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

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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].

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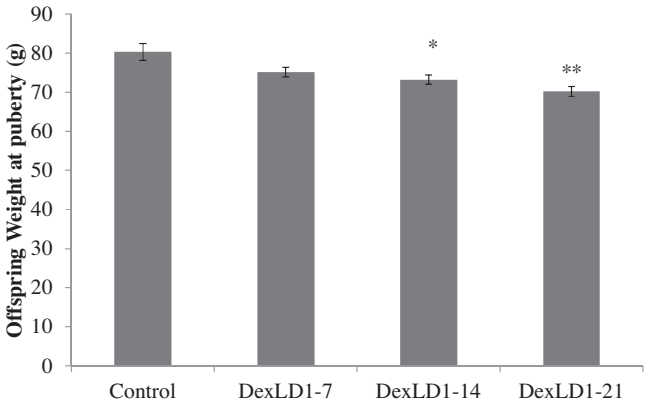

Fig. 1. Offspring weight decreases at puberty following maternal dexamethasone treatment during lactation.
Values are presented as mean ± SEM ($n = 5$), One way ANOVA revealed that there was a significant difference in the values. $^{*}p < 0.05$, $^{**}p < 0.01$ were significaltly different when compared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