



Original article

The impact of nandrolone decanoate administration on ovarian and uterine tissues in rat: Luteinizing hormone profile, histopathological and morphometric assessment



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ABSTRACT

The study had been conducted to evaluate the effects of nandrolone decanoate (abused repeated doses) on female rat's ovary and uterus during administration and withdrawal. The study included 18 rats that were divided into control group ($n = 6$) and treated group ($n = 12$). The treated group was injected intramuscular (IM) with nandrolone decanoate (7 mg/kg body weight) for three consecutive days, for two weeks. The study stated that nandrolone decanoate increases the weights of body, ovary, and uterus. Moreover, it has a tendency of bringing upon modifications in the biochemical, histopathological, and morphological makeup of the female reproductive aspects. In conclusion, nandrolone decanoate has been identified as deleterious element for the female rats, and it is suggested that keen observations must be made on the human abusers to control and manage the possible pathologies.

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1. Introduction

The use of androgenic anabolic steroids (AASs) is observed commonly among athletes, youth, and adults in contrast to the common perception. In clinical settings, these compounds are identified as the synthetic analogues of testosterone. Clinically, AASs are usually recommended for the treatment of various pathological conditions; including renal insufficiency, endometriosis, hereditary angioedema and breast cancer (Belardin et al., 2014).

Drug administration has been practiced since many years to enhance the ergogenic effects. AASs are amongst common 'drug of illicit nature' that are mainly taken by athletes along with other stimulants. Barceloux and Palmer (2013) have identified AAS abusers as the poly-drug users. AASs are typically administered via intramuscular route. The drug use cycle lasts for about 6–12 weeks, which is observed with abstinence between the periods to avoid any adversarial outcome (Barceloux and Palmer, 2013).

Women usually have irregularities in menstrual cycles, enlargement of clitoris, voice deepening, and growth of facial hairs with AASs usage. At cellular levels, testosterone has been identified as an inhibiting factor for the endothelial nitric oxide synthase (eNOS) that can ultimately reduce the production of nitric oxide (NO) in the cells and induce oxidative stress (Skogastierna et al., 2014).

Nandrolone decanoate is a popular AASs, which is used by athletes to boost their physical performance and body image. This compound is also known to induce aggression, depression, and many other adverse effects (Rainer et al., 2014). A study indicated that subcutaneously injection of nandrolone decanoate (7.5 mg/kg BW/week) for 30 consecutive days increases the count of atretic follicles and reduces the ovarian oocytes in female rats and mice (Camargo et al., 2014). The objective of this experimental study was to evaluate the effects of nandrolone decanoate (abused dose) among female rat's on the ovaries and uterus during administration and withdrawal for 3 consecutive days per week for two weeks by means of histopathological and morphometric assessment.

2. Material and methods

2.1. Chemicals

Nandrolone decanoate (17 β -hydroxy-19-nor-4-androstene-3-one) was purchased from local pharmacy. It is an injectable

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solution, containing 25 mg of the androgen in 1 ml, available as oily solution.

2.2. Animal models

Eighteen female adult rats with regular estrous cycle were obtained from the animal house of King Fahad Medical Research Center (KFMRC) with the approval of local university ethical committee on the study protocol. The average weight of the rats was 132 g. The settings, within the animal house, had been established for two weeks at the requisite laboratory conditions. The control group (n = 6) received IM injections of 0.1 ml of mineral oil as vehicle for 3 consecutive days each week for two weeks. The treated animals (n = 12) received a daily IM injection of ND diluted in vehicle for 3 consecutive days each week for two weeks. The dose employed in this study simulated the abusive dose of steroid utilized by youth at fitness centers. The first injection was administered at the estrus phase of the cycle in all animals. Blood samples were obtained from rats of the treated group after the first week (n = 3) and second week (n = 3) post-injection. While blood samples were obtained from the withdrawal group (n = 6) after the first (n = 3) and second week (n = 3) after stopping the ND injection.

2.3. Method and experimental protocol

The body weight; of both the control and treated groups, were recorded before the injection (initial), treated, and withdrawal. Moreover, the weight and size of ovary and uterus were also recorded at the end of the experiment. Blood samples from all the rats were obtained from the retro-orbital vein of the eye using capillary tube. The sera was separated for the estimation of luteinizing hormone (LH) using Rat Eliza LH kit (MyBioSource, San Diego, CA, USA).

Rats were exterminated by ethyl ether inhalation before dissection of the pelvis to obtain the ovaries and uterus. The organs were removed from all groups weighed and immediately fixed in 10% formalin, dehydrated, embedded in paraffin and sectioned at 4–5 μm . The stains used in the observations included Hematoxyline, Eosin and Mallory trichromatic stain. The slides were examined under light microscope for histopathological and morphometric assessment. The thickness of myometrium was measured using Figi software (Fiji co., Fiji Island).

2.4. Statistical analysis

The Statistical Package for Social Science (SPSS 20 for Windows) program was used for data analysis. The numerical data are presented as the means \pm standard error (SE). One-way analysis of variance (ANOVA) was used for the comparison of variables between control and other groups. Statistically significant results were considered at a P-value < 0.05.

3. Results

The initial body weight of adult control rats was (107 \pm 16.325 g), while in both treated and withdrawal groups it was around 132 \pm 16.28 g. The evaluation observed a marked increase by first week of the experiment, which has been about 147.6 \pm 17.18 g, followed by a decrease in the second week, which was 126 \pm 11.1 g. The withdrawal group presented that the body weight has returned closer to the initial body weight by the second week of experiment (Table 1).

The weight of ovary and uterus of adult control rats was 0.82 \pm 0.98 g in the 1st week. It has been observed that the weight

of organs was increased by 1.9 \pm 1.38 g in the first week of experiment, followed by a decline by 1.15 \pm 1.46 g in the second week in the ND treated groups. Moreover, withdrawal group displayed an increase in the weight of ovary and uterus by 1.44 \pm 1.22 g during second week of post administration (Table 2).

The LH profile in the test group of rats showed marked drop to 0 IU/ml as compared to the control group (1 IU/ml). However, withdrawal of the drug administration in the second week resulted in marked increase of LH hormone to 3.2 IU/ml (Fig. 1).

The thickness of myometrium of control group was found to be 120.33 \pm 12.25 μm . At the first week and second week; post injection, it became 144.97 \pm 0.56 μm and 179.80 \pm 5.33 μm , respectively. During the period of withdrawal, the thickness had been found to be decreasing to 166 \pm 6.0 μm and 157.07 \pm 8.67 μm , in first and second week of the experiment, respectively (Table 3).

Histopathological observations revealed that ovaries from the control group has a normal cortical follicles appearances at different stages of development (arrows), corpus luteum structures could also be observed (Fig. 2A).

Table 1

Body weights (grams) of rats injected IM, with 7 mg/kg body weight of nandrolone decanoate in all studied groups.

Groups	Initial (g)	1st week (g)	2nd week (g)
Control (n = 6)	107.00 \pm 16.325	107.00 \pm 10.30	102.30 \pm 10.11
Treated (n = 6)	132.00 \pm 16.28*	147.60 \pm 17.18*	126.00 \pm 11.10*
Withdrawal (n = 6)	132.00 \pm 16.28	125.34 \pm 8.00	131.34 \pm 11.45

* Significant versus initial weight of the same group.

Table 2

Weight of ovary and uterus (grams) of rats injected IM with 7 mg/kg body weight of nandrolone decanoate in all studied groups.

Groups	1st week (g)	2nd week (g)
Control (n = 6)	0.82 \pm 0.98	0.80 \pm 0.98
Treated (n = 6)	1.90 \pm 1.38*	1.15 \pm 1.46*
Withdrawal (n = 6)	1.39 \pm 1.00	1.44 \pm 1.22

* Significant at P < 0.01; 0.05.

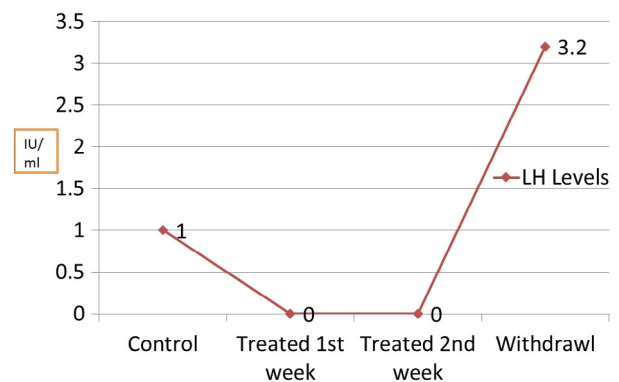


Fig. 1. LH fluctuation in the sera of rats injected IM with 7 mg/kg body weight of nandrolone decanoate in all studied groups.

Table 3

Thickness of myometrium (μm) of rats injected IM with 7 mg/kg body weight of nandrolone decanoate in all studied groups.

Groups	Initial (μm)	1st week (μm)	2nd week (μm)
Control (n = 6)	120.33 \pm 12.25	–	–
Treated (n = 6)	–	144.97 \pm 0.56	179.80 \pm 5.33*
Withdrawal (n = 6)	–	1166 \pm 6.00	157.07 \pm 8.67*

* Significant at P < 0.01; 0.05.

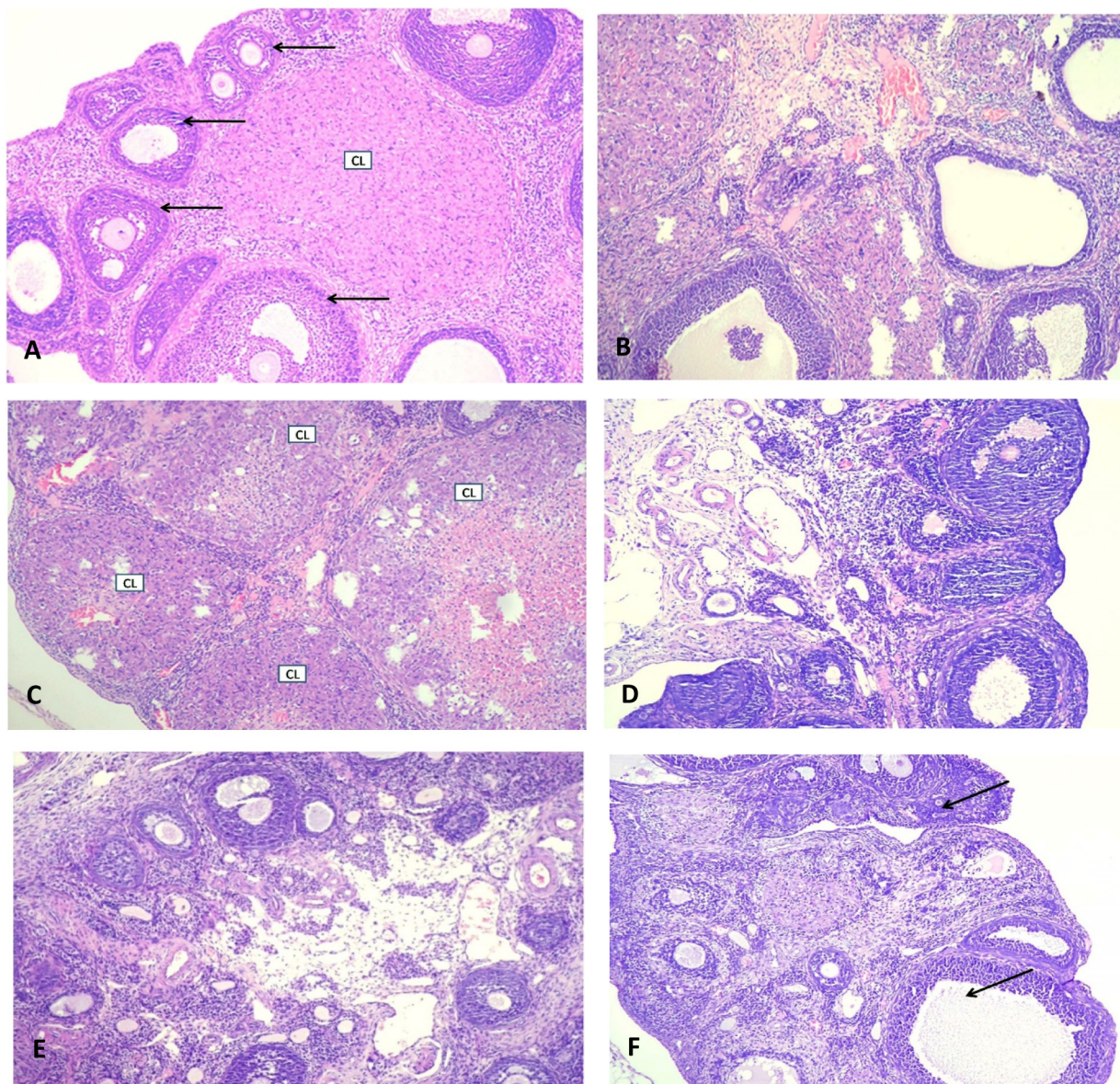


Fig. 2. Representative H&E stained histopathologic sections at $4\times$ magnification from ovarian tissues of all groups after the nandrolone decanoate (ND)-treatment period and withdrawal. Control (Fig. 1A indicates the ovary with normal appearance of cortex with different stages of ovarian follicles (arrows) and corpus luteum (CL)), one week post treatment (Fig. 1B indicates the absence of primary follicles) and (Fig. 1C indicates abundance of corpus luteum (CL), two weeks post treatment (Fig. 1D indicates different stages of ovarian follicles with relatively few number of primary follicles), one week of withdrawal (Fig. 1E indicates the restoration of the normal ovarian appearance), two weeks of withdrawal (Fig. 1F indicates normal appearance of the ovary with all stages of ovarian follicles (arrows)).

Rat ovary, which was injected with nandrolone decanoate, was collected after one week. It presented different stages of follicles, yet with relatively less primary follicles (Fig. 2B). Fig. 2C displays an abundance of corpora lutei (CL) on the expense of follicles.

The ovaries of rats injected with ND collected at two weeks post-injection showed different stages of follicles except relative less primary follicles (Fig. 2D). The predominance of CL at the expense of follicles has been consecutively noticed at the first week.

Withdrawal of ND for one week result in almost restoration of normal appearance of ovaries (Fig. 2E). At two weeks of withdrawal, the ovaries presented almost normal appearance with all stages of follicles (Fig. 2F).

The uterui of rats from the control group showed normal appearance of the different layers (Fig. 3A), the thickness of myometrium was $120.33 \pm 12.25 \mu\text{m}$.

After one week of ND injection the uteri showed pronounced papillary hyperplasia of endometrial epithelium (Fig. 3B). An increase in myometrium thickness has been observed with the measurement of around $144.97 \pm 0.56 \mu\text{m}$ (Fig. 3C).

Two weeks post injection, pronounced increase in the myometrium thickness as well as cystic dilatation of endometrial glands have been observed. The thickness of myometrium has been measured to be $179.80 \pm 5.33 \mu\text{m}$ (Fig. 3D). Moreover, enlargement in the nuclei of myometrial smooth muscle has also been noticed (Fig. 3E). After one week of ND withdrawal, the uterus displayed less papillary hyperplasia of endometrial epithelium and the thickness of the myometrium has been observed to decrease by $166 \pm 60 \mu\text{m}$ (Fig. 3F).

At two weeks of withdrawal, the uterus showed normal appearance of all uterine layers (Fig. 3G). Decrease in the myometrium thickness by $157.07 \pm 8.67 \mu\text{m}$ has been observed at two weeks post-injection (Fig. 3H).

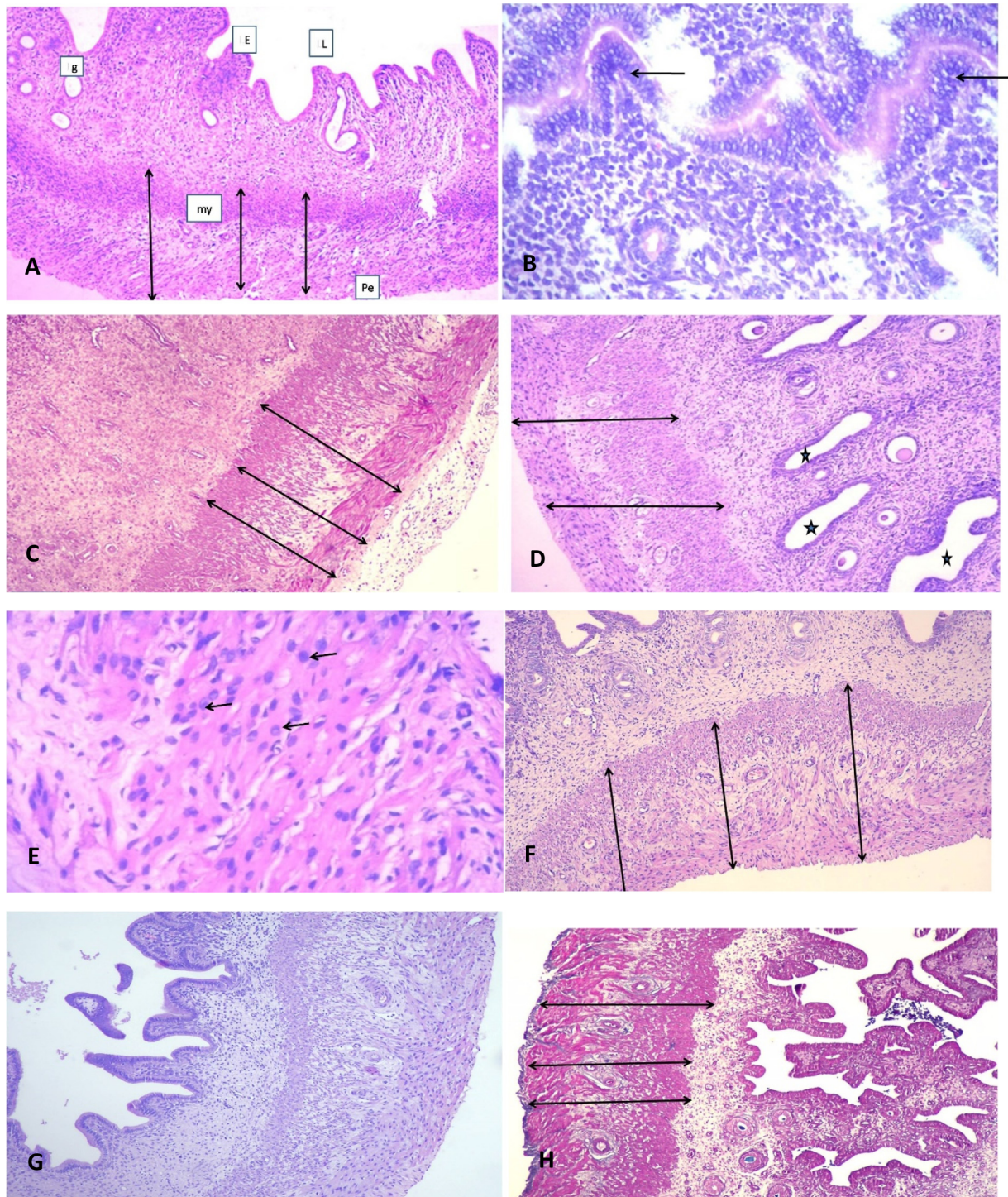


Fig. 3. Representative H&E stained histopathologic sections at 4 × magnification from uterine tissues of all groups after the nandrolone decanoate (ND)-treatment period and withdrawal. Control (Fig. 2A shows the different layers; endometrium (E), lumen (L), endometrial glands (g), myometrium (my), and perimetrium (pe) and the thickness of the myometrium (arrows)), one week post treatment (Fig. 2B shows hyperplasia of endometrial epithelium into the lumen (arrow) at 10 × and Fig. 2C shows pronounced increase in thickness of myometrium (arrows)), two weeks post treatment (Fig. 2D shows pronounced increase in the thickness of myometrium (arrow) as well as cystic dilatation of endometrial glands (*) and Fig. 2E shows enlargement of nuclei of myometrium (arrow) at 10 ×), one week of withdrawal (Fig. 2F shows decrease in the thickness of the myometrium (arrow)), two weeks of withdrawal (Fig. 2G shows normal appearance of uterine layers and Fig. 2H shows decrease in the thickness of the myometrium (arrows)).

4. Discussion

In the present study, the body weight of rats was increased in the first week of ND injection as compared to control group. Stopping the administration of ND showed that the body weight has returned close to initial body weight by the second week. The

increase in body weight of treated group can be attributed to increase in food intake, which was observed in the behavior of feeding of injected rats. Studies have identified that steroidal elements in ND have a tendency to influence the satiety level of the consumer; and thus, it increased the food demand. The increased and improved physical performance, provided by the influences

of the steroids, also elevated the energy demands of the body (Sato and Iemitsu (2015); Sato et al., 2014). Thus, the stimulated appetite can be the core reason behind the increase in weight of experimental group observed with the drug administration. Older studies have also stated that the nandrolone decanoate produces positive impacts on the body growth of a female rat (Brasil et al., 2015; Bisschop et al., 1997).

The ovary and uterus weight during treatment and withdrawal period had similar pattern to the body weight of rats. In this respect, Camargo et al. (2009) had evaluated the adult female rats that were injected intra-peritoneal with 6 mg/kg nandrolone decanoate (single injection per week for four consecutive days). The results indicated that there have been no significant impact on the uterine weight. However, it led to a significant decrease in the ovarian weight (Camargo et al., 2009). Different experimental designs, species, doses and routes of injection in the present study could be attributed to such disagreement. Nonetheless, the present study supported the morphological modification observed previously in the ovarian patterns (Camargo et al., 2009).

Observation done in the present study showed that the thickness of myometrium was increased by first and second weeks post-injection as compared to control group and decreased during the first and second week of withdrawal period. These results suggested that the discontinuation of drug administration may be responsible for the decrease in the thickness of myometrium in time dependant manner. Reproductive organs tend to respond efficiently to steroidal compounds. The ovaries and uterus primarily depend upon the dominant secretions of estrogen and progesterone in the body, along with a smaller fraction of the testosterone. It was reported that steroid administration can stimulate tissue growth which could be the possible cause of increased uterine thickness (Klyuchnikova and Voznessenskaya, 2015).

In the present study, drop of LH levels in the sera after treatment with nandrolone and then abrupt increase with the withdrawal of the drug had been observed. Bordbar (2014) stated that nandrolone decanoate has potential elements of male sex hormones. Nandrolone decanoate had been observed to reduce the secretions of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) through a negative feedback mechanism. These factors can subsequently lead to the menstrual and follicular disorders. Blasberg et al. (1997) and Bronson (1996) have reported that AAS compounds can markedly alter the normal functioning of hypothalamus–hypophysis–gonad axis. It has been noted that an increase in the circulating androgen levels can consequently inhibit the production and release of female reproductive hormones; including the LH, FSH, estrogen and progesterone (Liu et al., 2014; Blasberg et al. 1997; Bronson, 1996).

Histopathologically, control rat ovaries showed various stages of follicular development and corpora lutea. During the first and second week of treatment with ND, the presence of relatively less primary follicles were noticed; while more corpora lutea have been observed on the expense of ovarian follicles. These results may suggest that ND could result in alteration in the morphology of the ovaries. Several reports have indicated that altered morphology of the ovarian tissues in the form follicular atresia, destruction of follicular units, reduction in the number of ovarian oocytes, and increase in the cortical stroma by ovarian interstitial proliferation are the prevalent reactions to the steroid abuse on the female reproductive system (García-Manso and Esteve 2016; Camargo et al., 2009). The cessation of ND administration has resulted in the restoration of normal morphology of the ovarian tissues with the appearance of all stages of follicles and CL especially after the drug administration for two weeks. Such outcomes were attributed to the secretions of LH, FSH, and progesterone that may have return to the normal levels (Richards, 2013).

The uterine changes showed hyperplasia of endometrium and increased thickness of myometrium. Moreover, pronounced papillary hyperplasia was observed by the second week as well as glandular cystic dilatation. These changes suggested that repeated dose of ND could induce serious changes in the uterus. Mills et al. (2010) stated that endometrial hyperplasia is a heterogeneous set of pathologic lesions that range from mild reversible glandular proliferations to direct cancer precursors. It can be suggested that the abusive intervention of AAS can act as a possible risk factor for the reproductive cancer in females (Bansal & Acharya, 2014).

5. Conclusions

The results of this experimental study showed a serious health concern for the reproductive organs of adult females, who may use steroidal compounds for their physical enhancement. The abuse of these drugs can ultimately lead to deleterious histopathological changes in the uterus and ovaries and modifications in the hormonal makeup of the female biology; such as the decrease in the luteinizing hormone. It is evident that these compounds have a tendency to create reproductive dilemmas and can influence the childbearing capacity of female individuals. However, there is a need of performing keen observations among human population. The study has also indicated that the withdrawal of the drugs can be helpful in retaining back the normal physiology of the body.

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Conflict of Interest

This research holds no conflict of interest.

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