

Dexamethasone: Impact on Testicular Activity

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Abstract—Dexamethasone (Dex) is a synthetic glucocorticoid that is used in therapy. However prolonged treatments with high doses are often required. This causes side effects that interfere with the activity of several endocrine systems, including the gonadotropic axis.

The aim of our study is to determine the effect of Dex on testicular function in prepubertal Wistar rats.

Newborn Wistar rats are submitted to intraperitoneal injection of Dex (1µg of Dex dissolved in NaCl 0.9% / 5g bw) for 20 days and then sacrificed at the age of 40days. A control group received NaCl 0.9%. The rat is weighed daily. The plasmatic levels of testosterone, LH and FSH were measured by radioimmunoassay. A histomorphometric study was performed on sections of testis.

Treated groups showed a significant decrease in body weight ($p < 0.05$), testis weight ($p < 0.05$) and plasma levels of testosterone ($p < 0.05$), of LH ($P < .05$) and FSH ($p > 0.05$).

There is a reduction of seminiferous tubules average diameter and also of the seminiferous epithelium thickness with an increasing of lumen tubular. The diameter of the Leydig cells and Sertoli cell nucleus is also significantly reduced. Spermatogenesis is blocked at the stage round spermatid unlike witnesses or elongated spermatid stage is found. These results suggest that Dex administered during neonatal life influences testicular activity in the long term.

Keywords—Dexamethasone, FSH, LH, rat, testis, testosterone.

I. INTRODUCTION

DEXAMETHASONE is a synthetic glucocorticoid widely used in therapy for these immunosuppressive and anti-inflammatory effects. It is also used in the test evaluation of the reactivity of the hypothalamic pituitary adrenal axis [1]. Due to its long biological half-life (36h to 54h), Dex is particularly used for treating conditions which require a prolonged glucocorticoid action like inflammation, allergic and autoimmune disorders, leukemia, nausea, and endocrinology [2], [3]. Although glucocorticoids prevent neonatal respiratory distress syndrome and such treatment may cause hypertension, hyperglycemia and hyperinsulinemia and can affect behavior and neuroendocrine responses. These disorders can emerge throughout the lifespan after periods of apparent normality [4], [5]. The American Academy of Pediatrics (AAP) [6], recommends the limiting of the use of corticosteroids to exceptional clinical circumstances (for example, an infant on maximal ventilatory and oxygen support). In such circumstances, parents should be fully informed about the short and long-term risks that can occur and agree with the treatment. Despite the role of

glucocorticoids in organogenesis, consequences of glucocorticoids administration during prenatal and postnatal life remain uncertain. According Matthews et al. [7], using Dex treatment during pregnancy and neonatal period disrupts both brain development and reproductive function in adulthood.

The aim of this study was to investigate the effects of the Dex, on testis activity and testosterone, FSH, LH levels during a critical period of gonadotrope axis development.

II. MATERIAL AND METHODS

A. Experimental Animals

Pregnant females rats (14) were housed individually, under standard conditions (12:12 h light-dark cycle at $22 \pm 2^\circ\text{C}$) and offered food and water *ad libitum*. Experiments were conducted according to recommendations edited in the Guide to the Care and Use of Experimental Animals.

After birth, new born rats were randomized into control and experimental group, each consisting of eight animals. At 24 H *post partum*, experimental new born received intraperitoneally 1µg/5g b.w. Dex (Dexamethasone phosphate sodique, Soidal group, Algeria. dissolved in 0.9% saline). Control rats received the same volume of saline. Treatment has been maintained to day 21 and the male rats (control: n=8; experimental: n=8) were decapitated 40 days after birth. Blood was collected on 2% EDTA, plasma were separated by centrifugation and stored at -20°C for hormones measurements.

B. Tissue Preparation

The testis were excised, weighed, and then fixed in Bouin's solution for 48h and embedded in paraffin wax. Serial 3µm thick tissue sections were deparaffinized in xylene, rehydrated through a decreasing series of ethanol and stained with Masson's trichrome.

C. Histomorphological Analysis

Testis sections were observed under a Zeiss Microscope, and digital images were obtained with a digital camera attached to the microscope. The diameter of the tubular and luminal seminiferous tubules was measured in 50 circular tubules in each group using an Axio Visio Rel4.6. Software.

D. Hormone Assays

Plasma hormone concentrations were determined by radioimmunoassay (RIA) using Immunotech kit (Beckman Coulter Company). The RIA assay of testosterone is a competitive assay. Samples or calibrators are incubated in antibody-coated tubes with a tracer (hormone) labeled with ^{125}I . The assay of LH and FSH is a sandwich assay, using mouse monoclonal antibodies directed against two different

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epitopes of the molecule and reacting without competition. In tubes coated with a first monoclonal antibody, samples and calibrators are incubated in the presence of a second monoclonal antibody labeled with iodine 125. After incubation, the tube is emptied by aspirating the content and the bound radioactivity is measured. Levels are determined by interpolation using the standard curve. The amount of radioactivity is directly proportional to the concentration of hormone in the sample.

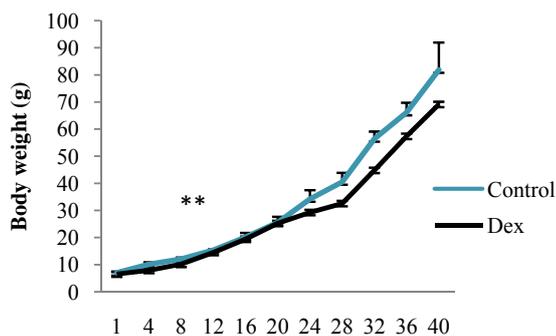
E. Statistical Analysis

Data are expressed as mean ± standard error of the mean (SEM). Groups were compared using Student's t-test, and values of $p < 0.05$ were considered to be significantly different. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

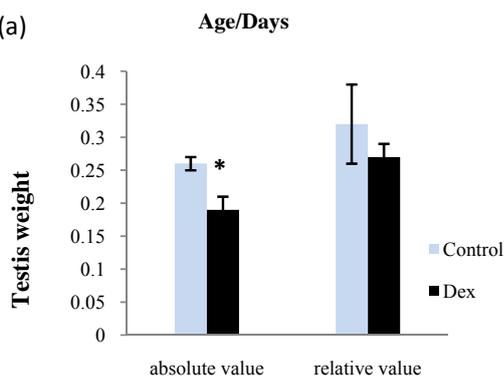
III. RESULTS

A. Body Weight (bw) and Testis Weight

Body weight of treated animals was significantly lower the first week (10.16 ± 0.79 g vs. 12.08 ± 0.44 g ; $p < 0.01$) and remained lower until from the 40 days; however, the differences were not statistically significant (69.13 ± 2.54 g vs. 81.81 ± 10.16 g ; Fig. 1 (a)).



(a)



(b)

Fig. 1 Body weight and testis weight in control and Dexamethasone treated rats. Bars represent mean ± SEM. * $p < 0.05$, ** $p < 0.01$, n=8

The absolute value of testis weight of treated rats was significantly decreased by 36% compared with control group (0.192 ± 0.02 g vs. 0.262 ± 0.01 g; $p < 0.05$). The relative weight (100g body weight) were not different in each group respectively in treated and control rats (0.27 ± 0.02 g/100g bw vs. 0.32 ± 0.06 g/100g bw; $p > 0.05$; Fig. 1 (b)).

B. Plasma Hormone Concentrations

Level of plasma testosterone in the treated group decreased significantly compared to control (0.123 ± 0.013 ng/ml vs. 0.23 ± 0.05 ($p < 0.05$).

Similarly, plasma concentrations of LH decreased significantly way ($p < 0.05$) compared with controls (7.92 ± 0.45 IU/L vs. 2.36 ± 0.91 IU/L).

Plasma FSH levels in treated rats was lower than those of control rats (6.83 ± 0.51 ng/ml vs. 9.70 ± 2.15 ($p < 0.05$) but did not show significant variation (Fig. 2).

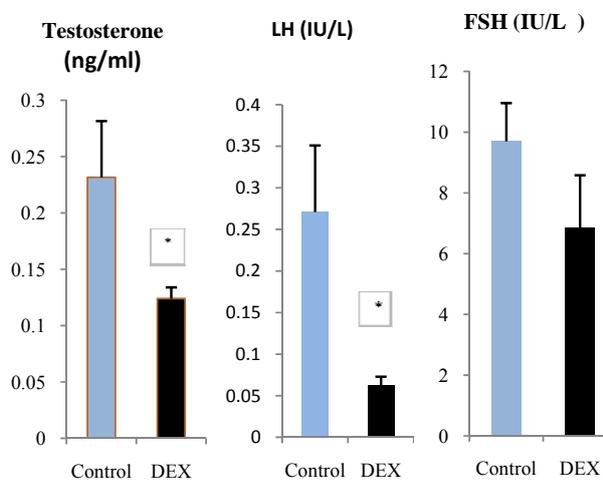


Fig. 2 Plasma Testosterone, LH, FSH in control and Dexamethasone treated rats. Bars represent mean ± SEM.

C. Morphometric Analysis

The morphometric evaluation of testis structure, showed a reduced diameter and global area of seminiferous tubules in rats treated, respectively about 17% and 22%, compared with controls animals ($189.5 \pm 2.67\mu\text{m}$ vs. $220.69 \pm 3.13\mu\text{m}$; $18973.56 \pm 690.36 \mu\text{m}^2$ vs. $23182.54 \pm 2.77 \mu\text{m}^2$; $p < 0.001$).

The superficie of tubule's lumen was 8.96% greater ($2904.1 \pm 0.03 \mu\text{m}^2$ vs. $2643.67 \pm 80.69 \mu\text{m}^2$; $p < 0.01$) while the height of seminiferous epithelium decreased significantly by 33% ($37.007 \pm 2.26\mu\text{m}$ vs. $49.33 \pm 0.35 \mu\text{m}$; $p < 0.001$).

The diameters of Leydig cells and nuclei of Sertoli cells were decreased respectively by 64% and 16% compared with the controls.

TABLE I

MORPHOMETRICS PARAMETERS IN TESTIS OF 40 DAYS OLD RAT, CONTROLS (C) AND TREATED BY DEXAMETHASONE (DEX) DURING THE NEONATAL LIFE

	Seminiferous tubules			Seminiferous epithelium height (μm)	Leydig cell diameter (μm)	Diameter cores Sertoli cells
	Diameter (μm)	Global area (μm^2)	Lumen area (μm^2)			
C	149.753 \pm 0.2	23182.54 \pm 2.77	2643.7 \pm 80.69	49.33 \pm 0.35	8.7 \pm 0.06	8.41 \pm 0.02
Dex	127.466 \pm 2.62	18973.56 \pm 690.36	2904.1 \pm 0.03	37.007 \pm 2.26	5.31 \pm 0.05	7.25 \pm 0.15
	**	***	**	***	***	*

All results are given as mean \pm SEM. *= p <0.05, **= p <0.01, ***= p <0.001, μm =micrometer

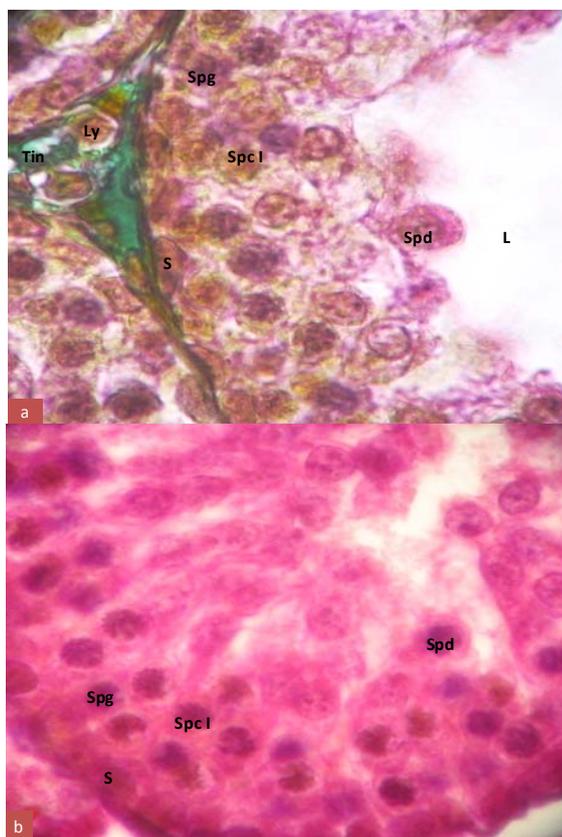


Fig. 3 Testis sections in control (a) and Dexamethasone (b) 40 day old rats. Tin: Interstitial tissue, L: Lumen, Ly: Leydig cell, S: Sertoli cell, Spc I: Spermatocyte I, Spg: Spermatogonium, Spd : spermatid. Magnification: \times 400

Spermatogenesis appeared to be slower in treated rats; the most advanced type of germ cell was round spermatid, while the tubules of control animals contained elongated spermatid (Table I, Fig. 3).

II. DISCUSSION

The prepubertal rats treated with the Dex during the neonatal life by intraperitoneal injection of a dose of $1\mu\text{g}/5\text{g}$ bw/days during 21 days, present during the development, a significant reduction in the body weight compared to the control. This effect on body growing was foreseen since numerous data demonstrate that glucocorticoids are strongly involved in the regulation of body growth during development periods through their complex metabolic, stimulation of somatostatin secretion and acceleration of catabolic process

according to Calogero et al., [8], muscular atrophy resulting from a decreased synthesis protein with an increased degradation [9], and an anorexic effect [9]-[11].

Our results demonstrate that the exposure to Dex during neonatal life has a deleterious effect on testis (weight and morphology) and plasmatic levels of testosterone, LH and FSH in 40day old rat. This indicates that change in the testis are not only a reflexion of whole body growth reducing, but rather indicates a specific effect of Dex on gonadal axis during neonatal development.

In our experimental conditions, rats new born were exposed to Dex in a period of intense proliferation of precursor and differentiate testis cells. Spermatogenesis is initiated in the testes of postnatal mammals from two gonocytes populations with distinct morphological characteristics (pseudopod and round gonocyte). Shortly after birth, the pseudopod gonocytes resume proliferation, migrate to the seminiferous tubule basement membrane, and give rise to stem cell spermatogonia while round cells undergo apoptosis [12].

Mendis-Handagama and Ariyaratne, [13] report, degeneration of fetal Leydig cells, a proliferation and differentiation of the peritubular mesenchymal cells into immature first, then into mature adult Leydig cells. These Leydig stem cells highlighted about the 10th post native day, expresses LH receptors and steroidogenesis enzymes (3 β HSD, cytochromes P450scc and P450c17). The immature cells of Leydig are dominant for the period starting from the 26th-28th days until the 45th to 50th days. These data may explain the decrease in Leydig cells volume and testosterone synthesis.

Several authors report the inhibiting action of the glucocorticoids on the function of reproduction [14], [15]. This action is related to the presence of the receptor of the glucocorticoids on the testicular level, highlighted by immunocytochemistry for the rat by Biagini et al., [16] and Weber et al. [17] in particular on the level of both tubular (germinal cells and Sertoli cells) and interstitial cells. Their rate is high until the 3rd week of development [17], [18] and decreases with adulthood. These receptors are also found in the Hippocampus, hypothalamus, and pituitary gland [19], [20] which explain the changes in plasma concentrations of LH and FSH in rats treated with Dex.

The glucocorticoids inhibit the testicular steroidogenesis by an indirect action on the hypothalamus and pituitary gland [21] and by a direct one on Leydig cells (action on P450scc, 3 β HSD and P450c17) [22], [23]. Leal et al. [24], suggests that the Dex acts on the gonadotrophic cells of pituitary gland former and has an influence on FSH regulation.

Ristic et al. [25] suggests that altered stereological parameters in pituitary gland after exposure to Dex in fetal period could be long-lasting.

Also, our results assumed that the exposure to Dex during neonatal life, vulnerable period in which changing's are unfolding in gonadal axis, proliferation, differentiation and maturing may probably slow down puberty.

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