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Sex steroid receptors profiling is influenced by nandrolone decanoate in the ampulla of the fallopian tube: Post-treatment and post-recovery analyses

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ABSTRACT

Anabolic androgenic steroids (AAS) are recommended for therapeutic clinic, but their use has increased in recent decades for aesthetic reasons. No study has evaluated the impact of AAS in the fallopian tube, after treatment and recovery periods. Herein, the aim of study was to investigate the effects of Nandrolone Decanoate (ND), administered in different doses (1.87; 3.75; 7.5 and 15 mg/kg) on the ampulla of the fallopian tube in rats, following post-treatment (PT; 15 consecutive days) and post-recovery (PR; 30 consecutive days) periods. The control group received mineral oil. Estrous cycle was monitored daily during both periods and in sequence the rats (n = 8/group/period) were killed. All ND-treated animals showed estral acyclicity during the PT and PR periods, but the histomorphometric changes in the fallopian tube varied according to the ND dose level. The expression of AR, ERα and ERβ varied in the nucleus and cytoplasm of epithelial cells. No AR expression was observed in the stroma. The muscle cells exhibited variation in immunostaining. In conclusion, ND promoted histomorphometric and immunohistochemical changes in the ampullary portion of the fallopian tube after treatment and recovery periods in a dose-independent manner.

1. Introduction

AAS are synthetic substances chemically related to testosterone (Rocha et al., 2014; Frizon et al., 2005), which present anabolic and androgenic activities, respectively correlated to stimulation of growth and maturation of non-reproductive tissues and maintenance of secondary sexual characteristics and reproductive function (Berne and Levy, 2000). Nandrolone decanoate (ND) is considered one of the most used synthetic steroids worldwide (Abrahin et al., 2013; Iriart et al., 2009), with moderate androgenic effects and strong anabolic properties (Kicman, 2008).

Regarding the therapeutic applications, AAS are recommended for the treatment of chronic diseases associated with the catabolic state of patient such as acquired immunodeficiency syndrome (AIDS), chronic obstructive pulmonary disease, cancer, severe burns, hepatic or renal failure and post-surgical recovery (Kicman, 2008; Shahidi, 2001). They are also prescribed for postmenopausal hormone replacement therapy (HRT) and in cases of age-related sarcopenia (Arlt, 2006; Evans, 2004). However, the non-therapeutic use of AAS increased in recent decades among men and women, exclusively for aesthetic reasons. In women, its indiscriminate use promotes various side effects that are dose and period-dependent (Bonetti et al., 2008; Kam and Yarrow, 2005) and include breast atrophy, body hair growth, voice deepening, oligo-amenorrhea, clitoris hypertrophy, anovulation and aggressiveness (Hoffman and Ratamess, 2006; Maravelias et al., 2005; Cannavo et al., 2001).

The adverse effects of ND on estrous cycle and ovarian/uterine architecture are well-described and reported to be associated with estrous cycle interruption (Chuffa et al., 2011; Bento-Silva et al., 2010; Gerez et al., 2005; Blasberg et al., 1998), suppression of the reproductive capacity (Belardin et al., 2014; Camargo et al., 2009) and long-lasting histopathological changes in the ovaries and uterus (Simão et al., 2015; Simão et al., 2016; Belardin et al., 2014; Camargo et al., 2014; Mobini Far et al., 2007). These effects can occur even after use of low doses and are mostly time-dependent (Simão et al., 2015; Belardin et al., 2014).

To date, the effect of AAS on the morphophysiology of the fallopian tube is still poorly explored. Because the fallopian tube plays important roles on bidirectional transport of gametes, as well as in nourishment and transport of the developing embryo towards the uterus at a precisely timed movement (Akison et al., 2014), an unexpected hormonal imbalance could affect its natural physiology resulting in low fertility rates.

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During the reproductive cycle, the mucosa of the fallopian tube undergoes morphological changes (Bylander, 2014; Patek et al., 1972), regulated by a set of sex steroid hormones (Akison et al., 2014; Goldberg and Friedman, 1995). After estradiol (E_2) and progesterone (P_4) bind to its receptors ER or PR expressed on the fallopian tube epithelium, a number of molecular and structural functions take place (Lyons et al., 2006; Mahmood et al., 1998). While E_2 stimulates epithelial cell hypertrophy, mucosa secretion and ciliogenesis (Donnez et al., 1985), P_4 regulates ciliary beating frequency (Bylander et al., 2013). Previous study showed that the administration of ethinylestradiol to fertile and postmenopausal women cause no ciliary alteration, whereas treatment with testosterone enanthate lead to decrease in the number of ciliated cells of the fimbrial and ampullary regions (Patek et al., 1973).

Despite extensive studies are conducted to evaluate the effects of AAS in the reproduction, no study has focused on the effects of ND regarding the morphology and expression of sex steroid receptors in the fallopian tube. Therefore, this study aimed to evaluate the effects of different doses of ND on the ampulla of the fallopian tube especially involving the expression of androgen receptor (AR), and estrogen receptors (ERα and ERβ subunits) after treatment and recovery periods.

2. Materials and methods

2.1. Animals

Eighty *Wistar* female rats (*Rattus norvegicus)*, 70-day-old, and approximately 250 g body weight, were housed in the Central Biotherium of School of Sciences, Humanities and Languages (UNESP, Assis, São Paulo, Brazil). All animals were individually kept in polypropylene cages with laboratory-grade pine shavings as bedding and maintained in a room with controlled temperature and luminosity (22 ± 2° C; 12 h-light/12 h-dark, respectively). Rat chow (Nuvital, Colombo, Paraná, Brazil) and tap water were provided *ad libitum*. Experimental protocol followed the ethical principles in animal research adopted by the Brazilian College of Animal and was previously approved by the Ethics Committee for Animals Use (Permit Number 005/2011).

2.2. Drug

Nandrolone decanoate (17β-hydroxi-19-nor-4-androsterone-3-one), also known as Deca Durabolin™, was purchased from Schering-Plough Laboratory (São Paulo, Brazil). It is commercially available as an injectable oily solution, containing 50 mg of the androgen.

2.3. Experimental design

Rats with regular estrous cycle were weighed and randomly divided into five groups (n = 16/group): control, treated with 0.1 mL of mineral oil, and ND-treated groups that received different doses (1.87, 3.75, 7.5 and 15 mg/kg b.w.). The injections were administered subcutaneously, once daily, for 15 consecutive days, at the same time (12:30–13:00 a.m). The doses were chosen based on our previous reports (Simão et al., 2015; Simão et al., 2016; Belardin et al., 2014). Briefly, the highest doses of ND (7.5 and 15 mg/kg) are usually employed in animal studies or used without therapeutic recommendation by young and adults. The lowest doses (1.87 and 3.75 mg/kg) are clinically recommended for various clinical treatments. Allometric calculations for animal dosing were performed as described by Belardin et al. (2014).

At the end of 15 days of experimental treatment, eight animals from each group were anesthetized and killed, corresponding to the post-treatment period (PT) of the study (Fig. 1A). Following the treatment period, the remaining rats from each group (n = 8/group), were kept without any treatment for a period of 30 days and killed at the end of this period, corresponding to the post-recovery period (PR) of the study (Fig. 1B).

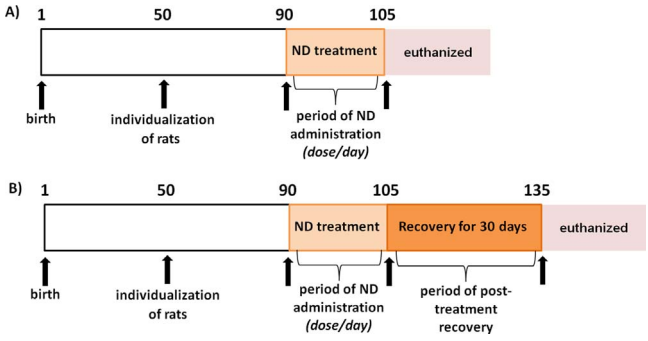

Fig. 1. Chronological experimental design in days. (A) At 90-days-old, eight rats per dose level (1.87, 3.75, 7.5 and 15 mg ND/kg) were killed at the end of the treatment period. (B) Eight rats per dose level (1.87, 3.75, 7.5 and 15 mg ND/kg) received the treatment for 15 days and were maintained in the recovery period for 30 days (105-135-days-old), followed by killing.

2.4. Assessment of estrous cycle

During the treatment and recovery periods (15 and 30 consecutive days, respectively), the estrous cycle was monitored daily by cytological examination (vaginal swabs). The time of collection was fixed at 9:00 a.m. The phases of cycle were identified under a light microscope (Olympys CX31 RBSFA, Tokyo, Japan), according to the criteria described by Goldman et al. (2007).

2.5. Histomorphometric and immunohistochemical analysis

At the end of each period (PT and PR), the animals from each group were killed using anaesthetic saturation in CO_2, followed by decapitation (Simão et al., 2016).

The right oviduct was collected and fixed in buffered 10% (v/v) formalin (0.2 M NaH_2PO_4, 0.2 M Na_2HPO_4, 37% formaldehyde LABYMPEX Ltda., Diadema, São Paulo, Brazil) for 24 h. Subsequently, the oviduct was dehydrated in ethyl alcohol, clarified in xylene, and embedded in paraffin (Oxford Labware, St. Louis, MO, USA). The blocks were cut into 4-μm thick sections using a microtome Leica RM2125 (Germany), and tissue sections were stained with hematoxylin-eosin (H &E) and Mallory's Trichromic.

For immunohistochemistry analysis, samples of the left oviduct were deparaffinized in xylene and hydrated in ethanol solutions. Antigen retrieval was performed in a microwave oven at 700–800 W for 15 min (3 × 5 min) in sodium citrate buffer 0.01 M, pH 6.0. After blocking peroxidase activity, the tissues were incubated with 3% bovine serum albumin (BSA) for 1 h to prevent nonspecific binding. The tissue sections were then incubated in a humidified chamber overnight at 4 °C with primary antibody anti-AR (dilution 1:100, NB300-551, Novus Biologicals, Littleton, CO, USA), anti-estrogen receptor alpha (ERα; 1:100 dilution, NB110-56961, Novus Biologicals) and anti-estrogen receptor beta (ERβ; dilution 1 μg/mL NB120-3577, Novus Biologicals). After reacting with the primary antibodies, the slides were washed in phosphate buffered saline (pH 7.0), and rapidly incubated with the secondary antibody (Anti-Mouse Polymer IgG or Anti-Rabbit – DAKO CYT) at room temperature for 1 h. After incubation, the slides were placed to react with the chromogen diaminobenzidine (Sigma, St. Louis, MO, USA) for 5 min. Finally, the sections were washed in deionized water, counterstained with Harris hematoxylin. Negative controls were used by omitting the primary antibody.

The criteria for the immunohistochemistry analysis were based on the following staining intensity levels: absent (−), low (+), moderate (++) and strong (+++). In cases in which the intensity of staining differed between the nucleus and the cytoplasm, the symbol (N) or (C) was used in superscript, respectively. When necessary, a vertical bar (/)