

# Gestational dexamethasone alters fetal neuroendocrine axis



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## ABSTRACT

This study tested whether the maternal transport of dexamethasone (DEXA) may affect the development of the neuroendocrine system. DEXA (0.2 mg/kg b.w., subcutaneous injection) was administered to pregnant rats from gestation day (GD) 1–20. In the DEXA-treated group, a decrease in maternal serum thyroxine (T4), triiodothyronine (T3), and increase in thyrotropin (TSH) levels (hypothyroid status) were observed at GDs 15 & 20 with respect to control group. The reverse pattern (hyperthyroid status) was observed in their fetuses at embryonic days (EDs) 15 & 20. Although the maternal body weight was diminished, the weight of the thyroid gland was increased at studied GDs as compared to the control group. The fetal growth retardation, hyperleptinemia, hyperinsulinism, and cytokines distortions (transforming growth factor-beta; TGF- $\beta$ , tumor necrosis factor-alpha; TNF- $\alpha$ , and interferon- $\gamma$ ; IFN- $\gamma$ ) were noticed at examined EDs if compared to the control group. Alternatively, the maternofetal thyroid dysfunctions due to the maternal DEXA administration attenuated the levels of fetal cerebral norepinephrine (NE) and epinephrine (E), and elevated the levels of dopamine (DA) and 5-hydroxytryptamine (5-HT) at considered days. These alterations were age-dependent and might damage the nerve transmission. Finally, maternal DEXA might act as neuroendocrine disruptor causing dysmorphogenesis and fetal cerebral dysfunction.

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## 1. Introduction

Glucocorticoids (GCs) and thyroid hormones (THs) regulate many features of fetal brain development (Manojlović-Stojanowski et al., 2008; Lanshakov et al., 2016). The dexamethasone (DEXA), a synthetic GCs, is now used in approximately one in every ten pregnancies in the U.S.A., and subsequently, even though thousands of preterm children are secure the adverse consequences of respiratory distress syndrome, hundreds of thousands of infants really receive treatment (Matthews et al., 2002; Dyer et al., 2010). Additionally, the use of multiple GC courses is common (Hattenbach et al., 2016; Hou et al., 2016; Yennurajalingam et al., 2016), in spite of the probability of subsequent metabolic, cardiovascular and behavioral anomalies (Yeh et al., 2004; Kosinski et al., 2015).

GCs also interrupt the expression of numerous growth factors, insulin, cytoarchitectural proteins, binding proteins and mechanisms of the intracellular signaling pathways (Fowden and Forhead, 2004; Bönnisch et al., 2016). Diminishing the growth has been a well-known side effect of long-term high-dose GC medication in childhood meanwhile its outline as anti-inflammatory and immunosuppressive treatment (Jux et al., 1998; Nagar et al., 2015; Mammi et al., 2016). Also, prenatal GC exposure altered the dopamine sensitivity and serotonergic activity (Slotkin et al., 2006), and the behavior of the hypothalamic-pituitary-adrenal axis (HPAA) (Diaz et al., 1998). This causes some gestational problems and diseases in the fetus/child (Taylor et al., 2015).

As the levels of THs were differentially affected by the development, this study examined the effect of maternal transport of DEXA on fetal thyroid-cerebral monoaminergic axis, fetal cytokines, and fetal neuroendocrine system, which is still a matter of debate.

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## 2. Materials and methods

### 2.1. Experimental animals

Mature white albino rats (*Rattus norvegicus*, Wistar strain) were purchased from the National Institute of Ophthalmology (Giza, Egypt). This study was carried out on twenty-four mature virgin females weighing approximately 160–180 grams and twelve mature males for mating only. To remove any intercurrent infections, I housed the animals in good aerated cages (stainless steel cages) in the department animal house for two weeks. The humidity ( $50 \pm 5\%$ ), standard atmospheric temperature ( $23 \pm 2^\circ\text{C}$ ), and exposure to 12 hrs-light/dark cycles were made constant during the experiment. The animals were fed a standard rodent pellet diet manufactured by an Egyptian company generating oil and soap and some vegetables (Ahmed et al., 2015a,b). The dams were allowed to drink tap water as *ad libitum*. All experimental procedures followed the rules and regulations of the Canadian Council in animal care (Olfert et al., 1993). All efforts were done to diminish the suffering of animals.

Daily investigation of vaginal smears of each female was carried out to detect the estrous cycle. Estrous females showed the presence of cornified cells in vaginal smears. Housing proestrous females mated with the males in a separate cage at a ratio of two females and one male overnight for one or two consecutive days. The manifestation of sperm in vaginal smears distinguished the first day of pregnancy (Marcondes et al., 2002). Then, the pregnant females were transported into separate cages without males to start the experimental study.

### 2.2. Experimental strategy

During whole gestation period, dams received subcutaneous injections of either saline vehicle or 0.2 mg/kg DEXA, a dose at the lower range recommended for therapeutic use in preterm labor (Levin et al., 2014). Dexamethasone sodium phosphate was obtained from Sigma Chemical Co. (St. Louis, MO). Before the decapitation, the maternal body weight gain and fetal body weight were observed. Dams and their fetuses were decapitated under mild diethyl ether anesthesia and sampled at GDs 15 and 20. At these days, the maternal blood samples (6 per group) were taken from a jugular vein while the fetal blood samples (6 per group) were collected rapidly from the umbilical cord at EDs 15 & 20. Centrifugations of the clotted blood were at 3000 rpm (1006.2 g) and  $15\text{--}24^\circ\text{C}$  for 30 min. Also, the fetal cerebrum was homogenized in methanol by using a Teflon homogenizer (Glas-Col, Terre Haute, USA). For each animal, the clear, non-hemolyzed supernatant serum and cerebrum were split into 3 portions and stored at  $-70^\circ\text{C}$  until use for different assays. All reagents were of the purest grades commercially available. Notably, the weight of maternal thyroid gland was recorded at studied GDs.

### 2.3. Maternofetal hormonal examination

Maternofetal serum T3 (Maes et al., 1997), T4 (Thakur et al., 1997), TSH (Mandel et al., 1993), and fetal GH (Reutens, 1995) levels were assessed quantitatively by RIA at the Diabetic Endocrine Metabolic Pediatric Unit, Center for Social and Preventive Medicine, New Children's Hospital, Faculty of Medicine, Cairo University, Egypt. The kits were purchased from Calbiotech INC (Spring Valley, CA, USA).

### 2.4. Fetal leptin, insulin, TNF- $\alpha$ , TGF- $\beta$ , and INF- $\gamma$ examination

Serum leptin, insulin, TNF- $\alpha$ , TGF- $\beta$ , and INF- $\gamma$  levels were identified by ELISA and measured with a microplate reader

(Spectra Max 190-Molecular Devices, Sunnyvale, CA, USA) in biochemistry department, faculty of medicine, Cairo University, Egypt. Commercial kits were applied for the measurement of leptin, insulin and TGF- $\beta$  (ELISA kit-Millipore, St. Charles, MO, USA). TNF- $\alpha$  and INF- $\gamma$  kits were purchased from Invitrogen Corporation 542 Flynn Road, Camarillo, CA 93012 (USA).

### 2.5. Fetal cerebral monoamines examination

The concentrations of monoamines were assessed by the fluorometric method (Ciarlone, 1978), and estimated in the Egyptian National Research Center. The fluorescence excitation was 380 nm for NE, 360 nm for E, 320 nm for DA and 355 nm for 5-HT, as well as the emission by Hitachi (F3010 model) spectrophotofluorometer was 480 nm for NE, E & DA, and 470 nm for 5-HT.

### 2.6. Statistical analysis

The results are evaluated using one-way analysis of variance (ANOVA) (PC-STAT, University of Georgia) followed by LSD analysis to distinguish the main effects and compare various groups with each other. F-probability for each marker expresses the general effect between groups. The data are offered as a mean  $\pm$  standard error (SE) and the values of  $P < 0.01$  and  $P < 0.001$  are considered statistically highly significant and very highly significant, respectively.

## 3. Results

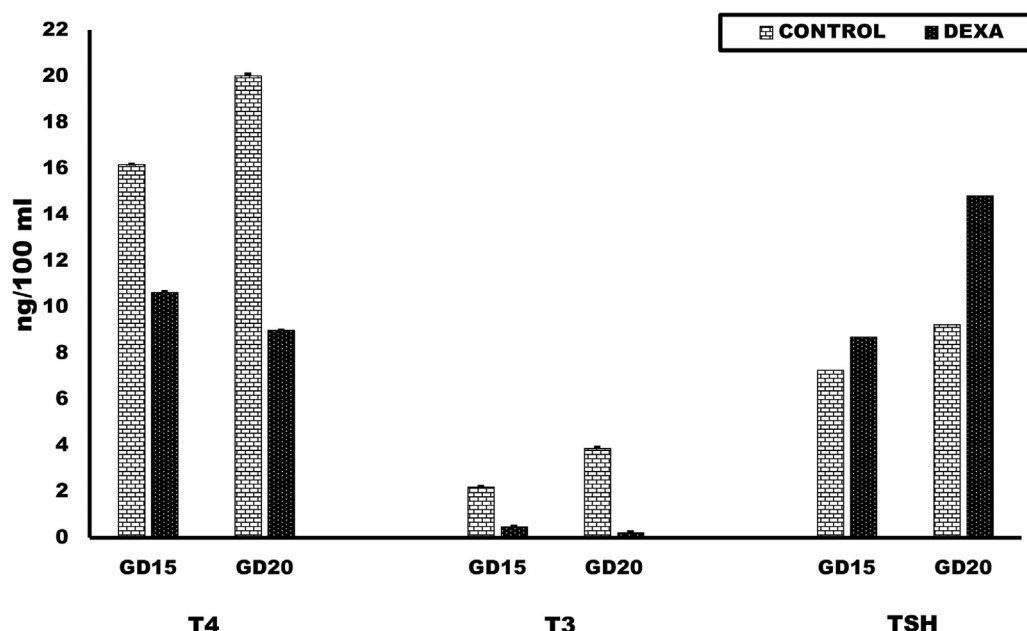
### 3.1. Maternofetal thyroid axis and weight gain

The concentrations of maternofetal serum T4, T3, and TSH in the control group revealed a stepwise increase from GD 15 to 20 (Figs. 1 and 2). At ED 20, the mean values of fetal serum GH of the control group were considerably higher than the corresponding values at ED 15 ( $1.92 \pm 0.44$  &  $0.66 \pm 0.49$ , respectively) (Fig. 2). The administration of DEXA to pregnant rats resulted in a marked decrease (LSD;  $P < 0.01$ ) of T4 and T3 levels and a significant increase (LSD;  $P < 0.01$ ) of TSH levels at GD 15 with respect to control (hypothyroid state). These deviations became more relevant at GD 20 (Fig. 1). Contrariwise, this maternal administration induced a pronounced hyperthyroid state in their fetuses with an increase in the levels of fetal serum T4 and T3 (LSD;  $P < 0.01$ ) and a decrease in the levels of fetal serum TSH and GH (LSD;  $P < 0.01$ ) from ED 15 to ED 20 (Fig. 2). Particularly, these inconsistencies became more profound at ED 20.

Also, the results indicate that the maternal body weight gain of the DEXA-treated group was lower (LSD;  $P < 0.01$ ) than that of the control group at GDs 15 ( $-19.80\%$ ) and 20 ( $-15.02\%$ ) (Fig. 3). However, the weight of maternal thyroid gland of the treated group was augmented (LSD;  $P < 0.01$ ) if compared to the control group at these days (Fig. 4). Additionally, the reduction in fetal body weight was apparent in the maternal DEXA-treated group as the age progressed from ED 15 to ED 20 as compared with the respective control (Fig. 3). Additionally, in both dams and fetuses, the general effect between the groups, for all studied parameters and days, was very highly significant ( $P < 0.001$ ), as evaluated by one-way ANOVA analysis.

### 3.2. Fetal serum leptin, insulin, TNF- $\alpha$ , TGF- $\beta$ , and INF- $\gamma$

In the control group, the gradual increase in the concentrations of the fetal leptin, insulin, TNF- $\alpha$ , TGF- $\beta$  and INF- $\gamma$  markers was profound from ED 15 to ED 20 (Fig. 5). In the maternal DEXA-treated group, the concentration of TNF- $\alpha$ , TGF- $\beta$ , and INF- $\gamma$  was

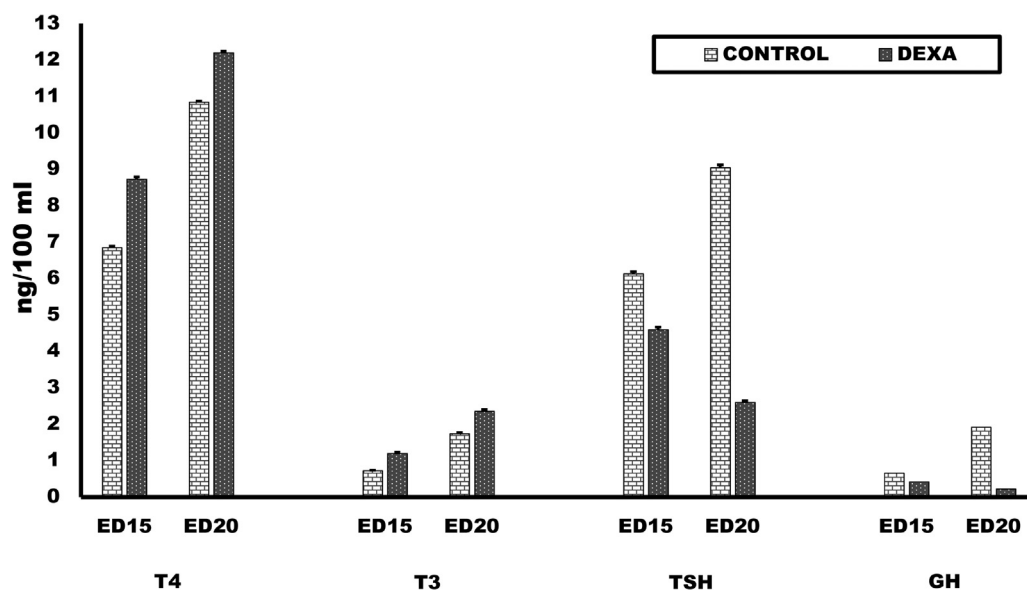


**Fig. 1.** Effect of dexamethasone on thyroid functions [thyroxine (T4), triiodothyronine (T3), and thyrotropin (TSH)] of pregnant rats during the gestational period. Bars represent mean  $\pm$  SE of six rats/group, where the change between DEXA and control/parameter/period is highly significant (\*\* $P < 0.01$ ) as determined by LSD analysis. ANOVA (F-Probability) shows that the effect between the groups/parameter and tested GDs is very highly significant ( $P < 0.001$ ).

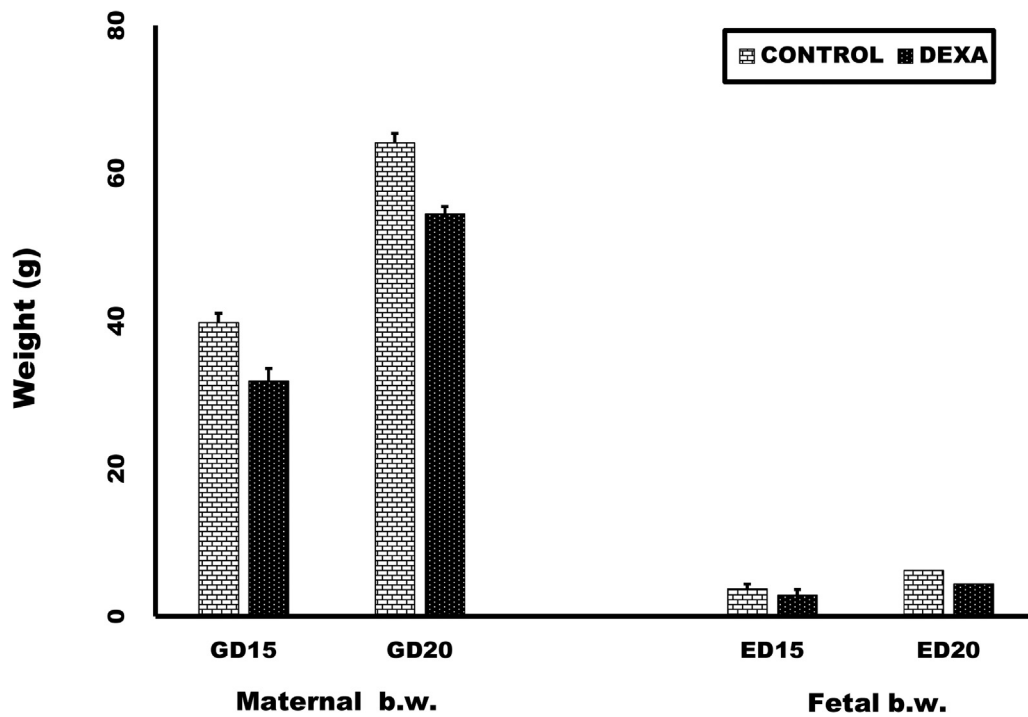
found to be declined (LSD;  $P < 0.01$ ), although the concentration of leptin and insulin was found to be increased (LSD;  $P < 0.01$ ) at both considered days if compared to their levels in the age-matched normal control (Fig. 5). According to one-way ANOVA analysis, the general effect between the groups for leptin, insulin, TNF- $\alpha$ , TGF- $\beta$  and INF- $\gamma$  markers was very highly significant ( $P < 0.001$ ) at all examined EDs.

### 3.3. Fetal cerebral monoamines

In the control group, the concentration of all monoamines [norepinephrine (NE), epinephrine (E), dopamine (DA) and serotonin (5-HT)] was noticeably increased in fetal cerebrum to reach maximum values at ED 20 (Fig. 6). In fetal cerebrum of maternal DEXA group, the levels of NE and E were significantly



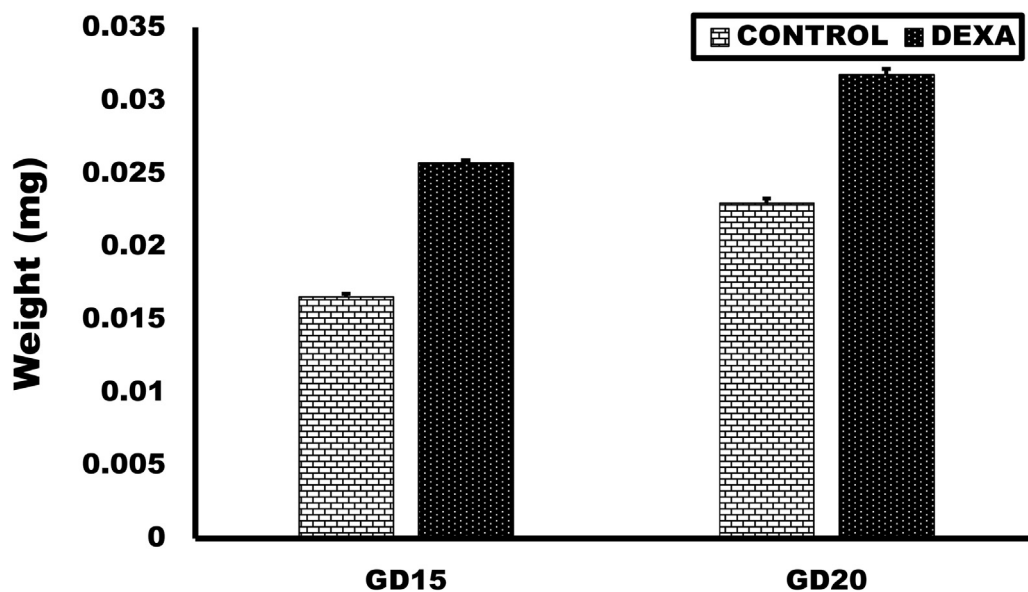
**Fig. 2.** Effect of maternal dexamethasone on fetal thyroid functions [thyroxine (T4), triiodothyronine (T3), and thyrotropin (TSH)], and growth hormone (GH) during the gestational period. Bars represent mean  $\pm$  SE of six rats/group, where the change between DEXA and control/parameter/period is highly significant (\*\* $P < 0.01$ ) as determined by LSD analysis. ANOVA (F-Probability) shows that the effect between the groups/parameter and examined EDs is very highly significant ( $P < 0.001$ ).



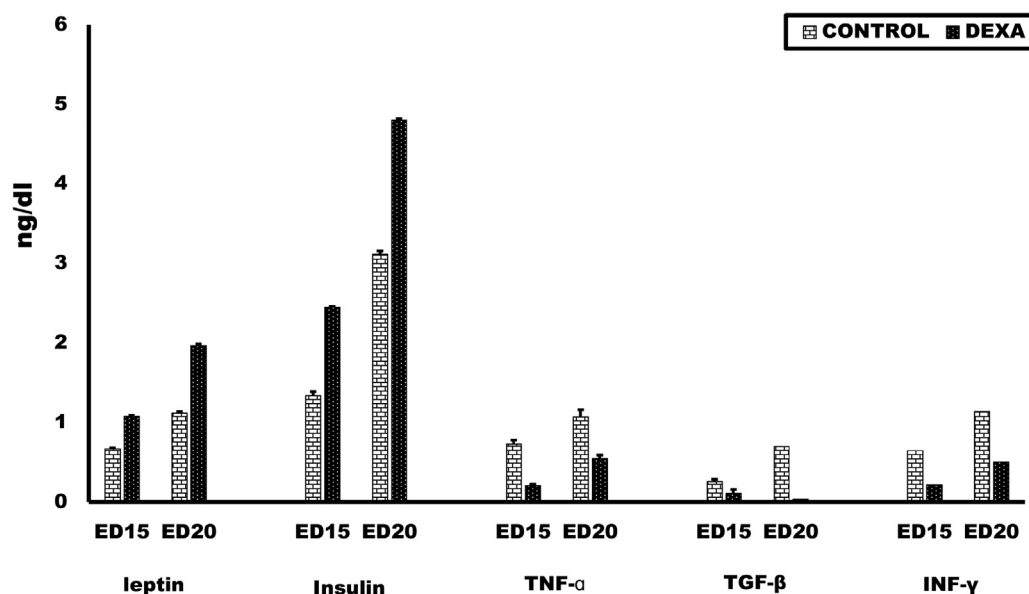
**Fig. 3.** Effect of dexamethasone on maternal body weight gain, and fetal body weight during the gestational period. Bars represent mean  $\pm$  SE of six rats/group, where the change between DEXA and control/parameter/period is highly significant (\*\* $P < 0.01$ ) as determined by LSD analysis. ANOVA (F-Probability) shows that the effect between the groups/parameter and studied GDs/EDs is very highly significant ( $P < 0.001$ ).

decreased (LSD;  $P < 0.01$ ) at ED 15 with respect to its own control. Minimal values for NE and E were noticed in the DEXA-treated group at ED 20 as compared to control (-97.43% & -97.02%, respectively). On the other hand, the elevation in the levels of fetal cerebral DA and 5-HT was observed in the maternal DEXA-treated

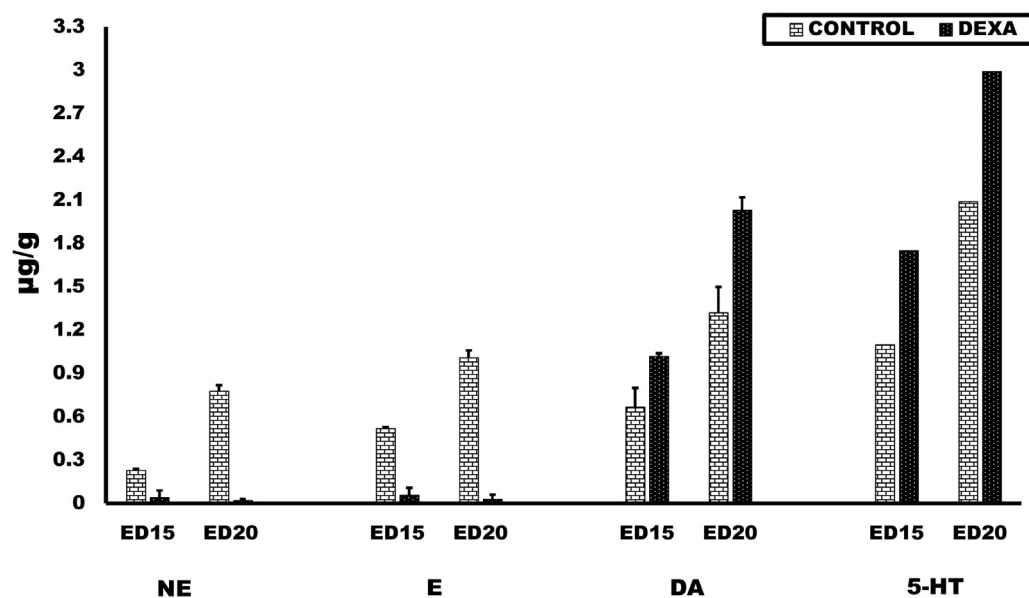
group at ED 15 if compared to control one. This elevation was more obvious at ED 20 as compared to control [DA (+53.78%) & 5-HT (+43.06%)]. The analysis of one-way ANOVA showed that the general effect between the groups for the fetal cerebral NE, E, DA, and 5-HT was very highly significant ( $P < 0.001$ ) at tested EDs.



**Fig. 4.** Effect of dexamethasone on maternal thyroid gland weight during the gestational period. Bars represent mean  $\pm$  SE of six rats/group, where the change between DEXA and control/parameter/period is highly significant (\*\* $P < 0.01$ ) as determined by LSD analysis. ANOVA (F-Probability) shows that the effect between the groups/parameter and studied GDs is very highly significant ( $P < 0.001$ ).



**Fig. 5.** Effect of maternal dexamethasone on fetal leptin, insulin, TNF- $\alpha$ , TGF- $\beta$ , and INF- $\gamma$  during the gestational period. Bars represent mean  $\pm$  SE of six rats/group, where the change between DEXA and control/parameter/period is highly significant (\*\* $P < 0.01$ ) as determined by LSD analysis. ANOVA (F-Probability) shows that the effect between the groups/parameter and considered EDs is very highly significant ( $P < 0.001$ ).



**Fig. 6.** Effect of maternal dexamethasone on fetal cerebral monoamines during the gestational period. Bars represent mean  $\pm$  SE of six rats/group, where the change between DEXA and control/parameter/period is highly significant (\*\* $P < 0.01$ ) as determined by LSD analysis. ANOVA (F-Probability) shows that the effect between the groups/parameter and tested EDs is very highly significant ( $P < 0.001$ ).

#### 4. Discussion

In the control group, the elevation in the concentrations of maternofetal serum T4, T3 and TSH was observed from GD 15 to 20. In the control group, the levels of serum fetal GH, leptin, insulin, TNF- $\alpha$ , TGF- $\beta$ , INF- $\gamma$ , maternofetal body weight gains and maternal

thyroid weight were noticeably elevated in an age-dependent way. These markers were found to be vital in the nutrition, maturation, and intrauterine conditions (Fowden and Forhead, 2004; Ahmed, 2011; Shved et al., 2011; Candelotti et al., 2015). Also, THs through genomic [thyroid receptors (TRs)] and non-genomic [membrane receptors such as integrin receptor ( $\alpha V\beta 3$ )] mechanisms regulate



the growth and immune response in part by changing the secretion and effects of pituitary GH via mRNA or growth factors (Saranac et al., 2013; Ahmed, 2015; De Vito et al., 2015). Additionally, many investigators reported that the GH, TNF- $\alpha$ , and TGF- $\beta$  could facilitate the actions of developing THs (Saranac et al., 2013; Ahmed et al., 2015a,b; Cremaschi et al., 2016).

The maternal administration of DEXA (0.2 mg/kg) during the whole gestation period has diverse effects on the THs axis of the pregnant rats and fetuses. The DEXA administration induced a hypothyroid state (decreased serum T4 and T3 levels, and increased serum TSH levels) in these dams at examined GDs compared to control group. However, the contrary action was noticed in their fetuses (increased serum T4 and T3 levels, and decreased serum TSH levels) at tested EDs. The alterations in maternofetal axis might result from the modifications in the developing hypothalamic–pituitary–thyroid axis (HPTA) or in the peripheral deiodinases to GCs. Also, the maternal DEXA administration raises the maternofetal glucogenic capability and alters the glucose availability during the perinatal period (Franko et al., 2007). In the placenta and kidney, the elevation in T3 may be due to both augmented hepatic productions of T3 from T4 by stimulation of deiodinase 1 (D1), and decreased clearance of T3 by suppression of D3 enzymes (Forhead et al., 2007). In parallel, the transport of DEXA through the fetoplacental barrier inhibited the proliferation of fetal TSH cells at ED 19 (Manojlović-Stojanoski et al., 2008), reduced the synthesis of thyroglobulin in follicular cells and produced a thyroid dysgenesis and dysfunction (Shaya, 2010). This revealed the negative action of DEXA on fetal TSH cells and on prenatal period (Manojlović-Stojanoski et al., 2008). My group postulated that thyroid functions during the development were vigorous, and even mild or transient fluctuations in maternal thyroid-transporters (THTs), TRs or TH levels can directly modify the fetal gene expression profile and the maternofetal HPTA (Ahmed, 2015). Thus, the maternal DEXA might designate some variations in fetal thyroid economy at numerous physiological levels (anti-agonistic on maternal HPTA and agonistic on fetal HPTA actions). This might be variable depending on the dose and on the fetal endocrine states.

The maternofetal body weight gains in the DEXA group were severely decreased while the weight of maternal thyroid was profoundly increased at studied days in relation to that of the control group. Although the reduction in the level of fetal serum GH was observed, the elevation in the levels of fetal serum leptin and insulin was recorded at EDs 15 and 20 in respect to the control group. Changes in these markers will, therefore, interrupt the partitioning of nutrients between the maternal and fetal tissues, and adjust the accessibility of substrates for the fetus. Concurrently, GCs prompt the permanent variations directly on genes and indirectly on physiological systems by varying hormone and growth factors bioavailability and the cellular expression of receptors, enzymes, ion channels, transporters and numerous cytoarchitectural proteins in the fetal tissues (Fowden and Forhead, 2004). The prenatal DEXA administration in rat reduces the birth weight and programs the hypothalamic–pituitary axis (O'Regan et al., 2004). This obstruction is supposed to be multifactorial. The reduction in the GH, insulin-growth factor-1 (IGF1) and glucose levels, and hypercortisolism, resistant or defective hormone receptor action contribute to the growth retardation and delay the sexual maturity (Gutch et al., 2013). A reduced hypothalamic growth hormone releasing hormone (GHRH) secretion and an enhanced somatostatin release together with a direct inhibition of pituitary somatotrophs are the hypothesized actions of the inhibitory consequence employed by GCs (Palmieri et al., 2014). Moreover, the GC-stimulated the release of leptin and insulin, by restricting the activity of the HPAA, might adjacent the feedback loop between this axis and the

adipose tissue during the stress response (Putignano et al., 2003; Bönisch et al., 2016). This elevation results in weakening the body weight gain and suppressing tissue accretion (Fowden and Forhead, 2004). It also changed the developmental endocrine activities (Fowden and Forhead, 2004; Hinds et al., 2016). Increased maternal GC levels have been suggested to be fundamental to the prenatal stress phenotype (Boersma and Tamashiro, 2015). These caused permanent hypertension, hyperglycemia, hyperinsulinemia, and hyperactivity of HPAA (a key neuroendocrine effector of the stress response) (Drake et al., 2005). These results support the view that the maternal DEXA might induce a permanent change in several developmental systems (growth retardation, hyperleptinemia, and hyperinsulinism) by altering THs bioavailability and numerous proteins in their fetuses. The timing of the stress is a significant variable in the prenatal metabolic disorder.

In considered EDs, the maternal DEXA-administration evokes the repression of fetal serum cytokines (TGF- $\beta$ , TNF- $\alpha$ , and IFN- $\gamma$ ) levels if compared to the control group. Several studies have shown that DEXA performs an anti-inflammatory (TNF- $\alpha$ ) (Nagar et al., 2015), anti-fibrotic (TGF- $\beta$ ) (Kosinski et al., 2015), and immunosuppressive (IFN- $\gamma$ ) (Boskabady et al., 2013) activities through down-regulating the expressions or varying the accessibilities of these cytokines (Mašlanka et al., 2013; Huang et al., 2016). Glucocorticosteroids have also been shown to act directly on leucocytes by suppressing cytokines produced by monocytes, macrophages, and T-cells (Steer et al., 2000; Pazdrak et al., 2016). Notably, excessive immunosuppression abolishes the immune system and leads to severe, even lethal infections (He et al., 2014). In parallel, fetal DEXA administration (even at the lowest doses examined) produces noticeable and persistent functional damage in cytokine biology (Dietert et al., 2003), which perseveres well beyond the fetal and infant periods (Coe et al., 2002). These vicissitudes will influence the basal functioning of the cell and its replies to endocrine, metabolic and other stimuli, with significances for its size, proliferation rate and terminal differentiation (Fowden and Forhead, 2004). Thus, it could be inferred that the administration of maternal DEXA might damage the maturation of these markers via the fetal HPTA dysfunction.

The concentration of fetal cerebral monoamines (NE, E, DA, and 5-HT) was regularly increased in the control group from ED 15 to 20. In the recent study (Ahmed et al., 2015a), the progressive behavior between the fetal cerebellar monoamines, THs, growth factors and adipocytokines was documented during the development. Also, the coordination between the THs, THTs and Ds is contributed to the control of developmental TRs-dependent CNS (Aszalós, 2007; Ahmed et al., 2008; Ahmed, 2015). In the maternal DEXA-treated group, a significant drop was observed in the concentration of fetal cerebral NE and E, and a marked increase was recorded in the concentration of DA and 5-HT at EDs 15 and 20 if compared to their respective control. In the developing brain, the reduction in the noradrenergic system due to prenatal DEXA might reproduce the variation in its turnover and compensation altering the dopaminergic or serotonergic signaling (Slotkin et al., 1994). Also, last-trimester DEXA administration raises 5-HT transporter expression in the rat brain, an effect expected to decrease 5-HT obtainability in the hippocampus and elsewhere (Seckl, 2004). There is a possibility that DEXA may exacerbate the neuronal injury by increasing the dopaminergic (Damsma et al., 1990) and serotonergic actions (Rothschild et al., 1985). These explanations are concomitant with the results of Mitsuyo et al. (2003) and Yaniv et al. (2008, 2010). It has also been proposed that the permanent variations in monoamines due to prenatal GCs may be associated with the alpha 2-adrenoreceptor dysfunction (Mokrani et al., 1997), the sensitive developmental stage (Venero et al., 1993) and the GR expression in the brain (Muneoka et al., 1997). Interestingly,

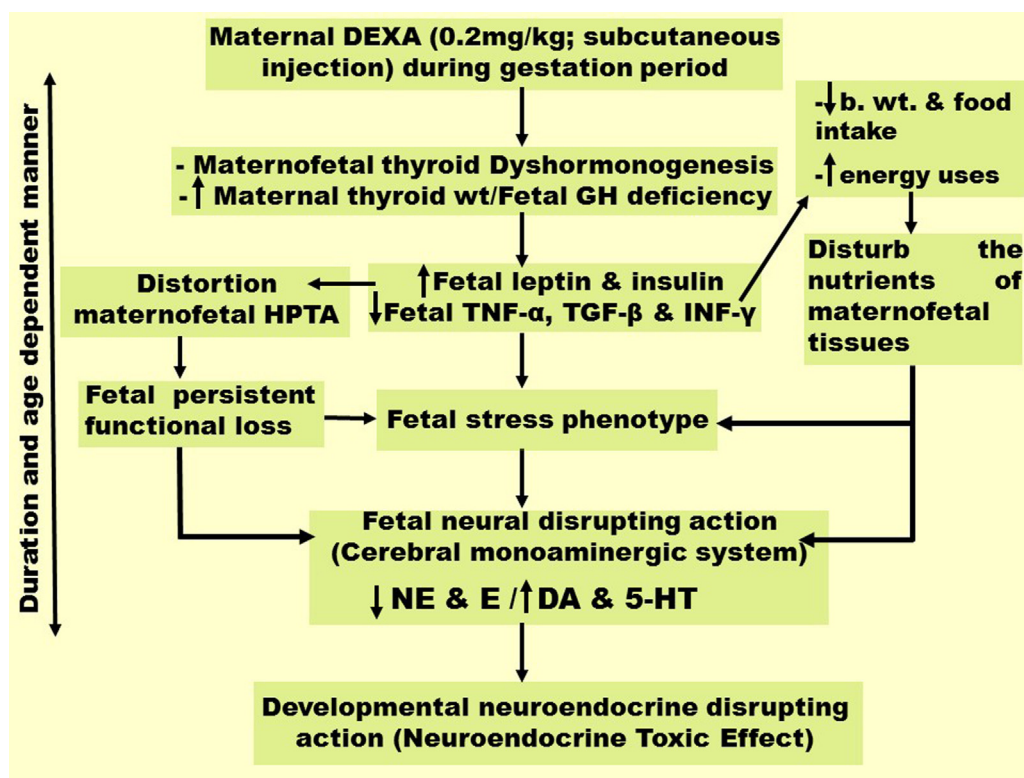


Fig. 7. Effect of DEXA on the developmental neuroendocrine system.

the cellular and molecular fluctuations induced by GCs in individual tissues caused some functional variations at a system level (Fowden and Forhead, 2004). There is considerable evidence that the excess GCs in prenatal period might contribute to HPA axis dysfunction, and numerous neurophysiological and neurodevelopmental disorders in adulthood (Reznikov et al., 2004; Yeh et al., 2004; Lanshakov et al., 2016). It is clear that the disorders in HPTA or Ds due to prenatal stress may impact the developing brain (Ahmed, 2015). These results support the mechanisms of maternal DEXA-induced fetal neurotoxicity (Lanshakov et al., 2016). It appears logic to presume that maternal DEXA might modify the fetal monoaminergic system indirectly through the hormonal imbalance.

## 5. Conclusion

The maternal transfer of DEXA was detected in fetus rat model caused impairment in the development of cerebrum via HPTA dysfunction and might delay the growth and differentiation of the neuroendocrine system. Thus, DEXA may act as endocrine- and neural-disrupting behaviors on the development of THs–cerebral axis (Fig. 7). The maternofetal response to DEXA is likely to vary depending on the intensity, and timing of the administration applied during the gestation. This disruption may limit the therapeutic efficiency of the DEXA because any slight variations in thyroid function during the development can result in brain injury. Additional studies are required to clarify the probable relations with human health.

## Conflict of interest

The author declares that no competing financial interests exist.

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