UDK 577.1 : 61 **ISSN 1452-8258**

J Med Biochem 33: 307–316, 2014

Review paper
Revijski rad

ANTENATAL TREATMENT WITH GLUCOCORTICOIDS AND THE HYPOTALAMIC-PITUITARY-ADRENAL AXIS

ANTENATALNA TERAPIJA GLUKOKORTIKOIDIMA I HIPOTALAMO-HIPOFIZNO-ADRENALNA OSOVINA

Milica Manojlović-Stojanoski[1], Nataša Ristić[1], Sandra Singh[2], Verica Milošević[2]

[1]Institute for Biological Research »Siniša Stanković«, University of Belgrade, Belgrade, Serbia
[2]Clinic for Endocrinology, Diabetes and Metabolic Diseases, Clinical Centre of Serbia, Belgrade, Serbia

Summary

Fetal development is a critical period in the life cycle which is why the placenta provides a structural and physiological barrier that protects the fetus from the outer fluctuations and inner disturbances. A variety of influences from the environment, however, might induce fetal overexposure to glucocorticoids that target the fetal hypothalamic-pituitary-adrenal (HPA) axis and influence the fetal growth trajectory. Development of the HPA axis starts in the early stages of pregnancy, but the timing of HPA axis maturation and the glucocorticoid receptor (GR) expression in relation to birth is highly species-specific. The functional state of the fetal HPA axis plays a key role in the maturation of many organs necessary for intrauterine development and existence after birth. A functional HPA axis in near-term fetuses provides an adequate response to stress and also affects the timing of parturition. Due to their potent effect on the maturation of fetal tissues, synthetic glucocorticoids are used in human pregnancy at risk of preterm delivery. Dexamethasone and betamethasone, as the ones most commonly used, cross the placental enzymatic barrier (11β-hydroxysteroid dehydrogenase type 2 – 11β-HSD2) and have 25-fold higher affinity to the GR than endogenous glucocorticoids, stimulating many aspects of fetal maturation. Despite the numerous positive effects, exposure to synthetic glucocorticoids during fetal development may result in intrauterine growth retardation and fetal programming of the HPA axis function which is associated with cardiovascular, metabolic and psychiatric disorders manifested later in life. Long-term consequences indicate the need for the implementation of new studies that will pro-

Kratak sadržaj

Fetalni razvoj predstavlja kritičan period tokom životnog ciklusa, zbog čega placenta obezbeđuje strukturnu i fiziološku barijeru koja štiti fetus od spoljašnjih kolebanja i unutrašnjih poremećaja. Brojni uticaji iz okruženja mogu dovesti do izlaganja fetusa povišenoj koncentraciji glukokortikoida koji deluju na fetalnu hipotalamo-hipofizno-adrenalnu (HPA) osovinu i utiču na rast. Razvoj HPA osovine počinje veoma rano tokom gestacije, ali vreme njenog sazrevanja i ekspresije glukokortikoidnog receptora (GR) u odnosu na rođenje je specifično za svaku vrstu. Fetalna HPA osovina ima ključnu ulogu u sazrevanju brojnih organa tokom intrauterinog razvoja neophodnih za preživljavanje nakon rođenja, obezbeđuje adekvatan odgovor fetusa na stres, a utiče i na vreme porođaja. Usled snažnog uticaja na sazrevanje fetalnih tkiva, sintetski glukokortikoidi se upotrebljavaju tokom rizičnih trudnoća kod kojih postoji opasnost od prevremenog porođaja. Deksametazon i betametazon, kao najčešće korišćeni lekovi u antenatalnoj terapiji, prolaze enzimsku placentalnu barijeru (11β-hidroksisteroid dehidrogenaza tip 2 – 11β-HSD2) i imaju 25 puta veći afinitet vezivanja za GR u odnosu na endogene glukokortikoide, stimulišući mnoge procese sazrevanja fetusa. Uprkos brojnim pozitivnim efektima, izlaganje sintetskim glukokortikoidima tokom fetalnog razvoja može rezultirati retardacijom rasta fetusa i programiranjem fetalne HPA funkcije. Nastale promene su povezane sa kardiovaskularnim, metaboličkim i psihijatrijskim poremećajima, koji se mogu manifestovati tokom životnog ciklusa. Dugotrajne posledice antenatalnog tretmana ukazuju na potrebu za novim studijama, koje bi obezbedile bolje razumevanje veze između izloženosti povišenim koncentracijama gluko-

Address for correspondence:

Verica Milošević, Ph.D.
Institute for Biological Research »Siniša Stanković«
142 Despot Stefan Blvd., 11060 Belgrade, Serbia
Tel: +381-11 2078-304; fax: +3811 2761-433
e-mail: dimi@ibiss.bg.ac.rs

vide a better understanding of the link between glucocorticoid overexposure during fetal development and adverse outcomes in adulthood.

Keywords: programming, fetal development, HPA axis, dexamethasone, betamethasone, antenatal therapy

kortikoida tokom fetalnog perioda i štetnih posledica u odraslom dobu.

Ključne reči: programiranje, fetalni razvoj, HPA osovina, deksametazon, betametazon, antenatalna terapija

Pregnancy and the HPA axis

In adults, psychological or physical stress activates the hypothalamic-pituitary-adrenal (HPA) axis. Hypothalamic corticotrophin-releasing hormone (CRH) stimulates adrenocorticotropic hormone (ACTH) release from the anterior pituitary, which, in turn, causes the release of adrenal glucocorticoids. Adrenal glucocorticoids are dominantly involved in protein, carbohydrate and lipid metabolism, and strongly affect immune response with the primary goal of achieving homeostasis. An elevated glucocorticoid level provoked by chronic stress exposure exerts long-term changes in negative feedback control at the hypothalamic and pituitary level, while rapid glucocorticoid feedback suppression of basal and stress-induced HPA axis activity is controlled through a central site of action and involves activation of the glucocorticoid receptor (GR) (1).

During pregnancy, numerous adaptations of maternal anatomy and physiology provide optimal conditions and maximal security for the developing fetus, from the environmental fluctuations and inner disturbances (2). The placenta represents a feto-maternal exchange barrier to many of the maternal factors, although sufficient amounts of nutrients and oxygen are delivered to the developing fetus, whereas fetal metabolic excretion products are removed, enabling optimal fetal growth (3). Although the placenta shows broad species-specific differences, the common histological structure includes the maternal blood vessels endothelium (endometrium) and the fetal blood vessels endothelium (chorion). At the earliest stage of development, the principal precursor of the fetal part of the placenta is the trophoblast, evident as a thin cellular layer surrounding the blastocyst. During implantation, the trophoblast becomes the chorion, which is in direct contact with uterine endometrial connective tissue and differentiates into syncytiotrophoblast, a multinucleated cytoplasmic mass or syncytium that arises from the fusion of separate cells of the underlying cytotrophoblast. The functional part of maternal placenta evolves from the uterine epithelium. The placental exchange area is villous in primates and sheep, and has the form of a labyrinth in mice, rats and guinea pigs (4).

Placental endocrine function creates a unique endocrine milieu providing the production of hypothalamic and pituitary hormones, as well as growth factors. The quantity of synthesized adrenal and gonadal hormones, using substrates of maternal and fetal origin, indicates intensive steroidogenic activity in the placenta. Placental CRH is secreted in large amounts into the maternal and fetal circulation influencing the maternal and fetal HPA axis function. In human CRH immunoreactivity was present in hypothalamic paraventricular nuclei (PVN) as early as at 12 weeks of gestation, but intensive hypothalamic stimulation of pituitary corticotropes started from week 16–30 of gestation (4, 5). Further trophic support of the adrenal gland growth and differentiation by the released ACTH becomes evident from around mid gestation towards the term, but there is no clear correlation between the level of plasma ACTH and glucocorticoid concentrations, suggesting immaturity of the negative feedback regulation during fetal development. In rats, significant hypothalamic support of ACTH synthesis and release and promotion of adrenal gland growth and functional differentiation started later, during the last third of gestation. The functioning of negative feedback control was verified by the decreased CRH expression before birth (4, 6).

Contrary to the hypothalamic CRH, placental CRH is positively stimulated by the increasing cortisol level that additionally up-regulates CRH gene expression establishing a feed forward loop (4). Circulating CRH influences fetal pituitary ACTH production and directly stimulates dehydroepiandrosterone (DHEA), dehydroepiandrosteronesulphate (DHEAS) and cortisol output from the fetal adrenals. The secreted DHEAS serves as a substrate for placental estrogen production due to aromatase activity. The created estrogen/progesterone ratio forces interconversion of the biologically active cortisol to inactive cortisone, thereby decreasing negative feedback drive of the higher control centers, and consequently hypothalamic CRH and pituitary ACTH output are enhanced and further stimulate the growth of fetal adrenal glands (7). In humans and primates, this mechanism has enabled the size of near-term adrenal glands to surpass several times the relative size of adult ones, primarily due to the enormously developed fetal zone, while the quantity of synthesized steroids 5 times surpasses the stimulated steroid production in adults. On the other hand, a centrally positioned fetal zone, responsible for androgen production during pregnancy, disappears during the postnatal period. In newborns, dramatic shrinkage of adrenal glands takes place due to involution of the fetal zone caused by apoptotic cell death (4, 8). In rodents, as short gestational period species, adrenal glands synthesize glucocorticoids and aldosterone, but they are unable to produce androgens (9). Instead, androgens are produced during gestation in the placenta, maternal cor-

pus luteum and in the fetal gonads (10). Thus, the placenta and the fetal adrenals make a unique feto-placental functional unit that controls and adjusts HPA axis functioning during *in utero* development.

HPA axis function during development

Prenatal presence of the GR has been demonstrated in the tissue derivatives of all three germ layers using *in situ* hybridization and immunocytochemical techniques. Strictly defined temporal and spatial expression of the GR, in fact, defines the target tissues and periods of glucocorticoid sensitivity during development. The established increase in GR mRNA amount just before the final differentiation step for each glucocorticoid target tissue, and the following decrease in GR mRNA amount upon differentiation, suggest that maturational events are controlled by glucocorticoids (11).

Activity of the fetal HPA axis and the released glucocorticoids direct the fetal growth trajectory to a considerable extent, according to the maternal energy resources. Glucocorticoids promote fetal maturation, as they affect the developmental and maturational processes of a variety of tissues and organs, in order to enable functional adaptations requested for extrauterine survival. Under constantly changing environmental conditions, the survival of newborns depends on the maintenance of homeostasis, thermogenesis and a stable energy supply, in view of the transition from placental to enteral nutrition (12). To achieve this, glucocorticoids influence the timely differentiation of vital organ systems, including the central nervous system, gastrointestinal system, as well as other endocrine axes and tissue hormones sensitivity. Fetal lung maturation and surfactant production, enhancement of gluconeogenic enzyme activities in the liver and glycogen deposition, as well as adaptive thermogenesis in brown adipose tissue are also controlled by glucocorticoid action (12–14).

Like in adults, where stress implicates an HPA axis response, adverse intrauterine conditions in fetuses also provoke fetal HPA axis activation (15). For example, fetal hypoxemia leads to an increase in the fetal ACTH concentration with up-regulation of mRNAs of the ACTH receptor and steroid-synthesizing enzymes in the adrenals that result in selective increase of cortisol synthesis in fetal sheep (16). Negative feedback control of the HPA axis by glucocorticoids begins to operate near the term, suggesting that active control of the glucocorticoid level is indispensable even during fetal development (17).

Coordinative activity of the HPA axis and the placenta in fetuses plays an important role in determining gestation length, which is certainly closely related to the maturation level of numerous organ systems. Although the exact mechanism remains unclear, an increase in fetal HPA activity has been established in humans and primates during late gestation. An increased cortisol concentration, that is generated locally in the placenta or derived systemically, stimulates prostaglandin synthesis, decreasing its metabolism, which results in an enhanced prostaglandin output. Huge amounts of DHEAS, produced by the fetal adrenals, undergo aromatization in the placenta and subsequently lead to elevated circulating estrogen levels (18). The created hormonal milieu and enhanced output of prostaglandins induce the expression of oxytocin receptors indispensable for uterine contractility. Late in gestation, an increase in unbound CRH fraction intensifies circulation in the feto-placental unit, potentiating the effects of local mediators and hormones that further up-regulate myometrial contractility and represent an important step in the initiation of birth (4, 19).

Antenatal treatment

Fetal growth and the development of fetal tissues and organs are dependent on many factors, including the hormonal environment. As mentioned, endogenous glucocorticoids produced by the fetal adrenal glands have a crucial role in these processes and exert beneficial effects on the maturation of fetal tissues where GR have been located. The timing of HPA axis maturation and expression of the genes encoding GR in relation to birth is highly species-specific. In species with a long gestational period (sheep, guinea pigs, primates and humans) that give birth to mature young, maximal brain growth and many aspects of neuroendocrine maturation take place *in utero*. But in species with short gestational periods (rats, rabbits and mice) that give birth to immature offspring, much of the neuroendocrine development, GR genes expression as well as the final maturation of endocrine axes occur in the postnatal period (20). Under normal circumstances, glucocorticoid levels are significantly lower in the fetus than in the mother and transplacental glucocorticoid diffusion is normally limited by the actions of the placental barrier enzyme, 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) (21). This enzyme plays a key role in regulating glucocorticoid concentrations in the fetal circulation by inactivating maternal glucocorticoids at the level of the placenta, and protects fetuses from glucocorticoid overexposure. In a rat and human study, it has been demonstrated that attenuated activity of 11β-HSD2 due to carbenoxolone application, maternal exposure to stress or antenatal treatment with synthetic glucocorticoids may expose the fetus to inappropriately high levels of glucocorticoids, which leads to reduced fetal growth followed by long-lasting consequences (12).

Due to their potency in maturating tissues and organs, synthetic glucocorticoids have been used for more than 40 years in human pregnancies at risk of preterm delivery. Antenatal glucocorticoid administra-

tion is perhaps one of the most effective prenatal interventions for the prevention of complications related to preterm birth, which include respiratory distress syndrome (RSD), intraventricular hemorrhage and, most importantly, neonatal mortality. The origin of this practice comes from Liggins and Howie (22), whose preliminary results indicated a reduction in the incidence of RSD and mortality in humans after maternal betamethasone treatment. Subsequent trials have confirmed the efficacy of this treatment in the reduction of complications associated with preterm delivery (23). According to the National Institutes of Health (NIH), all the fetuses between 24 and 34 weeks of gestation at risk of preterm delivery are potential candidates for antenatal glucocorticoid treatment. The recommended treatment consists of two doses of 12 mg betamethasone administered intramuscularly 24 h apart, or four doses of 6 mg dexamethasone every 12 h. They cross the placental barrier, have 25-fold higher affinity to the GR than endogenous glucocorticoids, and act in accordance with its nuclear transcription factor function (24). The question of the relative risk and benefit of repetitive courses of prenatal glucocorticoid administration is still open.

Antenatal glucocorticoid therapy is indicated in women with premature rupture of membranes and women with progestational or gestational diabetes at risk of preterm delivery, but contraindicated in cases of maternal systemic infections including tuberculosis (24).

During gestation, synthetic glucocorticoids are also used to treat several other clinical conditions of the mother, such as autoimmune diseases, allergies and asthma. The incidence of congenital adrenal hyperplasia (CAH) in fetuses is rare, but treatment with glucocorticoids is necessary to improve later outcome. In contrast to antenatal therapy applied in preparation for preterm birth, treatment under these clinical conditions often begins early in the pregnancy and lasts throughout gestation (25).

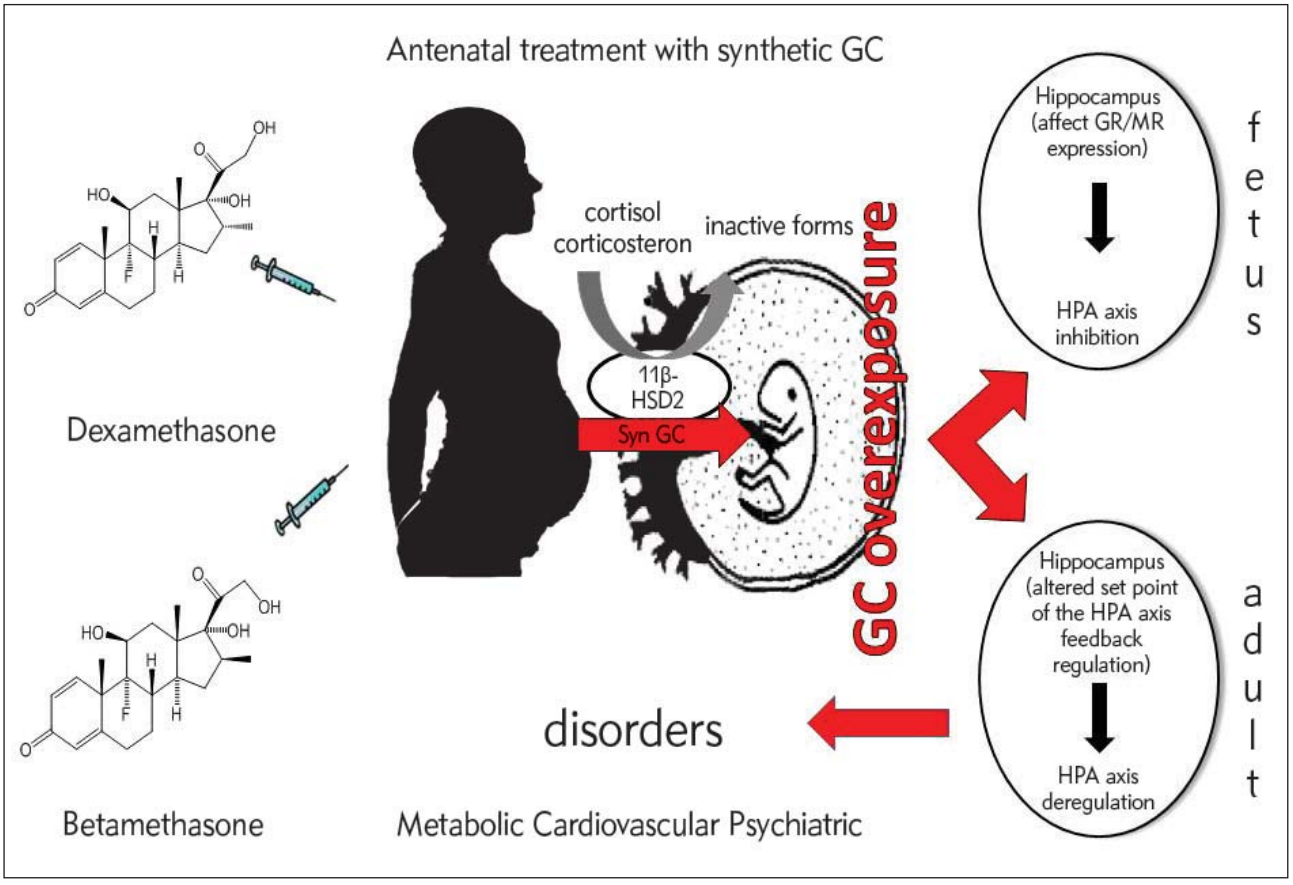

Figure 1 Synthetic glucocorticoids, such as dexamethasone and betamethasone, are used in antenatal therapy, cross the enzymatic placental barrier (11ß-hydroxysteroid dehydrogenase type 2 – 11ß-HSD2) and lead to fetal glucocorticoid overexposure. They affect GR/MR expression in the hippocampus and cause inhibition of the HPA axis function during fetal development. Molecular and cellular changes established during fetal development reflect on postnatal functioning of the HPA axis. The altered set point of HPA axis feedback regulation from the hippocampus leads to HPA axis deregulation under basal and stress conditions in offspring. Alterations of HPA axis functioning in adults, due to fetal glucocorticoid overexposure, are associated with an increased risk of cardiovascular, metabolic and psychiatric disorders.

Despite the mentioned positive effects, repeated doses of antenatal glucocorticoids have potentially negative effects on fetal growth and adverse effects on brain growth and development in animals and humans. Impaired intrauterine growth and low birth weight, as markers of an adverse *in utero* environment, are associated with hypertension, glucose intolerance, insulin resistance, type 2 diabetes, dyslipidaemia, obesity, reproductive and brain disorders in adulthood (20, 26). The phenomenon is known as programming. The concept of early life programming explains the link between prenatal environmental events, altered fetal growth and development, and later pathophysiology (27). Maternal undernutrition, placental insufficiency and exposure to glucocorticoids that includes maternal stress or antenatal glucocorticoid therapy are the most common conditions that lead to intrauterine growth retardation (IUGR) associated with programmed outcomes in adulthood. Such conditions ultimately result in fetal glucocorticoid overexposure, since they mediate the programming effects of nutritional and other environmental challenges during pregnancy (28). The nature of the modifications that occur as a consequence of glucocorticoid overexposure is dependent on the dose and timing of the exposure (*Figure 1*).

Antenatal glucocorticoid therapy reduces mortality and morbidity in preterm babies and at the same time increases the risks of dysregulation of the metabolic function and endocrine axes, including stress response, growth and reproduction (12). More randomized human follow-up studies are needed to better understand the short-term benefits and long-term consequences of antenatal exposure to synthetic glucocorticoids on development and health.

Antenatal treatment and fetal HPA axis function

Results from animal and human studies demonstrate that fetal glucocorticoid overexposure results in fetal growth retardation that persists for a prolonged period after birth. The developing HPA axis is especially sensitive to glucocorticoid overexposure, as they strongly affect the establishment of HPA axis structure and function (29). Glucocorticoids control the proliferative activity of cells at all the HPA axis levels, and influence the gene expression of receptors, enzymes, ion channels, as well as cytoarchitectural proteins. All these changes at the cellular and molecular level result in altered set points of the delicate hormonal feedback mechanisms that become evident during the life cycle, under basal or stress circumstances (12, 29, 30).

The hippocampus has a central role in the negative-feedback regulation of HPA axis by glucocorticoids which interact with the abundantly expressed GR and mineralocorticoid receptors (MR). Application of synthetic glucocorticoids during pregnancy provokes a sex-specific influence according to hippocampal mRNA GR and MR levels in fetuses. Single maternal Dx exposure results in significant increases in MR and GR mRNA in the CA1–2 region of the hippocampus, and MR mRNA in the dentate gyrus in female fetuses, while changes are absent in male guinea pig fetuses (31). After multiple maternal treatment with Dx, marked increases in MR mRNA level in the hippocampus of female fetuses were recorded, while in males enhancement of GR mRNA was shown in limbic structures indicating changed hippocampal feedback sensitivity to glucocorticoids, natural or synthetic (32). Quantitative *in situ* hybridization demonstrated that the MR mRNA level is transiently decreased after a single Dx injection in the mouse fetus (33). Synthetic glucocorticoids applied during pregnancy lead to reduction in brain weight in fetuses. Literature data show similar effects of fetal glucocorticoid overexposure in different species, including mice, rhesus macaques and humans, such as reduced hippocampal volume with a reduced number of pyramidal neurons and pronounced degeneration of neuronal perikarya and dendrites, resulting in a decreased number of synaptic contacts (34, 35). Epigenetic changes caused by antenatal treatment with synthetic glucocorticoids that alter genome-wide transcription and modify GR DNA binding in the fetal hippocampus during late gestation have been recently shown in guinea pigs (36).

The hypothalamus, as the main site for glucocorticoid negative feedback, represents a regulatory place where the stress response starts and terminates. Neurons in the parvocellular division of the hypothalamic paraventricular nuclei (PVN) synthesize both CRH and AVP, or only CRH, and their axons project to the median eminence where the produced neuropeptides are released. Repeated fetal glucocorticoid exposure to synthetic glucocorticoids, dexamethasone or betamethasone, inhibits fetal HPA function, as significantly decreased levels of CRH mRNA in the hypothalamic PVN in both male and female fetuses were established (32). Delay of CRH release in the external zone of the median eminence, shown by immunocytochemistry, might further confirm the decreased synthetic activity of CRH neurons in the fetus and neonatal offspring after fetal Dx exposure (37). Delicate cellular and molecular changes caused by the changed glucocorticoid milieu during development become evident at the level of hypothalamic neurosecretory cells morphometric parameters in fetuses and during the neonatal period (38). Analysis of neurosecretory parvocellular and magnocellular PVN neurons suggested that the area and diameter of neurosecretory cells nuclei at the levels were CRH neurons are dominantly positioned are significantly changed under the influence of dexamethasone, but the range of changes depends on the timetable and dosage of dexamethasone applied (39).

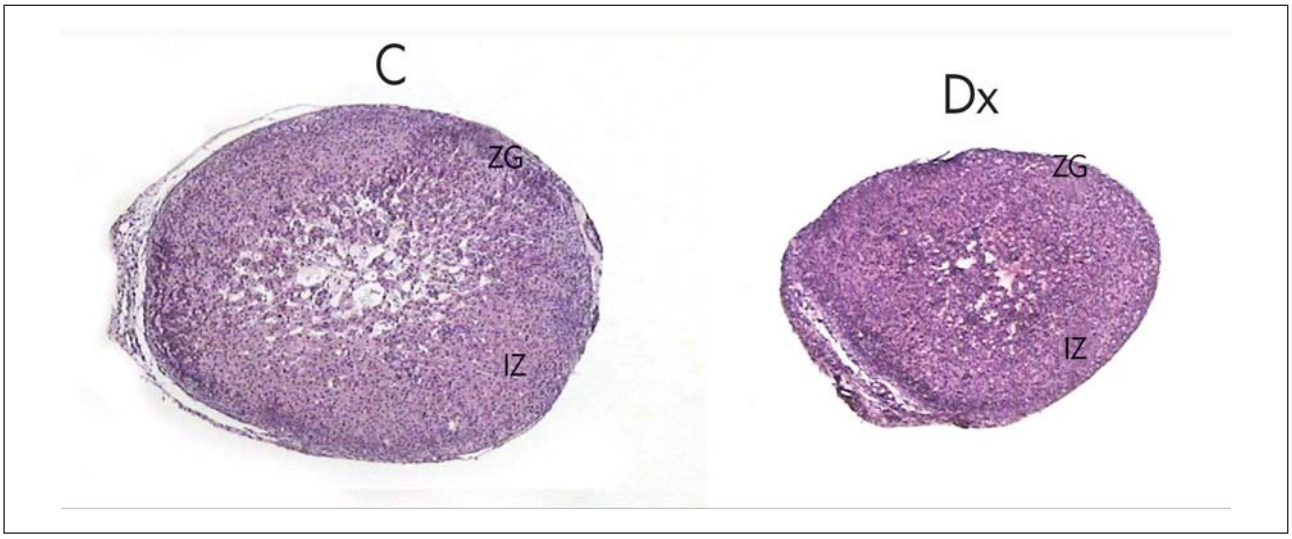

Figure 2 Central section of the adrenal gland of 21-day-old fetuses of control (C) and Dx treated (Dx) mothers during pregnancy. In control fetuses the main part of the adrenal gland is occupied by steroidogenic tissue composed of the zona glomerulosa (ZG) positioned at the periphery and inner zone (IZ), while in the central part formation of the adrenal medulla i.e. centripetal migration of chromoblast takes place. In the fetuses from Dx treated mothers the volumes of the adrenal gland and all its parts were significantly reduced.

As a consequence of the antenatal treatment, the quantity and time profile of hypothalamic CRH released into the hypophyseal portal blood along with other ACTH secretagogues such as AVP change, and this reflects on the differentiation and activity of anterior pituitary corticotropes and the secreted ACTH. Prenatal glucocorticoid exposure also directly influences the fetal pituitary, as GR are present in pituitary primordial cells. Again, the timing and regime of fetal glucocorticoid exposure *in utero* will determine the outcome, as regards the establishment of hormone-producing cell populations in the anterior pituitary. Glucocorticoid impact on the proliferative activity of immature and differentiated ACTH cells results in a decrease of their number in near-term rat fetuses (40, 41). Multiple Dx administration during the last third of rat gestation also provokes marked inhibitory ultrastructural changes, most prominent at the level of synthetic organelles such as the endoplasmic reticulum and Golgi complex, accompanied by reduced ACTH levels during the fetal and neonatal period (39). On the contrary, in sheep fetuses, Dx exposure in early pregnancy did not reduce the POMC mRNA pituitary level and circulating ACTH concentration (42).

Maternal glucocorticoid administration influences numerous processes during the establishment of fetal adrenal gland structure and function, including adrenal gland growth, formation of a centrally positioned medulla, expression pattern of the key steroidogenic enzymes, level of ACTH receptor expression, and consequently glucocorticoid output (43, 44). In rat fetuses, a marked decrease of the fetal adrenal gland volume as a consequence of the decreased proliferative activity in the adrenal gland periphery, which is the region of the adrenal cortex where most proliferating cells were found, has been established after intrauterine exposure to glucocorticoids (*Figure 2*). Even the mitotic activity of sympathoadrenal precursor cells – chromoblast that simultaneously migrate into the adrenal anlage and proliferate was inhibited under glucocorticoid influence (45, 46). Decreased expression of steroidogenic enzyme CYP17 after antenatal exposure to synthetic glucocorticoids has been reported, reflecting the persistence of the adrenal gland functional changes in guinea pigs (47). In fetal sheep, an acute effect of Dx exposure early in pregnancy resulted in a transient but significant decrease in plasma cortisol levels, followed by significant elevation in both female and male near-term fetuses. This may be attributed to an increased expression of the key steroidogenic enzymes in the adrenals, such as P450C 17 and 3-HSD mRNA in female fetuses. In the adrenal glands of male fetuses other types of changes have been reported including a reduced expression of ACTH receptor mRNA level (42).

Antenatal treatment, HPA axis function and long-term consequences

As presented, excessive glucocorticoid exposure during intrauterine development shapes the developmental profile at all the HPA levels, altering the set points of the offspring HPA axis feedback regulation. A decreased expression level of the GR and MR in limbic structures has been associated with reduced negative-feedback drive and increased HPA axis activ-

ity in offspring. Elevated basal corticosterone levels or a greater corticosterone response to stressful stimuli have been recorded, depending of the timing and dosage of fetal glucocorticoid exposure during pregnancy, in adult rats and sheep (48, 49). Sex-specific alterations in the HPA axis function and glucocorticoid output under basal or stress conditions during adulthood have been shown in guinea pigs as consequences of antenatal glucocorticoid exposure (50). In humans with low birth weight, increased cortisol response to stressful stimuli and ACTH hormone application as well as high fasting plasma cortisol levels were shown (51).

Growth retardation and the presented long-term deregulation of HPA axis function under glucocorticoid influence are the underlying mechanisms linking fetal development with postnatal health, including higher susceptibility to cardiovascular and metabolic diseases, as well as reproductive disorders (2, 52). Excessive glucocorticoid exposure causes major structural and metabolic alterations in numerous tissues during the period of tissue plasticity that actually represent an adaptive fetal response with permanent consequences (53). For example, glucocorticoids affect the development of the kidney by reducing the number of nephrons, influence the activity of the renin–angiotensin system and vascular responsiveness to angiotensin II, increasing the risk of cardiovascular diseases or hypertension in offspring subject to excessive glucocorticoid exposure during *in utero* development (54). Exposure to high glucocorticoid levels leads to a reduction of the insulin producing cells mass and their functional capacity during the fetal period. In addition, changed profiles of glucose tolerance and insulin sensitivity with enhanced glucose production and reduced glucose utilization in the major metabolic tissues, such as the liver, skeletal muscle and adipose tissue, become evident during the life cycle. Thus, the prenatal glucocorticoid environment might increase susceptibility to metabolic syndrome and metabolic diseases such as type 2 diabetes in adult offspring (55, 56).

Glucocorticoid exposure during early life creates an environment that the fetus recognizes as hostile conditions and this is followed by permanent changes in the HPA axis functioning. As a consequence, long-term neurodevelopmental changes might be provoked with adverse effects on neuromotor and cognitive functions, causing behavioral problems, even attention deficit hyperactivity disorders during adolescence (53–57). Even schizophrenia, anxiety or autistic disorders may be explained by the developmental origin of the disease hypothesis concept (58). Emerging evidence from animal studies and several human studies suggests that prenatal epigenetic changes are, at least in part, linked to an increased risk for the development of mental diseases during the life cycle (59).

Concluding remarks

The HPA axis begins functioning during fetal development and controls several of its important aspects, including fetal tissue maturation due to enable adaptations requested for extrauterine survival, fetal stress response and the initiation of birth.

Antenatal glucocorticoid administration is often used in clinical practice when the risk of preterm delivery persists. Short-term beneficial effects of prenatal glucocorticoids in fetal tissue maturation are, at the same time, the ones that increase the long-term risks of different types of dysregulation manifested in adulthood.

Exposure to synthetic glucocorticoids during pregnancy provokes a sex-specific influence according to hippocampal mRNA GR and MR levels in fetuses that reflects on HPA axis function during *in utero* development.

Molecular and cellular changes established during fetal development reflect on postnatal functioning of the HPA axis. Sex-specific alterations in HPA axis functioning with reduced negative feedback drive from the hippocampus lead to increased HPA axis activity in offspring, under basal or stress conditions, depending on the timing and dosage of glucocorticoids applied.

A growing amount of information obtained from animal and human studies has offered evidence that link alterations of the HPA axis functioning due to fetal glucocorticoid exposure with an increased risk of cardiovascular, metabolic and psychiatric disorders.

Acknowledgements. This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant number 173009 and 175036 We would like to thank Company Krka FARMA d.o.o. Belgrade, Serbia for their kind donation of Dexamethasone Phosphate.

Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

References

1. Andrews MH, Wood SA, Windle RJ, Lightman SL, Ingram CD. Acute glucocorticoid administration rapidly suppresses basal and stress-induced hypothalamo-pituitary-adrenal axis activity. Endocrinology 2012; 153: 200–11.
2. Negić N, Nestorović N, Manojlović-Stojanoski M, Filipović B, Šošić-Jurjevic B, Trifunović S, Milosević V, Sekulić M. Pregnancy and dexamethasone: Effects on morphometric parameters of gonadotropic cells in rats. Acta Histochem 2007; 109: 185–92.
3. Godfrey KM, Barker DJP. Fetal programming and adult health. Public Health Nutrition 2001; 4: 611–24.
4. Gerginov MK. Investigation and validation of animal models for the development of the human fetal and neonatal hypothalamic-pituitary-adrenal axis. A Dissertation Research, Institute of Psychobiology University of Trier, Germany 2011; p. 1–247.
5. Bresson JL, Clavequin MC, Fellmann D, Bugnon C. Human corticoliberin hypothalamic neuroglandular system: comparative immunocytochemical study with anti-rat and anti-ovine corticotropin-releasing factor sera in the early stages of development. Brain Res 1987; 429: 241–6.
6. Daikoku S, Okamura Y, Kawano H, Tsuruo Y, Maegawa M, Shibasaki T. Immunohistochemical study on the development of Crf-containing neurons in the hypothalamus of the rat. Cell Tissue Res 1984; 238: 539–44.
7. Ng PC. The fetal and neonatal hypothalamic-pituitary-adrenal axis. Arch Dis Child Fetal Neonatal Ed 2000; 82: F250–4.
8. Ben-David S, Zuckerman-Levin N, Epelman M, Shen-Orr Z, Levin M, Sujov P, Hochberg Z. Parturition itself is the basis for fetal adrenal involution. J Clin Endocrinol Metab 2007; 92: 93–7.
9. Conley AJ, Bird IM. The role of cytochrome P450 17 alpha-hydroxylase and 3 beta-hydroxysteroid dehydrogenase in the integration of gonadal and adrenal steroidogenesis via the delta 5 and delta 4 pathways of steroidogenesis in mammals. Biol Reprod 1997; 56: 789–99.
10. Le Goascogne C, Sananès N, Gouézou M, Takemori S, Kominami S, Baulieu EE, Robel P. Immunoreactive cytochrome P-450(17 alpha) in rat and guinea-pig gonads, adrenal glands and brain. J Reprod Fertil 1991; 93: 609–22.
11. Kitraki E, Kittas C, Stylianopoulou F. Glucocorticoid receptor gene expression during rat embryogenesis. An in situ hybridization study. Differentiation 1997; 62: 21–31.
12. Manojlović-Stojanoski M, Nestorović N, Milošević V. Prenatal glucocorticoids: Short-term benefits and long-term risks. In: Glucocorticoids – New recognition of our familiar friend Ed. Dr. Xiaoxiao Qian. In Tech Open Access Publisher, Rijeka, 2012; p. 337–90.
13. Gesina E, Blondeau B, Milet A, Le Nin I, Duchene B, Czernichow P, Scharfmann R, Tronche F, Breant B. Glucocorticoid signaling affects pancreatic development through both direct and indirect effects. Diabetologia 2006; 49: 2939–47.
14. Fowden AL, Li J, Forhead AJ. Glucocorticoids and the preparation for life after birth: Are there long-term consequences of the life insurance? Proc Nutr Soc 1998; 57: 113–22.
15. Mastorakos G, Ilias I. Maternal and fetal hypothalamic–pituitary–adrenal axes during pregnancy and postpartum. Ann NY Acad Sci 2003; 997: 136–49.
16. Braems G. Fetal hypoxemia on a molecular level: adaptive changes in the hypothalamic-pituitary-adrenal (HPA) axis and the lungs. Eur J Obstet Gynecol Reprod Biol 2003; 110: S63–9.
17. Reichardt HM, Schütz G. Feedback control of glucocorticoid production is established during fetal development. Mol Med 1996; 2: 735–44.
18. Deayton JM, Young IR, Hollingworth SA, White A, Crosby SR, Thorburn GD. Effect of late hypothalamo-pituitary disconnection on the development of the HPA axis in the ovine fetus and the initiation of parturition. J Neuroendocrinol 1994; 6: 25–31.
19. Challis JR, Sloboda D, Matthews SG, Holloway A, Alfaidy N, Patel FA, Whittle W, Fraser M, Moss TJ, Newnham J. The fetal placental hypothalamic-pituitary-adrenal (HPA) axis, parturition and post natal health. Mol Cell Endocrinol 2001; 185: 135–44.
20. Ristić N, Nestorović N, Manojlović-Stojanoski M, Filipović B, Šošić-Jurjević B, Milošević V, Sekulić M. Maternal dexamethasone treatment reduces ovarian follicle number in neonatal rat. J Microscop 2008; 232: 549–57.
21. Lindsay RS, Lindsay RM, Edwards CR, Seckl JR. Inhibition of 11-beta-hydroxysteroid dehydrogenase in pregnant rats and the programming of blood pressure in the offspring. Hypertension 1996; 27: 1200–4.
22. Liggins GC, Howie RN. A controlled trial of antepartum glucocorticoid treatment for prevention of the respiratory distress syndrome in premature infants. Pediatrics 1972; 50: 515–25.
23. Roberts D, Dalziel S. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. Cochrane Database Syst Rev 2006; 3: CD004454.
24. Miracle X, Di Renzo GC, Stark A, Fanaroff A, Carbonell-Estrany X, Saling E. Guideline for the use of antenatal corticosteroids for fetal maturation. Coordinators of world association of perinatal medicine prematurity working group. J Perinat Med 2008; 36: 191–6.
25. Tegethoff M, Pryce C, Meinlschmidt G. Effects of intrauterine exposure to synthetic glucocorticoids on fetal, newborn, and infant hypothalamic-pituitary-adrenal axis function in humans: a systematic review. Endocr Rev 2009; 30: 753–89.
26. Barker DJP. Mothers, babies and disease in later life. BMJ Publishing, London, 1994.
27. Seckl JR, Meaney MJ. Glucocorticoid programming. Ann NY Acad Sci 2004; 1032: 63–84.
28. Fowden AL, Forhead AJ. Endocrine mechanisms of intrauterine programming. Reproduction 2004; 127: 515–26.

29. Matthews SG. Early programming of the hypothalamo-pituitary-adrenal axis. Trends Endocrinol Metab 2002; 13: 373–80.

30. Slotkin TA, Lappi SE, McCook EC, Tayyeb MI, Eylers JP, Seidler FJ. Glucocorticoids and the development of neuronal function: effects of prenatal dexamethasone exposure on central noradrenergic activity. Biol Neonate 1992; 61: 326–36.

31. Dean F, Matthews SG. Maternal dexamethasone treatment in late gestation alters glucocorticoid and mineralocorticoid receptor mRNA in the fetal guinea pig brain. Brain Res 1999; 846: 253–9.

32. McCabe L, Marash D, Li A, Matthews SG. Repeated antenatal glucocorticoid treatment decreases hypothalamic corticotropin releasing hormone mRNA but not corticosteroid receptor mRNA expression in the fetal guinea-pig brain. J Neuroendocrinol 2001; 13: 425–31.

33. Noorlander CW, De Graan PN, Middeldorp J, Van Beers JJ, Visser GH. Ontogeny of hippocampal corticosteroid receptors: effects of antenatal glucocorticoids in human and mouse. J Comp Neurol 2006; 499: 924–32.

34. Uno H, Lohmiller L, Thieme C, Kemnitz JW, Engle MJ, Roecker EB, Farrell PM. Brain damage induced by prenatal exposure to dexamethasone in fetal rhesus macaques I. Hippocampus. Brain Res Dev Brain Res 1990; 53: 157–67.

35. Tijsseling D, Wijnberger LD, Derks JB, van Velthoven CT, de Vries WB, van Bel F, Nikkels PG, Visser GH. Effects of antenatal glucocorticoid therapy on hippocampal histology of preterm infants. PLoS One 2012; 7: e33369.

36. Crudo A, Suderman M, Moisiadis VG, Petropoulos S, Kostaki A, Hallett M, Szyf M, Matthews SG. Glucocorticoid programming of the fetal male hippocampal epigenome. Endocrinology 2013; 154: 1168–80.

37. Bakker JM, Schmidt ED, Kroes H, Kavelaars A, Heijnen CJ, Tilders FJ, van Rees EP. Effects of short-term dexamethasone treatment during pregnancy on the development of the immune system and the hypothalamo-pituitary adrenal axis in the rat. J Neuroimmunol 1995; 63: 183–91.

38. Kalafatić D, Plećaš B, Hristić M, Manojlović M. Manipulation of prenatal blood glucocorticoid level affects development of the hypothalamic paraventricular nuclei in rats. Biomed Res 1998; 19: 293–301.

39. Kalafatić D, Manojlović-Stojanoski M, Plećaš B, Hristić M. Development and differentiation of the nucleus paraventricularis and nucleus supraopticus of the hypothalamus during the perinatal period in rats. Arch Biol Sci 2000; 52: 19–20.

40. Manolović-Stojanoski M, Nestorović N, Negić N, Filipović B, Šošić-Jurjević B, Milošević V, Sekulić M. The pituitary-adrenal axis of fetal rats after maternal dexamethasone exposure. Anat Embryol 2006; 211: 61–9.

41. Stojanoski MM, Nestorović N, Filipović B, Milošević V. ACTH-producing cells of 21-day-old rat fetuses after maternal dexamethasone exposure. Acta Histochem 2004; 106: 199–205.

42. Braun T, Li S, Sloboda DM, Li W, Audette MC, Moss TJ, Matthews SG, Polglase G, Nitsos I, Newnham JP, Challis JR. Effects of maternal dexamethasone treatment in early pregnancy on pituitary-adrenal axis in fetal sheep. Endocrinology 2009; 150: 5466–77.

43. Miyamoto H, Mitani F, Mukai K, Suematsu M, Ishimura Y. Studies on cytogenesis in adult rat adrenal cortex: Circadian and zonal variations and their modulation by adrenocorticotropic hormone. J Biochem 1999; 126: 1175–83.

44. Mitani F, Mukai K, Miyamoto H, Suematsu M, Ishimura Y. Development of functional zonation in the rat adrenal cortex. Endocrinology 1999; 140: 3342–53.

45. Manojlović M, Kalafatić D, Hristić M, Plećaš B, Virag A, Čakić M. Treatment of pregnant females with dexamethasone influences postnatal development of the adrenal medulla. Ann Anat 1998; 180: 131–5.

46. Hristić M, Kalafatić D, Plećaš B, Manojlović M. The influence of prolonged dexamethasone treatment of pregnant rats on the perinatal development of the adrenal gland of their offspring. J Exp Zool 1997; 279: 54–61.

47. Owen D, Matthews SG. Prenatal glucocorticoid exposure alters hypothalamic pituitary-adrenal function in juvenile guinea pigs. J Neuroendocrinol 2007; 19: 172–80.

48. Sloboda DM, Moss TJ, Gurrin LC, Newnham JP, Challis JR. The effect of prenatal betamethasone administration on postnatal ovine hypothalamic-pituitary-adrenal function. J Endocrinol 2002; 172: 71–81.

49. Shoener JA, Baig R, Page KC. Prenatal exposure to dexamethasone alters hippocampal drive on hypothalamic-pituitary-adrenal axis activity in adult male rats. Am J Physiol Regul Integr Comp Physiol 2006; 290: R1366–73.

50. Liu L, Li A, Matthews SG. Maternal glucocorticoid treatment programs HPA regulation in adult offspring: sex-specific effects. Am J Physiol Endocrinol Metab 2001; 280: E729–39.

51. Reynolds RM. Glucocorticoid excess and the developmental origins of disease: two decades of testing the hypothesis–2012 Curt Richter Award Winner. Psychoneuroendocrinology 2013; 38: 1–11.

52. Negić N, Nestorović N, Manojlović-Stojanoski M, Filipović B, Šošić-Jurjević B, Milosević V, Sekulić M. Multiple dexamethasone treatment affects morphometric parameters of gonadotrophic cells in adult female rats. Folia Histochem Cytobiol 2006; 44: 87–92.

53. Cottrell EC, Seckl JR. Prenatal stress, glucocorticoids and the programming of adult disease. Front Behav Neurosci 2009; 7: 1–9.

54. Mesquita FF, Gontijo JA, Boer PA. Maternal undernutrition and the offspring kidney: from fetal to adult life. Braz J Med Biol Res 2010; 43: 1010–8.

55. Rose AJ, Vegiopoulos A, Herzig S. Role of glucocorticoids and the glucocorticoid receptor in metabolism: insights from genetic manipulations. J Steroid Biochem Mol Biol 2010; 122: 10–20.

56. Dumortier O, Theys N, Ahn MT, Remacle C, Reusens B. Impairment of rat fetal beta-cell development by maternal exposure to dexamethasone by maternal exposure

during different time-windows. PLoS One 2011; 6: e25576.

57. Yeh TF, Lin YJ, Lin HC, Huang CC, Hsieh WS, Lin CH, Tsai CH. Outcomes at school age after postnatal dexamethasone therapy for lung disease of prematurity. N Engl J Med 2004; 350: 1304–13.
58. Huang LT. The link between perinatal glucocorticoids exposure and psychiatric disorders. Pediatr Res 2011; 69: 19R–25R.
59. Kundaković M. Prenatal programming of psychopathology: the role of epigenetic mechanism. J Med Biochem 2013; 32: 313–24.

Received: January 15, 2014

Accepted: March 17, 2014