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# Maternal treatment with dexamethasone during lactation delays male puberty and disrupts reproductive functions *via* hypothalamic–pituitary–gonadal axis alterations

S.O. Jeje [a,b,*], O.O Akindele [a], M.E. Balogun [c], Y. Raji [a]

[a] Laboratory for Reproductive Physiology and Developmental Programming, Department of Physiology, University of Ibadan, Ibadan, Nigeria
[b] Department of Human Physiology, Cross River University of Technology, Okuku Campus, Cross River State, Nigeria
[c] Department of Physiology, Faculty of Medicine, College of Health Sciences, Ebonyi State University, Abakaliki, Nigeria

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ABSTRACT

The effects of maternal treatment with dexamethasone during lactation on pubertal timing, serum hormonal profile and sperm indices in the male offspring were assessed. Twenty lactating dams were divided into 4 groups ($n = 5$). Group 1 was administered subcutaneously 0.02 ml/100 g/day normal saline at lactation days 1–21. Groups 2–4 were administered subcutaneously 100 μg/kg/day dexamethasone (Dex) at lactation days 1–7, 1–14, and 1–21 respectively. Results showed that there was significant reduction in serum testosterone in the DexLD 1–7 ($p < 0.05$), DexLD 1–14 ($p < 0.01$) and DexLD 1–21 ($p < 0.001$) relative to control. In addition there was a significant reduction in serum FSH and LH in the DexLD 1–7 ($p < 0.01$), DexLD 1–14 ($p < 0.001$) and DexLD 1–21 ($p < 0.001$) when compared with the control. Treatment with dexamethasone during lactation significantly increased the days of preputial separation in the DexLD 1–7 ($p < 0.05$), DexLD 1–14 ($p < 0.05$) and DexLD 1–21 ($p < 0.001$) relative to control. Maternal treatment with dexamethasone throughout lactation period also significantly reduced sperm counts ($p < 0.001$), motility ($p < 0.01$) and increased percentage abnormal sperm ($p < 0.001$) in the offspring when compared with the control. In conclusion, maternal treatment with dexamethasone during lactation may induce delayed puberty and disrupt reproductive functions by altering activities at hypothalamic–pituitary–gonadal axis in the male offspring.



## 1. Introduction

Fetal exposure to stress and its glucocorticoids hormone mediator exerts influences on organ's growth, development, and subsequent offspring physiology and pathophysiology [1]. Sources of maternal exposure to glucocorticoids includes: maternal stress, treatment with synthetic glucocorticoid in threatening preterm delivery, treatment of medical condition such as asthma [2]. Pregnant women whose female offspring are at risk of being born with congenital adrenal hyperplasia are also likely to receive treatment of dexamethasone (a synthetic glucocorticoid that freely crosses the placenta) at doses that could result to a 60 fold higher blood concentration than the mid-gestation blood glucocorticoid level [3]. This is done in order to reduce genital virilization of the female fetus [4]. The programming effects of prenatal glucocorticoid treatment are centrally mediated through the programming of events at hypothalamic–pituitary–adrenal axis [5].

Reports have shown that in rodents and other mammals including non-human primates, prenatal glucocorticoid overexposure resulting from maternal stress or treatment with dexamethasone caused a reduction in birth weight and permanently altered offspring physiology [6–8]. Reduced birth weight is an established risk factor for testicular dysgenesis syndrome (TDS) in humans [9,10] which may also affect reproductive development, including alterations in fetal intratesticular testosterone (ITT) and anogenital distance [11,12].

Chronic exposure to glucocorticoid is known to affect gonadotropin activity in both male and female. In the males, it inhibits gonadotropin secretion and this results in subnormal plasma testosterone concentration. In the females, it also suppresses LH responsiveness to GnRH, resulting in suppression of estrogen and progestin with inhibition of ovulation and amenorrhea [13].

* Corresponding author at: Department of Physiology, Cross River University of Technology, Okuku Campus, Cross River State, Nigeria.
*E-mail addresses:* dhikrilat@yahoo.com, dhikrilat@gmail.com (S.O. Jeje).

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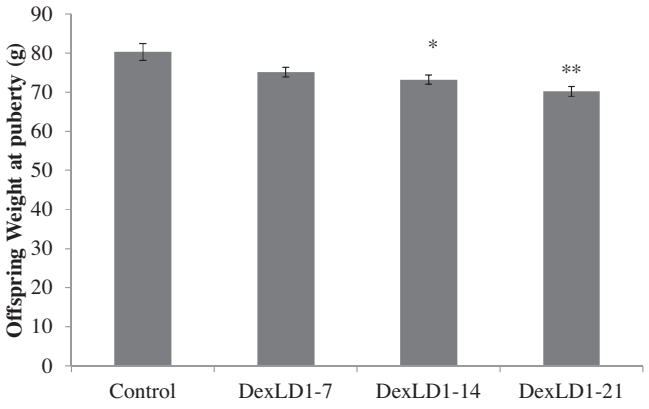


**Fig. 1.** Offspring weight decreases at puberty following maternal dexamethasone treatment during lactation.
Values are presented as mean ± SEM ($n = 5$), One way ANOVA revealed that there was a significant difference in the values. $^{*}p < 0.05$, $^{**}p < 0.01$ were significaltly different when compared with the control.
DexLD 1–7 (Dexamethasone exposure at lactation days 1–7).
DexLD 1–14 (Dexamethasone exposure at lactation days 1–14).
DexLD 1–21 (Dexamethasone exposure at lactation days 1–21).

Environmental compounds that possess steroidogenic and anti-steroidogenic activities affect onset of puberty and reproductive function in adulthood [14,15]. Drake et al. showed that exposure of pregnant rats at embryonic days 13.5–21.5 to combination of dexamethasone and dibutyl phthalate (endocrine disrupting agent) induced disruption of testosterone and male reproductive development [16].

Ostby and Gray [17] reported reproductive toxicity in rat offspring exposed to agents from gestation day 8 (Gd 8) (prior to onset of fetal gonadal differentiations) or on Gd 14 (near the onset of fetal testis steroid hormone syntheses). The exposure should continue through Gd 18 to cover the primary period of reproductive tract development. Pups may also be exposed *via* the mother's milk through postnatal day 3 (PND 3) (to encompass the period of sexual differentiation of the brain and CNS) or through the period of lactation [17]. The timing of maturation of the HPA axis relative to birth is highly specie specific and is linked to landmarks of brain development [18]. In animals that give birth to mature young (primates, sheep and guinea pigs) maximal brain growth and a large proportion of neuroendocrine maturation takes place *in utero* [19,20]. In contrast, in species that give birth to immature young (rats, rabbits and mice), much neuroendocrine development occurs in the postnatal period [5]. As a result, manipulations during lactation period will impact on different stages of neuroendocrine development.

Numerous studies on maternal dexamethasone exposure and programming of adult diseases have focused on exposure during prenatal life. The neonatal effects of dexamethasone exposure have only been observed through direct administration of dexamethasone in pups [21]. It is however not known whether administration of dexamethasone to mothers during lactation will affect the reproductive activities in the offspring. Tilbrook et al., reported that maternal stress during lactation suppresses hypothalamic pituitary adrenal activities in the mother [22]. The clinical use of the synthetic glucocorticoids as ant-inflammatory agents called for the understanding of the possible effect of maternal dexamethasone exposure during lactation on the programming of reproductive functions in the male Wistar rats. Therefore, this study examines the effects of maternal dexamethasone treatment (synthetic glucocorticoids) during lactation on reproductive functions in the male offspring of Wistar rats.

**Table 1**
Treatment of animals and number of offspring collected.

| Group | Treatment | Number of dams (no. of male offspring) |
|---|---|---|
| Control | 0.02 ml/100 g/day Normal saline (LD 1–21) | 5 (6) |
| DexLD 1–7 | 100 μg/kg/day Dexamethasone (LD 1–7) | 5 (6) |
| DexLD 1–14 | 100 μg/kg/day Dexamethasone (LD 1–14) | 5 (6) |
| DexLD 1–21 | 100 μg/kg/day Dexamethasone (LD 1–21) | 5 (6) |

Dex (Dexamethasone), LD (lactation days).

## 2. Materials and methods

### 2.1. Drug

Dexamethasone 21-phosphate disodium salt purchased from Sigma–Aldrich Chemical, UK was used for this study. A dose of 100 μg dexamethasone/kg/day was administered to the drug treated groups [1].

### 2.2. Experimental animal

Twenty female Wistar rats weighing 150–180 g purchased from Central Animal House of University of Ibadan were used for this study. The animals were housed in the Department of Physiology Animal House, University of Ibadan, Ibadan, Nigeria. After two weeks of acclimatization, animals in proestrus were exposed to male breeders overnight and the presence of sperm in their vaginal lavage on the morning after mating confirmed mating. The day on which spermatozoa were found in vaginal lavage was marked as gestation day 1 (Gd1). After mating had been established, animals were randomly divided into four groups of 5 animals each and they were treated during lactation as described below (Table 1). Administration was between 09.00am and 10.00am daily. 100 μg/kg/day dexamethasone was administered to the drug treated groups and 0.02 ml/100 g/day normal saline was administered to the control. All treatments were administered subcutaneously. The litter size was standardized to 5 pups/dam. All protocols involved in the animal experiments were conducted in accordance with ethical laws guiding animal care and use at the University of Ibadan.

The male offspring were allowed to grow to adulthood (12 week of age). Blood was collected from the ocular sinus into flint glass tubes for measurement of serum testosterone, FSH, LH and GnRH levels. Rats were thereafter sacrificed by cervical dislocation. During dissection, the testis and epididymis were carefully collected and fixed in 10% formalin for the preparation of tissue histology.

### 2.3. Experimental design

### 2.4. Evaluation of anogenital distance (AGD) and pubertal timing

Anogenital distance (AGD) at birth was determined by using a Vernier calliper to measure the distance between the posterior base of the papilla and the anterior anus at postnatal day 1. At necropsy, AGD was measured by placing the animal with the base of tail on the edge of a table. Then Vernier calliper was used to measure the distance between the posterior base of the phallus and the anterior rim of the anus [17].

To detect the preputial separation (PPS) (a measure of pubertal timing), male rats were checked daily beginning at 35 days of

age. Gentle pressure was applied to the prepuce to retract the prepuce and expose the glans penis. PPS is complete when the entire perimeter of the prepuce can be retracted evenly around the base of the glans penis [17].

### 2.5. Evaluation of serum testosterone, FSH, LH and GnRH

ELISA kits were used in the quantitative determination of serum levels of testosterone (Rapid lab, United Kingdom), FSH and LH (Dialab, Austria) and GnRH (Cloud Clone, United States).

### 2.6. Histopathology

Histology of testis and epididymis was performed following hematoxylin and eosin stains.

### 2.7. Evaluation of sperm indices

Sperm count and sperm motility were evaluated by microscopy. Epididymal spermatozoa were obtained by mincing the epididymis with anatomical scissors in 10 ml of physiological saline and incubated at 32 °C for 2 min. An aliquot of this solution was placed in Neubauer counting hemocytometer and spermatozoa were counted by using microscope at ×400 magnification. Non-motile spermatozoa were first counted, followed by counting of total number of spermatozoa. Sperm motility was the number of motile sperm cells expressed as percentage of the total sperm counted. Percentage of morphologically abnormal spermatozoa was determined by preparing two slides with H and E stains for morphological examination of live–dead ratio. A total of 400 sperm cells were counted on each slide under light microscope at ×400 magnification.

### 2.8. Statistical analysis

Data were expressed as mean ± Standard Error of Mean (mean ± SEM). Statistical comparisons were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to compare the means of the different treatment groups. Differences between the treatment groups with a $p$-value < 0.05 were considered significant. Data were analyzed with the use of Graphpad Prism Version 5.0 for Windows (GraphPad® Software, San Diego, CA, USA)

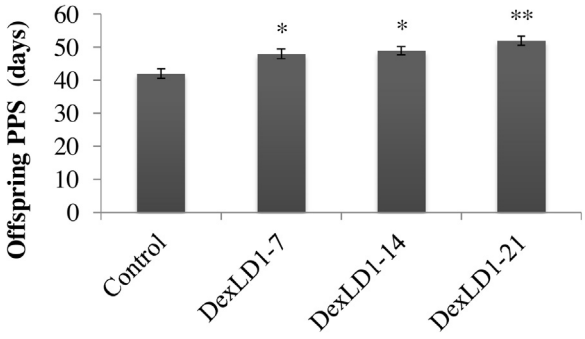


**Fig. 2.** Maternal dexamethasone treatment during lactation increases PPS (preputial seperation) in the male offspring.
Values are presented as mean ± SEM ($n = 5$), One way ANOVA revealed that there was a significant difference in the values. $^{**}p < 0.001$, $^{*}p < 0.05$ *were significant compared with control.*
DexLD 1–7 (Dexamethasone exposure at lactation days 1–7).
DexLD 1–14 (Dexamethasone exposure at lactation days 1–14).
DexLD 1–21 (Dexamethasone exposure at lactation days 1–21).

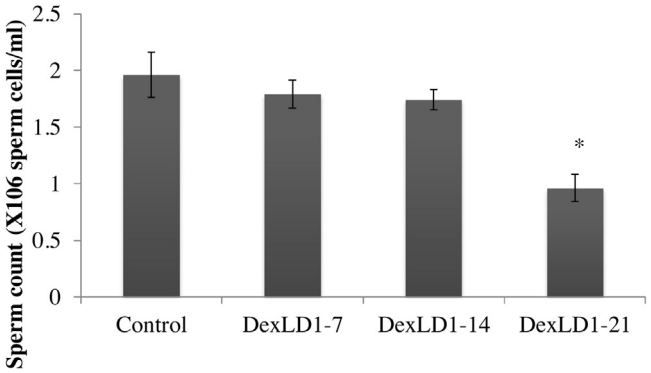


**Fig. 3.** Maternal dexamethasone treatment during lactation decreases sperm count ($\times 10^6$) in the caudal epididymis of the offspring.
Values are presented as mean ± SEM ($n = 5$), One way ANOVA revealed that there was a significant difference in the values. $^{*}p < 0.001$ *was significant compared with control.*
DexLD 1–7 (Dexamethasone exposure at lactation days 1–7), DexLD 1–14 (Dexamethasone exposure at lactation days 1–14), DexLD 1–21 (Dexamethasone exposure at lactation days 1–21).

## 3. Results

### 3.1. Effects of maternal dexamethasone treatment during lactation on anogenital distance pubertal timing and pubertal weight in the male offspring

The mean AGD at birth and at 12 weeks postnatal life following maternal treatment with dexamethasone during lactation were not significantly different in all the treatment groups when compared with the control. The mean days of preputial separation (PPS) increased significantly in DexLD 1–7 ($p < 0.05$), DexLD 1–14 ($p < 0.05$) and DexLD 1–21 ($p < 0.001$) when compared with the control (Fig. 2). The mean weight at puberty was significantly reduced in the DexLD 1–14 ($p < 0.05$) and DexLD 1–21 ($p < 0.01$) when compared with the control (Fig. 1).

### 3.2. Effects of maternal dexamethasone treatment during lactation on sperm indices in the male offspring

There was a significant ($p < 0.001$) reduction in the mean sperm count in DexLD 1–21 treatment group when compared with control (Fig. 3). The mean sperm motility was significantly ($p < 0.01$) reduced in DexLD 1–21 treatments group when compared with control (Fig. 4). There was also a significant ($p < 0.001$) increase in % abnormal sperm in DexLD 1–21 treatment group when compared with control (Fig. 5).

### 3.3. Effects of maternal dexamethasone treatment during lactation on serum hormone level in the male offspring

The mean serum testosterone concentration was also significantly reduced in the DexLD 1–7 ($p < 0.01$), DexLD 1–14 ($p < 0.01$) and DexLD 1–21 ($p < 0.001$) when compared with control (Table 3 ). The mean serum LH concentration was significantly reduced in the DexLD 1–7 ($p < 0.01$), DexLD 1–14 ($p < 0.001$) and DexLD 1–21 ($p < 0.001$) when compared with control. The mean serum FSH concentration was significantly ($p < 0.001$) reduced in the DexLD 1–7, DexLD 1–14 and DexLD 1–21 when compared with control. The mean serum GnRH concentration was significantly ($p < 0.01$) increased in the DexLD 1–21 when compared with control (Table 3).

**Table 2**
Relative organ weight of testis and epididymis in the offspring.

| | Control | DexLD 1–7 | DexLD 1–14 | DexLD 1–21 |
|---|---|---|---|---|
| Testis (%) | 0.398 ± 0.0013 | 0.464 ± 0.021* | 0.487 ± 0.001 ** | 0.5545 ± 0.011*** |
| Epididymis (%) | 0.162 ± 0.021 | 0.178 ± 0.009* | 0.182 ± 0.008* | 0.2112 ± 0.013* |

Values are presented as mean ± SEM ($n = 5$), One way ANOVA revealed that there was a significant difference in the values. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ were significaty different when compared with the control.
DexLD 1–7 (Dexamethasone exposure at lactation days 1–7).
DexLD 1–14 (Dexamethasone exposure at lactation days 1–14).
DexLD 1–21 (Dexamethasone exposure at lactation days 1–21).

**Table 3**
Effects of maternal treatment with dexamethasone during lactation on serum hormone concentration in the offspring.

| | Testosterone (nmol/l) | LH (mlU/ml) | FSH (mlU/ml) | GnRH (Pg/ml) |
|---|---|---|---|---|
| Control | 9.830 ± 1.102 | 4.20 ± 0.67 | 5.94 ± 0.112 | 2.874 ± 0.013 |
| DexLD 1–7 | 4.475 ± 1.31* | 2.23 ± 0.09* | 4.90 ± 0.109** | 1.655 ± 0.021 |
| DexLD 1–14 | 3.347 ± 1.09* | 1.34 ± 0.091** | 3.34 ± 0.21** | 3.21 ± 0.024 |
| DexLD 1–21 | 2.475 ± 0.23* | 0.76 ± 0.051** | 2.31 ± 0.02** | 5.035 ± 0.78* |

Values are presented as mean ± SEM ($n = 5$), One way ANOVA revealed that there was a significant difference in the values. ***p < 0.001, *p < 0.01 were significant compared with control.*
DexLD 1–7 (Dexamethasone exposure at lactation days 1–7).
DexLD 1–14 (Dexamethasone exposure at lactation days 1–14).
DexLD 1–21 (Dexamethasone exposure at lactation days 1–21).

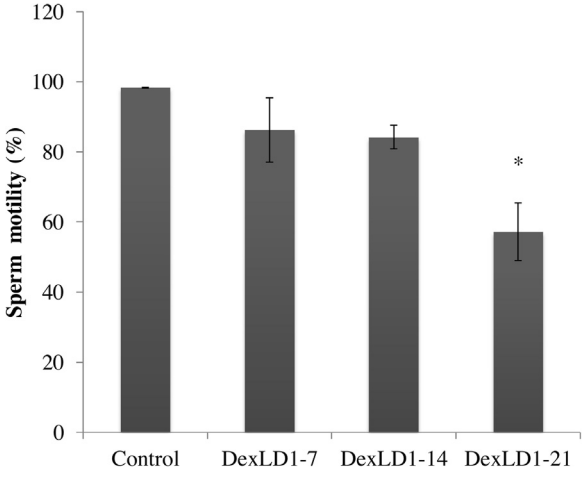


**Fig. 4.** Maternal dexamethasone treatment during lactation decreases percentage sperm motility in the caudal epididymis of the offspring.
Values are presented as mean ± SEM ($n = 5$), One way ANOVA revealed that there was a significant difference in the values. *$p < 0.01$ *was significant compared with control.*
DexLD 1–7 (Dexamethasone exposure at lactation days 1–7).
DexLD 1–14 (Dexamethasone exposure at lactation days 1–14).
DexLD 1–21 (Dexamethasone exposure at lactation days 1–21).

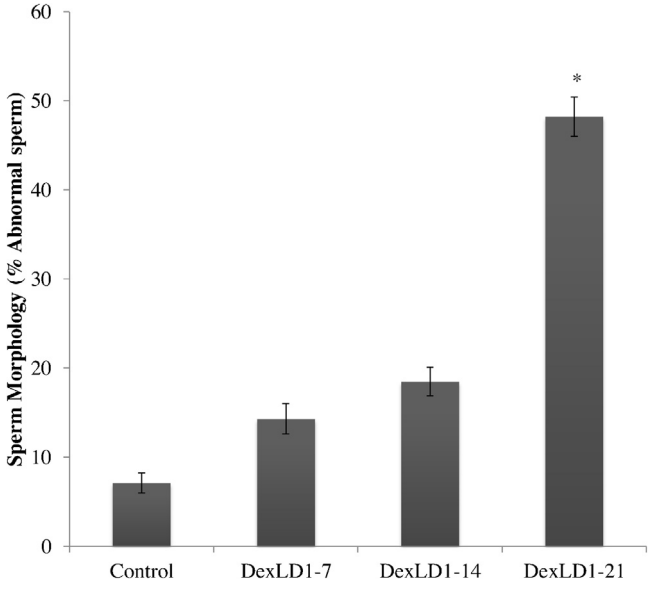


**Fig. 5.** Maternal dexamethasone treatment during lactation increases the percentage abnormal sperm in the caudal epididymis of the offspring.
Values are presented as mean ± SEM ($n = 5$), One way ANOVA revealed that there was a significant difference in the values. *$p < 0.001$ *was significant compared with control.*
DexLD 1–7 (Dexamethasone exposure at lactation days 1–7).
DexLD 1–14 (Dexamethasone exposure at lactation days 1–14).
DexLD 1–21 (Dexamethasone exposure at lactation days 1–21).

### *3.4. Effects of maternal dexamethasone treatment during lactation on relative organ weight*

The mean relative testis weight was significantly increased in the DexLD 1–7 ($p < 0.05$), DexLD 1–14 ($p < 0.01$) and DexLD 1–21 ($p < 0.001$) when compared with the control (Table 2). The mean relative epididymis weight was also significantly ($p < 0.05$) increased in the DexLD 1–7, DexLD 1–14 and DexLD 1–21 when compared with control (Table 2).

### *3.5. Effects of maternal dexamethasone treatment during lactation on tissue histology in the male offspring*

Fig. 6A shows photomicrograph of epididymis of control. Epididymal tubes were round to oval in shape and they were lined with pseodustratified stereociliated columnar epithelium. The epididymal tubes were filled with numerous spermatozoa.

Fig. 6B shows photomicrograph of epididymis of DexLD 1–7. Epididymal tubes were round to oval in shape and they were lined with pseudostratified stereociliated columnar epithelium. The epididymal tubes were filled with numerous spermatoza.

Fig. 6C shows photomicrograph of epididymis of DexLD 1–14. Epididymal tubes were round to oval in shape and they were lined with pseudostratified stereociliated columnar epithelium. The epididymal tubes were filled with abundant spermatoza.

Fig. 6D shows photomicrograph of epididymis of DexLD 1–21. Epididymal tubes were round to oval in shape and they were lined with pseudostratified stereociliated columnar epithelium. The epididymal tubes were filled with inadequate/scanty spermatozoa.

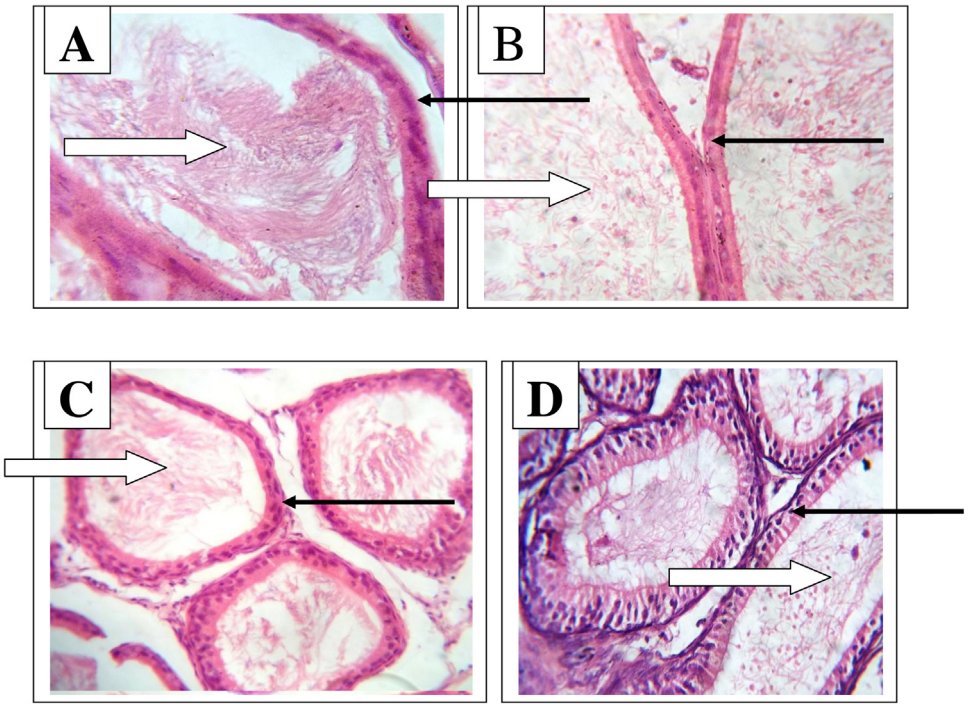


**Fig. 6.** Photomicrographs of the epididymis. Large white arrow shows spermatozoa in the lumen of epididymis and black arrow shows epididymal tubules. (A) control, (B) Dexamethasone administered group at lactation days 1–7, (C) Dexamethasone administered group at lactation days 1–14, (D) Dexamethasone administered group at lactation days 1–21) (**H&E X400**).

Fig. 7A shows the photomicrograph of the testis of control. As shown in the plate the seminiferous tubules size vary from elongated to round. The seminiferous epithelium consists of Sertolis cell, spermatogonia, spermatocytes and spermatids. There is active cell division and maturation as evidence in terminally differentiated spermatozoa.

Fig. 7B shows the photomicrograph of the testis of DexLD 1–7. As shown in the plate the seminiferous tubules size varies from elongated to round. The seminiferous epithelium consists of Sertolis cell, spermatogonia and spermatids. There is active cell division and maturation as evidence in terminally differentiated spermatozoa. The Sertolis cell are also seen to be undergoing mitosis. There is also thickening of the tunica albuginea in the testis.

Fig. 7C shows the photomicrograph of the testis of DexLD 1–14. The seminiferous tubules size varies from oval to round. The seminiferous epithelium consists of Sertolis cell, spermatogonia, spermatocytes and spermatids. There is moderate cell division and maturation as evidence by spermatozoa in the lumen of the seminiferous tubule. The Leydig cells look normal.

Fig. 7D shows photomicrograph of the testis of DexLD 1–21. The testis was characterized by degenerating seminiferous tubules with eosinophylic materials in the lumen. There are very few seminiferous tubules with spermatogonia, primary, secondary spermatids, Sertolis cells and in these there are eosinophilic substances inside the seminiferous tubules. Some seminiferous tubules show sloughing of germ cells into the lumen. The stratum of maturation is few.

## 4. Discussion

Reports from epidemiological study in human have suggested that early life dexamethasone exposure negatively affects the somatic growth [21]. Normal somatic growth is the result of the proper interactions between genetic, nutritional, metabolic and endocrine factors [23–26]. In agreement with this observation, the present study revealed that maternal treatment with dexamethasone during first two weeks of postnatal life and throughout lactation significantly reduced the body weight of offspring at puberty. Although the factors responsible for the growth retardation observed in dexamethasone treated offspring remain unclear, this adverse effect may be linked to alteration in the maternal HPA activity caused by dexamethasone treatment. Tilbrook et al., reported an alteration in HPA activity in the dams due to stress during lactation [22] and since the neuroendocrine system in the pups are still undergoing maturation [5], altered HPA activity in the dams may program stress related glucocorticoid secretion in the offspring. Chronic increase in the glucocorticoid level may raise tissue catabolism or protein breakdown [27,28]. This may lead to increase muscle waste and reduce muscle mass. Supporting this line of thought is the observation from this study that showed raised serum corticosterone level in the dexamethasone treated offspring (data not shown).

Maternal dexamethasone exposure during lactation had no effect on male offspring anogenital distance at necropsy in this study, although puberty was delayed. AGD is androgen dependent and is normally determined *in utero* [29]. Therefore maternal dexamethasone treatment during lactation does not affect AGD. The delayed puberty observed in offspring of all the dexamethasone treated groups may suggest possible alteration in the androgenic system or alteration in the HPG-activities in these offspring. According to Smith and Waddell, the mechanism involved in delayed puberty may be as a result of alteration in the androgen production from the testis or alteration in the hypothalamic–pituitary–gonadal axis [30].

Anti-androgenic agents have been reported to reduce sperm production [31]. In this study, it was also observed that dexamethasone treatment during lactation significantly reduced caudal epididymal sperm count and sperm motility. There was also an increase in the % abnormal sperm. However, the alteration in sperm indices was limited to the male offspring of group that was administered with dexamethasone throughout lactation. Photomicrographs of the testes revealed that maternal dexamethasone treatment throughout lactation induced gross abnormalities in

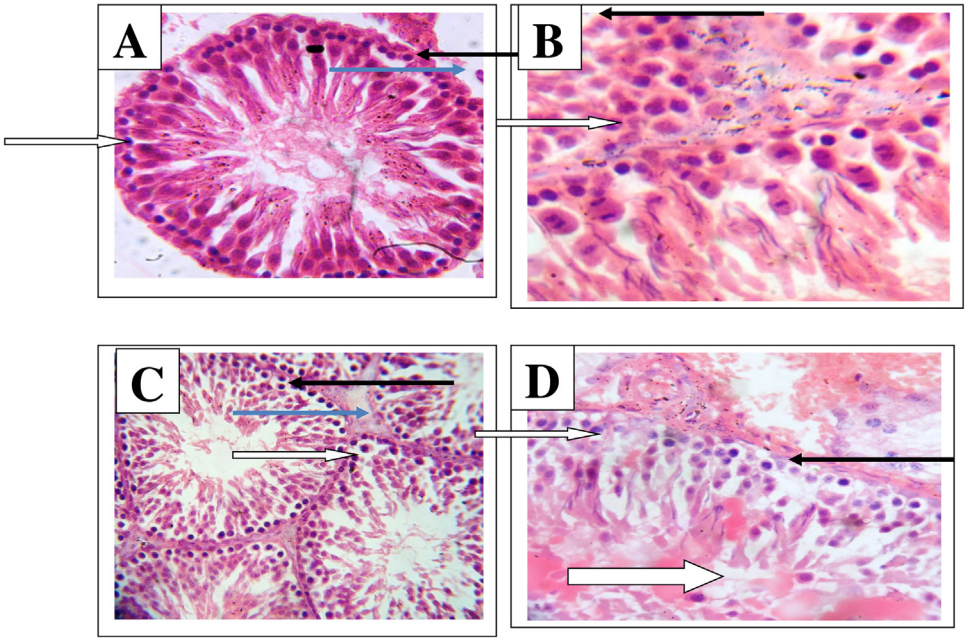


**Fig. 7.** Photomicrographs of testicular tissue showing seminiferous tubules, spermatogonia and Leydig cells. The large white arrow indicates areas of degenerating seminiferous tubules, black arrow shows the seminiferous tubules, white arrow shows spermatogonia and blue arrow shows the Leydig cells. (A) control, (B) Dexamethasone administered group at lactation days 1–7, (C) Dexamethasone administered group at lactation days 1–14, (D) Dexamethasone administered group at lactation days 1–21) (**H&E X400**).

the testes. Some areas of degenerating seminiferous tubules had sloughing off of the germinal epithelium into the lumen. Spermatogenesis takes place in the seminiferous tubule [32], therefore alteration in its structure may interfere with normal sperm production.

The fact that alteration in sperm indices and histology of the testis were only pronounced in the group exposed to dexamethasone throughout lactation suggests that prolonged duration of exposure was important to establish the mechanism involved. This requires further elucidation.

The results from this study also showed that serum testosterone level was significantly reduced by dexamethasone exposure during the first week, the first two weeks and throughout lactation. The Leydig cells do not seem to be responsible for the reduction in testosterone level as they were not anatomically affected. This then suggests that the reduction in serum testosterone may be secondary to alteration in neuroendocrine maturation in these groups. In rats the period of sexual differentiation of the brain encompasses postnatal day 3 [17]. Consequently, agents that alter neuroendocrine development may affect the neuroendocrine control of gonadal steroid production. It is therefore possible that dexamethasone exposure during lactation may program activities at hypothalamic–pituitary–gonadal axis. Similar programming of hypothalamic–pituitary–adrenal axis due to prenatal exposure to dexamethasone at late gestation has been observed [5]. Alteration in this feedback mechanism that regulates gonadal steroid hormone production could lead to disruption in reproductive functions.

Previous studies have reported abnormality in testis due to treatment with anti-androgenic agents *in utero* [33–35]. This may support the observation that maternal dexamethasone treatment during lactation have possible anti-androgenic effect on the offspring and this effect may be centrally mediated through the programming of HPG-axis during early neuroendocrine maturation in the pups.

In conclusion, maternal treatments with dexamethasone especially in the first two weeks of lactation and throughout lactation induced delayed puberty and altered reproductive functions in the male offspring of Wistar rats. This alteration may be associated with the programming of hypothalamic–pituitary–gonadal axis.

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