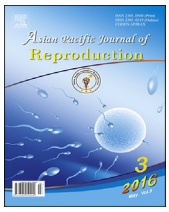
Contents lists available at ScienceDirect

Asian Pacific Journal of Reproduction

journal homepage: www.apjr.net



Original research http://dx.doi.org/10.1016/j.apjr.2016.04.015

Prenatal progesterone exposure of male rats induces morphometric and histological changes in testes

Samy Ismail Ahmed[1], Adil Salim Elsheikh[2*], Gamal Ali Attia[1], Tahir Osman Ali[3]

[1]*Department of Anatomy, Faculty of Medicine, Najran University, Saudi Arabia*

[2]*Department of Applied Medical Sciences, Community College Najran, Saudi Arabia*

[3]*Faculty of Graduate Studies & Scientific Research, National Ribat University, Sudan*

ARTICLE INFO

Article history:
Received 28 Feb 2016
Received in revised form 22 Mar 2016
Accepted 27 Mar 2016
Available online 19 Apr 2016

Keywords:
Prenatal
Hydroxyprogesterone caproate
Rat
Testis histology

ABSTRACT

Objective: To investigate the effects of prenatal progesterone exposure of male rats on testicular morphometry and histology.

Methods: Twenty male Wizard rats born to dams treated with hydroxyprogesterone hexanoate (HPH) during the first 2 weeks of gestation were employed to evaluate the effects of HPH on testicular morphometry, histology and cells quantities. Twenty male rats born to untreated dams were used as control. The rats were kept until they reached 90 days old, sacrificed, testes dissected, weighed and their dimensions recorded. Histological sections were prepared and stained with H & E. The diameter of the seminiferous tubule (ST), height of germinal epithelium, and thickness of the interstitial space (IS) were measured microscopically. Furthermore testicular cells were quantified.

Results: The results showed that experimental rats had significantly ($P < 0.001$) different testicular morphometry, histology and cells quantities compared to the control. The histological sections taken from testes of experiment group showed ST with unusual configuration, detached and/or folded basal lamina, thin germinal layer, wide IS with few LC, Sertoli cells (SC) experienced varying degrees of apoptosis and the lumen of ST contained cells debris and very rare few sperms.

Conclusions: Prenatal HPH exposure of male rats adversely affects their testicular morphometry and histological structure and presumably has crucial negative effects on their future fecundity.

1. Introduction

Recent data suggest deterioration of male reproductive health and this deterioration by no means is attributed to xenobiotic chemicals [1–3]. Xenobiotic hormones are widely used in human medicine. The xenobiotic hormones given to women during pregnancy are considered among the endocrine disrupting chemicals capable of modulating and disrupting the endocrine system of the male off-springs leading to deleterious effects on their reproductive health [4]. Prenatal exposure to steroid chemicals is known to induce testicular developmental disorders and consequently reduction in the fertility of adult males [5,6]. Irreversible testicular growth alteration, reduced sperm production and suppressed steroidogenesis have been reported after exposure to steroids [7,8].

Synthetic progesterone (P_4) is widely used in fertility clinics for many therapeutic purposes. It is traditionally used as contraceptive [9], ovulation inducer in primary infertility cases [10], and pregnancy support for threatened miscarriages during the first trimester, abnormal uterine bleeding inhibitor [2] and as luteal supporter for transplanted IVF embryos [11]. This situation entails that many women around the world are exposed to higher dose of xenobiotic P_4. These hormones are accused of eliciting adverse effects on reproductive health and fertility of future generations [9].

Prenatal exposure to P_4 reduced the body weight, testicular weight, total sperm count and damaged the ST and devoid them of sperm [12]. Histopathological study following in utero P_4 administration revealed severe testicular damage, decrease in

*Corresponding author: Adil Salim Elsheikh, Department of Applied Medical Sciences, Community College Najran, Saudi Arabia.
E-mail: adilelgarrai@yahoo.com
Peer review under responsibility of Hainan Medical College.

seminiferous tubules diameters, reduction in number of spermatogenic cells, widening of tubular lumen and interstitial spaces with increment of necrozoospermia [12,13]. Xenobiotic agents interfere with synthesis, secretion, transport, metabolism, binding, elimination of natural blood hormones causing disorder of homeostasis and consequently hinder reproduction and development [14].

Exposure of embryos to the xenobiotic HPC reduced the circulating testosterone levels, sperm cell count and increased necrozoospermia; indicating a probable inhibition of androgen synthesis [2,15]. In the light of the above mentioned studies; the current study investigated the effects of prenatal HPH exposure of male rats on testicular morphometry, histology and cells quantities.

2. Materials and methods

2.1. Animals' management

Eight to ten weeks old females (n = 14) and males (n = 4) of albino Wistar rats were obtained from the animal house of the Faculty of Pharmacy – Jazan University – Kingdom of Saudi Arabia. They were transferred to the animal house of the Department of Anatomy, Faculty of Medicine, Najran University and grouped into two groups. Each group consisted of seven females and two males. They were kept at 12:12 h light/dark cycle (28 ± 7) °C temperature, fed on commercial pellet and offered water *ad libitum*. The female rats were examined 3 times a day for the presence of cervical plugs and those proved mated were kept separated from males. The pregnant females were grouped into two groups and kept separate away from any stress in (90 cm × 45 cm × 15 cm) in sterilized polypropylene cages lined with woody husk. Group I served as control and groups II as the experimental group. The females in the experiment group were subcutaneously injected with 10 mg/kg BW of proluton depot (Hydroxyprogesterone hexanoate; Schering AG; Germany) on the 1st, 7th and 14th of gestation. The dams' body weights were taken on each day of injection to adjust the dose. The prescribed dose of P_4 is within the range of the regular clinical dose given to pregnant women [12]. The females in the control group were injected with a placebo.

2.2. Tissue collection and preparation

Animals were anesthetized with chloroform and sacrificed with cervical dislocation. The testes were dissected and left testes and epididymides were weighed then fixed immediately in aqueous Bouin's solution for 18 h, dehydrated into 70%, 90% and 100% ascending grades of alcohol, cleared with xylene and embedded in paraffin wax. Sections were cut at 5 µm thickness by using an American Optical microtome (A0-821. USA). The sections were mounted onto glass slides, deparaffinized and stained with H & E [16]. The testes were separated from their adjacent epididymides and their diameters and lengths were measured. Relative testes and epididymides weights were then calculated per final body weight [16].

2.3. Histometry

Morphometric measurers of testicles were done according to Thienpot *et al*. [17] and Batra *et al*. [18]. Longitudinal sections of each rat testis were stained with H & E. Then 10 round or nearly round ST were chosen randomly to measure their diameters, the height of germinal epithelium (GE), the thickness of interstitial space (IS) and the number of GE cells using Olympus BX-40 light microscope supported with an image Pro Plus program (100×). Two tubular diameters for each tubule were mapped and their mean recorded. The thickness of IS was measured by measuring three dimensions of spaces assumed to connect the center of each space to the basement membrane of the surrounding ST. The mean of the three dimensions was calculated and multiplied by 2 to obtain the whole thickness of the IS. The GE height was obtained for the same tubules used to determine tubular diameter. GE epithelium was assumed from the basement membrane to the latest stage of GE (spermatids).

2.4. Cells quantitation

The testes of each rat were prepared for light microscopy. After animal scarification, testes were fixed by perfusion with Bouin's fixative for 30 min. The testis was then cut into three vertical longitudinal slices, the middle slice including the mediastinum. After immersion and fixation in Bouin's for another 1.5 h, the slices were dehydrated in ethanol, cleared with xylene and embedded in paraffin wax. From each testis slice five sections of 5 µm were cut, thus 15 sections were obtained and mounted individually onto slides. LC of 15 IS of each rat testis were counted under Olympus BX-40 microscope supported with an Image Pro Plus program. The mean counts of LC were calculated for each group. Also mean counts of the SC and GE cells (spermatogonia type A & B and primary spermatocytes cells) were also recorded [19,20].

2.5. Experimental design

This experiment is a one factorial design to investigate the effects of prenatal exposure to HPH on pubertal rats' testicular morphometry, histology and cells quantities. Forty puppies born to the experiment group (n = 20 puppies) and control group (n = 20 puppies) were allowed to grow for 90 d where they reached maturity. The testes were collected and prepared as above. Morphometry, histometry and cell quantitation were carried as described above.

2.6. Statistical analysis

Statistical analyses for all obtained parameters were performed by using SPSS-16.020 (Chicago, USA). Data were subjected to one way ANOVA and were expressed as mean ± SD. The level of significance was set at $P < 0.05$.

3. Results

3.1. Morphometric aspects

3.1.1. Testicular and epididymal weights

The experimental rats group showed a significant ($P < 0.001$) reduction in testicular and epididymal weights (Figure 1) compared to control group. The mean testicular weights of the control and experimental groups were (2.11 ± 0.33) and (1.60 ± 0.14) g and the mean epididymal weights were (0.67 ± 0.10) and (0.43 ± 0.06) g in the same respective. The relative testicular weight for the control and experimental rats