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Testicular Changes in Male Albino Rat Pups Exposed to Medroxy-Progesterone Acetate during Lactational Period

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Abstract

There are great concerns regarding the use of synthetic progesterone during breastfeeding due to probable negative effects on future fecundity of male infants. Therefore the present study was conducted to evaluate the effects of exposing male rat pups to depot medroxyprogesterone acetate (DMPA) during lactational period on pubertal testicular histology, morphometry and cells quantitation. Twenty male Wistar rat pups reared to dams treated with DMPA (10 mg/kg BW) every other day during their early lactation period; were employed to achieve the objectives of this study. Other 20 male rat pups reared to untreated dams served as control. The pups were allowed to reach 90 days old, sacrificed, their testes were dissected and weighed and histological sections were prepared.

The results showed that exposing male rat pups to DMPA during the lactational period significantly ($P<0.001$) affected their testicular histology, morphometry and the quantities of testicular cells. The thickness of the germinal epithelium (GE) and the diameter of the seminiferous tubules (ST) were reduced; while the interstitial space (IS) thicknesses were increased. The testicular cells of the rats reared on dams treated with DMPA experienced varying degrees of apoptosis and count reduction. The ST appeared with unusual configuration with detached and/or folded basal lamina, it has few germinal layers, decreased Sertoli cells (SC) and their lumen contained cells debris and very few sperms; while the wide IS contained few Leydig cells (LC). Exposure to DMPA during the lactational period adversely affects the testicular structure. Thus foretells a negative impact on future fertility.

Keywords: Medroxyprogesterone acetate; Testicular structure; Fertility; Lactational period; Morphometry

Introduction

The synthetic progesterone is widely used in human reproductive clinics for many therapeutic purposes such as a contraceptive especially during lactation period [1,2] to reduce the risk of recurrent preterm birth, to treat endometrial hyperplasia, heavy menstrual bleeding, endometriosis, pelvic pain syndromes, breast and uterine cancer, loss of appetite and weight related to AIDS and cancer and topically in certain skin diseases [3-5]. This situation entails that male infants kept on breastfeeding will be exposed to these synthetic hormones; suggesting disorder of their reproductive health at puberty [6-8].

The effects of these synthetic hormones on the reproductive functions of the male human and animal cause considerable concerns among researchers who care to improve the male reproductive health [9-12]. Abnormal changes in the male reproductive health such as abnormal sexual development, alteration in testicular functions and increment of infertility rates were suggested by current studies [10,12-14]. Although, there is minimum evidences that exposure of male infants to exogenous hormones is harmful to their reproductive health, the concern of the use of DMPA during lactation remains [15,16].

Although many studies have asserted that exposure to synthetic progesterone hormone during pregnancy has critical effects on testicular structure and function [4,12-16], great controversies and doubts exist concerning its adverse effects on the male reproduction following their exposure to synthetic progesterone during suckling period. Thus, the aim of the current study is to evaluate the effects of exposing male rat pups during early suckling period to DMPA on pubertal testicular histology, morphometry and cells quantitation.

Materials and Methods

Materials

Experimental animals: Ten weeks old 8 pregnant female Wistar albino rats were grouped into two groups (4 rats each) and kept separate away from any stress in sterilized polypropylene cages (90 cm × 45 cm × 15 cm) lined with woody husk. They were kept at 28 ± 7°C temperature in light/dark cycle (12:12 h), fed on commercial pellet and offered water ad libitum. After delivery 4 dams with 20 male pups (Group I) were injected with a placebo to serve as controls; while 4 dams with 20 male pups (Group II) were subcutaneously injected with 10 mg/kg BW of depot medroxy-progesterone acetate (DMPA; Depo-Provera®) every other day (on the first fifteen days) of lactational period. The dam body weights were taken on each day of injection to adjust the dose. The 40 male rat pups were allowed to grow for 90 days where they reach maturity [2,4,10-12].

Study design: This one factorial experiment study was designed to investigate the effects of lactational exposure to DMPA on suckling male rat's pups' testis histological structure, morphometry, cells quantities at

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puberty. Twenty male rat pups born to the experiment group and 20 pups born to the control group were allowed to grow for 90 day where they reached maturity. The testes were collected and prepared as above. Morphometry and cell quantitation were carried as described above.

Methods

Tissue collection and preparation: Animals were anesthetized with chloroform and sacrificed with cervical dislocation [12,13,17]. The testis was dissected and separated from its adjacent epididymis and connective tissue and its relative weight was then calculated. Then the testes were immediately fixed in aqueous Bouin's solution for 18 h, dehydrated with 70%, 90% and 100% ascending grades of alcohol. The fixed testes were cleared with xylene and embedded in paraffin wax and micro-sections of 5 µm thickness were made by an American Optical microtome (A0-821. USA). Then the micro-sections were mounted onto glass slides, deparaffinized and stained with H&E stain [18].

Histomorphometry: Longitudinal sections of each rat's testis were prepared and stained with H&E as described elsewhere [19,20]. Then 10 round or nearly round STs were chosen randomly to measure their diameters, the height of GE, the thickness of IS and the number of GE cells using Olympus BX-40 light microscope supported with an image Pro Plus program ×100 [12]. Two tubular diameters for each seminiferous tubule were mapped and their mean recorded. The thicknesses of IS were measured by measuring three dimensions of each interstitial space from the space centre 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 interstitial space. The GE height was obtained for the same tubules used to determine tubular diameter. The GE height was assumed from the basement membrane to the latest stage of GE (spermatids).

Cells quantitation: After animal sacrification, testes were fixed by perfusion with Bouin's fixative for 30 min as above. They were then cut into 3 vertical longitudinal slices where middle slices include the mediastinum. After immersion and fixation in Bouin's fixative for another 1.5 h, the slices were dehydrated in ethanol, cleared with xylene and embedded in paraffin wax. From each slice 5 sections of 5 µm were cut, thus 15 sections were obtained and mounted individually onto slides**.** The testicular cells (spermatogonia type A & B, primary spermatocytes cells, LC and SC) of each rat testis were counted under Olympus BX-40 microscope supported with an Image Pro Plus program. The mean counts of each cell type were recorded per section for each group [12,21,22].

Statistical analysis: Data were subjected to one way ANOVA using SPSS-16.020 (Chicago, USA). The means that have been expressed as mean ± SD were compared with Dunnett's test. The level of significance was set at $P<0.05$.

Results

Testis histological observations

The cross sections of the control rats' testes showed compactly arranged, semi-round or oval ST with intact normal basement membrane, normal GE, and normal IS (Figure 1A and 1C). The different stages of spermatogenesis were observed in all the ST. The SCs were normal resting on a normal basement membrane and their lumens are contained masses of spermatozoa (Figure 2A). The IS is normal and contained normal clusters of LC (Figure 3A). The sections of testes of rats reared on dams treated with DMPA showed marked degenerative changes. The ST appear smaller disperse and lost its normal arrangement. The basement membrane of the ST are detached and/or folded and the GE were extremely reduced (Figure 1B). Obvious intercellular cavitations appeared in-between the GE lining the ST as a result of degenerative changes. The GE cells are few and the lumen of the ST is wider with few scattered spermatozoa and necrotic cells' debris (Figure 1D and Figure 2B). The SCs are few, lost their integration with the surrounding GE (Figure 1D and Figure 2B). The IS are wide due to

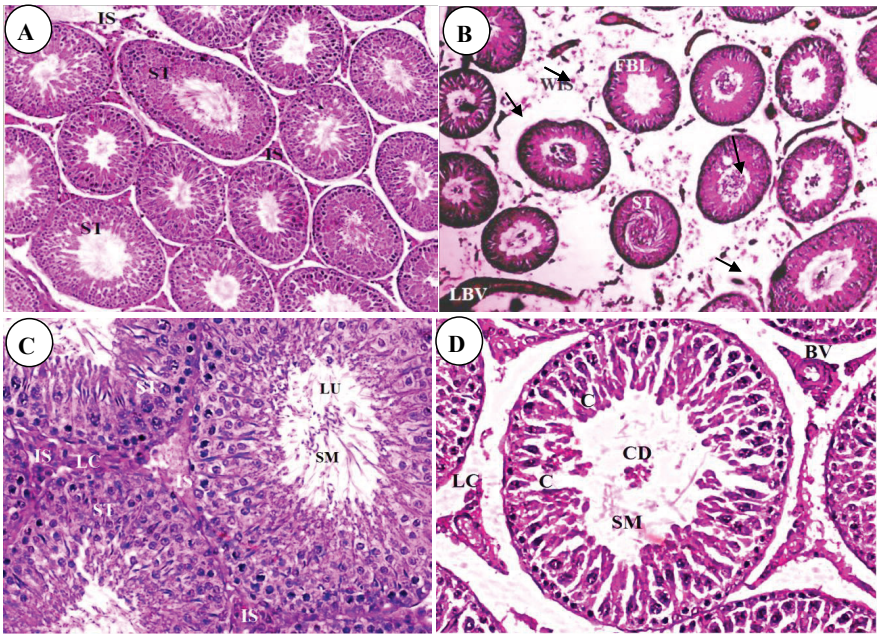

Figure 1: Light photomicrographs of rat's testes parenchyma. Control rat testis (A) and experimental rat testis (B) (H&E ×100). Control rat testis (C) and experimental rat testis (D) (H&E × 400). Seminiferous tubules (ST), interstitial spaces (IS), detached and folded basal laminae (arrowed; FBL) wide interstitial space (WIS), Lumen (LU), sperm mass (SM), Leydig cell (LC), cell debris in the lumen (CD) and large blood vessels (LBV).

ST hypoplasia and the LCs are very few and there are some vacuoles in the ISs (Figure 3B).

Histomorphometry

Relative testicular weights: The mean relative testicular weights of male rats reared on dams treated with DMPA were significantly ($P<0.001$) reduced compared to those of the control (Table 1 and Figure 4). The mean relative testicular weights of the control and experimental groups were (0.011 ± 0.001) and (0.008 ± 0.001) gm, respectively.

Measurements of Sts diameters, Ge And Iss thicknesses: The diameter of the ST, GE height and the thicknesses of the IS of the control and male rats reared on dams treated with DMPA varied significantly ($P<0.001$). The mean diameters of ST of the control and the treatment rats were 281.5 ± 17.2 and 253.2 ± 13.7 µm, respectively. The heights of the GE were 93.7 ± 9.6 and 71.4 ± 7.2 µm, in the same respective as above. The mean thickness of the IS of the control was 110.5 ± 11.3 µm and that of the treatment rats was 133.1 ± 5.5 µm (Table 1 and Figure 5).

Testicular cells quantitation: The counts of the different testicular cells per cross section varied significantly ($P<0.01$) with treatment. As in (Table 2 and Figure 6), the mean counts of spermatogonia type A of the control and male rats reared on dams treated with DMPA were 38.55 ± 3.47 and 27.00 ± 3.73 and type B mean counts were 36.50 ± 3.07 and 21.35 ± 4.11, respectively. The primary spermatocytes mean counts were 37.50 ± 4.02 and 28.10 ± 4.08, SC means were 17.65 ± 2.32 and 9.65 ± 2.50 and LC means were 12.35 ± 2.32 and 6.95 ± 2.35 in the same order as above.

Discussion

This study clearly demonstrates the negative effects of DMPA hormone on the testes of male rats that were breastfed on dams treated with this hormone during lactation period.

The marked abnormalities found in this study are comparable to the findings of Goyal et al. [2] who reported similar effects in neonatal male exposure to steroid hormones. One of the most serious effects of DMPA on the testis observed in this study is the reduction in the number of SC. The SC provide structural support and nutrition to the developing germ cells, produce proteins that regulate and/or stimulate pituitary hormones release and are responsible of phagocytosis of

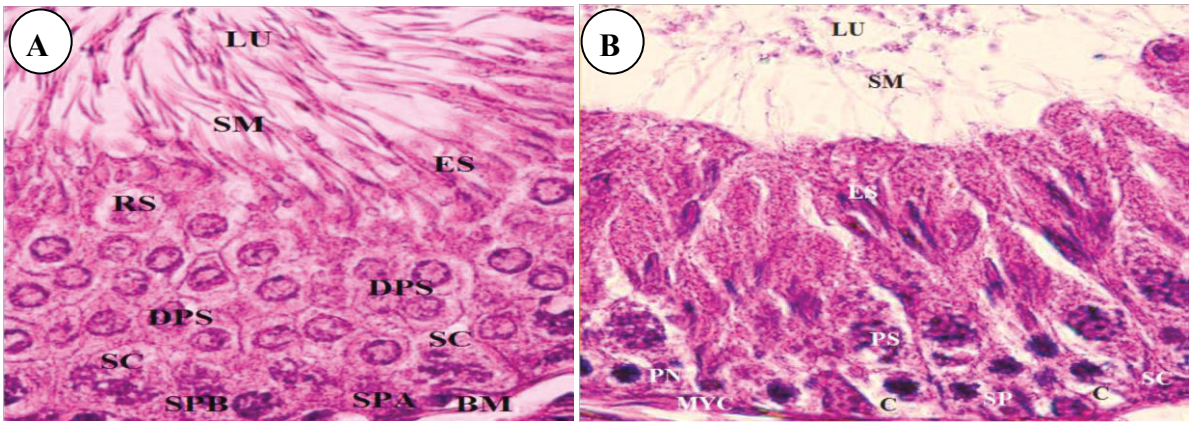

Figure 2: Light photomicrographs of rats Sertoli cells with germinal epithelium. Control rat (A) and experimental rat (B). Basement membrane (BM), myoid cell (MYC), Sertoli cell (SC), cytoplasmic vacuole (C), spermatogonia type A (SPA), spermatogonia type B (SPB), primary spermatocyte (PS), elongated spermatids (ES), rounded spermatids (RS), dividing primary spermatocyte (DPS), tubular lumen (LU) and SM: sperm mass (H&E × 1000).

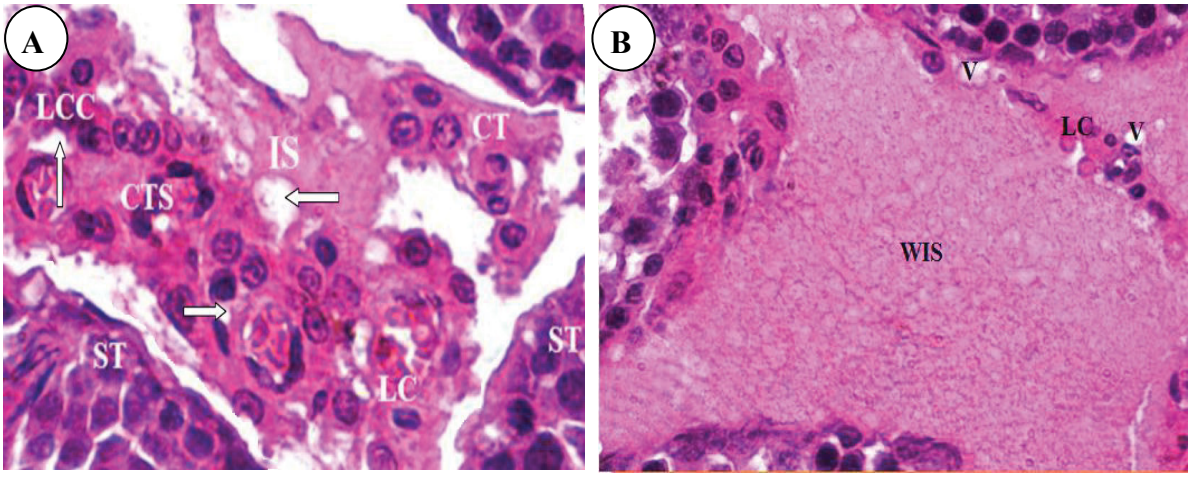

Figure 3: Micrographs of rat's testes interstitial space (IS) of the control (A) and experimental (B). Wider interstitial spaces with few cells (WIS), single Leydig cell (LC), Leydig cell clusters (LCC), vacuoles (arrowed and V), connective tissue (CT) with connective tissue cells (CTS) and surrounding seminiferous tubules (ST) (H&E × 1000).

Parameter	Synthetic medroxyprogesterone Injected(10 mg/kg body weight)	
	Control	Experiment
Relative Testicular Weight/gm	(0.011 ± 0.001)[a]	(0.008 ± 0.001)[b]
Seminiferous tubule diameter/µm	(281.5 ± 17.2)[a]	(253.2 ± 13.7)[b]
Height of germinal epithelium/µm	(93.7 ± 9.6)[a]	(71.4 ± 7.2)[b]
Thickness of interstitial space/µm	(110.5 ± 11.3)[a]	(133.1 ± 5.5)[b]

Data are presented as mean ± SD. Experiment: denotes male rats reared by females dams injected with medroxyprogesterone during lactation. [a, b.] Values with different superscripts in the same raw significantly different (P<0.001).

Table 1: Effects of synthetic medroxyprogesterone on the relative testicular weights, ST diameter, height of GE and thickness of IS.

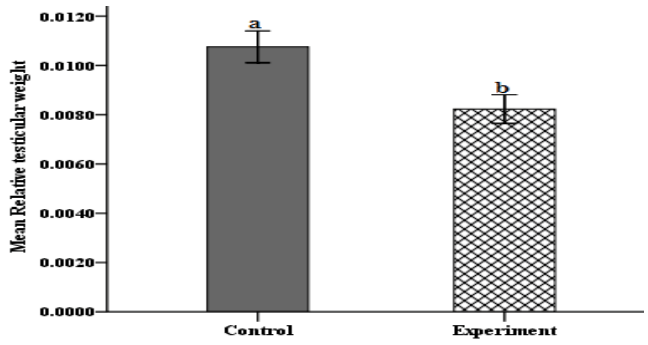

Figure 4: Mean relative testicular weights of control and experimental rats. Bars represent the mean ± SD of 20 replicates. [a,b] differ at P<0.01.

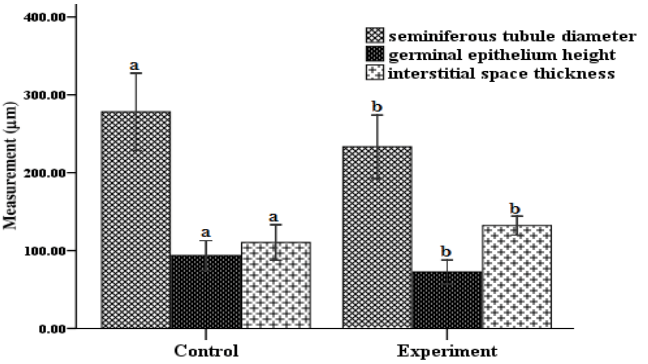

Figure 5: Compares the measurements of seminiferous tubule diameter, germinal epithelium height and interstitial space thickness of control and experimental rats. Bars represent the mean ± SD of 20 replicates. [a,b] P<0.01.

degenerating germ cells and residual bodies [23]. Also in male, SC maintain spermatogenesis whereby each SC is capable to support certain number of germ cells; such function makes the entire sperm production and the total sperm number produced dependent on the total number SC [23]. Furthermore, in this study the number of LC; which is dependent on SCs was reduced [24,25].

The reduction of sperm production and the presence of necrotic cellular debris in the lumen of ST of neonatal male rats exposed to DMPA is a clear indication for SC dysfunction [26]. Reduction in the SC and LC observed in this study indicates that exposure of suckling male to DMPA might affect the gonadal-pituitary hormonal axis and/or it impeded the processes of differentiation and maturation of LC and SC. Thus it might have potential hazardous effects on the testis [27]. The

Parameter	Synthetic Progesterone Injected (10 mg/kg body weight)	
	Control	Experiment
Spermatogonia Type (A)	(38.55 ± 3.47)[a]	(27.00 ± 3.73)[b]
Spermatogonia Type (B)	(36.50 ± 3.07)[a]	(21.35 ± 4.11)[b]
Primary Spermatocyte	(37.50 ± 4.02)[a]	(28.10 ± 4.08)[b]
Sertoli	(17.65 ± 2.32)[a]	(9.65 ± 2.50)[b]
Leydig cells	(12.35.77 ± 2.32)[a]	(6.95 ± 2.35)[b]

Data are presented as mean ± SD. Experiment: denotes male rats reared by females dams injected with medroxyprogesterone during lactation. [a, b.] Values with different superscripts in the same raw significantly different (P<0.01).

Table 2: Effects of synthetic medroxyprogesterone on the testicular cells count.

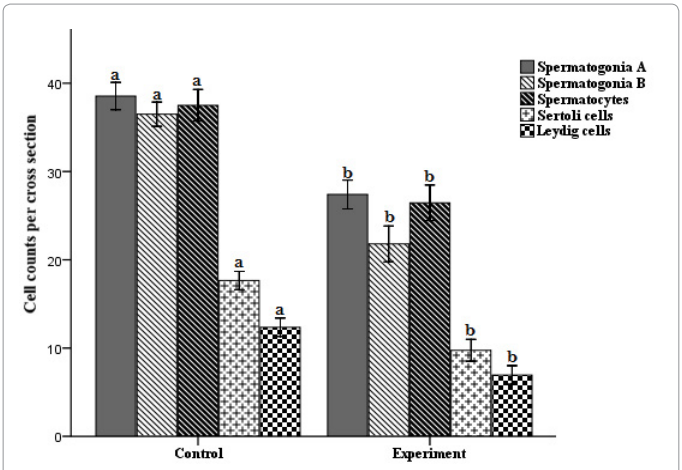

Figure 6: Comparison of testicular cells counts of control and experimental rats. Bars represent the mean ± SD of 20 replicates. [a,b] differ at P<0.01.

present study provides clear evidence that exposure of neonatal male rats to DMPA during lactation period has adverse effect on testicular structure that foretells poor fertility. This finding is in accordance with that of Patel et al. [28] and Truit et al. [29] who thought that newborns cannot metabolize and excrete the steroid hormones ingested during breastfeeding because of their immature liver and kidneys. Additionally they suggested a low plasma-binding capacity which leads to high levels of free biologically active hormones in their bodies.

It is known that the presence of toxic agents in the body enhances production of reactive oxygen species (ROS) in cells & tissues and exert oxidative stress (OS) [30]. It seems that DMPA and/or its metabolites; induced similar oxidative stress which affected the testicular antioxidant system and lipid peroxidation leading to the observed cellular changes in the rats testes [31].

In this study the relative testicular weight of male rats breastfed on dams treated with DMPA was reduced. The weight of the testis is largely dependent on the mass of differentiated spermatogenic cells and it has been used as a measure of spermatogenesis in rats [32]. Therefore, the decrement in relative testicular weight is presumably due to cells degeneration and low sperm production [4,12,16]. Also the degenerative processes are behind the low number of GE cells reported in this study.

Conclusion

Exposing male rat's pups to DMPA hormone have serious adverse effects on pubertal testis structures, morphometry and sperm production which in turn may affect male reproductivity.

Recommendations

Animals in this study were only observed till 90 days of age, and it is possible that other testicular disorders /recovery may become evident with ageing. Therefore, longer term studies may be necessary to identify the full effect of this synthetic drug.

More studies should also be conducted to help identify safety profile of medroxyprogesterone use as a contraceptive during lactation particularly in the presence of any testicular abnormality.

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