and prostate showed increase in their weight in rats injected with 10 mg/kg body weight (Fig. 1). Moreover, there was an increase in the organ body/weight ratio in the Nd treated group. While, the testes were decreased in weight in treated rats compared to the control as shown in Table 1.

Fig. 1. Gross appearance of seminal vesicles (SV) and Prostate gland (P) of both control and treated rats with Nandrolone decanoate.
3.1. Histopathology

3.1.1. Testes

Intramuscular with physiologic saline (control) was injected to testes of rats, which showed the normal appearance of seminiferous tubules with all spermatogenic cell layers. Morphometric measurements showed an average width of the seminiferous tubules of 234.25 ± 7.32 μm, whereas the height of the spermatogenic cell layers were 38.36 ± 1.95 μm (Figs. 2 and 3, Table 2).

Treated group injected intramuscular with Nd (10 mg/kg body weight) and collected at 4 weeks post-injection showed irregularity of the basement membrane (Fig. 4). Moreover, separation and dissociation of the primary spermatogenic cell layers was also observed (Fig. 5). Fig. 6 showed severe degeneration of the seminiferous tubules represented by presence of spermatid giant cell.

Vacuolation and scanty spermatids and its degeneration were also observed (Figs. 7 and 8). Morphometric measurements of rat testes were injected with Nd and collected at 4 weeks post-injection showed an average width of the seminiferous tubules of 189.08 ± 5.65 μm (Fig. 4; Table 2). While, the height of the spermatogenic cell layers were 23.83 ± 0.83 μm (Fig. 8).

Apoptosis was expressed by positive tunnel reaction of the spermatogenic cell layers either scattered or as clusters of cells (Figs. 9 and 10) in treated rats compared to the control rats which showed negative reaction with tunnel technique (Fig. 11). Moreover, positive signals were also detected in the interstitial cells of treated rat testes (Fig. 12).

3.1.2. Prostate glands

Control rat prostate gland showed the normal appearance of glandular acini, lined by cuboidal epithelium with eosinophilic secretion (Figs. 13 and 14). Prostate glands of rats injected with 10 mg/kg Nd and collected after 4 weeks post-injection showed nodular benign prostate hyperplasia (Fig. 15). Hyperplasia of the glandular epithelium was expressed by intercommunicated folds supported by stroma. Coagulated prostate secretion was also observed (Fig. 16).

| Organ          | Organ weight (gm)     | Body weight (gm)     | Organ body weight ratio | ns = Non-significant at P < 0.01; 0.05. * Significant at P < 0.01; 0.05. |
|----------------|-----------------------|----------------------|-------------------------|------------------------------------------------------------------------|
|               | Control (n = 10)       | Treated (n = 18)     | Control (n = 10)         | Treated (n = 18)                                                       | Control (n = 10)           | Treated (n = 18)         |
| Testes        | 2.08 ± 0.15           | 1.76 ± 0.06          | 100 ± 0.22               | 115 ± 0.5                                                             | 2.08 ± 0.68                | 1.52 ± 0.12              |
| Prostate      | 0.85 ± 0.25           | 2.2 ± 0.5*           | 100 ± 0.22               | 115 ± 0.5                                                             | 1.13 ± 0.85                | 1.91 ± 1.0               |
| Seminal vesicles | 0.68 ± 0.23          | 1.9 ± 0.4*           | 100 ± 0.22               | 115 ± 0.5                                                             | 0.68 ± 1.0                 | 1.65 ± 0.8               |

Table 2

The mean values of the width of seminiferous tubules and the height of spermatogenic cell layers as the mean ± SE.

| Groups       | Width of seminiferous tubules | Height of spermatogenic cell layers |
|--------------|-------------------------------|-------------------------------------|
| Control      | 234.25 ± 7.32 μm              | 38.36 ± 1.95 μm                     |
| Treated      | 189.08 ± 5.65 μm*             | 23.83 ± 0.83 μm*                    |

* Significant at P < 0.01.

Fig. 2. Control rat testes showing seminiferous tubules (SF) with an average width of 234.25 ± 7.32 μm. H&E. Bar = 100 μm.

Fig. 3. Higher magnification of Fig. 2 showing primary spermatogonia (PS), secondary spermatogonia (SP), spermatids (S), and spermatozoa (z). The height of the spermatogenic cell layer is 38.36 ± 1.95 μm. H&E. Bar = 50 μm.

Fig. 4. Treated rats injected intramuscular with Nandrolone decanoate (10 mg/kg bodyweight) and collected at 4 weeks post-injection showing irregularity of the basement membrane of seminiferous tubules with a width of 189.08 ± 5.65 μm. H&E. Bar = 100 μm.
3.1.3. Seminal vesicles

Control rat seminal vesicles showed the normal appearance and consisted of mucosal folds, lumen and muscularis layer (Fig. 17). The seminal vesicles of rats injected with 10 mg/kg BW Nd intramuscular and collected after 4 weeks post-injection had showed retained secretion, shortening of folds, and desquamation of the epithelial lining the folds (Figs. 18 and 19).

3.1.3. Seminal vesicles

Control rat testes showed negative reaction in the seminiferous tubules and the interstitial cells. Tunnel technique. Bar = 50 μm.

Fig. 5. Testes of rats injected with 10 mg/kg BW Nandrolone decanoate IM and collected after 4 weeks post-injection showing separation and dissociation of the primary spermatogonia (arrow) H&E. Bar = 50 μm.

Fig. 6. Testes of rats injected with 10 mg/kg BW Nandrolone decanoate IM and collected after 4 weeks post-injection showing severe testicular degeneration expressed by spermatid giant cell (arrow) and absence of spermatogenic cell layers (star.) H&E. bar = 50 μm.

Fig. 7. Testes of rats injected with 10 mg/kg BW Nandrolone decanoate IM and collected after 4 weeks post-injection showing severe vacuolation of seminiferous tubules (arrow) and scanty spermatids and spermatozoa. H&E. bar = 100 μm.

Fig. 8. Testes of rats injected with 10 mg/kg BW Nandrolone decanoate IM and collected after 4 weeks post-injection showing seminiferous tubules with severe vacuolation (head arrow), dense spermatogenic nuclei (arrow), degenerated spermatozoa (star). The height of spermatogenic cell layer is 23.73 ± 0.83 μm. H&E. Bar = 50 μm.

Fig. 9. Control rat testes showing negative reaction in the seminiferous tubules and the interstitial cells. Tunnel technique. Bar = 50 μm.

Fig. 10. Treated rat testes showing few scattered positive apoptotic cells within the seminiferous tubules (arrow) and interstitial cells (Head arrow). Tunnel technique. Bar = 50 μm.

3.1.3. Seminal vesicles

Control rat seminal vesicles showed the normal appearance and consisted of mucosal folds, lumen and muscularis layer (Fig. 17). The seminal vesicles of rats injected with 10 mg/kg BW Nd intra-
3.1.4. Lipid peroxidation assay

Nandrolone decanoate administration increased the concentration of malondialdehyde (MDA) in the testicular tissue to 15 ± 0.57 ng/g by the first week post-injection and 28 ± 1.55 ng/g by the 4th week post-injection compared to the control 10.97 ± 1.33; 10.4 ± 1.5 ng/g by the 1st and 4th weeks post-injection (Table 3).

4. Discussion

AAS are artificial equivalents of testosterone. Therefore, the AAS have functional and structural testosterone features (Westlye et al., 2017). AASs are utilized for treating different and essential pharmacological conditions, which include growth deficiency, osteo-
porosis, hereditary edema, renal insufficiency, and delayed puberty in men. In this study, both the width of the seminiferous tubules and the height of the spermatogenic cell layers were decreased in the Nd group compared to the control group. Degeneration of the testes was observed and manifested with vacuolation and the presence of spermatid giant cell. It has been reported that the presence of these cells may indicate hypospermatogenesis, or exposure of testes to heat and testicular degeneration.

Irregularity and thickening of the seminiferous tubules' basement membrane were consistently found in the present study. These results have suggested that altered spermatogenic dysfunction can occur due to the thickening of the basement membrane (Elsaed et al., 2018). Several authors have reported same results (Sasso-Cerri, 2009; Beltrame et al., 2011; Beltrame and Sasso-Cerri, 2017). An insignificant reduction has been reported by Beltrame et al. (2011) in the luminal region and demonstrated this reduction by the contraction of the tubular wall in the absence of germ cells. A contraction has been noted in such cells mutually with these changes (Sasso-Cerri, 2009; Beltrame and Sasso-Cerri, 2017).

Apoptosis was detected in the present study and expressed by immune-positive cells for Tunnel technique scattered signals or in clusters of cells in the seminiferous tubules. These results suggested that the mechanism of cell death is mediated through apoptosis (Galluzzi et al., 2018). The positive reaction of the interstitial cells suggested that there might be an occurrence of altered androgen hormone production (Zirkin and Papadopoulos, 2018). Shokri et al. (2010) posit that the rate of apoptosis increases in spermatogenic cells due to high dose of Nd administration, which can cause decrease in testes size. The findings of this study correlate with Shokri et al. (2010) and shows apoptosis in the spermatogenic cells and interstitial cell of the testes. However, this apoptotic effect has been reported to be ameliorated by antioxidants (Ahmed, 2015; Mohammed et al., 2020).

Nodular benign hyperplasia was reported in the treated rat prostate and manifested by hyperplasia of the glandular epithelium, proliferation of its stroma, and coagulation of its secretion. Godwin et al. (2017) stated that non-cancerous expansion of prostate gland was expressed through the term benign prostatic hyperplasia. It was more holistically explained as a non-malignant expansion of prostate gland classified by enlargement of the cellular aspects such as its stromal and epithelial cells into a discrete nodules or mass. Histological assessment is another method utilized for explaining BPH induction as recommended in the literature (Ergün et al., 2017). The findings are consistent with Kim et al. (2009). A contraction of approximately 40% has been reported in prostate size and a reappearance to the preliminary treatment size throughout almost 2 years (Crawford et al., 2011; Arnouk et al., 2012).

Morphometric assessment showed an elevation in the size of treated prostate in Nd group as compared to the control group. Foster (2000) reported that with respect to cytoplasmic volume, there is a substantial increase in smooth-muscle cell organelles, accompanied by an increase of the protein synthesis of the extracellular matrix. This explanation could also relate to the increased weight of treated prostate in the present study. Lipid peroxidation products (malonaldehyde as ng/g of testicular tissue) were increased significantly, especially by the 4th week post-injection at p < 0.01 and p < 0.05 using ANOVA and Duncan tests, respectively. It is a marker of oxidative stress and suggested the occurrence of oxidative damage which coincides with the results
reported by Mohamed and Mohamed (2014). Nd is a lipid deriva-
tive and increases the lipid peroxidation products which acts as me-
diator of disease and cell death in animal body (Gaschler and
Stockwell, 2017). Increased levels of MDA are indicators of various
male reproductive problems (Collodel et al., 2015). This suggests
that lipid peroxidation due to Nd is harmful for male reproductive
organs.

For spermatogenesis high levels of Leydig cells secreted intrat-
esticular testosterone is required (El Osta et al., 2016). The normal-
to-high serum androgen concentrations may not generate the test-
ticular concentration essential for maintaining spermatogenesis
even though those concentration were accomplished with anabolic
steroid use. The majority of male rats of anabolic steroids can
develop hypogonadothrophic hypogonadism with corresponding
testicular atrophy. Thereby, testicular concentrations are impor-
tant to maintain the contraction in tubule length and normal semi-
inferous tubule length to mitigate testes weight and volume.

5. Conclusion

In conclusion, within 4 weeks of administration into the body.
Nandrolone decanoate had harmful effects on the male genital
organs and had detectable changes. Degeneration in seminiferous
tubules caused by it can lead to spermatogenic dysfunction. In
testes it also commenced the cell death by causing cell apoptosis.
Benign prostatic hyperplasia was also caused by Nd which is a risk
factor for kidney and urinary tract infections. The roles of endoge-
nous gonadotropins cannot be mimicked through excess AASs.
Thereby, the functions of gonadotropin are affected by the admin-
istration of AASs, and in particular, the accessory reproductive tis-
sues, weight of testis, and fertility parameters. The results suggest
that the male genital organs are highly sensitive to the anabolic
steroids. All these outcomes have suggested a high extent of repro-
ductive risk associated with the use of AASs.

6. Future prospective

The significant changes were seen in testicular cells within four
weeks of Nd administration. However, its early effects should also
be seen in other organs through their biochemical, morphometric
and histopathological study. Keen observational studies should
be done among the human population. After the withdrawal of the
drug, the potential of retaining back to the normal physiology
should also be studied.

Declaration of Competing Interest

The authors declare that they have no known competing finan-
cial interests or personal relationships that could have appeared
to influence the work reported in this paper.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

References

Ahmed, M.A., 2015. Amelioration of nandrolone decanoate-induced testicular and
steroid toxicity in rats by taurine: effects on steriodogenesis, redox and
inflammatory cascades, and intrinsic apoptotic pathway. Toxicol. Appl.
Pharmacol. 282, 285–296. https://doi.org/10.1011/jcmcm.13092.
Arnouk, R, Suzuki Bellucci, C.H., Stull, R.B., de Besia Junior, J., Malave, C.A., Gomes, C.
M., 2012. Botulinum neurotoxin-a for the treatment of benign prostatic
hyerplasia: randomized study comparing two doses. Sci. World J. 2012.
https://doi.org/10.1100/2012/463574.
Beltrame, F.L., Caregini, B.H., Miraglia, S.M., Cerri, P.S., Sasso-Cerri, E., 2011.
Vitamin B12 supplement exerts a beneficial effect on the seminiferous
epithelium of cimetidin-treated rats. Cells Tissues Organs 193, 184–194.
https://doi.org/10.1159/000319171.
Beltrame, F.L., Sasso-Cerri, E., 2017. Vitamin B12-induced spermatogenesis recovery in
cimetidin-treated rats: effect on the spermatogonia number and sperm
concentration. Asian J. Androl. 19, 567. https://doi.org/10.4103/1008-
382X.179530.
Collodel, C., Moeretti, E., Micheli, L., Menchiari, A., Moltoni, L., Cerretani, D., 2015.
Semen characteristics and malondiuldehyde levels in men with different
reproductive problems. Andrology 3, 280–286.
Crawford, E.D., Hirst, K., Kusek, J.W., Donnell, R.F., Kaplan, S.A., McCary, K.T.,
Bruskewitz, R., 2011. Effects of 100 and 300 units of anabotuin toxin A on
lower urinary tract symptoms of benign prostatic hyperplasia: a phase II
randomized clinical trial. J. Urology 186, 965–970. https://doi.org/10.1016/j.
uro.2011.04.062.
Duraianjanayagam, D., 2018. Lifestyle causes of male infertility. Arab. J. Urol. 16, 10–
20. https://doi.org/10.1016/j.aju.2017.12.004.
El Osta, R., Almont, T., Diligent, C., Hubert, N., Eschwège, P., Hubert, J., 2016.
Anabolic steroids abuse and male infertility. Basic Clin. Androl. 26, 2.
https://doi.org/10.1080/1097682x.2016.102842-4.
Elsaeed, W.M., Bedeer, R.F., Eladl, M.A., 2018. Ameliorative effect of vitamin B12 on
seminiferous epithelium of cimetidine-treated rats: a histopathological,
immunohistochemical and ultrastructural study. Anat. Cell Biol. 51, 52–61.
https://doi.org/10.1111/acb.2018.51.1.52.
Ergun, O., Koşar, P.A., Onaran, I., Darici, H., Koşar, A., 2017. Lyszyme gene treatment
in testosterone induced benign prostate hyperplasia rat model and comparation of its’ effectiveness with botulinum toxin injection. Int. Braz. J.
Urol. 43, 1167–1175. https://doi.org/10.1590/s1677-5538.dju.2016.0677.
Ergun-Longmire, B., Wajnrajch, M.P., 2018. Growth and growth disorders. In
Endotext [Internet]. https://www.ncbi.nlm.nih.gov/sites/books/NBK279142/
(Accessed 4 June 2020).
Foster, C.S., 2000. Pathology of benign prostatic hyperplasia. The Prostate
Supplement 9, 4–14. https://doi.org/10.1007/1097-0045(2000)9+<4:AID-
PROS3>3.0.CO;2-Q.
Gabrielson, J.S., Tanrikut, C., 2016. Chronic exposures and male fertility: the impacts
of environment, diet, and drug use on spermatogenesis. Andrology 4, 648–661.
https://doi.org/10.1111/andr.12198.
Galluzzi, L., Vitale, I., Aaronson, S.A., Abrams, J.M., Adam, D., Agostinis, P.,
Amici, C., Pinton, P., 2015. Autophagy and cancer cells death: recommenda-
tions of the nomenclature committee on cell death 2018. Cell Death Differ.
25, 486–541. https://doi.org/10.1038/s41418-017-0012-4.
Gaschler, M.M., Stockwell, B.R., 2017. Lipid peroxidation in cell death. Biochem.
Biophys. Res. Commun. 482, 419–425. https://doi.org/10.1016/j.
bbrc.2016.10.086.
Jóźkow, P., Rossato, M., 2017. The impact of intense exercise on semen quality. Am.
J. Mens Health 11, 654–662. https://doi.org/10.1177/155798331669045.
Kim, J., Yagaghanaha, Y., Kikugawa, T., Ji, M., Tanji, N., Masayoshi, Y., Freeman, M.R.,
2009. A signaling network in phenylephrine-induced benign prostate
hyperplasia. Endocrinology 150, 3576–3583. https://doi.org/10.1210/en.2008-
1790.
Mcbride, J.A., Coward, R.M., 2016. Recovery of spermatogenesis following
testosterone replacement therapy or anabolic-androgenic steroid use. Asian J.
Androl. 18, 373. https://doi.org/10.4103/1008-682x.173933.
Mesbah, A.S., Shokri, S., Karbalaei, D.S., Mirkhan, H., 2007. The effect of nandrolone
decanoate on the body, testis and epididymis weight and semen parameters in
adult male rats. Toxicol. Lett. 144, 81. https://doi.org/10.1016/s0378-4274(03)
90292-x.
Min, T., Lee, K.H., 2018. Effects of nandrolone decanoate on expression of
steriodogenic enzymes in the rat testis. Asian-Australas J. Anim. Sci. 31, 658.
https://doi.org/10.5713/ajas.17.0899.
Mohammed, E.T., Radi, A.M., Aleya, L., Abdel-Daim, M.M., 2020. Cynara scolymus
leaves extract alleviates nandrolone decanoate-induced alterations in testicular
function and sperm quality in albino rats. Environ. Sci. Pollut. Res. 27, 5009–
5017. https://doi.org/10.1007/s11356-019-07302-4.
Mohamed, H.M, Mohamed, M.A.H., 2015. Effect of different doses of nandrolone
decanoate on lipid peroxidation, DNA fragmentation, sperm abnormality and
S.Y. Saddick Saudi Journal of Biological Sciences 28 (2021) 196–203
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histopathology of testes of male Wistar rats. Exp. Toxicol. Pathol. 67, 1–11. https://doi.org/10.1016/j.etp.2014.09.003.

Naraghi, M.A., Abolhasani, F., Kashani, I., Anarkooli, I.J., Hemadi, M., Azami, A., Shokri, S., 2010. The effects of swimming exercise and supraphysiologic doses of nandrolone decanoate on the testis in adult male rats: a transmission electron microscope study. Folia Morphol. 69, 138-146 https://pubmed.ncbi.nlm.nih.gov/21154283/.

Noorafshan, A., Karbalay-Doust, S.A.I.E.D., Ardekani, F.M., 2005. High doses of nandrolone decanoate reduce volume of testis and length of seminiferous tubules in rats. Apmis 113, 122–125. https://doi.org/10.1111/j.1600-0463.2005. apm1130205.x.

Saddick, S.Y., 2018. The impact of nandrolone decanoate administration on ovarian and uterine tissues in rat: Luteinizing hormone profile, histopathological and morphometric assessment. Saudi J. Biol. Sci. 25, 507–512. https://doi.org/10.1016/j.sjbs.2017.08.015.

Sasso-Cerri, E., 2009. Enhanced ERbeta immunoexpression and apoptosis in the germ cells of cimetidine-treated rats. Reprod. Biol. Endocrinol. 7, 127. https://doi.org/10.1186/1477-7827-7-127.

Shokri, S., Aitken, R.J., Abdolvahhabi, M., Abolhasani, F., Ghasemi, F.M., Kashani, L, Ejtemaeimehr, S., Ahmadian, S., Minaei, B., Naraghi, M.A., Barbarestani, M., 2010. Exercise and supraphysiologic dose of nandrolone decanoate increase apoptosis in spermatogenic cells. Basic Clin. Pharmacol. Toxicol. 106, 324–330. https://doi.org/10.1111/j.1742-7843.2009.00495.x.

Shokri, S., Mokhtari, T., Azizi, M., Abbaszadeh, H.A., Moayeri, A., 2014. Nandrolone decanoate administration can increase apoptosis in spermatogenesis cell lines in male rats. J. Basic Res. Med. Sci. 1, 21–31 http://jbrms.medilam.ac.ir/article-1-71-en.html.

Smith, L.B., Walker, W.H., 2014. The regulation of spermatogenesis by androgens. Semin. Cell Dev. Biol. 30, 2–13. https://doi.org/10.1016/j.semcdb.2014.02.012.

Soufr, J.C., 2017. Hormonal, chemical and thermal inhibition of spermatogenesis: contribution of French teams to international data with the aim of developing male contraception in France. Basic Clin. Androl. 27, 3. https://doi.org/10.1186/s12610-016-0047-2.

Sun, L., Lam, W.P., Wong, Y.W., Lam, L.H., Tang, H.C., Wai, M.S., Yew, D.T., 2011. Permanent deficits in brain functions caused by long-term ketamine treatment in mice. Hum. Exp. Toxicol. 30, 1287–1296. https://doi.org/10.1177/0960327110388958.

Takahashi, M., Tatsugi, Y., Kohno, T., 2004. Endocrinological and pathological effects of anabolic-androgenic steroid in male rats. Endocr. J. 51, 425–434. https://doi.org/10.1507/endocrj.51.425.

Tan, R.S., Scally, M.C., 2009. Anabolic steroid-induced hypogonadism—towards a unified hypothesis of anabolic steroid action. Med. Hypotheses. 72, 723–728. https://doi.org/10.1016/j.mehy.2008.12.042.

Tofighi, A., Shirpoor, M., Ansari, M.H.K., Shirpoor, A., Zerehpoosh, M., 2017. The effect of nandrolone treatment with and without enforced swimming on histological and biochemical changes in the heart and coronary artery of male rats. Anatol. J. Cardiol. 17, 176. https://doi.org/10.14744/AnatolJCardiol.2016.7333.

Törnqvist, E., Annas, A., Granath, B., Jalkesten, E., Cotgreave, I., Oberg, M., 2014. Strategic focus on 3R principles reveals major reductions in the use of animals in pharmaceutical toxicity testing e101638 PLoS ONE 9. https://doi.org/10.1371/journal.pone.0101638.

Torres-Calleja, J., Gonzalez-Unzaga, M., DeCelis-Carrillo, R., Calzada-Sanchez, L., Pedron, N., 2001. Effect of androgenic anabolic steroids on sperm quality and serum hormone levels in adult male bodybuilders. Life Sci. 68, 1769–1774. https://doi.org/10.1016/s0024-3205(01)00972-9.

Westlye, L.T., Kaufmann, T., Alnæs, D., Hulstein, I.R., Bjørnebekk, A., 2017. Brain connectivity aberrations in anabolic-androgenic steroid users. Neuroimage: Clin. 13, 62–69. https://doi.org/10.1016/j.nicl.2016.11.014.

Zirkin, B.R., Papadopoulos, V., 2018. Leydig cells: formation, function, and regulation. Biol. Reprod. 99, 101–111. https://doi.org/10.1093/biolre/ioy059.