Journal of Reproduction & Contraception doi: 10.7669/j.issn.1001-7844.2015.01.0001
2015 Mar.; 26(1):1-5 E-mail: randc_journal@163.com

• ORIGINAL PAPER •

Changes of testicular ultrastructure of rat after clenbuterol exposure

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Objective *To investigate effects of clenbuterol (CLB) on testicular ultrastructure of rat.*

Methods *Twenty adult male Sprague-Dawley rats were randomly divided into four groups (5 rats per group). CLB solved in normal saline solution was given at the dose of 0 mg/kg body weight (bw) (group A, as control), 0.4 mg/kg bw (group B), 2.0 mg/kg bw (group C), and 18.5 mg/kg bw (group D) for 14 d by gavage consecutively, respectively. Transmission electron microscopy was used to observe changes on testicular ultrastructure.*

Results *In group B, some small vacuoles were found in Sertoli cells. In groups C and D, vacuoles were common in Sertoli cells and spermatogonia. The phenomenon of vacuolation in group D was more severe than that in group C. In group D, basal membrane showed some irregular and wrinkled changes, Leydig cells had more vacuoles and increased lipid droplets.*

Conclusion *Testicular ultrastructure of rat had pathological changes after CLB exposure, and the alterations became more severe with the increasing doses.*

Key words: clenbuterol (CLB); ultrastructure; Sertoli cell; testis; rat

Over the past decade, there has been a shift in infertility population in most countries, and male infertility is reportedly up to 50% of infertile couples[1-3]. Decreased fertility in men has a variety of etiologies such as testicular or epididymal disorders, immunologic factor, varicocele, genital tract infection, endocrine disturbance and genetic defects. At present, it is

This study was supported by Science and Technology Planning Project of Guangzhou City, China (No. 2013 00000114)

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well recognized that the consequence of exposure to some chemicals can result in disruption of the programming of metabolic and reproductive processes, which contributes to male infertility[4-6].

Clenbuterol (CLB) is a member of the class of drugs called β-adrenergic agonists (βAR), which has the ability to promote an increase in lean muscle mass in meat-producing animals[7,8]. Because CLB can residue in the tissues of treated animals, which leads to adverse health effects in humans[9-12], it has been banned to use as a feed additive for food-producing animals in Europe, China and other countries. However, illegal use of CLB has existed in some places for economic purposes. Several reports have demonstrated that CLB has deleterious effects on reproductive organs or reproductive events[13-18]. Our previous investigations have showed that CLB inhibited the development of mouse embryo *in vitro*, and caused abnormal expression on testicular steroidogenic acute regulatory (StAR) protein and mRNA in rats[19,20]. In the present study, we observed effects of CLB on testicular ultrastructure of rats with transmission electron microscopy (TEM) in order to increase our understanding on testicular damage after CLB exposure.

Materials & Methods

Animals and grouping

Twenty adult male Sprague-Dawley rats (9−10 weeks old, weighing 200−220 g) were purchased from the Guangdong Medical Laboratory Animal Centre (Guangzhou, China). Rats were maintained under controlled temperature (23 ℃−25 ℃) and a 12 h light/dark cycle with free access to water and food throughout the experiment. The experiment was approved by the local ethics commission for the use of animals.

Rats were randomly divided into four groups (5 rats per group): a control group (group A), and three experimental groups (groups B, C and D; low, mid, and high doses, respectively). Each CLB (Bioo Scientific Co. Austin, TX, USA) dosage was dissolved 0.9% NaCl solution up to 1 ml. Based on our previous study and LD_{50} values [147−175 mg/kg body weight (bw)][19], rats in groups B, C and D were treated daily by gavage with CBL dosages of 0.4 mg/kg bw (1/500 of LD_{50}), 2.0 mg/kg bw (1/100 of LD_{50}), and 18.5 mg/kg bw (1/10 of LD_{50}), respectively. The animals in the control received an equivalent volume of 0.9% NaCl solution by gavage. The experimental period lasted 14 d.

Tissue samples and TEM

The rats were sacrificed by decapitation 24 h following the experimental period. Testicular tissues from the four groups were prepared for TEM analysis using a routine method as previously described[21]. Briefly, the samples were fixed in 2.5% glutaralsehyde in 0.1 mol/L sodium cacodylate/HCl buffer (pH 7.2−7.4), then postfixed in 1% osmium tetroxide, and dehydrated in graded alcohol and embedded in Emix resin.