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Prenatal Glucocorticoids: Short-Term Benefits and Long-Term Risks

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<http://dx.doi.org/10.5772/51106>

1. Introduction

Glucocorticoids are steroid hormones synthesized in the adrenal gland cortex, and most of their physiological effects are mediated by the glucocorticoid receptor (GR), that acts as a ligand-dependent transcription factor. Coordinate changes in metabolism under glucocorticoid influence provide energy that is instantly and selectively available to vital organs, and enables them to deal with immediate environmental demands, at the expense of anabolic pathways, such as bone formation, reproduction, immunological responses and other, that are being blunted or delayed, under glucocorticoid influence [1-3].

During fetal development the synthesis of adrenal glucocorticoids precedes the establishment of a definitive structure of the gland. In rats, secretion of the main glucocorticoid – corticosterone starts as early as on day 13 of development [4] (term=22 days, short gestation period), while in humans secretion of the main glucocorticoid – cortisol starts in the 8th week of pregnancy (term=40 weeks, long gestation period) [5]. Glucocorticoid receptor mRNA is present in the tissue derivatives of all three germ layers from fetal day 13 onwards, and increases gradually during rat fetal development [6]. Human fetal tissues express GR at the gestational age of 6 weeks, meaning that the machinery for hormone action is prepared at the early stages of development [5]. These facts suggest that endogenous glucocorticoids produced by the fetal adrenal glands have a crucial role in fetal growth and the development of individual fetal tissues [7]. In response to the parturition rise in glucocorticoids a wide variety of changes known as “preparation for birth” occurs, meaning that the maturational changes in many fetal tissues, essential for neonatal survival, are intensified during the last third of gestation. Namely, circulating glucocorticoids induce fetal lung maturation and surfactant production, trigger a variety of physiological effects on brain cell differentiation and synaptogenesis, stimulate the production of hepatic gluconeogenic enzymes, affect pancreatic β -cell development and

insulin content, influence renal development and affect the maturation of the immune system [8-10]. Metabolic, cardiovascular and immune adaptations under glucocorticoid influence are fundamental to successfully overcoming birth-related stress and postnatal adaptation of the newborn to environmental challenges [11, 12].

Environmental conditions influence the prevailing nutritional and endocrine status in mothers and fetuses. Numerous animal and human studies have shown that adverse environmental conditions during pregnancy, such as maternal undernutrition [13, 14], stress [15, 16], illness, placental insufficiency [17, 18], as well as prenatal glucocorticoid exposure [19, 20] affect fetal development and postnatal outcome. Changes in the maternal hypothalamic-pituitary-adrenal (HPA) activity, transplacental diffusion of nutrients, hormones and growth factor supply, potentially affect the fetal HPA axis influencing glucocorticoid output as well as other developing systems [21, 22]. Gestational age, at which an insult occurs, its nature and intensity, determines the specific tissue or organ which will be affected by the insult. Glucocorticoids are the key mediators between maternal environment and the fetus, and as such are involved in adaptations of the fetus to predicted postnatal environment. Even transient changes in glucocorticoid levels could have long-lasting consequences. The outcome might be growth retardation and change in the developmental trajectory, in the direction that best suited to the expected environment [23, 24]. This phenomenon is known as programming. The adaptations caused by suboptimal intrauterine conditions are appropriate if the predicted and actual postnatal environments match, and lead to survival to reproduce in a deprived environment [25, 26]. If there is a mismatch between the environment predicted and the actual environment experienced postnatally, adaptations are inappropriate and result in the development of disease like hypertension, ischemic heart disease, glucose intolerance, insulin resistance and type 2 diabetes [27-29].

In this chapter the latest findings, with clear statements from the literature, as well as own results regarding the endocrine mechanisms of intrauterine programming mediated by glucocorticoids will be analyzed. The causal relationship between a prenatally programmed endocrine axes and their postnatal functioning that affect growth, stress response, metabolism and reproduction will be discussed. In order to better understand mechanisms of fetal glucocorticoid programming of endocrine axes, special attention will be paid to key points of their development.

2. Development of endocrine axes

2.1. Development of hypothalamic-pituitary-adrenal axis

Functional differentiation of anterior pituitary cells is under the control of transcription factors and their cofactors. The transcription factors expressed in early pituitary development such as *Rpx/Hesx1*, *Ptx1*, *Ptx2*, *Lhx3* regulate the formation of Rathke's pouch and maintain the formation of the baseline cellular structure. Signaling between the developing hypothalamus and the Rathke's pouch is also involved in the initial formation of

pituitary primordia and further differentiation of the pituitary gland. Hypothalamic BMP 4 and FGF 8 are required for the activation of expression and maintenance of expression of the early transcriptional factors in the pouch. The lineage of adrenocorticotropin (ACTH) producing cells arises first during organogenesis, and thus represents a separate lineage. Pituitary homeobox 1 (Ptx1/Pitx1) was reported as factor for differentiation towards proopiomelanocortin (POMC) cells [30]. In the fetal pituitary the first cells that are immunopositive for ACTH can be found on fetal day 13 in the pars tuberalis anlage, whereas ACTH immunostaining is found 1 day later in the pars distalis [31]. The pars intermedia of the fetal rat pituitary is the last part to display ACTH staining [32]. Although pituitary precursor cells are influenced by spatial cues and extrinsic signals, for the initiation of ACTH synthesis in the fetal pituitary, a certain degree of autonomy exists. Moreover, ACTH immunostaining was found in 11-day-old fetal rat pituitary primordia cultured for 4 days in a serum-free medium, thus without endocrine or neuroendocrine signals [33]. Furthermore, ACTH-containing cells were detected in anencephalic human fetuses [34].

In the next stage of differentiation of ACTH-producing cells, the hypothalamic (corticotropin-releasing hormone) CRH control over ACTH cells has an indispensable role. In rats, the appearance of hypothalamic CRH-containing neurons occurs in lateral hypothalamic areas and in the paraventricular nucleus (PVN) on days 15.5 and 16.5 of gestation, respectively, whereas beaded fibers are visible in the external layer of the median eminence on day 17.5 of gestation [35]. Expression of CRH is correlated with a progressive rise in ACTH in the fetal circulation. A progressive 10-fold increase in ACTH concentration occurs in the pars distalis on days 17–20 of gestation [36], which suggests a crucial role of the developing hypothalamus. From the 20th day of gestation until term, the ACTH concentration remains unchanged. The existence of a mechanism that overcomes the negative feedback effect of elevated glucocorticoid levels on POMC gene expression during late pregnancy was demonstrated [37]. From that period onwards, ACTH is stored in the fetal pituitary gland as a readily releasable pool. Its location near the fenestrated capillary network enables momentary depletion of significant amounts of ACTH, and a subsequent considerable increase in ACTH concentration in the circulation, if physiologically demanded [38].

The cells of the adrenal cortex arise early in development due to the local proliferation of cells from the splanchnic mesoderm. The genes coding the orphan nuclear receptors SF-1 and DAX-1 control the early fetal adrenal cortex development. Knockout mice for these genes manifest adrenal and gonadal agenesis, gonadotropin deficiency and the absence of the hypothalamic ventromedial nucleus [39]. The potential for steroid synthesis occurs early, in 12-day-old rat fetuses [40]. In the later stages of fetal life, ACTH controls growth and development, as well as steroidogenic maturation of the adrenal glands [41]. Histological analysis of a near term rat fetal adrenal glands showed that the main part of the gland is steroidogenic tissue composed of a zona glomerulosa (ZG) and an inner zone (IZ), while the number of migrating chromoblasts is still modest [42]. Both cortical zones are functionally competent and able to produce aldosterone and corticosterone in 19-day-old fetuses [43]. The proliferative activity of adrenocortical cells is most intensive in the outer portion of the

glands, in the subcapsular ZG region and outer portion of IZ from where the cells migrated centripetally [44]. A balance between proliferation and cell death enables proper functioning and integrity of the developing adrenal glands. Programmed cell death appears to occur in the inner cortical layers, where many resident macrophages are present [4], as well as in the resorption zones and giant cells [45, 46].

The development of the human adrenal glands exhibits a number of important differences in histological organization and steroidogenic activity in relation to species with a short-term gestation period. The primordium of the human fetal adrenal glands can be recognized by 3–4 weeks of gestation, but by the 8th week of embryonic development the adrenal cortex is clearly identifiable with its characteristic zonal partitioning [47]. The principal steroids of primate fetal adrenal gland, i.e. fetal zone situated in the inner part of the gland, are dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS). DHEA serves as a substrate for placental estrone and estradiol production. Rapid growth of the fetal adrenal cortex, especially the fetal zone, with a significant increase in steroidogenic activity begins after week 10 of gestation and continues until term [47]. The definitive zone, positioned at the peripheral part of the gland, is steroidogenically inert, at least until the second trimester of pregnancy. After this period mineralocorticoid production was found, although aldosterone synthesis in the fetal adrenals is in poor correlation with plasma rennin activity. The transitional zone that becomes distinguishable between fetal and definitive zone at the end of the second trimester of pregnancy represents a precursor of the adult zona fasciculata (ZF). The expression of enzymes 3 β hydroxysteroid dehydrogenase (3 β HSD) and cytochrome P450 17 α -hydroxylase/lyase (CYP17) in the transitional zone enables the capacity for cortisol production [48]. In the initial phase, the fetal adrenals begin to produce cortisol between weeks 10 and 20 of gestation, possibly utilizing progesterone as a precursor. However, *de novo* synthesis of cortisol from cholesterol is established fairly late in gestation, leading to a remarkable increase in cortisol concentrations in the third trimester. Immediately after birth, the fetal zone of the adrenal cortex degenerates extensively, whereas the ZF matures in the subsequent period. [48]. Although the presence of ACTH is established in circulation from week 15 of gestation, cortisol and androgen production by the definitive and fetal zone are maximally stimulated by circulating ACTH from midgestation [49].

The functional state of the fetal HPA axis is important for several reasons. Firstly, glucocorticoids play a role in the maturation of numerous organs necessary for intrauterine development and extrauterine existence. Organ systems involved in reaching metabolic homeostasis, stress response and electrolyte balance in outer space are strongly controlled by glucocorticoids [50]. Secondly, the fetal HPA negative feedback mechanism begins to operate between days 15 and 17 of rat gestation [51]. Thus, near term fetuses are able to regulate their own homeostasis and glucocorticoid production in response to different maternal stressors [52] and adverse conditions [37]. Finally, the fetal HPA axis activity strongly affects the timing of parturition [48]. In a number of species at the end of gestation there is an increase in HPA activity, with increased plasma glucocorticoid levels that reflects on the placental trophoblast cells, causing enhanced output of prostaglandins [37]. The

effects of prostaglandins on the myometrium associated with increased oxytocin activity represent an important step in the initiation of birth [50].

2.1.1. *Effects of glucocorticoids during fetal development*

Strictly defined spatial and temporal effects of glucocorticoids are actually determined by the previous appearance of the GR. *In situ* hybridization histochemistry revealed GR gene expression in the tissue derivatives of all three germ layers. The facts that intense GR mRNA labeling happened just before the final differential step for each glucocorticoid target tissue, and that upon differentiation reduced amounts of GR mRNA were found further support the crucial morphogenic role of glucocorticoids during fetal development [6].

Sufficient glucocorticoid levels are essential for the normal maturation of many parts of CNS during the prenatal period. In general, glucocorticoids act on neuronal maturation, replication, differentiation, and programmed cell death [8, 12]. In parallel with reducing the rate of neuronal replication, glucocorticoids promote the differentiation of central noradrenergic, serotonergic and dopaminergic neurons, enhance axonal growth, dendritic arborisation and synaptogenesis in a regionally selective manner, and control programmed cell death [53, 54].

Glucocorticoids promote the differentiation of sympathoadrenal precursors, initially expressed multiple neuronal markers, into endocrine chromaffin cells in the adrenal gland medulla. As sympathoadrenal precursors invade the primordium of the fetal adrenal gland and migrate centripetally, they are exposed to adrenal steroids. The initial adrenaline synthesis occurs in parallel with a sharp rise in the adrenal tissue and plasma glucocorticoid concentrations, and the appearance of GR in the sympathoadrenal precursor cells in 17-day-old rat fetuses [55]. Thus, glucocorticoids represent an important signal for the induction and maintenance of adrenaline synthesis in the adrenal medulla, but initial induction of the adrenaline-synthesizing enzyme, phenylethanolamine-N-methyltransferase is rather determined by a cell-intrinsic timed process in the chromaffin precursors [56].

Glucocorticoids accelerate lung maturation as they speed up the thinning of the double capillary loop to form the thin gas exchanging walls of the alveoli. By enhancing the production of surfactant by type II pneumocytes, glucocorticoids allow the newborn to draw its first breath and enable the start of the breathing process [57].

In the fetal liver, glucocorticoids promote the activity of the key gluconeogenic enzyme systems and hepatic glycogen deposition in preparation for the nutritional transition at birth. Inability of the adrenalectomised sheep fetuses to induce gluconeogenesis during extreme circumstances such as maternal undernutrition with reductions in hepatic glycogen content was associated with lower circulating concentrations of cortisol [10]. It might be concluded that birth-related stress and subsequent environmental challenges trigger glucocorticoid actions essentially involved in the activation of fetal gluconeogenesis and glucose availability necessary for maintaining homeostasis after birth.

By combining *in vitro* studies with *in vivo* investigations in mice lacking the GR in the whole organism or in specific pancreatic cell populations, it has been shown that glucocorticoids are important hormones in pancreatic development. Acting before insulin expression onset, glucocorticoids decreased the differentiation of the embryonic pancreas into β -cells favoring acinar cells differentiation. Deletion of the GR in pancreatic precursor cells led to increased β -cell mass. Thus, glucocorticoids unable β -cells mass expansion in later stages, by modifying the balance of specific transcription factors, mostly Pdx-1 [9]. At birth, as the placental source of glucose is lost, tight glycemic control must be established. A prepartum rise in glucocorticoid levels in fetal horse and sheep increases pancreatic β cells sensitivity to glucose and influences the fetal insulin level, enabling active regulation of the glucose level after birth, and thus the transition to enteral supply [10, 22].

The highest expression of the GR mRNA was identified during early kidney development in the developing glomeruli, epithelial cells of the proximal and distal renal tubule, and the central collecting duct. Reduction of GR levels in the fully differentiated glomeruli pointed out the importance of glucocorticoids during a defined period of establishment of the definitive renal structure and function [5].

Effective thermoregulation in response to cold exposure in the extrauterine environment post birth is crucial to prevent hypothermia in newborns. The expression of mitochondrial uncoupling protein (UCP), that catalyzes adaptive thermogenesis in mammalian brown adipose tissue increases dramatically during the final week of gestation in fetal adipose tissue [58]. The late-gestation augment in fetal plasma glucocorticoid levels as well as application of synthetic glucocorticoids enhance mitochondrial UCP expression, suggesting that glucocorticoids are crucially involved in increasing the thermogenic potential of fetal adipose tissue near term [59, 60].

2.2. Development of the somatotropic axis

Growth hormone (GH) is secreted from the anterior pituitary gland under the control of two hypothalamic hormones: the releasing hormone is growth hormone-releasing hormone (GHRH), and the release-inhibiting hormone is somatostatin (SRIH). In addition to these two neurohormones, a number of factors such as free fatty acids, acetylcholine, amino acids, opiates, glucocorticoids and some neuropeptides also have direct or indirect effects on GH release. Most of the metabolic actions of GH are mediated by insulin-like growth factor I (IGF-I), which is produced in many different tissues, with most of the circulating IGF-I being derived from the liver. IGF-I has anabolic as well as metabolic effects in many cell types, acting through autocrine, paracrine and classical endocrine mechanisms. IGF signaling has been recognized as one of the major molecular regulators of cell growth and proliferation [61]. Moreover, it is generally accepted that GH, by controlling important aspects of IGF activity in many tissues and cell types of mammals, is able to coordinate somatic growth in a defined spatio-temporal manner at the whole body level [62]. IGF signaling, however, not only regulates growth but also affects differentiation and may, through epigenetic processes, steer adult cell function as a result of particular conditions during postnatal development [63].

In rat, GHRH neurons are detected at the 16th day of fetal development [64], while SRIH mRNA in the periventricular nucleus of the hypothalamus is expressed on the 14th fetal day [65]. Initial pituitary GH expression is detected on day 15 of gestation using sensitive methods such as the reverse transcriptase-polymerase chain reaction (RT-PCR) [66]. In the following phase of GH cell development the expression of Pit-1 occurs. Pit-1 is a pituitary-specific transcriptional factor that mediates cell proliferation and differentiation into specific hormone-producing cell types – thyrotropes, somatotropes or lactotropes [67]. During this period, the quantity of GH transcripts remains at an extremely low level. A marked increase in cell number and GH production occurs between days 18 and 19 of fetal development [68]. The expression of the GHRH receptor also occurs on fetal day 19 in rats [69, 70].

It has been considered that pituitary GH promotes and controls fetal development and body weight by stimulating the family of hepatic growth factors. Recent investigations showed that extrapituitary GH as well as local production of growth factors had great paracrine/autocrine influence on fetal developmental processes and differentiation. The expression of GH and GH receptor in a wide variety of tissues is established before the pituitary gland and circulatory system become functional [71]. In rats and mice a contribution of the pituitary GH to growth, development and body weight has been demonstrated postnatally, during the second week of life [72]. The influence of pituitary GH on normal growth and body weight in near-term fetuses, immediately after the GH cells become functional, is still difficult to understand and not well defined.

In humans, GH cells are evident at 8 weeks of gestation, with abundant immunoreactive cytoplasmic GH expression. Plasma GH concentrations are highest at midgestation and thereafter fall until term. The pattern of ontogenesis of plasma GH reflects the progressive maturation of hypothalamic–pituitary and forebrain function. The responses of GH to SRIH and GHRH are mature at term in human infants [73].

IGF-1 and IGF-2 mRNA transcripts are present in virtually all fetal tissues [74]. Both IGFs are also detected in the fetal circulation from early gestation, but the plasma concentrations of IGF-II are 3–10 fold higher than those of IGF-I during late gestation [75]. They are present in serum and other extracellular fluids associated with highly specific binding proteins (IGF binding proteins (IGFBPs)). In the fetus, IGFs are predominantly complexed with IGFBP-1 and -2, and the liver is the predominant production site for these IGFBPs [76]. Tissue and plasma IGF-II are higher in the fetus than in newborn or adult animals in most species [77]. In rodents, IGF-II expression disappears from most tissues except the brain by weaning, with the consequence that IGF-II is virtually undetectable in adult plasma [78]. In contrast, plasma IGF-I levels increase rapidly after birth, primarily as a result of the onset of GH stimulated IGF-I production by the liver [79], since IGF regulation is GH-independent during the fetal period [74]. There is, therefore, a shift in IGF predominance from IGF-II before birth to IGF-I after birth, which has led to the concept that IGF-II is the IGF primarily responsible for fetal growth [80].

2.3. Development of hypothalamic-pituitary-thyroid axis

The development of thyroid-stimulating hormone (TSH) cells in the fetal rat pituitary pars distalis is determined by the expression of Pit-1 and TEF transcription factors [67]. Differentiation from precursor cells enables detection of TSH cells mRNA on day 15 of gestation [81], while immunocytochemically recognized TSH cells can be observed in 16.5- to 17.5-day-old fetuses. TSH cells were few in 17.5-day-old rat fetuses, but their number increased thereafter, particularly during the 2nd week after birth [68].

In the rat fetal hypothalamus, thyrotropin-releasing hormone (TRH), which promotes prompt synthesis and secretion of anterior pituitary TSH, is first detected on the 16th day of gestation [82]. The destruction of paraventricular nuclei (PVN), which contain TRH neuronal cell bodies, results in a significant decrease in anterior pituitary TSH β - and α -subunit mRNA levels, as well as in serum TSH concentrations [83]. During the fetal period a major influence of TSH is to control the morphological and functional maturation of the fetal thyroid gland.

The thyroid gland is derived from the fusion of a medial outpouching from the floor of the primitive pharynx and bilateral evaginations of the fourth pharyngeal pouch, giving rise to the precursors of follicular (thyroxine-producing cells) and parafollicular (calcitonin-producing C cells) cells. Coordinate action of numerous transcription factors is involved in thyroid morphogenesis. *Titf1*, *Hhex*, *Pax8* and *Foxe1* are expressed in the rat just prior to the first appearance of the thyroid diverticulum on fetal day 9.5–10, controlling the proliferation, survival and migration of precursor cells [84]. Targeted disruption of *Titf1* in mice results in total absence of the thyroid tissue, while the lack of *Pax8* results in follicle agenesis, with the remaining tissue being composed almost exclusively of C-cells [85, 86].

In rats on fetal day 17 significant growth and rapid functional and structural development of the thyroid gland are established. The first appearance of follicles, iodine organification and thyroid hormonogenesis occur in parallel with a marked increase of TSH in fetal circulation and the expression of TSH receptors (TSHR) in thyroid tissue. Thus, upregulation of TSHR gene expression by TSH in fetuses is crucial for further maturation of the thyroid [81].

Deiodinase enzymes provide biologically active triiodothyronine (T3) to developing tissues by activating and/or deactivating systemic serum thyroid hormones (TH). Three types of iodothyronine deiodinases (D1, D2, D3) have been identified, which differ in tissue distribution, substrate specificity and sensitivity to inhibiting compounds [87]. Expression of D1 is low through gestation, while D2 and D3 are the major isoforms in the fetus [88]. D2 is the activating enzyme that catalyzes the removal of one iodide from the outer tyrosine ring of thyroxine (T4) and production of active T3. D3 is the inactivating enzyme that catalyzes the cleaving of one iodide from the inner tyrosine rings of T4 or T3, thus generating reverse T3 (rT3) or T2. Action of D2 and D3 preserves the safe level of T3 in the developing brain and the pituitary [87], while the activity of D3 in the utero-placental unit protects fetal tissues against high maternal T4 concentrations. Local tissue deiodinase

activity is essential for compensation and adaption to potential malfunctions in the fetal hypothalamic-pituitary-thyroid (HPT) axis, i.e. in the case of congenital hypothyroidism and normal maternal T4, the transfer of the latter, together with increased brain D2 activity, protects the fetal brain from T3 deficiency [89].

In humans, thyroid gland reaches maturity by the 11th–12th week of gestation, when tiny follicle precursors with thyroglobulin in follicular space can be seen and iodine binding is detected. In this period both T4 and T3 are measurable in fetal serum [90]. At this stage of early development maternal T4 transplacental passage contributes to the fetal hormonal status, which is essential for normal early fetal neurogenesis. In the later stages of development, if fetal thyroid function is normal, placental TH passage is relatively limited due to the presence of D3 [91]. The increase in total T4 and free T4 levels between weeks 18 and 36 of gestation indicates maturation of the HPT axis function, with the establishment of feedback control about midgestation.

2.4. Development of the endocrine pancreas

The embryogenesis of pancreas is mediated by a series of transcription factors involved in morphogenesis. The expression of Pdx1 has been found early in organ formation, in cells that give rise to endocrine and exocrine cells of the mouse neonatal pancreas. In Pdx1 null mice pancreas agenesis occurs. The appearance of insulin and glucose transporter GLUT2 is also regulated by this transcription factor [92]. Transcription factors Hlxb9 and Isl1 are necessary for the initial induction of Pdx1. Hlxb9 is observed during the formation of pancreatic anlage and later during the differentiation of β cells. Neurogenin 3 has been identified as the key regulator of endocrine development, giving rise to all pancreatic endocrine lineages [93].

The pancreas arises from a multipotent endodermal cell population that will produce ductal, exocrine and endocrine cells [94]. The human fetal pancreas develops during the 5th week of gestation, while endocrine cells are identifiable by the 8th or 9th week of gestation. Scattered single endocrine cells are recognized to produce insulin (β -cell), glucagon (α -cells), somatostatin (δ -cells) and pancreatic polypeptide (PP cells). Clusters of epithelial endocrine cells form primitive islets of Langerhans a few weeks later, in parallel with the expression of neural adhesion molecule (N-CAM) [95]. The largest expansion of the β -cell mass has been shown to take place in the second half of prenatal development, from approximately 20 weeks in humans. This developmental period is critical to achieve a β -cell mass required to ensure proper insulin secretion throughout life. It is thought to result from β -cell neogenesis from rapidly dividing undifferentiated progenitor cells [96]. Thereafter, some degree of β -cell expansion persists at least until adolescence due to β -cell neogenesis, mitosis and perhaps to transdifferentiation of α -cells, acinar or ductal cells [97].

During the development of insulin-expressing cells there is a changing phenotype, from progenitor cells to immature β -cells, and finally, to mature adult β -cells. Using gene expression and immunohistochemistry, differences among late-embryonic, neonatal, and adult β -cells were found in a series of markers with transient expression patterns during the

perinatal period. Of these, cytokeratin-19, matrix metalloproteinase-2 and surfactant protein-D can be considered as true markers of new and immature β -cells, as their expression is transient and not entirely synchronous, but absent in adults [98]. Sympathetic innervation and vascularization of islets play important roles in normal islet morphogenesis during prenatal life, and have trophic effects on β -cells survival, maturation and insulin secretion [99].

Human and rodent fetal islets are insensitive to glucose, as fetal β -cells do not discriminate between different glucose levels, despite adequate insulin reserves. Relative functional immaturity *in utero* was also recorded in response to circulating amino acids, particularly leucine, catecholamines and neural stimulation, with regard to the capacity to secrete both insulin and glucagon [100]. For blunted capacity for insulin and glucagon secretion several causes are proposed. Firstly, during fetal development in mammals glucose homeostasis is mainly achieved by the mother because of a constant supply of glucose by placental transfer through facilitated diffusion. Secondly, the mechanisms that mediate insulin secretion in adults are immature in fetuses, meaning that the production of cAMP is decreased, expression of glucose transporter GLUT2 is lower and expression of voltage-gated L-type Ca^{2+} channels is diminished in β -cells [100, 101]. Furthermore, generalized immaturity of the metabolic enzyme expression in pancreatic β -cells during the fetal and neonatal period in the rat has been recorded. Lower expression levels of the metabolic enzyme genes such as malate dehydrogenase, glycerol-3-phosphate dehydrogenase, glutamate oxaloacetate transaminase and pyruvate carboxylase were established and confirmed by quantitative PCR during fetal development and several weeks after birth than in adults [102]. During fetal life GH might be involved in the process of β -cell mass expansion and maturation that finally leads to an effective response to hyperglycaemia [103].

2.5. Development of the hypothalamic-pituitary-gonadal axis

Reproductive physiology in mammals is centrally regulated through the hypothalamic-pituitary-gonadal (HPG) axis and depends on gonadotropin-releasing hormone (GnRH). GnRH, a decapeptide, is released into the hypophyseal portal vasculature from axon terminals at the median eminence and binds to the GnRH receptor (GnRHR), which is specifically expressed in gonadotrope cells in the anterior pituitary gland. GnRH signaling controls the biosynthesis and release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which in turn regulate the development and activity of the ovaries and testes. LH and FSH, together with TSH, are heterodimeric glycoproteins composed of a common α -subunit (α GSU) and a hormone-specific β -subunit (LH β , FSH β).

The crucial maturation events at the hypothalamic-pituitary level are the onset of GnRH synthesis, access of GnRH to the hypothalamic-hypophysial circulation, appearance of GnRH receptors in the pituitary gonadotropes, and the onset of gonadotropin synthesis and secretion. Traces of GnRH in whole rat brain extracts were detected as early as on day 12 of gestation, and by the 17th day of gestation immunoreactive GnRH cells within the brain were distributed in a pattern similar to that of the adult, projecting neurosecretory axons to the median eminence [104].

Although gonadotrope is the last cell type in the anterior pituitary to reach maturation with the expression of terminal differentiation markers LH β , FSH β , and GnRHR, α GSU is the first pituitary hormone transcript expressed during development. In mice, it is first detected at fetal day 10.5 in the most ventral region of Rathke's pouch [105]. Gonadotrope specific expression of α GSU is regulated by SF1, which plays essential roles at multiple levels of the reproductive axis, reviewed in [106]. However, no single transcription factor has been demonstrated to be necessary and sufficient for gonadotrope lineage commitment [107]. In the rat pituitary, on fetal day 16 rare LH β were detected by *in situ* hybridization. Many more cells hybridizing LH β and FSH β were observed on day 17. At this stage, the fetal pituitary gland becomes GnRH responsive [104]. Recently, it has been shown that two types of gonadotropes may become responsive to GnRH at different time points during development [108].

In humans, fetal pituitary gonadotropins are secreted as early as at 12 weeks of gestation. Marked rises in the pituitary and plasma concentrations of FSH and LH are observed during the second trimester of gestation, and significantly higher levels of circulating gonadotropins are detected in female than in male fetuses [109].

Gonad development is a unique system in which a single rudimentary tissue can be induced to form one of two different organs, the ovary or the testis. The gonads originate from the thickening of the ventrolateral epithelium along the embryonic mesonephros surface, called the genital ridge, and in mice are visible at fetal day 10. Proliferation of these epithelial cells gives rise to somatic cells of the gonad. By contrast, the germ cell lineage arises outside the urogenital ridge before the formation of gonads. Mouse primordial germ cells (PGCs) are specified in the epiblast, and are first detected at about fetal day 7.25 using alkaline phosphatase as a marker [110]. PGCs proliferate and migrate through the gut mesentery into the urogenital ridge, populating the gonads between the 10th and 11th day of fetal development. The exact trigger that initiates PGCs migration to the genital ridge and the chemoattractants that are required for the directional movement toward the genital ridge are slowly beginning to be understood [111].

In mammals, the choice between the male or the female gonad, is initiated by a single gene on the Y chromosome, Sry (sex-determining region of the Y chromosome). Sry is expressed in the somatic cells of the XY gonad between 10.5 and 12.0 of gestation [112] and encodes a putative transcription factor that acts as a genetic switch for male development. The Sry protein is expressed in each pre-Sertoli cell during a narrow window of several hours in the period of gonadal differentiation, between fetal days 10.5 and 12.5, resulting in up-regulation of Sox9, the major gene transcriptionally downstream of Sry [113]. If Sry is expressed in the rudimentary gonad, either from the Y chromosome or from an ectopic transgene, a testis forms [114]. If Sry is not expressed, as in XX individuals or in cases where Sry is mutated or deleted, an ovary forms [115]. Based on this, it was believed that the presence of Sry actively caused testis development to occur, and that in the absence of Sry the ovary developed passively (i.e. the so-called "default" pathway). Recent discoveries have now made it clear that early ovarian development is an active process that involves the interaction and competition of multiple signaling pathways that specify male or female

development. The two alternative sex fates are thought to emerge through the antagonistic activities of sex-specific transcription factors in a restricted number of gonadal cells. This initial cell fate decision is further expanded by extracellular non-cell-autonomous signals that promote one developmental program, while at the same time suppressing the other [116]. Studies have identified two secreted factors, Wnt4 and follistatin, which are required during early gonad development to repress the aspects of testis differentiation in XX gonads, reviewed in [111].

By fetal day 13.5, germ cells in XX and XY gonads have taken different developmental paths. In XY gonads germ cells undergo mitotic arrest as prospermatogonia, whereas in XX gonads the germ cells enter the prophase of the first meiotic division. The fate of germ cells is dependent on the somatic environment, and not on the chromosomal sex of the germ cells [117]. Thus, signals from adjacent somatic cells must direct the differentiation of germ cells in the embryonic gonads, although these signals have not been identified.

The onset of gonadal endocrine activity is very clearly sexually dimorphic. During embryogenesis, male differentiation requires the secretion of three testicular hormones. Anti-Müllerian hormone (AMH), produced by fetal Sertoli cells, induces regression of the Müllerian ducts. Testosterone, produced by Leydig cells, promotes the development of Wolffian duct derivatives and masculinization of the external male genitalia. Finally, insulin-like 3 (Insl3) mediates transabdominal testicular descent into the scrotum [118]. The action of LH and production of testosterone start simultaneously in the rat testis on fetal day 15.5 [119]. In females, differentiation occurs when the absence of AMH allows development of Müllerian structures. The lack of androgens permits degeneration of Wolffian ducts, and the absence of Insl3 maintains the gonads in the abdomen. In the ovary, the responsiveness to LH appears postnatally at the end of the first week of life [120] concomitantly with the onset of steroidogenesis [121]. Functional FSH receptors can be detected some days earlier in the perinatal rat ovary, at the age of 4–5 days [122].

Steroid hormones play a crucial role in fetal gonadal development and ovarian cell wellbeing. Estrogen action is needed for normal ovarian development, follicle survival and the regulation of female reproduction. The role of estrogens in female sexual development has been demonstrated in many studies utilizing mice lacking functional estrogen receptors or estrogen-converting enzymes [123, 124]. However, the developmental role of estrogens in human fetal ovaries is not well known. In contrast, testicular hormones are crucial for testicular formation and function, i.e. induction and maintenance of the male phenotype at all stages of development.

In humans ovarian development starts at around the 5th week of gestation, when primordial germ cells migrate into the undifferentiated gonad. Thereafter, the germ cells undergo multiple mitotic divisions, and the number of oogonia reaches its peak by the 20th week of development. At this time about 7–8 million germ cells are present in the ovary [125]. Simultaneously with mitotic divisions, starting around the fetal age of 11–12 weeks, primordial germ cells begin to enter meiosis [126]. After the 10th week of gestation granulosa

cell precursors start to form, and at 24 weeks of development almost all oocytes are enveloped in a primordial follicle structure [127]. The human fetus is exposed to high concentrations of maternal and placental estrogens, and estrogens are produced in several fetal tissues [128, 129]. However, minimal amounts of fetal circulating estrogens are produced in the fetal ovarian follicles [109].

In the human male at 7 weeks of gestation, the presence of germ cells in the embryonic gonadal ridge and of coelomic epithelial cells that give rise to Sertoli cells was observed. This was followed by the appearance of Sertoli cells in the testicular tubules and of Leydig cells at 9 weeks, and also by the appearance of vascular endothelial cells and peritubular myoid cells at 12 weeks [130]. The production of testosterone peaks at around 11 or 12 to 14 weeks of gestation, as determined by measurements in the testis and fetal blood [131]. Between weeks 12 and 20, serum testosterone levels in the male fetus are from 3- to 8-fold higher than in the female [132].

3. Programming

During development, there are critical periods in the course of which a system or an organ has to mature. The critical periods are defined by the epochs of rapid cell division within an organ, and different organs develop at different rates and different times. At these critical periods organs are especially vulnerable to challenges such as decreased oxygenation, nutrient supply, and altered hormone exposure. If adverse conditions are experienced in the window of vulnerability, then the trajectory of development of the responding organ may be changed in ways that result in persistent malfunction. The concepts of “nutritional programming”, “fetal programming”, “fetal origins of adult disease”, “developmental origins of health and disease”, “developmental induction”, and “developmental programming” [26, 133-135] imply that some stimulus or an insult at these critical, restricted periods in development has long-lasting consequences, setting in train a series of events that culminate in the adult onset of disordered function, while the same environmental stimulus outside that critical period induces only reversible changes. The concept evolved from human epidemiological studies that have shown that impaired intrauterine growth is associated with an increased incidence of metabolic, cardiovascular and other diseases in later life. Low birth weight, in particular, has been linked to hypertension, glucose intolerance, insulin resistance, type 2 diabetes, dyslipidaemia, obesity and reproductive disorders in the adult (Figure 1) [133]. The Dutch famine, a unique “natural experiment” with a well-defined period of food shortage in an otherwise well-nourished population, has shown that maternal undernutrition during gestation compromises health in later life, and that these long-term effects depend on its timing during gestation [136]. Intrauterine growth retardation (IUGR) and/or delays in attaining motor, verbal and social skills were recorded in the offspring of mothers exposed to the influence of a large variety of “stresses” such as loud, unanticipated noise (as experienced by people living under the flight paths of busy airports) and living in a country preparing for and ultimately going to war (e.g. the six-day Israeli war) [137].

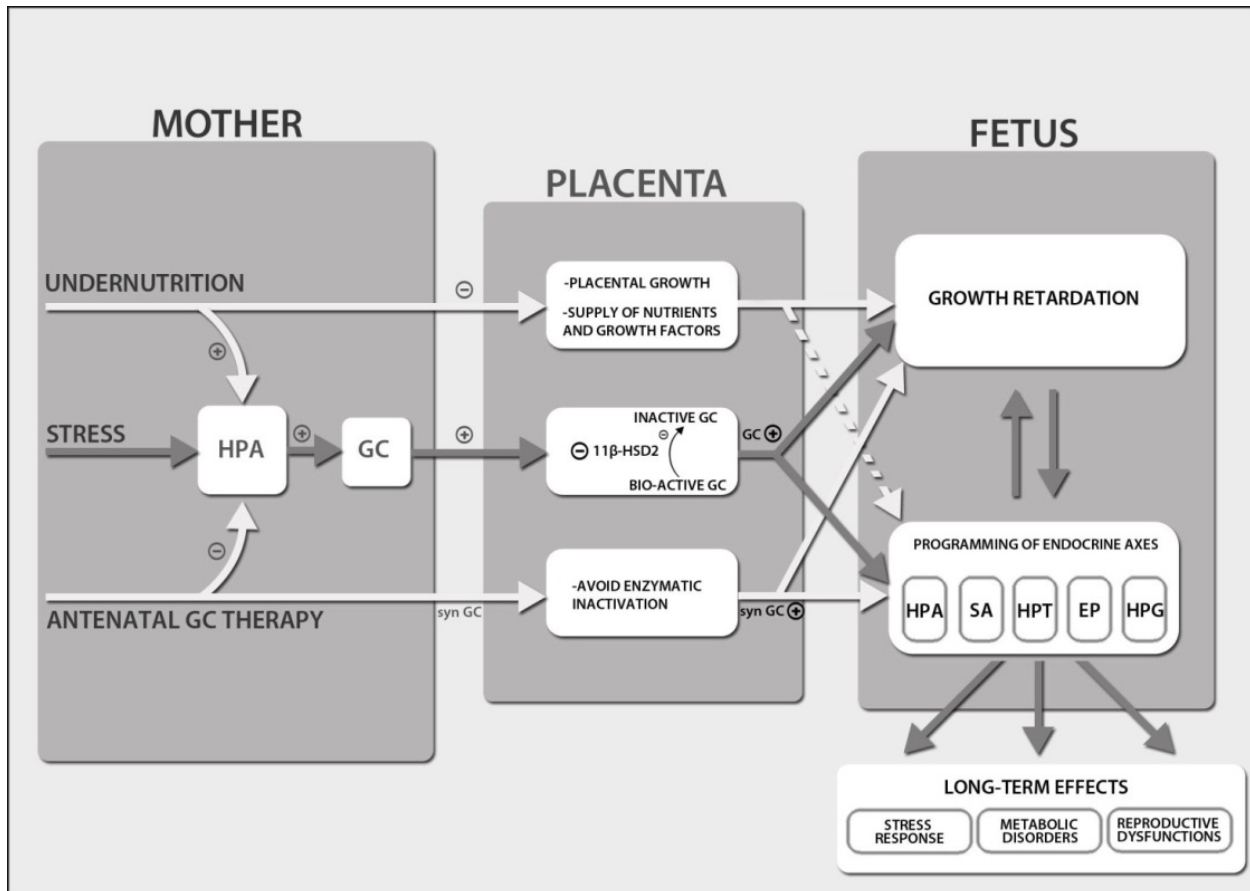


Figure 1. Maternal undernutrition or stress increase maternal glucocorticoid levels and decrease the rate of their inactivation by 11β -HSD2 in the placenta that results in fetal glucocorticoid overexposure. The synthetic glucocorticoids pass through the enzymatic placental barrier and reach the fetal circulation. Consequences of fetal glucocorticoid overexposure are growth retardation and programming of endocrine axes with long-term effects. HPA-hypothalamic-pituitary-adrenal axis; GC-glucocorticoids; synGC-synthetic glucocorticoids; 11β -HSD2- 11β -hydroxysteroid dehydrogenase type 2; SA-somatotropic axis; HPT-hypothalamic-pituitary-thyroid axis; EP-endocrine pancreas; HPG-hypothalamic-pituitary-gonadal axis.

The concept of programming has been tested experimentally in numerous species using a wide range of experimental approaches to impair fetal growth. Some of the most commonly used experimental models are maternal undernutrition (calorie restriction, protein deprivation, iron deficiency), placental insufficiency and exposure to glucocorticoids that includes maternal stress, maternal treatment with synthetic glucocorticoids and inhibition of placental 11β -hydroxysteroid dehydrogenase (11β -HSD2) (Figure 1) [138].

Majority of these experimental models ultimately result in fetal glucocorticoid overexposure, since they mediate the programming effects of nutritional and other environmental challenges during pregnancy [139]. Maternal low-protein diet and placental 11 β -HSD2 deficiency cause fetal growth restriction via distinct pathways but with a common component: overexposure of fetoplacental tissues to glucocorticoids. Whatever the source, glucocorticoids play an important role in the regulation of fetal growth, and through this in developmental programming [140]. Glucocorticoids are growth inhibitory and affect development of all the tissues and organ systems that are at increased risk of adult pathophysiology when fetal growth is impaired [11]. Glucocorticoids signal adverse intrauterine conditions and adapt fetal development to ensure the maximum chances of survival both *in utero* and at birth. They act at cellular and molecular levels to induce changes in tissue growth and differentiation by direct and indirect mechanisms. At the cellular level, glucocorticoid exposure *in utero* alters receptors, enzymes, ion channels and transporters in a wide range of different cell types during late gestation. They also change the expression of various growth factors, cytoarchitectural proteins, binding proteins and components of the intracellular signaling pathways [139]. These changes will influence the basal functioning of the cell and its responses to endocrine, metabolic and other stimuli, with consequences for its size, proliferation rate and terminal differentiation. In addition to these direct effects, glucocorticoids can act indirectly on tissue proliferation and differentiation through changes in the cellular secretion of proteins, hormones, growth factors and metabolites [139].

Another major mechanism by which glucocorticoids act on physiological systems is through changes in hormone bioavailability. They alter the production and secretion by the placenta and fetal endocrine glands of a number of hormones, such as estrogen, insulin, gastrin, neuropeptide Y, angiotensin II, T3, noradrenaline and adrenaline. They also regulate hormone receptor densities and the activities of several enzymes involved in activating and inactivating hormones in the fetal tissues. One of the recently proposed mechanisms of programming, with particular emphasis on glucocorticoids, is epigenetic programming. Glucocorticoids act as epigenetic signals that allow transgenerational transmission of non-genomic factors important in developing the optimal phenotype for survival to reproductive age [141]. The consequences of fetal overexposure to either endogenous or exogenous glucocorticoids lead to hypertension, glucose intolerance, insulin resistance, and abnormalities in the HPA function after birth [142].

3.1. Placental 11 β -hydroxysteroid dehydrogenase 2 (11 β -HSD2)

Transcriptional activation of GR is known to be determined by intracellular glucocorticoid availability that is regulated by two distinct isoforms of the enzyme 11 β -HSD. These enzymes control the first critical step for GR activation and target gene expression, while the process has been termed pre-receptor ligand control [2]. 11 β -HSD1 is NADPH-dependent with reductase activity, converting cortisone to the bioactive cortisol in the human, and 11-dehydrocorticosterone to corticosterone in the rat. 11 β -HSD2 is NAD-dependent and

catalyzes the rapid metabolism of cortisol and corticosterone to inactive 11-keto forms, cortisone and 11-dehydrocorticosterone, respectively [143]. These enzyme systems function in most fetal glucocorticoid sensitive tissues modulating ligand access to GR. This is especially important for mineralocorticoid sensitive tissues. In the developing kidney, for example, in the presence of 11 β -HSD2, cortisol was efficiently metabolised to inert cortisone, which does not bind to receptors allowing aldosterone action. As a consequence, sodium retention, potassium loss and hypertension are prevented [5].

11 β -HSD2 is highly expressed in the placenta, in the syncytiotrophoblast in humans, and in the labyrinthine zone in rodents, i.e. at the interface between maternal and fetal circulations. Besides the mentioned function, it has an additional role in the placenta: it selectively regulates passage of glucocorticoids from the mother to the fetus, since highly lipophilic glucocorticoid molecules are able to pass freely across the placenta [144, 145]. As glucocorticoid levels are significantly lower in the fetus than in the mother, the 11 β -HSD2 placental enzymatic barrier prevents most of the maternal glucocorticoids from reaching the fetus, protecting the very sensitive fetal tissues from high glucocorticoid levels during development. Although 11 β -HSD2 limits fetal exposure to maternal glucocorticoids, a certain amount avoids enzymatic inactivation and reaches the fetus [146]. 11 β -HSD2 maintains a gradient of glucocorticoids from the maternal to the fetal circulation, although its magnitude varies between species. There is a positive correlation between the materno-fetal gradient and the activity of placental 11 β -HSD2 [139].

Reduced 11 β -HSD2 placental activity results in higher levels of glucocorticoids reaching the fetus, which induces growth retardation and program later disease susceptibility (Figure 1). In rats the lowest placental 11 β -HSD2 activity caused the highest fetal exposure to maternal glucocorticoids is seen in the smallest fetuses with the largest placenta [146]. In addition, low levels of 11 β -HSD2 placental activity decrease birth weight in humans, and can lead to adult hypertension [143]. Furthermore, in 11 β -HSD2 knockout mice fetal weight is significantly reduced in relation to wild type controls, as a consequence, not only of the increased fetal exposure to maternal glucocorticoids, but also altered placental function, i.e. decreased transport of nutrients. Thus, absence of 11 β -HSD2 compromises not only fetal but also placental growth, function and morphology, representing an additional mechanism of fetal programming [147]. Since maternal glucocorticoid levels are significantly higher than those in the fetus, even modest perturbations of the placental 11 β -HSD2 levels or activity can have a profound impact on fetal glucocorticoid exposure [148]. Inhibition of 11 β -HSD2 activity by the application of carbenoxolone to gravid females, a potent inhibitor of 11 β -HSD2, reduces birth weight in rats and elevates blood pressure in the adult rat offspring. These effects require the presence of maternal adrenal products, since carbenoxolone given to adrenalectomized pregnant rats had no effect on birth weight or blood pressure [149].

Placental 11 β -HSD2 can be avoided by the application of synthetic glucocorticoids. It has been demonstrated that treatment of pregnant rats with dexamethasone and betamethasone, synthetic glucocorticoids that are poorly metabolized by the enzyme, results in reduced birth weight, higher activity of the HPA axis and elevated blood pressure in the adult

offspring (Figure 1) [143]. Synthetic glucocorticoids have been shown to up-regulate the activity of 11β -HSD2, and, hence, amplify the placental barrier for physiological glucocorticoids [150].

It can be concluded that fetuses are protected from environmental perturbations by an enzymatic placental barrier that, by regulating fetal exposure to maternal glucocorticoids, crucially determines foeto-placental growth. Its deficiency causes programming effects in the offspring.

3.2. Stress during pregnancy and maternal undernutrition

Exposure to stress during pregnancy has been associated with offspring behavior, morphology, physiology, and immunology [151]. Although the mechanisms by which stress during pregnancy can influence the development of the offspring are not entirely known, elevation of the maternal glucocorticoid levels after stress exposure could be the first step in early life programming that predisposes individuals to several illnesses and psychiatric disorders. Maternal exposure to alcohol, repeated restraint stress, electric tail shocks, and undernutrition during pregnancy induced a corticosterone increase [152, 153]. In the offspring of these mothers growth retardation with altered function of the HPA axis and glucocorticoid responses under stress challenge, hyperglycemia and other dysfunctions related to type 2 diabetes, as well as hypertension were established (Figure 1) [14, 15, 153].

Adrenalectomy, as a blockade of the maternal stress-induced corticosterone secretion, suppresses the changes established in the offspring of prenatally stressed dams. The effects of repeated restraint stress during pregnancy on the offspring HPA axis activity and hippocampal mineralocorticoid receptor (MR) level are suppressed by adrenalectomy, while the administration of corticosterone to adrenalectomised mothers reinstates the effects induced by prenatal stress [153]. Furthermore, a pharmacological blockade of the maternal glucocorticoid synthesis with metyrapone also prevented hypertension, which is induced by fetal exposure to maternal low-protein diet [154]. Adrenalectomy carried out during pregnancy (without stress exposure), although it causes the opposite result in relation to stress exposure due to circulating corticosterone levels, also affects offspring development. Maternal adrenalectomy performed during gestation results in a compensatory increase in fetal corticosterone levels [152], decreases body weight in both male and female offspring, while the HPA axis shows a sex-specific pattern of vulnerability. In females, a dramatic increase in hypothalamic CRH and GR mRNA levels was established on day 14 [155]. All this together points out that highly regulated maternal glucocorticoids are indispensable during normal fetal development.

Various types of stress applied during gestation cause a broad spectrum of effects in the offspring at any age. Effects of prenatal stress caused by the restraining of pregnant rats influence the development of fetal hypothalamic PVN neurons in a duration-dependent

manner. Long-lasting stress causes neurotoxic changes of the fetal PVN neurons, including CRH neurons that showed significantly shorter total length of the neuronal processes and an increased number of apoptotic cells. On the contrary, short-lasting stress facilitates the development of these fetal PVN neurons that showed enhanced CRH messenger RNA expression, while the varicosities of CRH-containing axons at the median eminence revealed more mature morphology. A greater degree of neuronal differentiation, as manifested by an increase in both the number of branch points and the total length of the processes from the cell body, was also demonstrated [156].

Chronic maternal restraint stress during late gestation decreases placental 11 β -HSD2 expression and activity, and reduces body weight in rat fetuses at term. These alterations were associated with reduced pancreatic β -cell mass, growth hormone level, and decreased glucose concentration in fetal plasma [18]. Other results established hyperglycaemia, glucose intolerance and decreased basal leptin levels in prenatally stressed aged male rats as dysfunctions related to type 2 diabetes mellitus [15]. These data suggest that maternal stress and later dysfunctions such as type 2 diabetes could be linked to the restricted fetal growth and the adverse glucocorticoid environment *in utero* as the consequences of decreased placental 11 β -HSD2 expression.

Observations from animal and human studies have linked maternal nutritional status and fetal growth retardation with the programming of hypertension and coronary heart disease in later life [27]. In the ewe, undernutrition in early pregnancy leads to placental enlargements, as adaptation to extract more nutrients. There is a correlation between placental weight and systolic blood pressure in adults that tends to rise as placental weight increases [157]. Mild protein restriction during pregnancy attenuates placental 11 β -HSD2 expression which leads to overexposure of the fetus to maternal glucocorticoids (Figure 1) [14, 143]. Intrauterine growth retardation and disturbed development of the HPA axis appear as an outcome [15]. In addition, in the adult offspring subjected to maternal undernutrition during pregnancy persistently elevated expression of GR and decreased expression of 11 β -HSD2 in the kidney, liver and brain mediated tissue-specific increases in glucocorticoid action. These changes represent potentially important mechanisms contributing to the programming of hypertension *in utero* [158].

As presented, it has so far been established that certain types of stress affect the reduction of placental 11 β -HSD2 expression in rats, suggesting that the fetus and placenta are exposed to excessive amounts of glucocorticoids. Thus, deficiency of the placental barrier to maternal glucocorticoids may represent a common pathway between the maternal environment and fetoplacental programming of later disease [12, 18]. Secondly, disturbances in placental growth and function, as a consequence of maternal stress exposure, decrease fetal nutrient supply and may further contribute to suboptimal fetal growth [14, 145]. Both changes, i.e. the reduced maternal glucocorticoid inactivation and decreased nutritional supply reflect on the HPA axis activity in fetuses and offspring, although the HPA axis response is differentially affected by the gestational stress procedure (Figure 1) [151]. Thus, the fetal HPA axis is a possible primary target and is

intricately involved in early life disturbance caused by maternal stress exposure with far-reaching physiological consequences.

3.3. Antenatal glucocorticoid therapy

Because glucocorticoids have potent influence on maturation of fetal lung and other tissues they have been used for more than 40 years in human pregnancies at risk of preterm delivery. Use of antenatal corticosteroid therapy reduced the complications associated with preterm delivery such as neonatal respiratory distress syndrome (RSD), periventricular hemorrhage, necrotizing enterocolitis and, most importantly, neonatal mortality [159]. According to the National Institute of Health [160] all fetuses between the 24th and 34th week of gestation at risk of preterm delivery should be considered as candidates for the beneficial effects of antenatal glucocorticoid treatment. The recommended treatment consists of two doses of 12 mg betamethasone given 24 h apart, or alternative regimen of four doses of 6 mg dexamethasone given 12 h apart [161]. However, antenatal glucocorticoid therapy may produce growth retardation, affective and cognitive disturbances as well as other disorders in children and adults [162], thus the question of the relative risk and benefit of repetitive courses of prenatal glucocorticoid administration is still open (Figure 1).

Furthermore, the effects of prenatal glucocorticoid administration in cases of congenital adrenal hyperplasia (CAH) that must begin early in the first trimester to be effective in preventing female genital ambiguity are not completely known. CAH is an inherited disease in which a disordered steroidogenic enzyme P450C21 diverts adrenal steroid synthesis away from cortisol toward androgen. As a consequence girls are masculinized, because the adrenal glands secrete large amounts of androgens during prenatal development. Dexamethasone treatment should be introduced very early in pregnancy, before the seventh week of gestation, with the aim to increase fetal glucocorticoid concentrations, thus suppressing the elevated ACTH level that drives adrenal androgen production [146, 163].

In obstetric practice different synthetic glucocorticoids are used: dexamethasone, betamethasone, or prednisolone. Synthetic glucocorticoids are slightly different from their endogenous equivalents in chemical structure. Dexamethasone and betamethasone both have the additional 9α -fluoro groups, and 16β - or 16α -methyl groups, respectively [164]. Prednisolone differs from cortisol by a 1δ -dehydro configuration (Figure 2). The choice of the concrete drug use will depend on its biological half-life, which represents the time that passes until one half of the initial drug concentration has disappeared from the blood [165]. Physiological and synthetic glucocorticoids have been divided into short-, medium- and long-acting substances dependent on the duration of measurable biological half-life [165]. Cortisol belongs to the short-acting category with the biological half-life of 8–12 h, prednisolone belongs to the medium-acting category with the half-life of 12–36 h, while dexamethasone and betamethasone have long-acting properties, ranging between 36 and 54 h [165].

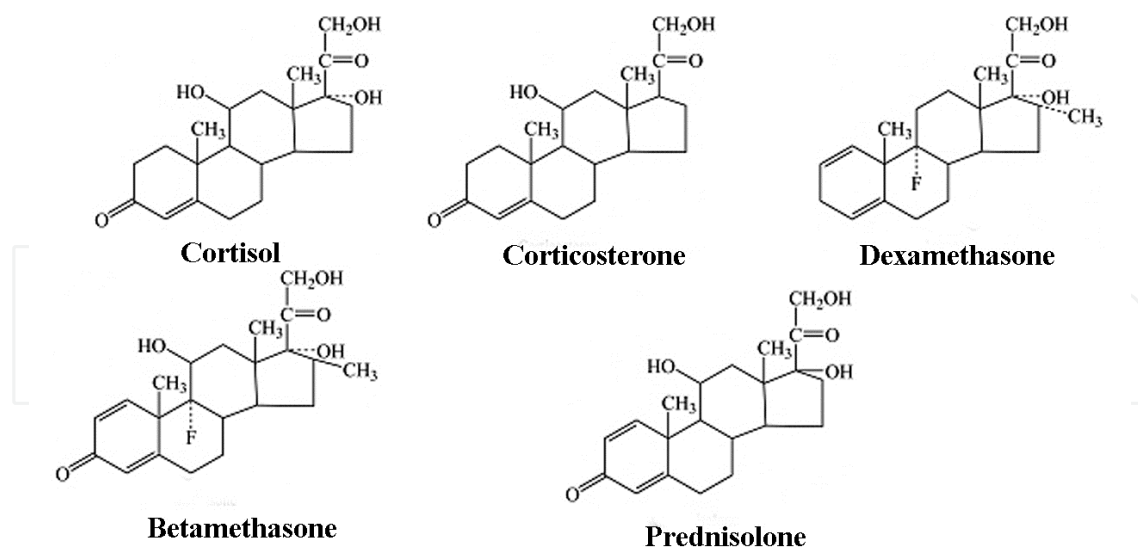


Figure 2. The structural formulas of natural (cortisol, corticosterone) and synthetic glucocorticoids.

The biological activity of glucocorticoids is partly determined by the rate and selectivity of protein binding, because only the unbound glucocorticoids fraction is biologically active. Gayrard et al. [166] have found that the plasma free cortisol concentrations (6% to 14%), corticosteroid-binding globulin (CBG)-bound (67% to 87%) and albumin-bound (7% to 19%) concentrations are similar within species. Cortisol binding decreases as its concentration increases [167]. In human plasma, betamethasone and dexamethasone bind predominantly to albumin, which has high capacity but low affinity for ligating, while both steroids bind only marginally to CBG [167]. Dexamethasone displays higher protein affinity than betamethasone. The potency of glucocorticoids in a biological system also depends on its affinity for its receptor. Genomic potency of betamethasone was reported to be moderately higher than that of dexamethasone, while both steroids have a 25-fold higher affinity to the GR than cortisol [168].

4. Programming of endocrine axes

4.1. Programming of the fetal hypothalamic-pituitary-adrenal axis

The HPA axis is particularly sensitive to glucocorticoid levels. Fetal exposure to excessive glucocorticoids, natural or synthetic, can occur via a number of mechanisms including maternal stress, undernutrition as well as maternal antenatal treatment. As previously noted, synthetic glucocorticoids such as dexamethasone and betamethasone pass easily through the placental barrier avoiding the placental enzyme 11β -HSD2 [7], while maternal stress and undernutrition affect the same enzyme, resulting in increased fetal exposure to maternal glucocorticoids [18]. As the increased fetal exposure to glucocorticoids occurs during a critical period of the HPA axis development, when its control is just setting up, permanent alterations in the basal and stress induced HPA axis activity and regulation occur in the offspring, and sustain throughout life. Crucial changes that underlie the programming of the HPA axis will be presented below. Additionally, disturbances of the

complex maturational process such as HPA axis development that have far-reaching immediate and delayed physiological effects will be discussed later.

The hippocampus represents a major inhibitory input to the HPA axis function. This is the point where glucocorticoid feedback, via GR and MR in the hippocampus and GR in the hypothalamic PVN and anterior pituitary, inhibits further HPA activity [169]. Thus, the balance between GR and MR in the hippocampus is an important factor in determining the HPA axis feedback sensitivity. Prenatal dexamethasone exposure alters GR and MR expression in the developing limbic system of guinea pig fetuses in both a region-specific and a sex-specific manner. After a single dexamethasone dose, female fetuses exhibited a significant increase in MR and GR mRNA levels in the CA1 and CA2 regions of the hippocampus and MR mRNA in the dentate gyrus [170]. Other results showed that multiple dexamethasone administration during pregnancy led to a marked increase in hippocampal GR and MR mRNA levels in male fetuses [171]. In mice, a single course of dexamethasone transiently reduced MR mRNA expression in the fetal hippocampus [172]. In addition, prenatal stress, or dexamethasone exposure are implicated in the development of rat hippocampal GR and MR in the offspring. In rat offspring exposed to glucocorticoid excess during late pregnancy permanently attenuated GR and MR mRNA expression in specific hippocampal regions reduced sensitivity to glucocorticoids [173]. The administration of betamethasone to pregnant sheep resulted in significant increases in MR and 11- β HSD2 gene expression in adult animals, reflecting a possible role for the locally produced glucocorticoids within the hippocampus, and the potential for long term alterations in HPA function [20].

At the level of the hypothalamic PVN, significantly decreased amounts of CRH mRNA were seen in male fetuses and female offspring after treatment of guinea pig mothers with dexamethasone or betamethasone, supporting the idea that synthetic glucocorticoids enter the fetal brain and inhibit central drive to the fetal HPA axis [171]. In addition, it has been shown that prenatal dexamethasone treatment induces a clear delay in increment of CRH in the external zone of the median eminence [174]. Morphometric analyses of rat PVN neurosecretory cells at eight distinct subdivisions indicate that dexamethasone given to pregnant dams causes significant changes in PVN neurosecretory cells in 20-day-old fetuses as well as in neonatal offspring. Significantly decreased neurosecretory cell nuclei volume and number in PVN, due to decreased proliferative activity, were found at the levels where parvocellular neurons are present, i.e. where CRH neurons are dominant [175, 176]. On the other hand, removal of maternal adrenals at day 16 of gestation significantly affected the size of neurosecretory cells in different subgroups of fetal PVN. These effects persisted during the neonatal period [177], confirming that prenatal glucocorticoid exposure alters the development and function of prenatal and neonatal PVN.

Prenatal glucocorticoid application alters the monoaminergic transmitter systems involved in the regulation of GR expression in the brain. Significant differences in the turnovers of serotonin, dopamine and noradrenaline contents between the weeks 3 and 14 of life were found in a wide area in the rat brain [54]. Thus, developmental alterations of

monoaminergic neurons, that represent major modulators of the HPA axis function, influence endocrine response in the adult offspring. The data suggest that key targets for programming include GR gene expression and the CRH system [178].

Antenatal treatment with synthetic glucocorticoids affects pituitary development and the differentiation of hormone-producing cell types during the fetal period as well as after birth. A significant reduction in fetal ACTH cell volume and number was demonstrated in 19 and 21-day-old fetuses after multiple prenatal dexamethasone administration (Figure 3) [38, 42]. Dexamethasone decreased the rate of division of both immature cells and the existing fetal ACTH in the period when its proliferation was most intensive, on day 19 of fetal development [179], thus leaving long lasting consequences. Multiple dexamethasone exposure during pregnancy affects the ultrastructure of ACTH cells, which in the Golgi complex show much lower presence of specific granules as well as dilation of the endoplasmatic reticulum [180]. Decreased morphometric parameters of the ACTH cells and their changed ultrastructure resulted in significantly reduced plasma ACTH levels in fetuses and neonatal offspring after multiple dexamethasone administration during pregnancy [180, 181]. On the contrary, a single dose of dexamethasone, given to pregnant rats on day 16 of gestation, suppressed the synthetic activity of fetal ACTH cells, but in the early neonatal period this suppression was followed by stimulation of ACTH secretion and increased circulating ACTH levels [182]. Other results showed that following antenatal exposure to synthetic glucocorticoids in juvenile males POMC mRNA and CRH receptor mRNA on the pituitary level were increased [183].

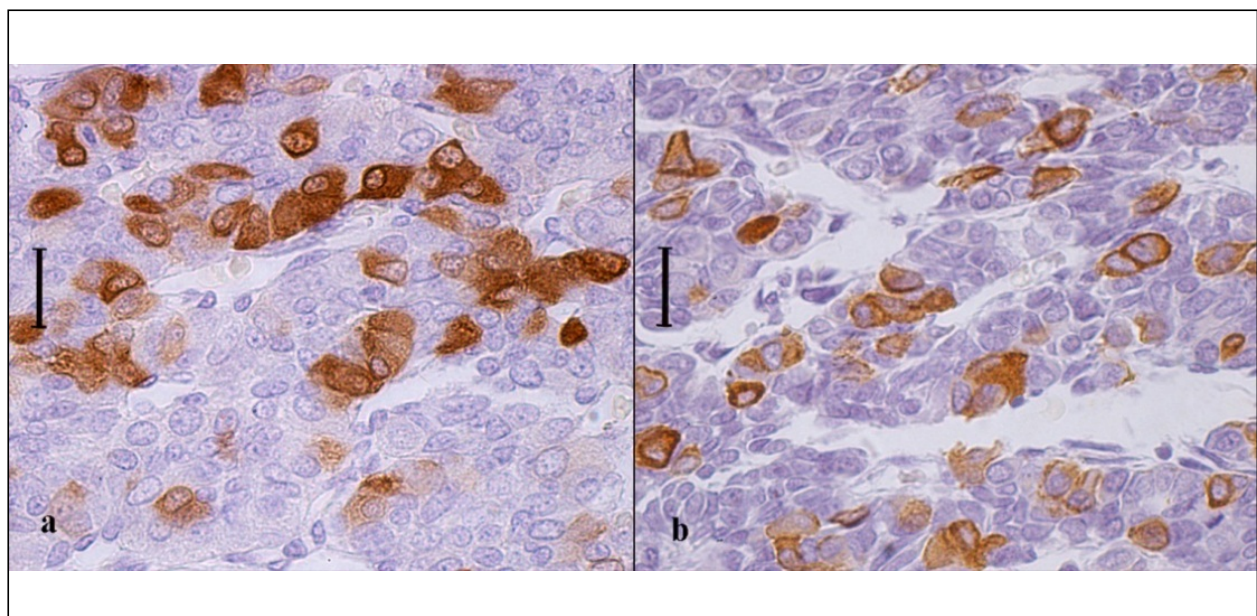


Figure 3. a) Intensive immunopositivity of ACTH cells located near the capillary network is characteristic for 21-day-old fetus. b) Decreased size, immunopositivity and number of ACTH cells in 21-day-old fetuses after maternal dexamethasone administration. Bar - 25 μm .

After maternal glucocorticoid exposure the absence of peaks in ACTH blood concentration in near term (19-day-old) fetuses reduces ACTH-trophic support and reflects on the adrenal glands structure and functional activity [42]. Administration of a single or multiple dexamethasone dose to pregnant rats induced a significant decrease in adrenal glands weight, volume of whole adrenal glands, as well as average volume and total number of cells in near term fetuses and neonatal rat offspring, a consequence of the decreased proliferative activity of adrenocortical cells [45, 46]. Interestingly, in 19-day-old fetuses the proliferative activity of adrenocortical cells that is most intensive in the outer portion of the fetal adrenal glands is markedly reduced in ZG. The proliferation rate of adrenocortical cells in IZ was not affected by prenatal dexamethasone application [42], suggesting that different sensitivity and/or responses of the proliferating cells in ZG and the outer portion of IZ to external stimuli could be a possible mechanism for the formation and maintenance of the zonal structure of the adrenal cortex [184].

In the rat adrenal glands of fetuses and pups of dexamethasone treated dams, during during the early neonatal period adrenocortical cells in various stages of degeneration were abundant, especially near the central part of the gland where zona reticularis (ZR) begins to differentiate. Resorption zones with lymphocytic infiltrations and presence of macrophages and multinuclear giant cells were observed, indicating that remodeling of the adrenal gland

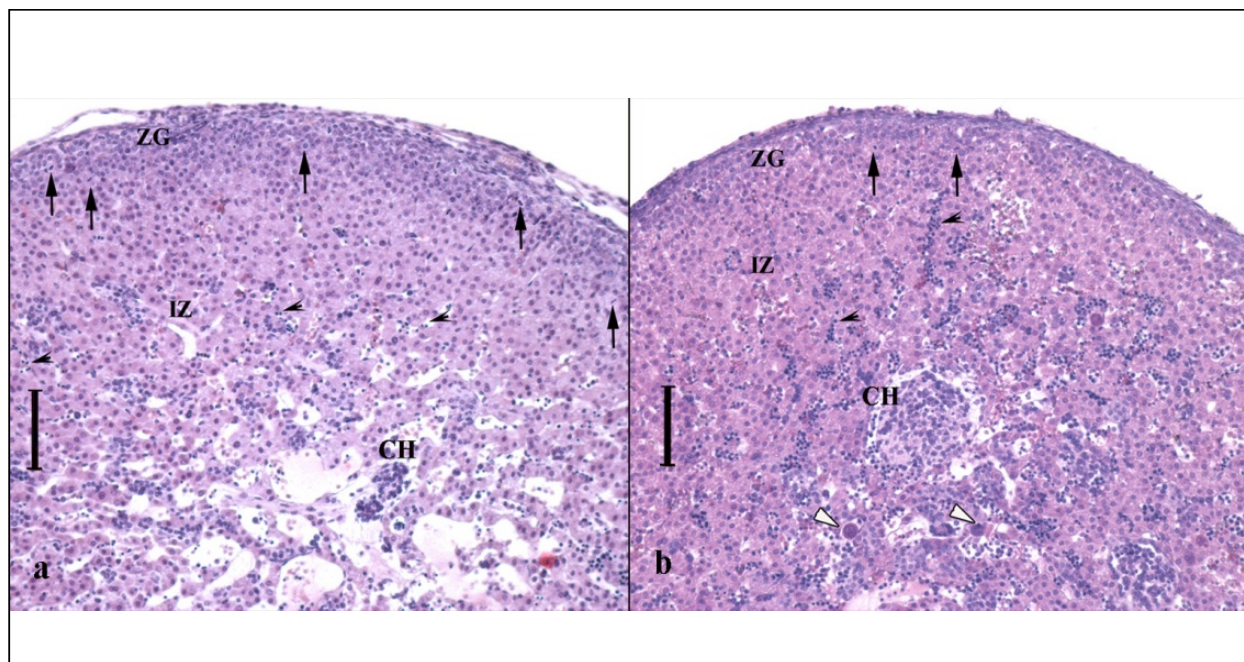


Figure 4. a) Zona glomerulosa (ZG) with numerous dividing cells (\rightarrow), inner zone (IZ) with lymphocytes (black arrowheads), cellular interspaces, and centrally positioned group of chromoblasts (CH) are seen in adrenal gland of 21-day-old fetus. b) Decreased number of proliferating cells (\rightarrow) in the adrenal gland of 21-day-old fetuses from gravid females treated with dexamethasone. Infiltration of lymphocytes (black arrowheads) and giant cells (white arrowheads) are indications of intensive tissue remodeling. Bar - 100 μ m.

structure is affected by prenatal glucocorticoid exposure (Figure 4) [45, 46]. In the juvenile period, decreased expression of steroidogenic enzyme CYP17 after antenatal exposure to synthetic glucocorticoids has been established, reflecting the persistence of the adrenal glands functional changes [183].

The influence of a single dexamethasone treatment given to gravid females resulted in the decreased volume of adrenal medulla and the number of chromaffin cells that persisted during the fetal and neonatal period. Decreased proliferation of chromaffin cells during the fetal and early neonatal period was followed by significantly higher values in relation to controls during the second neonatal week, indicating the capacity of the adrenal gland medulla to recover [185]. Multiple dexamethasone doses applied during pregnancy exert a more potent inhibitory effect. A reduced number of chromaffin cells and significantly decreased adrenaline content in the adrenals were seen in 14-day-old neonatal offspring [181].

As pointed out, the consequences of fetal glucocorticoid exposure occur at the level of central regulation, pituitary ACTH cells and the adrenal gland, causing programming effects on HPA axis function in later life. In offspring HPA axis activity may be changed in different directions under basal conditions and after stress challenge [186]. Permanently elevated basal plasma glucocorticoid levels [178, 187], greater glucocorticoid response to stress [54] as well as blunted HPA axis response to stress [183] have been established in offspring following antenatal exposure to synthetic glucocorticoids. In addition, antenatal glucocorticoid treatment programs HPA function in the adult offspring in a sex-specific manner [188]. Programming of the fetal HPA axis, although it could have had an adverse postnatal outcome, actually demonstrated the amazing plasticity of the HPA axis.

Exposure to stress or glucocorticoids, exogenous or endogenous, causes fetal growth retardation and low birth weight in parallel with deregulation of the HPA axis during the life cycle [139]. Additionally, there is a correlation between the natural variation in body weight and the HPA axis function in offspring. In adult pigs that were low-weight at birth and remained small after birth altered HPA axis function has been recorded in later life, i.e. elevated adrenal responsiveness to insulin-induced hypoglycaemia [189]. Thus, it can be concluded that growth retardation and programming of the HPA axis are two mutually dependent processes that actually represent the modality by which prenatal environment influences adult stress-related diseases (Figure 1).

It has been shown that in rodents, effects of programming can be induced by insults even in neonatal period of life. One of the striking characteristics of the HPA axis is the stress hypo-responsive period during the first 2 weeks of life for species that are immature at birth, such as rats and mice. During the stress hypo-responsive period there is low basal corticosterone secretion and the inability to increase corticosterone in response to mild stressors, in order to protect the developing nervous system from glucocorticoid excess. Thus, neonatal glucocorticoid exposure and early life experience that activate the HPA

axis have programming effects on HPA axis organization and functioning during the life cycle. Postnatal handling attenuated HPA response to stress in adult animals. Most likely this is an indirect effect, caused by altered maternal behavior which results in increased licking and grooming of pups by the dam. It is considered that serotonin plays a crucial role in the persistence of the handling effect through increased hippocampal GR levels [190]. Similarly, as adults, the offspring of mothers that exhibited more licking and grooming of pups during the first 10 days of life showed reduced HPA axis stress response due to increased hippocampal GR mRNA expression and decreased levels of hypothalamic CRH mRNA [191]. On the other hand, maternal separations during the critical periods of hippocampal development can disrupt hippocampal cytoarchitecture and neurogenesis in a stable manner, with stress hyper-responsiveness observed in these animals as adults [192, 193].

4.2. Programming of the somatotrophic axis

Programming of the somatotrophic axis (GH-IGF axis) is known to be induced by transient events in early postnatal life. The best described example is the effect of transient neonatal manipulation of sex steroids to permanently alter subsequent GH secretion to resemble the pattern of GH secretion of the opposite sex in rodents [194]. Intrauterine programming of the somatotrophic (GH-IGF) axis is still not fully understood, despite its importance in postnatal growth and metabolism. Synthetic activity, storage, and proliferation of rat pituitary GH cells, indicated by the significant increase in GH cell immunopositivity, size, and number per volume and unit of area, rise markedly from the 19th till the 21st fetal day [195]. This corresponds with an increase in plasma corticosterone concentration in near term rat fetuses [46]. It has been shown that dexamethasone administered during the last week of pregnancy has a maturational effect on pituitary GH cells in rats [196]. Dexamethasone induced GHRHR mRNA expression and accumulation in the fetal rat pituitary gland [70] and amplified the stimulatory influence of GHRH. As a consequence, dexamethasone induced GH cells to synthesize and release more GH, leading to increases in GH cell size and immunopositivity (Figure 5) [196]. Corticosterone-induced GH cell differentiation involves GH expression in cells not expressing GH mRNA previously [197]. Moreover, dexamethasone can induce GH progenitors to start GH synthesis one day earlier than in normal fetuses. *In vitro* findings suggested that incubation of the pituitary gland with dexamethasone for 24 h increased GH mRNA on fetal day 18 to a level nearly identical to that in intact 19-day-old fetuses [70]. In humans, low-weight babies have high basal GH and low IGF-I concentrations at birth, with an increased GH response to GHRH [198]. These altered concentrations are maintained during early childhood and are accompanied by changes in the pattern of GH secretion. By early adulthood, urinary GH excretion, which reflects GH secretion, is low in men and women with low birth weights, but in old age birth weight is unrelated to either urinary GH excretion or the GH secretory profile [198]. Low birth weight is associated with decreased IGF-I, IGF-II and IGFBP-3, and elevated levels of IGFBP-1 [199].

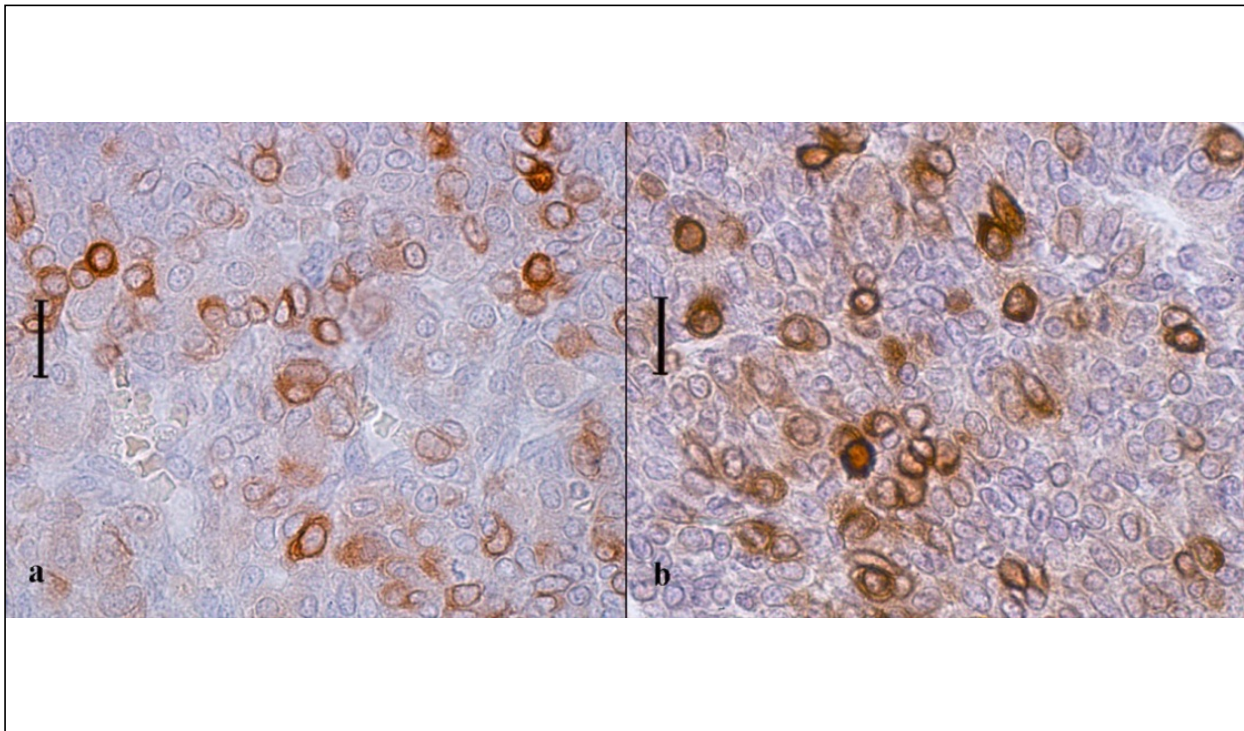


Figure 5. a) Pituitary GH cells of control 21-day-old fetus. b) Intensive immunostaining of GH cells in 21-day-old fetuses after maternal dexamethasone treatment. Bar - 25 μ m.

In the fetus, IGF-I together with insulin, acts as a signal of nutrient plenty at the cellular level and promotes tissue growth in line with substrate availability in the fetus [24]. In fetal sheep, concentrations of insulin and IGF-I rise with increasing fetal concentrations of glucose over the normal range of values induced by variations in maternal nutritional state. The rise in fetal plasma IGF-I probably reflects overspill of IGF-I produced by a number of different fetal tissues, since IGF-I is primarily a paracrine growth factor *in utero*. In contrast, the concentrations of cortisol rise as fetal glucose levels decline [200]. Fetal undernutrition induced by maternal dietary manipulation, placental insufficiency and restriction of uterine blood flow, all reduce the circulating levels and tissue expression of IGF-I [200]. Glucocorticoids affect the expression of *Igf1* and *Igf2* genes, although their effects are tissue and *Igf*-specific. In fetal sheep, cortisol up- and down-regulates *Igf1* gene expression in the liver and skeletal muscle, respectively, whereas it down-regulates *Igf2* gene expression in these tissues. These changes in tissue expression occur both in response to exogenous cortisol infusion before term, and when fetal cortisol levels rise endogenously during the immediate prepartum period [200]. The cortisol-induced changes in tissue *Igf* gene expression are also accompanied by decreases in the fetal growth rate and, close to term, by a fall in plasma IGF-II levels [77, 201]. Cortisol, therefore, appears to initiate the switch from paracrine IGF production *in utero* to the hepatic production of endocrine IGF-I characteristic of the postnatal animal. Glucocorticoids may act on *Igf* gene expression either directly or indirectly, through changes in the GH receptor gene expression [79] and/or via other transcription factors or cortisol-dependent hormones, such as T3 [202]. This premature transition from IGF-II to

IGF-I production has beneficial effects on tissue differentiation, should delivery occur before full term. However, if delivery is not stimulated prematurely, the glucocorticoid-induced switch from the fetal to the adult mode of somatotrophic regulation may lead to inappropriate changes in cell proliferation and differentiation *in utero* with adverse sequelae both at birth and much later in life [200]. The reduced axial growth and reduced femur and tibia length reported in juvenile rats prenatally exposed to dexamethasone could serve as an illustration [203]. Altogether, the long-term consequences of such fetal changes in the GH-IGF axis are yet not fully understood in terms of functional adaptation or diseases. However, glucocorticoid-induced alterations might appear as potentially beneficial for short-term survival in an environment of shortage of nutritional resources. After birth, normalization of insulin, IGFs and IGFPs occurs. During this period, when suddenly exposed to increased concentrations of insulin and IGF-1, tissues chronically depleted of these two hormones during fetal life may counteract the hike by developing insulin resistance as a metabolic defense against developing hypoglycemia [204]. Therefore, infants with low birth weight who show early and complete growth recovery could be at higher risk for the occurrence of the metabolic syndrome in adulthood. Indeed, recent results in rats have shown permanent and sexually dimorphic changes in the expression of genes involved in the GH-IGF axis in animals that were weaned on to a high fat diet [203].

4.3. Programming of the hypothalamic-pituitary-thyroid axis

Glucocorticoid milieu strongly influences HPT axis activity during critical developmental periods. Prenatal alterations in glucocorticoid levels, caused by the application of synthetic glucocorticoids, maternal undernutrition or adrenalectomy, reflect on the fetal, neonatal and adult HPT axis structure and function.

Unbiased estimation of the cell number applying a design-based modern stereological approach revealed that maternal dexamethasone treatment significantly decreased pituitary TSH cell number in near term fetuses. This result together with the strong immunopositivity of TSH cells, and the fact that the decreased number of TSH cells sustains serum TSH concentrations at the control level, indicates that glucocorticoids exert a maturation-promoting effect on fetal TSH cells enhancing TSH synthesis (Figure 6) [205]. In sheep fetuses, antenatal glucocorticoid administration induced an increase in the circulating T3 concentration. Tissue-specific changes in deiodinase enzyme activities show stimulation of hepatic D1 activity with consequent increases in hepatic T3 production, as well as decreased T3 clearance by suppression of D3 enzymes in the kidney and placenta [206]. In the brain, glucocorticoid application stimulated TH activity during a period between gestational day 20 and neonatal day 12 that largely overlaps with the transient window in time during which brain development is TH sensitive [207]. On the contrary, maternal undernutrition during the gestational period results in lower serum T3 and higher serum reverse T3 concentrations in neonatal pups [208].

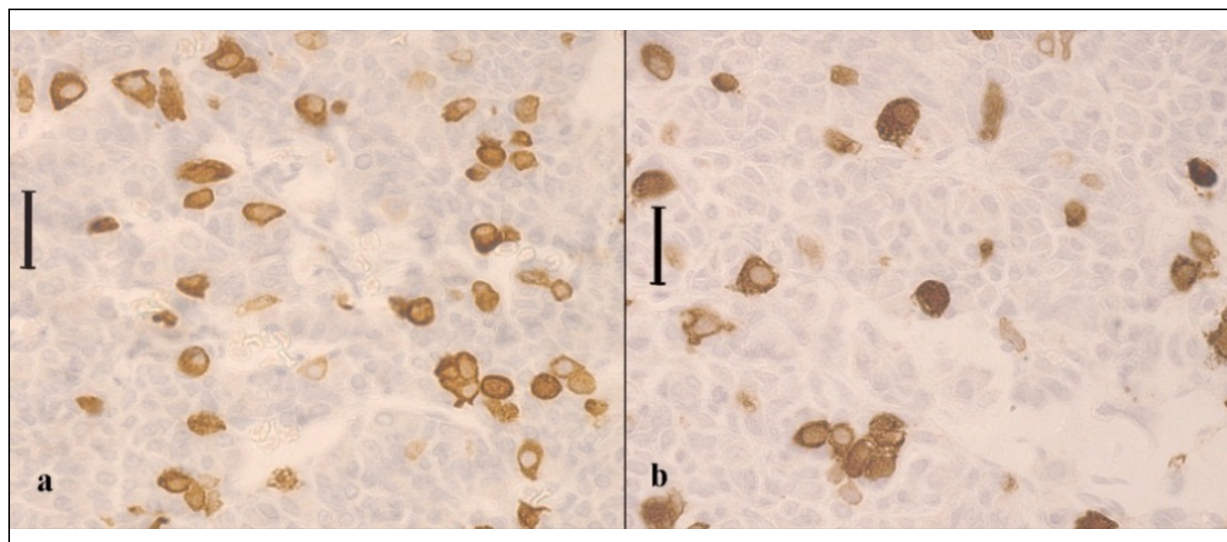


Figure 6. a) Numerous TSH cells characteristic for pituitary of 21-day-old fetus. b) Decreased number of TSH cells, with intense immunopositivity was observed in 21-day-old fetuses after maternal dexamethasone administration. Bar - 25 μ m.

Alteration of the glucocorticoid milieu caused by maternal adrenalectomy influences HPT axis functioning in adult offspring. Decreased hypothalamic TRH mRNA levels and increased plasma TSH levels recorded in both male and female adult offspring of adrenalectomized dams were reversed by the administration of corticosterone to the pregnant adrenalectomized dam. The decreased plasma T3 concentrations in female offspring, which were reversed by the administration of higher levels of corticosterone to the adrenalectomized pregnant rats, suggest that the adult HPT axis responded to variations in maternal glucocorticoid milieu in a sex-specific manner [209].

Importantly, TH per se are potent programming factors. Fetal and neonatal hyperthyroidism or hypothyroidism results in programming of the HPT function. During critical periods, TSH secretion is suppressed by an excess of TH, but cannot be increased despite the marked lowering of circulating TH caused by perinatal propylthiouracil administration. More importantly, perinatal thyroid status "programs" its own future reactivity, so that early hypothyroidism results in reduced T4 and T3 levels in adulthood, despite normal levels of TSH [210].

TH influence the accretion, differentiation and metabolism of many tissues and cell types during development in a time-dependent manner. The effects of its deficiency during critical periods, when the tissues still have some plasticity and are in a higher proliferating and differentiating stage, are thus notable, often permanent. Fetal hypothyroidism leads to asymmetrical growth retardation, with reduction in muscle mass [211]. Fetal metabolism and utilization of oxygen, as well as bone tissue growth were adversely affected by TH deficiency in utero [211]. A well known example is that hypothyroidism during the period of thyroid-dependent brain development, in fetuses and during infancy, causes permanent mental retardation [212]. Thus, the structure and function of TH-dependent tissues, determined during critical periods by the striking effects of TH action [139], might be the

cause of different (patho)physiological alterations which manifest during the life cycle. The potent influence of glucocorticoids on serum TH concentrations and the TH tissue bioavailability in the same period represents an important additional cause of the programming events recorded in different tissues.

4.4. Programming of endocrine pancreas

A number of epidemiological and clinical studies demonstrate an association between low birth weight and an increased incidence of metabolic, cardiovascular and other diseases in adult life. Adverse intrauterine environment caused by inadequate maternal nutrition status [13], poor placental function [17], maternal stress [15] or treatment with synthetic glucocorticoids [187] is linked with impaired intrauterine growth and increased rates of metabolic diseases such as type 2 diabetes in adulthood.

The increased fetal glucocorticoid exposure observed during and after suboptimal conditions, triggers cell differentiation in many of the tissues, resetting the set points of metabolic homeostasis and endocrine axes and in most individual fetal tissues leads to weight reduction, restricted fetal growth and decreased birth weight [24]. Glucocorticoids therefore switch the cell cycle from tissue accretion to tissue differentiation in preparation for delivery. At the same time, glucocorticoids are involved in the programming of the HPA axis during critical periods, causing structural and functional changes specified in the above section. Alterations in the feedback sensitivity of the fetal HPA axis, as adaptation to suboptimal conditions, mostly result in enhanced HPA axis activity postnatally, under basal conditions or after stress challenge, with elevated glucocorticoid levels [21]. These changes are in close association with the programming of susceptibility in the fetus to develop metabolic syndrome in later life. Indeed, hyperactivity of the HPA axis with chronically elevated glucocorticoids is positively correlated with the metabolic syndrome, which includes a cluster of symptoms such as hyperglycemia, hyperinsulinemia, or insulin resistance. Dyslipidemia, hyperleptinemia, raised serum triglycerides, lowered serum high-density lipoprotein cholesterol, and high blood pressure have also been recorded. All of those risk factors are a prelude to the development of diseases such as type 2 diabetes, atherosclerosis and cardio-vascular complications [28].

Suboptimal conditions *in utero* lead to changes in the endocrine environment which influence fetal development so that its nutrient requirements are decreased and a thrifty phenotype is produced to maximize its chances for survival. These short-term beneficial adaptations may be maladaptive in postnatal life, contributing to poor health outcomes [26]. If postnatal nutrient availability is better than predicted, metabolic dysfunctions occur, as the organism is not adapted to cope with excessive caloric intake in later life. The association of low birth weight with early postnatal catch-up growth, in situations where discrepancies between the pre- and postnatal environment are significant, adversely affects body composition, producing increased susceptibility to non-insulin dependent type 2 diabetes. But if environmental conditions remain unchanged, and the offspring of mothers on a low protein diet continue with the low protein diet during lactation, development of the

metabolic phenotype is prevented. The “predictive adaptive response” hypothesis proposes that the degree of mismatch between the pre- and postnatal environments is a major determinant of subsequent disease, and leads to the premise that adult disease arises *in utero* [28, 213].

The thrifty phenotype is not able to respond to unexpected environmental conditions because the changes in metabolic tissues established during critical periods are directed towards low nutritional demands. The fetus adapts to an adverse intrauterine milieu through changes that permanently affect the pancreas, muscles, adipose tissue, and liver structure and function, which are involved in the pathogenesis of obesity and type 2 diabetes [25].

Progressive reduction in insulin-producing β -cell mass is observed in rats with restricted fetal growth [214]. There is evidence that prenatal caloric restriction during pregnancy causes alteration in pancreatic islet neogenesis by decreasing the β -cell precursor pool [215], while maternal protein restriction in rats lowers β -cell proliferation and/or increases apoptosis rates in the fetal endocrine pancreas [215, 216]. Permanent reductions in β -cell mass and its functional efficiency, although achieved by different mechanisms, result in glucose intolerance in adulthood [214]. Nutritional deprivation as severe stress induces a rise in both maternal and fetal corticosterone levels, which in turn are responsible for the observed effects [217].

Prenatal stress that induces a restriction in intrauterine growth in aged male rats causes hyperglycemia, glucose intolerance, and decreased basal leptin levels. Again, an adverse glucocorticoid environment during critical periods might be the underlying mechanism that mediates long-lasting disturbances in feeding behavior and dysfunctions related to type 2 diabetes [15].

Overexposure to exogenous glucocorticoids during different stages of development reduces β -cell mass in the fetal endocrine pancreas: impairment of β -cell commitment is recorded in fetuses exposed to glucocorticoid during the last week of gestation, while glucocorticoids treatment throughout gestation lowers β -cell proliferation and impairs islet vascularization [218]. Glucocorticoid excess during the last week of gestation leads to lower levels of insulin expression in the β -cells of 3-week-old offspring via a mechanism that involves down-regulation of Pdx-1, the transcription factor that initiates and promotes β -cells development [219]. Programming of the functional capacity of pancreatic β -cell mass by adverse intrauterine conditions increases susceptibility to type 2 diabetes during adulthood that is especially evident if offspring when they are challenged with nutritional abundance. As during adulthood the majority of β -cells are formed through proliferation of the existing cells [220], smaller β -cell mass in the newborn means fewer β -cells will be available for renewal during life, which increases the risk of developing glucose intolerance or diabetes [221].

Impaired insulin action at the major sites of glucose utilization, such as skeletal muscles, liver and adipose tissue, further predisposes to a later diabetic state. Excess prenatal

glucocorticoid exposure, uteroplacental insufficiency as well as maternal low protein diet in the perinatal period prepare skeletal muscle metabolism for poor metabolic conditions in later life [22, 25, 213]. These changes include up-regulation of GR expression that determines higher muscle glucocorticoid sensitivity, with the promotion of protein breakdown and blunted protein synthesis in muscles [222]. Prenatal growth restriction caused by adverse intrauterine conditions of different etiology has a long-term influence on adiposity. Redistribution of body fat from the periphery to the central or visceral deposits that have a relatively higher level of GR expression and are thus more sensitive to glucocorticoid action is established in adult rats and sheep prenatally exposed to glucocorticoid excess [223-225], contributing to decreased insulin sensitivity and blunted glucose intake [2]. Adipocytes from 15-month-old low-protein rat offspring are also resistant to the antilipolytic action of insulin and insulin-induced glucose uptake [25]. It can be concluded that adverse conditions during critical periods may program adipocyte metabolism to give rise to later obesity and type 2 diabetes, especially when challenged postnatally with a hypercaloric diet [226]. Suboptimal conditions during fetal development program an increased level of liver GR expression that enables much higher glucocorticoid impact in diabetic animals [227]. Down-regulation of glucokinase activity in parallel with decreased liver glucose uptake, and up-regulation of gluconeogenic enzyme activities, notably phosphoenolpyruvate carboxykinase which catalyzes a rate-limiting step in gluconeogenesis, have been established in rats exposed to excessive glucocorticoids in utero [25, 228]. The programming effects established in glucocorticoid overexposed fetuses with restricted growth are thus directed toward enhanced glucose production and reduced glucose utilization in the liver and other peripheral tissues in adulthood, and represent the structural and physiological basis of the development of type 2 diabetes [19].

4.5. Programming of hypothalamic-pituitary-gonadal axis

Steroid hormone excess during fetal life, including glucocorticoids and sex hormones, is well known to induce permanent alterations in the physiology of the adult HPG axis in both sexes [229]. The majority of data describing the effects of elevated levels of glucocorticoids on the HPG axis and possible mechanisms, come from studies in adults, and there are only limited data on fetal effects. It has been known that the HPA axis, when activated by stress, exerts an inhibitory effect on the female and male reproductive system. Reallocation of resources during the stress response suppresses the reproductive axis, which gives higher priority to an individual's survival rather than the maintenance of species. This effect is responsible for the "hypothalamic amenorrhea of stress" in females, which is observed in anxiety and depression, malnutrition, eating disorders and chronic excessive exercise, and the hypogonadism in Cushing's syndrome [230]. Stressors trigger a rise in glucocorticoids that suppress reproductive functions along the HPG axis [3]. Glucocorticoids decrease expression of GnRH mRNA [231] in the hypothalamus, and

are associated with alterations in both FSH and LH cells [232, 233]. Glucocorticoids also affect gonads directly. It has been reported that dexamethasone inhibits ovarian function in immature female rats and the differentiation of granulosa cells by FSH [234]. In the testis, elevated levels of glucocorticoids suppress testosterone biosynthesis [3]. Additionally, dexamethasone induces apoptosis of tubules and germ cells in adult rat testis [235].

Elevation of maternal glucocorticoids induced by maternal stress, undernutrition or exogenously administered dexamethasone or betamethasone, along with IUGR cause alterations in HPG axis function in male and female offspring. The major alterations reported were related to changed sexual behavior, delayed puberty, and delayed development of the gonads. In rats, exposure to prenatal stress demasculinizes and feminizes the behavior of the male offspring. When dams are restrained under bright light from days 14 to 21 of gestation, the male offspring display reduced anogenital distance and lower testis weight at birth compared to controls [18, 236], which could predict impaired sexual activity at adulthood [237]. Prenatal treatment with glucocorticoids caused the disappearance of sexual dimorphism of aromatase activity in the brain preoptic area of rat pups in early postnatal life [238]. Prenatal bethamethasone treatment diminished the testosterone peak in male pups, a peak crucial for brain sexual differentiation. As a consequence, this prenatal treatment may have impaired the hypothalamus–pituitary axis, thus reducing production of testosterone in adulthood and altering the partner preference and sexual behavior [239].

It has been reported that maternal protein restriction altered the key components of pregnant maternal steroid endocrinology, as well as the endocrinology of the offspring. Maternal corticosterone and testosterone levels were elevated, which resulted in an increased anogenital distance in males [240]. In females exposed to protein restriction during development the onset of puberty was delayed and the cycle length was increased [241]. The decrease of LH and slight, but not significant, decrease of FSH levels was detected in adult females that experienced maternal protein restriction at some stage of development. Together with the increases in testosterone levels at 1 year, this presage potential reproductive problems, including changes in the ovarian cycle [241]. Maternal protein restriction leads to similar changes in reproductive hormones in the male offspring [240], indicating that a major effect of the challenge imposed on the developing offspring is to alter hypothalamic–pituitary endocrine function. The reproductive function aged more rapidly in females that had been exposed to protein restriction during development [241]. Testicular and ovarian growth was drastically retarded, and the onset of puberty was delayed in male and female rats prenatally exposed to maternal food restriction [242]. In addition, the ovulation rate in adulthood was reduced in female sheep that experienced undernutrition during the prenatal period [243]. The suggested main mechanism by which maternal calorie restriction induced delay of puberty in the female offspring is that decreased function of the kisspeptin system retards the development of

reproductive function and the onset of puberty. Hypothalamic levels of Kiss1 mRNA were decreased in prenatally undernourished rats, and the replacement of kisspeptin normalized the timing of vaginal opening in these females [244]. However, this mechanism is not responsible for delaying puberty in dexamethasone-induced IUGR females, since the levels of Kiss1 mRNA were not altered [245]. On the other hand, alterations of ovarian functions found in dexamethasone-induced IUGR rats, can affect sexual maturation [246]. As ovarian weight in the dexamethasone-induced IUGR rats was lower than in the controls during the prepubertal period (postnatal day 28), but not on the day of vaginal opening, the retardation of ovarian function development might be involved in the delayed onset of puberty [245]. Smith and Waddell [247] have shown that variations in fetal glucocorticoid exposure across the normal physiological range are capable of influencing the timing of subsequent puberty. Puberty was substantially delayed by increased exposure to glucocorticoids, which was most clearly evident in female offspring. Of particular importance were the observations that increased exposure of the fetus to endogenous maternal glucocorticoids (via inhibition of placental 11 β -HSD by carbenoxolone treatment) delayed puberty in the female offspring, whereas an experimental reduction in fetal glucocorticoid exposure (by maternal metyrapone treatment) advanced puberty in the male offspring [247].

When higher multiple doses of dexamethasone were administered to dams between the 16th and 18th day of gestation, a significant reduction in body weight was recorded in near-term fetuses that persisted till the peripubertal period of life. The volume of pituitaries of the exposed females were also significantly reduced till the peripubertal period. The absolute number of both types of gonadotropic cells, obtained by design-based stereological methods, was decreased in the pituitaries of exposed females (Figure 7). As the pituitaries, the ovaries of exposed females were smaller than that of controls (Figure 8). Significant decrease in healthy, but an increase in atretic primordial follicles was observed in neonatal period (at 5 days of age) [246]. Alterations in the number of healthy and degenerated germinative cells were evident in fetuses as well, and sustained till the peripubertal period of life. Since the puberty was delayed in females exposed prenatally to dexamethasone, no *corpora lutea* were seen in their ovaries. In contrast, 3-5 *corpora lutea* were present in the ovaries of control females (Figure 8). However, the process of folliculogenesis remained unchanged, since the follicles at all stages of development seen in the ovaries of control females in the neonatal [246], infantile and peripubertal period, were present in the ovaries of females prenatally exposed to high levels of glucocorticoids. Therefore, a clear programming effect of dexamethasone was detected in the female HPG axis. It has been shown that glucocorticoids mediate changes in the dynamic balance between mitosis and apoptosis [248], and may be a mechanism for the control of total cell number in developing tissues and organs [249, 250]. This could be one of the mechanisms by which glucocorticoid overexposure affects the hypothalamic-pituitary-ovarian axis.

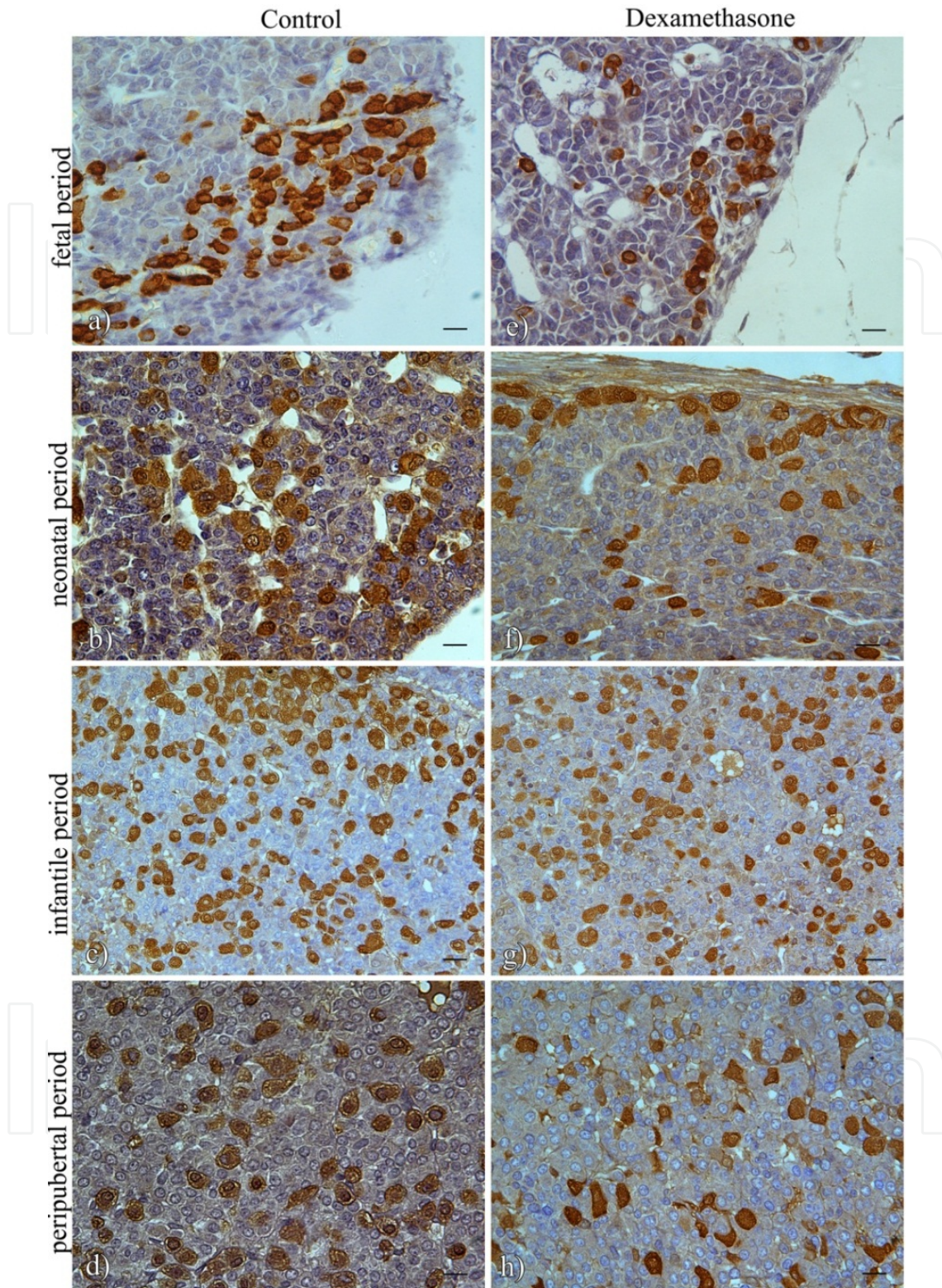


Figure 7. Immunohistochemically stained FSH cells in the pituitaries of control (a-d), and females prenatally exposed to dexamethasone (e-h). FSH cells were examined in different periods of life: in nearm-term fetal period (a, e), neonatal (b, f), infantile (c, g) and peripubertal period (d, h). In all examined periods the number of FSH cells was lower in the pituitaries of dexamethasone exposed females compared to controls. Bar - 20 μ m.

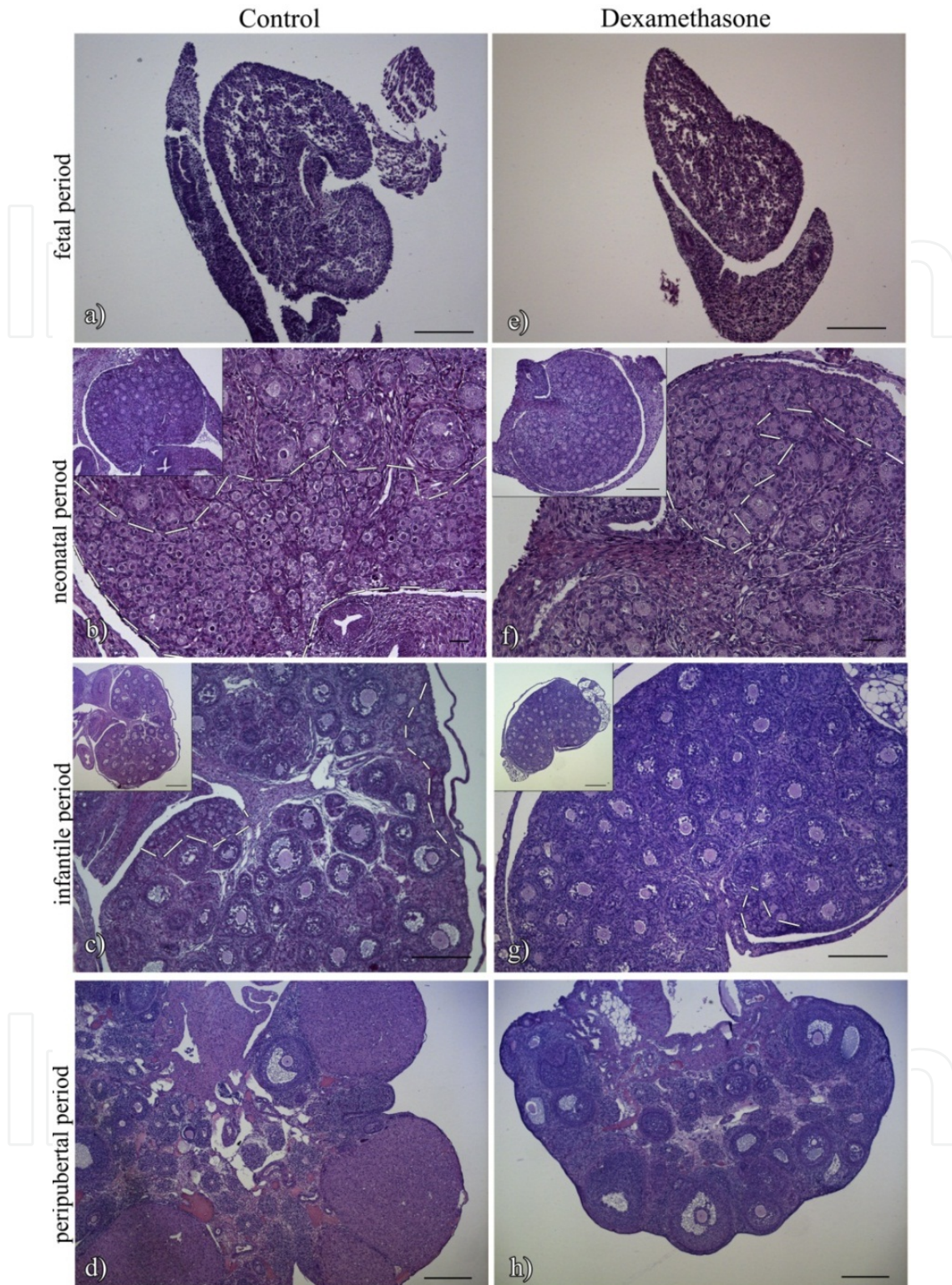


Figure 8. Ovaries of control (a-d), and females prenatally exposed to dexamethasone (e-h). Ovaries were examined in different periods of life: in nearm-term fetal period (a, e), neonatal (b, f), infantile (c, g) and peripubertal period (d, h) and they were smaller in dexamethasone exposed females. Numerous primordial follicles (dashed line) are present in the ovaries of control females, while they were fewer in number in the ovaries of dexamethasone exposed rats in all examined periods of life. Bar - 200 μm . In b) and f) bar - 20 μm .

Maternal bethamethasone administration affected the morphological development of the testes in male sheep fetuses, by reducing the length of testicular cords, the amount of interstitial tissue and testicular weight. Because interstitial tissue is primarily made up of Leydig cells, it is possible that betamethasone altered Leydig cell development. In contrast, there was no inhibitory effect on Sertoli cell number. This could be a result of the direct influence of the glucocorticoid used, since the presence of glucocorticoid receptor was demonstrated in ovine fetal Leydig cells, while the level of glucocorticoid receptor expression in Sertoli cells was low [251].

Fetal overexposure to glucocorticoids without any doubt has programming effects on the HPG axis, and reproduction in later life is thus impaired in both sexes. However, the mechanism of HPG programming is yet to be elucidated. The time interval between the exact insult and a fully functioning HPG axis is long, and prone to influences and interplay with other endocrine axes that are also altered by glucocorticoid overexposure. For example, an impaired somatotrophic axis negatively affects reproduction. Somatostatin treatment inhibits pituitary gonadotropic cells and initial folliculogenesis in the ovaries of infant, peripubertal and adult females [252-257]. Polycystic ovary syndrome (PCOS) is of great importance, owing to its prevalence in up to 10% of the women population of reproductive age. Besides being characterized by perturbed gonadotropin secretion and excess production of androgens, PCOS shares a lot of commons with the metabolic syndrome. Metabolic syndrome is also believed to be of fetal origin and the result of programming in which glucocorticoids play a crucial role [213]. The short-term benefits of glucocorticoid exposure are also difficult to establish due to physiological dormancy of the system till puberty. The maturational effect of glucocorticoids is evident in the pituitary and in the ovary, since fetal overexposure induces a decreased volume of these glands, and of the absolute number of gonadotrops and ovarian somatic and germinative cells till puberty (Figure 1).

5. Conclusion

Glucocorticoids have a powerful influence on growth, maturation and tissue remodeling during fetal development. Their use in human pregnancies at risk of preterm delivery reduces neonatal mortality and morbidity. Glucocorticoids are also the key mediators between the maternal environment and the fetus, and their levels rise, in the mother and in the fetus, when the conditions are suboptimal. They reduce fetal growth, force maturational processes and provoke permanent changes in physiological systems in order to adapt the fetus to an adverse postnatal environment and ensure the maximum chances of survival at birth. These short-term beneficial effects of prenatal glucocorticoids are, at the same time, the ones that increase the long-term risks of dysregulation of the metabolic function and endocrine axes, including stress response, growth and reproduction.

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Acknowledgments

This work was supported by the Ministry of Education and Science of the Republic of Serbia, Grant No. 173009

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