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Ultra-structure of testes of rats born to dams treated with hydroxy-progesterone hexanoate

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ABSTRACT

Objective: To investigate the effects of prenatal exposure of male rats to hydroxy-progesterone hexanoate (HPH) on ultra-structures of testis.
Methods: Ten male rat puppies born to dams treated with 10 mg/kg body weight of HPH on the 1st, 7th and 14th day after conception were allowed to grow for 90 d; sacrificed, their testes dissected and prepared for electron microscope examination.
Results: Results showed that the testes of all rats born to dams treated with HPH had degenerated germ cells that lost their attachment to surrounding Sertoli cells (SCs) and the basement membrane. The germinal cells were abnormal with cytoplasmic cavitations. SCs had small invaginated nucleus with peripheral nucleolus, folded cell membrane and large lipid droplets. The cytoplasm of SCs had small cavities and the mitochondriae appeared distended and had lost their cristae. The interstitial spaces were wider with few Leydig cells (LCs) that had elongated nucleus with indented nuclear membrane and three prominent nucleoli with dense scattered chromatin. LCs cytoplasm contained abundant lipid droplets, numerous mitochondriae and cytoplasmic vacuoles. The seminiferous tubules germinal epithelium suffered severe degree of degeneration and there were no stages of spermatogenesis.
Conclusion: Prenatal exposure of male rats to HPH induces ultra-structural changes in the seminiferous tubules, SCs and LCs as well as it hampers spermatogenesis. Thus the future fecundity of males born to females treated with HPH during pregnancy will inevitably be affected.

1. Introduction

The male reproductive health disorders are steadily increasing among humans [1]. Many manmade chemicals and medicines are accused of these disorders [2]. Synthetic progesterone is one of the medicines that have been accused of this problem, which is widely used in fertility clinics [3–5]. Homeostasis of progesterone within the body of the pregnant woman is very critical for maintenance of normal pregnancy. Accordingly worldwide, many females are being extensively exposed to this synthetic progesterone which is thought to elicit adverse effects on development and fertility of the next generation [6]. Many studies have confirmed that males born to females exposed to steroid hormones during pregnancy suffered varying degrees of testicular developmental disorders [7–10].

Males born to dams medicated with synthetic progesterone during pregnancy were reported to have small testicles and damaged seminiferous tubules (ST) with large lumen and devoid of sperm [11]. Histopathological studies of the testes of rats exposed to synthetic progesterone during prenatal life revealed decrement in STs diameters, reduction in the number of spermatogenic cells, widening of tubular lumen with necrozoospermia and widening of the interstitial space [11–13].

Exposure of males to hydroxyl-progesterone caproate during embryonic development was associated with increment in the levels of FSH & LH hormones and reduction in the level of circulating testosterone suggesting a probable interference in the synthesis of this hormone [14,15]. Also prenatal exposure to hydroxy-progesterone resulted in permanent alteration in the spermatogenic cells function and suppressed the testicular steroidogenesis by decreasing the steroidogenic enzyme activity

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which in turns may suppress the reproductive activities in male rat [4]. Although many studies were conducted to study the influences of prenatal exposure to synthetic progesterone on the function and histology of the testes of rats; ultramicroscopic studies are absent. Therefore the objective of the current study is to elucidate the effects of prenatal exposure of male rats to hydroxy-progesterone hexanoate (HPH) on the ultra-structures of STs, Sertoli cells (SCs) and Leydig cells (LCs).

2. Materials and methods

2.1. Animals and treatments

Eight to 10 weeks old female albino Wistar rats ($n = 14$) were grouped into two groups of 7 females. Every 7 females were kept with a male of proven fertility at 12:12 h light/dark cycle at 28 °C ± 7 °C temperature, fed on commercial pellets and offered water *ad libitum*. The female rats were daily examined for the presence of cervical plugs and those with cervical plugs were grouped into 2 groups and kept in sterilized polypropylene cages (90 × 45 × 15) cm bedded with woody husk. Group I females were injected with HPH, 10 mg/kg body weight; subcutaneously on the 1st, 7th and 14th day after the day of cervical plug formation (the probable day of pregnancy). The dose of the hormone was adjusted daily to the body weight of the dams. Group II females were injected on the same days with a placebo to serve as control. Ten male puppies born to HPH treated females and 10 male puppies born to the controls were allowed to grow for 90 d; thereafter they were sacrificed to collect the testicles.

2.2. Tissue collection and preparation

After 90 d of rearing the puppies were sacrificed with cervical dislocation and the testes were dissected [16]. The testes were decapsulated and cut into small pieces and rapidly fixed in 0.1 M cacodylate buffered in 3% glutaraldehyde at 4 °C then washed in the 0.1 M cacodylate buffer overnight before they were post-fixed in 1% osmium tetroxide in cacodylate buffer for 2 h. Thereafter they were dehydrated in an ascending series of ethyl alcohol and cleared in propylene oxide and embedded in epoxy resin in an oven at 60 °C for 20 h to produce affirm block. Ultrathin sections were cut from the desired parts, mounted on perforated copper grids (Plano, Wetzlar, Germany) and stained

with uranyl acetate and lead citrate [17]. The sections were examined under a transmission electron microscope with 80 KV acceleration voltages (JEOL, 1200 EXII, Tokyo, Japan).

3. Results

Electron microscopic examination of the STs and the interstitial spaces of the control rats revealed normal spermatogenic cells, SCs and LCs cells. As in Figure 1(A) the spermatogenic cells were in various normal stages of spermatogenesis. The testes of all rats born to dams treated with P_4 had degenerated germ cells that lost their attachment to surrounding SCs and the basement membrane. The spermatogonia cells of rats born to dams treated with HPH were degenerated with vacuolated cytoplasm and small pyknotic nuclei. As in Figure 1(B) there were empty spaces left by the atrophied degenerated germ cells and occasionally the nuclear envelops of the primary spermatocytes had invaginations. The pachytene stage primary spermatocytes appeared smaller in size and the basal lamina appeared thick, folded and contained hypertrophied myoid cells. The germinal cells were abnormal with cytoplasmic cavitations (Figure 2). SCs had small invaginated nucleus with peripheral nucleolus, folded cell membrane and large lipid droplets. The cytoplasm of SCs had small cavities as a result of the degenerative processes that affected its micro-organelles. Figure 3(A) shows that the mitochondriae appeared distended and they had
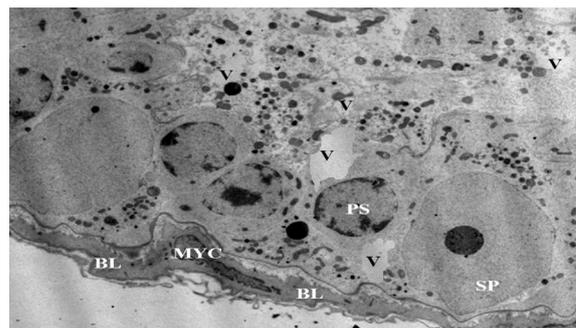

Figure 2. An electron photomicrograph of ST of a rat from the experimental group showing small size pachytene primary spermatocyte (PS) separated from adjoining cell by empty spaces (V), spermatogonia (SP), thick folded basal lamina (BL) and hypertrophied myoid cell (MYC). ×3 000.
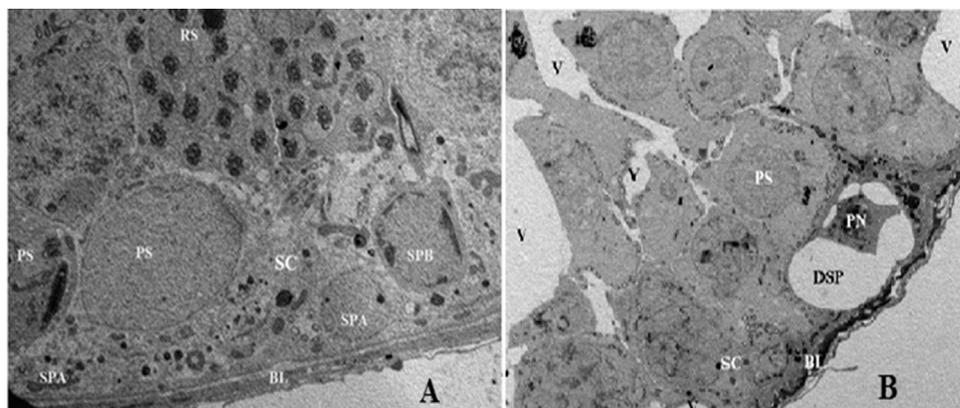

Figure 1. (A), (B): An electron photomicrograph of normal ST of a control rat with normal ultrastructure. SPA: spermatogonia type A; SPB: spermatogonia type B; PS: primary spermatocyte; SC: Sertoli cell; RS: round spermatids (RS), and BL: normal basal lamina. B: An electron photomicrograph of ST of a treatment rat with abnormal ultrastructure. V: vacuoles; PS: small size primary spermatocyte; DSP: degenerated spermatogonia with pyknotic nucleus (PN); SC: Sertoli cell and BL: folded basal lamina (×3 000).
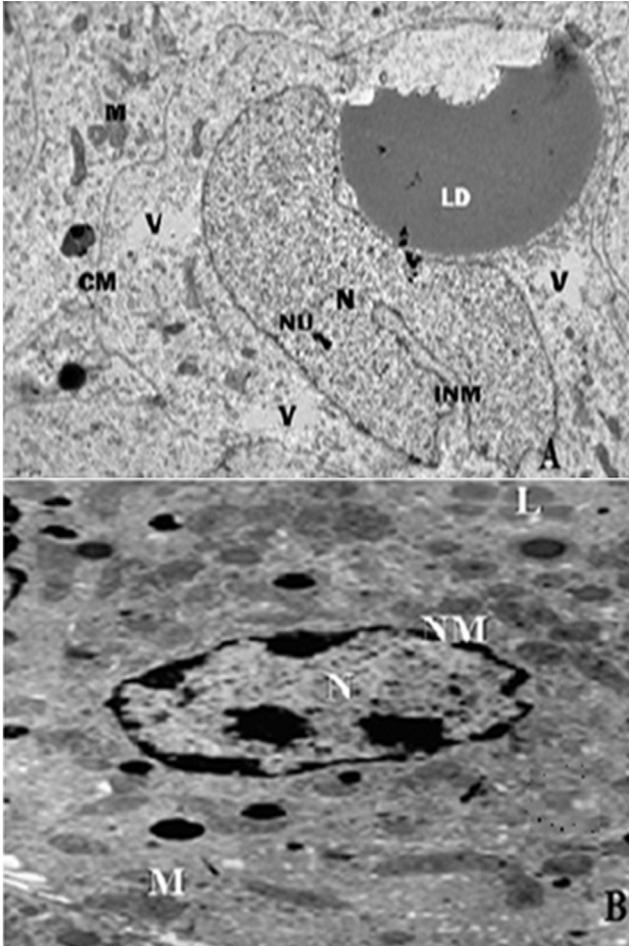
Figure 3. (A), (B): An electron photomicrograph of SC of a rat born to dams treated with P4. The nucleus (N) is small in size, invaginated with ill-defined nucleolus (NU) and the cell membrane (CM) is folded. The cytoplasm contains large & small vacuoles (V), distended mitochondria (M) and lipid droplet (LD). B: LC of a rat born to dams treated with P 4 with elongated nucleus (N), indented nuclear membrane (NM), numerous mitochondria (M) and large amounts of lipid droplets (L) within the cytoplasm (×4 000).

lost their cristae and the LCs had elongated nucleus, indented nuclear membrane, three prominent nucleoli and dense scattered chromatin. Additionally Figure 3(B) revealed the cytoplasm of LCs had abundant lipid droplets, numerous mitochondria and cytoplasmic vacuoles.

4. Discussion

The electron microscope examination of the testes of rats born to dams treated with HPH revealed that the testes of the rats had undergone different ultra-structural defects in the STs, SCs and LCs. The STs had undergone serious apoptotic changes especially the germinal epithelium, SCs and LCs.

Some authors reported that the STs of rats born to dams treated with HPH had undergone different degrees of degenerative histopathological changes [12,13,18–20]. The current study agrees to and confirms these findings. The most serious findings of this study are the apoptotic changes of SCs and LCs. It is well known that the development of the male reproductive traits and reproductive tract depends on normal differentiation and development of LCs and SCs during the prenatal life [21–23]. The function of the fetal LCs during

prenatal life is to synthesize the androgens that are necessary for the development of the masculine characteristics and play an important role in development of the STs and LCs [6,24,25]. Also the androgens that are produced by the fetal LCs are essential for the healthiness of SCs that nurses the spermatogenic cells. Since in this study SCs and LCs were seriously affected by prenatal exposure to HPH and developed serious degenerative changes; the germination of the spermatogonia cells and spermatogenesis process were also affected. A similar phenomenon was reported in testes of rats born to dams treated with HPH during pregnancy [13,26]. The SCs is responsible of orchestrating spermatocytogenesis and spermatogenesis through sequential phases of mitosis, meiosis, and differentiation through hormonal, nutritional, and physical support.

The SCs of the experimental rats had undergone various apoptotic changes such as an ill-defined nucleolus, folded cell membrane, cytoplasmic vacuoles, distended mitochondria and increment of lipid droplet. Furthermore empty vacuolar spaces were observed in the places between SCs where spermatogonia and spermatocytes should differentiate and germinate. These vacuoles indicate the failure of spermatocytogenesis. The ultrastructural apoptotic changes of the germinal epithelium found here support the findings of Blanco *et al.* [20] who described apoptosis in hamster testis following treatment with anabolic androgenic steroids. It is well known that the elimination apoptotic process develop when drugs and toxic agents injure the SCs and reduce their supportive role in spermatogenesis [15]. These apoptotic and degenerative changes that occur in cells and tissues are due to oxidative stress caused by toxic agents [27]. From the results of the current study probably HPH and/or its metabolites reached the fetal testicular circuit and induced an oxidative stress on the fetal testes. Consequently the antioxidant and peroxidation mechanisms within the testes were affected leading to the observed cellular changes [28].

Conflict of interest statement

The authors declare that they have no conflict of interest.

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