Acta Biologica Hungarica 60 (3), pp. 253–261 (2009)
DOI: 10.1556/ABiol.60.2009.3.2

OVARIAN HISTOLOGY AND FOLLICULAR SCORE IN FEMALE RATS TREATED WITH NANDROLONE DECANOATE AND SUBMITTED TO PHYSICAL EFFORT

Isabel Cristina Cherici Camargo,[1]* Roberta Barreiros de Souza,[1]
Suzana de Fátima Paccola Mesquita,[2]
L. G. A. Chuffa[1] and F. Frei[1]

[1] Department of Biological Sciences, Faculty of Sciences and Letters,
São Paulo State University – UNESP, Assis, SP, Brazil
[2] Department of General Biology, State University of Londrina – UEL, Londrina, PR, Brazil

(Received: June 19, 2008; accepted: October 2, 2008)

The study was conducted to analyze the histology of the ovaries of adults rats treated with steroids, and submitted or not to physical effort. The control group consisted of females submitted to physical effort and sedentary females, both of which received a physiological solution of 0.9% saline. Treated females, sedentary or not, received 6 mg/kg of body weight of nandrolone decanoate. The steroid and physiological solution were administered intraperitoneally, with a single injection per week for 4 consecutive weeks. The applied physical effort was swimming (20 minutes daily, 5 days/week, for the 4 weeks of treatment). Serial sections (5 μm) of ovaries were prepared for histological evaluation and follicular score. The weight of ovaries and hypophysis, the number of antral and atretic follicles, and the area of corpus luteum were all affected by the steroids. In the ovaries of the control groups, well-developed corpus luteum was observed. In the treated groups, the cortical stroma was occupied by ovarian interstitial tissue. The females treated with steroids presented estral acyclicity. The use of nandrolone decanoate, whether associated with physical effort or not, affected the morphological pattern of the ovaries.

Keywords: Anabolic steroid – ovaries – histology – follicular score – female rat

INTRODUCTION

The use of androgenic anabolic steroids (AAS) is a common practice among youth and adults, whether athletes or not [14, 19]. These compounds are synthetic analogues of testosterone, recommended for the treatment of many conditions, including renal insufficiency, endometriosis, hereditary angioedema and breast cancer [1]. However, AAS has been abusively and indiscriminately used by many professional athletes and non-athletes, including adolescents, to increase muscular mass, strength and dexterity [11].

* Corresponding author; e-mail: camargo@assis.unesp.br

Little is known about the dosage of anabolic steroids of female users, except that, like men, women have used five steroids simultaneously [20]. The doses generally are supraphysiologic and are associated with facial hair growth, deepening voice, clitoris enlargement and irregular menstrual cycle [20]. In men, anabolic steroids can affect spermatogenesis, promote testicular atrophy, and cause breast growth and impotency [12, 20, 21].

Many authors [9, 22] have reported that the potential benefits of AAS are followed by undesirable side effects. The frequency and severity of the side effects depends on many factors such as drug type, dosage, and duration of use as well as individual sensitivity and response [15]. According to Yu-Yahiro et al. [22], nandrolone decanoate presents fewer side effects than most other AAS available and has more anabolic than androgenic effects, with a anabolic/androgenic ratio of 8:1, compared to the testosterone steroid (1:1).

Among many commercial versions of AAS, nandrolone decanoate, sold as Deca-Durabolin (in Brazil), is considered the most popular steroid on the market [10]. Considering, first, that AAS has been indiscriminately used by youth and adults, and second, that are few morphological studies about the effects of AAS on feminine gonadal structure, the present study was designed to evaluate the effects of steroids on ovarian histology and follicular score in female rats either submitted or not to physical effort.

MATERIALS AND METHODS

Adult female rats from the Wistar lineage were obtained from Paulista State University (UNESP-Botucatu, SP, Brazil) and kept at the Faculty of Sciences and Letters (UNESP-Assis, SP, Brazil) under adequate conditions of temperature (22–24 °C) and luminosity (12 h light/dark photoperiod). The experimental protocol followed the ethical principles in animal research adopted by the Brazilian College of Animal Experimentation.

Nandrolone decanoate (4-estren-17β-ol-3-one 17 decanoate), known as Deca-Durabolin, was purchased from Organon Industry (São Paulo, Brazil) in an injectable preparation containing 50 mg of the androgenic substance.

The females rats were weighed and distributed at random in four experimental groups (n = 5/group): A) control-sedentary; B) control-submitted to physical effort; C) steroid-sedentary, and D) steroid-submitted to physical effort.

The drug was administered intraperitoneally, in a single dose of 6 mg/kg of body weight weekly, over four consecutive weeks. The dose employed in this study simulated the abusive dose of steroid utilized by youth at fitness centers. The females of control groups received a physiological solution of 0.9% saline in the same procedure that was used on the treated groups.

Swimming was chosen as the model of physical effort. The females were incrementally adapted to the exercise before the start of the experimental period, begin-

ning with 5 minutes on the first two days, 10 minutes on the third and fourth days, 15 minutes on the fifth and sixth days and 20 minutes on the seventh day. After this training period, the scheme of 20 minutes of daily swimming was adopted for five consecutive days each week over the four week treatment. During the experimental period, the estrous cycle of rats was daily monitored by vaginal cytology [12].

At the end of the fourth week of treatment, the females were weighed and killed by ethyl ether inhalation. The ovaries, uterus and hypophysis were removed and weighed. The gonad was fixed in Bouin's solution and embedded in Paraplast (Labware-Oxford, St. Louis, MO, USA). The 5-μm-thick serial sections were stained by hematoxylin and eosin (HE), for light microscope analysis.

In the analysis of the follicular score, one serial section was evaluated and the three consecutive others were discarded for each ovary until the final section of the organ, resulting in 4 repetitions/rat/group. The identification of follicle types was based on the classification proposed by Pedersen and Peters [16], according to Plowchalck et al. [17]. Scoring and analysis were performed to evaluate growth follicle (of more than 3 layers of follicular cells), the antral follicle (with antral areas), the atretic follicle and the corpus luteum. The area of corpus luteum was obtained through the computerized image analysis system Image-ProPlus®Media Cybernetics (Media Cybernetics, Silver Spring, MD, USA), using the X10 objective.

The weight of the body, ovary, uterus and hypophysis of five rats in each group were statistically analyzed by means of non-parametric Kruskal-Wallis variance analysis, complemented by the Dunn test, and the results were expressed as median ± interquartile deviation values. The data of ovarian follicle count and corpus luteum area were analyzed by ANOVA followed by the Tukey test, and the results were expressed as means ± standard deviation. Significance was set at $p < 0.05$.

RESULTS

The use of steroids whether associated or not with physical effort, did not significantly affect body and uterine weights (Table 1). However, there was a significant decrease ($p < 0.05$) in the ovarian weight of sedentary rats treated with steroids in comparison to the other experimental groups. The females of the two groups treated with steroids, whether submitted or not to physical effort, presented a decrease ($p < 0.05$) in hypophysis weight when compared to the control groups (Table 1).

There was no significant difference in the number of ovarian growth follicles and corpus luteum in the four experimental groups (Table 2). The rats treated with steroids presented higher ($p < 0.05$) number of antral and atretic follicles than the females of control groups.

Histologically, the ovaries of the rats in the two control groups presented various follicular types and well-developed corpus luteum (Table 2 and Figs 1A, 1B).

In the experimental groups, the ovaries presented a significantly reduced area of corpus luteum (Table 2) when compared to the control groups, and a cortical stroma occupied by cellular strings, which characterizes the interstitial tissue (Figs 2A, 2B).

Table 1
Final body weight and weights of the reproductive organs and hypophysis of the females in the four experimental groups (Median ± interquartile deviation)

Experimental groups	Body weight (g)	Ovaries weight (g)	Uterus weight (g)	Hypophysis weight (g)
Control A	251.2 ± 24.8[a]*	0.1180 ± 0.0163[a]	0.5502 ± 0.1030[a]	0.0112 ± 0.0018[a]
Control B	235.6 ± 12.44[a]	0.1144 ± 0.0174[a]	0.5912 ± 0.2306[a]	0.0110 ± 0.0025[a]
Treated A	264.6 ± 7.76[a]	0.0774 ± 0.0137[b]	0.4124 ± 0.0402[a]	0.0082 ± 0.0019[b]
Treated B	253.8 ± 24.81[a]	0.0982 ± 0.0106[a]	0.4356 ± 0.1128[a]	0.0084 ± 0.0011[b]

* In the same columm, median with identical letters do not differ statistically among themselves ($p > 0.05$).

Control A and Treated A: sedentary animals (n = 5/group)

Control B and Treated B: animals submitted to physical effort (n = 5/group)

Based on exfoliative cytology, the females of the control groups, sedentary or not, presented estral cyclicity, with typical estrous characterized by presence of many cornified epithelial cells. In the groups treated with steroids, the vaginal smear revealed that three sedentary females and five females submitted to physical effort presented estral acyclicity after the third week of treatment. These females showed persistent diestrus, with predominance of leukocytes in the vaginal smear.

Table 2
Follicular score and area of corpus luteum in the four experimental groups (Mean ± standard deviation)

Experimental groups	Growing follicle	Antral follicle	Atretic follicle	Corpus luteum	Area of corpus luteum (μm^2)
Control A	10.2 ± 4.6[a]*	29.0 ± 8.0[a]	43.4 ± 10.0[a]	17.0 ± 6.6[a]	384,310 ± 85,294[a]
Control B	8.6 ± 7.8[a]	15.4 ± 13.3[b]	32.8 ± 17.2[b]	22.6 ± 14.8[a]	448,651 ± 67,578[a]
Treated A	12.6 ± 6.7[a]	46.4 ± 17.9[c]	82.8 ± 19.1[c]	38.4 ± 17.0[a]	230,633 ± 43,918[b]
Treated B	10.0 ± 5.0[a]	54.6 ± 17.6[c]	102.6 ± 21.3[c]	32.6 ± 10.5[a]	197,490 ± 31,427[c]

* In the same columm, mean with identical letters do not differ statistically among themselves ($p > 0.05$). The mean represent the value obtained in both ovaries.

Control A and Treated A: sedentary animals (n = 5/group)

Control B and Treated B: animals submitted to physical effort (n = 5/group)

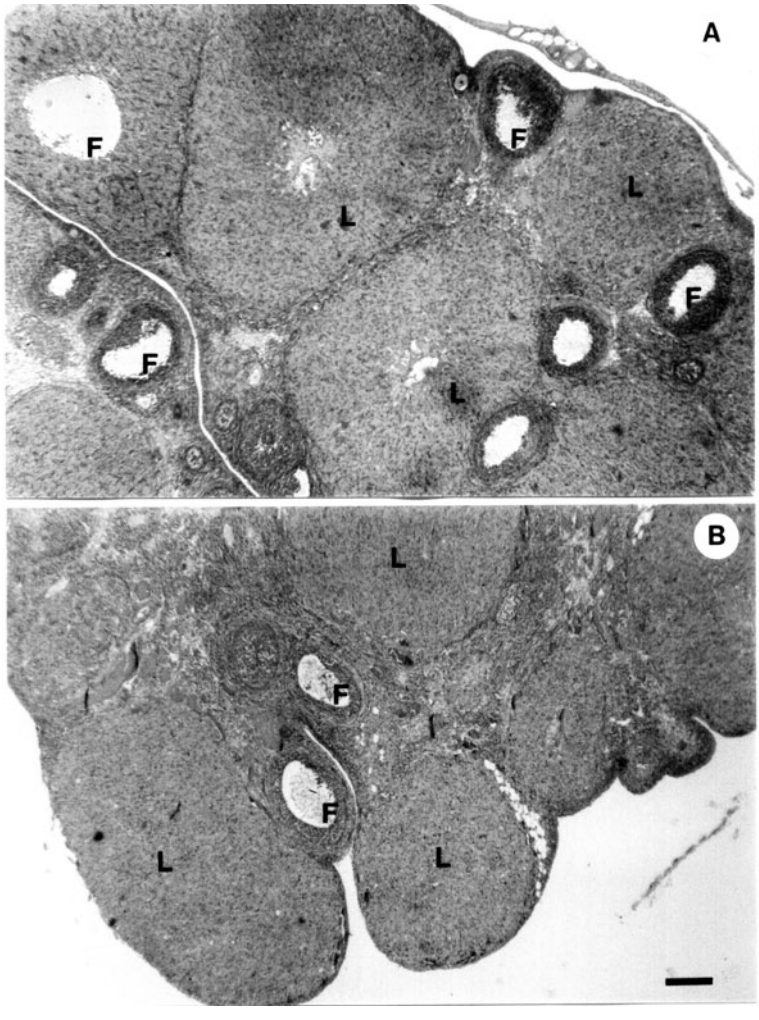

Fig. 1. Photomicrographs of the ovaries. (A) control-sedentary group and (B) control-submitted to physical effort group. Note that the ovaries presented well-developed corpus luteum (L) and antral follicles (F). Hematoxylin-eosin. Bar = 110 μm

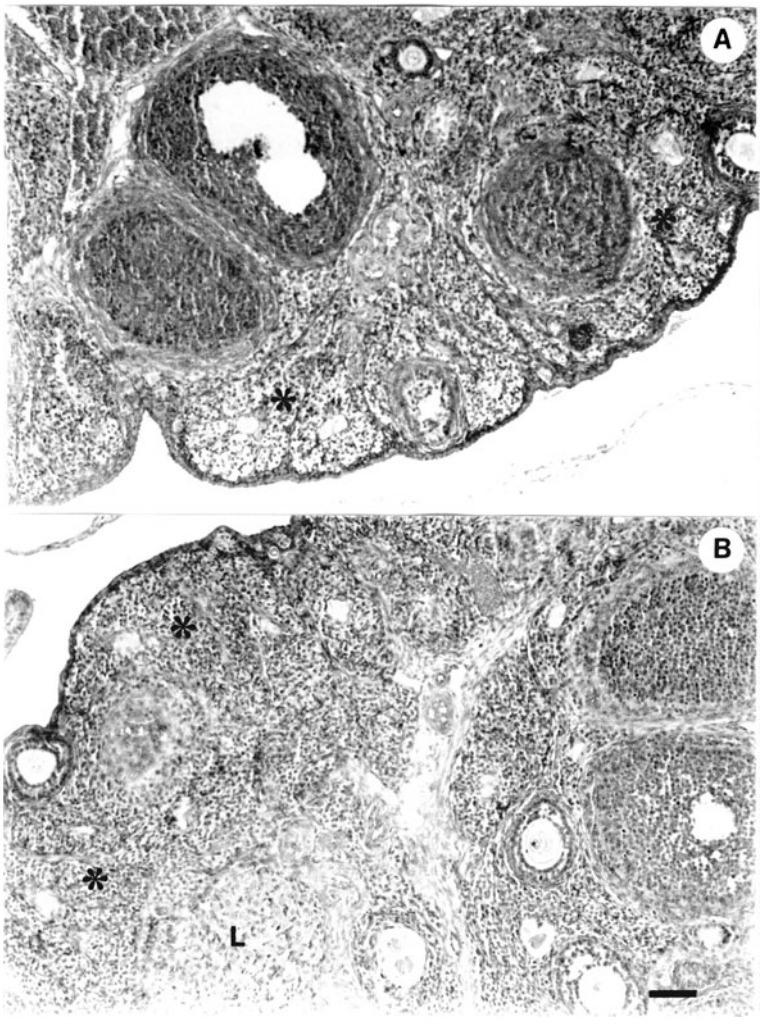

Fig. 2. Photomicrographs of the ovaries. (A) steroid-sedentary group and (B) steroid-submitted to physical effort group. Note that the ovaries presented interstitial tissue (*) in the stroma and reduced area of corpus luteum (L). Hematoxylin-eosin. Bar = 110 μm

DISCUSSION

In the present study, anabolic steroid did not demonstrate efficacy for body mass gain in females, whether submitted or not to physical effort. This result agrees with that obtained by Blasberg et al. [1] in sedentary female rats treated with different doses of nandrolone decanoate. Other studies [4–7] demonstrated that the body weight of androgenized females increased significantly in comparison with the control group.

The weights, however, of the ovaries, uterus and hypophysis are affected by administration of AAS [3–6]. The reproductive capacity of treated female rats with nandrolone decanoate might be suppressed by alteration in uterine morphology (myometrium hypertrophied and endometrium atrophied) [5]. Treatment with steroids decreases hypophysis weight, indicating a negative effect on the ovaries. In the androgenized females, submitted or not to physical effort, morphological alterations to the ovaries were evidenced.

Many authors [1–4] reported that AAS compounds alter the function of hypothalamus–hypophysis–gonad axis. An increase in the levels of circulating androgen inhibits the production and release of LH, FSH, estrogen and progesterone. This can result in the inhibition of ovarian follicle formation, ovulation and sexual cycle irregularity. The results obtained in the present study confirm the effects of AAS on the neuroendocrine function of the hypothalamic–hypophysis–gonadal axis. A higher frequency of atretic follicles occurred in the ovaries of rats treated with steroids. The corpus luteum was evident in the ovaries of females of four experimental groups, but there was a decrease in the area this structure in the ovaries of androgenized females. Gao and Short [6] verified that the treatment of female rats with methyltestosterone promoted ovarian quiescency characterized by absence of mature follicle or corpus luteum, indicating that the females had not ovulated. The same treatment applied to female mice resulted in the presence of mature follicles in the ovaries, but in atresia [6]. Rats treated with nandrolone decanoate showed destruction of follicular units and absence of corpus luteum in the ovaries [7].

In the current study, the treatment with nandrolone decanoate induced the formation of ovarian interstitial tissue in sedentary and trained females. According to Ross and Pawlina [18], there is a contribution by theca interna cells of atretic follicles to the formation of strings of interstitial cells.

According to Giordano-Lanza et al. [8], the psychophysical stress that occurs during intense sporting activity determines in female athletes the elevation of certain specific hormones such as cortisol and prolactin, which interfere with the pulsatile rhythm of GnRH, leading to a state of hypogonadotrophy, which promotes irregularity of the sexual cycle. Although the determination of hormone levels in rats was not carried out in this study, reports of other authors confirm our results, since high levels of circulating androgen promoted estral acyclicity and induced histological alterations in gonadal tissue. The pattern of the diestrus observed in the rats treated with anabolic steroids corroborates the results obtained by many authors [1, 3, 6, 7, 9].

Women who use compounds of AAS might present disturbances in their menstrual cycle [1] in the same way as the rats treated with nandrolone deacanoate in this study.

The results allow the conclusion that steroid treatment, whether associated or not with physical effort, affects ovarian histology and follicular score, through the disruption of female neuroendocrine function.

ACKNOWLEDGEMENT

The authors express their gratitude to the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), São Paulo, SP, Brazil.

REFERENCES

1. Blasberg, M. E., Langan, C. J., Clark, A. S. (1997) The effects of 17 α-methyltestosterone, methandrostenolone, and nandrolone decanoate on the rat estrous cycle. *Physiol. Behav. 61,* 265–272.
2. Blasberg, M. E., Clark, A. S. (1997) Anabolic-androgenic steroid effects on sexual receptivity in ovariectomized rats. *Horm. Behav. 32,* 201–208.
3. Bronson, F. H. (1996) Effects of prolonged exposure to anabolic steroids on the behavior of male and female mice. *Biochem. Behav. 53,* 329–334.
4. Bronson, F. H., Nguyen, K. Q., de La Rosa, J. (1996) Effect of anabolic steroids on behavior and physiological characteristics of female mice. *Physiol. Behav. 59,* 49–55.
5. Far, H. R., Agren, G., Lindqvist, A. S., Marmental, M., Fahlke, C., Thiblin, I. (2007) Administration of the metabolic androgenic steroid nandrolone decanoate to female rats causes alterations in the morphology of their uterus and a reduction in reproductive capacity. *Eur. J. Obstet. Gynecol. Reprod. Biol. 131,* 189–197.
6. Gao, Y., Short, R. V. (1993) Use of an oestrogen, androgen or gestagen as a potential chemosterilant for control of rat and mouse populations. *J. Reprod. Fertil. 97,* 39–49.
7. Gerez, J. R., Frei, F., Camargo, I. C. C. (2005) Histological assessment of ovaries and uterus of rats subjected to nandrolone decanoate treatment. *Contraception 72,* 77–80.
8. Giordano-Lanza, G., Tafuri, D., Guerra, G. (2003) Sporting activity and male reproduction. *Med. Sport 56,* 47–56.
9. Howe, G. R., Morello, C. J. (1985) Effects of an anabolic steroids on reproduction in female rats. *Steroids 45,* 497–501.
10. Ichihara, I., Kawamura, H., Nakano, T., Pelliniemi, L. J. (2001) Ultrastructural, morphometric, and hormonal analysis of effects of testosterone treatment on Leydig cells and other interstitial cells in young adult rats. *Ann. Anat. 183,* 413–426.
11. Iriart, J. A. B., de Andrade, T. M. (2002) Musculação, uso de esteróides anabolizantes e percepção de riscos entre jovens fisiculturistas de um bairro popular de Salvador, Bahia, Brasil. *Caderno de Saúde Pública 18,* 1379–1387.
12. Irving, L. M., Wall, M., Neumark-Sztauner, D., Story, M. (2002) Steroid use among adolescents: findings from project EAT. *J. Adolescent Health 30,* 243–252.
13. Junqueira, L. C. U., Junqueira, L. M. M. S. (1983) *Técnicas Básicas de Citologia e Histologia*. Santos, São Paulo.
14. Korkia, P., Stimsom, G. V. (1997) Indication of prevalence, practice and effects of anabolic steroid use in Great Britain. *Intern. J. Sports Med. 18,* 557–562.
15. Kuipers, H., Wijnen, A. G., Hartgens, F., Willelms, S. M. (1991) Influence of anabolic steroids on body composition, lipid profile and liver functions in body builders. *Intern. J. Sports Med. 12,* 413–418.

16. Pedersen, T., Peters, H. (1968) Proposal for a classification of oocytes and follicles in the mouse ovary. *J. Reprod. Fertil*. *17,* 208–212.
17. Plowchalck, D. R., Smith, B. J., Mattison, C. R. (1993) Assessment of toxicity to the ovary using follicle quantitation and morphometrics. In: Heindel, J., Chapin, R. E. (eds) *Methods in Toxicology. Female Reproductive Toxicology*. Academic Press Inc., San Diego, pp. 57–68.
18. Ross, M. H., Pawlina, W. (eds) (2008) *Histologia – Texto e Atlas*. Guanabara Koogan S/A, Rio de Janeiro.
19. Scott, D., Wagner, J., Barlow, T. (1996) Anabolic steroid use among adolescents in Nebraska schools. *Am. J. Health-System Pharmacol*. *53,* 2068–2072.
20. Strauss, R. H., Liggett, M. T., Lanese, R. R. (1985) Anabolic steroid use and perceived effects in ten weight-trained women athletes. *JAMA 253,* 2871–2873.
21. Wood, T. Q., Cooke, P. H., Goodship, A. E. (1988) The effect of exercise and anabolic steroids on the mechanical properties and crimp morphology of rat tendon. *Am. J. Sports Med. 16,* 153–158.
22. Yu-Yahiro, J. A., Michael, R. H., Nasrallah, D. V., Schofield, B. (1989) Morphologic and histologic abnormalities in female and male rats treated with anbolic steroids. *Am. J. Sports Med. 17,* 686–689.