# INVESTIGATION AND VALIDATION OF ANIMAL MODELS FOR THE DEVELOPMENT OF THE HUMAN FETAL AND NEONATAL HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

A Dissertation

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by

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TABLES

| | |
|---|---|
| A. | artery |
| Aa. | arteries |
| ACTH | Adrenocorticotropic Hormone |
| ADHD | Attention Deficit Hyperactivity disorder |
| ATP | Adenosine Triphosphate |
| AVP | Arginine-Vasopressin |
| BCRP | Breast Cancer Resistance Protein |
| CBG | Corticosteroid-Binding-Globulin |
| CNS | Central Nervous System |
| CRH | Corticotropin-Releasing-Hormone |
| CRH-BP | Corticotropin-Releasing-Hormone-Binding Protein |
| CYP11A1 | Cholesterol side-chain cleavage enzyme |
| CYP11B1 | Steroid 11$\beta$-hydroxylase |
| CYP11B2 | Aldosterone synthase |
| CYP17 | Steroid 17$\alpha$-hydroxylase |
| CYP21A2 | Steroid 21-hydroxylase |
| day | gestational day |
| DHEAS | Dehydroepiandrosterone-sulfate |
| DHEA | Dehydroepiandrosterone |
| DZ | Definitive Zone |
| e | embryonic day |
| 11$\beta$-HSD | 11$\beta$-hydroxysteroid dehydrogenase |
| FZ | Fetal Zone |
| GR | Glucocorticoid Receptor |
| HPA | Hypothalamic-Pituitary-Adrenal |
| IP3 | Inositol Triphosphate |
| IR | Immunoreactivity |
| LHPA | Limbic-Hypothalamic-Pituitary-Adrenal |
| mp | medial parvocellular |
| MR | Mineralocorticoid Receptor |
| mRNA | messenger ribonucleic acid |
| NAD+ | Nicotinamide Adenine Dinucleotide |
| NADP+ | Nicotinamide Adenine Dinucleotide Phosphate (oxidized) |
| NADPH | Nicotinamide Adenine Dinucleotide Phosphate (reduced) |
| P | Postnatal day |
| PC-1 | Proconvertase-1 |
| PC-2 | Proconvertase-2 |
| P-gp | Multidrug resistance Phospho-glycoprotein |
| PND | Postnatal day |
| POMC | Pro-opiomelanocortin |
| PVN | Paraventricular Nucleus |
| RIA | Radioimmunoassay |
| SHRP | Stress Hyporesponsive Period |
| SON | Supraoptic Nucleus |
| 3$\beta$-HSD | 3$\beta$-hydroxysteroid dehydrogenase/isomerase |
| TZ | Transitional Zone |
| V. | vein |
| Vv. | veins |
| Vmax | Maximum rate of catalysis in Michaelis-Menten equation |
| w | weaning |
| wk | week |
| zF | zona Fasciculata |
| zG | zona Glomerulosa |
| zR | zona Reticularis |

# CHAPTER 1

## Introduction, Materials and Methods

### 1.1 Introduction

A particular phenotype (neuroendocrinological, behavioral) is determined by the individual's genetic background and its personal experience. In other words, based on a given genotype, the expression of a trait can vary across a range of environments [216]. Environmental programming presumably prepares the organism for the world into which it will be born. Already since the fifties and sixties, it is established that the early environment influences the developing hypothalamic-pituitary-adrenal (HPA) axis [246, 282]. Glucocorticoids play a major role in fetal programming. Here, events during gestation determine the long-term outcome of life [318]. Recently, alteration in the activity of the HPA axis moved in the center of attention for stress vulnerability and stress-related disorders and gene x environment interactions were traceable in prevalent stress related disorders such as human depression and posttraumatic stress disorder (PTSD), as well as antisocial behavior [80, 415, 422, 458]. It was possible to verify epigenetic modifications as a link between gene and environment interactions. Here, environmental variations alter the gene expression and protein physiology, which then affect the HPA response to stress [292].

The mother is able to signal the fetus via glucocorticoids information about her environment. All communications between mother and fetus are conveyed through the placenta. The maternal and the fetal HPA axes, as well as the placenta, play critical roles in fetal growth and health. The regulation between these three key players is very complex. Glucocorticoid transfer across the placenta from the mother into the fetus is determined by species-specific placental 11$\beta$ hydroxysteroid dehydrogenase (11$\beta$HSD) expression [421, 478]. Placental corticotropin-releasing hormone (CRH) stimulates maternal and fetal adrenocorticotropic hormone (ACTH), cortisol and androgen release. In the following, fetal cortisol induces placental CRH synthesis in a feed forward loop [85, 384, 385]. Hypothalamic CRH and arginine vasopressin (AVP) elicit the synthesis of ACTH in the pituitary. ACTH stimulates in the adrenal cortex glucocorticoid and in some species androgen secretion [134, 234]. Glucocorticoids induce negative feedback via the brain and self-regulate their own secretion by binding to mineralocorticoid- (MR) and glucocorticoid-receptors (GR) in the hippocampus, to GR in the hypothalamic paraventricular nucleus (PVN) and in the anterior pituitary, inhibiting further HPA activity [337, 471]. Among the many parameters known to affect the neuroendocrine stress response, the HPA axis is closely influenced by noradrenergic [187, 337], serotonergic [12, 31], thyroidal [293] and gonadal input [27].

During pre- and postnatal brain maturation, especially over periods of accelerated growth, early adverse experiences such as maternal stress, synthetic glucocorticoid exposure, placental 11$\beta$HSD2 inhibition, fetal malnutrition, inflammation or inadequate mother-offspring interactions after birth, are able to modify neuroendocrine function later in life. Aside from the physiological ability of glucocorticoids to generate fetal tissue maturation (e.g. lung, liver, adrenal), exposure of the fetus to enhanced glucocorticoid level is associated with preterm delivery and growth retardation. The impact of maternal synthetic glucocorticoid administration on the developing HPA axis can vary according to the applied dose and frequency, with gender and in relation to the highly species-specific timing of HPA axis maturation relative to birth. Further, the impact seems to change with age [128, 282, 318, 421, 478]. Apparently, the female HPA axis is more vulnerable to these programming effects as higher level of glucocorticoids seem to pass through the placenta into the female fetus compared to the male fetus [309]. While prenatal stress increases corticosterone levels, the latter binding to both GR and MR, synthetic glucocorticoids have a low affinity to MR and primarily elicit their effects by binding to GR. Still the administration of synthetic glucocorticoids to the mother increased hippocampal MR expression in the sheep and in the male guinea pig offspring

but decreased MR expression in the female guinea pig and in the rat offspring as well as in the fetal mouse. GR expression in the hippocampus following maternal treatment was increased in female guinea pig and decreased in rat offspring but synthetic glucocorticoids showed no effect on MR or GR expression in the hippocampus of the human fetus during midgestation [121, 227, 255, 329, 442, 490]. In addition, antenatal synthetic glucocorticoid treatment of the mother decreased the CRH expression in the PVN of the fetal guinea pig and the neonatal rat but increased the CRH expression in the adults‘ rat hypothalamus [68, 285, 431], increased the human ACTH response to stress [147], and decreased the expression of adrenal steroid enzymes in guinea pigs [336]. Exposure to synthetic glucocorticoids during gestation caused elevated plasma basal and stimulated cortisol concentrations in the rhesus monkey offspring and a blunted salivary cortisol response to stress in human babies and infants [114, 115, 469]. Besides these direct impacts on the offspring's limbic-hypothalamic-pituitary-adrenal (LHPA) axis, antenatal synthetic glucocorticoid administration to the mother decreased the passage of maternal glucocorticoids through the placenta into the fetus in baboons while maternal stress increased the passage into the rat [258, 262]. In humans, the exposure to increased glucocorticoid concentrations or an early adverse environment is correlated in the fetus with a reduction in birth weight, and in infants with altered affective reactivity to novelty and symptoms analogous to the attention-deficit hyperactivity disorder (ADHD) [116, 157, 308]. The inconsistent results in the response of different animal species to early adversity can be explained to some extent by the species-specific differences in maturational status of the LHPA axis during exposure and a comparison between species might be determined by the timing of the impact in relation to the gestational length [278, 282]. It can be assumed that depending on the mentioned effects during development, environmental programming activates or deactivates the HPA axis and increases the risk of disorders related to hyper- and hypocortisolism, such as anxiety, depression and metabolic diseases [89].

The examples given below, associating early programming in connection with stress vulnerability later in life, possibly under the influence of epigenetic mechanisms, will lead us to the research question at hand: Fibromyalgia, a disease with a strong gender disposition (women/men: 9/1), involves muscle pain, depression, fatigue and anxiety. Stress exposure during prenatal periods might cause the inability of fibromyalgia patients to adequately mobilize cortisol during stress (hypocortisolism), apparently due to adrenal insufficiency. Hellhammer and his group showed that patients with a shorter gestational length exhibit a significantly lower cortisol awakening response and both factors occur more often in women, whose mothers were exposed to adverse life events. Low cortisol levels presumably fail to restrain prostaglandin and pro-inflammatory cytokines, mediators for pain perception and memory, and with ‘Sickness behavior‘ associated fatigue and lack of initiation [188, 196, 239]. Human research suggests that early adversity increases the risk of chronic diseases in adulthood, in connection with changes in HPA axis activity [89]. The often retrospective studies in humans rely on reported adversity during gestation. Manipulations during human gestation are limited and occur primarily in connection with therapeutic treatment, termination of pregnancy, preterm and term birth. Research on animals permits a timely programming by applying adverse environmental stimuli during specific, sensitive windows of development to cause alterations (endocrinological, behavioral) in the offspring's stress response [421]. To investigate the programming effects inside brain regions like hippocampus and hypothalamus, animals are often used as a model for the human. Depending on the species, specific alterations occurred, and Matthews‘ research group at the university of Toronto, Canada associated these programming effects with the maturational status of the HPA axis during the time of exposure [278, 282]. The individual's resilience and vulnerability to stress can be affected by polymorphisms in certain genes (e.g. serotonin transporter gene, monoamine oxidase A gene) and by epigenetically altered gene expression (e.g. GR gene expression) inside specific brain regions. By using rhesus monkeys as a human model for gene x environment interactions, Suomi‘s group at the National Institute of Health in Maryland demonstrated that early adversity (peer rearing), in combination with a specific allelic variation (s-allele) in the promoter region on the serotonin transporter gene, impaired serotonin metabolism and could lead to delayed neurobiological development, altered HPA axis reactivity, High-Risk Aggression and excessive alcohol consumption. A similar polymorphism in humans was associated with depression

[80, 415, 458, 459]. Meaney and his coworkers at the McGill University in Montreal established their fundamental epigenetic research, by showing that in rats during a very tight time windows after birth, behavior of the mother can change methylation status and gene expression of the GR in the pups. High licking and grooming increases hippocampal GR expression and dampens the HPA axis activity later in life. Vice versa low licking and grooming is not only associated with the phenotype of increased HPA axis stress response but also with behavioral modification in form of anxiety, learning and memory disabilities [292, 487, 488]. The impact of maternal licking and grooming on the methylation status of her pups was only present in the first but not in the second week after birth [87], indicating changes in the neuroendocrine maturation over time.

The complex LHPA axis of mammals features prominent species-specific differences in development and regulation, e.g. the apparently primate-specific regulation by placental CRH. It was assumed that the discrepancies across species occur because environmental input affects the developing organism during different, specific for the involved species, level of neuroendocrine maturation [282]. During accelerated growth periods, the organism is especially vulnerable to programming effects. There are significant differences in the period of brain growth spurt, taking place at the end of the second trimester in guinea pigs and rhesus monkeys, at the end of the third trimester in sheep but only after birth in rats [128, 478]. Species-specific differences in the development of the LHPA axis can be assumed in various brain regions and might involve a number of hormones and receptors. Comparative research between species is rare. A source of data about neuroendocrine development in sheep and guinea pig is derived again from Matthews research group. It appeared for example, that fetal CRH expression in the hypothalamic PVN of the sheep increases toward term, while in the guinea pig, the expression seems to decrease during gestation [281, 337]. Environmental programming of the HPA axis often involves alterations in the GR expression. The first appearance of GR expression inside the hippocampus shows species-specific development differences. This event is verifiable in the mouse after birth but already in the second half of gestation in the sheep and the rat [127, 329, 442].

In conclusion, it can be assumed that modifications of the environment, like the administration of glucocorticoids, might have an entirely different impact on one species compared to another, because one species could be extremely vulnerable, due to its currently rapid developing stress system, while the stress system of the other species could be in a recovery phase, where it might be quite immune against programming effects. How stress of the mother influences the fetal HPA axis additionally depends on the amounts of glucocorticoids crossing the placenta and possibly on the activity of the fetal stress axis at the time of the impact. Maternal glucocorticoids might cause more damage to fetal maturation during times of reduced glucocorticoid synthesis in the fetal adrenal than during periods, when maternal glucocorticoid just adds to the already high fetal glucocorticoid production.

To estimate the implications of the programming effects on the developing HPA axis, concerning the risk of stress vulnerability and stress related disorders later in life, it is necessary to know during which physiological stage of maturation the neuroendocrine system is exposed to programming. Concretely, knowledge about species-specific pre- and postnatal development of the HPA axes could be essential to interpret environmentally-induced behavioral and neuroendocrine modifications, and to transfer results from animal to humans and between animal species. Additionally, such a comprehensive work could help to distinguish which species and which time period during its development is most promising to examine with regards to the research question at hand. This thesis provides a comparative analysis of research concerning pre- and postnatal development of the HPA axis across different species. This study is intended to help evaluating the transferability of animal research from animal to human, or to other animal species. We compared the development of the neuroendocrine stress system in six animal models (rhesus monkey, baboon, sheep, guinea pig, rat, mouse) commonly used to gain insight into the human stress axis during antenatal or postnatal periods, with the fragmental information about the development of the human HPA axis. After this introduction, the first chapter presents outline of the thesis and material and methods of the research. In *Chapter 2*, an overview of factors, influencing steroidogenesis in the fetal adrenal cortex under the control of higher brain centers (fetal hypothalamus, fetal pituitary) as well as from the

placenta, is given. In this context, glucocorticoid negative feedback on these higher brain centers is addressed. *Chapter 3* presents data about glucocorticoid synthesis in the fetal and neonatal adrenal cortex. *Chapter 4* attempts to relate the findings presented in the previous two chapters and gives a comprehensive picture of the fetal and neonatal HPA axis across the different species. *Chapter 5* encompasses the summary, the general discussion and the outlook.

## 1.2 Materials and Methods

### 1.2.1 Selected species

The following seven species will be investigated in this theoretical study. Besides the human (*Homo sapiens*), the primate species rhesus monkey (*Macaca mulatta*) and baboon (*Papio anubis*), as well as the sheep (*Ovis*), the guinea pig (*Cavia porcellus*), the rat (*Rattus*) and the mouse (*Mus*) are selected to study, as most commonly used species serving as models for human pre- and postnatal development of the hypothalamic-pituitary adrenal axis.

### 1.2.2 Normalization process

#### 1.2.2.1 Prenatal normalization

- Prenatal normalization of the timing of an event

To compare the timing of an event, during the prenatal development across these species or of different events inside a species, all data from the utilized literature sources are normalized to equally long gestational periods. Of the two different systems to time the prenatal period in the human fetus, we decided to use 'gestational age' (about 40 weeks or 280 days of gestation), starting with the first day of the last menstrual period of the conception cycle. This system begins two weeks earlier than the 'conceptional age' (from fertilization until delivery, encompassing 38 weeks or 267 days post conceptionem). The here used gestational age is more conventional for clinical purposes [212] and is more commonly used in recent research, while the second system is mainly used in anatomy.

In the single species, the following gestational periods are used:

TABLE 1.1

Gestational periods

| Species | Weeks |
|---|---|
| Human | 40 weeks |
| Rhesus monkey | 165 days |
| Baboon | 184 days |
| Sheep | 150 days |
| Guinea pig | 68 days |
| Rat | 22 days |
| Mouse | 19.5 days |

Second, to compare the time of an event directly between single species, the percentage of gestational period will be calculated with 100% of gestation equal to day of birth. For example: In human 4 weeks of gestation equal 4/40 weeks and 10% of gestation while 15 days of gestation in the sheep resemble 15/150 days and again 10% of gestation.

- Prenatal normalization of the quantity of an event

To compare the quantity of an event prenatal, the data will be expressed in relation to their values at the end of gestation (term). When displaying data between different species, or different events in one species, the amplitudes of the data from a single species or a single event are adjusted (divided or multiplied) to be able to present the data graphically across different species or different events. These data then can only depict the trends of an event over time.

#### 1.2.2.2 Postnatal normalization

- Postnatal normalization of the timing of an event

To compare time of an event during postnatal development across these species, percent of weaning will be used, with weaning defined as 'getting accustomed to take food otherwise than by nursing'. Using a common developmental time point between the species is necessary, because of the much slower development in primates than in sheep and compared to much faster development after birth in guinea pig, rat and mouse. Weaning takes place in guinea pig, rat and mouse approximately at postnatal day 21, and in the sheep, 90 days after birth. For human we will use weaning at 12 months of life as an estimation [189]. In the rhesus monkey, weaning takes place latest by 10-11 months of life, when the lactation in the mother ceases [501] and the weaning in the baboon similarly happens after 10-12 months [81]. Thus, we will use for both primate species postnatal day 300 as an approximation.

TABLE 1.2

Postnatal periods

| | Human | Rhesus monkey | Baboon | Sheep | Guinea pig | Rat | Mouse |
|---|---|---|---|---|---|---|---|
| days | 365 | 300 | 300 | 90 | 21 | 21 | 21 |

- Postnatal normalization of the quantity of an event

To compare the quantity of an event postnatal, the data will be expressed in relation to their values in adults. In case data for adults are not available, values will be expressed in relation to value at birth. As with the prenatal data, when displaying data between different species, or different events in one species, the amplitudes of the data from a single species or a single event are adjusted (divided or multiplied) to be able to present the data graphically across different species or different events. These data then can only depict the trends of an event over time.

### 1.2.3 Statistical analyses

All results are presented as means ± standard error of the mean. A $P$ value of $<0.05$ is considered significant.

# CHAPTER 2

## Adrenal regulation by higher brain centers and placenta

In the following, we will briefly compare the most important factors, influencing the fetal adrenal cortex, from higher brain centers and the placenta, before we will focus on the fetal adrenal cortex itself. The HPA axis is a neuroendocrine system, encompassing the hypothalamus and the pituitary gland as part of the diencephalon/CNS and the adrenal cortex as a peripheral endocrine gland. The fetal HPA axis is influenced by factors of fetal, maternal and placental origin. As there is no direct anatomical connection between the mother and the fetus, the placenta is responsible for the dialog between both. From the mass of placental factors affecting the fetal HPA, we will focus on:

- placental 11$\beta$HSD, as the enzyme responsible for conversing active glucocorticoids into inactive glucocorticoids and vice versa

,

- the multidrug resistance protein P-glycoprotein (P-gp), in charge of substrate transport across the placenta, by carrying glucocorticoids actively out of cells

and

- placental CRH and its plasma binding protein (CRH-BP). Placental CRH is released into fetal and maternal circulation and most likely affects both the HPA axis of the mother and the child.

Inside the fetal HPA axis, we will investigate in the hypothalamus

- CRH

and

- AVP, together triggering in the pituitary POMC expression and ACTH release;

and further

- hypothalamic GR expression, to investigate the hypothalamus as one of the primary sites of glucocorticoid negative feedback.

In the pituitary,

- ACTH with its precursor POMC will be examined, as ACTH is the direct stimulator for adrenal steroid production

and in addition,

- pituitary GR expression, as a further target of glucocorticoid negative feedback will be analyzed.

The regulation of HPA axis is extremely complex. Besides its tight regulation in itself, the fetal HPA axis is influenced by factors of fetal, maternal and placental origin. The placenta is necessary to shelter and nourish the developing embryo, to remove fetal metabolic excretion products, and is responsible for the gas exchange and the protection from outer and inner deleteriousness. This linking organ transmits the maternal environment to the fetus and plays a detrimental role in sending out programming stimuli. Fetal input arises from peripheral organs such as fetal gonads, thyroid gland, liver, kidneys, brain areas such as the fetal hippocampus, locus coeruleus, raphe nuclei, amygdala, bed nucleus of stria terminalis and further the fetal systemic/adrenomedullary sympathetic nervous and immune system. Maternal nutrition, physical activity, but also stress, toxins, drugs and illness, have their impact on the newly developing organism, mediated through the placenta. As a neuroendocrine organ, the placenta synthesizes a multitude of hormones, influencing both the HPA axes of mother and fetus. The developing fetal HPA axis is affected by the synthesis of placental CRH, prostaglandins, progesterone, and in some species, placental estrogen and androgens. Oxygen and nutrition supply have their input on the developing stress system. Placental transporters like the multidrug resistance P glycoprotein (P-gp) and the breast cancer resistance protein (BCRP), as well as 11$\beta$ hydroxysteroid dehydrogenase (11$\beta$HSD) can influence transplacental glucocorticoid transfer (for further reading see [3, 187, 192, 209, 223, 264, 282, 299, 318, 324, 350, 378, 478].

In the following, we will give a short overview of the structural differences in the placenta, will subsequently investigate placental 11$\beta$HSD, placental P-gp and placental CRH as three important factors directly or indirectly influencing the fetal glucocorticoid synthesis.

## 2.1 Placental 11$\beta$HSD and P-gp

### 2.1.1 Species-specific placenta structure

The location of the feto-maternal exchange inside the placenta plays a decisive role, with regard to the transfer of glucocorticoids across the placenta from the mother to the fetus and vice versa. At the feto-maternal barrier, between maternal and fetal blood, the glucocorticoid inactivating enzyme 11$\beta$HSD2 as well as P-gp, with its ability to transport glucocorticoids out of the cell, are able to reduce fetal exposure to maternal glucocorticoids [15, 70, 223, 354].

The morphology and composition of the placenta shows broad species-specific differences. Following the impregnation in the ampulla of fallopian tube, the zygote begins to cleave while traveling toward the uterus. During the implantation, the now developed trophoblast connects with the uterus epithelium, which under the influence of estrogens and progesterone, evolves into the functional placenta maternal or endometrium. In the following the fetal part of the placenta, the chorion, originates from the trophoblast and the mesoderm, the latter one of the three primary germ cell layers.

In humans, baboons, guinea pigs, rats and mice, the placenta has the form of a disk, while in the rhesus monkey, two disk-like placentas are present, located opposite each other. The anatomy of the sheep placenta is different. Here roughly 100 discrete areas of attachment called placentomes are distributed over the fruit sack. The human chorion only forms in the disk-like area villi and is in contact with maternal blood. Outside this area the chorion is free of villi. The yolk sac is part of the mammal placenta, but does not contain yolk, rather a serous fluid with no nutritional function. In humans, the yolk sac wall builds transiently fetal blood cells and degenerates with the building of the intestine to the slim yolk sac stem and subsequently becomes part of the umbilical cord. While in sheep, the yolk sac becomes quickly meaningless, in guinea pigs, rats and mice it seems to be an important second feto-maternal exchange area additional to the main placenta, and operates throughout the pregnancy. In the guinea pig, 95% of the fetal membrane in contact with the maternal endometrium is presented by the yolk sac, and only the very small disk-like placenta occupies the remaining 5%. Circumstances are similar in the mouse where outside the placenta, the chorion degenerates together with the maternal epithelium, so that here the yolk sac is in direct contact with the decidua. The placental exchange area is villous in primates and sheep and has the form of a labyrinthine in mice, rats and guinea pigs. The labyrinth feto-maternal exchange

area of mouse and rat has one lobe, while in the guinea pig it is presented by the multilobular syncytiotrophoblast traversed by a system of channels containing maternal blood lacunae and fetal capillaries [78, 131, 228, 346, 413, 463].

Looking at the histological structure of the feto-maternal exchange area, without any tissue degradation, the following layers between fetal and the maternal blood circulation exist:

Endometrium -Maternal blood vessel endothelium (maternal) -Uterus epithelium

Chorion -Chorion epithelium (fetal) -Fetal blood vessel endothelium

The blood vessels in the endometrium and the chorion are located very superficial, which facilitates the feto-maternal exchange.

The sheep is the only investigated species where all four layers of the feto-maternal barrier are maintained. Microvilli (membrane protrusions of a cell that increase the surface area) of the maternal and fetal epithelium interlock. The chorion epithelium consists of one layer of single cells. In all other species, both maternal parts of the barrier are degraded and the chorion epithelium is in direct contact with the maternal blood. In primates and guinea pigs, the epithelium of the chorion has only one layer, where the cells fuse together to form a multi-nucleated barrier, the syncytium. In mice and rats, the chorion epithelium encompasses three cells layers. The outer layer consists of single cells while the cells of the inner two layers are fused in each layer to a syncytium. The syncytial layer of the trophoblast (also referred to as the syncytiotrophoblast) is assumed to be important in order to limit the exchange of cells between the mother and the developing embryo. This syncytiotrophoblast is the actual place where hormones are exchanged between mother and fetus. The placenta develops during gestation and changes its appearance. The placenta of the mice has reached its maximal volume around 85% (e16.5) of gestation, still the labyrinth zone volume increases further at the expense of the other parts of the placenta [103, 113, 223, 268, 413, 491, 506].

*Recapitulatory, in primates and sheep, only the main placenta (two in the rhesus monkey) act as a feto-maternal exchange barrier, while in the guinea pig, rat and mouse, beside the small actual placenta, the large yolk sac operates as a second area of placental exchange. In the latter species, this difference also means that factors, regulating the transplacental glucocorticoid transport, need to be additionally detected in the yolk sac placenta. Also the possibility exists that this large yolk sac increases the exchange area compared to species without a well-developed yolk sac, but it has to be kept in mind, that the dimension of the exchange area is also dependent on the magnitude of the existing folding in the primates. Due to its placentome-like placenta and four-layered feto-maternal barrier, the sheep does not present well the situation in the human. In the other species, the feto-maternal barrier is reduced to the two layers of chorion, with the chorion epithelium directly bathing in the maternal blood. In primates and the guinea pig, the chorion epithelium has a single layer of cells, united to one multiple nuclei syncytium. In rat and mouse, the chorion epithelium exhibits three layers, and the possibility exists that two additional epithelium layers hinder the exchange compared to human and guinea pig.*

### 2.1.2 Location of placental 11$\beta$HSD and P-gp

The syncytiotrophoblast is the location of feto-maternal exchange. In the human placenta, 11$\beta$HSD2 protein as well as ABCB1/P-gp were located in the syncytiotrophoblast cells and 11$\beta$HSD2 mRNA was expressed in the chorion villi [15, 457]. The baboon syncytiotrophoblast expressed both 11$\beta$HSD1 and 11$\beta$HSD2 mRNA [354]. In the placenta of the sheep, 11$\beta$HSD1 and 11$\beta$HSD2 mRNA were detected. 11$\beta$HSD1 mRNA was located in the luminal epithelium of the endometrium and 11$\beta$HSD1 protein was present in the fetal trophoblast cells [74, 510]. The guinea pig placenta expressed 11$\beta$HSD2 mRNA and ABCB1 protein was detected in the syncytiotrophoblast cells [225, 400]. The rat and mouse labyrinth zones not only expressed both 11$\beta$HSD1 and 11$\beta$HSD2 mRNA, but also Abcb1a and Abcb1b mRNA. In the mouse, 11$\beta$HSD2 mRNA was additionally localized in the yolk sac [65, 274, 446, 463]. More specifically, in the mouse labyrinth zone, Abcb1a and Abcb1b mRNA as well as ABCB1 protein/P-pg were present in the syncytial trophoblast cells. The chorion epithelium of the mouse consists of three cell layers, an outer layer of single cells and two inner syncytia. ABCB1 protein/P-gp was detected in the syncytial layer II, the outer syncytial

layer that is in contact with maternal blood [223, 413].

### 2.1.3 Placental 11$\beta$HSD

Two isoforms of 11$\beta$HSD exist. Type 1 (11$\beta$HSD1) and type 2 (11$\beta$ HSD2) are members of the short chain alcohol dehydrogenase super family of enzymes. Each isoform is encoded by its specific gene HSD11B1 and HSD11B2. The unidirectional NAD (+)-dependent 11$\beta$HSD2 works as a dehydrogenase, by catalyzing the inactivation of cortisol to cortisone and corticosterone to 11-dehydrocorticosterone. 11$\beta$HSD1 is thought to act bidirectional. Beside its NADP (+)-dependent dehydrogenase ability, 11$\beta$HSD1 primarily has reductase activity in cells providing NADPH, and generates cortisone and 11-dehydrocorticosterone to their bioactive forms cortisol and corticosterone [301, 345].

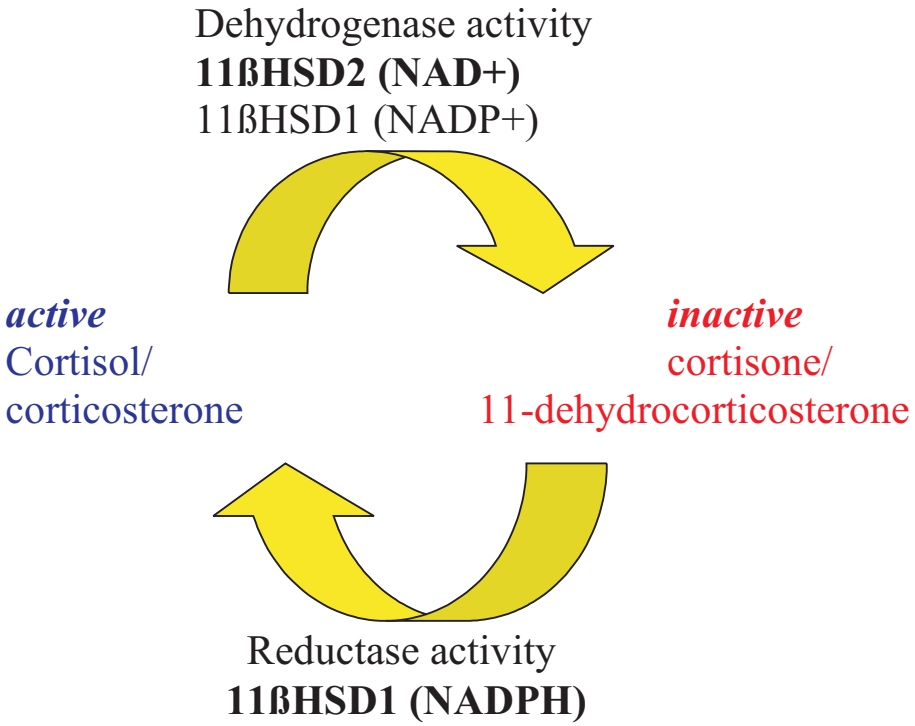


Figure 2.1. Glucocorticoid conversion by 11$\beta$HSD

11$\beta$HSD2 had a higher affinity to cortisol than 11$\beta$HSD1 [451, 454]. The affinity of 11$\beta$HSD1 to its substrate (cortisone in primate, guinea pig and sheep; 11-dehydrocorticosterone in rat and mouse) differed between the species. While the affinity of 11$\beta$HSD1 seemed similar in human and guinea pig, the relative affinity of 11$\beta$HSD1 to 11-dehydrocorticosterone was more than 2-fold higher in mouse than in rat [13].

#### 2.1.3.1 Species-specific 11$\beta$HSD isoform expression

While in the entire human placenta, both isoforms were present, in the syncytiotrophoblast, as the location of feto-maternal exchange, only 11$\beta$HSD2 was detectable [132, 454, 456]. No infor-

mation was available about placental 11$\beta$HSD in rhesus monkeys. Other than in humans, both isoforms were located in the baboon syncytiotrophoblast [355]. In the whole placenta of the sheep, both isoforms were active [507], but unlike in humans and guinea pigs, the 11$\beta$HSD1 gene was predominantly expressed [74]. Surprisingly, the 11$\beta$HSD1 dehydrogenase activity was always predominant, assuming that in sheep, 11$\beta$HSD1 may act like 11$\beta$HSD2 in other species [506]. In guinea pigs, 11$\beta$HSD1 mRNA was completely absent in the placenta, leading to the assumption that 11$\beta$HD2 is the predominant, if not exclusive, enzyme type expressed [400, 511]. In the placenta of rats and mice, both 11$\beta$HSD1 and 11$\beta$HSD2 mRNA are expressed in the labyrinth zone, the feto-maternal exchange location [70].

TABLE 2.1

11$\beta$HSD isoforms at the side of feto-maternal exchange (sheep whole placenta). [(1)]Sheep 11$\beta$HSD1 has predominantly dehydrogenase activity.

| | **Human** | **Rhesus monkey** | **Baboon** | **Sheep** | **Guinea pig** | **Rat** | **Mouse** |
|---|---|---|---|---|---|---|---|
| 11$\beta$HSD1 | - | ? | + | +[(1)] | - | + | + |
| 11$\beta$HSD2 | + | ? | + | + | + | + | + |

*In summary, in humans and guinea pigs, only 11$\beta$HSD2 is present in the syncytiotrophoblast. Both isoforms are present at the side of feto-maternal exchange in baboons (syncytiotrophoblast), rats and mice (labyrinth zone). The sheep is an exception, by predominantly expressing 11$\beta$HSD1, which in this special case has mainly dehydrogenase activity similar to 11$\beta$HSD2.*

#### 2.1.3.2 Regulation of placental 11$\beta$HSD2 and 11$\beta$HSD1

Various factors influence 11$\beta$HSD isoforms in the placenta. Most of the studies investigated the regulation of 11$\beta$HSD2, as the isoform that protects the fetus from high maternal glucocorticoid levels. In the following, glucocorticoid, catecholamine, P-gp, progesterone and estrogen will be reviewed, as regulation factors of placental 11$\beta$HSD. By increasing in the fetal sheep cortisol levels at 86% (day129) of gestation to physiological cortisol concentrations at term, placental 11$\beta$HSD2 activity decreased to term 11$\beta$HSD2 levels [92]. On the other hand, administration of dexamethasone to the baboon mother between 65-62% (day120-134) of gestation dramatically increased 11$\beta$HSD2 mRNA and protein levels, but had no effect on 11$\beta$HSD1 mRNA or protein concentrations [258]. Stress during gestation, similar to increased placental glucocorticoid transfer, leads to adverse fetal effects. Physiological stress of the mother results in secretion of norepinephrine and epinephrine. Interestingly, both catecholamines down-regulated, via alpha-adrenergic signaling, human placental 11$\beta$HSD2 mRNA levels at 15-23% (wk6-9) of gestation and at term [405]. ABCB1 protein/P-gp in the placenta is assumed to work together with placental 11$\beta$HSD2 to augment the glucocorticoid barrier against maternal transfer into the fetus [273]. Progesterone reduced human syncytiotrophoblast 11$\beta$HSD2 activity and mRNA, possibly in an autocrine or paracrine way, and inhibited the inactivation of cortisol in human and baboon term placenta [347, 455]. Mark et al. 2009 showed that the physiological prepartum progesterone withdrawal in rats increased 11$\beta$HSD1 mRNA and protein expression in the labyrinth zone [274]. In humans, estrogen inhibited placental trophoblast 11$\beta$HSD2 activity at term, while in baboons, 11$\beta$HSD2 activity increased with increasing estrogen levels. Progesterone and estrogen had no effect on 11$\beta$HSD1 activity, assuming a different regulation system for this isoform [23, 455].

*Progesterone decreases 11$\beta$HSD2/dehydrogenase activity in the placenta at term. Cortisol in-*

*hibits 11βHSD2 activity at term in sheep, but increases 11βHSD2 expression in baboons around midgestation. While estrogen decreases 11βHSD2 in the human placenta at term, estrogen levels rise in parallel with placental 11βHSD2 activity in baboons. Stress induces catecholamine release in the mother, which down-regulates 11βHSD2 expression in the human placenta. P-gp is assumed to reduce the fetal exposure to maternal glucocorticoid and seems to work together with 11βHSD2. High cortisol concentrations, stress, catecholamines and progesterone, as well as estrogen in humans, decrease placental 11βHSD2 levels at term.*

#### 2.1.3.3 Species-specific ontogeny of 11βHSD

a) Human placental 11βHSD

The human syncytiotrophoblast exhibited 11βHSD2 protein as early as 15% (wk6) of gestation [15]. Between 15-36% (wk 6-14.5) of gestation, IR-11βHSD2 increased in the syncytiotrophoblast. 11βHSD2 protein increased significantly ($P<0.001$) by 1.6 fold in the whole placenta between 20-36% (wk 8-14.5) of gestation [6]. By combining placental tissue from 33-45% (wk13-18) of gestation, Murphy et al. 1974 detected that most maternal cortisol is inactivated to cortisone when crossing the placenta [316]. Significantly higher 11βHSD2 enzyme activity was present at term compared to 20-30% (wk8-12) ($P<0.01$) and 33-50% (wk13-20) ($P<0.001$) of gestation [426]. Placental 11βHSD2 mRNA expression significantly ($P=0.0002$) increased from 50% (wk 20) of gestation on with gestational age [414]. Murphy et al. 2003 detected a significant ($P<0.01$) decrease of 2 fold in placental 11βHSD2 activity between 95-100% (wk38-40) of gestation [317]. In line with the previous data, Murphy et al. 1977 detected a dramatic decrease in the placental cortisol to cortisone ratio between 98-100% (wk39-40) of gestation, indicating decreasing 11βHSD2 levels [312]. Neither the process of labor nor vaginal delivery compared to caesarean section significantly changed placental 11βHSD2 expression, protein abundance or activity [317, 414, 426, 454].

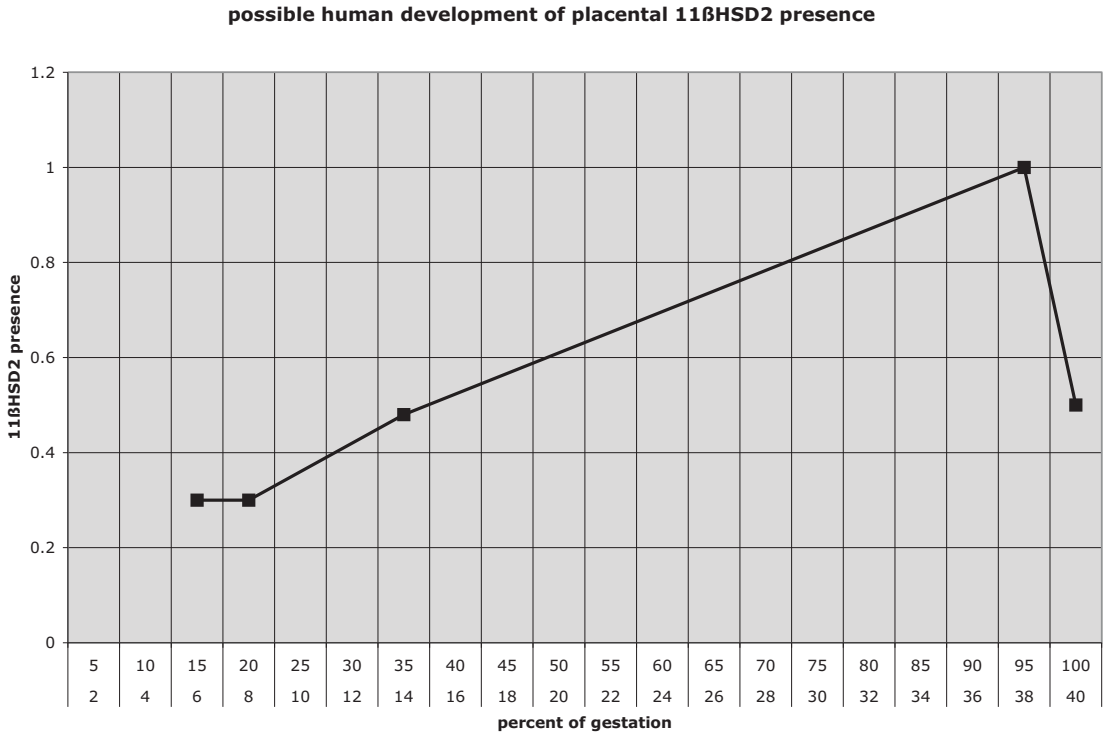


Figure 2.2. Possible human development of placental 11$\beta$HSD2

*In conclusion, by combining placental 11$\beta$HSD2 immunoreactivity, enzyme activity and placental cortisol to cortisone ratio, human placental 11$\beta$HSD2 development suggests an early increase between 20-36% of gestation. Around 40% of gestation, the placental barrier seems relatively tight and maternal cortisol transfer into the fetus is prevented. Placental 11$\beta$HSD2 seems to increase during most of gestation until high levels are reached by 95% of gestation. Over the last 5% of gestation, 11$\beta$HSD2 levels dramatically decrease again.*

b) Rhesus monkey placental 11$\beta$HSD

Even though the literature about placental 11$\beta$HSD in humans and baboons is extensive, it is surprising that there seems to be not a single study investigating 11$\beta$HSD directly in the rhesus monkey placenta. Still, from the work of Kittinger and Mitchell, it can be assumed that 11$\beta$HSD is present in the rhesus monkey placenta and is involved in the maternal and fetal cortisol metabolism. Mitchell et al. 1981 showed that 44% of fetal plasma cortisol and 72% of fetal plasma cortisone derived from maternal sources at 80-83% (day132-137) of gestation, while at 93-96% (day154-159) of gestation, Kittinger et al. 1974 detected that 58% of fetal plasma cortisol and 76% of fetal plasma cortisone were of maternal origin. On the contrary, only 4% of maternal cortisol was of fetal origin [237, 305]. This is in the range of fetal plasma cortisol, originating from maternal circulation in the baboon around 90% of gestation, where it was shown that placental cortisol inactivation was abundant in late gestation [347, 352, 355]. Beside the 15 times greater maternal cortisol synthesis compared to the fetus in late gestation, the rates of cortisol transfer across the placenta in both directions were not significantly different in rhesus monkeys. Between 80-83% (day132-137) of gestation, most of maternal cortisol is converted to cortisone during placental transfer. Comparing reported data, Mitchell et al. 1981 concluded that placental cortisol transfer is more extensive in

rhesus monkeys and humans than in the sheep [36, 37, 237, 305].

*This could be interpreted as marginal increasing maternal cortisol transfer across the placenta between approximately 82-95% of gestation or little decreasing 11βHSD2 concentrations over that period. In total, beside the huge maternal cortisol production, placental cortisol inactivation is relatively high during the whole period.*

c) Baboon placental 11$\beta$HSD

In baboons, available data for placental 11$\beta$HSD were restricted to a very small time window of investigation. Already at 33% (day60) of gestation, both 11$\beta$HSD1 and 11$\beta$HSD2 mRNA were expressed in the syncytioblast of the baboon. At 54% (day100) of gestation, all fetal baboon cortisol was of maternal origin, indicating high cortisol transfer across the placenta, due to low placental 11$\beta$HSD2 levels. By 90-92% (day165-170) of gestation, only roughly 50% of fetal cortisol originated from the mother, which could result from increasing 11$\beta$HSD2 levels in the placenta. The fetal adrenal cortexes start to produce more and more of their own cortisol and the placental barrier is relatively tight for cortisol transfer. Early in gestation, placental activation to cortisol (11$\beta$HSD1) exceeded inactivation to cortisone (11$\beta$HSD2), while in late gestation, inactivation to cortisone was 7-fold greater than activation of cortisol. This could be interpreted as decreasing levels of 11$\beta$HSD1 reductase and increasing levels of 11$\beta$HSD2 dehydrogenase in the course of gestation. In the baboon placenta at 54% (day100) and 98% (day180) of gestation, abundant 11$\beta$HSD1 expression was localized inside the syncytioblast close to the microvilli membrane (adjacent the maternal blood), while 11$\beta$HSD2 expression was limited in this location. On the other hand, adjacent to the basal membrane, which is close to the fetal circulation, 11$\beta$HSD2 expression was excessive and 11$\beta$HSD1 expression was significantly lower [347, 352, 354].

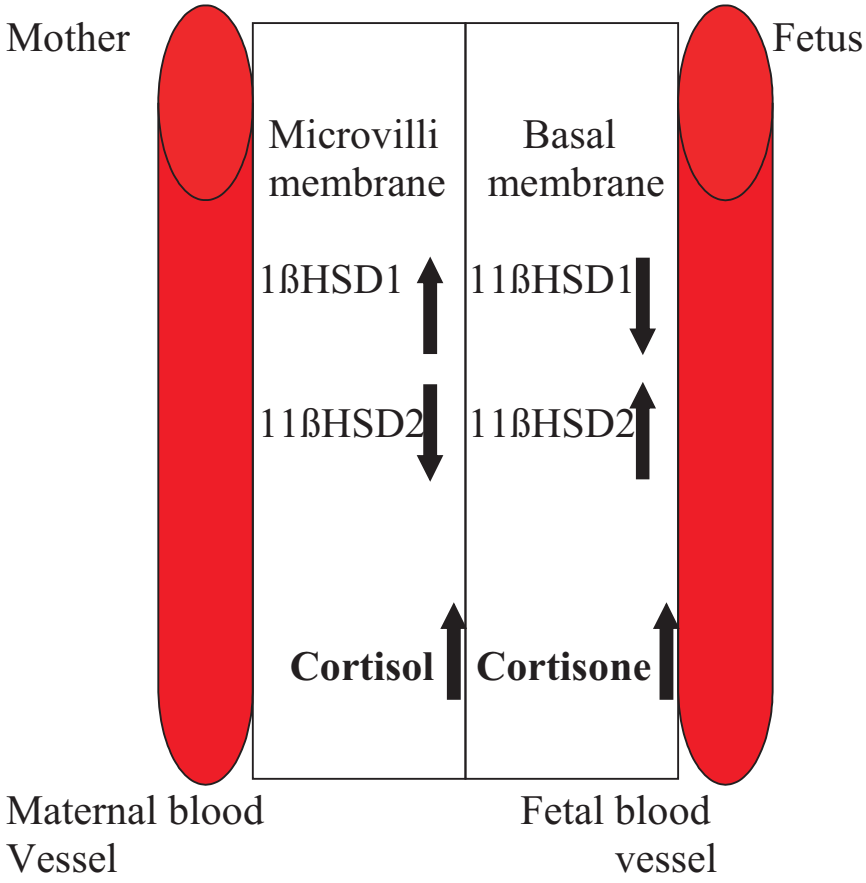


Figure 2.3. Syncytioblast 11$\beta$HSD1 and 2 expression

The protein concentrations of 11$\beta$HSD1 and 11$\beta$HSD2 changed in the baboon basal membrane over the course of gestation, while their concentrations did not significantly changed in the microvilli membrane. In the basal membrane (close to fetal blood vessel), levels of 11$\beta$HSD1 protein decreased significantly ($P<0.05$) between 54-98% (day100-180), leading near term to a significant ($P<0.05$) increase of 2.2 fold in the ratio of 11$\beta$HSD2 to 11$\beta$HSD1 protein [355]. Venihaki et al. 2000 assumed (in mice) that only during the maternal glucocorticoid circadian peak (in day active species like primates in the morning and in night-active species like mice in the evening) are the circulating levels so high that 11$\beta$HSD2 could be saturated, allowing the transfer of active glucocorticoids into the fetal blood [475]. Increasing 11$\beta$HSD2 activity late in gestation, not only protects the fetus from high maternal cortisol, but also might stimulate the fetal HPA axis. With advancing gestation, placental 11$\beta$HSD2 prevents maternal cortisol from reaching fetal circulation. Lower fetal plasma cortisol concentrations are assumed to prevent inhibition of the fetal HPA axis and to activate maturation of the DZ [349, 414].

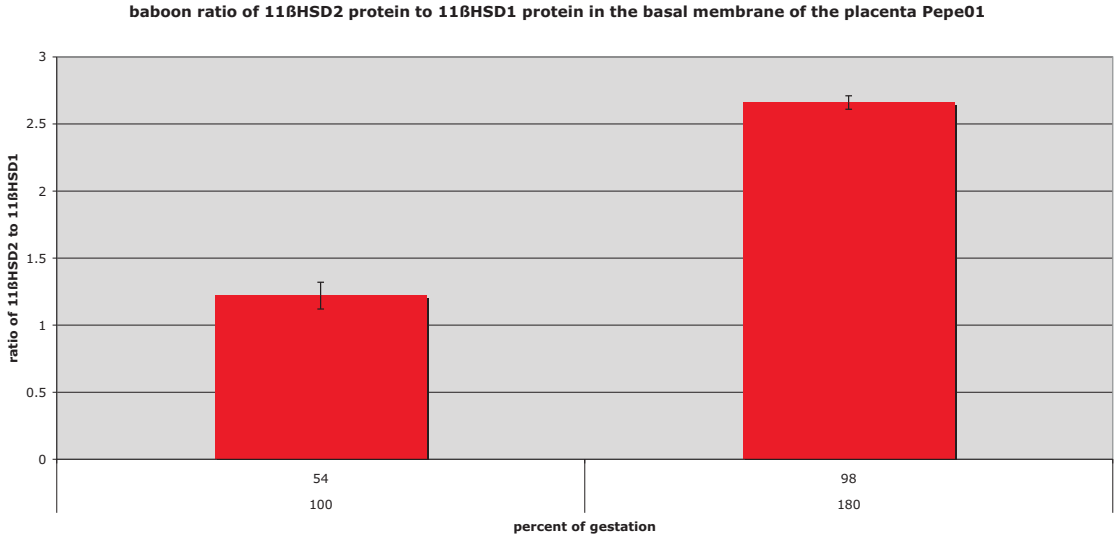


Figure 2.4. Baboon ratio of placental 11$\beta$HSD2 to 11$\beta$HSD1 protein [355]

*Both 11$\beta$HSD1 and 2 are expressed in the placental syncytiotrophoblast of the baboon already at 33% of gestation. The syncytiotrophoblast can be divided into the microvilli membrane close to maternal blood and the basal membrane, close to fetal blood vessels. In the basal membrane, the ratio of 11$\beta$HSD2: 11$\beta$HSD1 protein increases significantly after 54% of gestation. This could explain the decreasing maternal contribution to fetal cortisol levels with advancing gestation. Increasing placental inactivation of maternal cortisol protects the fetus and dis-inhibits the fetal HPA axis.*

d) Sheep placental 11$\beta$HSD

In the sheep placenta, both 11$\beta$HSD1 and 11$\beta$HSD2 were expressed, but surprisingly, the 11$\beta$HSD1 gene was predominant [74, 507]. Due to Yang et al. 1997a, in sheep placental 11$\beta$HSD1 seemed to function similar to 11$\beta$HSD2, and showed a 3-4 times higher dehydrogenase activity than reductase activity, with a net effect (11$\beta$ dehydrogenase minus 11-oxoreductase) assumingly leading to cortisol inactivation [511]. Expression of 11$\beta$HSD1 mRNA was already detected in the placenta by 10-15% (day15-22) of gestation [510]. High placental 11$\beta$HSD1 mRNA levels were detected at 43% (day64), but the decrease of 1.8 fold between 70-88% (day105.5-131.5) of gestation failed to reach statistical significance. On the other hand, dehydrogenase and reductase activity of 11$\beta$HSD1 significantly ($P<0.05$) decreased between 70-88% (day106-132) of gestation by 2 fold. One has to keep in mind that dehydrogenase activity is 3-4 times higher than reductase activity [511]. 11$\beta$HSD2 mRNA was present in the fetal and maternal component of the placenta at 53% (day80), but was undetectable in both locations between 98-99% (day147-149) of gestation [495]. Clarke et al. 2002 investigated the activity of 11$\beta$HSD2 in sheep placenta. A significant ($P<0.05$) decrease by 1.6-1.7 fold in placental 11$\beta$HSD2 activity between 90-97% (day135-146) of gestation was detected [92].

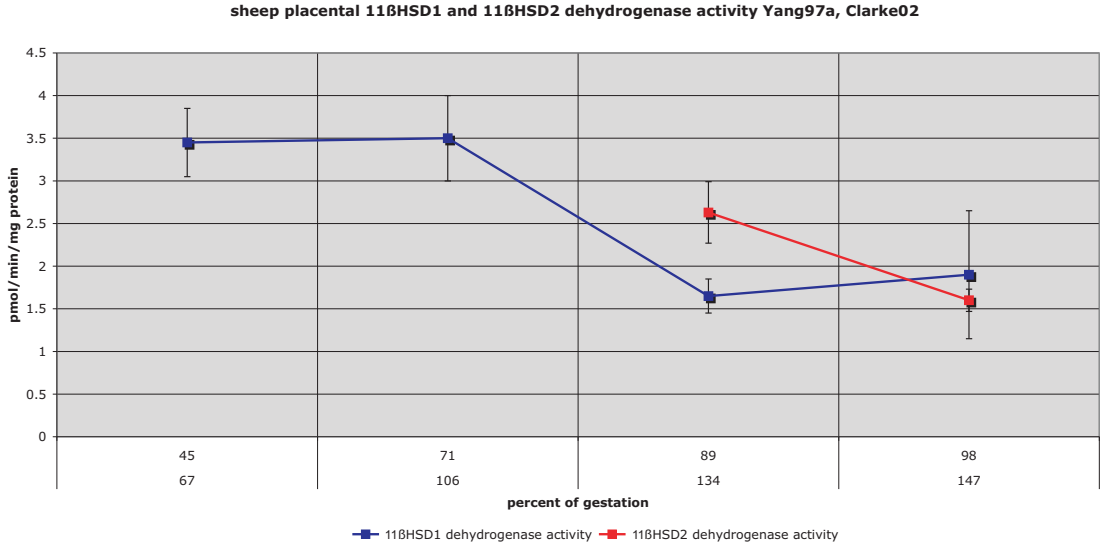


Figure 2.5. Sheep placental 11$\beta$HSD1 and 11$\beta$HSD2 dehydrogenase activity Yang97a, Clarke02

*Taken together, the sheep placenta expresses mainly 11$\beta$HSD1 mRNA, but 11$\beta$HSD1 shows in the sheep placenta predominantly dehydrogenase activity. 11$\beta$HSD1 expression is already detected by 10-15%, but 11$\beta$HSD2 expression is present as well latest by 53% of gestation. The expression of 11$\beta$HSD1 is high at 43% of gestation. Both dehydrogenase and reductase activities decrease by similar amounts between 70-88%, but as dehydrogenase activity is dominant, the net result is a stronger decrease in cortisol inactivation that cortisone activation. 11$\beta$HSD2 activity in still high at 90%, but decreases significantly between 90-97% of gestation, which is in line with the absence of 11$\beta$HSD2 expression in maternal and fetal parts of the placenta at 98-99% of gestation. So in the end, while around 43-51%, the cortisol inactivating 11$\beta$HSD1 mRNA is high and 11$\beta$HSD2 mRNA is present, between 70-99% of gestation, the sheep placenta 11$\beta$HSD1/2 dehydrogenase activity decreases, with 11$\beta$HSD2 mRNA expression disappearing close to term, assuming high cortisol transfer across the placenta after approximately 90% of gestation.*

e) Guinea pig placental 11$\beta$HSD

In guinea pigs, maternal plasma cortisol concentrations were in general markedly higher than cortisol in fetal circulation, but the fetal to maternal plasma cortisol ratio increased from 0.04 to 0.25 between 91-100% (day62-68) of gestation [105]. Between 91-99% (day62-67) of gestation, maternal contribution to fetal plasma cortisol slightly decreased from 96% to 83%. Fetal contribution to maternal plasma cortisol stayed low between 91-97% (day62-66), but increased significantly ($P<0.05$) by 1.8 fold to 23% between 97-99% (day66-67) of gestation [106]. The transfer of cortisol across the placenta increased in both directions near term [399], assuming decreasing 11$\beta$HSD2 concentrations in the placenta. The ratio of maternal to fetal transfer rate increased marginally from 1.6 to 2.3 between 92-94% (day62-64), but then decreased to 0.9 at 99% (day67) of gestation, showing slightly higher fetal to maternal transfer rates than vice versa. The sudden change in the net transfer direction is due to the abrupt increase in fetus to mother transfer between 97-99% (day66-67) by 2.9 fold, while between 92-97% (day62-66) of gestation, transfer into the mother was low. Interestingly, around 87% of cortisol, transferred from mother to fetus, originated from the maternal adrenals, but only 14% of fetal cortisol transferred into the mother, derived from fetal adrenal production,

the rest was recycled cortisol of maternal origin. It can be assumed that the fetus is not able to use all the cortisol transferred to him from the mother [106]. Only 11$\beta$HSD2 expression (dehydrogenase activity), but not 11$\beta$HSD1 expression was apparent in the guinea pig placenta. 11$\beta$HSD2 dehydrogenase activity did not significantly change between 63-81% (day42.5-55), but significantly ($P<0.01$) decreased by 2.4-2.8 fold until 100% (day68) of gestation. In parallel, 11$\beta$HSD2 mRNA expression decreased significantly ($P<0.01$) by 3.7 fold between 81-100% (day55-68) of gestation. The decrement of 11$\beta$HSD2 mRNA and activity between 81-100% (day55-68) of gestation would explain the increased cortisol transfer across the feto-maternal barrier late in gestation [399, 400].

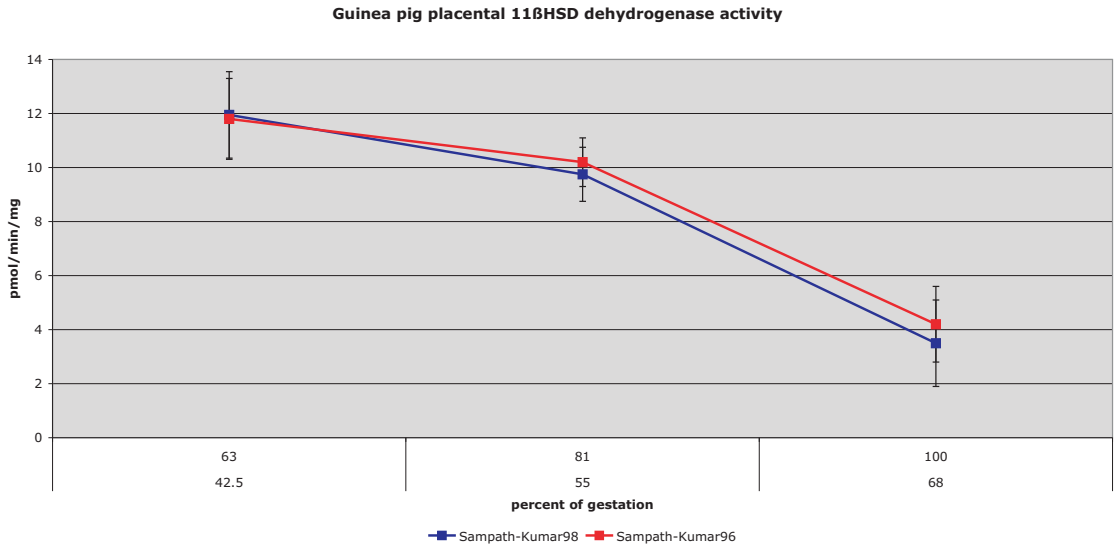


Figure 2.6. Guinea pig placental 11$\beta$HSD dehydrogenase activity

*In summary, the difference in maternal and fetal cortisol levels decreases strongly over the last 12% of gestation, in parallel with increased placental cortisol transfer. While maternal contribution to fetal plasma cortisol is roughly 90% over that period, fetal contribution to maternal plasma cortisol suddenly increases between 97-99% of gestation, but still only reaches 23%. While at 91-97% of gestation, the mother transfers approximately twice as much cortisol across the placenta into the fetus than the fetus into the mother, the transfer rate is roughly similar in both directions at 99% of gestation. Even though maternal transfer rate continuously increases, fetal transfer rate suddenly and dramatically increases between 97-99% of gestation. The fetus mostly transfers recycled cortisol back into the mother. The guinea pig placenta does not express 11$\beta$HSD1. Only dehydrogenase activity by 11$\beta$HSD2 expression is apparent and is detectable at least by 63% of gestation. Both 11$\beta$HSD2 mRNA and dehydrogenase activity decreases significantly over the last 19% of gestation, which explains the sudden increase in cortisol transfer across the placenta.*

f) Rat placental 11$\beta$HSD

The rat labyrinth zone expressed both 11$\beta$HSD1 and 11$\beta$HSD2 mRNA [274]. In the rat placenta, 11$\beta$ dehydrogenase activity with a predominant NAD+ dependent reaction, which is typical for 11$\beta$HSD2, was detectable already at 55% (e12) of gestation [127]. In the labyrinth zone between 68-96% (e15-21) of gestation, 11$\beta$HSD2 mRNA decreased significantly ($P<0.01$) by 15 fold, while 11$\beta$HSD1 mRNA expression increased ($P<0.01$) by more than 20 fold [274]. Labyrinth zone 11$\beta$HSD2 mRNA expression significantly ($P<0.05$) decreased by 11.8 fold from high levels at 68%

(e15) to very low levels at 96% (e21) of gestation. Similarly, 11$\beta$HSD2 dehydrogenase activity (Vmax) in the labyrinth zone was maximal at 68% (e15), remained high at 82% (e18), but significantly ($P<0.01$) decreased by 3.8 fold between 82-96% (e18-21) of gestation. On the other hand, 11$\beta$HSD1 mRNA expression increased significantly ($P<0.05$) by 19 fold between 68-96% (e15-21) of gestation, from very low levels to medium levels [70, 477]. Mark et al. 2009 showed that consistent with decreasing 11$\beta$HSD2 and increasing 11$\beta$HSD1 expression between 68-96% (e15-21) of gestation, corticosterone levels in the labyrinth zone increased by 10 fold over the same time [274].

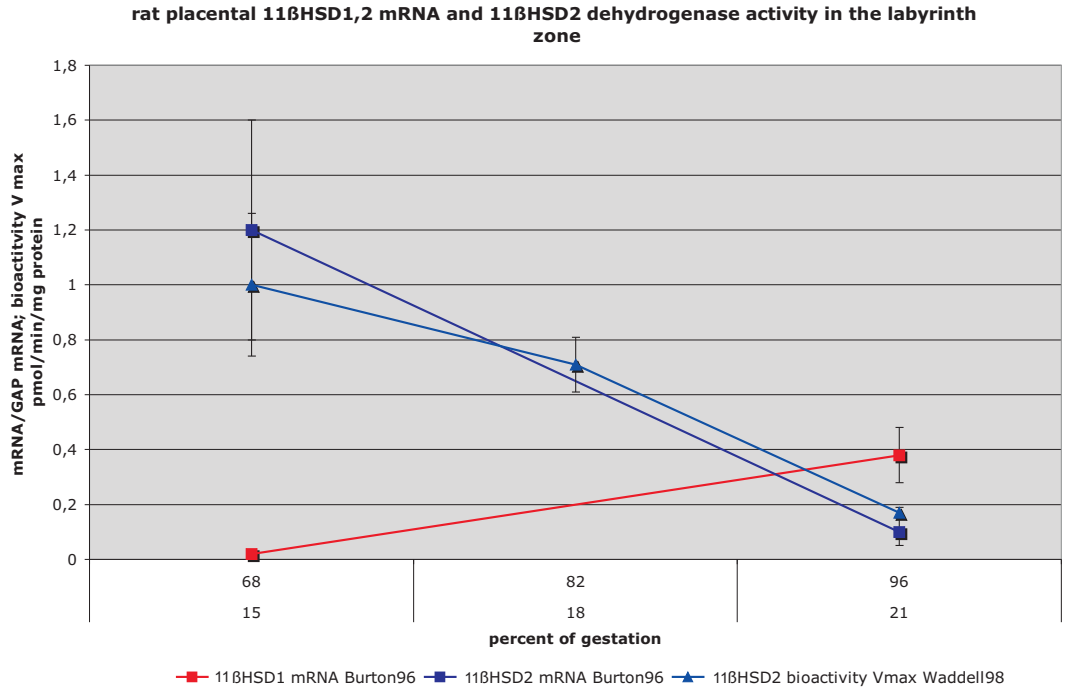


Figure 2.7. Rat placental 11$\beta$HSD1,2 expression and 11$\beta$HSD2 dehydrogenase activity in the labyrinth zone

*In summary, at the site of maternal-fetal cortisol exchange (labyrinth zone), the expression of 11$\beta$HSD2 decreases between 68% and 96% of gestation to very low levels, while the expression of 11$\beta$HSD1 increases. In parallel, dehydrogenase activity decreases over that period. By including another time point, it becomes visible that the decrease of 11$\beta$HSD2 bioactivity to low levels takes place between 82% and 96% of gestation. At term, 11$\beta$HSD2 expression and activity can be assumed to be very low, and corticosterone inactivation at the side of maternal fetal exchange might be insignificant. On the other hand, activation of corticosterone by 11$\beta$HSD1 is increasing toward term, assuming high levels of corticosterone transfer across the placenta into the fetus.*

g) Mouse placental 11$\beta$HSD

Expressions of both 11$\beta$HSD1 and 11$\beta$HSD2 mRNA were present in the mouse labyrinth zone, the major site of maternal-fetal exchange, while the yolk sac, as a second site of exchange, only expressed 11$\beta$HSD2 mRNA and 11$\beta$HSD1 mRNA was absent. At 51-77% (e10-15), 11$\beta$HSD1 mRNA was absent in the placental labyrinth zone, but strongly increased to high levels at 87% (e17), and further maintained at 97% (e19) of gestation. 11$\beta$HSD2 mRNA in the labyrinth zone

was abundant between 51-82% (e10-16), but absent at 87% (e17) of gestation. Thompson et al. 2002 detected high 11$\beta$HSD2 mRNA expression in the labyrinth zone at 67% (e13), moderate expression at 77% (e15), but at 87% (e17), 11$\beta$HSD2 expression was barely detectable and absent at 97% (e19) of gestation [65, 446, 463].

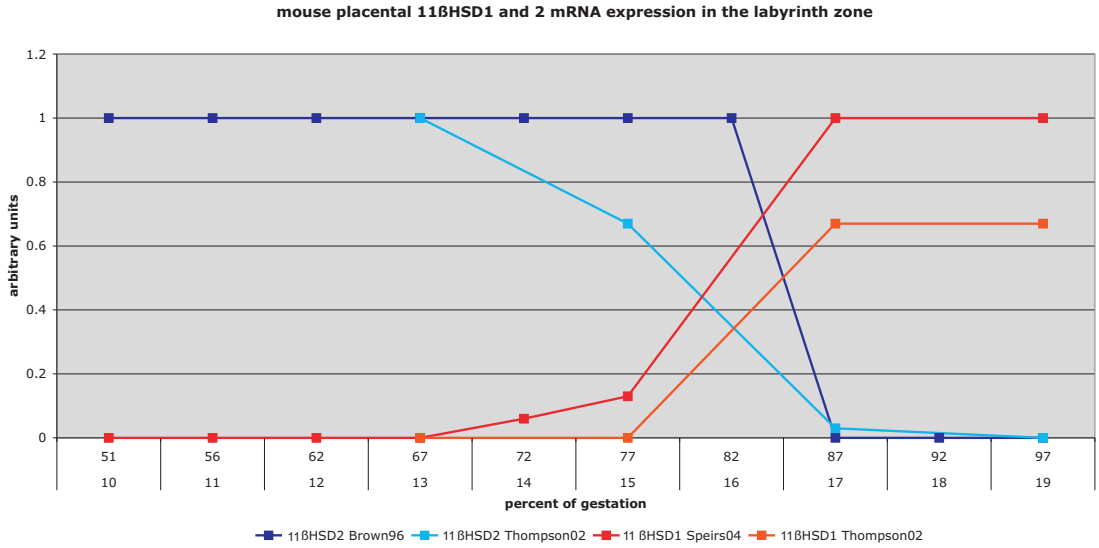


Figure 2.8. Mouse placental 11$\beta$HSD1,2 expression in the labyrinth zone

The yolk sac placenta showed a similar profile of decreasing expression than the labyrinth zone. At 67-77% (e13-15), 11$\beta$HSD2 mRNA was expressed (weakly), but absent at 87-97% (e17-19) of gestation. Thompson et al. 2002 assumed that the abundant expression of 11$\beta$HSD2 and nearly absence of 11$\beta$HSD1 expression in the mouse labyrinth zone between 67-77% (e13-15) of gestation, might assure maximal protection from maternal corticosterone, during a sensible time in fetal development. Between 87-97% (e17-19) of gestation, the vice versa is present. High 11$\beta$HSD1 expression and the absence of 11$\beta$HSD2 expression guaranteed maximal maternal glucocorticoid transfer during the period of final organ maturation in the mouse fetus [463]. Measuring the transport of 3[H] corticosterone across the placenta from maternal to fetal blood exhibited a 15% higher fetal-maternal ratio in female fetuses compared to male fetuses at 90% (e17.5) or gestation, suggesting a greater placental transport from mother to the female fetus than to the male fetus at that time [309].

*Recapitulatory, in the mouse labyrinth zone, 11$\beta$HSD1 and 11HSD2 mRNA develop vice versa in late gestation. 11$\beta$HSD2 mRNA was strongly expressed between 67-77% of gestation, but was absent between 87-97%, while 11$\beta$HSD1 mRNA was absent or low between 67-77% and increased to high levels between 87-97% of gestation. This indicates a dramatic change from high placental corticosterone inactivation before 77%, to very low corticosterone inactivation after 77% of gestation. In parallel with the disappearance of 11$\beta$HSD2 expression in the labyrinth zone at 87%, 11$\beta$HSD2 expression disappeared between 77-87% in the yolk sac placenta, as a second place of feto-maternal exchange. While labyrinth zone 11$\beta$HSD1 mRNA expression increases in the rat from very low levels to moderate levels between 68-96% of gestation, the increase in the mouse from low to moderate levels takes place between 77-87% of gestation.*

### 2.1.3.4 Summary placental 11$\beta$HSD

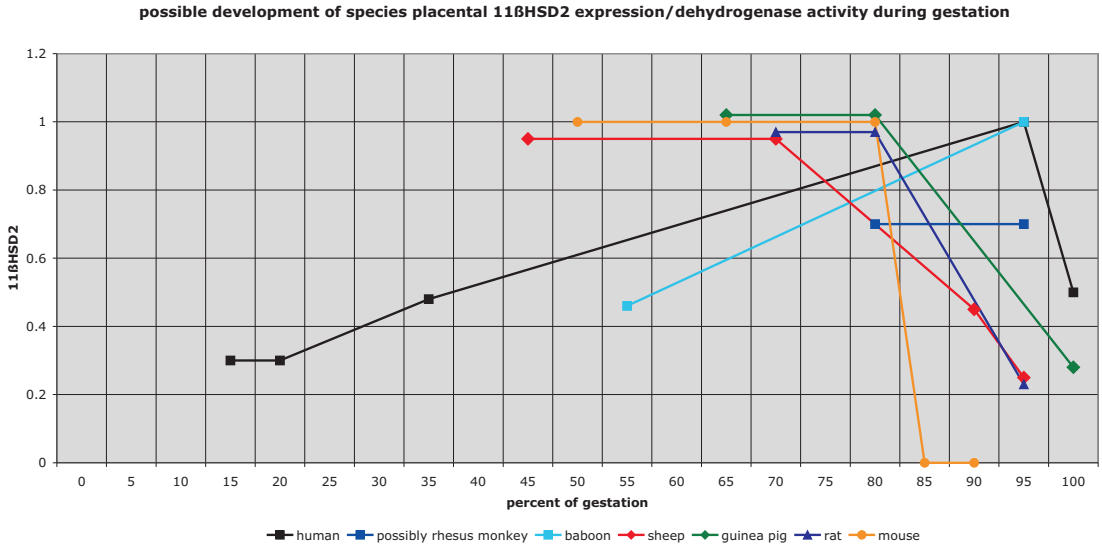


Figure 2.9. Possible development of species placental 11$\beta$HSD2 expression and activity during gestation

*After integrating all available information, the human placental 11$\beta$HSD2 expression could develop the following way. Between 20-36% of gestation, 11$\beta$HSD2 protein levels increase and from 50% of gestation until term, 11$\beta$HSD2 expression increases. As 11$\beta$HSD2 activity is 2 fold higher at 95% than at 100% of gestation, and 11$\beta$HSD2 levels increase after 50% of gestation with age, we assume increment of 11$\beta$HSD2 level until 95%, then a dramatically decrease at term and no further change during labor. In rhesus monkeys, high placental 11$\beta$HSD2 levels are assumed between 80-95% of gestation. Placental 11$\beta$HSD2 levels increase in baboons between 55-95% of gestation. In the sheep, cortisol inactivation in the placenta remains high between 45-90% of gestation, but decreases thereafter. On the other hand, guinea pigs, rats and mice show high 11$\beta$HSD2 levels around 60-80% of gestation and subsequently a dramatic decrease to low levels at 85-100% of gestation.*

## 2.1.4 Placental P-gp

Phospho-glycoprotein (P-gp), the multidrug resistance P-gp ATP-binding cassette subfamily B (ABCB1), is a member of a superfamily of proteins working as an adenosine triphosphate (ATP) dependent efflux pump. ABCB1 is encoded in humans by the single gene Abcb1 and in rodents by two genes, Abcb1a and Abcb1b. In rodents, the two genes translate into the two isoforms of the protein, ABCB1A and ABCB1B, which are similar in function and distribution to the human ABCB1 protein [10, 126]. ABCB1/P-gp actively transports a wide range of substrates, among them endogenous and synthetic glucocorticoids, e.g. cortisol and dexamethasone, out of cells and is able to prevent substrate transport across the placenta from mother to fetus. This was shown when a decrease in ABCB1 expression and P-gp levels corresponded with an increment in the accumulation of the ABCB1 substrate [(3)H]digoxin in the fetal compartment and an increased transfer across the placenta [225, 363, 364, 408, 450, 467]. P-gp is highly expressed in the placenta at the side of feto-maternal exchange (see also Chapter 2.1.2) and is assumed to reduce fetal exposure to glucocorticoids together with 11$\beta$HSD2. In the blood-brain barrier, P-gp can prevent synthetic

glucocorticoids from entering the hippocampus, PVN or cerebellum in adult mice [223, 273, 296].

#### 2.1.4.1 Species-specific ontogeny of placental P-gp

In the human placenta, ABCB1/P-gp mRNA and protein were detectable at 14/16-31% (wk5.5/6.5-12.5) and at 59-86% (wk23.5-34.5) of gestation, as well as at term before and after labor. ABCB1 protein was confirmed in the syncytiotrophoblast at all investigated gestational times. Placental ABCB1 mRNA expression was significantly ($P<0.05$) higher at 16-31% (wk6.5-12.5) and at 59-86% (wk23.5-34.5) of gestation compared to term values before and after labor. AbCB1 protein levels were significantly ($P<0.05$) higher at 14-31% (wk5.5-12.5) compared to values at term before and after delivery and at 59-86% (wk23.5-34.5) of gestation in comparison to the time at term after delivery. Both ABCB1 mRNA expression and protein levels did not significantly differ between 14/16-31% (wk5.5/6.5-12.5) and 59-86% (wk23.5-34.5) of gestation [457]. At term, ABCB1 protein is decreased at the side of feto-maternal exchange and ABCB1 expression is decreased in the placenta, compared to levels earlier in gestation. This development is in concert with the significant decrease of placental 11$\beta$HSD2 activity just before term between 95-100% (wk38-40) and the increasing placental cortisol to cortisone ratio between 98-100% (wk39-40) of gestation [312, 317]. We can expect an increased exposure of the fetus to maternal cortisol at term. Similar to the human, the guinea pig placental expression of Abcb1 mRNA and protein levels decrease toward late gestation. ABCB1 protein was present in the syncytiotrophoblast and Abcb1 mRNA expression was apparent in the placenta of the guinea pig between 57-86% (day40-60) of gestation. The highest Abcb1 mRNA expression was detected at 57% (day40) of gestation. The expression significantly ($P<0.001$) decreased already until 71% (day50) by 37% or even by nearly 50% at 86% (day60) of gestation. There was no significant change in the expression between 71-86% (day50-60) of gestation or a gender-specific development. ABCB1 decreased toward late gestation in a similar pattern [225]. Again this decline is mirrored by the decrement in placental 11$\beta$HSD2 mRNA and dehydrogenase activity in late gestation. Abcb1 mRNA already decreased between 57-71% (day40-50) and did not significantly change between 71-86% (day50-60) of gestation. In contrast, placental 11$\beta$HSD2 expression and activity remained high during 63-81% (day42.5-55) and only significantly decreased by 100% (day68) of gestation [399, 400]. This evokes the question whether ABCB1/P-gp and 11$\beta$HSD2 protect the fetus from strong maternal cortisol levels at different times. The possibility exists that an increment of fetal exposure by the earlier decrease in P-gp is still dampened by high 11$\beta$HSD2 levels and only the decrease of the latter before term fully expose the fetus to maternal cortisol. In the placenta of the rat, the expression of Abcb1a and Abcb1b mRNA was present in the labyrinth zone but no significant change occurred between 68% (e15) and 96% (e21) of gestation. It is surprising that no significant change in Abcb1a and Abcb1b mRNA expression takes place in the rat, as 11$\beta$HSD2 mRNA expression significantly decreased over that period in the labyrinth zone [274]. In mice, both Abcb1a and Abcb1b mRNA were expressed in the placenta, but the predominant placental isoform was Abcb1b. Abcb1a and Abcb1b mRNA were already detected at 49% (e9.5) of gestation at the placental-fetal interface that will late differentiate into labyrinth trophoblast cells. Abcb1a and Abcb1b mRNA expression peaked in the labyrinth zone at 64% (e12.5), then Abcb1b mRNA significantly ($P<0.05$) decreased by 80% (e15.5) of gestation. By 95% (e18.5) of gestation, both Abcb1a and Abcb1b mRNA were dramatically reduced ($P<0.0001$ compared to 64% (e12.5)) and remained very low at 97% (e19) of gestation. Placental ABCB1 protein developed according the development of its mRNA expression and significantly ($P<0.05$) decreased between 64-80% (e12.5-15.5) of gestation [223]. Like Abcb1a and Abcb1b mRNA, 11$\beta$HSD2 mRNA was present in the mouse labyrinth zone at midgestation and decreased in late gestation [65, 446, 463]. Similar to the situation in the guinea pig, Abcb1a mRNA decreased earlier than 11$\beta$HSD2 mRNA expression in the labyrinth zone and again different regulatory function can be assumed. This assumption has to be investigated in the context of fetal adrenal glucocorticoid synthesis and maternal plasma glucocorticoid levels. Additionally it should be mentioned that not only the labyrinth zone of the mouse placenta, but also the yolk sac express another member of the ABC superfamily, the Breast Cancer Resistance Protein (BCRP). BCRP is assumed to be involved in placental substrate trans-

port, and its expression decreases in both locations in late gestation. This opens the possibility of increased substrate transport into the fetus [224]. Taken together, the late gestational increase in 11$\beta$HSD1 mRNA, but the decrement in 11$\beta$HSD2 mRNA, Abcb1a, Abcb1b mRNA and BCRP mRNA expression at the side of feto-maternal exchange in the mouse could all contribute to a radical diminution of fetal protection against maternal glucocorticoids at the end of gestation. Dexamethasone treatment of the rat's dam significantly decreased Abcb1a expression in the labyrinth zone in late gestation [274]. Increasing levels of corticosterone in the dam's circulation seems to be involved in the increased exposure of the fetus.

*P-gp seems to be another important regulator of feto-maternal glucocorticoid transfer across the placenta. Control of transplacental glucocorticoid exchange by P-pg, additionally to 11$\beta$HSD, might be a crucial factor in the liberation of fetal adrenal production under the inhibition of maternal glucocorticoids. Surprisingly, in all investigated species, including the human, but with the exception of the rat, a late gestational decrease in ABCB/P-gp levels at the side of feto-maternal exchange were detectable. This assumingly increases the transport of glucocorticoids from the mother into the fetus but does not exclude the possibility of an increment of exchange from fetus to mother as well. In the guinea pig, mouse and maybe human placenta, P-gp expression decreases earlier in gestation than 11$\beta$HSD2 levels. A sophisticated interplay of both 11$\beta$HSD2 and ABCB/P-gp at the feto-maternal barrier is expected. It might be possible that P-pg is involved in the early phase of fetal adrenal glucocorticoid surge and is later replaced or assisted by 11$\beta$HSD2. The undetectable decrease of P-gp in the rat could be caused by the selected sampling times, which might have been too late in gestation, where 11$\beta$HSD2 rather than P-gp is responsible for placental transfer inhibition. Together with 11$\beta$HSD1/2 (and maybe BCRP) levels, developmental changes of P-gp expression at the side of feto-maternal exchange has to be investigated in the face of species-specific differences in the timing of the fetal adrenal glucocorticoid synthesis surge and maternal plasma glucocorticoid levels.*

## 2.2 Placental CRH and CRH-BP

Placental CRH is secreted in large amounts into the maternal and fetal circulation, and seems to influence the maternal and fetal HPA axis, as well as playing an important role in mediating human parturition [158, 221, 480]. Both maternal and fetal pituitaries are physiological targets for placental CRH [407]. Maternal and umbilical cord plasma CRH stimulated in vitro secretion of pituitary POMC derived peptides, while plasma from non-pregnant women failed to elicit secretion [165]. Additionally to inducing pituitary ACTH secretion, CRH is known to directly stimulate the human fetal adrenal DHEAS and cortisol production, and CRH receptor type 1 is present in fetal adrenal gland and pituitary [439, 443, 445]. The preferential secretion of DHEAS over cortisol increases placental estrogen production and the higher estrogen/progesterone ratio induced the expression of oxytocin receptors for uterine contractions before birth [221, 287, 445]. Placental and hypothalamic CRH are differentially regulated. While glucocorticoids inhibited hypothalamic CRH, synthesis and secretion of human placental CRH was increased in vitro by synthetic glucocorticoids [158, 384]. Via the umbilical arteries, increasing fetal cortisol concentration (and possibly additionally maternal cortisol increment) stimulated placental CRH production and release in the syncytiotrophoblast [443]. In a positive feed forward loop, placental CRH then, via the umbilical vein, reached fetal circulation and on its part stimulated fetal cortisol release [384]. It should be mentioned that Challis 2000 mentioned the possibility of a different regulation, were placental CRH stimulates ACTH production in the placenta and subsequently both peptides are secreted back in fetal circulation [85]. Placental CRH output is increased by AVP, prostaglandin, glucocorticoid, preeclampsia related stress and possibly intrauterine growth retardation, but is inhibited by progesterone, estrogen and nitric oxide [85, 167, 168, 220, 325, 362, 384, 453].

*Placental CRH stimulates maternal and fetal pituitary ACTH secretion, as well as cortisol and DHEAS synthesis directly in the fetal adrenals. Fetal adrenal androgens increase placental estrogen synthesis, which then elicits preparations for labor. Fetal cortisol increment stimulates placental CRH production in a feed forward loop.*

### 2.2.1 Species-specific placental CRH expression and plasma CRH

Since the discovery of CRH-like activity in the human placenta in 1982, CRH mRNA expression could not be verified in the placenta of every species. CRH mRNA was demonstrated additionally to the human in the rhesus monkey placenta. Robinson et al. 1989 failed to detected CRH mRNA in the placenta of rat and guinea pig by using Northern blot. When applying the more sensitive Radioimmunoassay (RIA) method, a small amount of CRH appeared to be present in extracts from the rat placenta [85, 385, 427]. The presence of CRH expression in the placenta of the rat, and assumingly also the guinea pig, cannot be excluded and these results are in need to be rechecked with the now available more sensitive methods of detection. CRH-like activity was detected in the uterine and umbilical vein of the sheep [220]. Mastorakos et al. 2003 suggested placental CRH stimulated pituitary ACTH production in the fetal sheep [276]. No information about placental CRH expression in baboons is available. Beside placental CRH, endometrial CRH seemed to play an important physiological role by promoting and protecting implantation. While endometrial CRH assisted in implantation and early maternal tolerance, placental CRH was involved in fetal adrenal steroidogenesis, feto-placental circulation and the onset of parturition [266, 267, 276]. CRH was detected in maternal circulation of human, rhesus monkey and baboon and in fetal plasma of human and rhesus monkey [61, 165, 166, 385]. As plasma CRH of fetus and mother might derive entirely from the placenta [443], the baboon placenta possibly is able to synthesis CRH as well.

*It was assumed that placental CRH expression might be a primate-specific affair after its discovery in the human and the rhesus monkey. On the other hand, the expression of placental CRH cannot be excluded in the placenta of rat, sheep and guinea pig, and indicators for its expression in the placenta of these species necessitate remeasurements with more sensitive methods of detection. As endometrial CRH is involved in implantation, detection of placental CRH could be mistaken, depending on the exact location of examination inside the placenta. Plasma CRH can be assumed*

*to derive from placental CRH. CRH is present in the circulation of pregnant human, rhesus monkey and baboon, and in the fetuses of the human and the rhesus monkey. The existence of plasma CRH in the baboon indicates placental CRH expression in this species as well.*

#### 2.2.1.1 Human placental CRH, plasma CRH, CRH-BP

Human placental CRH mRNA had a similar size and identical transcription initiation site as the hypothalamic CRH mRNA. Human CRH was encoded by a single locus on the long arm of chromosome 8, assuming placental and hypothalamic CRH transcription from the same gene. Immunoreactive-CRH (IR-CRH) was primarily detected in the human placental syncytiotrophoblast, but it should be kept in mind that the detection of IR-CRH cannot equate with CRH mRNA expression in a location, as the present IR-CRH could be produced at a distant site. In the whole human placenta, both CRH mRNA and peptide were apparent. Between 15-20% (wk6-8) of gestation, IR-CRH was undetectable in the placenta. Low but detectable CRH mRNA was present in placenta at 18-38% (wk7-15) of gestation, but CRH peptide was not traceable during that time. Between 38-85% (wk15-34), CRH mRNA expression and protein were apparent but low, while from 88-100% (wk35-40) of gestation, placental CRH mRNA in parallel with CRH peptide increased by more than 20 fold [14, 158, 382, 385, 503].

*Placental CRH mRNA seems to be identical to CRH mRNA from the hypothalamus and is present at the location of feto-maternal exchange. Expression of human placental CRH mRNA remains low between 18-85%, and placental CRH peptide persists in low levels between 38-85% of gestation. Between 85-100% of gestation, both CRH mRNA expression and CRH peptide increase strongly in the human placenta.*

- Human placental CRH release into circulation

Placental CRH was secreted into maternal plasma and via the umbilical vein into fetal circulation [165, 167, 328, 406]. CRH in maternal and fetal plasma was assumed to be entirely of placental origin without contribution from the portal circulation of the fetal pituitary [161, 276, 443]. Secretion into the fetal compartment was 2 times less than into maternal compartment [73, 276]. Increment of human placental CRH expression assumingly increased maternal plasma CRH exponentially, as the late gestational increase of CRH mRNA expression in the placenta seemed to parallel with the maternal plasma CRH surge [83, 158, 165].

*Maternal and fetal plasma CRH might originate entirely from the placenta. Placental CRH release into maternal circulation is considerably higher than into the fetal plasma. Plasma CRH of the mother increases in parallel with the expression of placental CRH.*

- Human maternal and fetal plasma CRH

Human maternal plasma CRH was undetectable before 60% (wk24) and increased significantly ($P=0.027$) to moderately low levels between 60-78% (wk24-31) of gestation. Subsequently, maternal plasma CRH slowly increased ($P=0.018$) until 90% (wk36) of gestation by 5 fold, when CRH in maternal circulation exponentially increased ($P<0.001$) until 98-100% (wk39-40) by mean 3.7 fold [165, 288]. At term, CRH concentration in umbilical arteries was only 1% of maternal plasma CRH, assuming much lower CRH levels in fetal than maternal plasma at birth. Between umbilical arteries and vein, plasma CRH did not significantly differ [73]. Early measurement of maternal plasma CRH predicted the timing of parturition. Preterm delivery was accompanied by a faster rise of plasma CRH, while delayed parturition went along with a slower increment of CRH in circulation [287, 288]. Already at 50% (wk20) of gestation, pregnant women at risk for preterm birth had significantly elevated plasma CRH concentrations and lower levels of plasma CRH binding protein (CRH-BP). Mothers suffering from pregnancy-induced hypertension also showed significantly elevated plasma CRH levels [73, 198]. Human fetal plasma CRH levels were 1.3 fold higher in umbilical plasma from preterm than from term fetuses. Fetuses with growth retardation showed significantly ($P<0.01$) higher levels of umbilical cord plasma CRH compared to normally grown fetuses [165, 167].

*CRH is undetectable in maternal circulation before 60% of gestation. Maternal plasma CRH remains low until 78% of gestation, in parallel with to low CRH mRNA expression and peptide synthesis in the placenta. Maternal plasma CRH levels slowly increase between 61-89% and dramatically increase thereafter until term, which reflects the strong increase in placental CRH synthesis from 85-100% of gestation. Fetal plasma CRH levels are very low in comparison to the mother and all fetal plasma CRH might derive from the placenta, as umbilical blood vessels from and to the placenta shows the same CRH plasma concentration. Increased CRH levels in maternal circulation already after 50% of gestation can be used to predict preterm birth. Fetal growth retardation and pregnancy-induced hypertension are associated with increased plasma CRH concentration.*

- Human maternal and fetal plasma CRH-BP

Beside CRH, the human placenta additionally synthesized CRH binding protein (CRH-BP) and released it into the fetal and maternal circulation [221, 357]. CRH-BP was also detected in plasma of rhesus monkey, but not of baboon or sheep [61, 444]. During most of gestation, human maternal plasma CRH was inactivated to a large extent by plasma CRH-BP, when the levels of the latter were much higher than CRH concentrations in circulation [221, 254, 288].

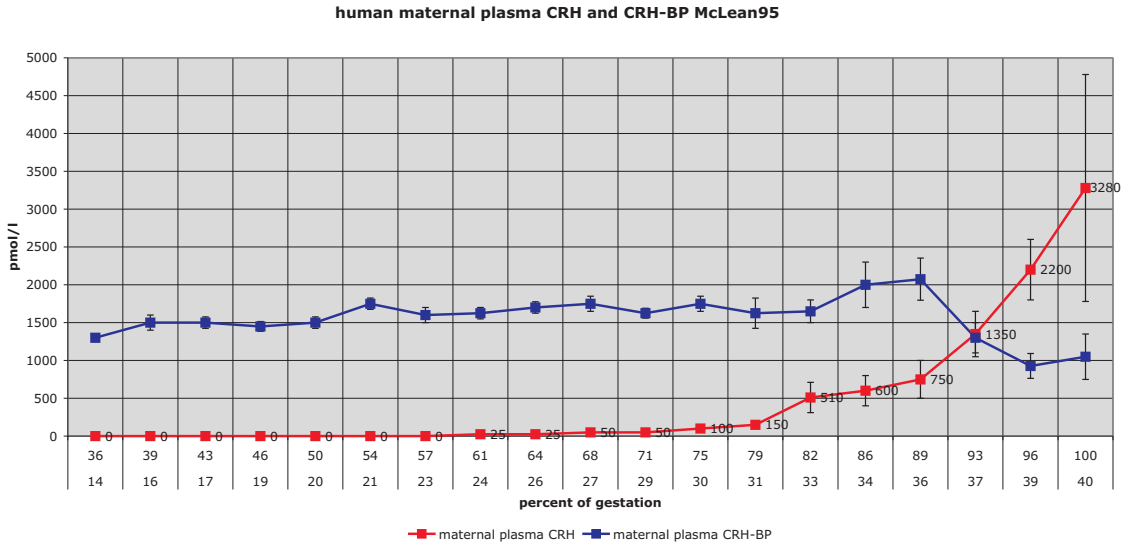


Figure 2.10. Human maternal plasma CRH and CRH-BP McLean95

Maternal plasma CRH-BP levels did not differ from non-pregnant levels for most of gestation. Only between 83-98% (wk33-39) of gestation, maternal plasma CRH-BP levels have significantly ($P<0.001$) decreased by 2.5 fold. Levels remained low at 100% (wk40) of gestation and recovered again in 48 hours after birth [254, 288]. The late gestational decrease in maternal plasma CRH-BP levels coincided with a sudden and dramatic increase in maternal plasma CRH concentration from 89% (wk36) of gestation on. At 93% (wk37), maternal plasma CRH and CRH-BP levels had reached equivalence, while at 96-100% (wk38-40) of gestation, CRH-BP concentration reached its nadir and plasma CRH levels achieved their maximum [288]. The increasing CRH levels together with the dramatic decline in binding protein led to fully saturated CRH-BP and resulted in a sudden significant ($P=0.013$) increment of free plasma CRH from low levels before 83% (wk33) to high levels at 85-98% (wk34-39) of gestation [35]. The availability of free CRH in late gestation is thought to trigger parturition in humans [287, 288]. Fetal plasma CRH-BP concentration at 50-83% (wk20-33)

of gestation (from cordocentesis) and at term (from umbilical cord) did not significantly differ from simultaneously collected maternal plasma CRH-BP levels. Between 50-83% (wk20-33) of gestation and term, CRH-BP concentration and CRH-BP binding capacity significantly ($P<0.001$) decreased in fetal plasma in parallel with decreasing levels in maternal plasma [357]. The expression of both CRH and CRH-BP in the primate placenta might have reflected an adaptation against inappropriate pituitary stimulation by placental CRH during most of gestation [371]. It is assumed that CRH-BP protects the anterior pituitary from placental, but not from hypothalamic CRH [253].

*CRH-BP is synthesized in the human placenta and is present in maternal and fetal circulation. Plasma CRH-BP is present in the plasma of rhesus monkey, but could not be detected in baboon or sheep circulations. High plasma CRH-BP levels inactivate CRH in circulation, assumingly to prevent untimely pituitary stimulation.*

*Only close to term, maternal plasma CRH dramatically increases and CRH-BP decreases. The provoked increase in free plasma CRH is assumed to trigger birth. Fetal plasma CRH-BP concentrations do not differ from CRH-BP levels in maternal circulation approximately in the second half of gestation and plasma CRH-BP concentrations decrease in parallel in mother and fetus toward term.*

#### 2.2.1.2 Rhesus monkey placental CRH, plasma CRH, CRH-BP

CRH mRNA expression was detected in the placenta of the rhesus monkey and, as in human, CRH mRNA was of similar size in placenta and hypothalamus. CRH mRNA and peptide were expressed in the rhesus monkey syncytiotrophoblast [385, 503]. During labor, both placental CRH gene expression and peptide increased significantly ($P<0.05$) by 2.5 fold compared to control animals not in labor. A local role of placental CRH on myometrial contractibility during parturition was assumed [503]. Plasma IR-CRH was present in rhesus monkey mothers and their fetuses between 74-96% (day122-158) of gestation. Fetal plasma IR-CRH levels were approximately only 1.5% of CRH concentration in maternal circulation. Half of the tested fetuses even had undetectable plasma CRH levels [61]. Maternal plasma CRH increased nearly 2 fold in pregnant rhesus monkeys between 78-100% (day129-165), and fetal plasma CRH levels were elevated during spontaneous labor [162, 385]. CRH-BP was detected in rhesus monkey plasma [61]. No information of CRH-BP during rhesus monkey pregnancy is available.

*The rhesus monkey synthesizes CRH mRNA and assumingly CRH-BP in the placenta. The CRH expression and peptide syntheses increase during labor. Maternal and fetal plasma CRH are present from 74% of gestation on. CRH in maternal circulation increases during late gestation and fetal plasma CRH is, as in the human fetus, very low, though shows an increment at delivery.*

#### 2.2.1.3 Baboon plasma CRH and CRH-BP

Plasma IR-CRH levels are undetectable in male and non-pregnant female baboons [444]. Maternal plasma CRH was not present at 8-12% (day14.5-21.5), but was detected between 17-20% (day30.5-36.5) and increased significantly ($P<0.001$) until 24% (day43.5) of gestation. CRH in maternal circulation remained elevated from 27% (day50.5) until at least 96% (day176.5) of gestation, but disappeared within the first day after birth [166]. Maternal plasma CRH levels remained high throughout pregnancy, but were significantly higher ($P=0.003$) by 1.9 fold from 1-50% (day1-93) compared to 50-100% (day94-184) of gestation [444]. Between 74-91% (day136.5-166.5) of gestation, maternal plasma CRH was only 2-3 fold higher ($P=0.01$) than in the plasma CRH of chronically catheterized fetal baboon. Plasma CRH in the baboon fetus did not change significantly during that period [166]. CRH-BP was absent in the circulation of non-pregnant or pregnant baboons [61, 444].

*Placental CRH expression in the baboon can be assumed, as plasma CRH is present in the female only during pregnancy. In contrast to rhesus monkey and human, plasma CRH-BP is not detected in baboon. Plasma CRH in the baboon mother is detected already very early in gestation. Maternal plasma CRH levels are elevated for most of gestation, but seem not to increase toward term,*

*assuming a different role in parturition than in humans and rhesus monkey. While in human and rhesus monkey, fetal plasma CRH levels are approximately 1% of CRH concentration in maternal circulation, in the fetal baboon, levels are much higher, making up 33-50% of maternal plasma CRH.*

#### 2.2.1.4 Summary

*Placental CRH synthesis is verified in the human and the rhesus monkey, assumed in the baboon, rat and sheep and cannot be excluded in the guinea pig. CRH from placenta and hypothalamus seems to be identical. Placental CRH and CRH-BP is released in maternal and fetal circulation. Plasma CRH is assumed to entirely originate from the placenta. Compared to maternal concentration at term, fetal plasma CRH levels are negligible in human and rhesus monkey. In fetal baboon, plasma CRH levels make up 33-50% of maternal levels during late gestation. Placental CRH in fetal and maternal circulation is assumed to stimulate the pituitary of mother and fetus. In human and rhesus monkey, placental CRH expression and maternal plasma CRH is detected in late gestation and increase before term, supposedly to stimulate delivery. In the baboon, maternal plasma CRH does not increase in late gestation and its role during gestation and delivery is unknown. Additionally, while human and rhesus monkey present CRH-BP in circulation, no CRH-BP is detectable in baboon plasma. In human maternal plasma it is shown that for most of gestation, plasma CRH-BP inactivates CRH. Late in gestation an increase in CRH levels go along with a decrease of CRH-BP concentration and the increased unbound CRH fraction is assumed to trigger birth. Increment in maternal plasma CRH can predict preterm delivery and is present in connection with pregnancy induced maternal hypertension and fetal growth retardation.*

REGULATORY FACTORS FROM THE HYPOTHALAMUS

In the hypothalamus, we will investigate CRH and AVP expression, as the major secretagogues for pituitary ACTH. Subsequently hypothalamic GR expression is looked into, because the hypothalamus is a main site for glucocorticoid negative feedback.

Inside the hypothalamus, cell bodies synthesized hormones behind the blood-brain barrier and transported them outside the blood-brain barrier to the pituitary [339]. The PVN was located adjacent to the third ventricle. Ventro-rostral from the PVN and above the optic chiasm laid a small longish hypothalamic nucleus, the supraoptic nucleus (SON). The PVN contained two different neurosecretory cells types, the magnocellular and the parvocellular neurons, while the SON only harbored magnocellular neurons [471]. The magno- and the parvocellular neurons inside the PVN belonged to two different systems. In the parvocellular division of the PVN, neurons produced either CRH and AVP or only CRH and their axons projected to the median eminence. Magnocellular neurons of both hypothalamic nuclei produced AVP, which was mainly transported down the axons into the posterior pituitary and released into circulation [493, 496] (for further information see Chapter 2.4). At the median eminence, PVN axons released CRH and AVP in the portal blood vessels of the pituitary, which connected the median eminence with the anterior pituitary. Hypothalamic AVP and CRH bound to their anterior pituitary receptors V1b and CRH1 and led to synthesis of the precursor molecule POMC and the release of ACTH into the blood stream [150, 496]. Activation of the CRH neurons in the PVN occurred through the ascending noradrenalin and adrenalin neurons from brain stem nuclei through glutamate and also angiotensin II, which increased expression of CRH1 receptor and CRH release. Serotonin, released during stress from the medial and dorsal raphe nuclei, activated hypothalamic serotonin receptors and facilitated CRH release [118, 183]. On the other hand via Gamma-Amino Butyric Acid (GABA) nergic circuits, the hippocampus exerted inhibitory control over CRH neurons in the PVN [471].

## 2.3 Hypothalamic CRH

### 2.3.1 First CRH expression

As early as 30% (wk12) of gestation, CRH was detected in the fetal human hypothalamus and CRH-like immunoreactivity was present in the human PVN and pituitary [2, 64, 276]. No information is available for rhesus monkeys. In the baboon PVN, CRH mRNA was present latest at 54% (day100) of gestation [113]. In the sheep fetus, CRH labeled neurons (IR-CRH) and nerve fibers were apparent in the PVN and the median eminence already at 33% (day49) of gestation [485]. Fetal guinea pig CRH mRNA was highly expressed in the PVN at 59% (day40) of gestation [337], assuming an earlier start of CRH expression. Rat CRH mRNA was first detected in the parvocellular portion of the fetal PVN at 77-82% (e17-18) of gestation [28, 171]. By help of antiserum against rat CRH, Daikoku et al. 1984 detected IR-CRH containing neurons in the PVN at 73% (e16) and immunoreactive fibers in the external layer of the median eminence at 77% (e17) of gestation [104]. At 77% (e17) of gestation, endogenous CRH started to play a role in the secretion of ACTH and corticosterone in fetal rats [60]. In the fetal mouse PVN, CRH mRNA was absent at 64% (e12.5), but lowly expressed by 69% (e13.5) of gestation [230].

TABLE 2.2

First CRH expression in hypothalamus

| CRH in PVN | CRH in wk/day | CRH in % |
|---|---|---|
| Human | wk 12/40 | 30 |
| Rhesus monkey | % | % |
| Baboon | <100/184 | < 54 |
| Sheep | 49/150 | 33 |
| Guinea pig | <40/68 | < 59 |
| Rat | 16-17/22 | 73-77 |
| Mouse | 12.5-13.5/19.5 | 64-69 |

*The first expression in humans and sheep is surprisingly congruent. It can be assumed that baboons and guinea pigs start to earlier express CRH than the detected times, due to the fact that baboon CRH in PVN is already moderate at that point and guinea pig CRH expression is extremely high. The first expression of CRH in rats and mice is more than two times later than in sheep and humans. CRH expression is still earlier in mice than in rats.*

### 2.3.2 Prenatal CRH expression

After the detection of IR-CRH around 30% (wk12) in the human fetal PVN, hypothalamic CRH bioactivity showed a significant (P<0.01) increase with gestational age between 30-68% (wk12-27) of gestation. Pituitary ACTH release stimulated by hypothalamic tissue was significantly (P<0.005) higher per adult hypothalamic tissue than per fetal tissue by 4.4 fold. Between 40-75% (wk16-30) of gestation, CRH positive fibers in the median eminence increased steadily in number [2, 63, 64]. In fetal baboons at 54% (day100), CRH mRNA expression in the PVN was high. CRH mRNA level did not change in the baboon PVN between 54% (day100) and 90% (day165) of gestation, but due to a 3-fold increase in the weight of the hypothalamus over that period, the content of hypothalamic CRH increased (P<0.01) markedly by 3.9 fold [113]. In the PVN of the fetal sheep, CRH mRNA was present in low levels at 43% (day64) of gestation. Paraventricular CRH expression did not significantly change between 49-76% (day74-114) of gestation. From 76% (day114) till 97% (day145.5), CRH mRNA expression significantly (P<0.05) increased by 1.9 fold and further between 97% (day145.5) and spontaneous labor (P<0.05) by another 1.6 fold. The highest CRH mRNA level was present at term, in the phase of active labor [281]. Myers et al. in 1993 investigated CRH mRNA expression exclusively in the midrostral PVN. Between 71-86% (day106-129) of gestation, CRH mRNA expression significantly (P<0.05) increased in the midrostral PVN by 3.7 fold [319]. Guinea pig CRH mRNA was highly expressed in the PVN at 59% (day40) and at 74% (day50) of gestation. No gender difference in PVN CRH mRNA expression was apparent during that time. The medial parvocellular region (mp) of the PVN harbored most of the CRH neuron cell bodies. Here, CRH mRNA levels were roughly 2 fold higher than in the whole PVN. In the whole as well as in the mp PVN, CRH mRNA levels decreased significantly (P<0.05) between 74% (day50) and 94% (day64) of gestation by approximately 3.5 respectively 2.6 fold, during a time of dramatically increasing plasma ACTH and cortisol levels. This development seemed to be counterintuitive and Owen et al. 2005 assumed an increase of CRH in the PVN of fetal guinea pigs sometime before 94% (day64), which could remain undetected due to their sampling times at 74% (day50) and at 94%

(day64) of gestation [164, 337]. At 73% (e16) of gestation, CRH expression was absent in the rat's hypothalamus. Between 77% (e17) and 82% (e18) of gestation, CRH mRNA expression in the PVN had increased significantly ($P<0.001$) by roughly 2 fold to maximal levels. CRH gene expression did not significantly change between 82% (e18) and 91% (e20) of gestation. Between 91-96% (e20-21), CRH mRNA expression significantly ($P<0.001$) decreased by 2.1 fold, but did not significantly change between 96% (e21) of gestation and PND1. Between 96-100% (e21-22) of gestation, CRH mRNA level significantly ($P<0.01$) decreased by 1.4 fold, but remained unchanged between term and PND1. A significant ($P<0.05$) increase took place by PND4 of 1.5 fold [28, 29, 171]. In fetal mice, CRH mRNA expression increased from its appearance on 69% (e13.5) to maximal expression at 80% (e15.5), stayed high till 90% (e17.5), and then suddenly dropped by 2.8 fold ($P<0.05$) to low level at 95% (e18.5) of gestation, with similar levels at birth [230].

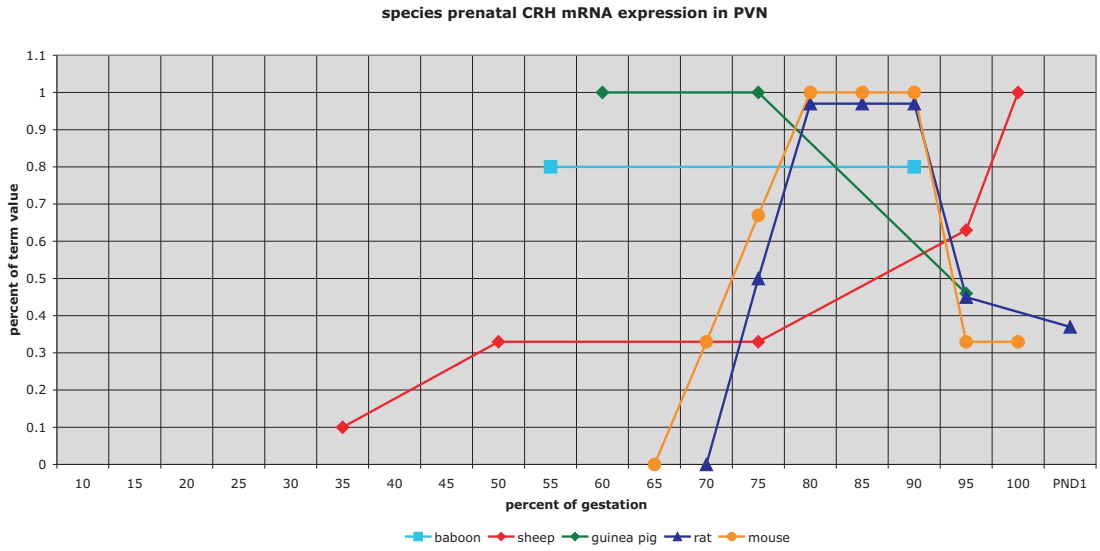


Figure 2.11. Species prenatal CRH expression in PVN

*Not much information is available about human fetal CRH expression in the hypothalamus. In the human fetus, hypothalamic IR-CRH bioactivity increases significantly between 30% and 68% of gestation, while IR-CRH positive fibers significantly increase in number in the median eminence between 40% and 75% of gestation. No information is available about CRH in the hypothalamus of fetal rhesus monkeys. Baboons' fetal CRH content increases between 54% and 90% of gestation, but CRH mRNA level remains high and unchanged over that period and no further information is available. In the sheep, low expression of CRH mRNA in the PVN is present from 42% to 76% of gestation, subsequently increases to high levels at term, especially over the last 3% of gestation. Guinea pigs' CRH mRNA expression seem to follow the opposite pattern, with high levels between 59% and 74% of gestation, and a significant decrease to moderate levels at 94% of gestation. An undetected increase between 74-94% before the decrease could be possible. CRH mRNA expression in the PVN of rats and mice follow a similar trend, from undetectable level at 73% respectively 64% of gestation, CRH mRNA expression increases to maximal level on 80-82% with continuously high expression until 90-91% of gestation. Afterwards, CRH mRNA expression in the PVN of rats and mice decreases to moderately low levels between 95% of gestation and term. In total, while CRH expression increases in sheep and possibly humans toward high levels at term, in guinea pig, rat and mouse CRH expression decreases to low or moderate levels at the end of gestation.*

### 2.3.3 Postnatal CRH expression

No data were available about postnatal CRH expression in the primate hypothalamus. In the newborn sheep at 4% (PND3.5) of weaning, levels were comparable to labor values. In comparison to term values, the expression significantly ($P<0.001$) decreased by 2 fold at 50% (PND45) of weaning, to adult-like values [281]. It is difficult to estimate postnatal development of CRH mRNA in the PVN of guinea pigs, due to very little data. After the significant decrease at the end of gestation to low levels, the data point at 33% (PND7) of weaning was equally low as the value at 94% (day64) of gestation [337]. In adult guinea pigs, CRH mRNA levels in the PVN were similarly high in both genders [255]. In rats, CRH mRNA levels remained low from term to 5% (PND1) of weaning, but had significantly ($P<0.01$) increased by 1.5 fold at 19% (PND4) of weaning [28]. A significant ($P<0.0005$) increase of CRH mRNA levels in the PVN took place between 5% (PND1) and 33% (PND7) of weaning by approximately 3.4 fold, but no further change occurred by 67% (PND14) of weaning [171]. At the age of 3, 6 or 9 months, CRH expression in the PVN of rats was unchanged [110]. In neonatal mice at birth, CRH mRNA expression in the PVN was low [230]. Schmidt et al. 2002/2003 and Enthoven et al. 2009 detected elevated levels of CRH mRNA in the PVN at 5-43% (PND1-9) of weaning, followed by declining ($P<0.001$) levels until 57-67% (PND12-14) of weaning. CRH mRNA expression remained low at least until 76-91% (PND16-19) of weaning, which might represent adult values. Schmidt et al. 2003 assumed two phases of postnatal HPA development in the mouse. The first phase corresponds to the rat stress-hypo-responsive-period (SHRP) and lasts between 5-43% (PND1-9) of weaning, were high CRH/PVN expression is exhibited. The second phase after 57% (PND12) of weaning features significantly decreasing CRH expression in the PVN [146, 410, 411]. Between 5-67% (PND1-14) of weaning, rats underwent the SHRP, with inhibited corticosterone and ACTH stress responses, while certain stressors were still able to produce a CRH and AVP response. The SHRP of rats was assumed to be mainly caused by altered hippocampal MR/GR ratio, CRH deficit in PVN neurons, GR feedback on pituitary ACTH release, and diminished adrenal sensitivity to pituitary stimulation [471]. A diminished input from higher brain areas on CRH in the PVN decreased CRH secretion and stimulation of the pituitary ACTH release [118].

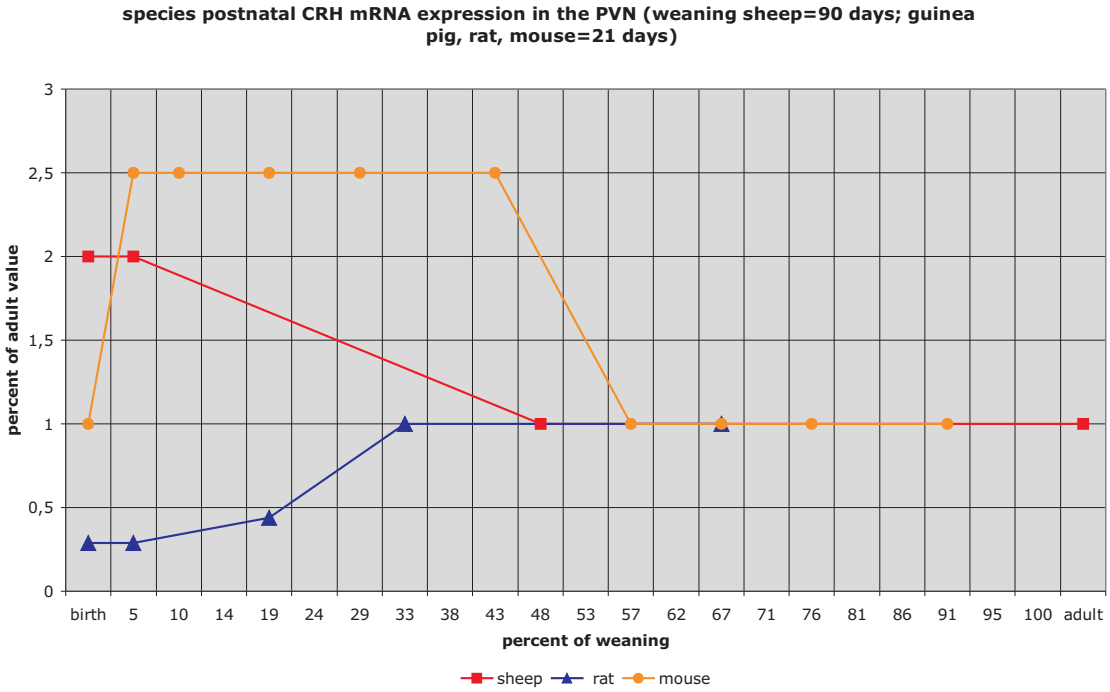


Figure 2.12. Species postnatal CRH expression in the PVN (weaning sheep=90 days; guinea pig, rat, mouse=21 days)

*No data about CRH expression in the neonatal period were available for primates. After birth, CRH mRNA expression in the sheep at 4% of weaning is similar to levels during labor, and slowly further decreasing levels are assumed, until adult levels are reached around 50% of weaning. In the guinea pig at 33% of weaning, CRH mRNA expression is low similarly to that at 94% of gestation. In adult guinea pigs, CRH mRNA expression was similarly high across gender. CRH expression in newborn rats is very low at 5% of weaning, subsequently increases significantly to high levels at 33%, similar to the level at 67% of weaning. No further information of CRH mRNA expression in the PVN of rats after 67% of weaning is available. In newborn mice, CRH mRNA expression were low at birth, but is very high at 5-43% of weaning, then continuously decreases until 57-67% of weaning, remaining on this level at least until 91% of weaning and possibly even in adulthood. The possibility that there is a comparable decrease of CRH mRNA expression in the PVN of the neonatal rat after 67% to that in the mouse at 57% of weaning cannot be excluded. The assumed increasing CRH expression, from very low levels at birth to high levels at 5% in mice and at 33% of weaning in the rat, could represent the immature CRH system during the SHRP.*

## 2.4 Hypothalamic AVP

Arginine vasopressin (AVP) is also known as vasopressin, adiuretin, argipressin or antidiuretic hormone [17]. The name 'vasopressin' comes from its ability to raise the blood pressure by inducing vessel constriction while the expression 'antidiuretic hormone' derived from its ability to increase water absorption in the kidneys to concentrate the urine. AVP is released in response to decreasing plasma volume, increasing extracellular osmolarity, stress, emesis and sexual arousal. Its secretion is inhibited by Gamma-Amino Butyric Acid (GABA) and increased through dopamine, endorphin and angiotensin II [176]. Most AVP was synthesized in magnocellular and parvocellular neurons of the PVN and in magnocellular neurons of the SON. Other brain locations of AVP synthesis were the suprachiasmatic nucleus, the bed nucleus of stria terminalis and the amygdala [311, 496]. Inside the PVN, AVP originating from the magnocellular and parvocellular neurons had to be treated independently, as AVP from each of the two locations was assigned to its pituitary lobe for different functions. Magnocellular AVP from the PVN and the SON was transported along the axons through the interior part of the median eminence to the posterior pituitary. Subsequently, AVP was released into the circulation to regulate the water balance [17, 496]. Parvocellular neurons of the PVN, co-expressing CRH and AVP, transported both substances axonal to the outer layer of the median eminence. Here they were released into the hypothalamo-pituitary portal system and in cooperation play their role in the stress regulation by stimulating ACTH secretion in the anterior pituitary. The total AVP production in the magnocellular nerve bodies (PVN and SON) was much higher than in the parvocellular neurons. By only looking at the PVN, magnocellular AVP synthesis was already 5 times higher than in the parvocellular neurons of the PVN in the fetal sheep at birth [17, 281] (see also Chapter 2.3).

### 2.4.1 AVP and CRH stimulated ACTH release in the pituitary

Parvocellular AVP from the PVN alone was only a weak stimulator for ACTH. Together with CRH, AVP potentiated CRH stimulated ACTH release by binding to its V1b receptors on corticotrophs [183, 193, 486]. Other than CRH stimulated ACTH secretion, AVP stimulated ACTH release seemed not to be restricted by glucocorticoid negative feedback [183]. Between 35-58% (wk14-23) of gestation, human fetal pituitary cells secreted ACTH after CRH administration. AVP elicited also ACTH secretion increment, but to a lesser extent than CRH. Administration of both secretagogues synergistically induced higher ACTH secretion than either substance alone [51]. Butler et al. 1999 presented fetal sheep ACTH secretion stimulated by AVP to be less sensitive to glucocorticoid negative feedback inhibition than stimulated by CRH. In the fetal sheep anterior pituitary, 70% of ACTH was stored in CRH responsive corticotrophs. It seems that hypothalamic stimuli on ACTH synthesis must counteract glucocorticoid negative feedback on CRH responsive corticotrophs [71]. ACTH producing corticotrophs in the fetal sheep changed during their maturation from being more responsive to CRH than to AVP at 77% (day115) to being more AVP-responsive than CRH-responsive at 96% (day144) of gestation and adulthood [154]. The same pattern occurred for a CRH and AVP stimulated increase in the number of ACTH secreting corticotrophs in the fetal sheep anterior pituitary. At 70% (day105) of gestation, CRH but not AVP was responsible for the increase in corticotrophs secreting ACTH. At 83% (day125), both secretagogues alone or in combination were responsible, while at 93% (day140) of gestation and in adult, AVP alone or together with CRH but not CRH alone triggered the increase of ACTH secreting cell numbers [356]. At 82% (day123) of gestation, simultaneous administration of CRH and AVP produced a synergistic increase in fetal sheep ACTH response. At 75% (day113) or 89-95% (day133-143) of gestation the response was only additive. The response to AVP was significantly ($P<0.05$) higher at 89% (day133) and at 95% (day143) than between 75-82% (day113-123) of gestation [77]. The increased pituitary ACTH secretory responsiveness to AVP in late gestation might have been due to heightened second messenger inositol triphosphate (IP3) generation. IP3 levels significantly ($P<0.05$) increased in response to AVP between 69-95% (day103-143). Hypothalamo-pituitary disconnection significantly ($P<0.05$) decreased IP3 formation and ACTH secretion, assuming hypothalamic impact on

late gestational increment in IP3 and ACTH release [75]. In late gestation, the increase in ACTH synthesis seemed to be differentially regulated by CRH and AVP. While both hormones seemed to be involved in ACTH release before 87% (day130), AVP may have stimulated the rise through 87-100% (day131-150) of gestation. At term, the additive effect of AVP and CRH on ACTH release might have maintained high ACTH levels [494].

*ACTH release stimulated by AVP is, other than by CRH, not inhibited by glucocorticoids. It can be assumed that AVP counteracts the negative feedback on CRH in stimulating pituitary ACTH secretion. During fetal maturation in sheep and human, the responsiveness of ACTH release toward the two secretagogues CRH and AVP changes. In human fetus between 35-58% of gestation, ACTH response is stronger toward CRH than to AVP, but together both hormones elicit a stronger increase than one hormone alone. In the fetal sheep, ACTH cell number increment and ACTH response is stronger in the presence of CRH approximately before 80% of gestation, and stronger in the presence of AVP after roughly 90% of gestation, as well as in adults. Between 80-90% of gestation, the responsiveness to the presence of both secretagogues is synergistic. The late gestational increase in AVP responsiveness seems to be caused by the increment of the second messenger IP3 synthesis in response to AVP.*

### 2.4.2 Fetal and neonatal AVP expression in the hypothalamus

Assuming that AVP in the pituitary originates from hypothalamic nuclei, we will use both the pituitary and the hypothalamus as locations for detection of AVP expression. Preferably, we would only use data about AVP expression from the parvocellular portion of the PVN, and AVP content in the anterior pituitary as the locations in connection with AVP stimulated ACTH release during stress regulation. Due to the scarcity of data from these two locations, data about AVP in the whole hypothalamus and in the whole pituitary had to be included. This is a definite limitation, as magnocellular AVP (from the SON and the PVN of the hypothalamus) is released into the posterior pituitary and is involved in the regulation of the water balance rather than the HPA stress regulation. Additionally the magnocellular AVP synthesis is much higher than AVP production in the parvocellular neurons. By investigating AVP content in the whole pituitary, the data rather reflect magnocellular AVP in the posterior pituitary and mask the here important parvocellular AVP from the anterior pituitary regulating ACTH release (see also above). Some consolation might come from the information that a substantial amount of AVP in the hypothalamo-pituitary portal system, which connects the median eminence with the anterior pituitary, originates from projections from the SON to the median eminence. Further, it was assumed that AVP from the posterior pituitary might also reach the anterior pituitary by vessels connecting the two lobes of the pituitary [193, 280].

#### 2.4.2.1 First AVP expression

Immunoreactive AVP was present in the fetal human hypothalamus by 28-33% (wk11-13) and in the posterior pituitary by 30% (wk12) of gestation [2, 67, 276, 440]. Due to these data, Mai et al. 1997 came to the conclusion that the hypothalamo-hypophyseal system was already functioning at the end of the first trimester (33% of gestation) in the human fetus [261]. No information is available about AVP appearance or ontogeny in the fetal rhesus monkey and baboon. In the fetal sheep, already at 30% (day45) of gestation, AVP cell bodies were mainly present in the SON, but a few weakly stained cells were detected in the PVN as well. Additionally, AVP fibers in the median eminence were apparent [245]. Burton et al. 1972 detected AVP in the fetal guinea pig pituitary around 29% (day20) of gestation [69]. In the fetal rat, AVP was apparent in the pituitary at 82-86% (e18-19) and in SON and PVN at 86% (e19) of gestation [8, 88]. In the fetal mouse at 74% (e14.5) of gestation, only a very small amount of AVP was detectable in the hypothalamus-pituitary unit [505]. Hyodo et al. 1992 detected IR-AVP in the median eminence by 74% (e14.5), in the SON by 80% (e15.5) and in the PVN by 85% (e16.5) of gestation [204].

TABLE 2.3

First AVP expression in hypothalamus. *The guinea pig data originate from the pituitary.

| | AVP in wk/day | AVP in % |
|---|---|---|
| Human | wk 12/40 | 30 |
| Rhesus monkey | % | % |
| Baboon | % | % |
| Sheep | 45/150 | 30 |
| Guinea pig* | (20/68) | (29) |
| Rat | 18-19/22 | 82-86 |
| Mouse | 14.5/19.5 | 74-85 |

*It is surprising how timely conform the first expression of AVP in the hypothalamus-pituitary system appears across human, sheep and guinea pig. Looking only at the PVN, AVP is detected in rat and mouse at the same time, but much later in gestation compared to the sheep.*

#### 2.4.2.2 Prenatal AVP expression

Over the period of 30-45% (wk12-18) of gestation, IR-AVP significantly ($P<0.01$) rose in human fetal pituitary gland by 5.1 fold [440]. Mastorakos and Ilias in 2003 reported a dramatic increase (over 1000-fold) of IR-AVP in the fetal posterior pituitary from 28-30% (wk11-12) until 60-70% (wk24-28) of gestation [276]. Between 35-58% (wk14-23) of gestation, AVP elicited ACTH secretion in human fetal pituitary cells [51]. No information is available about AVP ontogeny in the fetal baboon and rhesus monkey. In the sheep pituitary, no significant change in AVP content was detected between 59% (day88) and 98% (day147) of gestation [404]. In the fetal sheep PVN and SON, low levels of AVP mRNA were identified at 30% (day45), respectively at 43% (day64) of gestation [245, 281]. In the PVN, AVP mRNA was detected in both parvocellular and magnocellular fields, but 5 fold higher in the latter. In the parvocellular portion of the PVN, the location decisive for AVP induced stress regulation and ACTH release, AVP mRNA levels did not change significantly between 49% (day73) of gestation and spontaneous term labor. In the magnocellular PVN, AVP mRNA increased significantly ($P<0.05$) between 47% (day74) and 97% (day145.5) by 2.2 fold and from 76% (day114) of gestation to spontaneous labor ($P<0.05$) by 1.7 fold. No significant change was present between 97% (day145.5) and labor. In the SON, AVP mRNA was exclusively present in magnocellular neurons and increased significantly ($P<0.05$) in the course of gestation [281]. In the guinea pig, rat and mouse, AVP data are measured in the whole pituitary, with their limited informative value. Pituitary AVP content in the guinea pig remained low from 29% (day20) to 71% (day48), but then started to increase significantly ($P<0.001$) between 71-85% (day48-57.5) by 6 fold, and between 85% (day57.5) of gestation and 2.4% (PND 0.5) of weaning ($P<0.001$) by another 5 fold [69]. Owen et al. 2005 assumed that hypothalamic AVP levels might have risen in late gestational fetal guinea pigs, when CRH levels have fallen [337]. In the fetal rat, AVP content in the hypothalamus and the pituitary dramatically increased between 82-100% (e18-22) of gestation. Between 82-95% (e18-21), AVP peptide content in the posterior pituitary increased by 25 fold ($P<0.001$) and between 95-100% (e21-22) of gestation by another 2.3 fold ($P<0.001$). Pattern of AVP content were similar in hypothalamus, pituitary and the entire brain. During

the course of labor, AVP content in the pituitary of fetal rats did not change significantly, but had significantly decreased by 2.4% (PND0.5) of weaning (P=0.007) by 1.3 fold [52, 436]. In the fetal mouse, magnocellular IR-AVP level in SON and PVN increased between 80-85% (e15.5/16.5) of gestation and term, especially in the SON [204]. Between 80-85% (e15.5-16.5) of gestation, pituitary AVP content did not change, but hypothalamic AVP content significantly (P=0.001) increased by 5 fold. This made 85% (e16.5) of gestation the only gestational time point, when AVP levels were significantly (P<0.05) higher in the hypothalamus than in the pituitary, assuming extremely enhanced AVP synthesis in the magnocellular neurons of the hypothalamus between 80-85% (e15.5-16.5) of gestation. Between 85-90% (e16.5-17.5) of gestation, AVP content did not change in the hypothalamus, but the content of AVP in the pituitary rapidly increased (P<0.001) in the pituitary by 4.3 fold. The latter might be due to activation of AVP transport during that time. Pituitary content significantly increased between 90-95% (e17.5-18.5), and further between 95-100% (e18.5-19.5) of gestation by 3.7 fold (P<0.001), respectively, while hypothalamic increments were only 1.7 fold (P=0.028) and 1.9 fold (P=0.026) [505].

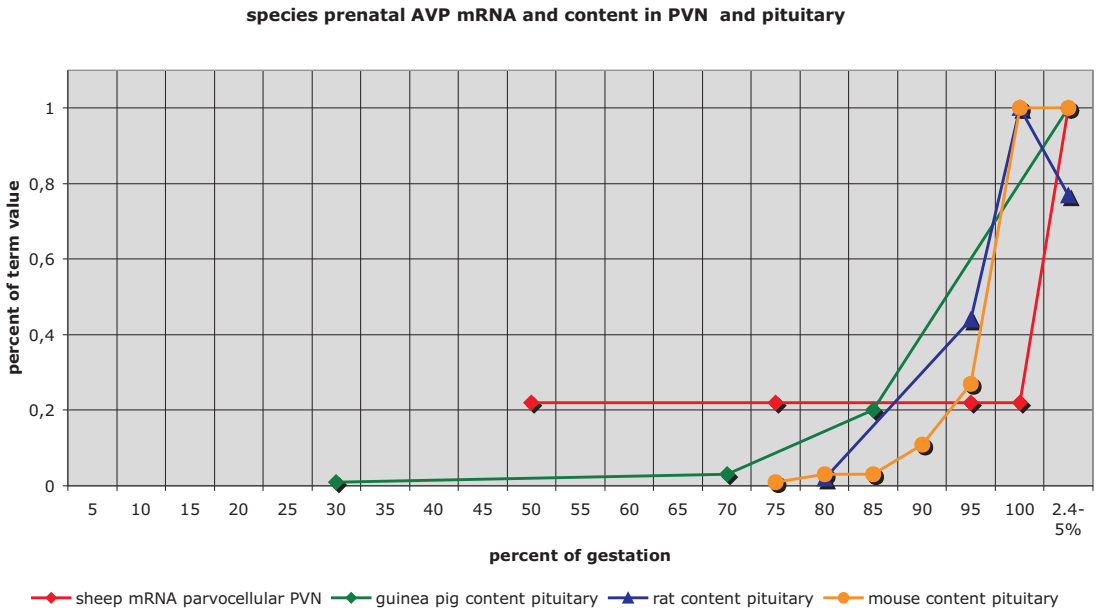


Figure 2.13. Species prenatal AVP expression and content in PVN and pituitary

*We were able to use AVP expression in the parvocellular PVN of the fetal sheep but due to the scarce data, we had to use AVP content from the pituitary for the other species. We are aware of the limitations of AVP detection in the pituitary as these data reflect mainly the content in the posterior pituitary rather than in the much more significant anterior pituitary regarding stress regulation. With the possibility that posterior AVP might also reach the anterior pituitary and that the pattern of AVP contents in hypothalamus, pituitary and the entire brain seems often congruent, we will include these data with their limitations into our study. In the human pituitary, AVP levels increase at least between 30-70% of gestation. No information is available for rhesus monkey or baboon. In the sheep, AVP from the parvocellular PVN remains unchanged during gestation. The possibility cannot be excluded that the increasing AVP expression from magnocellular PVN during the second half of gestation might add up to the amount from the parvocellular PVN. In the guinea pig, AVP is expressed in the pituitary from 30% of gestation on, but remains very low until 70%*

*and dramatically increases between 85% of gestation and 2.4% of weaning. AVP is detected in the PVN and pituitary of the fetal rat only at 82-86%, and increases at least in the pituitary most dramatically from 82-100% of gestation. Subsequently, pituitary AVP levels remain constant during labor. In the mouse, AVP levels increase in both hypothalamus and pituitary strongly from the first appearance in the SON and PVN around 80-85% until term.*

#### 2.4.2.3 Postnatal AVP expression

Again we do not have data of hypothalamic or pituitary AVP levels in primates. In the newborn sheep, AVP mRNA significantly ($P<0.05$) increased between birth and 4% (PND3.5) of weaning in the whole PVN by 1.6 fold, but more important is the dramatic increment in the parvocellular PVN by 4.6 fold. AVP mRNA expression in the parvocellular PVN remained unchanged on high levels until adulthood. As CRH in the PVN did not significantly changed between term and 4% (PND3.5) but significantly decreased by 50% (PND45) of weaning [281], the significant increase of AVP in the parvocellular PVN between birth and 4% (PND3.5) of weaning might be especially important in controlling ACTH release from the pituitary in younger lambs. In the guinea pig pituitary between 85% (day57.5) of gestation and 5% (PND1) of weaning, AVP has dramatically ($P<0.001$) increased by 5 fold. Pituitary AVP content increased ($P=0.018$) between 5-41% (PND1-8.5) of weaning by another 1.5 fold [69]. In the newborn rat between birth and 2.4% (PND0.5) of weaning, AVP level in hypothalamus and pituitary decreased by 1.3 fold ($P=0.007$) [436]. A significant ($P<0.01$) increase by 1.5 fold took place between 14-19% (PND3-4) of weaning in the hypothalamus. Between 67-100% (PND14-21) ($P<0.001$), and particularly again between 100% (PND21) of weaning and PND40 ($P<0.001$), the main increases in the hypothalamic AVP content were by 2.5 fold and respectively by 5.9 fold. At 100% (PND21) of weaning, only 17% of the adults level is achieved in hypothalamic AVP [397]. In the rat pituitary, AVP content significantly ($P<0.01$) increased by 2.6 fold between 24-48% (PND5-10), with no further changes by 95% (PND20) of weaning [518]. AVP was crucial for the expression of ACTH stress response in the postnatal period of rats. Homozygous deficient AVP rats were unable to present an ACTH stress response, while heterozygous controls showed increasing ACTH plasma levels to stressors. Surprisingly, in homozygous deficient AVP rats, corticosterone stress response remained intact, assuming that neither AVP nor ACTH are necessary to induce corticosterone stress reaction. Additionally, CRH mRNA levels in the PVN at 48% (PND10) of weaning did not increase in response to maternal separation, like it happened in adult rats, assuming immature CRH secretion and a more important role of AVP [518]. Neonatal mouse AVP content in the hypothalamus between 5-10% (PND1-2) increased significantly ($P=0.005$) by 2.8 fold and further by 33% (PND7) of weaning ($P<0.001$) by another 1.9 fold. Between 33-67% (PND7-14), hypothalamic AVP content increased by 3.8 fold ($P=0.003$) but no further change occurred by 100% (PND21) of weaning. AVP mRNA did not show any age-related change from 3-9 month of age in the PVN magno- and parvocellular subfields and in both locations, AVP mRNA was expressed in similar amounts [110]. AVP pituitary content in the neonatal mouse between birth and 5% (PND1) did not change, but increased significantly ($P<0.001$) by 2.3 fold between 5-10% (PND1-2) of weaning. Between 10-100% (PND2-21), AVP content in the pituitary rapidly increased ($P<0.001$) by 18 fold and significantly decreased between 100% (PND21) of weaning and PND30 ($P<0.05$). Afterwards AVP gradually increased again to adult levels on PND90 [505].

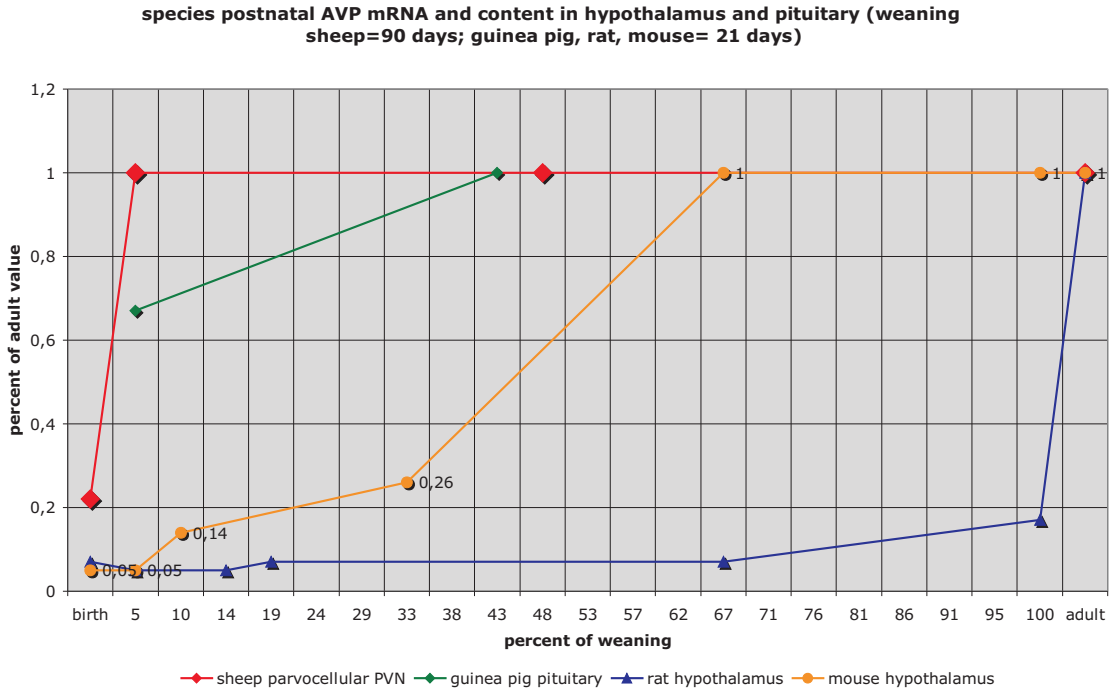


Figure 2.14. Species postnatal AVP expression and content in hypothalamus and pituitary (weaning sheep=90 days; guinea pig, rat, mouse= 21 days)

*As not much data are available, postnatal AVP level in the guinea pig can only be presented in the pituitary, while in all other species, the levels from the hypothalamus are used. For the guinea pig, AVP content in adults is unknown, so 41% (PND8.5) of weaning is used as adult value. At least for the neonatal sheep we can present AVP mRNA expression from the parvocellular PVN. While AVP mRNA expression in this location remains low and unchanged during gestation, between birth and 4% of weaning AVP expression in the parvocellular PVN increases dramatically already to adult-like levels. As shown before, CRH mRNA expression remains unchanged over that early neonatal period, but decreases by 50% of gestation to low levels, which persists into adulthood. We assume a more important role in the regulation of ACTH release after birth by AVP than CRH in the neonatal sheep, a condition, which might even remain into adulthood. Aware of the limitation due to the use of data from the whole pituitary in the guinea pig as well as from the whole hypothalamus in the rat and mouse, the following assumption might be possible. The guinea pig pituitary AVP content increases moderately between 5-41% of weaning. This most likely expresses an increase in the posterior pituitary. But under the assumption that AVP from the posterior pituitary might also reach the anterior pituitary by vessels connecting the two lobes of the pituitary, an increase in the posterior lobe might also lead to an increment in anterior lobe AVP. While AVP levels in the hypothalamus remain very low in the rat until weaning and only reaches 17% of adult value at that point, hypothalamic AVP levels in the mouse remain relatively low until 33% of weaning but increase to adult values already by 67% of weaning. Again, we have to be aware of the limitation of these data, as in both species AVP content was measured in the whole hypothalamus, which most likely masks the content in the parvocellular portion of the PVN. The possibility exists that an increasing hypothalamic AVP content also reaches the anterior pituitary and elicits AVP regulated ACTH release. As the increase in the mouse happens much earlier than in the rat, earlier AVP*

*stress regulation in the mouse than in the rat might be present.*

## 2.5 Hypothalamic GR

The hypothalamus expresses CRH and AVP and regulates ACTH secretion in the pituitary. The hypothalamus is the primarily site of glucocorticoid negative feedback. Stress-induced release of cortisol/corticosterone from the adrenal cortex, which then binds to GR on CRH neurons, decreases CRH synthesis [118]. No MR was expressed in the hypothalamic PVN of human, rhesus monkey, guinea pig, rat, mouse and assumingly sheep [54, 277, 358, 374, 375, 403, 474].

### 2.5.1 Adult GR expression in the hypothalamus

No information is available about human or baboon GR expression in the PVN. The adult rhesus monkey expressed high GR mRNA level in the PVN [374, 403]. In the intracellular fluid of hypothalamic cells in the adult sheep, far less GR binding sites were expressed compared to pituitary cells. Between fetal/neonatal and adult sheep hypothalamus, GR binding sites in the intracellular fluid decreased ($P<0.01$) approximately by 6 fold [391]. In the adult guinea pig PVN, GR mRNA was expressed in high levels in the PVN. GR mRNA expression was significantly higher in the PVN of adult guinea pig females than adult males [27, 255]. In adult rats, GR expression in the anterior pituitary was slightly lower than in the PVN [265]. It seemed to be that GR-like immunoreactivity was present in rat parvocellular PVN but not in magnocellular PVN [82]. The adult mouse expressed moderate levels on PVN GR mRNA, compared to high expression in the rat and rhesus monkey [374].

*Rhesus monkey, guinea pig and rat express high levels of paraventricular GR mRNA in adulthood, while the mouse GR expression is only moderate. The adult sheep expresses much less GR binding sites in the hypothalamus compared to fetus and neonate. Sheep hypothalamic GR binding sites are much lower than in the pituitary in adulthood. GR expression in the PVN of the female guinea pig is higher than in the male guinea pig. The rat expresses slightly higher GR mRNA levels in the PVN than in the anterior pituitary. It is possible that GR-like immunoreactivity is absent in the magnocellular PVN, but present in the parvocellular PVN. This would makes sense when we remember that negative feedback seems to only inhibit CRH but not AVP, and that CRH is only apparent in parvocellular but not in magnocellular PVN cells (see also hypothalamic AVP expression).*

### 2.5.2 Fetal and neonatal GR expression in hypothalamus

#### 2.5.2.1 First GR expression

No information is available about GR expression in the fetal primate PVN. In the sheep whole hypothalamus as well as in the PVN, GR mRNA expression was present at least at 43% (day65) of gestation. At that time, GR mRNA expression in the PVN was already high [11, 509] and an earlier start of expression in this location can be assumed. In the guinea pig PVN at 59% (day40), GR mRNA was highly expressed, indicating a start of expression before 59% of gestation [277, 335]. In the whole hypothalamus of the fetal rat at 59% (e13) of gestation, GR mRNA expression was detected. At 55% (e12), GR expression was apparent in the fetal rat anterior hypothalamus and while still absent at 68% (e15), GR expression was present in the PVN at 73% (e16) of gestation [90, 127, 236, 512]. Mouse GR mRNA expression was first clearly detected at 64% (e12.5) of gestation in the whole hypothalamus [446], but no information is available about the first expression in the mouse PVN. Reichardt and Schuetz in 1996 detected negative feedback regulation on CRH expression (but not on AVP expression) in the PVN of the fetal mouse by at least 90% (e17.5) of gestation [377], assuming GR expression in the PVN at or before that time.

TABLE 2.4

First GR expression in the hypothalamus

| | GR in wk/day | GR in % |
|---|---|---|
| Human | ? | ? |
| Rhesus monkey | ? | ? |
| Baboon | ? | ? |
| Sheep | <65/150 | <43 |
| Guinea pig | <40/68 | <59 |
| Rat | 15-16/22 | 68-73 |
| Mouse | $12.5 \leq X < 17.5/19.5$ | $64 \leq X < 90$ |

*With the presence of GR expression in the PVN, functional negative feedback on CRH synthesis can be assumed. No information is available about the first expression of GR in the PVN of fetal human, rhesus monkey or baboon. As GR mRNA is already highly expressed in sheep and guinea pig at 43% respectively 59% of gestation, an earlier start of expression can be expected. In the fetal rat, GR expression in the PVN starts at 73% of gestation, and in the fetal mouse, first expression assumingly is present at or after 64% but before 90% of gestation.*

#### 2.5.2.2 Prenatal GR expression

No information is available for prenatal GR expression in the PVN of primates. Inside the sheep's PVN, GR mRNA was mainly expressed in the parvocellular region. High levels were present between 49-86% (day74-129) of gestation. Subsequently levels significantly (P=0.017) decreased by approximately 2.8 fold to low levels at 96% (day144) of gestation. Constantly low levels seemed to be present between 96% of gestation and term as well as between term and 9% (PND7) of weaning [11]. In the guinea pig, GR mRNA expression was present in the total PVN as well as in the medial parvocellular (mp) PVN of both genders. Between 59-94% (day40-64) of gestation in the total PVN, GR mRNA expression significantly (P<0.05) decreased by 1.7 fold in the female guinea pig, while a decrease by 1.5 fold in the male fetus failed to reach significance. Looking more precisely at the mp PVN, GR mRNA levels decreased between 59-94% (day40-64) of gestation significantly (P<0.05) by 1.75 fold in both genders [335]. Matthews 1998 presented a significant (P<0.05) decrease of guinea pig GR expression in the mp PVN in two steps, from 63-77% (day42.5-52.5) by 1.5 fold and further between 77-92% (day52.5-62.5) of gestation by 1.4 fold [277]. In the rat, from its first expression in the PVN at 73% (e16) of gestation, GR mRNA was moderately to strongly expressed until term, while GR immunoreactivity emerged 1-2 days after the appearance of GR mRNA and was weak to moderate between 82-100% (e18-22) of gestation. From moderate levels at 73-82% (e16-18), GR mRNA expression increased to moderate-strong levels at 86% (e19) and further to strong expression at 95% (e21) of gestation and at term [90]. Yi et al. in 1994 detected equally robust GR mRNA hybridization in the rat at 77% (e17), 86% (e19) and 5% (PND1) of weaning [512]. No information is available about GR mRNA development in the PVN of the fetal mouse. GR mRNA expression was present in the whole hypothalamus of the fetal mouse by 64% (e12.5) of gestation. At 90% (e17.5) of gestation, sufficient GR mRNA expression in the PVN can be assumed due to functional negative feedback in this location [377, 446].

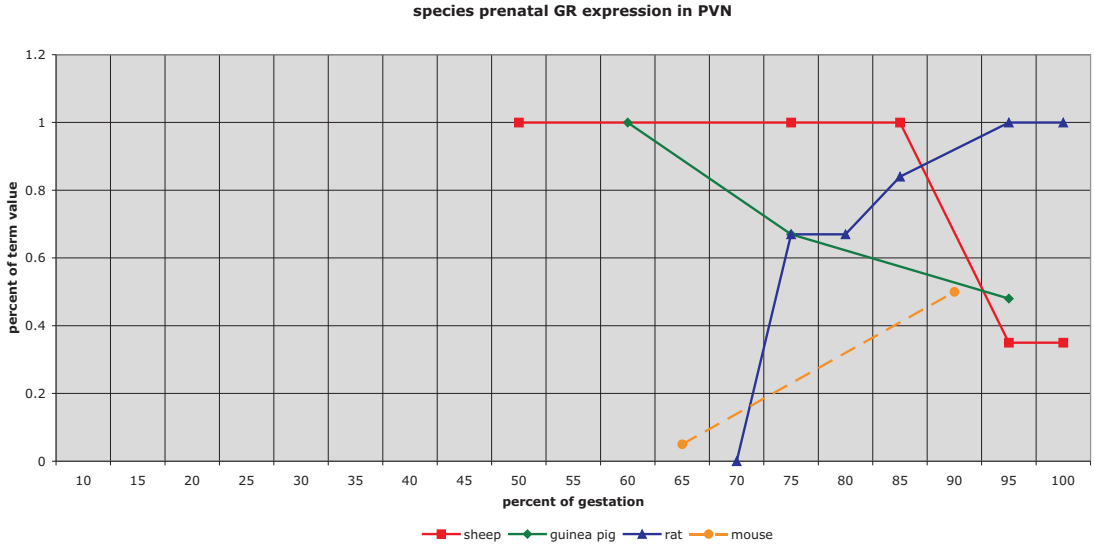


Figure 2.15. Species prenatal GR expression in PVN

*In the fetal sheep PVN, GR expression remains constantly high between 49-86%, but dramatically decreases to low levels at 96% of gestation and is still low at term. The fetal guinea pig expresses high GR levels in the whole PVN as well as in the mp PVN at 59% of gestation. GR expression strongly decreases by 94% of gestation. In both sheep and guinea pig close to term, a decrease in glucocorticoid negative feedback on CRH expression can be assumed. In the PVN of the fetal rat, GR expression is absent by 68%, but is moderate by 73-82% of gestation. By 86% of gestation, expression is moderate to strong and increases to high levels at 95-100% of gestation. GR expression in the mouse PVN is present around 64-90% and is expressed in sufficient amount for functional negative feedback at least at 90% of gestation. In the rat and possibly also in the mouse, GR expression strongly increases towards term, assuming very high glucocorticoid feedback inhibition on the expression of CRH around birth.*

#### 2.5.2.3 Postnatal GR expression

In the neonatal period, no information about GR expression in the hypothalamus is available for humans, rhesus monkeys or baboons. By 8% (PND7) of weaning, GR mRNA expression in the PVN of the sheep was low, and did not significantly differ from term values [11]. In the whole hypothalamus, GR receptor binding was lower in the newborn sheep at 1% (PND1) of weaning than in adult [391]. By 33% (PND7) of weaning, guinea pig GR mRNA expression in the mp PVN was similarly low as the levels shortly before term [277, 335]. At 86% (PND18) of weaning, GR mRNA expression in the PVN was significant ($P<0.005$) greater in female than in male guinea pigs [122]. In the rat PVN, GR mRNA expression was similarly strong at 82% (e18) of gestation, 5% (PND1), 19% (PND4), 29% (PND6), 48% (PND10) and 76% (PND16) of weaning [512]. Van Eekelen et al. 1991 confirmed abundant GR expression in the parvocellular division of the rats PVN at 10% (PND2) of weaning [474]. GR mRNA expression in the neonatal mouse was similarly high in the PVN between 19-33% (PND4-7) of weaning. Additionally, GR mRNA in PVN was moderate to high at 43% (PND9) of weaning and might have increased slightly in animals 3 month of age [110, 146, 411].

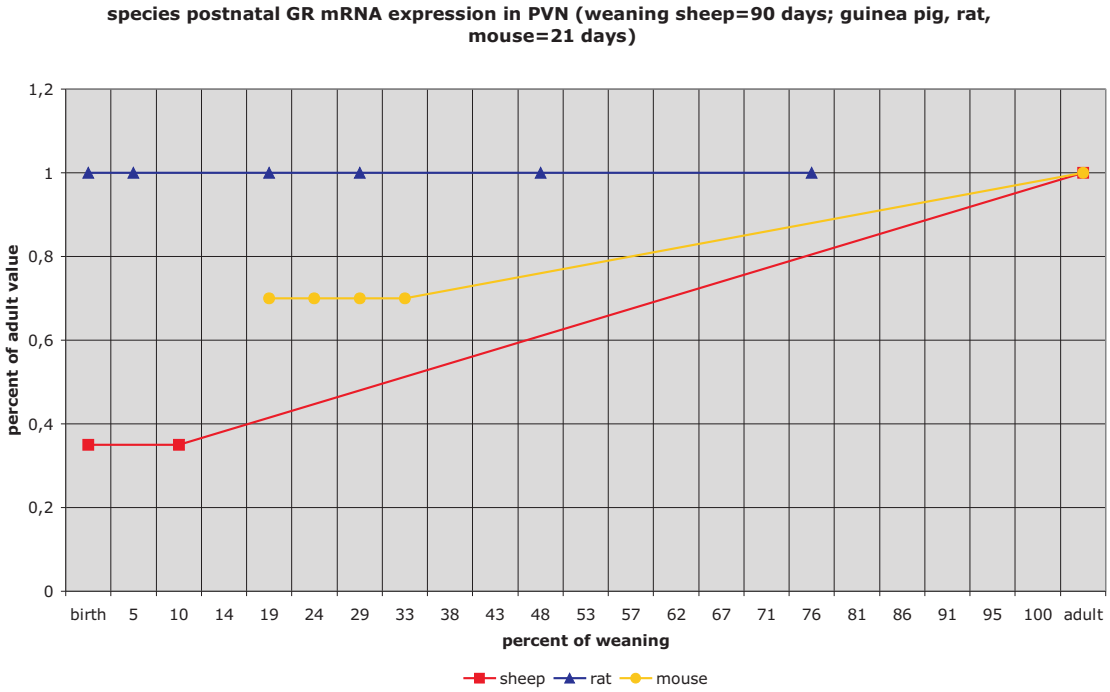


Figure 2.16. Species postnatal GR expression in PVN (weaning sheep=90 days; guinea pig, rat, mouse=21 days)

*In the neonatal sheep, GR expression is similarly low at term and at 8% of weaning, but seems to increase toward adulthood. After birth, the guinea pig expresses GR mRNA in the PVN in analogical low levels at 33% of weaning as in late gestation. Already at 86% of weaning, the gender differences with significantly higher GR mRNA expression in the PVN of females compared to males occur.*

*The newborn rat shows continuously strong PVN GR expression from birth until at least 76% of weaning. The mouse exhibits moderately high GR mRNA expression in the PVN from 19-33% of weaning, which might subsequently slighty increase to adult level. In sheep and guinea pig, low GR expression after birth indicates low feedback inhibition on CRH level. Later in life, negative feedback on PVN CRH expression increases in the sheep. Negative feedback on hypothalamic CRH is stronger in female than male guinea pigs from at least 86% of weaning on. In rat (mouse) neonates, negative feedback on CRH expression is very strong ( respectively strong) and remains such until at least 76% of weaning in rat (and 43% of weaning in mouse).*

## 2.6 Pituitary POMC and ACTH

In the following we will investigate POMC expression and ACTH synthesis in the fetal pituitary as well as ACTH concentration in the fetal circulation, which directly stimulates glucocorticoid but also androgen release from the fetal adrenal cortex.

### 2.6.1 ACTH and POMC synthesis

Proopiomelanocortin (POMC) is produced in two types of pituitary cells, corticotrophs and melanotrophs [272]. It is a large protein of 265 amino acids that is cleaved into smaller peptides like ACTH, $\beta$-endorphin, lipoprotein and $\alpha$-melanocyte-stimulating hormone by help of proconvertase-1 (PC-1) and proconvertase-2 (PC-2) [199, 496]. Adrenocorticotropic hormone (ACTH) or corticotropin is a peptide hormone of 39 amino acids [388]. Already in 1942, Li et al. isolated ACTH protein from the sheep pituitary, which selectively stimulated the adrenal cortex [249]. Both CRH and AVP stimulate synthesis of ACTH from its precursor POMC in the anterior pituitary [369]. ACTH and POMC were expressed in the pars distalis of the fetal sheep. PC-1 expression increased in late gestation, assuming that increased PC-1 expression led to increased POMC expression [199]. As many researchers focused on the precursor molecule POMC, rather than its derivate ACTH, we will center on both hormones for a more comprehensive picture. Beside pars distalis cells, pituitary pars intermediate cells of the fetal sheep responded to CRH and AVP and released POMC and ACTH precursors, which could modulate fetal adrenal function [154, 416]. Hawkins et al. 2001 reported 26-32 times higher POMC mRNA expression in the intermediate lobe of the fetal sheep compared to the total anterior lobe and to the inferior region of the anterior lobe [184]. The intermediate lobe in the fetal guinea pig represented elevated POMC mRNA expression at the time of parturition and Owen et al. 2005 suggested that the intermediate lobe might represent a significant source of ACTH [337]. On the other hand, the fetal human pituitary only has a rudimentary pars intermedia [19] and this part of the pituitary might not be involved in glucocorticoid negative feedback, due to a lack of GR expression [62, 337, 379].

### 2.6.2 Fetal and neonatal POMC expression/ACTH synthesis

#### 2.6.2.1 First POMC expression/ACTH synthesis

In the human fetal pituitary, IR-ACTH was not detected at 20% (wk8), but was present at 23% (wk9), and first signs of pituitary ACTH activity appeared at 23-25% (wk9-10) of gestation [25, 170, 344]. Goto et al. 2006 were able to show that ACTH secretion was present at the beginning of 25% (wk10) of gestation, together with ACTH receptor expression in the human fetal adrenal gland [170]. POMC, as the precursor molecule of ACTH, is assumed to be present at least at the same time than ACTH in the pituitary. In the fetal rhesus monkeys‘ anterior and intermediate lobe of the pituitary, POMC containing cells were detectable at 30% (day50) and IR ACTH was detected in the whole pituitary at 30-33% (day50-55) of gestation [7, 182]. POMC mRNA is expressed in the fetal baboon pituitary at least by 54% (day100) of gestation [5, 353]. In the fetal sheep pituitary, POMC mRNA and IR-ACTH were absent at 23% (day35) but were detected in the pars distalis and pars intermedia at 30% (day45) of gestation. In the basal region of the pars distalis, POMC mRNA was already highly expressed at that point [174]. Latest at 59% (day40) of gestation, POMC mRNA was expressed in the fetal guinea pig anterior pituitary and intermediate lobe and plasma ACTH was apparent in the guinea pig circulation [277, 335, 337]. In the fetal rat at 59% (e13) of gestation, POMC mRNA was first detected in the ventral Rathke‘s pouch, which will later in gestation become the anterior pituitary. At 68% (e15) of gestation, POMC mRNA was present in the pars intermedia [257]. IR-ACTH labeled cells were absent in the pars distalis at 55-59% (e12-13), but were first detected at 64% (e14) in the ventral periphery of the pars distalis, and at 73% (e16) of gestation in the pars intermedia [322]. At 70% (e15.5) of gestation, only a few cells reacted weakly to anti-ACTH labeling [462]. In the fetal mouse, POMC mRNA appeared at 64% (e12.5) in the anterior lobe, and at 74% (e14.5) of gestation in the intermediate lobe [143, 210].

Rius et al. 1991 suggested that 23-30% of the processed POMC peptides appeared one day after the expression of POMC mRNA and showed that between 64-74% (e12.5-14.5) of gestation, nearly all corticotrophs of the anterior pituitary developed staining for ACTH [383].

TABLE 2.5

First ACTH/POMC expression in anterior pituitary

| Anterior pituitary | POMC in wk/day | POMC in % | ACTH in wk/day | ACTH in % |
|---|---|---|---|---|
| Human | wk 9 | 24 | wk 9 | 24 |
| Rhesus monkey | 50/165 | 30 | 50-55/165 | 30-33 |
| Baboon | <100/184 | <54 | | |
| Sheep | 35-45/150 | 23-30 | 35-45/150 | 23-30 |
| Guinea pig | <40/68 | <59 | <40/68 | <59 |
| Rat | 13/22 | 59 | 14/22 | 64 |
| Mouse | 12.5/19.5 | 64 | 12.5-14.5/19.5 | 64-74 |

*Only in mouse and rat are different time points detectable for the beginning of POMC expression in the anterior and intermediate lobe.*

TABLE 2.6

First POMC expression in anterior lobe (AL) and intermediate lobe (IL)

| | POMC in day AL | POMC in day IL | POMC in % AL | POMC in % IL |
|---|---|---|---|---|
| Rat | 13/22 | 15/22 | 59 | 68 |
| Mouse | 12.5/19.5 | 14.5/19.5 | 64 | 74 |

*In fetal anterior pituitary, POMC expression can be assumed to appear slightly earlier than ACTH, and hormone peptide to follow in its appearance mRNA expression. We can expect a slightly earlier appearance of POMC first expression in the human anterior pituitary than the occurrence of ACTH at 24% of gestation. In human, rhesus monkey and sheep, POMC and ACTH appear in the anterior pituitary between 23% and 32% of gestation. No valid information about the first appearance of POMC or ACTH is available for baboon and guinea pig. Rat and mouse express POMC and ACTH at 59-64% respectively 64-74% of gestation. As expected, POMC appears slightly earlier than ACTH. The first occurrence of POMC mRNA takes place earlier in the rat than in the mouse. In both species, POMC expression is apparent approximately 10% of gestation earlier in the anterior lobe than the intermediate lobe of the pituitary.*

#### 2.6.2.2 Prenatal POMC expression and plasma ACTH

It is not assumed that maternal ACTH which crosses the placenta is released into fetal circulation, as umbilical artery and vein concentrations of ACTH are similar [331]. Already at 25% (wk10) of gestation in the human fetal adrenal gland, ACTH receptors were expressed and ACTH stimulated cortisol synthesis was apparent. Additionally, GR expression in the pituitary and glucocorticoid negative feedback on ACTH synthesis was apparent at this point. From its first detection in the human anterior pituitary at 23% (wk9), ACTH expression increased between 25-28% (wk10-11), with similar findings at 30% (wk12) of gestation [169, 170]. An early peak in pituitary ACTH concentrations and possibly plasma ACTH levels in the human fetus might be apparent, as ACTH concentrations in the anterior pituitary decreased between 31-39% (wk12.5-15.5) by 1.7 fold. Similarly high fetal plasma ACTH levels were present at 43% (wk17) and 71% (wk28.5) of gestation [344, 498], and a depression between this two time points might be apparent. Between 45-53% (wk18-21), the pituitary ACTH levels had significantly increased ($P<0.001$) by 2.5 fold and by another 3 fold ($P=0.001$) between 53-60% (wk21-24) of gestation. Pituitary ACTH concentrations and content continued to grow until 66% (wk26.5) of gestation by another 2.8 fold. Subsequently the levels decreased until term by 2.2 fold to similar levels than at 60% (wk24) of gestation [344]. Winters et al. 1974 presented significantly ($P=0.002$) decreasing fetal plasma (umbilical cord) ACTH values from 73% (wk29) of gestation until term by 1.6 fold. By 1% (PND3.5) of weaning, neonatal plasma ACTH had significantly decreased ($P=0.034$) again by 1.2 fold [498].

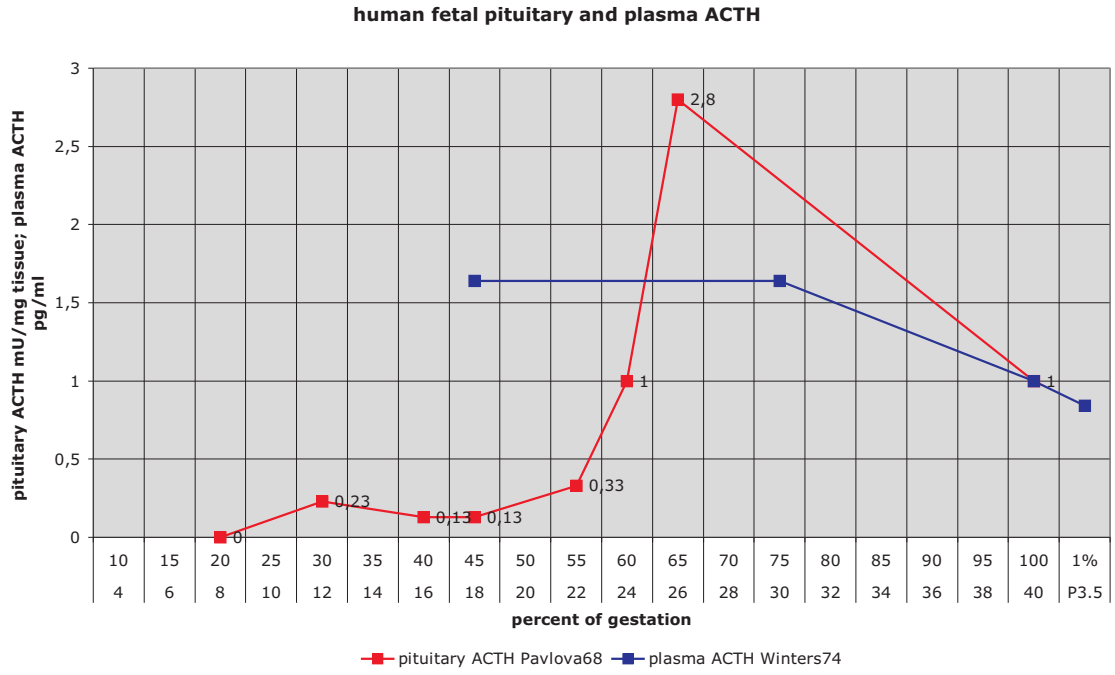


Figure 2.17. Human fetal pituitary and plasma ACTH

For the fetal rhesus monkey, no information is available about the development of ACTH or POMC expression in the pituitary or plasma ACTH from their appearance at 30-32% (day50-55) until 80% (day132) of gestation. Between 81-87% (day133-143) of gestation, fetal plasma ACTH levels were low [98]. At 82% (day136) of gestation, physiologically low ACTH level were assumed, as metyrapone-induced ACTH secretion caused steroid enzyme expression in the adrenal

gland, which was normally absent at this time of gestation [97]. Between 81-87% (day133-138) and 89-96% (day147-158) of gestation, fetal plasma ACTH levels increased significantly ($P<0.001$) by 3 fold. Maternal and fetal plasma ACTH level were similar at 84% of gestation but 3 fold ($P<0.001$) higher in fetal circulation compared to the mother at 92% of gestation. During that period, fetal plasma ACTH levels significantly ($P<0.05$) increased in response to stress, but the maternal plasma ACTH did not [98]. Between 'not in labor' at 93% of gestation (day154) and spontaneous term labor, plasma ACTH in the fetal rhesus monkey increased significantly ($P<0.05$) by 2.1 fold [162]. In the fetal baboon, the earliest information concerning pituitary POMC or ACTH development is available from 54% (day100) of gestation onwards. Between 54% (day100) and 89% (day164) of gestation, POMC mRNA expression increased significantly ($P<0.05$) by 2-2.3 fold, and the number of pituitary cells expressing ACTH increased ($P<0.05$) by 1.6 fold. At 89% (day164) of gestation, POMC mRNA was 3-4 fold lower than in adult baboons [5, 353]. POMC in the fetal sheep pituitary was higher expressed at the base of the pars distalis (inferior region) than in the superior region of the pars distalis, and did not change in the latter during gestation [62, 283]. In the inferior region, high POMC mRNA expression was present at 30% (day45) of gestation. A significant ($P<0.01$) decrease of 2.2 fold by 37% (day55) took place, followed by a significant ($P<0.01$) increase of 2.4 fold until 93-97% (day139-146) of gestation [174]. Between 49% (day74) and 76% (day114), POMC mRNA expression increased ($P=0.004$) by 2.5 fold, remained constant until 98% (day146.5) and further increased ($P=0.001$) by 1.7 fold until term, with no further change during labor [199, 283]. In the pars intermedia, POMC mRNA expression increased significantly ($P<0.05$) between approximately 37-76% (day55-114) of gestation and levels remained constant thereafter [174, 283]. In the female fetal sheep, Braun et al. 2009 detected significantly ($P=0.008$) increasing plasma ACTH levels between 33-83% (day50-125) of gestation by 3.5 fold [62]. IR-ACTH concentrations increased significantly ($P=0.011$) between 82-85% (day123-128) by 1.7 fold, remained constant until 92% (day138), then significantly ($P=0.03$) increased by 3.9 fold between 92-99% (day138-148) of gestation [370]. Between not in labor and labor, plasma ACTH significantly ($P<0.05$) increased by another 2.5 fold [199]. In the fetal guinea pig pituitary, POMC mRNA was also higher in the inferior than in the superior region of the anterior pituitary. In the total anterior pituitary, POMC expression significantly ($P<0.05$) decreased in female guinea pigs between 59-94% (day40-64) by 2 fold, but in male guinea pigs already between 59-74% (day40-50) by 1.8 fold. In the inferior region, POMC mRNA significantly ($P<0.05$) decreased in male guinea pigs between 59-94% (day40-64) by 2.3 fold. Surprisingly in the intermediate lobe of the guinea pig pituitary, POMC mRNA expression behaved anti-parallel and increased significantly ($P<0.002$) in both genders between 59-74% (day40-50) by 2.2 fold, then remained high by 94% (day64) of gestation [337]. In parallel with POMC mRNA expression in the intermediate lobe, plasma ACTH concentrations in the fetal guinea pig were relatively high at 63% (day42.5) and remained constant by 77% (day52.5) of gestation. Between 77% (day52.5) and 92% (day62.5) of gestation, plasma ACTH significantly ($P=0.03$) increased by 1.6 fold [277]. In agreement, Jones et al. 1980 detected constant fetal plasma ACTH levels between 66-81% (day45-55), but an increase by 3 fold by 91% (day62) and constantly high levels until 100% (day68) of gestation [218]. Owen et Matthews 2003 did not detected gender difference in the developing fetal guinea pig plasma ACTH concentrations [335].

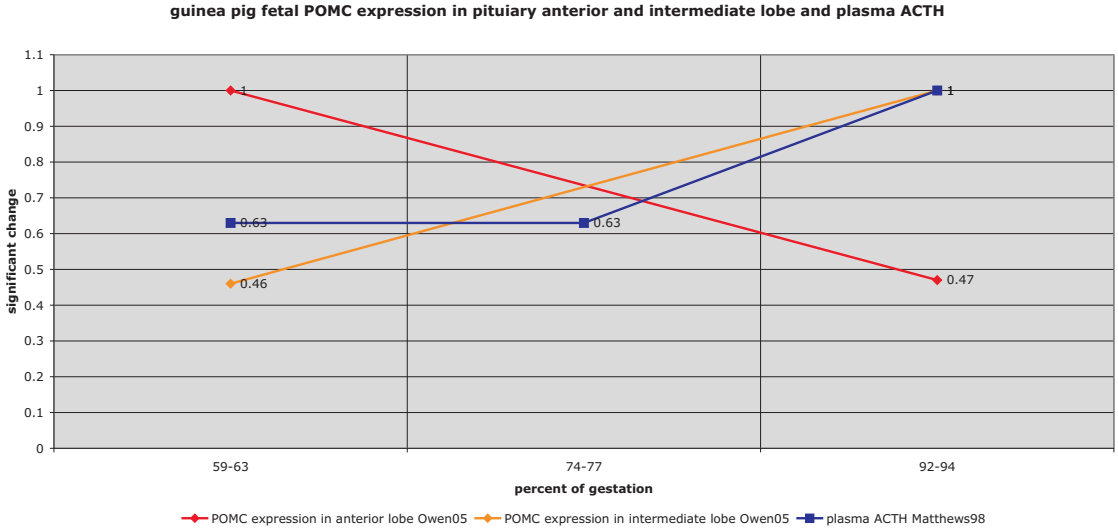


Figure 2.18. Guinea pig fetal POMC expression in pituiary anterior and intermediate lobe and plasma ACTH

From its first detection at 59% (e13), POMC mRNA expression in the fetal rat increased in the whole pituitary dramatically (P<0.001) by 8.2 fold from 77% (e17) until its peak at 96% (e21) of gestation. Subsequently, expression dropped at the time of birth by approximately 1.5 fold or until 5% (PND1) of weaning significantly by (P<0.001) 3.5 fold [171, 257]. Plasma ACTH was detectable in the fetal rat at 73% (e16) and IR ACTH was absent in the fetal anterior pituitary at 55-59% (e12-13), but detectable by 64% (e14) of gestation [60, 322]. Fetal plasma ACTH concentrations increased steadily from 73% (e16) until the peak at 86% (e19) (P<0.001) by 5.3 fold or between 77-86% (e17-19) of gestation (P=0.02; P=0.001) by 1.4-2.7 fold. From 86% (e19) until 96% (e21) of gestation, plasma ACTH decreased again by 3 fold (P=0.001). Between 96% (e21) of gestation and 5% (PND1) of weaning, plasma ACTH decreased significantly (P<0.001) by 3.9 fold [60, 139].

In the fetal mouse from its first detection in the anterior lobe (pars distalis) at 64% (e12.5), POMC mRNA expression increased at 69% (e13.5) by 2 fold and between 74-80% (e14.5-15.5) of gestation by another 1.5 fold. Until 88% (e17.5) of gestation, POMC expression remained constantly high. By 5% (PND1) of weaning, POMC expression increased by another 1.3 fold. In the intermediate lobe of the fetal mouse, POMC mRNA is first expressed at 74% (e14.5) and increased similarly until 5% (PND1) of weaning [143, 210]. POMC expression is differentially regulated in the anterior lobe and the intermediate lobe in the mouse. In GR knock-out mice, POMC expression increased in the anterior lobe and decreased in the intermediate lobe compared to wild type mice from 85% (e16.5) on until 95% (e18.5) of gestation and in adult [377]. In the mouse embryo, POMC was processed to ACTH already at 60% (e11.5) of gestation. The amount of ACTH increased at 64% (e12.5) and a major increase took place at 74% (e14.5) of gestation. The anterior pituitary processed POMC to ACTH and $\beta$-endorphin at 74% (e14.5) of gestation and almost all corticotrophs in the anterior pituitary stained exclusively for ACTH. Fetal plasma ACTH levels increased between 82-92% (e16-18) of gestation by 5.7 fold and might be similarly high at birth [201, 519].

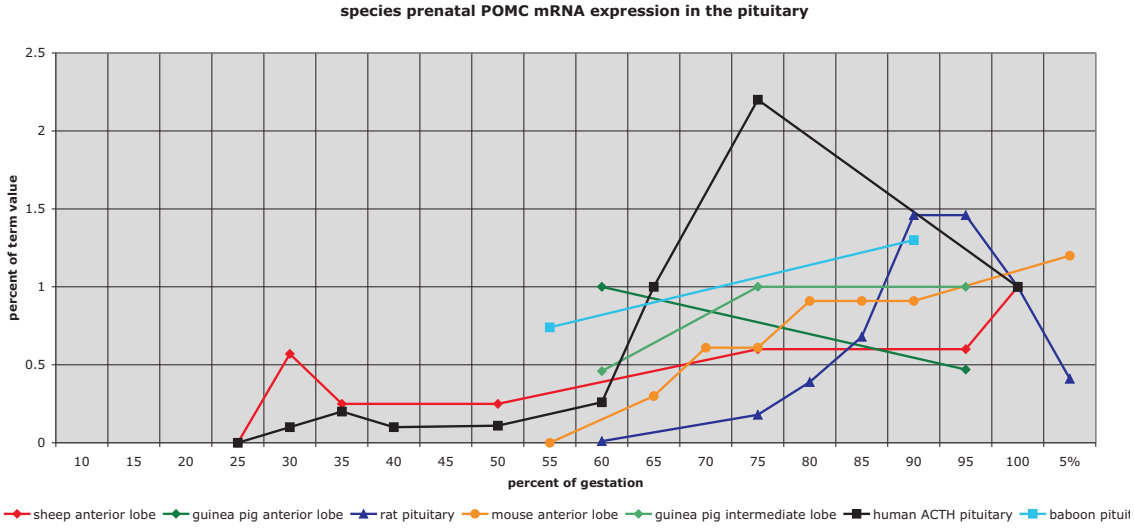


Figure 2.19. Species prenatal POMC expression in the pituitary

*Due to unavailable data about human fetal pituitary POMC expression, ACTH concentrations in the pituitary are used instead. There seems to be an early small peak around 31% of gestation and dramatically increasing pituitary ACTH concentrations from 53-70% with a subsequent decrease to moderate levels at term. No information about POMC expression in the rhesus monkey is available. In the fetal baboon, POMC expression increases between 54-89%, but is at 89% of gestation still 3-4 times lower than in adult baboons. In the fetal sheep, anterior lobe (inferior region) POMC expression shows a peak around 30%, constantly low levels between 35-50% and then increasing levels until 75% of gestation. Expression remains constant until 95% of gestation and increases further by term. In the intermediate lobe, POMC expression increases from very low to maximal levels between 37-76% of gestation. The guinea pig is the only species that shows inverse development of POMC expression in anterior and intermediate lobe. In the intermediate lobe, POMC expression increases sufficiently in parallel with plasma ACTH levels between 59-63% and 92-94% of gestation, while in the anterior lobe, POMC expression decreases over the same period by similar amounts. The rat pituitary POMC expression remains low until 75% and then increases dramatically to maximal levels at 90-95% of gestation. By 5% of weaning, POMC expression dramatically decreases again. In the fetal mouse, POMC expressions in both lobes show similar development and increase strongly until 90% of gestation. No information is available about POMC expression at term but the level has further increased between 90% of gestation and 5% of weaning.*

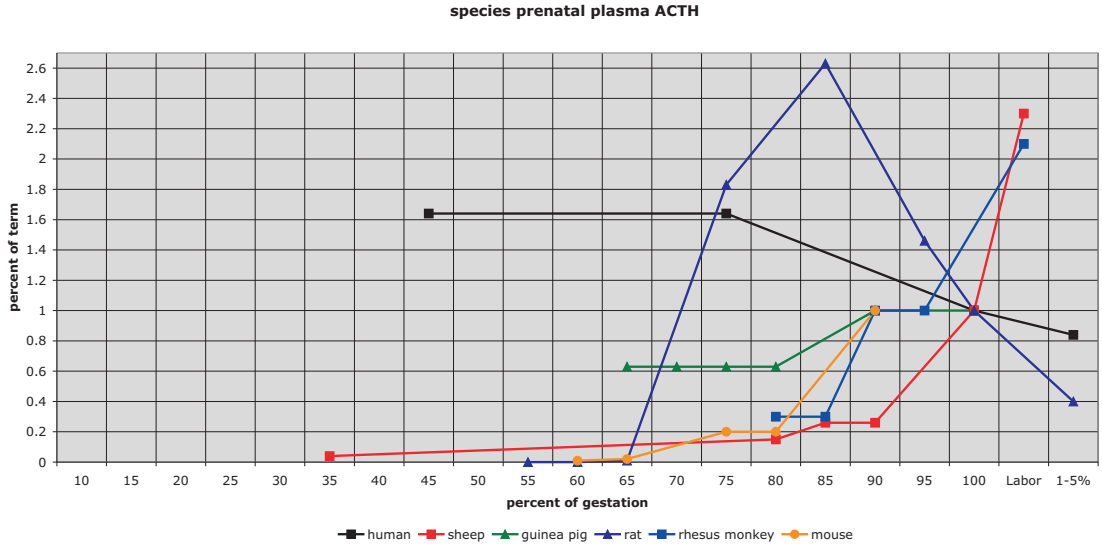


Figure 2.20. Species prenatal plasma ACTH

*Plasma ACTH concentrations in the human fetus are similarly high at 45% and 75% of gestation, but decrease dramatically at term and further by 1% of weaning. These data might be misleading, as a possible increase in plasma ACTH levels between 45-66% of gestation, similar to pituitary ACTH levels, would remain unnoticed. Both plasma ACTH concentrations and ACTH content in the pituitary are assumed to decrease in late gestation from 75% of gestation until term. Plasma ACTH levels in the rhesus monkey remain low at 80-85%, then increase strongly to moderate levels at 90-95% and dramatically further during labor. No information is available about plasma ACTH in the fetal baboon. In the sheep, plasma ACTH levels remain low assumingly from 35-80%, increase slightly by 85% and increase dramatically from 90% of gestation until term and further during labor. A possible smaller peak around 30% of gestation, similar to POMC expression, cannot be excluded. In the guinea pig, plasma ACTH levels are already moderate at 65% and remain constant until 80%, to further increase by 90% of gestation. No more changes are apparent from 90-100% of gestation. In the fetal rat, the plasma ACTH concentrations develop in general very similar to POMC expression in the pituitary. But while POMC expression slowly increases from 60-75% and then faster until a peak at 90-95%, plasma ACTH concentrations remain very low from 55-65%, then strongly increase by 75% and peak by 85% of gestation. POMC expression remains maximal at 95% of gestation, but plasma ACTH concentrations already decrease to moderate levels at this time. In parallel with POMC expression, plasma ACTH decreases further until birth and 5% of weaning. In the fetal mouse, plasma ACTH concentrations seem to remain very low at 60-65%, slightly increase at 75% and show a strong increment between 85-90% of gestation. POMC expression in the fetal mouse shows in general a similar development, but increased already between 55-70% and further between 75-80% to remain constant until 90% of gestation and increased slightly by 5% of weaning.*

#### 2.6.2.3 Postnatal POMC expression and plasma ACTH

In the human neonate, plasma ACTH concentration decreased by 2.3 fold between term and 0.3% (PND1) or by only 1.2 fold (P=0.0034) between birth and 1% (PND3.5) of weaning. Between 0.3-2% (PND1-7) of weaning, human plasma ACTH levels decreased by another 1.8 fold. At 25% (PND90) of weaning, plasma ACTH concentration had not changed but decreased by another 2.3

fold after 50% (PND180) of weaning. Adult (male) plasma ACTH levels are not more than 4 fold higher compared to 50% (PND180) of weaning [497]. No information is available about pituitary POMC expression or plasma ACTH levels in the newborn rhesus monkey or baboon. In adult baboons, POMC expression in the pituitary was 3-4 fold greater than in late gestation [353]. In the sheep, POMC expression in the inferior pars distalis and in the pars intermedia did not change significantly between labor and 4% (PND3.5), 50% (PND45) of weaning or adulthood [283]. No information is available about plasma ACTH concentrations in the newborn sheep. In the guinea pig at 33% (PND7) of weaning, POMC mRNA expression significantly ($P<0.05$) increased compared to 94% (day64) of gestation in the whole anterior pituitary as well as in its inferior region in both genders, while levels are similarly high at both time points in the intermediate lobe [337]. Plasma ACTH concentrations significantly ($P<0.05$) decreased by 4.1 fold in both genders at 33% (PND7) of weaning compared to 94% (day64) of gestation [335]. Unfortunately there is no study, investigating the guinea pig pituitary POMC expression or plasma ACTH concentration more in detail after birth. In the newborn rat, after a decrease of POMC mRNA expression between birth and the 5% (PND1), the expression in anterior pituitary increased from 10% (PND2) to a minor peak at 14% (PND3) ($P=0.02$) by 3 fold, then decreased ($P=0.01$) until 23% (PND5) of weaning by 5.6 fold. From 33-100% (PND7-21) of weaning, POMC mRNA expression in the anterior pituitary increased dramatically ($P<0.001$) by roughly 15 fold to a level twice as high as at the peak at 96% (e21) of gestation. The expression of POMC mRNA in the intermediate lobe differed slightly. In this location POMC mRNA remained low from 14-23% (PND3-5), then increased steadily until 100% (PND21) of weaning by roughly 9 fold [171]. Plasma ACTH concentrations in the neonatal rat had continued to decrease between 96% (e21) of gestation to 5% (PND1) by 3.9 fold ($P<0.001$) and remained low at least until 14% (PND3) of weaning [139]. Subsequently, plasma ACTH concentrations continuously increased ($P<0.001$) by 2.2 fold to very high levels at 67% (PND14), afterwards decreased significantly ($P=0.007$) by 1.5 fold to adult-like levels at 100% (PND21) of weaning [481]. In the newborn mouse, POMC mRNA levels were high in both anterior and intermediate lobe. POMC was similarly expressed in the pituitary at 43% (PND9) and 86% (PND18) of weaning [210, 410]. A decrease of 5.7 fold in plasma ACTH concentrations between birth and 10-14% (PND2-3) of weaning failed to reach significance ($P=0.05$) [201]. Adult like basal plasma ACTH level were already represent at 1% (PND1) of weaning in the neonatal mouse. Plasma ACTH concentrations remained unchanged between 5-76% (PND1-16) but between 43-86% (PND9-18) of weaning significantly ($P<0.0001$) increased by 2 fold [410, 411].

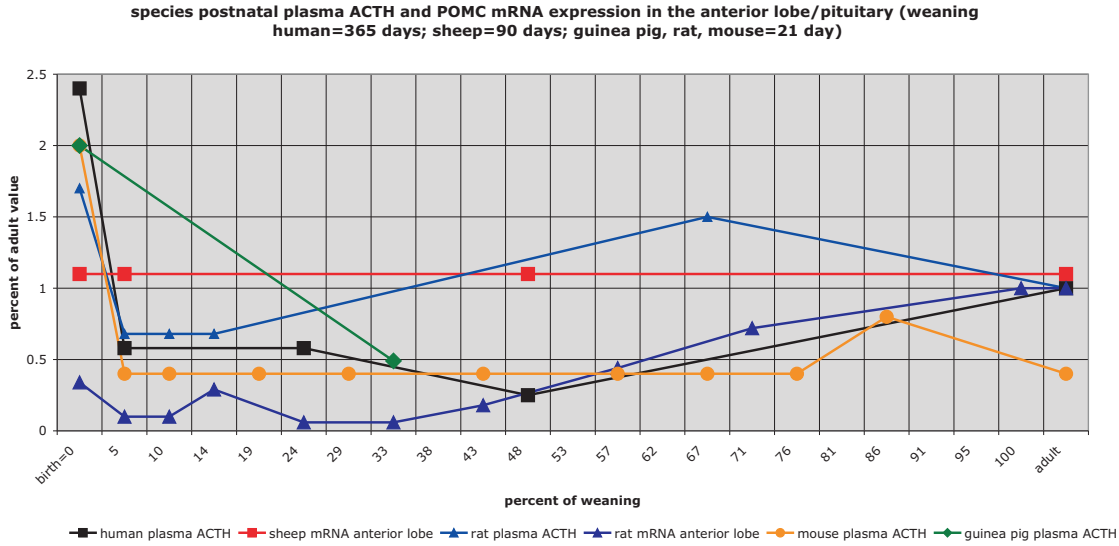


Figure 2.21. Species postnatal plasma ACTH and POMC expression in the anterior lobe/pituitary (weaning human=365 days; sheep=90 days; guinea pig, rat, mouse=21 day)

*The data for the human neonatal plasma ACTH concentrations show a strong decrease from birth to adult-like levels at 0.3% of weaning. Human plasma ACTH concentrations continuously decrease at 2% to remain low at 25%, then decrease even further at 50% of weaning. Until adulthood, human plasma ACTH levels increase strongly again. No data are available for rhesus monkey or baboon. For the newborn sheep, only data for POMC expression are available, showing similarly strong expression at labor, 4%, 50% of weaning and adulthood. In the neonatal rat, both POMC expression in the anterior lobe of the pituitary and plasma ACTH concentrations decrease between birth and 5%, subsequently remain low until 10% respectively 14% of weaning. POMC expression shows a small peak at 14% and returns to very low levels at 14-23% of weaning. From here on, POMC expression increases dramatically to very high levels at the end of weaning and adulthood. Neonatal rat plasma ACTH concentration increases in parallel with POMC level until 67% of weaning then decreases in adults. In the newborn mouse, plasma ACTH levels can be assumed to decrease dramatically between birth and 5% of weaning. Between 5-76% of weaning plasma ACTH concentrations show low adult like level. POMC expression in the pituitary is unchanged between 43-86% of weaning, while plasma ACTH concentrations increase by 2 fold.*

## 2.7 Pituitary GR

Our next step in the HPA cascade considers GR expression in the fetal pituitary. The anterior pituitary synthesizes and secretes the POMC derivate ACTH, under regulation of CRH and AVP from the hypothalamic PVN. ACTH is released into the general circulation and modulates adrenal hormone synthesis [150]. Hypothalamic PVN cells and pituitary corticotrophs are the primary sites for glucocorticoid negative feedback [118]. The pituitary cells contain both GR and MR, but no specific function for MR was described in this location [119]. Other than in the hypothalamic PVN, CBG was expressed in the fetal pituitary corticotrophs of several species, and is assumed to compete with GR for glucocorticoids [41, 117, 359]. Maternal deprivation in rats decreased GR expression in the anterior pituitary and the PVN, but did not change hippocampal GR and MR levels [394].

### 2.7.1 Adult GR expression in the pituitary

GR mRNA was highly expressed in the anterior lobe of the adult rhesus monkey pituitary. Expression here was more than twice higher compared to the PVN and four times higher than in the hippocampus [403]. In the fetal guinea pig, rat and sheep pituitary, GR mRNA was exclusively expressed in the pars distalis/anterior lobe and was absent in the pars intermedia/intermediate lobe [62, 337, 379]. As the pars intermedia is rudimentary in humans and seems to be not or to a lesser extend involved in negative feedback on pituitary ACTH synthesis, we will not investigate GR expression in this location [19, 377].

### 2.7.2 Fetal and neonatal GR expression in pituitary

#### 2.7.2.1 First GR expression

GR expressing cells were detected in the human fetal anterior pituitary at 25% (wk10), but were still absent at 20% (wk8) of gestation [170]. No information seems to be available about GR expression in the fetal rhesus monkey and baboon pituitary. In the fetal sheep, GR was expressed relatively early in the pituitary and was present in the anterior pituitary at least by 43% (day65) and more precisely in the pars distalis not later than 42% (day63) of gestation [85, 284, 509]. Unfortunately, no study investigates GR expression in the fetal sheep pituitary before 42% (day63), but as expression is still very low at 49% (day73), 42% (day63) of gestation might be not too far away from the day of first appearance. In the fetal guinea pig, GR mRNA was apparent in the anterior pituitary at 59% (day40) of gestation [337]. An earlier start of expression is assumed, because at 59% (day40), levels are already on average preterm strength. In the whole rat embryo, GR mRNA was absent at 50% (e11) of gestation [379]. At 55-59% (e12-13) of gestation, GR mRNA was expressed in the fetal rats‘ Rathke‘s pouch, the primordium of the pituitary [127, 236]. GR gene expression encompassed the mouse Rathke‘s pouch by 51-62% (e10-12) or the primitive anterior pituitary by 64% (e12.5) of gestation. At this time, GR mRNA expression is already abundant [446].

TABLE 2.7

First GR expression in the pituitary

| In anterior pituitary | GR in wk/day | GR in % |
|---|---|---|
| Human | wk 8-10/40 | 20-25 |
| Rhesus monkey | ? | ? |
| Baboon | ? | ? |
| Sheep | ≤63/150 | ≤42 |
| Guinea pig | <40/68 | <59 |
| Rat | 12-13/22 | 55-59 |
| Mouse | 10-12/19.5 | 51-62 |

*In the fetal human anterior pituitary, GR mRNA is expressed extremely early, between 20-25% of gestation. Information about rhesus monkey and baboon is missing. Again, it is not possible to give the starting point of GR expression in the anterior pituitary of sheep and guinea pig, but as the GR levels are very low around the given time in the sheep, though already at average level in the guinea pig, it can be assumed that the sheep starts its pituitary GR expression around 40% of gestation. Rat and mouse first express GR mRNA in the anterior pituitary respectively the pituitary primordium around the same time, at the beginning of the second half of gestation.*

#### 2.7.2.2 Prenatal GR expression

GR expression in the human anterior pituitary appeared between 20-25% (wk8-10) of gestation. Pituitary GR expression can be assumed to increase to sufficient levels for functional glucocorticoid feedback already at 25% (wk10) of gestation, as dexamethasone was able to inhibit ACTH secretion from the anterior pituitary in vitro in a dose-dependent manner [170]. No information is available about the development of GR expression in the pituitary of fetal rhesus monkey or baboon. In the fetal sheep during gestation, GR expression, number and binding sites were higher in the pituitary than in hypothalamus, hippocampus or adrenals [391, 508]. In the pars distalis, after the detection of the GR mRNA expression at 42% (day63), no significant change was verifiable between 49-97% (day74-145) of gestation [284]. Recently, gender differences were detectable at 67% (day100) of gestation, with significantly ($P<0.001$) higher GR expression in the pars distalis of the male fetus compared to the female fetus. In the female fetus, GR mRNA levels in the pars distalis remained constant between 67-83% (day100-125), subsequently slightly but significantly ($P<0.001$) decreased by 1.2 fold at 93% (day140) of gestation. In the male fetus, GR mRNA levels decreased significantly ($P<0.001$) by 1.6 fold between 67-93% (day100-140) of gestation [62]. Keller-Wood et al. 2006 also presented a significant ($P<0.05$) decrease by 2.3 fold in pituitary GR expression between 55-98% (day82-147) of gestation [233]. Between 97% (day145) of gestation and term labor, GR mRNA levels significantly ($P<0.05$) increased by 3.7 fold [284]. Additionally, Holloway et al. 2000 detected increased GR mRNA expression in the pars distalis ($P<0.05$) by 2.1 fold between 92% (day138) of gestation and 'term not in labor'. During labor, GR mRNA expression dramatically decreased ($P<0.05$) again by 1.7 fold compared to 'term not in labor' values [199]. Challis et al. 2000 assumed that the decrease in pars distalis GR mRNA expression in the course of labor indicates a potential decrease of glucocorticoid negative feedback in the pituitary during that time [85]. In the anterior pituitary (pars distalis) of the fetal guinea pig, GR mRNA expression did not change

significantly between 59% (day40), 74% (day50) and 94% (day64) of gestation. Additionally, no gender differences in GR expression were apparent during that period [337]. In the fetal rat at 59-64% (e13-14), only weak GR labeling in the whole Rathke's pouch was present. At 68% (e15) of gestation, the GR mRNA signal could be allocated to the basolateral anterior pituitary, with POMC mRNA labeling in the same area [379]. Already at this time, Dexamethasone was able to prevent an increase in CRH-induced POMC expression in vitro, indicating functional glucocorticoid negative feedback on the pituitary at this point [418]. GR protein was present at 59% (e13), and substantially increased between 59/64% (e13/e14) and 68% (e15) of gestation [236]. Strong IR-GR, together with IR-ACTH, was detected in nuclei of anterior pituitary cells by 77% (e17) of gestation [90]. In the basal anterior pituitary, the GR mRNA signal increased between 73-82 (e16-18) and was strong at 82-91% (e18-20) of gestation [379]. In the whole pituitary of the fetal rat, GR mRNA expression clearly increased between 86-100% (e19-22) of gestation [127]. In the fetal mouse, GR mRNA seemed to appear in the Rathke's pouch around 51% (e10) and was abundantly expressed at 64% (e12.5) of gestation. By 67% (e13) of gestation, GR mRNA expression could be assigned to the anterior pituitary. GR gene expression had increased by 82% (e16) and rose dramatically again from 97% (e19) of gestation to 2.4% (PND0.5) of weaning [446]. Weak GR protein levels were detected in the pituitary between 87-97% (e17-19) of gestation and at 2.4% (PND0.5) of weaning [464]. Between 72% (e14) and 82% (e16) of gestation, glucocorticoid negative feedback regulation appeared in the anterior pituitary [148, 377].

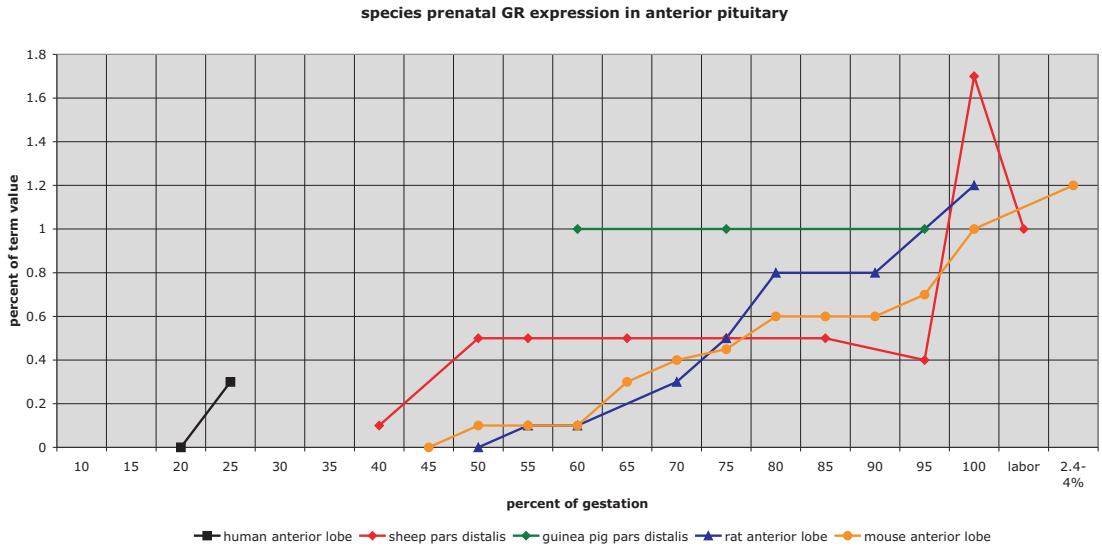


Figure 2.22. Species prenatal GR expression in anterior pituitary

*In the human anterior pituitary, GR expression is detectable at the extremely early age of 20-25 % of gestation. In the sheep pars distalis, GR expression is detected around 40% and remains constant from 50% till 67-83%, when GR expression slightly but significantly decreases toward 93% of gestation. GR expression in the sheep pars distalis dramatically increases at term but decreases again during labor. In guinea pig, data for GR expression in the anterior pituitary are only present from 50% of gestation onwards, with constant expression until 94% of gestation. Rat and mouse GR expression in the anterior pituitary remains low at 55-60% respectively 50-60%, then increase in parallel to a plateau between 80-90% and further until term. In the mouse, GR expression increases even further by 2.4% of weaning. Functional negative feedback on pituitary ACTH synthesis is*

*detected in the human fetus already at 25% of gestation. In the sheep, decreased negative feedback on pituitary ACTH levels can be assumed around 83-93% of gestation and during labor. In the guinea pig, strong glucocorticoid feedback seems to be present at least between 59-94% of gestation and no gender differences appear in this location. Glucocorticoid negative feedback in the rat pituitary is apparent at least by 68% and in the mouse pituitary at 72-82% of gestation. This does not exclude the possibility that negative feedback by glucocorticoids on the expression of pituitary ACTH/POMC is present during a much broader time frame, encompassing the whole species-specific time of GR presence in the pituitary.*

#### 2.7.2.3 Postnatal GR expression

No information is available about GR expression in the pituitary of human, rhesus monkey or baboon after birth. In the sheep, GR mRNA expression in the pars distalis decreased significantly ($P<0.05$) by 1.7 fold during term labor, but a subsequent increase by 4% (PND3.5) of weaning and a further increment by 50% (PND45) of weaning, followed by a decrease toward adulthood, did not reach significance [199, 284]. Gender-specific GR mRNA expression in the anterior pituitary of guinea pigs seemed to be absent from 59% (day40) of gestation to adulthood. Guinea pig GR mRNA expression did not change significantly in the anterior lobe of the pituitary between 94% (day64) of gestation and 33% (PND7) of weaning. GR expression in the pars distalis of the pituitary was present at 86% (PND18) of weaning and in adults [122, 255, 337].

In the rat at 100% (e22) of gestation, 19% (PND4) and 100% (PND21) of weaning, GR mRNA expression was strong in the anterior pituitary, but was only faintly labeled in adult animals [379]. At 5-48% (PND1-10) of weaning, Scott and Pintar 1993 verified functional glucocorticoid negative feedback on the anterior pituitary [418]. High inhibition through pituitary GR seemed to be responsible for low ACTH levels during the SHRP [120]. In adult adrenalectomized rats, corticosterone exposure led to down regulation of GR mRNA in the anterior pituitary, but only after 8-24 hours exposure and not already after 4 hours, assuming a relative insensitivity of GR expression in adult rats anterior pituitaries to absence or excess of glucocorticoids [202]. In the newborn mouse, GR mRNA expression increased by 1.2 fold between birth and 2.4% (PND0.5) of weaning. Still GR protein levels were relatively low at 2.4% (PND0.5) of weaning [446, 464]. In neonatal mice at 38% (PND8) of weaning, treatment with a GR antagonist strongly enhanced pituitary POMC expression, as well as circulating ACTH and corticosterone concentrations, while CRH expression in the PVN decreased [412], assuming glucocorticoid negative feedback impairment during the SHRP on the level of the PVN but a functional negative feedback in the pituitary. At 85% (e16.5) of gestation and in adult mice, glucocorticoid negative feedback decreased POMC expression in the anterior lobe, and this decrease was stronger at the end of gestation than in adults [377].


Figure 2.23. Species postnatal GR expression in the anterior pituitary (weaning sheep=90 days; guinea pig, rat, mouse= 21 days)

*In the sheep, the strong GR mRNA expression does not show a significant difference between term labor, 4%, 50% of weaning or adulthood. Strong negative feedback on the sheep pituitary can be assumed after birth until adulthood.*

*The anterior pituitary of guinea pigs show no gender differences in GR mRNA expression during gestation until adulthood. GR expression in the anterior pituitary remains unchanged between late gestation and 33% of weaning.*

*In the rat, GR mRNA is highly expressed in the anterior pituitary from birth until 100% of weaning. GR mRNA expression seemed to be lower in adulthood. Strong negative feedback on pituitary ACTH synthesis is indicated by high pituitary GR expression and low plasma ACTH levels until 14-19% and maybe 48% of weaning. At 67% of weaning, plasma ACTH concentration shows a moderate peak, and a transient drop in pituitary GR expression at this time cannot be excluded. Negative feedback is strong again by the end of weaning but weaker in adults.*

*The newborn mouse shows a slight increase in pituitary GR mRNA expression between birth and 2.4 % of weaning, during a time when the mouse neonatal plasma ACTH concentration dramatically decreases. Sufficient negative feedback is present at 38% of weaning. As negative feedback on pituitary POMC expression is lower in adulthood than at the end of gestation, there might be decreasing GR levels toward adulthood.*

## 2.8 Summary of Chapter 2

In the following we will summarize the data of *Chapter 2*, regarding the first appearance of the single investigated factors from hypothalamus and pituitary, concerning fetal HPA axis. The possible advent of glucocorticoid negative feedback in these locations will be discussed. In *Chapter 4*, the development of these factors will be debated in the presence of the data about fetal adrenal steroidogenesis from *Chapter 3*.

The first appearance of CRH, AVP and GR expression in the fetal hypothalamus (PVN), POMC and GR expression as well as ACTH content in the anterior pituitary:

TABLE 2.8

First appearance of fetal CRH, AVP, POMC, ACTH, and GR

| | Human | Rhesus monkey | Baboon | Sheep | Guinea pig | Rat | Mouse |
|---|---|---|---|---|---|---|---|
| CRH/PVN | 30 | | <54 | 33 | <59 | 73-77 | 64-69 |
| AVP | 30 | | | 30 | 29 | 82-86 | 74-85 |
| GR/PVN | | | | <43 | <59 | 68-73 | 64≤X<90 |
| POMC | 24 | 30 | <54 | 23-30 | <59 | 59 | 64 |
| ACTH | 24 | 30-33 | | 23-30 | <59 | 64 | 64-74 |
| GR/pituitary | 20-25 | | | ≤42 | <59 | 55-59 | 51-62 |

In the human hypothalamus, CRH and AVP expression are present by 30% of gestation. No information about the appearance of GR expression in this location is available and subsequently no conclusion of the advent glucocorticoid negative feedback on CRH expression can be drawn. In the human pituitary, POMC expression and ACTH content appear already at 24% of gestation. Here functional glucocorticoid negative feedback could be apparent by 24% of gestation with the advent of pituitary GR expression. The data for the rhesus monkey are very scarce. POMC expression in the fetal pituitary is detectable by 30% and ACTH content by 30-33% of gestation. No information about the first expression of CRH and AVP, as well as GR in PVN or pituitary is available. In the fetal baboon, CRH and POMC expression in the PVN, respectively the pituitary, are apparent latest at 54% of gestation. Again the first appearance of the remaining factors is not certifiable. The fetal sheep expresses CRH in PVN by 33% and AVP by 30% of gestation. GR gene expression in the PVN appears latest by 43% of gestation, so functional glucocorticoid feedback on CRH expression assumingly begins sometime between 33-43% of gestation. POMC mRNA expression and ACTH synthesis in the pituitary are already present by 23-30% of gestation. GR expression in the pituitary is apparent at least by 42% of gestation. Glucocorticoid negative feedback begins in the fetal sheep pituitary most likely between 23-30% and 42% of gestation. Data for the fetal guinea pig are only available in the second half of gestation. An exception is the presence of AVP in the pituitary, which is verified already by 29% of gestation. All other, here investigated, factors are apparent at least by 59% of gestation, but earlier appearance can be assumed. In the fetal rat PVN, CRH expression appears between 73-77% and AVP expression begins significantly later at 82-86% of gestation. GR expression in the PVN is present by 68-73% of gestation, expecting the appearance of functional feedback on CRH expression around this time. POMC expression in the developing anterior pituitary is already detectable by 59% and ACTH content is present by 64% of gestation. Functional negative feedback by the appearance of pituitary GR is assumingly

present directly from the start of POMC expression at 59% of gestation. In the fetal mouse, the PVN starts to express CRH by 64-69% and AVP is present in the hypothalamus at 74-85% of gestation. Functional negative feedback on CRH expression appears sometime between 64-90% of gestation due to the presence of GR expression. POMC mRNA is expressed in the anterior lobe of the pituitary at 64% and ACTH is apparent in corticotrophs by 64-74% of gestation. Again glucocorticoid negative feedback on pituitary ACTH secretion seems to be functional right from the beginning of ACTH synthesis, with the appearance of pituitary GR expression already between 51-62% of gestation.

*In summary, the data verify the appearance of GR expression and assumingly functional glucocorticoid negative feedback in the pituitary with the presence of ACTH synthesis at least in human, rat and mouse. Similarly in the PVN, GR expression/glucocorticoid negative feedback appear simultaneously with CRH expression in the fetal rat and most likely in the mouse fetus. For the remaining species the data are too scarce to draw conclusions.*

# CHAPTER 3

## Fetal and neonatal adrenal cortex

*After the brief survey of factors from higher brain centers and the placenta, directly or indirectly influencing the fetal adrenal cortex (see Chapter 2), we will finally cover this important fetal endocrine organ itself. Our study will focus mainly on the development of the glucocorticoid synthesis but will refer to the adrenal synthesis of androgens and aldosterone, when the production of these hormones have an effect on the glucocorticoid synthesis.*

### 3.1 Introduction

The adrenal gland is a morphologically and functionally astonishing organ. Located at the cranial pole of the kidney, the gland encompasses two different endocrine fractions, the adrenal cortex and the adrenal medulla. The adrenal cortex takes up roughly 80% of the whole organ and produces in close proximity glucocorticoids, mineralocorticoids and, depending on the species, androgens [326]. The adrenal medulla belongs to the vegetative as well as the endocrine system and synthesizes the catecholamines epinephrine and norepinephrine [176]. Bornstein was able to show close interweavement and interaction between adrenal medulla and cortex in mammalian species including the human [56]. The functions of the adrenal cortex are indispensable to life. Destruction, due to illness or experimental protocol, can lead sooner or later to death (e.g. Morbus Addison in humans). An exception here is the adult rat, which has plenty of accessory adrenal glands at different location in the abdomen. In general, the left adrenal gland of the adult mammal is heavier than the right one. Gender differences in weight of the adrenal gland occur and principally, the adrenals of females are heavier than the ones of males [326]. In the fetus, the adrenal gland is the largest endocrine gland. After 50% (wk20) of gestation, the human adrenal gland matches the size of the fetal kidney. By 75% (wk30) of gestation, it possessed 10-20 fold the relative size of the adult adrenal [231]. In the human fetus, the bulk of the adrenal gland is occupied by the androgens producing fetal zone [221]. Close to term, the fetal adrenal gland produced 5 times more steroids than the unstimulated gland in adults [433]. Glucocorticoids, synthesized by the fetal adrenal cortex, particularly corticosterone in rat and mouse and cortisol in other mammals, play an essential role in the maturation of fetal organs systems (e.g. lungs, liver, gonads) in preparation for extrauterine life [445, 492]. Near term, fetal glucocorticoid synthesis induces in the fetal lungs the production of surfactant proteins. These proteins are essential for reduction of the surface tension in the pulmonary alveoli, to initiate breathing at birth [124, 155, 203]. Glucocorticoid synthesis during the development enables the fetus to respond to intrauterine stress [98]. In some species like in the sheep, fetal adrenal cortisol also triggers the onset of parturition [97, 447]. This seems not to be the case in primates. Instead, the rapid rise of adrenal androgens toward term might affect the advent of delivery in primates [295]. Because the primate placenta is incapable to produce its own androgens, fetal adrenal androgen synthesis is essential as substrate for placental estrogen synthesis and demands a large area inside the adrenal cortex [43, 299, 417]. Fetal adrenal androgen production is of less importance in the sheep and in the guinea pig. The sheep placenta is able to synthesize its own androgens as estrogen precursors. In the guinea pig, androgens derive from maternal and fetal gonads additionally to fetal adrenal production [32, 85, 333]. The adrenals of rats and mice are even incapable to produce androgens. Instead, they are produced during gestation in placenta, maternal corpus luteum and in fetal gonads [159, 243, 484]. The time of achieving steroidogenic capacity, with its diverse input on fetal organs, is essential for maintaining intrauterine homeostasis [97].

## 3.2 Development

*Before we will investigate the physiology of the fetal adrenal, we will study the morphological development of the fetal adrenal gland between the different species, to gain an inside into the species-specific velocity of development and the completion at term. We will begin with a broad comparison of degree of development at birth, in terms of autonomy and self-sufficiency of the newborn from its mother. Standard factors, normally employed to evaluate newborns, will be here used, for comparison between the different species. The following indicators will be taken into account: eye opening, hairiness (for independency of maternal warmth), penetration of milk teeth, locomotion, and additionally, autonomy from maternal food supply (time of weaning).*

### 3.2.1 Degree of development at birth between species

The seven studied species differ dramatically at term, concerning the ability to be autonomous from their mother. From the degree of development at birth, guinea pig and sheep show common characteristics. Both are born with milk teeth, the guinea pig is even able to digest firm food right from the beginning. Nevertheless, the guinea pig mother provides milk roughly until PND21. Newborn of guinea pigs and sheep are completely haired, have far developed sense organs (taste, smell, hearing, vision), and can walk immediately (guinea pig), respectively after the first two hours (sheep). But most important, the stress systems of the newborn guinea pig and sheep can be assumed to be functional very early. Autonomous they soon have to follow their mothers and, in case of emergency, need to be able to escape from predators [151, 396, 473]. Primates, here human, rhesus monkey and baboon, are born without teeth, but with open eyes. They depend on protection, nutrition and, especially the human, on warmth from the mother. Compared to the human, the non-human primates are born with neonatal fur, and the thermoregulation will soon work. The rhesus monkey brain has reached already 76 % of the adults‘ brain weight at term, compared to only 27% in humans [112, 128]. The weight of the brain might not be very significant. Both newborn rhesus monkey and human are able to imitate facial gestures already within the 1% (PND3) of weaning [149, 298]. The newborn baboon has the ability to hold its head erected, soon clings to its mother with only a little maternal support and starts to move around by itself at least by 1% (PND7) of weaning, actively searching for mothers‘ protection [430]. The human baby cannot hold its head up by itself and is long unable to move sufficiently. Nevertheless, from birth until 25-33% (wk12-16) of weaning, the human baby shows the clinging or ‘moro‘ reflex, which requires well-developed motor function [215, 219, 241]. Also, the human newborn can see, hear, smell and taste, response to touch and voice, can follow objects with its eye and can maintain eye-to-eye contact [16]. Like humans at birth, rat and mouse neonates are unable to escape from danger. Born blind, deaf and naked, but in a secure nest, rat and mouse pups are completely defenseless and rely on their mother for warmth, nutrition and protection [26, 151]. The newborn rat had only 12% of its adult brain weight accomplished at birth [128]. This short excursion should give a rough impression, how the species-specific stress systems need to be equipped and to which degree they have to work in order for the newborn to survive.

TABLE 3.1

Degree of development at birth

| | Warmth | Vision | Nutrition | Teeth | Locomotion |
|---|---|---|---|---|---|
| Human | Naked | Yes | Milk | No | No |
| Rhesus monkey | Fur | Yes | Milk | No | Yes |
| Baboon | Fur | Yes | Milk | No | Yes |
| Sheep | Fur | Yes | Milk | Yes | Yes |
| Guinea pig | Fur | Yes | Milk, Firm food | Yes | Yes |
| Rat | Naked | No | Milk | No | No |
| Mouse | Naked | No | Milk | No | No |

*From Table 3.1, we can conclude that rat and mouse are completely helpless when born, and by that a sufficient stress reaction at this point seems unnecessary. The human cannot move and is naked, but possesses sophisticated motor function in form of the 'moro' reflex and well developed sense organs that allow a form of communication and even learning. Rhesus monkey and baboon are further developed with the ability to move and flee soon after birth. They would require a sufficient stress reaction in the face of danger. The guinea pig is the most independent, with the ability to digest also firm food and immediate walking skills, while the also quite self-sufficient lamb still completely relies on maternal milk. Both latter species would need full stress reactivity when threatened.*

### 3.2.2 Pre- and postnatal development of the adrenal gland

Subsequently, the development of the adrenals during gestation will be described more in detail in the human, then only discrepancies in the other species will be shown. Especially the fetal period (time where the organ differentiates, gains functionality and grows [212, 413]) will be compared between the species. Enlargement of an organ occurs in consequence of two different events, by increment in cell size (hypertrophy) or by increase in cell number (hyperplasia or proliferation) [388]. Hyperplasia/proliferation proceeds by division of the mother cell into two daughter cells, which are genetically identical to each other and their mother cell (cell division) [491]. It was shown that cell proliferation was inhibited by glucocorticoids [402].

#### 3.2.2.1 Human adrenal development

The human fetal period started around 23% (wk9) of gestation [211] and lasted until birth. The primate fetal adrenal cortex showed a unique morphological appearance [40, 231] in form of a disproportionately large fetal zone, which produced enormous amounts of androgens [179]. Around 18% (wk7) of gestation, cells concentrated at the cranial end of the primitive kidney, to form the 'adrenal blastema'. By that it separated itself from the common adrenal-gonadal primordium [178, 211]. The first adrenal cortex zone to appear was the fetal zone (FZ, fetal reticularis). Soon the adrenal cortex contained an undifferentiated outer zone and a central network of FZ cords. The outer zone developed into the definitive cortex (DZ), so that by 20% (wk8) of gestation, the human fetus exhibited a distinct adrenal cortex of two zones, the FZ and the DZ [153, 175, 177, 299]. Already at this time, the FZ possessed characteristic organelles that indicated active steroid production [153]. Both DZ and FZ cells seemed to originate from the same small epithelial progenitor cells.

The cells of the prospective FZ then differentiated into large, eosinophilic cells and the DZ cells still resembled the appearance of their progenitors, being much smaller with dark stained nuclei [211, 231]. At 20-23% (wk8-9) of gestation, future medulla cells invaded the cortex anlage from medial and built small islands between the cortical cells [175, 211]. At least in adults, hormones from the medulla were able to stimulate steroidogenesis in the adrenal cortex [56]. A capsule surrounded the adrenal cortex at 23% (wk9) of gestation and a large net of sinusoidal capillaries had developed in the FZ, which made the adrenal cortex one of the most highly vascularized fetal organs and ensured the access of adrenal hormones to the circulation. By 25% (wk10) of gestation, a third zone was distinguishable between the FZ and the DZ. Mesiano et al. 1993 referred to the new zone as the 'transitional zone' (TZ), while Jirasek et al. 1980 described it as the fetal zona fasciculata (zF). Later in gestation, by examining the location of the key steroidogenic enzymes in the primate adrenal cortex, assumingly androgens were produced in the FZ, which was analogous to the adult zona reticularis (zR). Apparently the DZ produced mineralocorticoids and resembled the adult zona glomerulosa (zG) and the TZ produced cortisol and was analogous to the adult zona fasciculata (zF) [99, 211, 299, 300]. Proliferation in DZ cells increased significantly ($P<0.05$) by 2.6 fold between 25-35% (wk10-14) and 38-50% (wk15-20) and remained high at least until 60% (wk26) of gestation [447]. Proliferation decreased in the DZ to low levels at 68% (wk27) of gestation and disappeared by 90% (wk36) of gestation [152]. Taking into account, that adrenal growth is mostly due to enlargement of the FZ, Mesiano et al. 1997 assumed that the DZ grew mainly by hyperplasia and the FZ growth occurred by hypertrophy and less hyperplasia [299]. The FZ started to regress from the 50% (wk20) of gestation on [153]. In primates, the separation of adrenal medulla and cortex did not takes place until postnatal when, with the involution of the cortical FZ, the medulla cell islands melted into a solid central organ [213]. From 0.3% (PND1) till 12% (PND42) of weaning, the human fetal gland shrank dramatically due to the regression of the FZ. The decrease in adrenal gland size was even more rapidly that previously thought. Until 4% (PND14) of weaning, the large human adrenal gland shrank to its normal infantile size [38]. First it was believed that this occurred by necrosis (premature cell death) and hemorrhage (bleeding) [231]. Later it got clear that the decrease in weight attributed to apoptosis (naturally occurring programmed cell death), which was greatest immediately after birth between 0.8-8% (PND3-30) of weaning, particularly in the FZ [194, 447]. Angiotensin II was involved in apoptotic processes and large numbers of Angiotensin II receptors were present from 40% (wk16) of gestation onward in the fetal human adrenal gland [86]. The zR, as a derivate of the FZ, regressed in early childhood, was absent in children age 1-8 years, but reappeared between the ages of 8-12 years to develop into the adult like zR. The adult zonation is achieved between 10-20 years of age [175, 211, 213].

*Regarding the achievement of steroidogenesis, the following information about fetal human adrenal development is of importance. Between 18-25% of gestation, all three zones of the fetal adrenal cortex evolved. At 20% of gestation, the FZ possess organelles for active steroidogenesis and at 23%, a large net of blood vessels appears in the FZ. The DZ cells show lower proliferation at 25-35% than at 38-60% of gestation. Proliferation in the DZ decreases to low levels at 68% and disappears at 90% of gestation. The FZ begins to regress due to apoptosis from 50% of gestation onward but most dramatically between birth and 4-8% of weaning.*

#### 3.2.2.2 Rhesus monkey and baboon adrenal development

The fetal period in both rhesus monkey and baboon started around 27% (day45) of gestation [190, 470]. At 27% (day45) of gestation, the rhesus monkey FZ cells showed signs of active steroidogenesis. By 36% (day59) of gestation, the DZ was a discontinuous band of mitotic cells underneath the capsule and medulla cells had not yet invaded the cortex [291]. At what time the TZ appears is not clear in both species. In the rhesus monkey the TZ was present latest at 66% (day109) of gestation [99]. In the baboon fetus, the TZ was detected by enzyme expression after ACTH administration at 54% (day99) of gestation [5, 242]. It cannot be excluded that the TZ appears in both rhesus monkey and baboon much earlier in gestation. Between 45-53% (day74-87) of gestation, proliferation was prevalent in the DZ of the rhesus monkey adrenal cortex. Around 73-82%

(day120-135) of gestation, the DZ possessed more capillaries and steroidogenic organelles and no evidence of cellular hyperplasia was present in the adrenal [99, 286]. Cell proliferation in the baboon adrenal cortex was much higher in the DZ than in the FZ and dramatically decreased in the former between 54% and 90% (day99-166) of gestation [135]. Leavitt et al. 1999 suggested that in baboon in late gestation, growth of the TZ but not the DZ was dependent on ACTH [242]. Due to the FZ involution, the weight of the rhesus monkey adrenal gland decreased by about one third until 5% (PND14) of weaning [291]. Compared to the fetus, in the baboon adrenal cortex at 5% (PND14) of weaning, the FZ cell diameter was much smaller and the sinusoidal spaces were reduced, which are signs of decreased secretory capacity [134].

*The appearance of all three zones in the adrenal cortex of the fetal rhesus monkey and baboon is not ascertainable, due to difficulties in indicating the emergence of the TZ. In the rhesus monkey fetus at 27% of gestation, the FZ organelles indicate active steroid synthesis. At 36% of gestation, the DZ is built out of mitotic cells. Between 45-53%, proliferation is apparent in the DZ, while at 73% of gestation, the DZ organelles and blood vessels indicate steroid synthesis. The rhesus money adrenal gland decreases after birth due to the involution of the FZ. It the fetal baboon, growth of the TZ is dependent on ACTH and mitosis decreases in the DZ between 54-90% of gestation. At 5% of weaning, hormone secretion in the FZ has decreased.*

#### 3.2.2.3 Sheep adrenal development

The embryonic period in the sheep lasted until 24% (day36) of gestation, when the fetal period began [173, 413]. During gestation, the cortex of the fetal sheep only seemed to consist of two zones, the zF and the zG, due to the absence of the zR [489]. By 25% (day38) of gestation, an outer layer of mesothelial cells was separated from the mass of inner cells through connective tissue and sinusoidal capillaries were apparent. Already between 25-29% (day38-43) of gestation, organelles for steroidogenesis were present in the zF. At 29% (day43) of gestation, a thin capsule appeared. Around 31% (day47) of gestation, the invasion of future medulla cells into the cortex had started [386]. Boshier et al. 1989 divided the adrenal development of the sheep after 37% (day56) of gestation into three periods. The first period of rapid growth between 37-69% (day56-104) of gestation was mainly due to cell hyperplasia and the development of the zG [57, 380]. During that time, the volume of steroidogenic tissue in the zF was very high. A period of reduced adrenal growth followed. The third period from 89% (day134-150) of gestation until term was associated with the final adrenal growth by hypertrophy/hyperplasia and maturation of the zF [57, 59, 387]. This trinomial pattern can be applied to zF steroidogenesis and the steroidogenic organelles. At 37% (day56) of gestation, the zF was steroidogenic active and showed moderate to high levels of smooth endoplasmic reticulum and Golgi apparatus. Steroidogenesis in the zF was absent at 69% (day104) of gestation and this zone exhibited very low levels of both organelles. Between 89% and 98% (day134-147) of gestation, when cortisol production increased to its most, both steroidogenic organelles increased to their maximal development. By 2% (PND2) of weaning, steroidogenic organelles were still maximal developed [57, 58]. While by 2% (PND2) of weaning, there was still no histological evidence of the zR, this zone became apparent at 33% (PND30) of weaning [57, 380].

*By 25% of gestation, the zF and the zG are distinguishable and sinusoidal capillaries appear. In the zF at 25-29% of gestation, steroidogenic organelles are present. At 37% of gestation, moderate to high levels of steroid producing organelles are detected in the zF, but at 69% of gestation, these organelles have decreased to very low levels. Between 89-98% of gestation, steroidogenic organelles increase to maximal levels and remain very high at least until 2% of weaning. The zR only appears latest at 33% of weaning.*

#### 3.2.2.4 Guinea pig adrenal development

At 37 % (day25) of gestation, the embryonic period ended and the fetal period began [470]. Compared to rat and mouse, the guinea pig had a much longer fetal development, but still shorter than

human and baboon [205]. Until late gestation, when the zR was distinguishable, the fetal adrenal cortex of the guinea pig only encompassed the zG and the zF [47]. Around 35% (day24) of gestation, gonads and adrenal cortex were separated. Already at 32% (day22) of gestation, steroidogenic organelles were detected in the cells of the cortical blastema, and their amount increased dramatically by 38% (day26) of gestation in the inner zone cells (most likely referring to zF+zR) [48, 333]. The appearance of smooth endoplasmic reticula coincided with the earliest evidence of adrenal steroid production (androgens), even before zonation occurred. At 37% (day25) of gestation, histologically two zones of cells were recognizable, the small zG and the large zF. The zF built a broad inner band, while the zG presented a narrow outer layer. By 38% (day26) of gestation, medulla cell progenitors began to invade the cortex [47, 333, 514]. Between 35-40% (day24-27), the inner zone doubled its width and tripled in width between 40-81% (day27-55) of gestation. During that period, the inner zone grew strongly by hypertrophy, and proliferation was present in the zG [47]. The female adrenal weight remained nearly constant between 88-100% (day60-68) and the weight of the male adrenal did not change between 94-100% (day64-68) of gestation [310]. The amount of smooth endoplasmic reticula increased in the zG after 50% (day34) of gestation. Between 44-74% (day30-50), the lipid droplets of the inner zone became larger and more numerous in the future zF and by 82% (day56) of gestation, the arrangement of cell cords allowed the demarcation of the zF from the zR [47]. Close to term and at birth, proliferation was equally low in zG and zF [207]. During 5-14% (PND1-3) of weaning, high cell proliferation was mainly present in the zF, but decreased to low levels by PND30 [207, 222]. Postnatal, the zR increased progressively. By 15% (PND7) of weaning, the zR made up 7-11% of the total cortex area, at a time when the zF still encompassed the bulk of the cortex. The zR increased to more than 50% of the total cortical area after PND100 [514, 515].

*At 32% of gestation, androgen synthesis is present in the fetal adrenal, but the zR can only be distinguished from the zF by 82% of gestation. The term 'inner zone' might describe both zF and zR. Between 32-38% of gestation, the amount of steroidogenic organelles increases dramatically in inner zone. By 37% of gestation, the zF and the zG are separable. Especially between 40-81% of gestation, the inner zone grows dramatically by hypertrophy, while the zG shows proliferation. From 50% of gestation onward, the zG exhibits increasing numbers of steroidogenic organelles. Steroidogenic organelles increases in the zF between 44-74% of gestation. Close to term and at birth, proliferation is equally low in zF and zG, but increases to high levels in the zF at 5-14% of weaning, to decrease again to low levels at PND30. After birth, the zR width increases dramatically and encompasses more than half of the cortical area after PND100.*

#### 3.2.2.5 Rat adrenal development

The fetal period started in the rat at 73% (e16) of gestation [470]. The rat had, together with the mouse, a very long embryonic period but a very short fetal period [205]. In rat and mouse, due to missing enzyme expression in the adrenal cortex, androgen synthesis was restricted to the gonads [96]. The common adrenocortical and gonadal cell population was first detected in the fetal rat around 55% (e12) and by roughly 65% (e14) of gestation, primordia of adrenal cortex and gonad were completely separated [181]. The immigration of future medulla cells into the adrenal cortex primordium began around 70% (e15.5) of gestation and continued until term [53]. At 73% (e16) of gestation, the adrenal appeared for the first time as an ovoid mass with a capsule. Cells with the enzymatic features of zF cells, small clusters of functionally competent zG cells and many proliferating cells were scattered throughout the gland. In the periphery, zF cells were fewer in number. Over the next days, cells expressing the zG phenotype became less and less in the central areas of the gland [304, 502]. At 82% (e18) of gestation, hardly any development of a capillary network had taken place [303]. By 86% (e19) of gestation, the zG appeared as a band adjacent to the capsule [304, 502]. The zF encompassed the bulk of the adrenal cortex, taking possession of 76% of the adrenal cortex area, while the zG hold the remaining 24% [452]. Even when the number of proliferating cells in the zG was decreasing, proliferation was 5 times higher in the zG compared to the zF [303, 304, 452]. Wotus et al 1998 detected a lipid free zone harboring proliferating cells between zF and zG at this time [502]. Medulla and cortex started to separate by

91% (e20) of gestation and functional zonation began. Some capillaries became visible. Signs for apoptosis (DNA fragmentation and macrophages) were scattered throughout the adrenal gland in late gestation and neonatal period. After birth, apoptosis was detected in the innermost cortex. At 5% (PND1) of weaning, mitotic cells were mainly found in the zG and by PND30, the low number of proliferating cells had shifted inward to the undifferentiated zone between zG and zF. At 14% (PND3) of weaning, a well-defined medulla had developed and the capillary network had an adult like appearance. By 48% (PND10) of weaning, cortical zonation was established [303, 304]. The zG width increased in size from birth until PND40 [366]. In the zF after PND49 and for the zG from PND70 on, the volumes of both zones were larger in females than in males [263].

*The fetal rat synthesizes glucocorticoids and aldosterone in the adrenal cortex during gestation. At 73% of gestation, with the beginning of the fetal period, steroid producing zF and zG cells are present. Medulla cells start their immigration into the cortex, which continues until birth. By 86% of gestation, the zF encompasses the bulk of the cortex and zG cells build a band under the capsule. A layer of proliferating cells appears between both zones. While between 73-82%, many proliferating cells are present in the adrenal cortex, proliferation decreases until 86% of gestation and is much lower in the zF than in the zG. Shortly after birth, medulla and cortex are separated and apoptosis appears in the innermost cortex. By 14% of weaning, the capillary net has an adult like appearance and the width of the zG increases after birth. After PND49-70, both zones are larger in the female rat than in the male rat.*

#### 3.2.2.6 Mouse adrenal development

Mice did not have a functionally distinct zR and could not synthesize androgens in the adrenal gland [229]. The mouse had a very short fetal period compared to human, baboon and guinea pig, lasting from 72% (e14) of gestation till term [205, 470]. After 46% (e9) of gestation, the common primordium of adrenal and gonad separated and the DZ was formed by 56% (e11) of gestation. Between 62-72% (e12-14), future medulla cells migrated into the adrenal gland and by 72% (e14) of gestation, a definitive capsule was present [175, 229, 232]. By 82% (e16) of gestation, the cortex was divided into two zones, zG like outer part and the inner zF, the latter encompassing 60-70% of the cortical area [483, 519]. Between 82-92% (e16-18) of gestation, proliferation was decreasing in both zones, still proliferation was much stronger in the zG than the zF. Over that period, the zF showed much higher levels of steroidogenic organelles than the zG [519]. The adult zonation of the adrenal cortex developed between 5-33% (PND1-7) of weaning [175, 229]. Unique in the mouse adrenal cortex was the transient development of the X zone between the cortex and the medulla after birth [195]. Keegan and Hammer 2002 suggested, that the human FZ and the mouse X zone might have been analogous in their position and regression. The X zone was histological evident by 48-67% (PND10-14) and enlarged until 100% (PND21) of weaning [175, 229]. In males mice it degenerates around puberty (before PND35), in females during the first pregnancy [229, 460]. Only after the degeneration of the X zone, a fibrous tissue band encapsulated the medulla and separated it from the cortex [175].

*At 62-72%, medulla cells invade the cortex and by 82% of gestation, the cortex is divided into zG and zF. Proliferation decreases between 82-92% of gestation in both zones, but is still much stronger in the zG than in the zF. On the other hand, amounts of steroidogenic organelles are markedly higher in the zF during that time. The X zone develops around 57% of weaning and disappears in males around puberty and in females during the first pregnancy.*

TABLE 3.2

Comparison of major events during adrenal development, given in percent (%) of gestation and in percent (w%) of weaning

| | Human | Rhesus monkey | Baboon | Sheep | Guinea pig | Rat | Mouse |
|---|---|---|---|---|---|---|---|
| Separation adrenal gonad | 18 | | | | 35 | 65 | 46 |
| **Organelles for steroidogenesis** | **20** | **27** | | **25-29** | **32** | **73** | |
| 2 zones | 20 | >36 | | 25 | 37 | 91-w48 | 82-w33 |
| TZ in primates | 25 | ≤66 | ≤ 45 | | | | |
| **Invasion medulla cells** | **20-23** | **>36** | | **31** | **38** | **70** | **62-72** |
| Capsule | 23 | | | 29 | | 73 | 72 |
| **Sinusoids** | **23** | | | **25** | | **91-w14** | |
| zR or X zone (mouse) | | | | w2<X<w33 | 81 | | X zone w48-67 |
| **Start fetal period** | **23** | **27** | **27** | **24** | **37** | **73** | **72** |
| **Estimated start of steroidogenesis** | **20-23** | **27-36** | | **25-31** | **32-38** | **70-73** | **62-72** |

*It has to be kept in mind, that the presented data are especially dependent on the time points investigated by the authors and often only give a rough estimation of the time frame, were an event happens. For functional development of fetal adrenal cortex, the first appearance of steroidogenic organelles is a valuable indicator for steroid synthesis and the appearance of sinusoids might be of importance, to assure the arrival of regulatory hormones and the release of adrenal hormones into the circulation. The invasion of medulla cells could be of relevance, as it is shown, that steroidogenesis in the adrenal cortex can be stimulated, independently from the hypothalamus-pituitary axis, by the adrenal medulla hormones epinephrine and norepinephrine. Separation of adrenal and gonads, appearance of the capsule and the distinction into single zones is assumed to be mostly of anatomical importance. The timing of cortical zonation is difficult to use for functional evaluation, because of the challenge to separate the single zones early in gestation and the fact that steroidogenic cells can start hormone production before they reach there final location. We should be aware, that the detection of the TZ in primates is extremely dependent of the investigated time periods and the advent of cortisol production might happen in specific cells intermingled or present in other zones. By integrating the three more important events (appearance of sinusoids and steroidogenic organelles, as well as invasion of medulla cells), the following picture for estimating functional maturation occurs:*

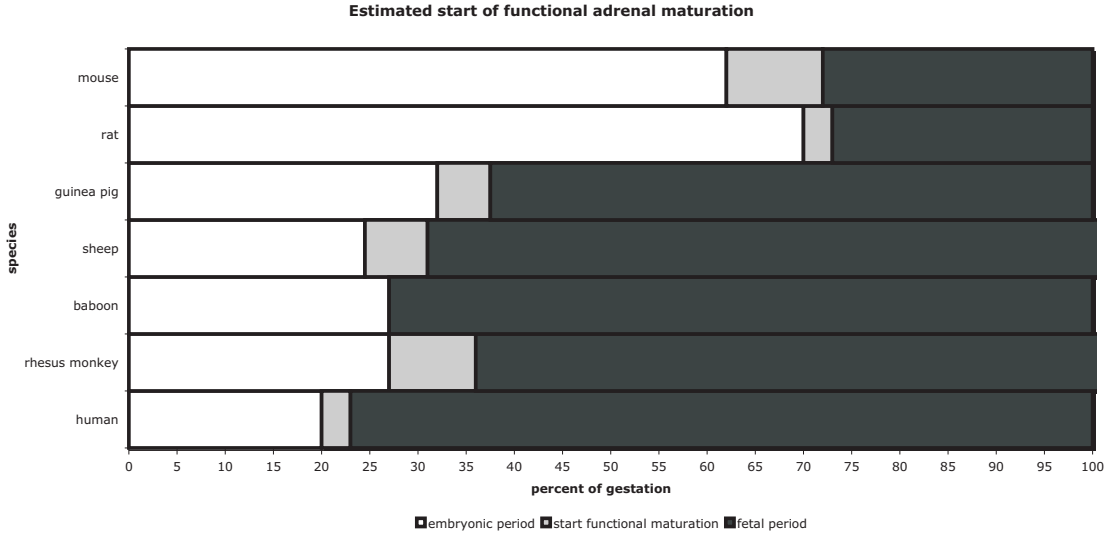


Figure 3.1. Estimated start of functional adrenal maturation

*Drawing conclusions from adrenal development regarding steroidogenesis, the adrenal cortex might reach sufficient maturation for hormone synthesis in the human by 20-23%, the sheep between 25-31%, the rhesus monkey by 27-36%, the guinea pig by 32-38%, the rat between 70-73% and the mouse between 62-72% of gestation. For the fetal baboon, no data are available concerning these three events. As the start of the fetal period lays approximately in the estimated time frame for the other species, the beginning of the fetal period in the baboon at 27% of gestation might indicate functional maturation of the adrenal cortex in this case.*

### 3.2.3 Adrenal zonation

Mesiano et al. 1997 concluded that the primate fetal adrenal cortex encompasses the DZ as the site of aldosterone synthesis, the TZ, synthesizing cortisol and the FZ as the location of DHEAS

production [299]. There might be not such a distinctive interaction between a single zone and a specific steroid produced here in all species. It is known, that after birth the guinea pig adrenal synthesizes cortisol additionally to the zF (corresponding to the TZ in primates) in the zR (corresponding to FZ in primates) [94]. Even when a distinctive zR in the fetal guinea pig only appears at 82% of gestation, detection of fetal adrenal androgen synthesis is already possible by 32% of gestation [333, 514]. On the other hand, fetal rat and mouse seem to be unable to synthesize androgens in the adrenal cortex and a zR is missing [96, 229].

TABLE 3.3

Assumed main adrenal locations of pre- and postnatal steroid synthesis

| Steroid synthesis | Human/Rhesus monkey/Baboon | Sheep/Guinea pig/Rat/Mouse |
|---|---|---|
| Glucocorticoid | Transitional Zone **TZ, zF** | Zona fasciculata **zF** |
| Mineralocorticoid | Definitive Zone **DZ, zG** | Zona glomerulosa **zG** |
| Androgen | Fetal zone **FZ, zR** | Zona reticularis **zR** Zone absent in rat, mouse, and in prenatal sheep |

### 3.2.4 Stem cell reservoir/proliferation

According to the cell migration model, a common pool of lipid containing progenitor cells is located in the periphery of the adrenal cortex (stem cell reservoir). While these cells migrate centripetally, they differentiate into the specific cells of the cortical zones and undergo senescence in the center [299]. In the rhesus monkey adrenal cortex, actively proliferating DZ cells were detected in early gestation and were referred to as progenitor cells. These cells moved centripetally to populate the rest of the adrenal gland [97]. After 75% (day123) of gestation, the rhesus monkey DZ cells changed in their appearance from proliferating to hormone secreting cells [286]. In guinea pigs, the highest proportion of proliferating cells was found in the zF, at the border to the zG [222]. Vinson et al. 2003 in rats referred to the zG as the site of cell proliferation and differentiation. As cells migrate inward, they change their phenotype from glomerulosa to fasciculata [476]. Mitani et al. 1999 described a new zone in the rats adrenal cortex, with cells neither staining with enzymatic antibody markers for zG and zF. These cells were present in an undifferentiated zone between zG and zF. They migrate inward in a time dependent manner [304]. Keegan et Hammer 2002 suggested a pluripotent stem cell zone, with cells able to differentiate in the specific zonal steroid producing cells. Cells might migrate outward from this undifferentiated zone to the zG and inward to the zF/zR [229]. Arola et al. 1994 showed that in rats, adrenocortical cells grown in the absence of ACTH resemble zG cells. In the present of ACTH, these cells then differentiate into the zF cell type [18].

*In summary, a progenitor cell zone or layer seems to exist in the periphery. The location is assumed in the zG or between zG and zF. Form here, undifferentiated cells/zG progenitor cells migrate into the rest of the cortex and differentiate into specific steroid producing cells of their future homeland. In the end, their programmed cell death occurs most likely in the center of the cortex.*

## 3.3 Fetal adrenal steroid enzyme expression

*After researching the morphological development of the adrenal gland, we will now investigate the steroid synthesis in the adrenal cortex of the species. This first part will focus on the expression of the different required enzymes for adrenal steroid steroidogenesis. Especially important is the presence and absence of enzymes under the assumption, that no steroid production is possible in the absence of a required enzyme. The second part will present the available information about the actual steroid detection in the fetal adrenals. These data will be completed with the data about steroid enzyme expression and morphological adrenal development. It will give us the potential to frame the time of the first hormone production in the fetal adrenal cortex and the development of fetal adrenal steroid synthesis.*

*While focusing on adrenal glucocorticoid synthesis, aldosterone and androgens are influencing factors as both hormones are produced in the adrenal cortex in close proximity to glucocorticoids. In early gestation it is very difficult to spatially separate the different zones inside the adrenal cortex and the possibility exists that glucocorticoids as well as androgens and maybe even aldosterone are produced in more than one zone. Adrenal syntheses of all three hormones share and compete for common essential precursor metabolites. For example, cholesterol is metabolized to pregnenolone, the precursor for glucocorticoid, androgen and aldosterone synthesis and corticosterone can be metabolized to cortisol but also to aldosterone. Additionally, adrenal aldosterone and androgen syntheses require partially the same enzymes that are involved the production of glucocorticoids. Not only a close location of synthesis, competition for precursor metabolites and enzymes, but also mutual dynamic regulations seems to be present among adrenal hormones. Androgens are believed to play an important control function in adrenal glucocorticoid synthesis, as androgen and glucocorticoid synthesis show strong associations. ACTH does not only induce cortisol but also androgen secretion in the adrenal cortex. Due to this important regulatory interaction between adrenal glucocorticoids and androgens, enzyme expression for androgen synthesis needs to be shortly reviewed here in the primate species, where large amounts of adrenal androgens are synthesized. Enzymes for aldosterone synthesis will only be mentioned when their detection cannot be separated from cortisol enzyme detection or the presence of a common enzyme cannot be clearly assigned to glucocorticoid and aldosterone synthesis.*

*Before looking into the specific enzymes, necessary for steroid synthesis in the adrenal cortex, a short overview, over hormones produced in the fetal adrenal cortex of the different species will be given.*

In the primate fetal adrenal cortex, three major steroids are produced, the glucocorticoid cortisol, the mineralocorticoid aldosterone and the androgen dehydroepiandrosterone (DHEA) [299]. The sheep fetal adrenal cortex synthesizes primarily cortisol and aldosterone. In the absence of a substantial zR, less fetal adrenal DHEAS synthesis is assumed [251, 321]. The fetal guinea pig produces in particular cortisol. Aldosterone and the androgen androstenedione are also synthesized in the adrenal cortex, but no or only negligible amounts of DHEA [96, 465]. Fetal rat and mouse especially produce the glucocorticoid corticosterone as well as aldosterone in the adrenal cortex. Both species are unable to produce androgens in the adrenal cortex [96]. It has to be mentioned that the fetal rat and mouse seem transiently able to synthesize cortisol, as an additional glucocorticoid to corticosterone [123, 232].

TABLE 3.4

Major steroids synthesized in the fetal adrenal cortex

| | **Glucocorticoid** | | Mineralocorticoid | Androgen | |
|---|---|---|---|---|---|
| | **Cortisol** | **Corticosterone** | Aldosterone | DHEAS | Androstenedione |
| Human | **Yes** | Yes | Yes | Yes | (Yes) |
| Rhesus monkey | **Yes** | Yes | Yes | Yes | (Yes) |
| Baboon | **Yes** | Yes | Yes | Yes | (Yes) |
| Sheep | **Yes** | Yes | Yes | Yes | Yes |
| Guinea pig | **Yes** | Yes | Yes | No | Yes |
| Rat | **?** | **Yes** | Yes | No | No |
| Mouse | **?** | **Yes** | Yes | No | No |

### 3.3.1 Necessary enzymes for adrenal steroid synthesis

Which hormone will be produced in a cell of the fetal adrenal cortex depends among others on the equipment with enzymes. Three enzymes have been thought to be particularly important for the adrenal biosynthesis of glucocorticoids but are also involved in androgen and aldosterone production:

- Cholesterol side chain cleavage enzyme **CYP11A1**,
- Steroid 17$\alpha$-hydroxylase **CYP17** [279],

and

- 3$\beta$-hydroxysteroid dehydrogenase/isomerase **3$\beta$HSD** [300].

Later, further enzymes, belonging to the cytochrome P450 super family, were added to this list, including

- Steroid 21 hydroxylase **CYP21A2**,

and

- Steroid 11$\beta$-hydroxylase **CYP11B1** [97, 206].

TABLE 3.5

Shared enzymes in glucocorticoid, aldosterone and androgen syntheses

| | Cortisol | Corticosterone | Aldosterone | DHEAS | Androstenedione |
|---|---|---|---|---|---|
| CYP11A1 | Yes | Yes | Yes | Yes | Yes |
| CYP17 | Yes | | | Yes | Yes |
| 3$\beta$HSD | Yes | Yes | Yes | | Yes |
| CYP21A2 | Yes | Yes | Yes | | |
| CYP11B1 | Yes | Yes | | | |

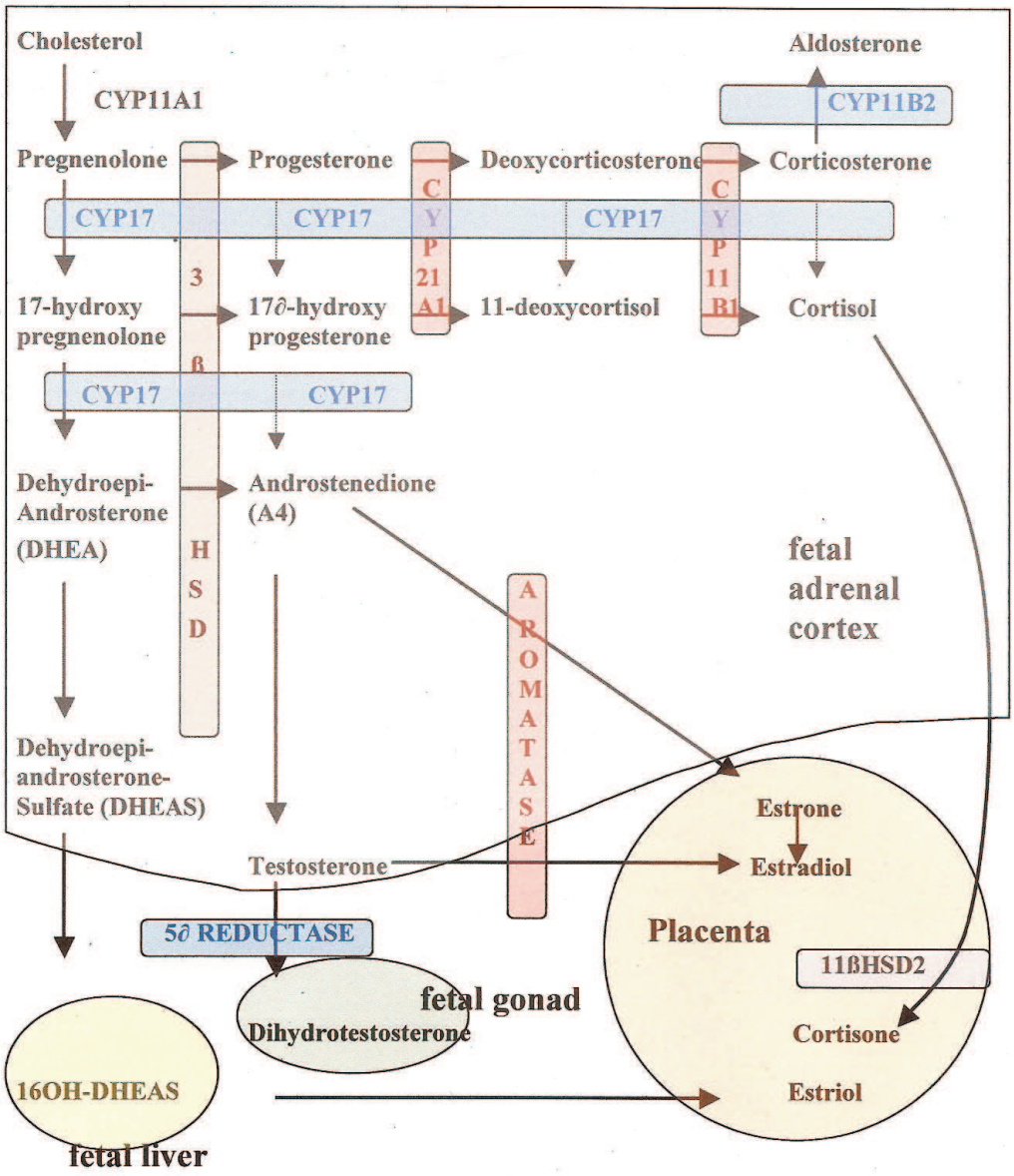


Figure 3.2. Human steroid synthesis in the adrenal cortex

The necessary enzymes are located in different cell organelles, particularly the membranes of the smooth endoplasmic reticulum and the matrix side of the inner mitochondrial membrane including the cristae [206]. There are species-specific peculiarities in the adrenal enzyme equipment.

#### 3.3.1.1 CYP11A1 (Cholesterol side chain cleavage enzyme)

The first step in the synthesis of each of the three groups of steroids (gluco- and mineralocorticoid, androgen) is the conversion of cholesterol into pregnenolone by CYP11A1. The enzyme is responsible for hydroxylation of the cholesterol side chain at the positions c20 and c22 and the cleavage of the side chain. Both reactions take place in all species in mitochondria [206, 302].

#### 3.3.1.2 CYP17 (17$\alpha$-hydroxylase)

CYP17 expression is necessary for cortisol and androgen synthesis, but the enzyme is not involved in corticosterone or aldosterone production [96]. The enzyme converts pregnenolone to 17$\alpha$-hydroxy pregnenolone and progesterone to 17$\alpha$-hydroxy progesterone [206, 429]. Further, CYP17 can catalyze the synthesis of cortisol from corticosterone, of DHEA from 17$\alpha$-hydroxy pregnenolone and of androstenedione from 17$\alpha$-hydroxy progesterone. The enzyme is located in the smooth endoplasmic reticulum and adds a hydroxyl group (-OH) to the c17 atom [206]. There are differences between the species, to express CYP17 in the adrenal cortex, causing species-specific discrepancies in the ability for adrenal androgen and cortisol synthesis. In adults, only primates, sheep and guinea pigs are able to express CYP17 in the adrenal cortex, but not adult rats and mice [24, 243, 299, 513]. Due to the absence of CYP17 in the adult adrenal cortex of rats and mice, these species are not able to produce cortisol or androgens in this location [24, 96]. It should be mentioned that CYP17 was transiently detectable in the fetal adrenal cortex of the mouse [24, 185, 232].

#### 3.3.1.3 3$\beta$HSD (3$\beta$-hydroxysteroid dehydrogenase/isomerase)

3$\beta$HSD type 2 is the only enzyme in the adrenal steroid pathway that is not a member of the CYP450 super family [111]. It is necessary for cortisol and corticosterone as well as androstenedione and aldosterone synthesis. 3$\beta$HSD converts pregnenolone into progesterone as well as 17$\alpha$-hydroxy pregnenolone into 17$\alpha$-hydroxy progesterone. 3$\beta$HSD also converts DHEA into androstenedione in the zR [96, 206]. In the primate placenta, 3$\beta$HSD type 1 (an isozyme distinct from the adrenal 3$\beta$HSD type 2) is present, transforming DHEA into androstenedione [492]. 3$\beta$HSD adds a carbonyl group (COOH) to the c3 position and is located in the smooth endoplasmic reticulum of adrenal cortex cells [206].

#### 3.3.1.4 CYP21A2 (steroid 21 hydroxylase)

CYP21A2 is necessary for glucocorticoid and mineralocorticoid synthesis. It converts progesterone into deoxycorticosterone and 17$\alpha$-hydroxy progesterone into 11-deoxycortisol (not in rat and mouse) by adding a hydroxyl group (-OH) to the c21 atom. CYP21A2 is apparent in the smooth endoplasmic reticulum of the cell [97, 206].

#### 3.3.1.5 CYP11B1 (steroid 11$\beta$-hydroxylase)

Sheep CYP11B catalyzes the final step of cortisol synthesis but also the last steps of aldosterone production. In rodents and humans, the related enzyme CYP11B1 cannot synthesize aldosterone [55]. In the mitochondria, CYP11B1 (CYP11B in sheep) is responsible for 11$\beta$ hydroxylation of deoxycorticosterone to retain corticosterone in rodents and is involved in cortisol production from 11-deoxycortisol in primates, sheep and guinea pigs [206, 302].

#### 3.3.1.6 CYP11B2 (Aldosterone synthase)

Other than in sheep, CYP11B2 is necessary for aldosterone synthesis and has to be mentioned as it is often simultaneously detected together with CYP11B1 [96].

*Under these circumstances, the expression of CYP11A1, CYP17, 3βHSD, CYP21A2, CYP11B1/2 will be investigated in primates, sheep and guinea pigs regarding cortisol (and androgen) synthesis and in rat and mouse with respect to corticosterone (and additionally CYP17 for possible cortisol) production. In the fetal sheep, instead of CYP11B1/2, the expression of CYP11B will be investigated.*

In general, the following problems have to be taken into account. The allocation of steroid production to different cortical zones contains

- difficulties in spatial separation of the different zones, especially early in gestation
- the possibility that a single zone could synthesize different hormones, due to dissimilar enzyme expression in individual cells
- the danger of changing hormone expression over time in a zone or individual cell.

*The pattern of enzyme expression in different locations of the adrenal cortex still gives valuable information about which hormone might be present at what time point during gestation.*

### 3.3.2 Human fetal adrenal steroid enzyme expression

#### 3.3.2.1 Human CYP11A1 expression

Due to its central role, CYP11A1 is required in each zone of the primate fetal adrenal cortex, in the DZ for mineralocorticoid synthesis, in the TZ for glucocorticoid synthesis and in the FZ for androgen synthesis [243, 302]. CYP11A1 immunoreactivity was absent in the fetal human adrenal cortex at 20% (wk8), but was robustly present in the FZ and weaker in the DZ at 25-40% (wk10-16) of gestation [170]. The difficulty to distinguish the TZ from the FZ at this early age needs to be mentioned again in this context. CYP11A1 mRNA was present centrally in the adrenal cortex at 20-23% (end of wk8) of gestation [179]. CYP11A1 immunoreactivity was apparent in the TZ at least from 35% (wk14) of gestation (the earliest date investigated) and continued to be present in both TZ and FZ until term [170, 320]. Due to its continuous presence in the FZ and the TZ from early on, CYP21A1 seems not to play a rate-limiting role in the cortisol synthesis [97, 299].

*CYP11A1 is necessary for the synthesis of cortisol, DHEAS and aldosterone and is expressed in the human FZ at 20-23% and IR-CYP11A1 is present in the TZ at least at 35% of gestation until birth.*

#### 3.3.2.2 Human CYP17 expression

CYP17 expression is necessary for DHEAS and cortisol synthesis in the fetal human adrenal cortex. No CYP17 expression was detected in the adrenal blastema at 13% (wk5) of gestation. IR-CYP17 and CYP17 expression was detected as early as 20% (wk8) respectively 20-23% (wk8-9) of gestation in the center of the human adrenal anlage. By 25% (wk10) of gestation, the FZ cells strongly expressed CYP17 [142, 179]. In Goto et al. study from 2006, IR-CYP17 was not present at 20% (wk8) in the fetal human adrenal cortex, but was robustly apparent at 23-24% (wk9-10) of gestation in the inner cortex. From 25-28% (wk10-11) until 40% (wk16) of gestation, CYP17 continued to be present in the FZ, and the DZ stained weakly positive [170]. Narasaka et al. 2001 showed that CYP17 protein was present in the human fetal FZ and TZ, but absent in the DZ from 35% (wk14) of gestation until term [320].

*Cortisol and DHEAS synthesis both require CYP17 expression. CYP17 is expressed weakly at 20-23% of gestation in the FZ. At 25% of gestation, CYP17 is strongly expressed in the FZ and immunoreactivity remains present until birth. In the DZ (maybe rather referring to the TZ as the enzyme is not required for aldosterone synthesis), CYP17 is absent at 20%, but is weakly present from 26-40% of gestation. CYP17 protein is apparent in the TZ but absent in the DZ from 35% of gestation until term.*

#### 3.3.2.3 Human 3$\beta$HSD expression

3$\beta$HSD is necessary for de novo cortisol synthesis from cholesterol [170] but also for aldosterone synthesis. IR-3$\beta$HSD was absent in the fetal human adrenal cortex at 20% (wk8) of gestation. At 23% (wk9) of gestation, some IR-3$\beta$HSD cells were apparent, mostly at the interface between DZ and FZ (assumingly the location of the TZ). In particular, abundant and more expanded IR-3$\beta$HSD was present at 25-28% (wk10-11) of gestation. During this time, 3$\beta$HSD staining seems not only to cover the location of the TZ but also the FZ (personal observation). From this age on, 3$\beta$HSD IR declined to low levels on 30-33% (wk12-13) and was absent at 40% (wk16) of gestation [170]. IR 3$\beta$HSD was not detectable in all three zones between 35-58% (wk14-23) of gestation [320]. Mesiano et al. 1993 showed the absence of 3$\beta$HSD expression in the fetal human adrenal cortex between 48-65% (wk19-26) of gestation [300]. From 58-60% (wk23-34) of gestation until term, 3$\beta$HSD IR was present in the TZ and the DZ [320]. Goto 2007 reviewed the literature about 3$\beta$HSD absence and presence in the human fetal adrenal cortex by integrating the data of Mesiano et al. 1993 [300], Parker et al. 1995 [341], Narasaka et al. 2001 [320] and Goto et al. 2006 [170] and summarized the presence of 3$\beta$HSD from approximately 23% (wk9) until its disappearance by 40% (wk16) and again reappearance of 3$\beta$HSD from 65% (wk26) of gestation until term [169]. After birth at 16%

(PND60) and 66% (PND240) of weaning, with two years of age and in adults, 3$\beta$HSD staining was present in the zF (TZ) [137].

*From these quite extensive data about the presence of 3$\beta$HSD in the human fetal adrenal cortex, it can be concluded that the enzyme is absent in the whole cortex at 20%, appears between 23-24%, is apparent in high levels at 25-28% and decreases from 28% until its absent at 38-40% of gestation. 3$\beta$HSD is not present from 38-40% until 58-60% of gestation, when weak immunoreactivity is recognized again. It is then apparent until term. Regarding zonal distribution, 3$\beta$HSD is present at the border between FZ and DZ (assuming the future location of the TZ) at 23-24% of gestation. Immunoreactivity is stronger and broader at 25-28% of gestation, which seems to include the FZ. It might be possible that there is no clear zonation for hormone presence in early gestation, so that first, 3$\beta$HSD could be used for aldosterone and cortisol synthesis at that point and second, cortisol could be synthesized in both FZ and TZ cells. With the disappearance of 3$\beta$HSD immunoreactivity around 38-40%, cortisol synthesis is assumed to be transiently absent, until the reappearance of the enzyme at 58-60% of gestation in the TZ and its presence until birth.*

#### 3.3.2.4 Human CYP21A2 expression

The presence of CYP21A2 is indispensable for cortisol and aldosterone synthesis. CYP21A2 immunoreactivity was absent at 20% (wk8), but robustly present at 25% (wk10) of gestation more centrally in the cortex [170]. Folligan et al. 2005 detected CYP21A2 immunoreactivity from 30% (wk12) onward in the FZ, and Narasaka et al. 2001 from 35% (wk14) of gestation until term in the TZ and FZ [152, 320]. Between 38-65% (wk15-26) of gestation, CYP21A2 immunoreactivity was strongly present in TZ and FZ [97].

*CYP21A2 is a necessary enzyme for cortisol and aldosterone but not DHEAS synthesis. Its presence is detected from 25% of gestation until term in the fetal human adrenal cortex. At 25%, CYP21A2 immunoreactivity is detected more centrally, and is detectable in the FZ and the TZ from 30% or 35% of gestation until birth. The detection in the FZ again let us question the clear regional allocation of cortisol synthesis to the TZ, as it is very difficult to distinguish the TZ from the other two zones early in gestation and it is possible that cortisol producing cells are located in the FZ as well.*

#### 3.3.2.5 Human CYP11B1/2 expression

CYP11B1 is necessary for cortisol synthesis and CYP11B2 is required for the production of aldosterone. IR-CYP11B1/CYP11B2 was detected together at 25% (wk10) of gestation, more weakly in the periphery than the center of the cortex. The same pattern was still apparent by 40% (wk16) of gestation [170]. Between 38-65% (wk15-26) of gestation, CYP11B1/CYP11B2 was present in the FZ and TZ [97].

*From 25% of gestation on, CYP11B1/CYP11B2 is present in the adrenal cortex, what would allow early cortisol synthesis. Approximately between 38-65% of gestation, CYP11B1/CYP11B2 is continuously expressed in the FZ and TZ, despite the fact that both enzymes are not involved in androgen synthesis.*

#### 3.3.2.6 Summary human enzyme expression

*To draw conclusions, the summarization of steroid enzyme expression/immunoreactivity is necessary. By presence of absence of required enzymes, indication for cortisol synthesis in the fetal adrenal cortex will be given. Due to the close regulation of cortisol with androgens, enzyme expression for DHEAS synthesis will be additionally discussed. Especially the verified absence of an enzyme in the entire cortex at a specific time is crucial, because steroid synthesis can be assumed to be absent simultaneously.*

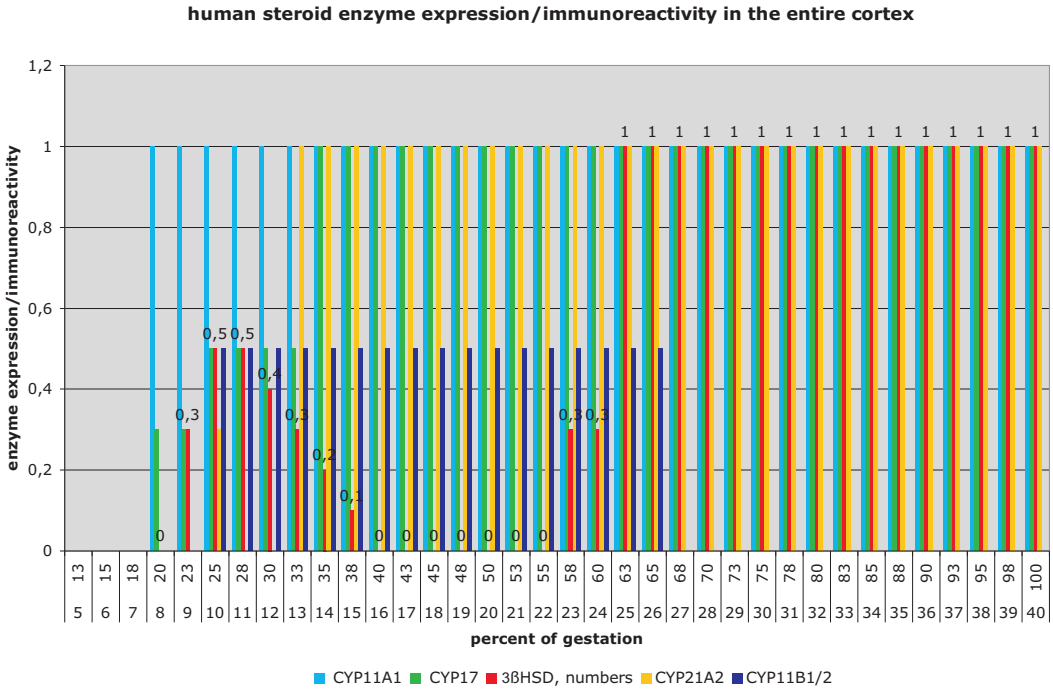


Figure 3.3. Human steroid enzyme expression in the entire cortex

*As 3βHSD expression/immunoreactivity is one of the best researched enzymes in the human fetal adrenal cortex, its presence and absence can be used as an indicator for cortisol synthesis, but is also involved in aldosterone production. Independent from zonal distribution, 3βHSD is absent at 20%, but apparent between 23-38% of gestation. Again enzyme expression/immunoreactivity disappears between 40-55%, but then is present from 58% of gestation until term. This development of 3βHSD is assumed to cause a similar pattern of cortisol synthesis. CYP17, a telltale for DHEAS synthesis, is apparent from 20% of gestation until term.*

*To arrive at a conclusion of fetal adrenal cortisol and DHEAS synthesis due to their enzyme expression/immunoreactivity, the presence of enzymes in the single zones of the fetal adrenal cortex will be analyzed, being aware, that due to difficulties in zonal separation and the possibilities of synthesis of a hormone in more than one zone, the interpretation might be complicated.*

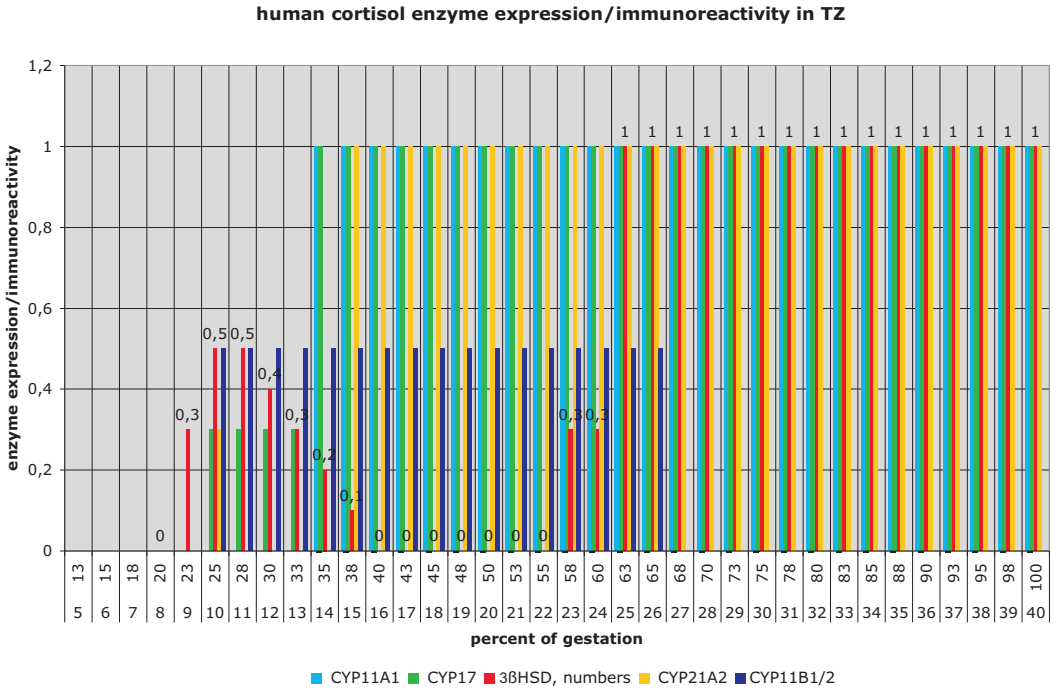


Figure 3.4. Human cortisol enzyme expression in TZ

*At 20% of gestation, CYP11A1, CYP17, CYP21A2 and 3βHSD expression/immunoreactivity are absent in the TZ, not indicating cortisol synthesis at that early time. By 23%, 3βHSD expression/immunoreactivity is detected and by 25% of gestation, additionally the presence of moderate levels of CYP17, CYP21A2 and CYP11B1/2 suggests cortisol synthesis. Moderate cortisol synthesis might remain until 28% of gestation, when 3βHSD starts to decrease and disappears at 40% of gestation. 3βHSD remains absent, until low levels are detected again at 58-60% of gestation and stronger levels are present until birth. Between 40-58% of gestation, moderate CYP11B1/2 levels are detected and the presence of CYP11A1, CYP17 and CYP21A2 is verified. No information is available about CYP11B1/2 after 68% of gestation in the TZ, but the other three enzymes remain present. Less cortisol might be synthesized during 23/25-40% than after 63% and over the period of 40-55% of gestation, cortisol production in the fetal human adrenal cortex is assumed to be low or absent.*

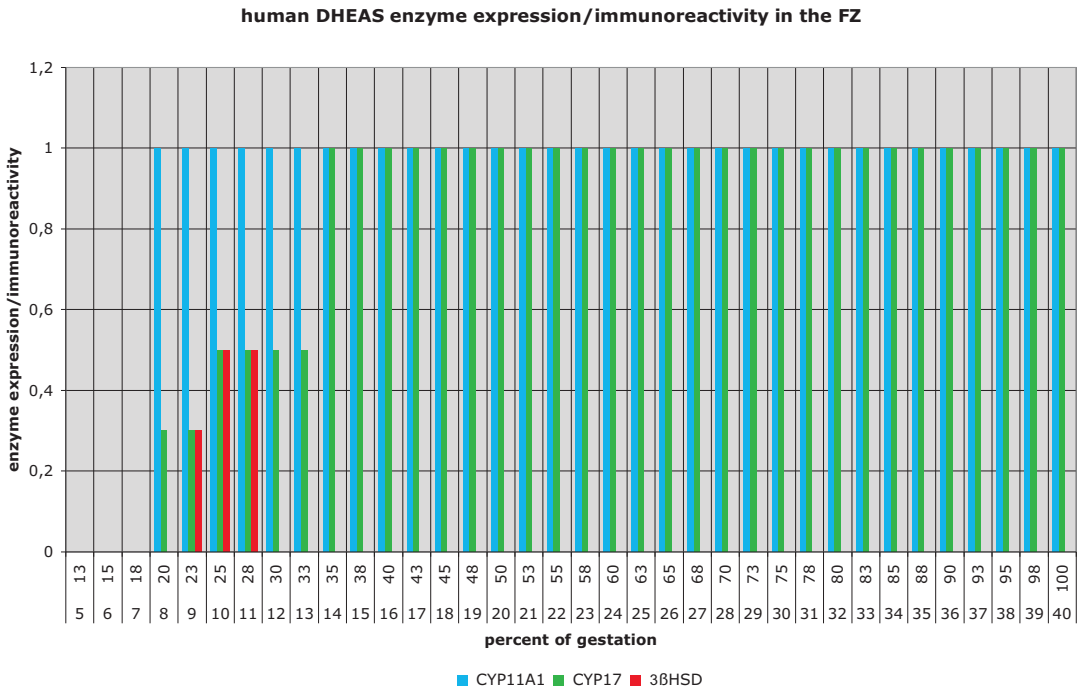


Figure 3.5. Human DHEAS enzyme expression in the FZ

*Regarding the expression/immunoreactivity of the two required enzymes for DHEAS synthesis, CYP17 and CYP11A1, it can be assumed that DHEAS is synthesized already at 20% of gestation and its production remains until term. DHEAS synthesis might be moderate from 20-33% of gestation, and subsequently can be assumed to increase. Surprising, 3$\beta$HSD seems to be apparent in the FZ around 23-28%, which maybe rather lead to cortisol than DHEAS synthesis and could cause a decrement in DHEAS production during that time.*

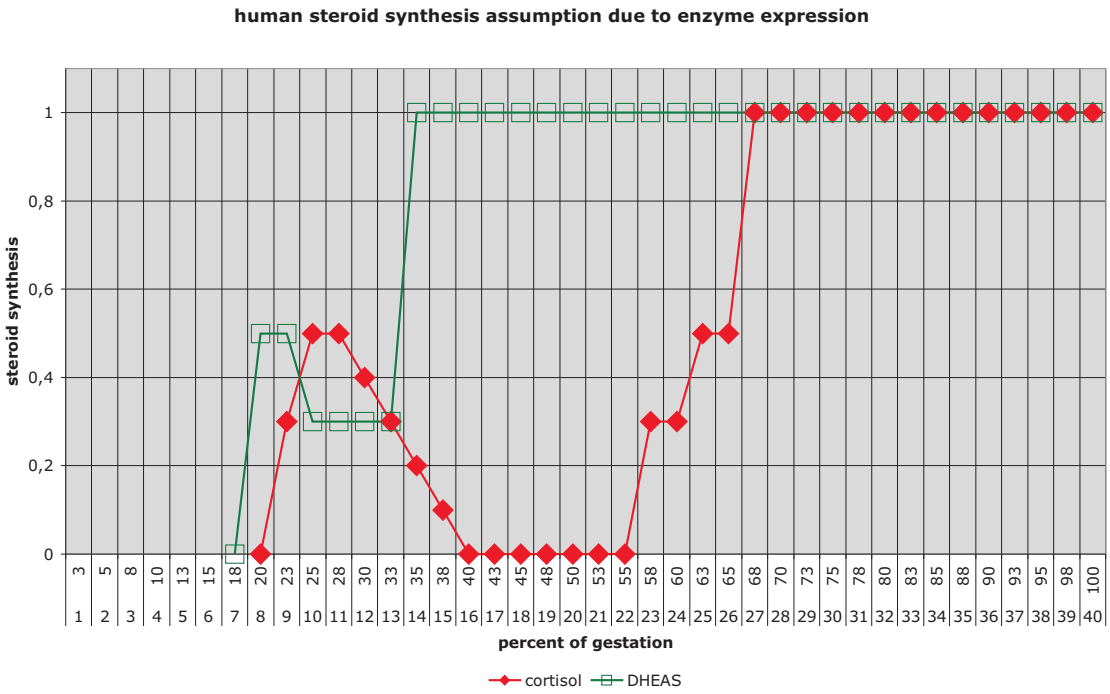


Figure 3.6. Human steroid synthesis assumption due to enzyme expression

*Due to the presence of cortisol, and additional DHEAS enzymes, the following picture of steroid synthesis in the fetal human adrenal cortex could occur. As often the only information about enzyme expression/immunoreactivity states presence or absence of an enzyme, the magnitude and the changes in the abundance become lost when the extent of expression/immunoreactivity reaches higher levels. DHEAS synthesis might be present by 20-23% and subsequently might decrease during peaking cortisol synthesis at 25-28% of gestation. By 30% of gestation, cortisol synthesis could have decreased again and DHEAS synthesis seems to remain moderately low. Cortisol synthesis seems to decrease to low level by 40% and DHEAS increases by 35% to remain apparent until term. Cortisol synthesis due to enzyme expression is assumed to remain very low or absent between 40-55%, then gradually increase between 55-68% of gestation to stronger levels thereafter until term.*

### 3.3.3 Rhesus monkey fetal adrenal steroid enzyme expression

#### 3.3.3.1 Rhesus monkey CYP11A1 expression

Between 79% and 90% (day130-148) of gestation, CYP11A1 mRNA and protein were present in the FZ and the TZ of the rhesus monkey fetus [99, 300].

#### 3.3.3.2 Rhesus monkey CYP17 expression

At 30% (day50) of gestation, CYP17 was strongest expressed in FZ cells just underneath the DZ, which possibly also involves the location of the developing TZ. Between 49-79% (day80-130) of gestation, IR-CYP17 appeared to have decreased and can be only found in a few TZ cells [271]. Mesiano et al. 1993 and Coulter et al. 1996 detected CYP17 expression between 79-90% (day130-148) of gestation in the FZ and TZ. The abundance was higher in the TZ than in the FZ [99, 300]. Subsequently between 91-97% (day150-160) of gestation, CYP17 immunoreactivity was more intensive again, especially in the TZ and in the outer half of the FZ. At 97% (day160) of gestation, the enzyme was strongly expressed, but lower in FZ than in TZ [271].

*At 30% of gestation, the CYP17 expression is present in the FZ and assumingly also in the TZ. Between 49-79% of gestation, CYP17 immunoreactivity is absent in FZ and only very low expression is apparent in the TZ. After 79% of gestation, CYP17 again is strongly expressed, but more intensive in the TZ than in the FZ and further increases between 91-97% of gestation. At 97% of gestation, CYP17 immunoreactivity is very strong, especially in the TZ.*

#### 3.3.3.3 Rhesus monkey 3$\beta$HSD expression

Mapes et al. 2002 detected light staining of 3$\beta$HSD between 30-88% (day50, 80, 100, 115,130 and 145) of gestation especially in the FZ, but strong expression in late gestation in the TZ [271]. 3$\beta$HSD immunoreactivity was lacking in TZ at 66-76% (day109-125), but was weakly present between 79-90% (day130-148) and stronger between 94-100% (day155-165) of gestation [99, 100, 300]. Immunoreactivity seemed to be weak between 66-76% (day109-125) and had increased by 85-90% (day140-148) of gestation. Between 91-97% (day150-160) of gestation, IR-3$\beta$HSD seemed to increase to very high levels in the TZ [99, 271].

*The data about 3$\beta$HSD expression/immunoreactivity in the fetal rhesus monkey adrenal cortex are not coherent. At 30%, 49% and 61% of gestation, low levels are present in the inner cortex. In the TZ, IR-3$\beta$HSD is absent from 66-76% of gestation, then levels are weak at 79% until 90%, increasing from 91% till 97% of gestation and possibly further until term.*

#### 3.3.3.4 Rhesus monkey CYP21A2 expression

CYP21A2 was detected between 66-100% (day109-165) of gestation. Immunoreactivity was present in all three zones, but stronger in the TZ than in the FZ. Levels increased in the TZ over the investigated period [97].

*CYP21A2, required for cortisol and aldosterone synthesis, is present between 66% of gestation and term. Over the whole period, CYP21A2 is low in the FZ, but increases in the TZ to high levels until term.*

#### 3.3.3.5 Rhesus monkey CYP11B1/2 expression

Between 66-100% (day109-165) of gestation, IR-CYP11B1/2 was present in the adrenal cortex. Surprisingly even in the FZ, the enzyme is moderately present between 66-94% (day109-155) and stronger from 97-100% (day160-165) of gestation. CYP11B1/2 levels were already strong in the TZ from 66-94% (day109-155), but reached very high levels from 97% (day160) of gestation until term. The administration of Metyrapone (a substance increasing ACTH secretion), induced maximal CYP11B1/2 staining in the TZ already between 83-89% (day137-143), instead of the physiological

appearance of very high staining after 97% of gestation. It also caused higher staining in the FZ [97].

*Unfortunately, no information is available about CYP11B1/2, a necessary enzyme for cortisol synthesis, before 66% of gestation. In the TZ, strong levels are apparent between 66-94% of gestation and maximal levels are present from 97% until term. The moderate and later higher levels in the FZ are surprising, as CYP11B1/2 is not required for DHEAS synthesis. Between 83-89% of gestation, CYP11B1/2 immunoreactivity can be increased by higher ACTH concentrations.*

#### 3.3.3.6 Summary rhesus monkey enzyme expression

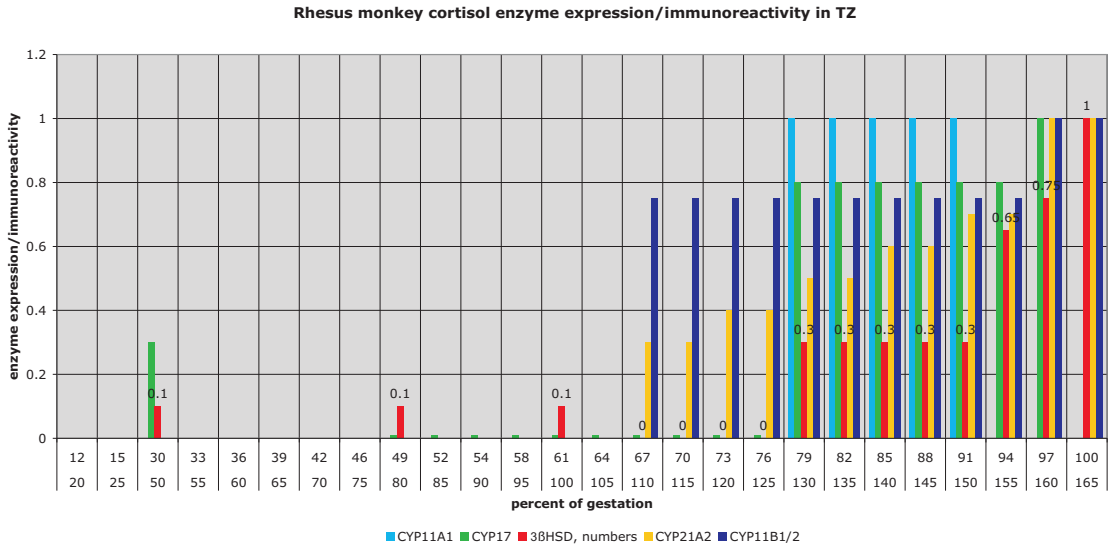


Figure 3.7. Rhesus monkey cortisol enzyme expression in TZ

*Looking at CYP17 and 3βHSD in the TZ of the rhesus monkey, there could be cortisol synthesis at 30% of gestation, due to low CYP17 and 3βHSD immunoreactivity. CYP17 is very low between 49-76%, while 3βHSD is low at 49% and 61% and absent at 67-76% of gestation. We assume between 49-61% low cortisol synthesis, but it seems to be absent at least between 67-76% of gestation. From 79-91% of gestation on, 3βHSD is modest together with CYP17, CYP11A1, CYP21A1 and CYP11B1/2, indicating moderate cortisol synthesis. By 94% 3βHSD levels have increased and by 97% of gestation, CYP17 and CYP11B1/2 reach high levels, so fetal cortisol production can be assumed to increase to very strong levels until term.*

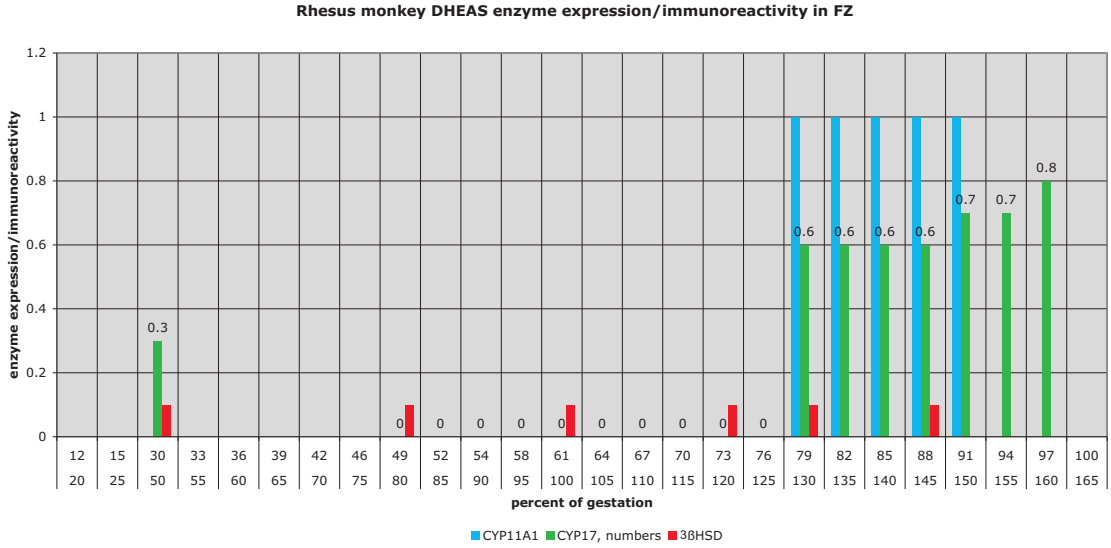


Figure 3.8. Rhesus monkey DHEAS enzyme expression in FZ

*According to enzyme levels in the FZ around 30% of gestation, DHEAS synthesis could be possible due to the presence of CYP17. CYP17 immunoreactivity is absent from 49-76% of gestation, indicating no DHEAS synthesis during that period. By 79%, both required enzymes for DHEAS production, CYP17 and CYP11A1, show strong levels and CYP17 immunoreactivity seems to increase by 97% of gestation.*

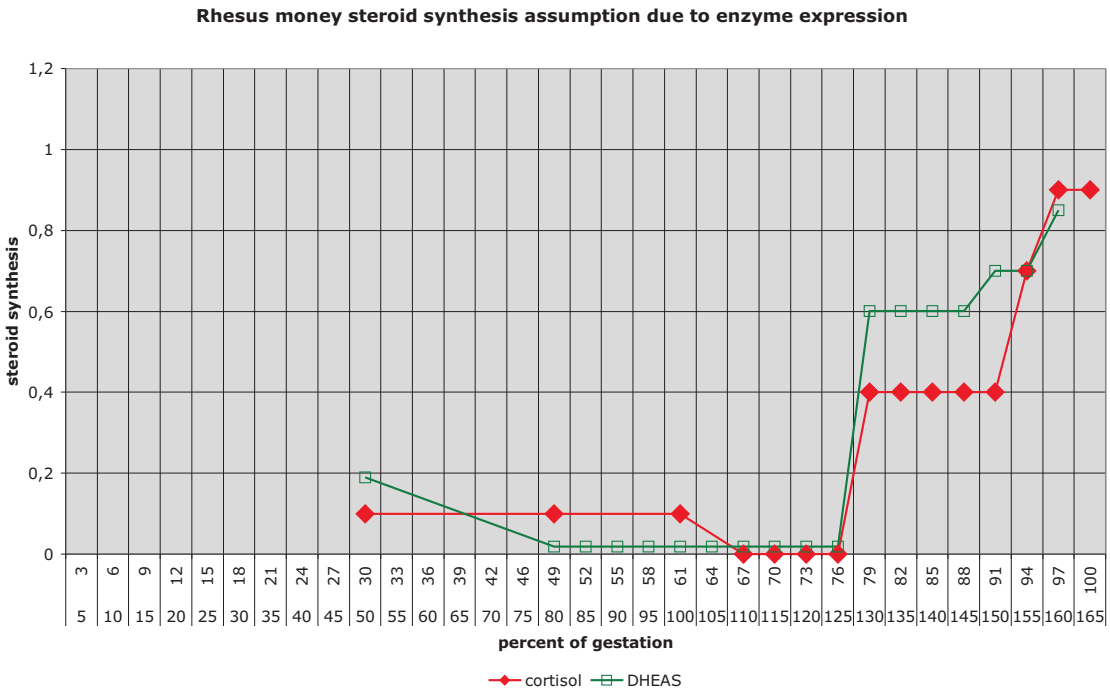


Figure 3.9. Rhesus money steroid synthesis assumption due to enzyme expression

*According to enzyme pattern, low fetal adrenal cortisol synthesis could be present between 30-61% and is absent between 67-76% of gestation. From there on, cortisol synthesis can be assumed to increase to moderate levels at 79-91% and further to very high levels at 97-100% of gestation. DHEAS synthesis, in correlation to its enzyme expression/immunoreactivity, could be sufficiently present at 30%, but might be absent or very low between 49-76% of gestation. Between 79-91% of gestation, DHEAS synthesis seems to be moderately strong again and further increases until birth.*

### 3.3.4 Baboon fetal adrenal steroid enzyme expression

#### 3.3.4.1 Baboon CYP11A1 expression

CYP11A1 mRNA expression in the baboons‘ fetal adrenal cortex did not significantly changed between 32-35% (day58-64), 54-56% (day99-103) and 89-92% (day164-170) of gestation [1]. Unfortunately it is unknown in which zone of the fetal adrenal baboon cortex CYP11A1 was expressed.

*CYP11A1 seems to be present in the adrenal cortex at least from 32% of gestation onward. The abundance of enzyme expression does not change between 32-92% of gestation. As the enzyme is required for cortisol, DHEAS and aldosterone synthesis, it could be present in each zone.*

#### 3.3.4.2 Baboon CYP17 expression

The enzyme was expressed in the baboon fetal adrenals in relative constant levels at 32-35% (day58-64), 54-56% (day99-103) and at 89-92% (day164-170) of gestation [1]. At 54 % (day100) of gestation, CYP17 protein was present in the FZ, but not in the TZ. Between 86-92% (day160-170) of gestation, the CYP17 immunostaining was present in both FZ and TZ [5, 135, 242].

*At 32-35% of gestation, it is not possible to establish, whether CYP17 is expressed in the FZ, the TZ or both. Enzyme protein is present in the FZ but absent in the TZ at 54-56% and present in both zones at 86-92% of gestation.*

#### 3.3.4.3 Baboon 3$\beta$HSD expression

In fetal baboon, 3$\beta$HSD mRNA, involved in cortisol and aldosterone synthesis, was hardly detectable at 32-35% (day58-65), but increased to low expression at 53-57% (day98-104) and significantly ($P<0.001$) increased by 13-fold to high levels at 89-92% (day164-170) of gestation. In the TZ, 3$\beta$HSD immunostaining was absent at 54% (day100) but was apparent between 87-92% (day160-170) of gestation. The enzyme was absent in the FZ at least at 54% (day100) and 87-92% (day160-170) of gestation [5, 135, 242].

*3$\beta$HSD seems to be absent in the FZ. The enzyme is very low expressed at 32-35% of gestation. By 53-57% of gestation, 3$\beta$HSD protein is absent in the TZ but present in the DZ. A dramatic increment is present at 87-92% of gestation, possibly in both TZ and DZ.*

*No information about CYP11A1, CYP21A2 or CYP11B1/2 expression is available in the fetal baboon adrenal cortex.*

#### 3.3.4.4 Summary baboon enzyme expression

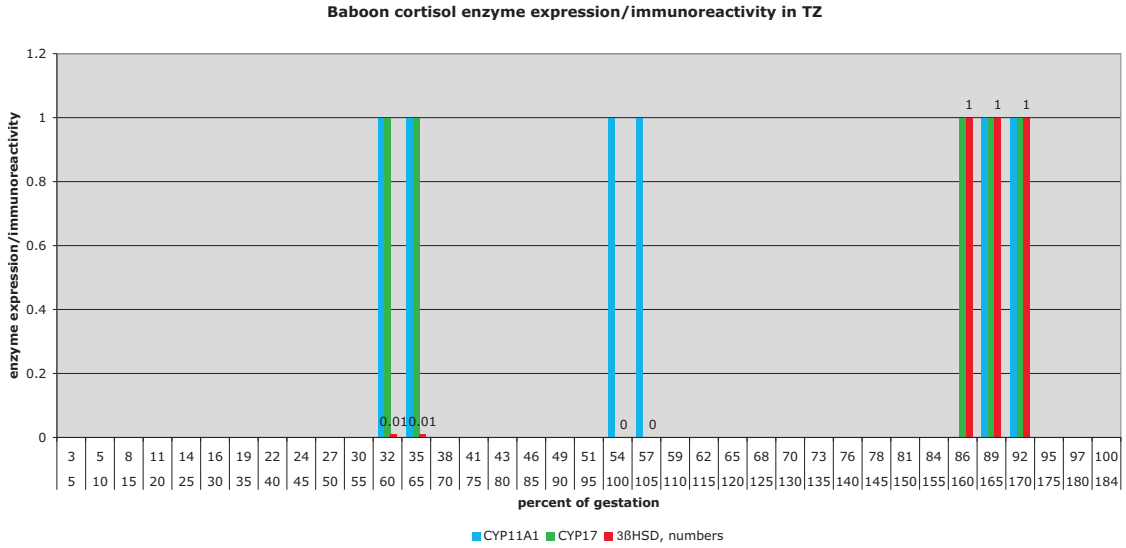


Figure 3.10. Baboon cortisol enzyme expression in TZ

*The information on steroid enzyme expression/immunoreactivity in the fetal baboon adrenal cortex is not extensive. Still, the expression of 3βHSD at 32-35% of gestation, although very low, together with CYP11A1 and CYP17 expression allows cortisol synthesis at that stage. By 54-57% of gestation, protein of both 3βHSD and CYP17 are absent in the TZ, indicating no cortisol production at that point. At least between 87-92%, 3βHSD and CYP17 expression are present in the TZ and CYP11A1 mRNA is apparent between 89-92% of gestation. The abundance of 3βHSD mRNA has increased dramatically in late gestation, assuming strong cortisol synthesis toward term.*

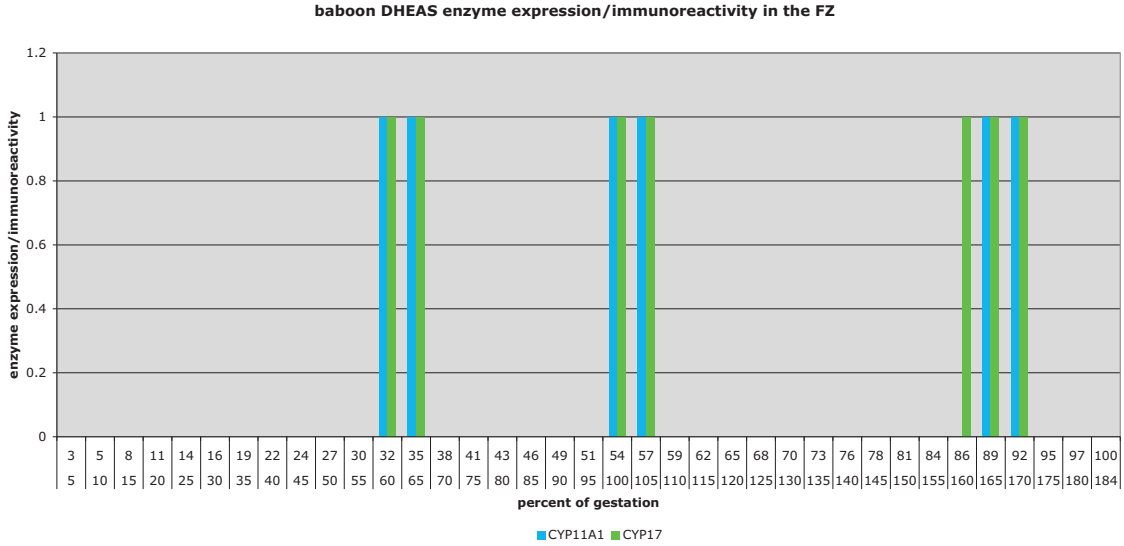


Figure 3.11. Baboon DHEAS enzyme expression in the FZ

*Both required enzymes for DHEAS synthesis, CYP11A1 and CYP17, are expressed at 32-35%, 54-57% and 86-92% of gestation. Protein of the latter can be allocated to the FZ, assuming the presence of sufficient fetal DHEAS production over the whole period.*

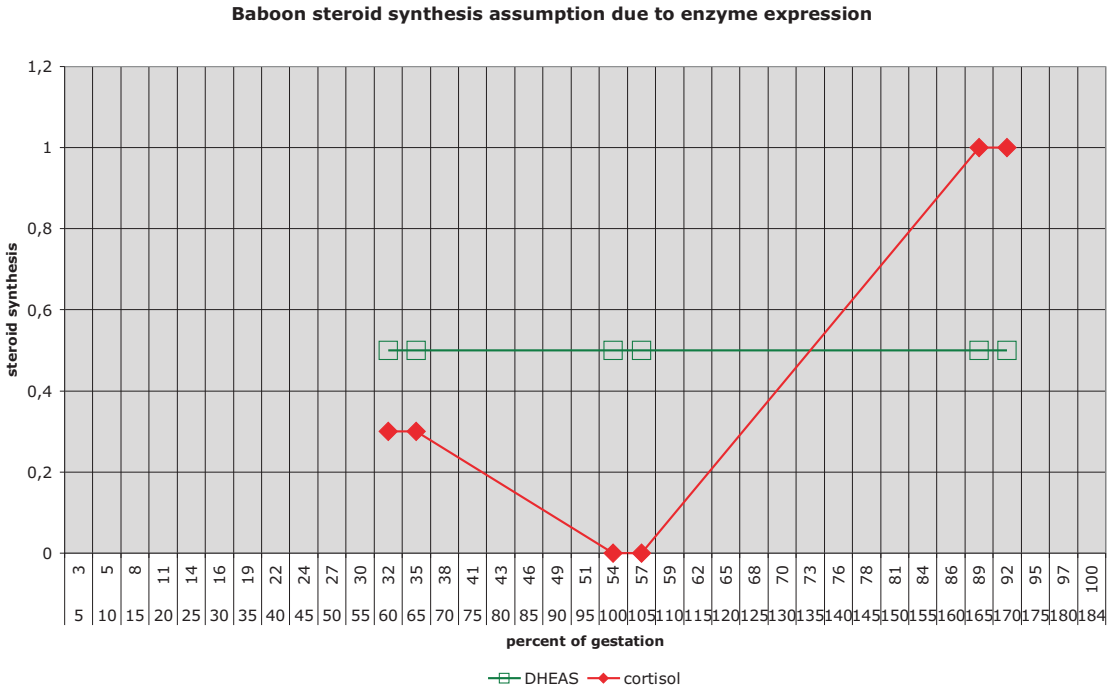


Figure 3.12. Baboon steroid synthesis assumption due to enzyme expression

*Enzyme expression/immunoreactivity in the fetal adrenal cortex of the baboon is not very comprehensive. Out of the available data the following adrenal cortisol and DHEAS syntheses in the baboon fetus during gestation can be assumed. Low cortisol synthesis could be present at 32-35%, but decreases by 54-57% of gestation to very low or possibly undetectable levels. Between 54-57% and 86-92% of gestation, cortisol synthesis could increase strongly. DHEAS synthesis is assumed to be sufficiently present from 32% until at least 92% of gestation.*

### 3.3.5 Sheep fetal adrenal steroid enzyme expression

#### 3.3.5.1 Sheep CYP11A1 expression

Strong expression of CYP11A1 was present between 30-43% (day45-65) and again between 83-100% (day125-150), but very low levels were apparent between 63-83% (day95-125) of gestation. Assumingly sufficient CYP11A1 expression was present from 43% (day65), until very weak expression was verified around 63% (day95) of gestation. After zonation took place after 63% (day95) of gestation, CYP11A1 expression was assigned to both zF and zG, as expected for its involvement in cortisol and aldosterone synthesis [461]. CYP11A1 mRNA expression significantly ($P<0.05$) increased by 3.1 fold between 89-92% (day133-138) and 93-95% (day139-143) of gestation. Expression remained high at 96-99% (day144-148) of gestation [365]. Simmonds et al. 2001 detected a significant ($P<0.05$) increase in CYP11A1 mRNA expression by 3.7 fold between 90-100% (day135-150) of gestation [434].

*CYP11A1 is sufficiently expressed between 30-43% and again between 83-100%, but very low between 63-83% of gestation. From 63% of gestation onward, CYP11A1 expression can be assigned to both zF and zG, as it is necessary for cortisol and aldosterone synthesis. Additionally, it is a required enzyme for DHEAS production. From 89-92% to 93-95% of gestation, expression increases strongly and remains high thereafter until term.*

#### 3.3.5.2 Sheep CYP17 expression

Like CYP11A1, CYP17 mRNA was sufficiently expressed in the fetal adrenals between 30-43% (day45-65) and between 83-100% (day125-150), but expression is low between 63-83% (day95-125) of gestation. After 63% of gestation, CYP17 could be confined to the zF [461]. Phillips et al. 1996 revealed lower levels of CYP17 mRNA at 89-95% (day133-143) and significantly ($P<0.05$) increasing expression at 96-99% (day144-148) of gestation by 3.2 fold [365]. Simmonds et al. 2001 only detected an increase ($P<0.05$) by 1.6 fold between 90-100% (day135-150) of gestation [434]. CYP17 mRNA expression was regulated among others factors by ACTH and increased from 90% to 100% (day135-150) of gestation in parallel with the fetal plasma ACTH concentration [365]. Wintour et al. 1995 correlated the pattern of cortisol synthesis to the presence and absence of CYP11A1 and CYP17 expression [500].

*Expression of CYP17 is present solely in the zF. CYP17 is strongly expressed between 30-43% and between 83-100% of gestation but nearly absent in between. Between 89-95% and 96-99% of gestation, CYP17 expression in the zF increases by more than 3 fold.*

#### 3.3.5.3 Sheep 3$\beta$HSD expression

After Phillips et al. 1996 detection of constantly high 3$\beta$HSD expression between 89-99% (day133-148) of gestation [365], it was suggested that CYP17 is the rate-limiting enzyme during cortisol synthesis of the fetal sheep in late gestation, rather than 3$\beta$HSD like in the primate fetal adrenal cortex. On the other hand, Simmonds et al. 2001 detected significantly ($P<0.05$) increased expression of 3$\beta$HSD mRNA by 4.4 fold between 90% and 100% (day 135-150) of gestation [434]. This questioned the exclusiveness of 3$\beta$HSD expression as an important factor in late gestational cortisol synthesis of the sheep fetus. The mRNA expression for all three enzymes CYP11A1, CYP17 and 3$\beta$HSD were significantly correlated with each other and with plasma cortisol concentration [434]. Boshier et al. 1989 were the only source that looked at the presence of 3$\beta$HSD in the different zones. At 89% (day133) and 98% (day147) of gestation as well as at 2% (PND2) of weaning, 3$\beta$HSD immunostaining was only present in the zF. In the zF, 3$\beta$HSD protein was present right to the medulla. There is no differentiation inside the zF into a part that lacks 3$\beta$HSD, which would indicate a developing zR. A zR is at least absent until the early neonatal period [57].

*The expression of 3$\beta$HSD mRNA is not investigated in the fetal sheep adrenal cortex before 89% of gestation. This raises the question whether fetal sheep 3$\beta$HSD has an additional period of early expression comparable to CYP17 and CYP11A1 expression around 30-43% of gestation and*

*as it occurs in the primate. From the available data, 3βHSD expression increases strongly by 4 fold between 90 and 100% of gestation in the sheep fetus. 3βHSD immunoreactivity was only detected in the zF at 89% and 98% of gestation and at 2% of weaning. Enzyme expression does not indicate the presence of a zR until at least 2% of weaning.*

#### 3.3.5.4 Sheep CYP21A2 expression

Tangalakis et al. 1989 detected a steady increase in CYP21A2 expression from 30% (day45) of gestation until term [461]. Phillips et al 1996 investigated CYP21A2 mRNA expression in late gestation. Enzyme expression increased significantly ($P<0.05$) by 1.8 fold between 89-92% (day133-138) and 93-95% (day139-143) and remained high at 96-99% (day144-148) of gestation [365].

*CYP21A2 expression, necessary for cortisol and aldosterone synthesis, increases from 30% of gestation until term. A strong increment takes place between 89-92% and 93-95% of gestation and remains high until birth.*

#### 3.3.5.5 Sheep CYP11B expression

Immunoreactivity of CYP11B, necessary for cortisol synthesis, was at least present at 63% (day95) of gestation in the zF. CYP11B immunoreactivity was intense at 63% (day95), 83% (day125), 98% (day147) of gestation and in adulthood [102]. John et al. 1987 detected CYP11B protein as early as 61% (day92) of gestation in the fetal sheep adrenal gland. Again, the levels of CYP11B protein were constant between 61-99% (day92-148) of gestation [214].

*CYP11B, necessary for cortisol production in the fetal adrenal cortex, is present at least from 61% of gestation in the zF. Immunoreactivity seems not to change until term or in adult.*

#### 3.3.5.6 Summary sheep enzyme expression

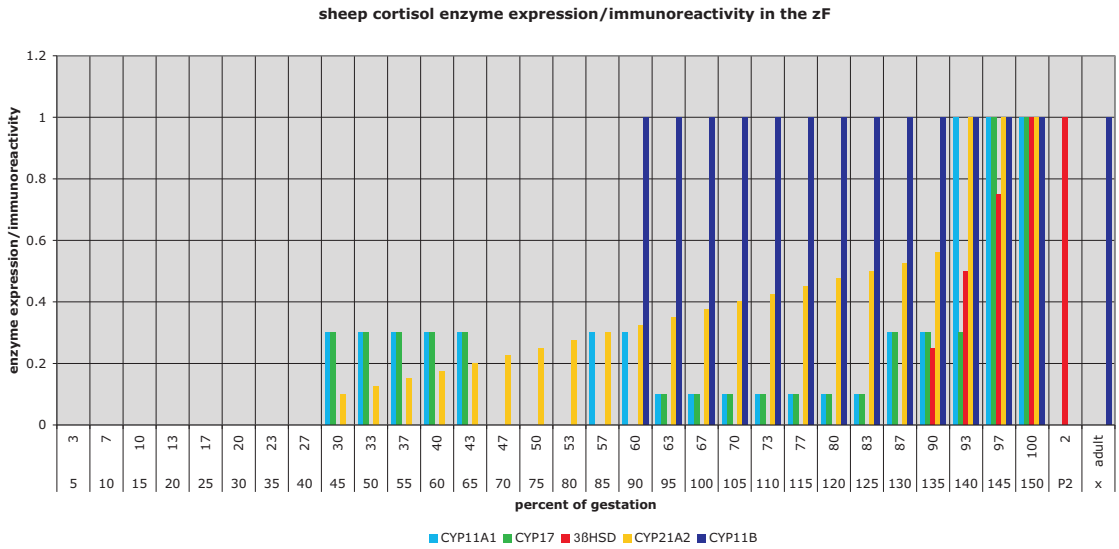


Figure 3.13. Sheep cortisol enzyme expression in the zF

*Cortisol enzyme expression/immunoreactivity in the fetal sheep zF presents a picture of early as well as late steroid synthesis, but a period of very low expression in between. Between 30-*

*43%, both CYP11A1 and CYP17 are sufficiently expressed and adequate expression of CYP11A1 seems to remain until 57-60% of gestation, which enables cortisol synthesis between 30-60% of gestation. The expression of both enzymes is very low between 63-83% of gestation and suggests at best low fetal cortisol production during that time. Between 87-90% of gestation, expression of CYP17 and CYP11A1 have moderately increased and 3βHSD as well as CYP21A2 expression are present in sufficient levels. CYP17 expression remains moderately around 93%, thereafter, 3βHSD expression increases dramatically to high levels at term. By 100% of gestation, all required enzymes for cortisol synthesis are strongly expressed and a dramatic increment in cortisol synthesis due to enzyme expression can be assumed in late gestation.*

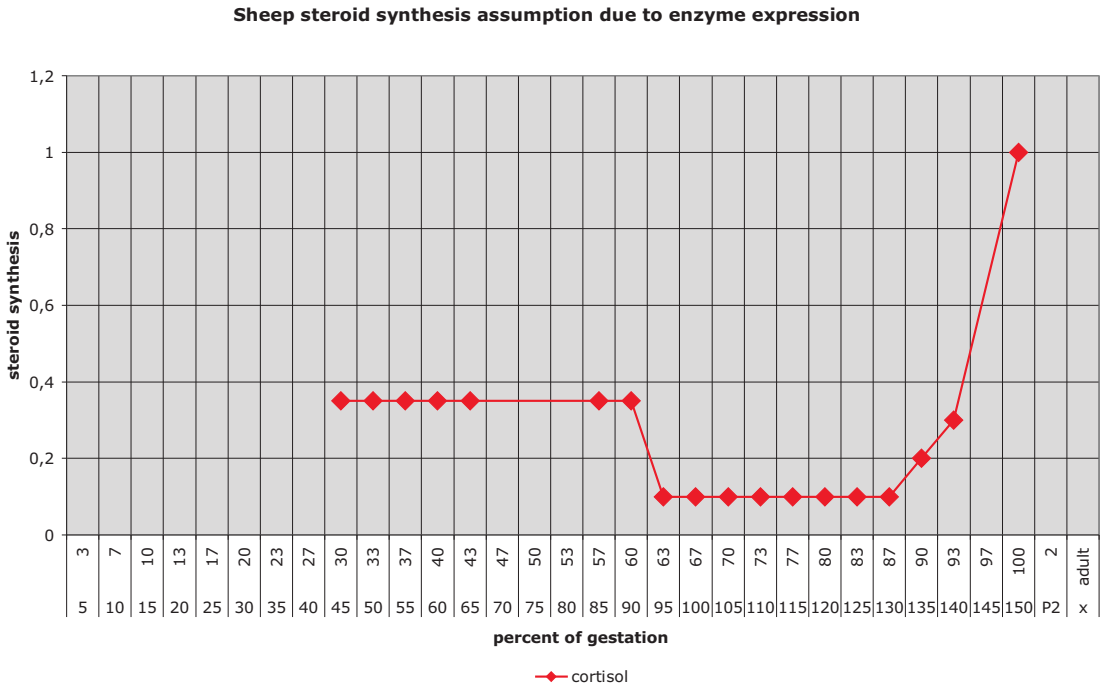


Figure 3.14. Sheep steroid synthesis assumption due to enzyme expression

*Like in primates, the fetal sheep seems able to produce adrenal cortisol early and late in gestation with an intermediate period of low or absent synthesis. From 30% to 60% of gestation, moderate cortisol synthesis can be assumed. Between 63-83% of gestation, very low enzyme expression indicates at best low cortisol synthesis. In late gestation between 87-93%, cortisol synthesis can be assumed to gradually increase and continue with a dramatic increment between 93-100% of gestation.*

### 3.3.6 Guinea pig fetal adrenal steroid enzyme expression

*Investigating adrenal enzyme expression in the fetal guinea pig is problematic, due to the very rare data. Postnatal the amount of valuable data is slightly better. The only information about fetal steroid enzymes regards 3βHSD.*

#### 3.3.6.1 Guinea pig CYP11A1 expression

No data are available in this respect for the fetal adrenal cortex of the guinea pig. After birth, at 71% (PND15) of weaning, IR-CYP11A1 was present in the zG, but no information is available about the other cortical zones [243]. Between wk5-14, CYP11A1 activity decreased significantly ($P<0.05$) in the zR, but did not change in the outer zone (zF+zG). This decrement was proportionally greater than the zR age matching decrease of CYP17 or CYP11B activity. The decrease in CYP11A1 activity in zR was closely correlated to the decrease in adrenal cortisol secretion in the zR over the same time period [94]. In the adult guinea pig adrenal cortex, immunostaining for CYP11A1 was higher in the outer zone (zF+zG) than in the inner zR, or more precisely CYP11A1 activity was greater in zF than in zR [94, 140, 141].

*After two weeks of life, CYP11A1 is present at least in the zG. Enzyme activity can be assumed in zF and possibly in the developing zR as well. Between wk5-14, CYP11A1 activity decreases dramatically in the zR, together with cortisol synthesis in this location. In adulthood, IR-CYP11A1 levels are higher in zF than zR.*

#### 3.3.6.2 Guinea pig CYP17 expression

No information was existent about fetal guinea adrenal CYP17 expression. CYP17 activity significantly ($P=0.032$) increased between 33% (PND7) of weaning and wk7 in the whole adrenal cortex of the male guinea pig [514, 515]. At 71% (PND15) of weaning, CYP17 staining was present in zF and zR. Intensity of staining was stronger in the zF than the zR [243]. Looking at the single cortical zones, Colby et al. 1993 detected a significant ($P<0.05$) decrease in CYP17 activity in the male zR between wk5-29 [94]. In adult guinea pigs, CYP17 was present in the zF and zR cells. Immunostaining was stronger in zF than zR [94, 141, 206].

*At least after two weeks of life, CYP17 is present in the zF and zR. Staining intensity of CYP17 is stronger in the zF than in the zR. From 33% of weaning until wk7, CYP17 staining increased in both genders in the whole adrenal cortex. Subsequently activity decreases significantly from wk5 until adulthood in the male zR.*

#### 3.3.6.3 Guinea pig 3βHSD expression

For the enzyme 3βHSD, we are finally able to present data of the fetal adrenal cortex. Unfortunately in the guinea pig, 3βHSD is not only involved in adrenal cortisol and additionally in aldosterone synthesis like in the other species, but also in androstenedione production. 3βHSD activity was detected in the fetal guinea adrenal at 41% (day28) of gestation [49]. In the adrenal cortex of the fetal guinea pig, 3βHSD activity was present in moderate levels at 46% (day31), 66% (day45) and 73% (day50) of gestation [372]. In the adult guinea pig, 3βHSD was apparent in all cortex cells, with strong immunostaining in zF and zR and weak intensity in zG [137, 206].

*In the fetal guinea pig adrenal cortex, 3βHSD activity is present at least between 41% and 73% of gestation, which is necessary for adrenal cortisol, but also aldosterone and androstenedione synthesis. In adult guinea pigs, enzyme immunostaining is strong in zF and zR, but week in zG.*

#### 3.3.6.4 Guinea pig CYP21A2 expression

No information is available about fetal adrenal CYP21A2 expression. Between wk5-29, CYP21A2 activity was higher in the zR than in the zF+zG, but did not change over time [94]. ACTH did not

affect CYP21A2 activity in any cortical zone [275]. CYP21A2 was detectable in all adult cortex cells. Its immunoreaction was slightly stronger in zR than in zF [94, 141, 206].

*Between wk5 and adulthood, CYP21A2 activity is strongest in the zR, but does not change in any zone over that period. The enzyme activity does not respond to ACTH.*

#### 3.3.6.5 Guinea pig CYP11B expression

Again data are missing for the fetal period. Between wk5-29, CYP11B activity was greater in the zF+zG than in the zR. Over this period, CYP11B activity decreased in the zR significantly ($P<0.05$), but not dramatically [94].

*CYP11B activity is stronger from 5 weeks of life until adulthood in the zF+zG than in the zR. Over that period, the enzyme activity decreases moderately in the zR.*

#### 3.3.6.6 Summary guinea pig enzyme expression

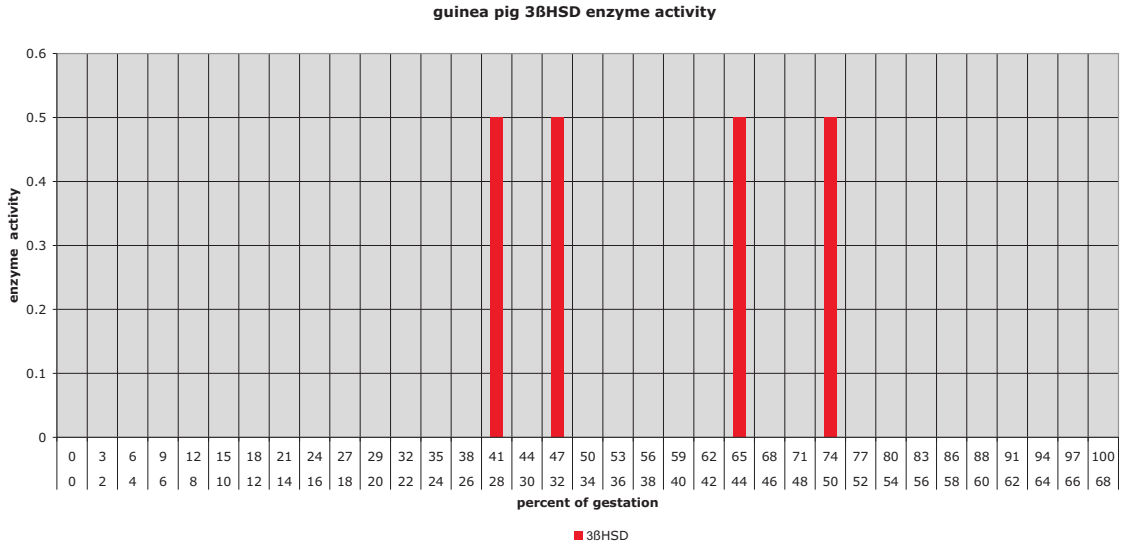


Figure 3.15. Guinea pig 3$\beta$HSD enzyme activity

*The data about adrenal steroid enzyme expression in the fetal guinea pig are more than scarce and only covers the presence of 3$\beta$HSD in assumingly moderate levels between 41-47% and again 65-74% of gestation. Unfortunately, nothing is known about the zonal distribution of this enzyme. In the guinea pig, androstenedione instead of DHEAS is the major androgen synthesized. This makes the enzyme necessary for cortisol, aldosterone and androstenedione synthesis.*

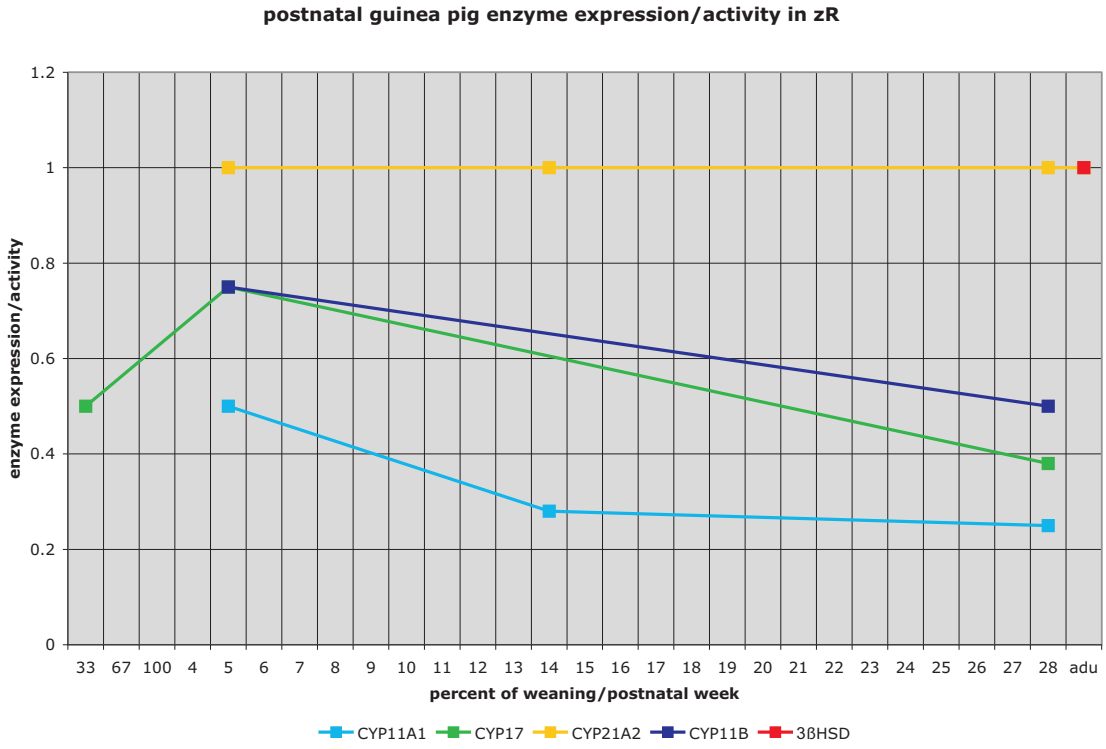


Figure 3.16. Postnatal guinea pig enzyme expression/activity in zR

*No postnatal change in enzyme activity is apparent in the zF. On the other hand, postnatal in the zR, CYP17 activity increases significantly in male guinea pigs between 33% (PND7) of weaning and wk5. Subsequently, activity of CYP11A1, CYP17 and CYP11B decrease between wk5-29, respectively already by wk14 for CYP11A1. As cortisol is the only of the three adrenal steroids requiring all three enzymes, assumingly cortisol synthesis dramatically decreases in the zR over that period.*

*After birth, cortisol synthesis seems to be possible in both the zR and the zF, due to the presence of all required enzymes (CYP11A1, CYP17, 3βHSD, CYP21A1 and CYP11B) in either zone. The synthesis of cortisol seems to be stronger in the zF than in the zR, because activity of CYP17 and CYP11A1 is less in the zR than in the outer zone. Other than DHEAS synthesis, which requires only CYP11A1 and CYP17 expression, the guinea pig typical androgen androstenedione requires additionally 3βHSD.*

*After birth, androstenedione synthesis could be possible in zF and zR, but due to the lower activity of CYP11A1 and CYP17 in the zR, androstenedione might be produced to a lesser extent in the zR than the zF. With the decrease of CYP17 and CYP11A1 activity in the zR between wk5-29, androstenedione production in the zR could decrease as well.*

*Between wk5-14 and possibly further until wk29, cortisol production can be assumed to decrease in the adrenal cortex, due to the decrement in CYP11A1, CYP17 and CYP11B activity. During the same time, androstenedione synthesis in the zR might decrease as well.*

### 3.3.7 Rat fetal adrenal steroid enzyme expression

#### 3.3.7.1 Rat CYP11A1 expression

The enzymatic requirements for the first step in adrenal steroidogenic activity, CYP11A1 expression, seemed to be already present in rat fetal adrenal gland as early as 55% (e12) of gestation. The enzyme expression had markedly increased between 68-73% (e15-16) by 2.4 fold, and remained high at least through 82% (e18) of gestation [389]. At 73% (e16) and 82% (e18) of gestation, stronger CYP11A1 mRNA was present in zF than in the zG. At 5% (PND1) of weaning, enzyme expression was located in the zF cells. The intensity of CYP11A1 mRNA signal at 5% (PND1) of weaning was weaker than at 82% (e18) of gestation, but had intensified again by PND25 [304]. In Malee et al. 1999, CYP11A1 mRNA expression was very low at 5% (PND1) and increased significantly ($P<0.001$) by 4 fold until 33% (PND7) of weaning. At 67% (PND14), there was a slight but significant ($P=0.013$) increase by 1.4 fold but no significant change by 100% (PND21) of weaning [269].

*CYP11A1 is detected in the fetal rats adrenal gland as early as 55% of gestation. Between 68-73%, the expression increases and remains high at 82% of gestation. During that period, CYP11A1 expression is stronger in the zF than the zG. After birth, the enzyme expression has markedly decreased by 5% but has increased strongly again by 33% and further by 67% of weaning. There is no more change at 100% of weaning.*

#### 3.3.7.2 Rat CYP17 expression

The expression of CYP17 is necessary for cortisol synthesis. The fetal mouse was able to express adrenal CYP17 during a short period in the second half of gestation [232]. However, between 55-75% (e12 and e16.5) of gestation, Rogler et al. 1993 failed to detect CYP17 expression in the fetal rat adrenal gland [389]. CYP17 immunoreactivity was undetectable in the adrenals at 5% (PND1) of weaning and at PND31 [243].

*CYP17 expression is not detected in the fetal rats adrenal cortex between 55-75% of gestation nor is CYP17 protein measurable directly after birth or at the end of the first month of life.*

#### 3.3.7.3 Rat 3$\beta$HSD expression

Due to Schlegel et al. 1967, 3$\beta$HSD activity was apparent in the rat fetal adrenal cortex between 77-100% (e17-22) of gestation [409]. 3$\beta$HSD mRNA expression was absent at 64-68% (e14-15), but enzyme expression was first apparent at 73% (e16) of gestation. At 77% (e17) of gestation, first 3$\beta$HSD protein was detected in the fetal rat adrenal cortex. Between 82-95% (e18-e21) of gestation, enzyme mRNA and protein were detectable. Over that period, 3$\beta$HSD expression and immunoreactivity was strong in the zF. At 2.4% (PND0.5), 14% (PND3), 24% (PND5) and 71% (PND15) of weaning the same picture occurred, with strong expression and immunoreactivity in the zF [138].

*3$\beta$HSD mRNA expression in the fetal adrenal cortex of the rat starts at 73% and 3$\beta$HSD protein is discovered at 77% of gestation. Between 82-95% of gestation, both mRNA and immunoreactivity are strong in the zF. After birth, expression in the zF remained strong.*

#### 3.3.7.4 Rat CYP21A2 expression

CYP21A2, an enzyme necessary for glucocorticoid but also aldosterone synthesis, was present at 73% (e16) and 82% (e18) of gestation [303, 304].

#### 3.3.7.5 Rat CYP11B1 expression

CYP11B1 is merely necessary for glucocorticoid production in the zF. CYP11B1 mRNA and protein were present as early at 77% (e17) (the first day investigated) and were detectable between 77-95% (e17-21) of gestation and 5% (PND1) of weaning in the adrenal [502]. CYP11B1 mRNA

expression was present at 77% (e17) and 91% (e20) of gestation only in the zF. At 91% (e20) of gestation, CYP11B1 positive cells now built a zone under the zG. At 100% (e22) of gestation, CYP11B1 was detectable in the adrenals [297]. At 5% (PND1) of weaning, CYP11B1 mRNA signal intensity was weaker than at 91% (e20) of gestation, but has intensified again at PND25 [304]. Malee et al. 1999 detected significantly (P=0.002) lower CYP11B1 mRNA expression at 5% (PND1) than at 33% (PND7) of weaning by roughly 2 fold, but expression did not change significantly further at 67% (PND14) and at 100% (PND21) of weaning [269]. Amounts of CYP11B1 protein were similar at 77% (e17), 96% (e21) of gestation as well as at 5% (PND1) and 48% (PND10) of weaning. The intensity of CYP11B1 staining did not differ between 91% (e20) of gestation and 5% (PND1) of weaning [304].

*CYP11B1 expression and protein in the fetal adrenal cortex is confirmed from 77% of gestation onward. Staining intensity is mostly present in the zF, as the enzyme is required for glucocorticoid synthesis. Enzyme expression is strong at 82-91% and weak at 77% and 100% of gestation as well as 5% of weaning. By 33%, enzyme expression has increased but remains constant until 100% of weaning, to intensify again at PND25.*

#### 3.3.7.6 Summary rat enzyme expression

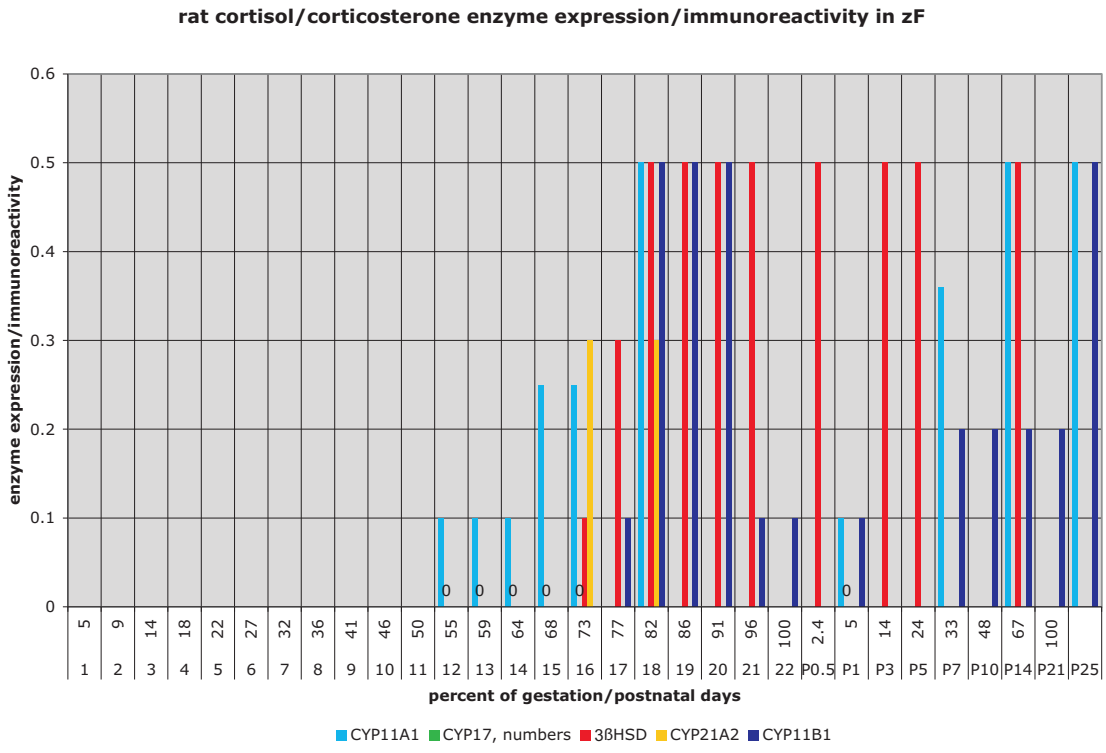


Figure 3.17. Rat cortisol/corticosterone enzyme expression in zF

*Between 55-73% of gestation and at 5% of weaning, cortisol synthesis due to undetectable CYP17 expression is assumingly absent, but cannot be excluded between 77-100% of gestation. 3βHSD expression is undetectable at 64-68%, but at 73% of gestation, corticosterone synthesis is assumed due to the appearance of 3βHSD, and the presence of CYP11A1 and CYP21A2. After 73% of gestation,*

*CYP11A1, 3βHSD and CYP11B1 expression seems to intensify and to be robustly expressed around 82-91% of gestation. Around 100% of gestation, CYP11B1 expression assumingly decreases. Both CYP11A1 and CYP11B1 expression are low at 5% and have increased at 33% of weaning. By 67% and 100% of weaning, expression of CYP11B1 remains moderate to increase again by PND25. Due to its enzyme expression, corticosterone synthesis could start at 73% at low levels, increase by 82%, is robust around 86-91% and has decreased again towards term. At 5%, corticosterone synthesis can be assumed to be low, increases slowly by 33%, remains constant until 100% of weaning to increase again by PND25.*

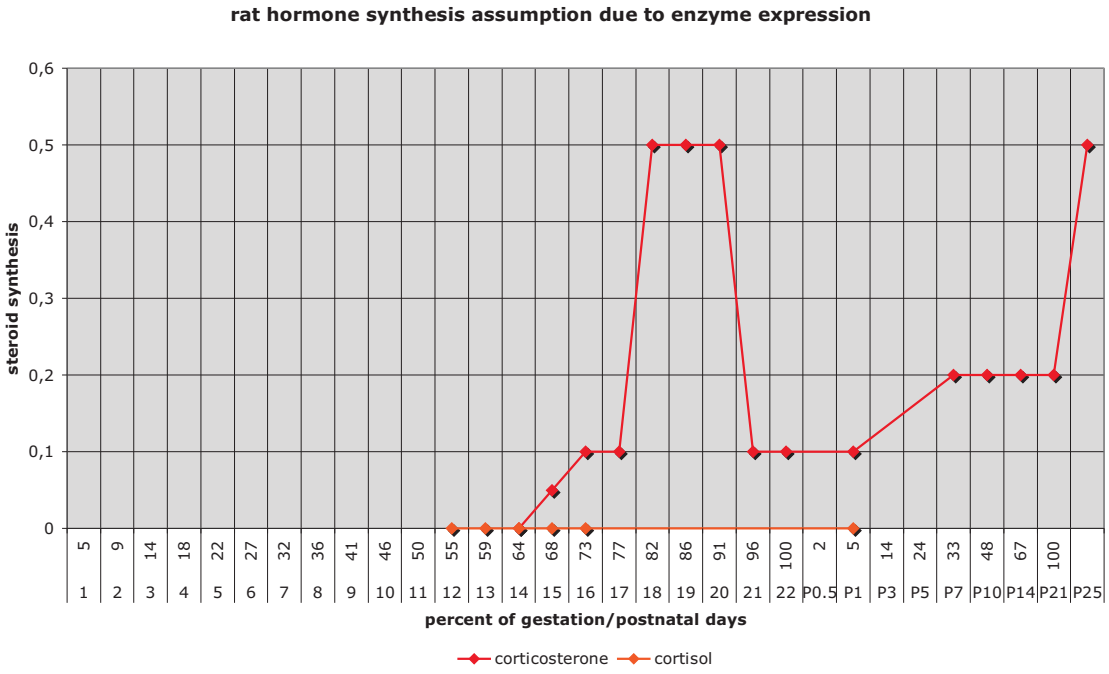


Figure 3.18. Rat hormone synthesis assumption due to enzyme expression

*A possible adrenal steroid synthesis due to enzyme expression could look like this. Cortisol synthesis is absent between 55-73% of gestation and at 5% of weaning, but production cannot be excluded between 77-100% of gestation. Corticosterone synthesis begins after 68%, remains low until 77%, and could dramatically increase to high levels by 82-91% of gestation. Subsequently enzyme expression could indicate a decrease to low levels toward term to remain low until 5% of weaning. In the following a small increase at 33% of weaning and a dramatic increase to high levels by PND25 could take place.*

### 3.3.8 Mouse fetal adrenal steroid enzyme expression

#### 3.3.8.1 Mouse CYP11A1 expression

The fetal mouse started to express CYP11A1 between 62-67% (e12-14) of gestation. At 74% (e14.5) of gestation, CYP11A1 expression was nearly uniformly distributed throughout the cortex and showed that pattern until birth and even in adults [232]. Postnatal, in both genders, CYP11A1 protein levels in the adrenal gland were similar at 100% (PND21) of weaning and at PND49, but increased significantly ($P<0.05$) between PND49 and PND63 [45].

*Fetal mouse CYP11A1 expression is present from 62-67% of gestation until term and is uniformly expressed in the cortex. Between PND49-63, CYP11A1 protein significantly increases.*

#### 3.3.8.2 Mouse CYP17 expression

In the adult mouse, there is no CYP17 expression in the adrenal gland, causing the glucocorticoid pathway to end in the mouse, similar to the rat, with the synthesis of corticosterone and not in cortisol production like in guinea pigs, sheep and primates [96]. CYP17 expression was apparent in the fetal mouse adrenal [185, 232], so adrenal cortisol production might be transiently possible in mice during gestation. Keeney et al. 1995 detected (in male fetuses) CYP17 expression first at 69% (e13.5) of gestation. CYP17 mRNA increased between 69-79% (e13.5-15.5) of gestation. At 79% (e15.5) of gestation, CYP17 expressing cells circumscribed the gland and CYP17 activity was detected, showing the presence of translation into functional protein. No CYP17 expression was detectable at 90% (e17.5) of gestation, in neonates or adult mice [232]. Heikkila et al. 2002 found a gender difference in the expression of this cortisol enzyme. CYP17 expression was present at 69% (e13.5), peaked at 79% (e15.5) (which was weak in males and more intensive in females) and disappeared in the male adrenal gland between 90-100% (e17.5-19.5) of gestation. In the female adrenals, CYP17 expression continued strongly until birth, was less abundant in newborns and absent in the adult female adrenal gland [185].

*In the fetal mouse, adrenal CYP17 is transiently expressed, which could indicate cortisol synthesis during that period. Strong gender differences are apparent. In both genders, CYP17 appears at 69% and increases until its peak at 79% of gestation, but levels are much stronger in the female compared to the male fetus. While in the male fetus, CYP17 expression disappears around 90% of gestation, in the female, it continues until term, where CYP17 is still strongly expressed. In the newborn female, CYP17 expression has decreased to low levels, and is absent in adult females. In the male newborn, CYP17 expression is absent and is not detectable in adult animals.*

#### 3.3.8.3 Mouse 3$\beta$HSD expression

Traces of 3$\beta$HSD activity were found in the fetal adrenal gland of the mouse already from 54-64% (e10.5-12.5) of gestation on. Stronger activity was present at 69-74% (e13.5-14.5) of gestation until term [180]. 3$\beta$HSD was strongly expressed in the adrenal gland at 79% (e15.5) of gestation in both genders [185]. From birth until PND70, 3$\beta$HSD activity is moderate in the zF [180]. Postnatal 3$\beta$HSD was detected in the zF of the male mouse at 100% (PND21) of weaning and PND75 and in the female mouse at PND60 [195].

*3$\beta$HSD is present in the fetal adrenal gland from around 60% of gestation. Expression increases at 69-74% and is high at 79% of gestation. After birth, 3$\beta$HSD is moderately expressed until adulthood.*

#### 3.3.8.4 Mouse CYP21A2 expression

CYP21A2 was already detected in the adrenal gland at 64% (e12.5) of gestation. It was present at least until 85% (e16.5) of gestation and was expressed in the newborn mice throughout the cortical layers [185, 232]. Raschella et al. 1989 detected in the entire cortex a steady increase in CYP21A1 transcription levels from 74% (e14.5) of gestation until expression reached a plateau on

high levels from 95% (e18.5) of gestation until term. Expression had significantly dropped at 24% (PND5) but had increased again by 48% (PND10) of weaning. The increase continued again to high levels at PND90 [376].

*CYP21A2 is present at 64% of gestation and increases to high levels from 74% until it has reached a plateau at 95% of gestation until birth. Subsequently, expression has decreased by 24%, increased again at 48% of weaning and further increased in adulthood.*

#### 3.3.8.5 Mouse CYP11B1 expression

Between 79-85% (e15.5-e16.5) of gestation, CYP11B1 expression was detected in the fetal mouse adrenal gland [232]. Moderately weak IR-CYP11B1 was predominantly observed in the zF on 82% (e16) and 92% (e18) of gestation. Especially at 82% (e16) of gestation, staining was not homogenous and was more intense in cells near the medulla. At 92% (e18) of gestation, staining was more equally distributed inside the inner zone and made up a larger area. Already at 72-77% (e14-15) of gestation, ACTH 1-24 treatment increased the number of cells expressing CYP11B1 and the staining intensity [519].

*CYP11B1 expression, necessary for glucocorticoid synthesis, is present by 79-85% of gestation, but can be induced already at 72-77% of gestation by ACTH administration. The enzyme is expressed in the zF.*

#### 3.3.8.6 Summary mouse enzyme expression

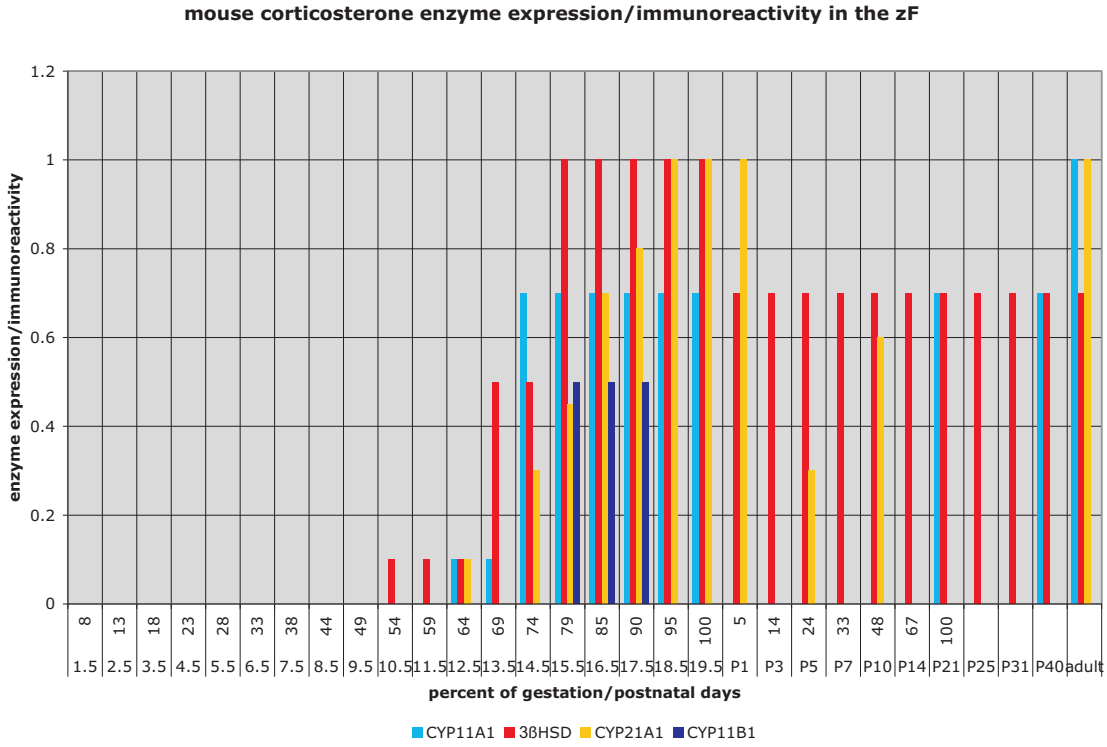


Figure 3.19. Mouse corticosterone enzyme expression in the zF

*Low corticosterone synthesis could be apparent at 54-59% due to 3βHSD expression but by 64% of gestation, additionally CYP11A1 and CYP21A2 indicate fetal adrenal corticosterone production. All three enzymes are necessary for cortisol synthesis in the zF as well. Corticosterone synthesis seems to increase from 64-79% of gestation and might be sufficiently apparent during 79-90% of gestation, due to the presence of the glucocorticoid specific enzyme CYP11B1. After birth, 3βHSD expression in the zF is lower than in late gestation. CYP21A2 expression is low at 24% and has increased slightly at 48% of weaning, but is stronger in adulthood, which together indicates decreased corticosterone synthesis after birth followed by an increase.*

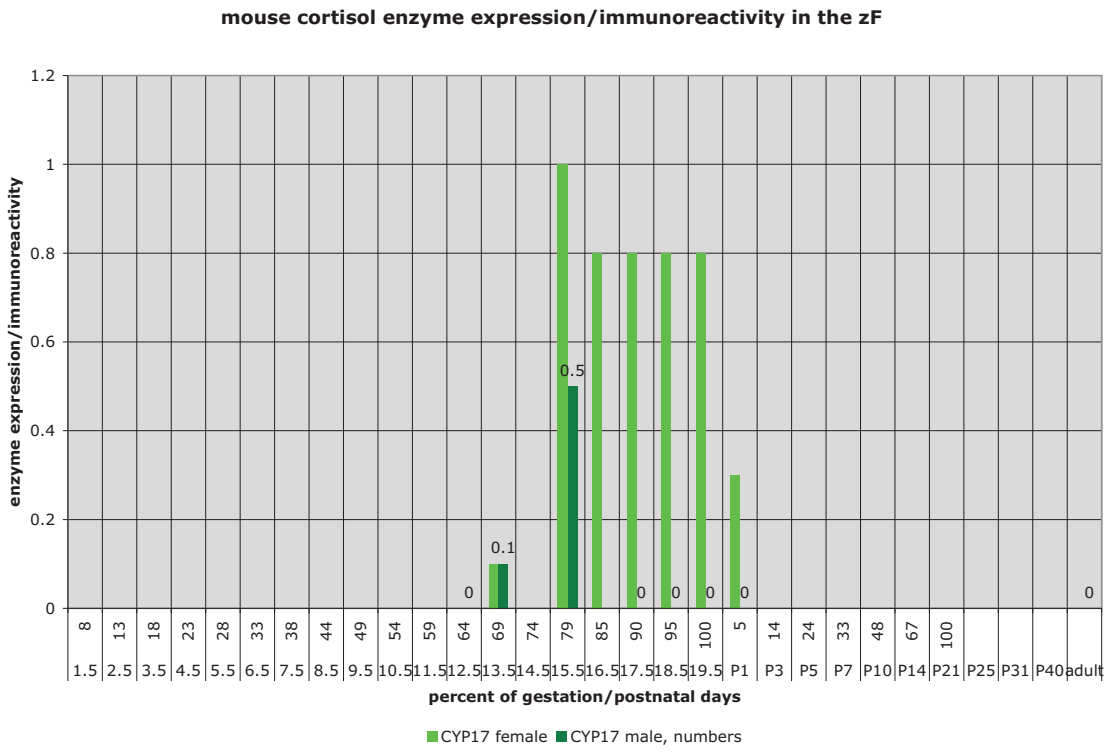


Figure 3.20. Mouse cortisol enzyme expression in the zF

*Expression of CYP17 is used as an indicator for cortisol synthesis. By that, cortisol synthesis could be absent at 64%, and start at 69% of gestation in both genders. CYP17 expression is maximal at 79% of gestation, but stronger in female fetuses than in males. In females, a possible cortisol synthesis could remain high until birth, decrease at 5% of weaning and is absent in adult. After the peak at 79% similarly to the female, cortisol production in the male fetus could decrease and be absent by approximately 90% of gestation until birth. At 5% of weaning and in adult males, no cortisol synthesis due to the lack of CYP17 expression is assumed.*

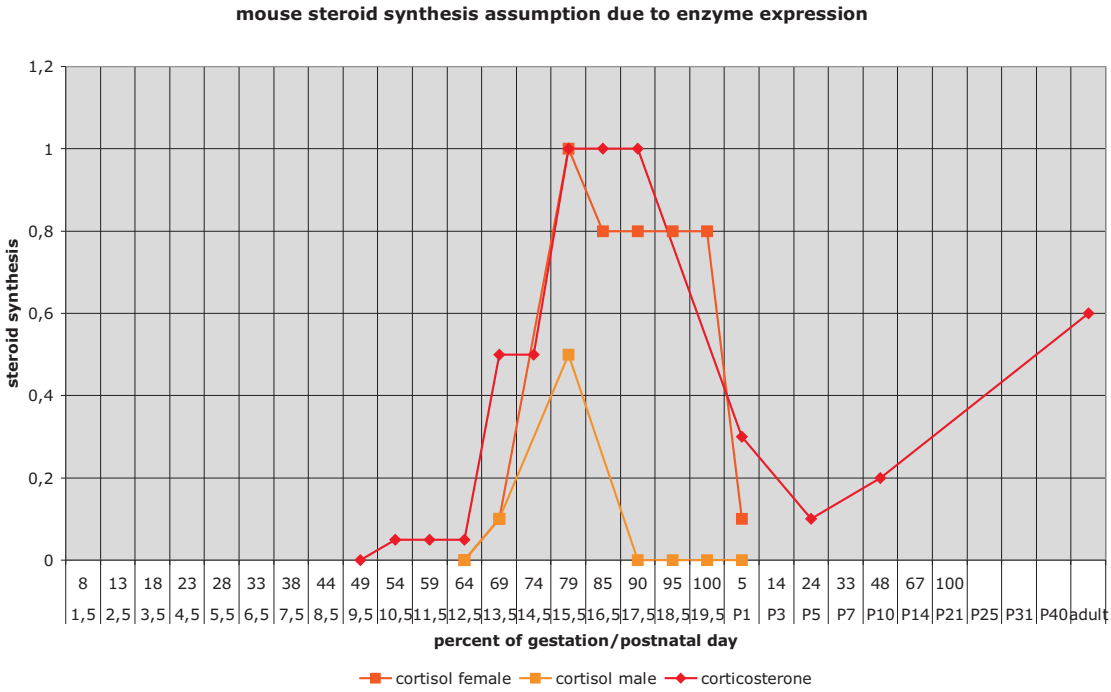


Figure 3.21. Mouse steroid synthesis assumption due to enzyme expression

*Fetal adrenal steroid synthesis due to enzyme expression could look like this. Corticosterone synthesis could be present by 54% and remain low until 64%, then increase to high levels between 79-90% of gestation. Enzymes indicate a decrease in corticosterone synthesis by 5%, low levels at 24-33% of weaning, but an subsequent increase toward adulthood. Cortisol synthesis due to CYP17 expression could be absent at 64% and low at 69% of gestation. In both genders, cortisol synthesis could peak at 79% of gestation, but at much higher levels in females than in males. In the male fetus, cortisol synthesis could disappear between 90-100% of gestation and would be absent in newborns. In the female fetus, levels might remain moderately high until term and decrease in newborns, according to CYP17 enzyme expression.*

## 3.4 Glucocorticoid synthesis in species

To examine the steroidogenic activity in the fetal adrenal gland, two different approaches are commonly used. One approach distinguishes the appearance of enzymes for adrenal steroid production (see Chapter 3.3). The second approach now will analyze the actual detection of adrenal steroid output.

### 3.4.1 Detection of fetal adrenal glucocorticoid synthesis

Measuring fetal plasma steroid concentrations as an indicator for fetal adrenal steroid synthesis involves the problem, that maternal glucocorticoids pass through the placenta into the fetal circulation [33, 316]. The same placental transfer is suspected for DHEAS and androstenedione [279]. Fetal cortisol/corticosterone concentrations can be detected in blood, taken from the umbilical arteries (Aa. umbilicales). These arteries transport blood, low in oxygen, from the fetus back to the placenta. The concentrations of plasma cortisol/corticosterone here can be compared with the levels in the venae umbilicales, which bring blood from the placenta to the fetus [388]. Even better, fetal adrenal cortisol production can be measured in the fetal adrenal vein or directly in fetal adrenal homogenates by radioimmunoassay (RIA). Maternal cortisol transfer across the placenta assumingly regulates fetal adrenal cortisol synthesis. It was suggested that maternal cortisol inhibits fetal ACTH release in the pituitary, which successively reduces fetal cortisol synthesis [347, 349, 352, 353].

### 3.4.2 Human cortisol synthesis

#### 3.4.2.1 Human fetal adrenal cortisol

Beside ‘de novo‘ cortisol synthesis from pregnenolone (requires the enzyme 3$\beta$HSD), cortisol can be produced from placental progesterone [169].

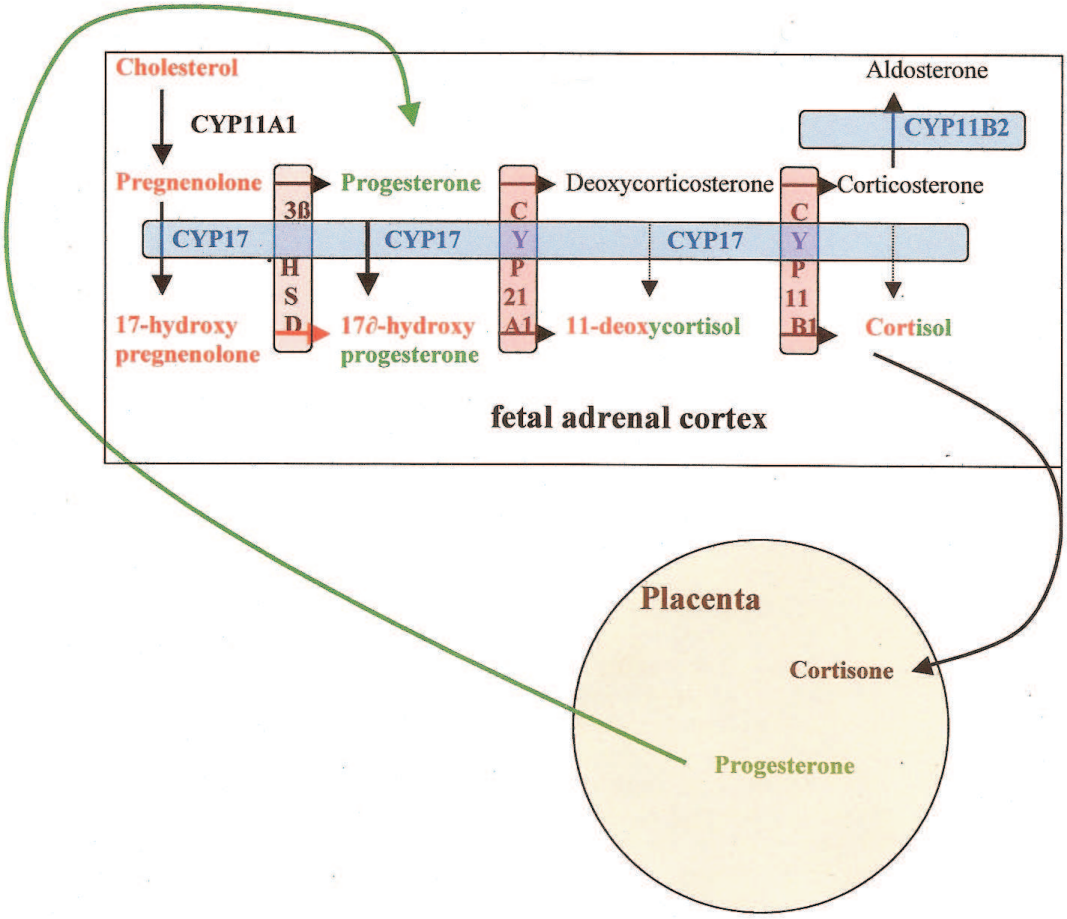


Figure 3.22. *De novo* cortisol synthesis

For 'De novo' cortisol synthesis see red writing, for cortisol synthesis from progesterone see green writing.

In the developing human fetal adrenal cortex, the FZ and the DZ can be distinguished by 20% (wk8) and the TZ by 25% (wk10) of gestation [211, 299]. By 20-23% (wk8-9) of gestation, medulla cells invade the fetal adrenal cortex and adrenalin and noradrenalin might stimulate steroidogenesis [56, 211] (see also Chapter 3.2.2.1). In fetal whole body homogenates, no cortisol was measurable at 14% (wk5.5) of gestation. Adrenal cortisol and cortisone were detectable at 21% (wk8.5) of gestation. At 28% (wk11), fetal adrenal cortisol concentrations were high and remained present until 45% (wk18) of gestation [315]. Recently, Goto et al. 2006 detected ACTH regulated 'de novo' cortisol synthesis in the fetal human adrenal already at 25% (wk10) of gestation. ACTH receptors were present in the fetal adrenal at this time point. Cortisol synthesis was maximal at 25% (wk10), but cortisol content per adrenal weight decreased significantly ($P<0.02$) by 2 fold between 25-30% (wk10-12) of gestation [169, 170].

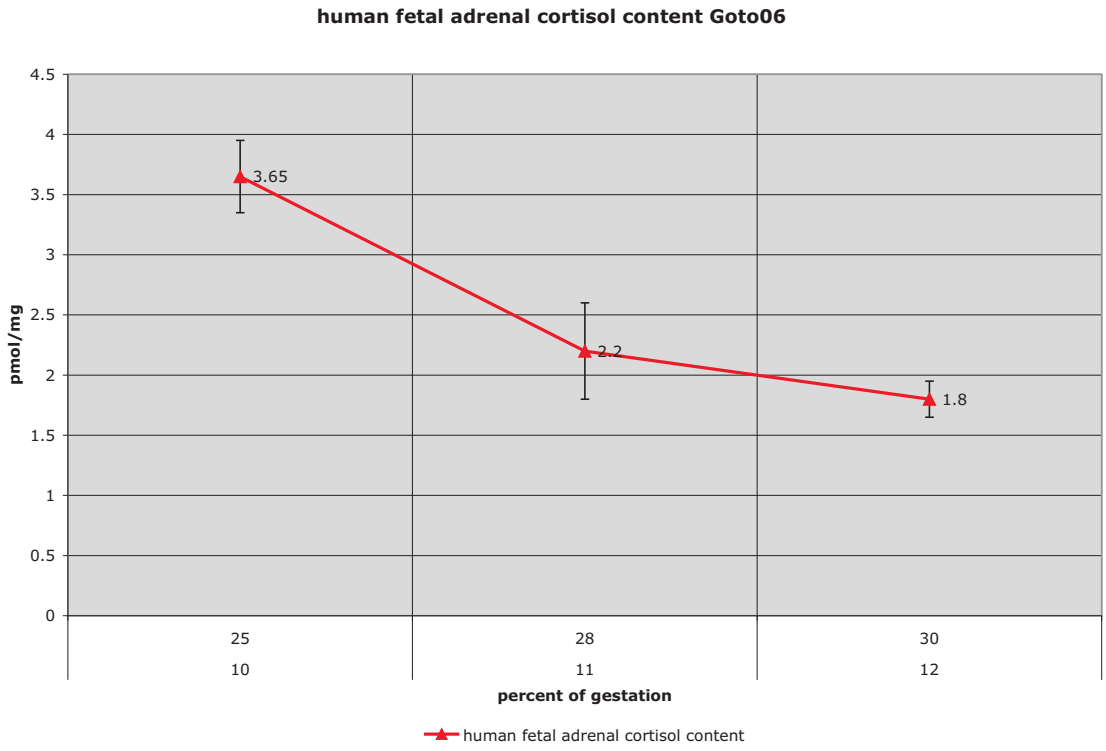


Figure 3.23. Human fetal adrenal cortisol content [170]

White 2006 illustrated the impact of early transient fetal adrenal cortisol synthesis on gonad maturation in humans (see also Chapter 3.5.1). The transient cortisol production between 25-35% (wk10-14) of gestation was necessary to inhibit the fetal adrenal DHEAS synthesis during the critical time of sexual differentiation, were high androgen levels could virilize the female fetus [492]. Goto et al. 2007 suggested that a decrease in human fetal adrenal cortisol synthesis in midgestation may prevent preterm labor, as active labor is associated with a significant increment in fetal plasma cortisol/DHEAS ratio [169, 332]. Between 40-45% (wk16-18) of gestation, cortisol synthesis from infused progesterone was present [259]. But this would imply that first, placental progesterone reaches the fetus during this period and second, placental progesterone is not solemnly used for fetal adrenal DHEAS synthesis but can also be used for cortisol production. Increasing fetal cortisol production is assumed approximately from 55% (wk22) of gestation until term [169, 235, 492].

*Fetal adrenal cortisol synthesis is detected as early as 21% of gestation. By 25% of gestation, fetal adrenal cortisol synthesis is high and ACTH dependent. This transient early fetal cortisol production seems to be necessary to constrain fetal adrenal androgen synthesis during sexual differentiation. The dramatic decrease in fetal adrenal cortisol synthesis between 25% and 30% of gestation and a possible low fetal cortisol production during midgestation could prevent premature organ maturation and initiation of labor. From 55% of gestation onward the fetal cortisol production is assumed to increase toward high levels at term.*

#### 3.4.2.2 Human maternal adrenal and plasma cortisol

During pregnancy, the maternal adrenal gland became hypertrophic and maternal zF cortisol production was increased, due to relative increasing maternal ACTH secretion. Still, diurnal plasma

cortisol variations were maintained. Maternal total and free plasma cortisol gradually increased during gestation, increased during labor and decreased approximately at 1% (PND4) of weaning. During the third trimester, maternal plasma cortisol concentrations were approximately 2-3 times higher compared to non-pregnant women. Maternal plasma cortisol concentrations significantly increased ($P<0.01$) from non-pregnant to 33% (wk11-15) of gestation by 1.6 fold [125, 276]. Plasma cortisol in maternal circulation was low at 18-23% (wk7-9), and increased significantly ($P<0.001$) by 2.8 fold between 23% (wk9) and 25% (wk10) of gestation. Maternal plasma cortisol levels were not significantly different between 28% (wk11) and 40% (wk16) of gestation. While the increment between 40-50% (wk16-20) by 2.3 fold failed to reach statistical significance ($P=0.058$), between 28% (wk11) and 50% (wk20) of gestation, a significant ($P=0.035$) increase was detectable [76]. After 58% (wk23) of gestation, maternal plasma cortisol did not change significantly until term or 0.3-1% (PND1-4) of weaning [125]. In Carr et al. 1981, maternal plasma cortisol levels remained constant from 50% (wk20) until 95% (wk38) of gestation. Between 95% (wk38) of gestation and labor (wk40), maternal plasma cortisol increased markedly ($P=0.05$) by 1.8 fold. Between labor and 0.5% (PND2) of weaning, maternal plasma cortisol decreased ($P=0.018$) by 2.4 fold [76].

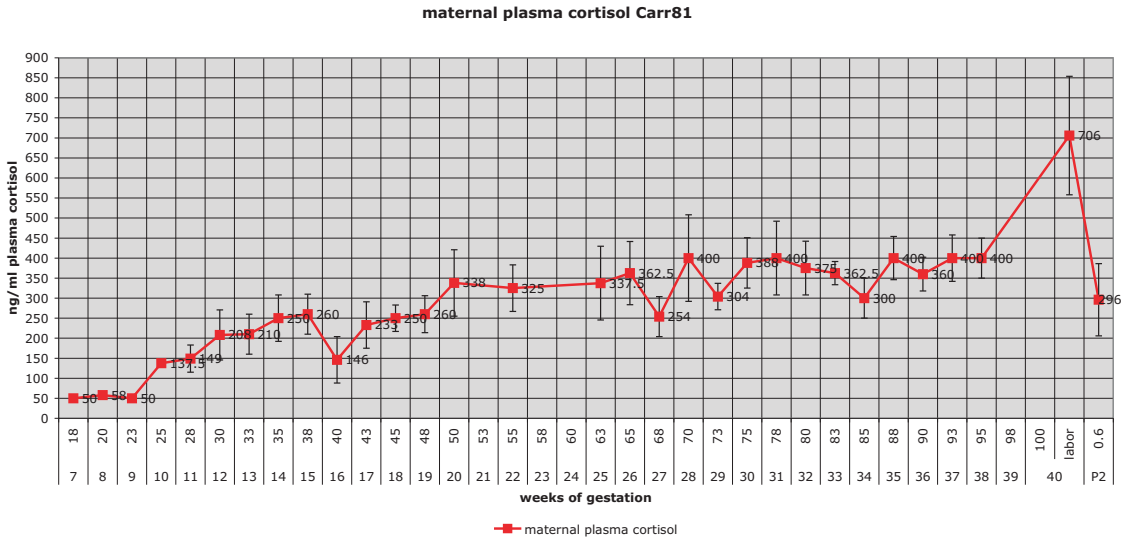


Figure 3.24. Maternal plasma cortisol Carr81

*Maternal plasma cortisol seems to remain on non-pregnant levels until 20-23% of gestation. Then the concentration increases until 50% of gestation. From 50-95% of gestation, maternal plasma cortisol concentrations remain on moderate levels, but increase dramatically between 95% and labor, to decrease again after delivery.*

### 3.4.2.3 Human fetal and neonatal plasma cortisol

The human (fetal) plasma cortisol concentrations from umbilical cord showed a significant ($P=0.005$) decrease by 2.1 fold between 40% (wk16) and 47% (wk18.8) of gestation [314]. Fetal plasma cortisol remained low (around 1 mug/100ml) and constant from 44% until 49% (wk17.5-19.4), then it increased significantly ($P<0.001$) by roughly 3 fold between 49-60% (wk19.4-23.8), reaching similar values than at 29-41% (wk11.5-16.5) of gestation [37]. Murphy et al. 1982 showed that by 89% (wk35.5) of gestation, mean fetal plasma cortisol had significantly ($P<0.001$) increased by 4.8 fold compared to 47% (wk18.8) of gestation. A further rise ($P=0.002$) took place between

94-100% (wk37.5-40) of gestation by 2 fold [314, 332]. Similarly, after 90% (wk36), fetal plasma cortisol increased until 100% (wk40) of gestation significantly (P<0.0001) by 2 fold. Active labor significantly (P<0.0001) increased fetal plasma cortisol by nearly 2 fold compared to comparable gestational time not in labor [332].

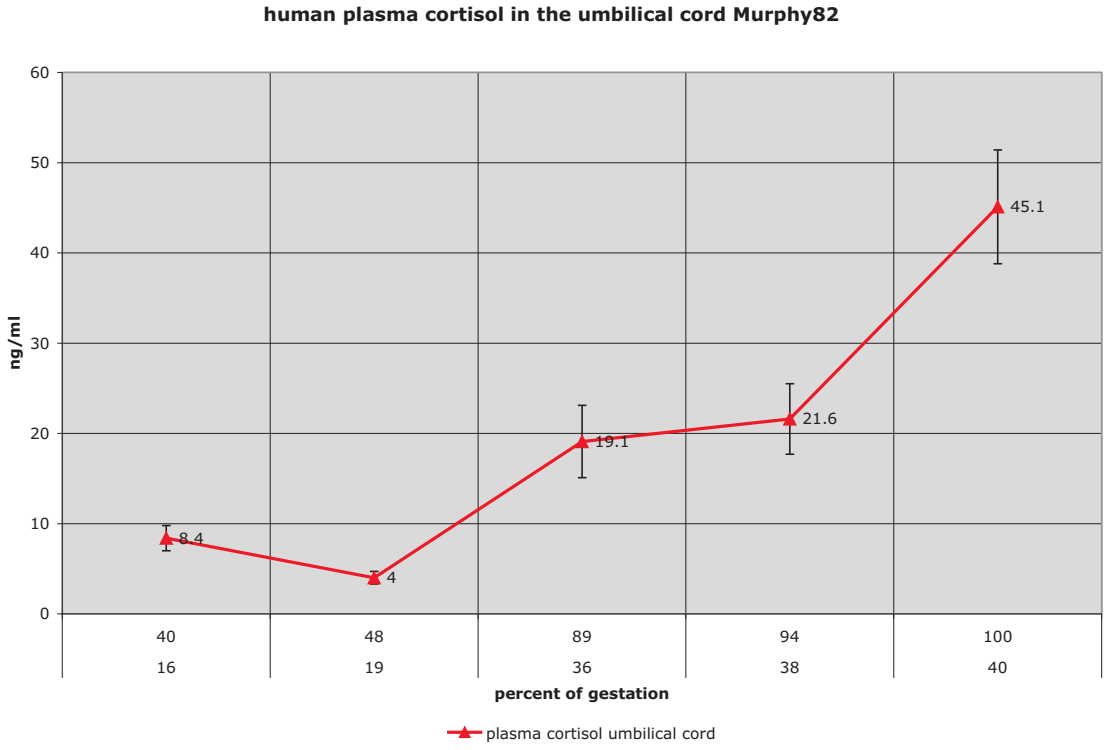


Figure 3.25. Human plasma cortisol in the umbilical cord [314]

Human neonatal mean plasma cortisol concentrations had decreased by 5.3 fold between birth and 0.3% (PND1) of weaning [497]. Sippell et al. 1978 and Doerr et al. 1992 investigated the early postnatal development of neonatal plasma cortisol from birth to 2% (PND7) of weaning and found surprisingly similar values [129, 438]. At birth, cortisol concentrations from the umbilical vein were not significantly different to neonatal plasma cortisol levels 2 hours after birth. Between 2-6 hours after birth, neonatal plasma cortisol decreased significantly (P<0.01) by 3.7 fold to a minimum. At 12 hours (PND0.5), there was another significant (P<0.05) increase by 2.7 fold, followed again by a significant decrease (P<0.05) at 24 hours after birth (PND1) to roughly the same levels than at 6 hours. These fast but significant changes in neonatal plasma cortisol concentrations might be due to circadian changes after birth [438]. Cortisol levels did not significantly change between 0.3% (PND1), 1% (PND4) and 2% (PND7) of weaning [129, 438, 497]. By 25% (PND90), cortisol concentrations were still unchanged compared to 2% (PND7), but at 49% (PND180) of weaning, mean plasma cortisol levels had increased by 1.9 fold and further increased by 2.2 fold to mean adult values [497].

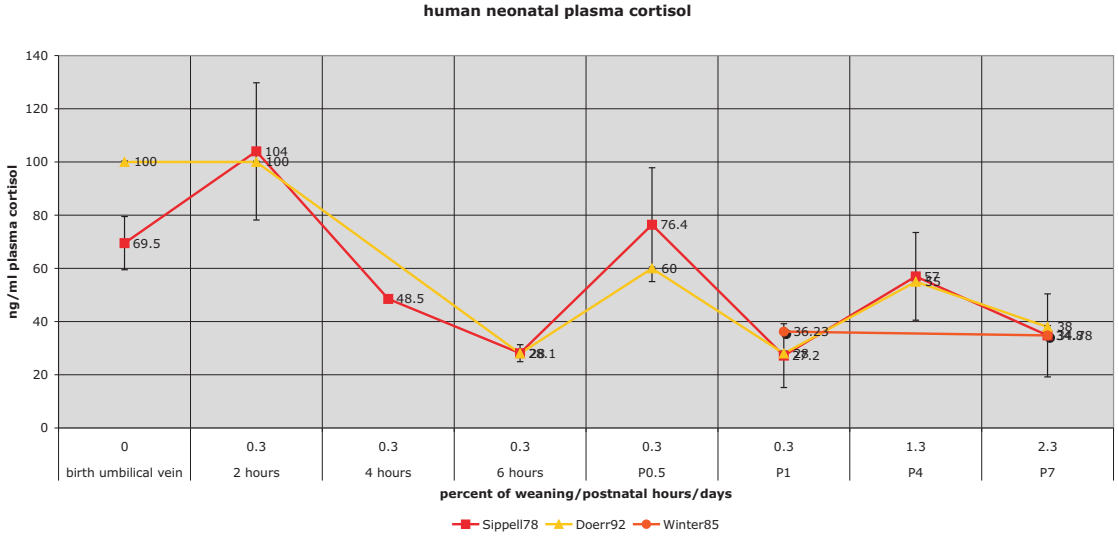


Figure 3.26. Human neonatal plasma cortisol

*Fetal plasma cortisol is always a mixture out of fetal and maternal adrenal cortisol released into the circulation. Still, the cortisol concentration in fetal plasma is the amount that reaches fetal organs, and elicits negative feedback in hippocampus, hypothalamus and pituitary. Maternal placenta transfer of cortisol is dependent on the impenetrability of the placenta barrier due to the expression of 11βHSD2. As direct measurement of fetal plasma cortisol is not possible, blood from the umbilical cord is used, excepting the problem of actually measuring fetal and maternal shares of cortisol in circulation. Beneficial, it opens the possibility to compare fetal with maternal plasma cortisol. Fetal plasma cortisol levels (umbilical cord blood) have significantly decreased between 40-47% of gestation. The concentration remains constantly low until it has increased again at 60% to similar levels than at 29-41% of gestation. From here on, fetal plasma cortisol concentrations seem to remain constant until 94-96% of gestation, when they increase dramatically until term and further during labor. After birth, plasma cortisol is a more valuable indicator for neonatal cortisol synthesis, as no maternal contribution is present. In the first two hours after birth, plasma cortisol concentrations seem to remain high, but by 6 hours of life, neonatal plasma cortisol levels decrease by 4 fold. Over the next 6 hours, plasma cortisol concentrations increase again but are back to 6 hours concentrations at the end of the first day. Plasma cortisol levels remain constant between 0.3-2% of weaning. The concentrations are similar at 25% but have increased by 49% of weaning, to 2 fold lower levels than in adulthood.*

#### 3.4.2.4 Human plasma cortisol ratio

At least between 43-88% (wk17-35) of gestation, there was a significant correlation ($P<0.0001$) between fetal (from fetal circulation) and maternal plasma cortisol. This correlation was assumed to be due to maternal cortisol transfer into the fetal compartment. Fetal plasma cortisol levels were about 13 times lower than the maternal plasma cortisol concentrations and around one third of the variation in fetal cortisol was attributable to maternal cortisol levels [160].

Between 33-45% (wk13-18) of gestation, cortisol in maternal circulation was 54 times higher than the concentrations of cortisol, after it passes through the placenta into the fetus (cortisol in umbilical vein). High levels of placental cortisol inactivation can be assumed between 33-45% (wk13-18) of gestation, because 85 % of cortisol infused into the mother is converted to cortisone in

the placenta [316]. The ratio of cortisol in umbilical artery to umbilical vein was 1.9 between 28-40% (wk11-16) and decreases to a ratio of 1 between 43-55% (wk17-22) of gestation. The plasma cortisol ratio in umbilical artery to umbilical vein decreased from 3.4 at 89% (wk35.5), to 1.7 at 93-99% (wk37-39.5) and 1.4 on 100% (wk40-40.5) of gestation [314]. Near term, maternal contribution to fetal cortisol was approximately 25 % and almost 90% of fetal cortisone originated from maternal transfer [37], while the fetal contribution to maternal cortisol was unknown. Following spontaneous term labor, plasma cortisol concentrations were significantly (P=0.004) higher in umbilical artery than vein, while the plasma cortisol ratio of artery to vein was only 1.3 [244].

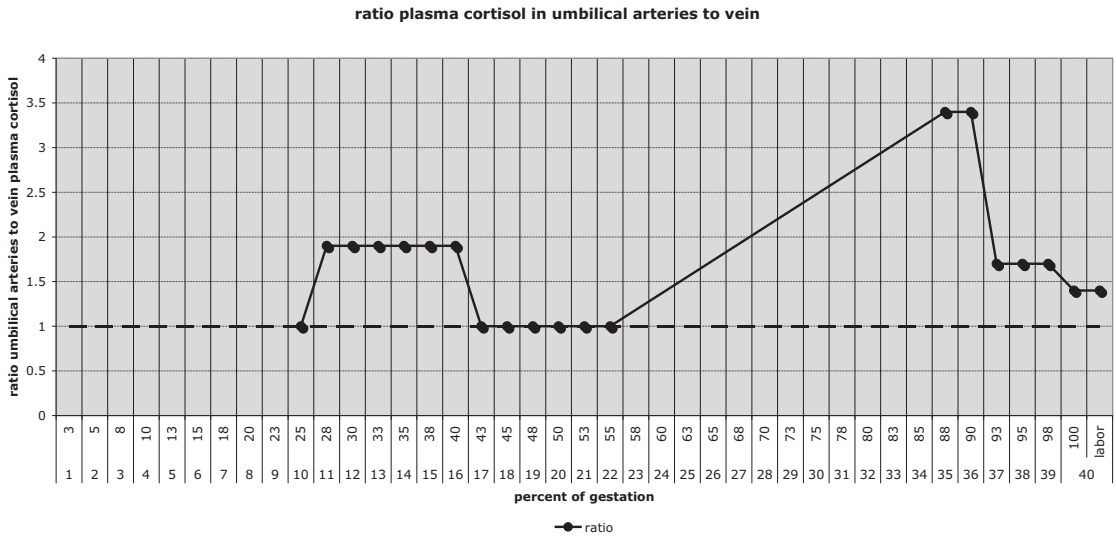


Figure 3.27. Human ratio plasma cortisol in umbilical arteries to vein

*It is very surprising to find from at least 25% of gestation until labor equally high or higher plasma cortisol levels in the umbilical artery compared to the umbilical vein, indicating at least similar transfer of cortisol in and out of the fetus. Between 43-88% of gestation, maternal plasma cortisol levels are 13 times higher than plasma cortisol in fetal circulation, and after placental transfer, maternal plasma cortisol between 33-45% of gestation is 54 times lower in the umbilical vein than in maternal circulation, indicating dramatic cortisol inactivation in the placenta before the hormone is released into the fetus. Early in gestation and later during pregnancy, more cortisol is transferred from the fetus to the placenta than vice versa. More preciously, during 28-40% of gestation, the fetus transfers nearly twice more cortisol to the placenta than the placenta into the fetus, assuming that the majority of maternal cortisol is inactivated in the placenta, before it can reach the fetus. After 40% until at least 55% of gestation, equal amounts of cortisol are transferred to and from the placenta. Unfortunately, there is no information about cortisol levels in umbilical vessels for the period from 55% to 89% of gestation. At 89% of gestation, it seems to be that 3.4 times more cortisol is transferred from the fetus to the placenta than vice versa. Until labor, this ratio decreased to approximately 1.3. Close to term, only 25% of fetal plasma cortisol originates from the mother, not knowing how much the fetus contributes to maternal cortisol.*

#### 3.4.2.5 Human plasma CBG and free plasma cortisol

When we investigate plasma cortisol and its active share of free plasma cortisol, we are bound to make a short excursion into the carrier protein for glucocorticoids. Transcortin or corticosteroid

binding globulin (CBG) is a 1-glycoprotein with high affinity to cortisol and corticosterone [388]. While hydrophilic hormones are mostly able to circulate in plasma in an unbound state, lipophilic hormones like cortisol and CRH require carrier proteins. Only a small fraction of a lipophilic hormone is soluble in water, their unbound or free fraction. Free and bound hormone concentrations together form the total hormone concentration in the plasma. In human circulation, 4% unbound (=free) cortisol and 89.3 % CBG bound cortisol is present. Free hormone is considered to be the biologically active portion, being able to bind to its receptors inside the cells after penetrating the cell membrane [221]. CBG knock out mice showed, beside the expected strong increase in free plasma corticosterone, increased pituitary activity, indicating an inability to respond to elevated free corticosterone levels via negative feedback in the absence of CBG [361]. During pregnancy, increasing estrogen production in the placenta induces hepatic CBG production [252].

- Human fetal plasma CBG and free cortisol

The CBG affinity constant for cortisol was significantly lower in fetal blood than in maternal circulation. At term, plasma CBG levels were 5 fold higher in maternal than in fetal circulation [39]. Fetal plasma CBG concentrations were extremely low during gestation compared to adult values. Fetal plasma CBG levels remained roughly constant between 55-78% (wk22-31), but increased by 85% (wk34) of gestation. By 1-8 hours after birth, neonatal plasma CBG was similar to 85% (wk34) of gestation. Neonatal plasma CBG values slowly but continuously increased, reaching adult levels between 12-22% (PND46-80) of weaning [448]. The low binding capacity for cortisol in fetal plasma at term remained until 8% (PND30), after which there was a significant ($P<0.001$) increase by 2.2 fold to adult levels between 16-100% (PND60-365) of weaning [172]. Percent of free cortisol in umbilical arteries increased significantly ($P<0.001$) by 2.2 fold from 0.35% of total cortisol at 25-50% (wk10-20) of gestation to 0.75% of total cortisol at spontaneous term labor [72].

*Fetal plasma CBG is extremely low during gestation compared to adults, assuming high levels of free cortisol in fetal circulation. Fetal plasma CBG increases from 78% to 85% of gestation. At term, fetal plasma CBG is still fivefold lower compared to maternal plasma levels. Free cortisol in fetal circulation increases during gestation. Between 16-22% of weaning, plasma CBG levels slowly and continuously increase to adult levels. During the first month of live, binding capacity for cortisol is extremely low but until the end of weaning, the capacity increases to adult levels.*

- Human maternal plasma CBG and free cortisol

CBG in maternal circulation increased from non-pregnant levels until 33% (wk13) of gestation, then reached a plateau from 33-45% (wk13-18) of gestation [125]. Ho et al. 2007 presented significantly ($P<0.001$) increasing CBG concentrations in maternal circulation by 1.8 fold between 45-95% (wk18-38), but a significant ($P<0.05$) decrement between 95-100% (wk38-40) of gestation [197]. Until 45% (wk18) of gestation, maternal free plasma cortisol did not significantly change from non-pregnant values [125]. In parallel with plasma CBG, maternal free plasma cortisol concentrations progressively and significantly ($P<0.001$) increased by 1.8 fold between 45-95% (wk18-38) of gestation. Between 95-100% of gestation, maternal free plasma cortisol concentrations increased significantly ($P<0.05$) by roughly 1.5 fold, due to both increased cortisol and reduced CBG levels [197].

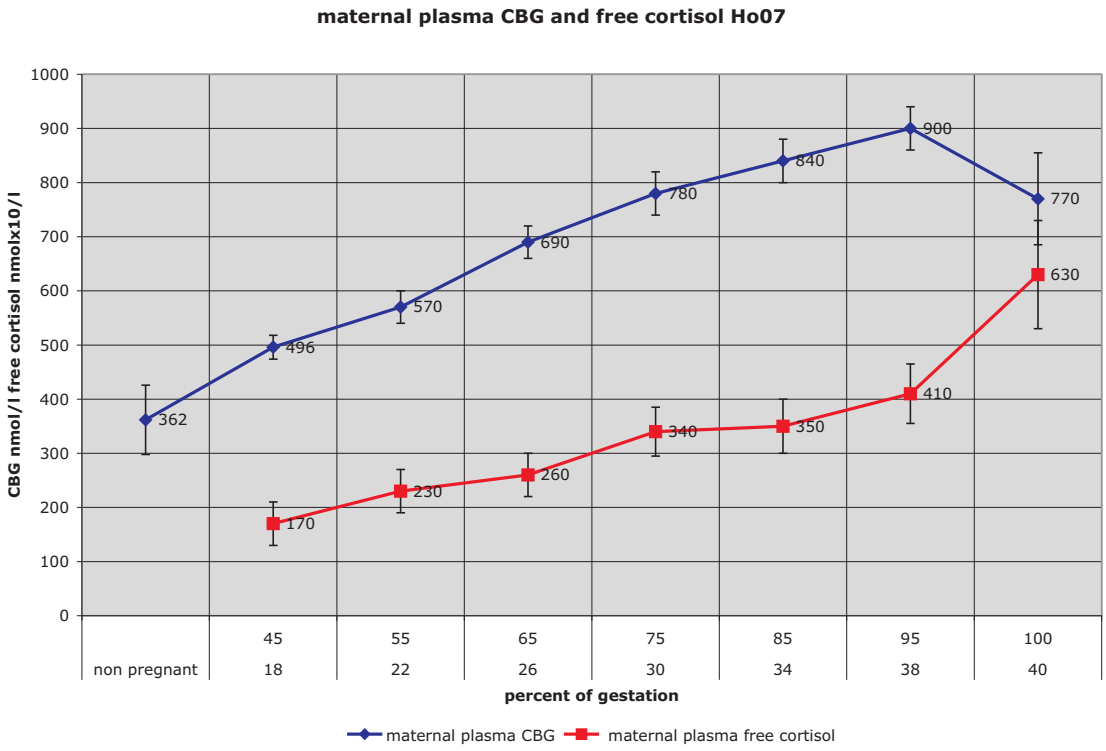


Figure 3.28. Human maternal plasma CBG and free cortisol [197]

*Maternal plasma CBG and free cortisol values increase in parallel during gestation. Over the last two weeks of gestation, a sudden decrease in plasma CBG levels causes an increase in free plasma cortisol levels.*

### 3.4.3 Summary human fetal cortisol

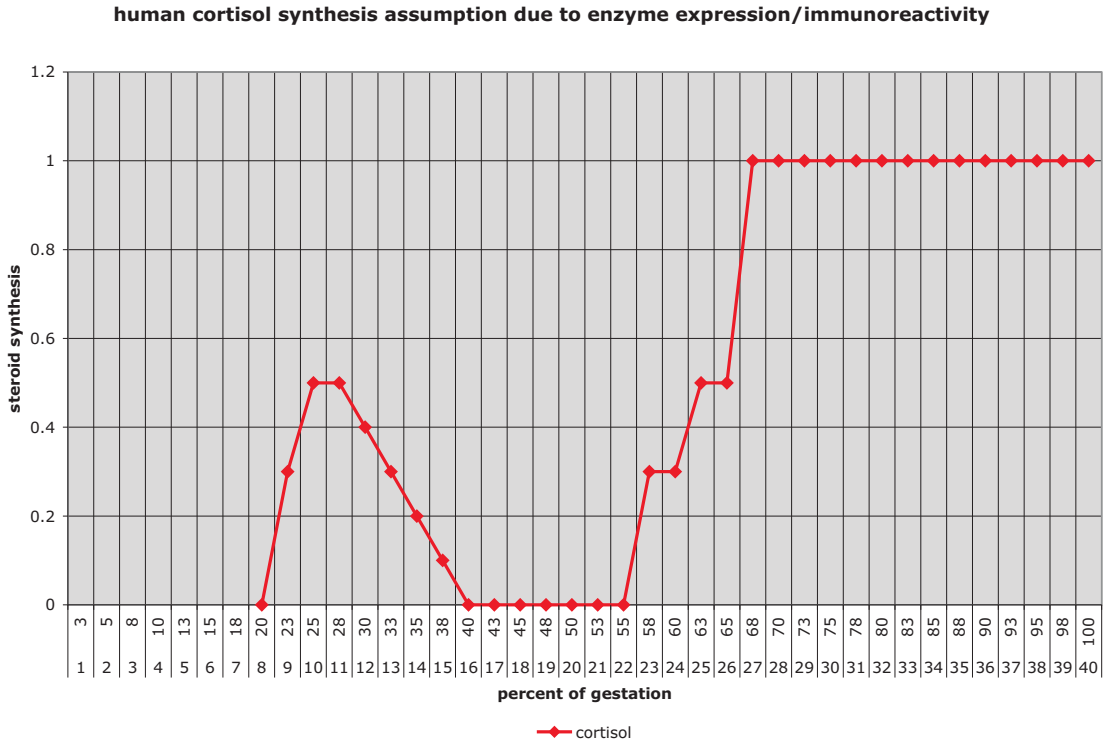


Figure 3.29. Human cortisol synthesis assumption due to enzyme expression

*Before summarizing the presented information, the data of cortisol enzyme expression/immunoreactivity will be shortly illustrated again. At 20% of gestation, most of the required enzymes for cortisol synthesis are absent in the TZ, not indicating cortisol synthesis at that early time. By 23%, IR-3βHSD is detected and by 25% of gestation, the additional presence of CYP17, CYP21A1 and CYP11B1/2 protein indicates cortisol synthesis. Moderate enzyme levels might remain until 30% of gestation, when 3βHSD protein starts to decrease and disappears at 40% of gestation. 3βHSD remains absent until low immunoreactivity is detected again at 58-60% of gestation and stronger expression is present thereafter until birth. Less cortisol might be synthesized during 23/25-40% of gestation than after 65% of gestation and over the period of 40-55% of gestation, cortisol production in the fetal human adrenal cortex seems to be absent or very low.*

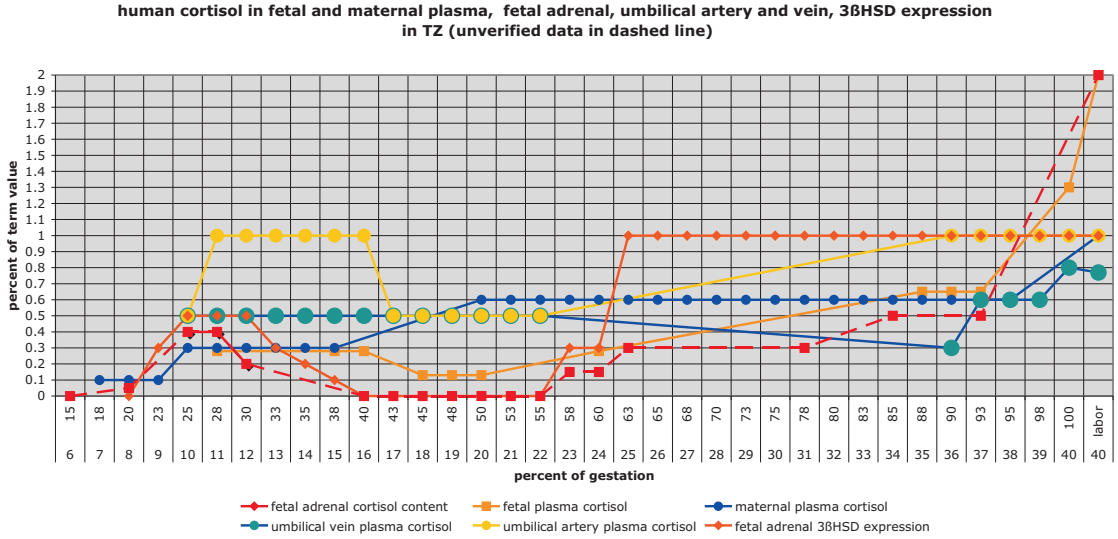


Figure 3.30. Human - summary of adrenal and plasma cortisol

*By trying to integrate fetal and maternal adrenal and plasma cortisol, free cortisol and plasma CBG as well as plasma cortisol in umbilical artery and vein and adrenal 3βHSD expression in the TZ, the following picture occurs. Maternal free cortisol fraction decreases by 33% of gestation. This might trigger the activation of fetal adrenal cortisol synthesis, as the fetal adrenal cortisol content increases assumingly between 20-25% in parallel with 3βHSD expression in the TZ and subsequently decreases between 28% and 30% of gestation. Surprisingly, while a decrease in maternal plasma cortisol around 40% of gestation fails to reach significance, fetal plasma cortisol decreases significantly between 40% and 47% of gestation. The ratio of plasma cortisol in umbilical artery to umbilical vein is very high (approximately 2) from 28% till 40% of gestation, which indicates higher transfer of cortisol from the fetus to the placenta than vice versa. Whether cortisol transport from the placenta into the fetus is low due to very high placental cortisol inactivation, due to fetal adrenal cortisol synthesis or both cannot be answered at this point. From 43% until at least 55% of gestation, plasma cortisol levels in umbilical arteries and vein are similarly high. Maternal plasma cortisol increases to moderate levels approximately by 50% of gestation, while cortisol in fetal circulation recovers to levels before the decrement by at least 60% of gestation. Fetal adrenal cortisol synthesis is assumed to increase again from 55% of gestation until term. Maternal plasma cortisol concentrations seem to remain constant until 95% of gestation. Already by 90% of gestation, plasma cortisol concentrations in umbilical artery are again much higher than in umbilical vein, which again gives the two possibilities of high fetal adrenal cortisol production and/or placental inactivation of maternal cortisol. From 93% of gestation till term, the ratio of plasma cortisol in umbilical artery to vein decreases, maybe due to increasing maternal cortisol levels or decreasing maternal cortisol inactivation in the placenta. Still, even at labor, the ratio of plasma cortisol in umbilical artery to vein is above 1, indicating more cortisol transfer from the fetus toward the placenta than vice versa, which suggests strong fetal cortisol production in late gestation. Both fetal and maternal plasma cortisol starts to increase dramatically from 93% respectively 95% of gestation until term labor. Between 95-100% of gestation, CBG concentrations in maternal plasma suddenly decrease, causing a dramatic increment in maternal plasma free cortisol fraction at that time. In the fetus, plasma CBG content increases between 55-78% and 85% of gestation. In general, CBG concentrations in fetal circulation are low and it can be assumed that all CBG molecules are saturated due to high fetal*

*plasma cortisol concentrations. Fetal free cortisol in circulation increases at labor. So at delivery, both maternal and fetal adrenal glands seem to produce high amounts of cortisol, with a large fraction of free plasma cortisol. A large magnitude of maternal cortisol reaches the fetus, but seems to be incapable to inhibit the firing fetal adrenal cortisol synthesis.*

### 3.4.4 Rhesus monkey cortisol synthesis

#### 3.4.4.1 Rhesus monkey fetal adrenal cortisol

By 27% (day45) of gestation, at least the FZ seemed to be actively steroidogenic and the DZ was composed of proliferative cells [291]. Latest by 44% (day73) of gestation, the fetal adrenal cortex was able to produce cortisol (measured in fetal adrenal vein) and cortisol synthesis resided from 53% (day87) of gestation till term. Fetal cortisol production significantly increased in presence of ACTH [238, 423]. Between 78% (day129) and 82% (day135) of gestation, minimal fetal cortisol synthesis from fetal plasma progesterone was present, and fetal progesterone secretion decreased with gestational age [133, 423], indicating increasing 'de novo' cortisol synthesis in the fetal adrenals toward term. In the last third (>67%) of gestation, the fetus produced 5 times more cortisone than cortisol. Nearly 50% of plasma cortisol originated from cortisone and roughly 80% of cortisol was metabolized to cortisone. The reduction of cortisone back to cortisol was assumed to be an important source of fetal cortisol, especially in late gestation, when fetal cortisol levels increase [305, 306]. At 80-83% (day132-137) of gestation, adrenal cortisol production rate and secretion rate (mg/day) was significantly ($P<0.001$) lower in the fetus than in the mother by 7.3 fold respectively 10.4 fold [305]. Between 93-96% (day154-159) of gestation, fetal adrenal cortisol production rate and secretion rate was even 15.3 fold respectively 33 fold lower ($P<0.001$) compared to its mother [237]. On the other hand, cortisol production rate was similar in the fetus at 80-83% (day132-137) of gestation and in the neonate at 0.3-2% (PND1-7) of weaning. Compared to adults, cortisol production in fetus and neonate was significantly ($P<0.05$) higher by 5.7 fold, respectively 4.4 fold [305].

*The rhesus monkey fetus is able to produce adrenal cortisol at least by 44% of gestation. Cortisol production is responsive to ACTH. Around 80% of gestation, cortisol is not synthesized from progesterone, so any cortisol synthesis in the fetal adrenal cortex at that time must use pregnenolone as a precursor (de novo cortisol). The synthesis of progesterone in the fetal adrenal gland decreases with gestational age. At least at 80-96% of gestation, the mother produces and secretes very high levels of cortisol compared to the fetus. Fetus and neonate produce equal amounts of cortisol but several times more cortisol than the adult rhesus monkey. The fetus in late gestation produces more cortisone than cortisol and the reduction of cortisone to cortisol might be an additional source of cortisol for the fetus.*

#### 3.4.4.2 Rhesus monkey fetal plasma cortisol

In the last trimester, the concentration of fetal plasma cortisol is 2.2 fold lower compared to cortisone [306]. Using fetal catheter plasma cortisol, Jaffe et al. 1978 and Serron-Ferre et al. 1978 did not detect a significant change between 78-87% (day129-144) of gestation. A significant increase ($P<0.05$) was detected in plasma cortisol between 87-90% (day144-149) of gestation by 1.4 fold. Another increase between 90% (day149) of gestation and labor of 2 fold failed to reach significance ($P=0.052$) [208, 424]. Coulter et al. 1993 confirmed the significant ($P<0.05$) increase between 84-93% (day138-153) of gestation by 2.1 fold [98]. Fetal plasma cortisol increased significantly ($P<0.05$) by 2 fold between 84-92.5% (day138-152.5) and again ($P<0.01$) by 2.5 fold between 96-99% (day159-164) of gestation [482]. Beside similar cortisol production rates, the plasma cortisol concentration was 3.2 fold lower ($P<0.001$) in the fetus at 80-83% (day132-137) of gestation, than in the neonates between 0.3-2% (PND1-7) of weaning, due to a very high metabolic clearance rate ($P<0.05$) in the fetus (3x higher compared to neonates, 4x higher compared to the mother). Plasma cortisol levels decreased between neonates and adults by 1.6 fold but the decrement failed to reach statistical significance. The cortisol metabolic clearance rate was significantly ($P<0.001$) higher by 2.3 fold in neonates compared to adults [305].

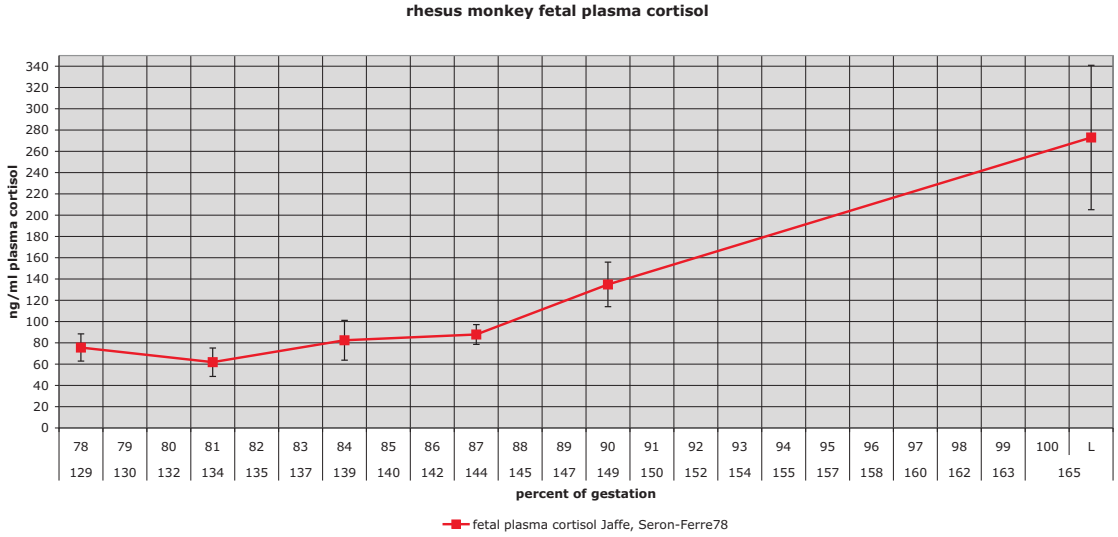


Figure 3.31. Rhesus monkey fetal plasma cortisol

*Unfortunately, no data are available for the fetal plasma cortisol concentration before 78% of gestation. Still, by using chronic catheterized fetal rhesus monkeys, relying on plasma cortisol from umbilical blood vessels can be avoided. Fetal plasma cortisol does not change between 78% and 87%, subsequently increases by 90% of gestation and seems to further increase until labor. While fetus and neonate synthesize similar amounts of cortisol, cortisol in circulation of the neonate is much higher, due to the very high metabolic clearance rate in the fetus. In the neonate, plasma cortisol concentration does not significantly differ from adult levels, but the metabolic clearance rate is still higher in neonates than in adults.*

#### 3.4.4.3 Rhesus monkey maternal plasma cortisol

Maternal plasma cortisol concentration showed a diurnal pattern with significantly higher values in the morning than in the evening [482]. Cortisol metabolic clearance rate was similar in pregnant and non-pregnant females [305]. Maternal plasma cortisol seemed to remain constant during gestation and hours before delivery [441, 482]. No significant differences in maternal plasma cortisol levels were detected between 18% (day30), 36% (day59) and 79% (day130), nor between 64-88% (day105-145) of gestation. Similarly no significant change was detectable between 72-93% (day119-153), or between 95% (day157) of gestation and 10 hours before delivery. After delivery until PND15, maternal plasma cortisol remained unchanged [84, 432, 441, 468].

*Surprisingly, maternal plasma cortisol seems to remain constant at least during the measured periods and until hours before birth as well as during roughly the first two weeks of lactation. Maternal plasma cortisol shows the normal diurnal variation with higher values in the morning as well as a similar metabolic clearance rate compared to non-pregnant females.*

#### 3.4.4.4 Rhesus monkey fetal-maternal plasma cortisol ratio

The approximate ratio of 0.3 between fetal and maternal cortisol was believed important in determining the length of gestation [237]. The fetal to maternal plasma cortisol ratio was 0.34 at 79% (day130) of gestation [441]. Jaffe et Seron-Ferre 1978 showed a ratio of fetal to maternal plasma cortisol of 0.23-0.38 between 78-87% (day129-144) and an increase in the ratio to 0.57 by 90%

(day149) of gestation, due to significantly increasing fetal plasma cortisol concentration [208, 424]. At 84% (day138), the fetal to maternal plasma cortisol ratio was 0.27 but at 93% (day154) of gestation, the ratio increased to 0.59 [98]. During labor, the ratio of fetal to maternal plasma cortisol had increased to 0.95 [208, 424].

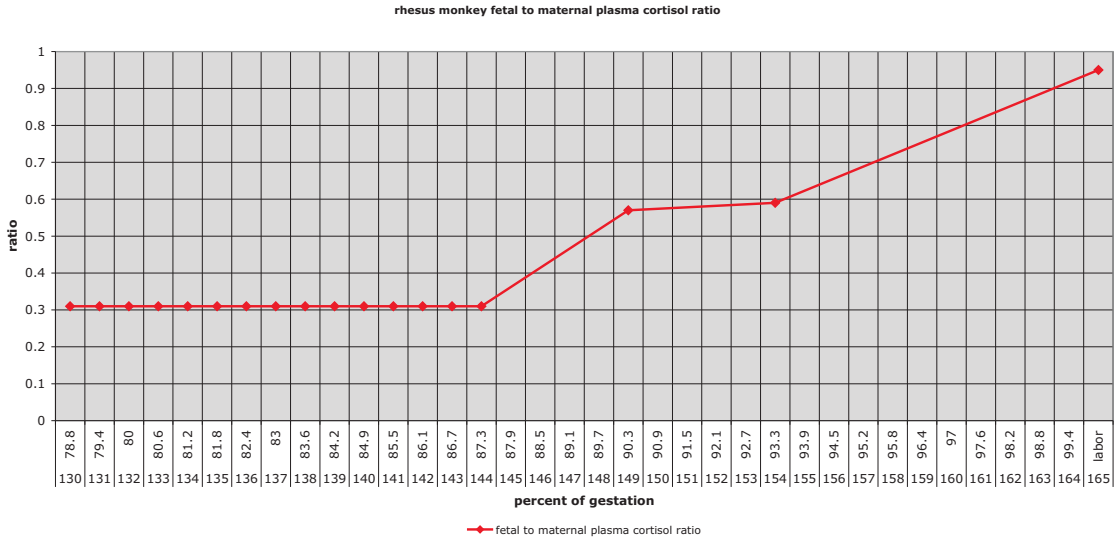


Figure 3.32. Rhesus monkey fetal to maternal plasma cortisol ratio

*Maternal plasma cortisol levels are approximately 3 times higher than cortisol levels in fetal circulation. The ratio of fetal to maternal plasma cortisol increases from 0.3 at 87% of gestation to nearly similar cortisol levels in maternal and fetal circulation at term or a ratio of roughly 1.*

#### 3.4.4.5 Rhesus monkey fetal and maternal free plasma cortisol

Fetal free cortisone fraction was significantly higher than maternal free cortisone fraction [237]. No further information is available about fetal plasma CBG or free plasma cortisol. The CBG concentration in maternal plasma was low around 21-30% (day35-50) of gestation, then increased to maximal levels by 48-64% (day79-105) of gestation. From 64% (day105) of gestation until term, maternal plasma CBG decreased continuously [449].

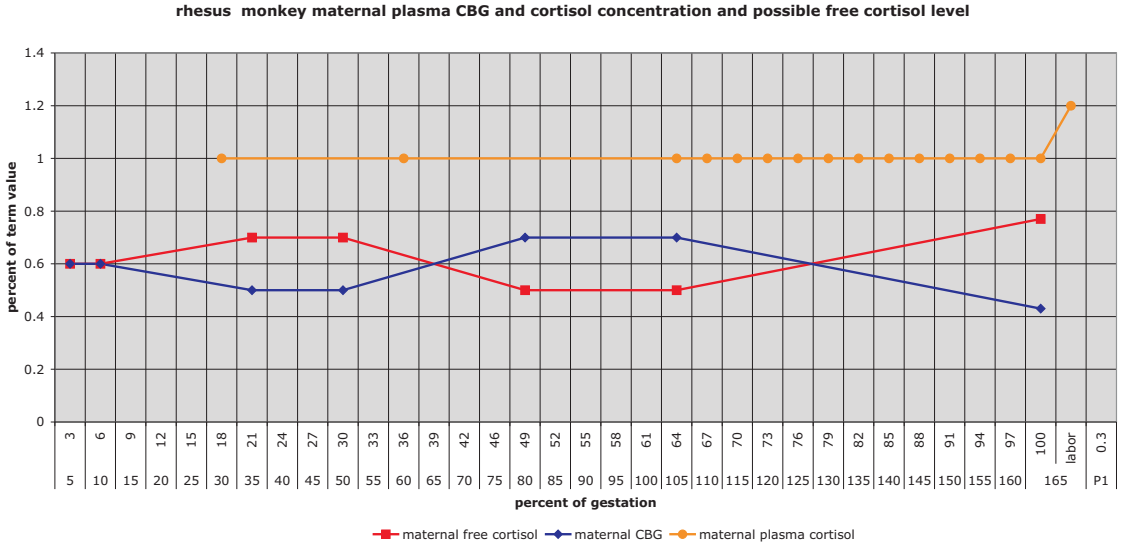


Figure 3.33. Rhesus monkey maternal CBG, plasma total and free cortisol

*The free cortisol fraction in maternal circulation can be assumed to remain elevated between 18-30% of gestation, then decrease strongly in consensus with increasing maternal plasma CBG levels until 48% of gestation. CBG levels might remain high until 64% of gestation, when the decreasing maternal plasma CBG concentration is assumed to increase the free fraction of cortisol in maternal circulation.*

### 3.4.5 Summary rhesus monkey fetal cortisol

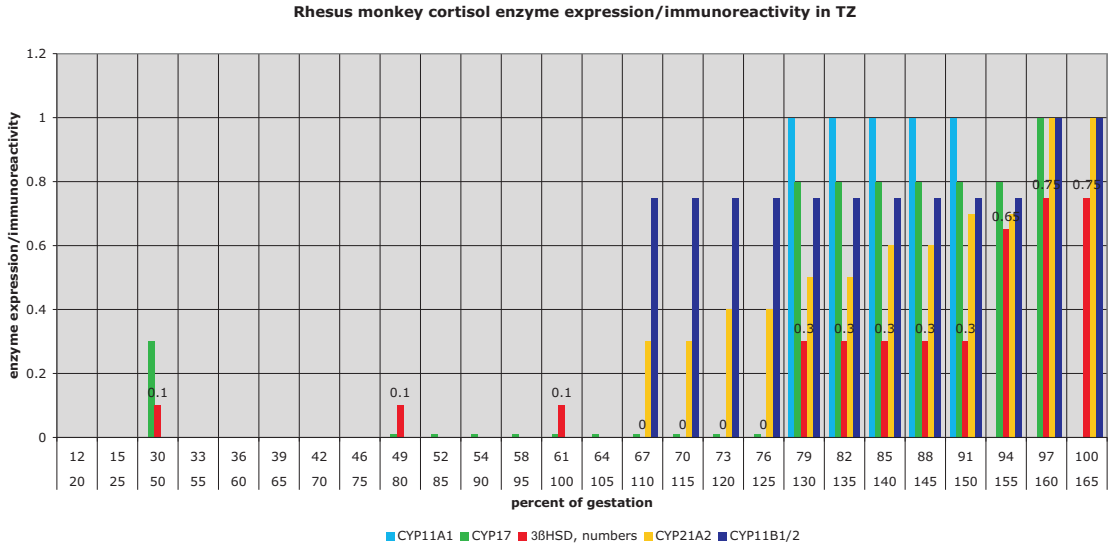


Figure 3.34. Rhesus monkey cortisol enzyme expression in TZ

*By investigating the expression/immunoreactivity of cortisol enzymes in the TZ of the fetal rhesus monkey, fetal cortisol synthesis might be apparent around 30% of gestation, due to the presence of low CYP17 and 3βHSD protein levels. CYP17 protein is very low between 49-79% of gestation, while 3βHSD immunoreactivity is low at 49% and 61% and absent at 67-76% of gestation. So between 49-61% of gestation, low cortisol synthesis could be present, but cortisol production is assumingly absent at least between 67-76% of gestation. From 79-91% of gestation on, 3βHSD is moderately expressed together with CYP17, CYP11A1, CYP21A1 and CYP11B1/2, indicating sufficient cortisol production. By 94% of gestation, 3βHSD protein levels have increased and further increase until term. Between 97-100% of gestation, CYP17 and CYP11B1/2 immunoreactivity reach high levels, leading to the assumption, that fetal cortisol synthesis increases strongly from 91% of gestation until term.*

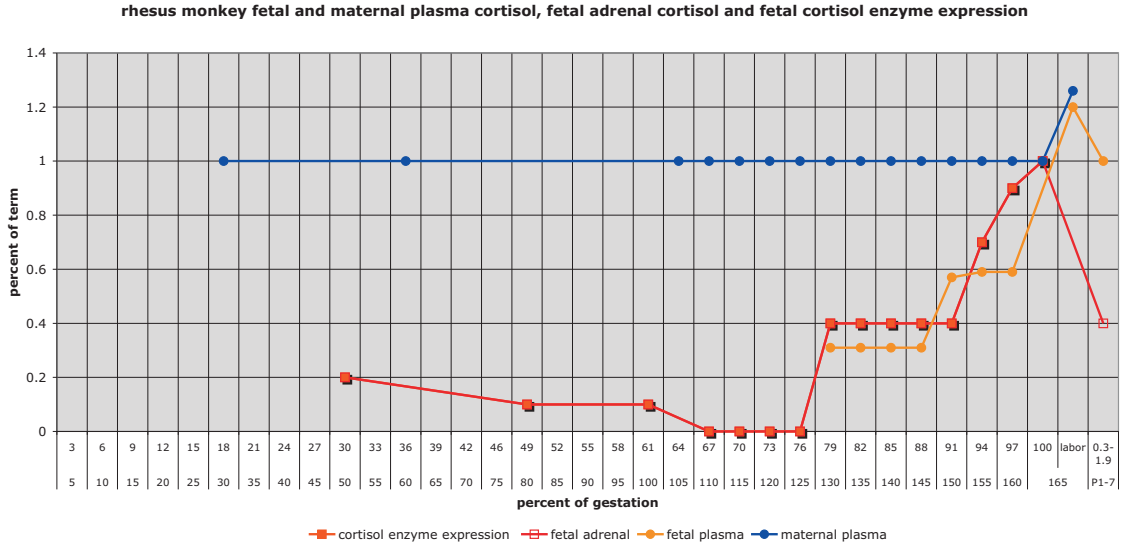


Figure 3.35. Rhesus monkey - summary adrenal and plasma cortisol

*It is unknown when the rhesus monkey fetal adrenal gland starts to produce cortisol by its own. It is only possible to state that fetal adrenal gland cortisol production is present at 44% of gestation and increases in response to ACTH. Whether there is an earlier period of fetal cortisol production is unknown but, at 30% of gestation, enzyme expression in the fetal TZ indicates fetal adrenal cortisol production. Between 30-48% of gestation, the free cortisol fraction in maternal plasma decreases strongly, due to increasing CBG levels, which might dis-inhibit the fetal adrenal cortisol synthesis. Fetal cortisol enzyme expression indicates fetal adrenal cortisol synthesis between 30-61% and a very low or absent synthesis between 67-76% of gestation. Maternal plasma cortisol levels are similarly high at 18%, 36% and 64-100% of gestation. Fetal plasma and adrenal cortisol levels (due to enzyme expression) continued to be moderate between 79-88% of gestation, maybe due to the increasing maternal free cortisol fraction after 64% of gestation. The fetal plasma cortisol concentration increases between 89-91%, then remains constant again between 91% and assumingly 97% of gestation, while fetal cortisol production can be assumed to continuously increase to maximal levels at term. Fetal plasma cortisol concentration increases strongly from 97% of gestation until labor, and an increment of maternal plasma cortisol during labor can be calculated according to plasma levels and fetal to maternal ratio. At labor, fetal plasma cortisol concentration is approximately similar to maternal plasma cortisol levels. Over the first week of life, neonatal cortisol synthesis and plasma levels decrease again.*

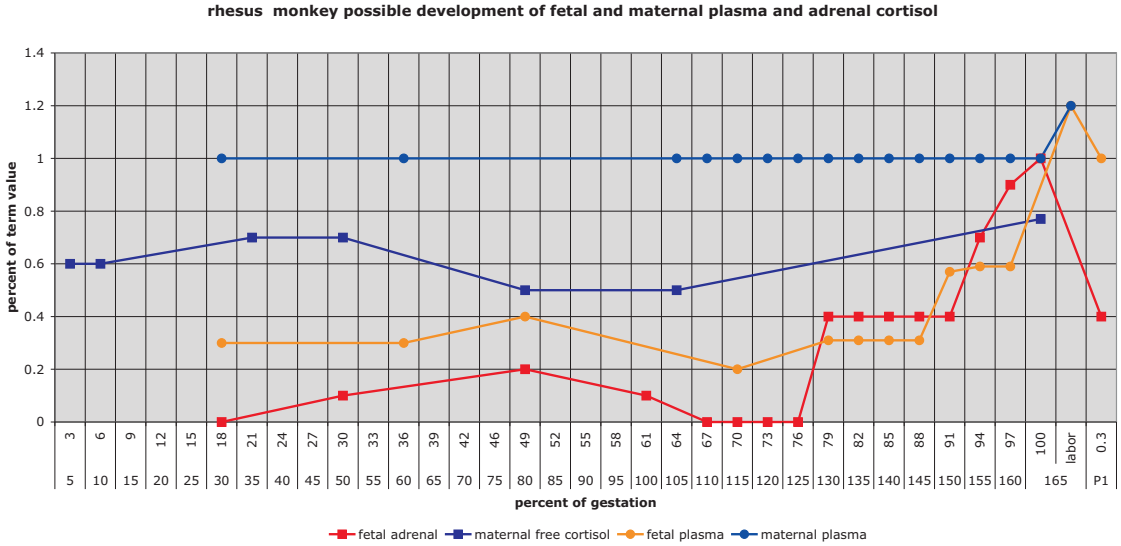


Figure 3.36. Rhesus monkey estimated fetal and maternal cortisol

*Looking at maternal plasma CBG levels and assuming an inversely proportional development in maternal free plasma cortisol, the following scenario could be possible in early gestation. Fetal cortisol synthesis could be present by 30% and might increase with decreasing free maternal plasma cortisol levels until 49% of gestation. Maybe fetal plasma cortisol would increase in parallel. At least with increasing maternal free cortisol level after 64% of gestation, fetal adrenal cortisol synthesis could decrease and be absent or extremely low at 67% of gestation. Changing placental 11$\beta$HSD2 levels could influence maternal transfer and fetal cortisol synthesis as well during that time. Another possibility could be changes in maternal adrenal cortisol synthesis and plasma cortisol concentration between 18-36% of gestation or between 36-64% of gestation, periods where no data confirm the assumed constant maternal plasma cortisol levels.*

### 3.4.6 Baboon cortisol synthesis

#### 3.4.6.1 Baboon fetal adrenal cortisol

The fetal period started in the baboon at 27% (day50) of gestation [470], and the beginning of adrenal steroidogenesis can be assumed to take place around that time. The baboon fetal adrenal expressed ACTH receptor mRNA at least by 32-35% (day58-65) of gestation [1, 4]. At 54% (day100) of gestation, there was no fetal adrenal cortisol production and all fetal plasma cortisol arose from maternal transfer [349]. Pepe et al. 1994 appraised that estrogen influences the timing of baboon fetal adrenal 'de novo' cortisol production. Low estrogen levels during early and midgestation resulted in low placental 11$\beta$HSD2 expression, high maternal cortisol transfer into the fetal circulation and the low fetal ACTH output, which might not sufficiently trigger fetal 'de novo' cortisol synthesis. Increasing estrogen production with advancing gestation increases placental 11$\beta$HSD2, shields the fetus against maternal cortisol and dis-inhibits the fetal 'de novo' cortisol production [353].

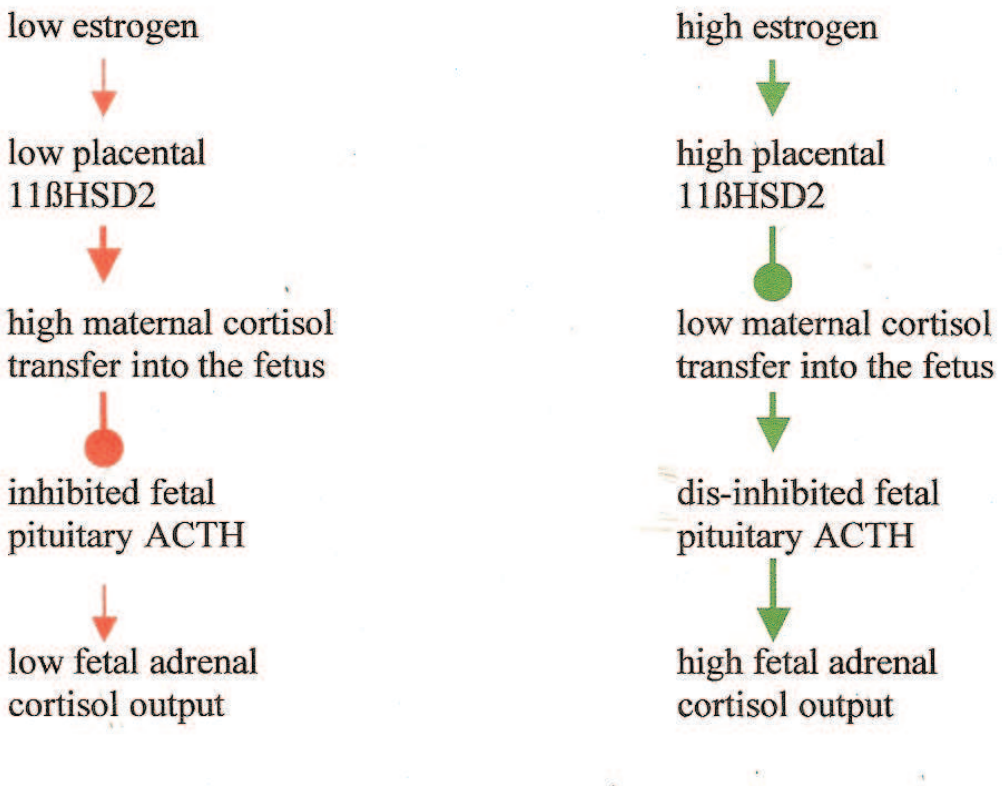


Figure 3.37. Interaction of estrogen, placental 11$\beta$HSD expression and fetal cortisol synthesis

It was show that between 54-90% (day100-165) of gestation, the placental capacity to convert cortisol into cortisone increased significantly ($P<0.01$) by 3-3.6 fold, indicating increasing placental 11$\beta$HSD2 expression during a period, where maternal plasma estradiol increased significantly ($P<0.05$) by approximately 3 fold [23, 349, 355].

Basal fetal cortisol production increased significantly (P<0.05) by 9.4 fold between 54-90% (day100-165) of gestation. ACTH increased cortisol production (P<0.05) by 1.4 fold at 90% (day165) but failed to elicit an increase at 54% (day100) of gestation [42]. In vitro cortisol production in the presence of ACTH (22nM) compared to production in the absence of ACTH, revealed a significant (P<0.05) increase by 9.6 fold at 84% (day155) of gestation, respectively by 6.6 fold at 98% (day180) of gestation [134]. Between 54-71% (day100-130) of gestation, the ability for 'de novo' cortisol production (synthesis from pregnenolone) was very small. As predicted, fetal 'de novo' cortisol production increased significantly (P=0.031) by 4.7 fold from 54-71% (day100-130) to 87-91% (day160-167) of gestation [351]. By 90% (day165) of gestation, 49 % of fetal cortisol derived from fetal 'de novo' cortisol production and 50% of plasma cortisol in the umbilical vein was of fetal origin [349]. From 87-91% (day160-167) of gestation until 0.3% (PND1) of weaning, 'de novo' cortisol production increased significantly (P=0.008) by another 3.6 fold [351]. From 98% (day180) of gestation until 3% (PND10) of weaning, in vitro cortisol production, even in the absence of ACTH, increased significantly (P=0.008) by 1.8 fold [134].

*The start of the fetal period at 27% and the presence of ACTH receptor expression in the fetal baboon indicate the beginning of steroidogenesis in the fetal baboon adrenal around that time. No cortisol production is apparent in the fetal baboon at 54% of gestation and ACTH is unable to elicit cortisol synthesis. All fetal plasma cortisol originates from the mother. From 54% till 71 % of gestation, it is known even more specific that the ability to produce 'de novo' cortisol is very small.*

*The strong increment in maternal plasma estrogen concentrations between 56-90% of gestation is assumed to inhibit maternal cortisol transfer and dis-inhibit fetal ACTH and cortisol synthesis. It is shown that between 54-90% of gestation, maternal plasma estrogen increases, assumingly increasing placental 11$\beta$HSD2 expression. Less maternal cortisol is then able to transfer into the fetus and the maternal contribution to fetal plasma cortisol decreases from 100% to 50% over that period. Less maternal cortisol in fetal circulation dis-inhibits the fetal ACTH synthesis and fetal adrenal cortisol output dramatically increases in late gestation.*

*Between 84-98% of gestation, fetal cortisol synthesis is highly responsive to ACTH. Fetal adrenal cortisol secretion increases significantly between 54-90% of gestation and the ability to synthesize cortisol from pregnenolone increases by 5 fold. From moderate levels at 90% of gestation, cortisol production rate increases by 3.4 fold until 0.3% of weaning and from 98% of gestation until 3% of weaning by 1.8 fold.*

#### 3.4.6.2 Baboon fetal plasma cortisol

Oakey et al. already in 1975 showed that fetal plasma cortisol (after caesarian section) was very low at 61% (day112) and 70% (day128), but had increased at 89% (day164) of gestation by 3.8 fold and again at term by another 3.3 fold. Term values were in the rage of plasma cortisol levels of non-pregnant adult females [330]. Fetal plasma cortisol (from umbilical arteries) increased significantly (P<0.001) from very low levels at 54% (day100) until 92% (day170) of gestation by 5.6 fold [135]. After birth, neonatal plasma cortisol concentrations increased dramatically between 0.7-1.3% (PND2-4), but had decreased again by 1.7% (PND5) and further decreased to low levels by 4.3% (PND13) of weaning. Afterwards neonatal plasma cortisol concentrations showed fluctuation until roughly 27.3% (PND82) of weaning, reaching very low plasma cortisol levels. A diurnal rhythm was apparent at 2.3% (PND7) of weaning [134].

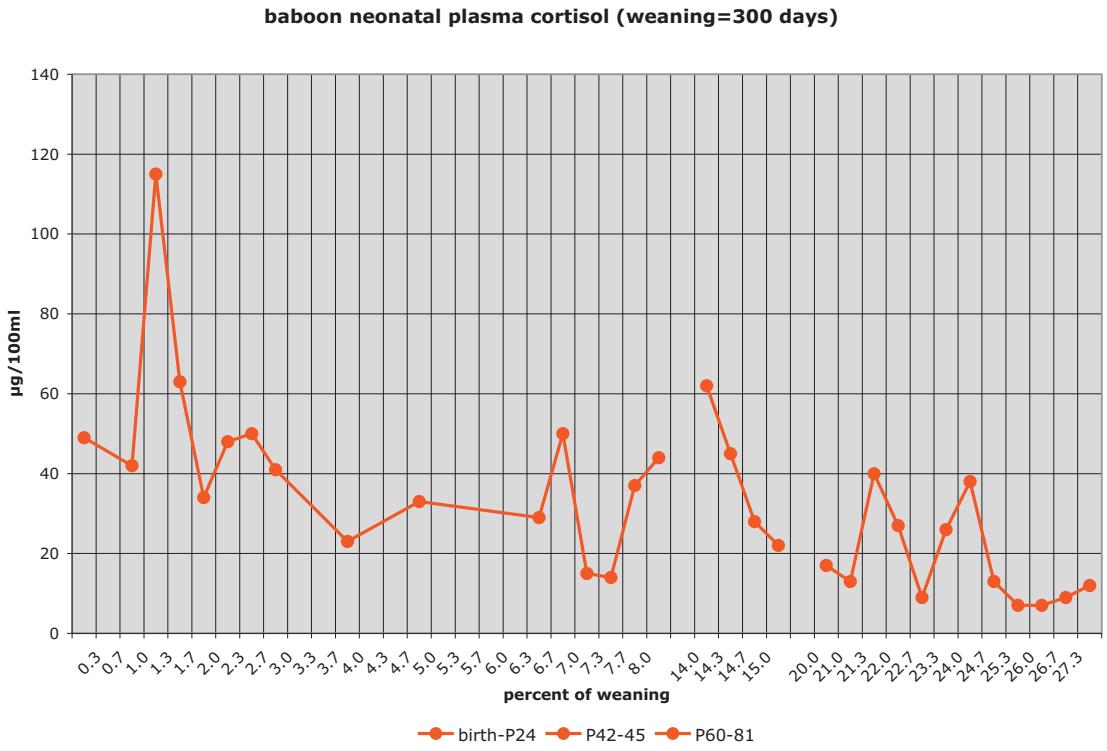


Figure 3.38. Baboon neonatal plasma cortisol (weaning=300 days)

*The fetal plasma cortisol concentrations in the umbilical artery, as well as in fetal blood vessels after caesarean section, are very low between 54% and 70%, but have already increased by 3.8 fold at 89% of gestation and increased by another 3.3 to very high levels at term. After birth, neonatal plasma cortisol concentrations increase dramatically between 0.7-1.3%, to decrease again by 1.7% and with fluctuations, show in general a decrement to very low levels by 27% of weaning.*

#### 3.4.6.3 Baboon maternal cortisol, plasma cortisol ratio

Maternal cortisol production rate did not differ significantly between 54-90% (day100-165) of gestation [349]. Maternal plasma cortisol concentrations were constant between 52% (day96), 61% (day112), 70% (day129) and 89% (day164) of gestation [330]. Pepe et al. 1990 and 1996 detected equally constant cortisol concentrations between 54-90% (day100-165) of gestation in maternal circulation [349, 354]. More recently, Dumitrescu et al. 2007 presented constant levels between 33% (day60) and 54% (day100), but at 92% (day170) of gestation, cortisol in maternal circulation had significantly ($P<0.001$) increased by 1.8 fold [135].

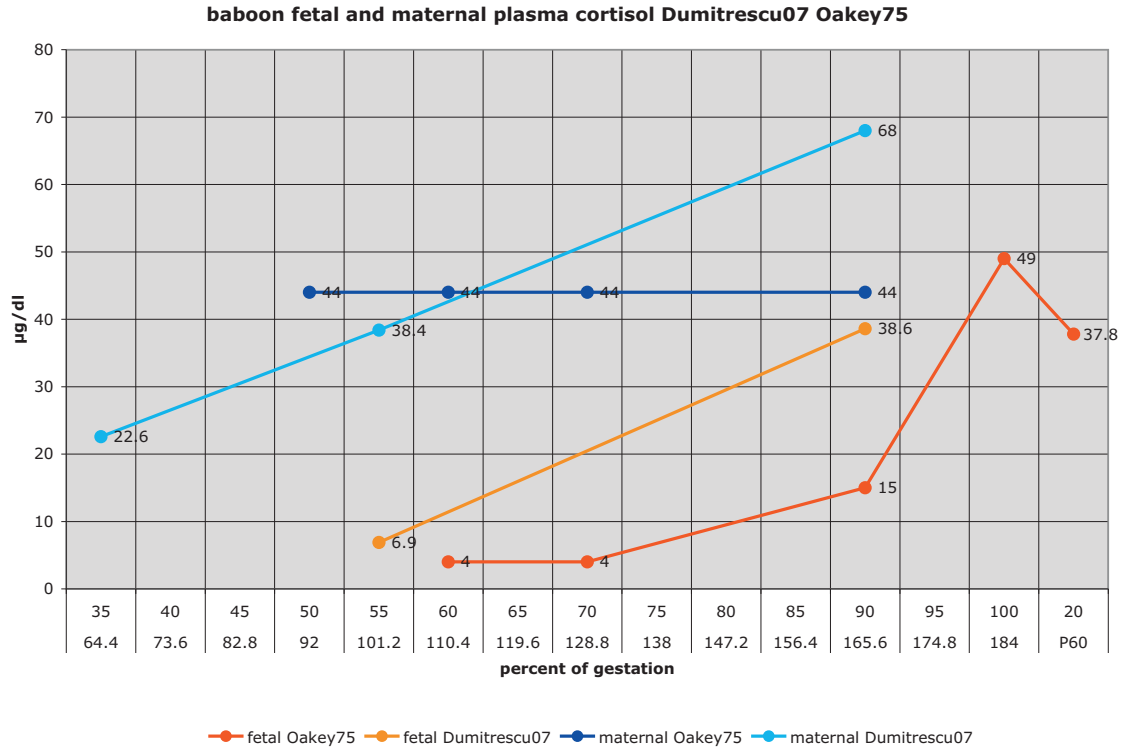


Figure 3.39. Baboon fetal and maternal plasma cortisol [135, 330]

Between 54% (day100) and 90% (day165) of gestation, the metabolic clearance rates for cortisol in maternal plasma were similar [349]. The ratio of fetal to maternal cortisol is only 0.1-0.2 at 55-70% (day101-129), but increases to 0.34-0.57 at 89-92% (day164-169) of gestation (data from [135, 330] ).

*Maternal adrenal cortisol production is constant between 54-90% of gestation. It can be assumed that maternal plasma cortisol concentrations remain constant from 32% until roughly 89%, but there might be a sudden dramatic increase in maternal plasma cortisol levels between 89-92% of gestation. Cortisol metabolic clearance rate does not change between 54-90% of gestation. The ratio of fetal to maternal plasma cortisol is very low at 55-70% of gestation, due to very low fetal plasma cortisol levels. By 90% of gestation, the ratio has increased to mean 0.46, caused by strongly increasing fetal plasma cortisol levels.*

#### 3.4.6.4 Baboon fetal and maternal plasma CBG

Fetal plasma CBG levels decreased significantly ($P<0.05$) between 54-90% (day100-165) of gestation by 1.9 fold. Maternal plasma CBG did not significantly change between the two-time points [354]. Between 54-74% (day100-136) of gestation, fetal plasma CBC (cortisol binding capacity) concentrations were similar to maternal levels, but at term, fetal concentrations were only 50% compared to maternal [330].

*While maternal plasma CBG levels remain unchanged between 54-90% of gestation and possibly until term, fetal plasma CBG concentrations decrease sufficiently over that period. Fetal and maternal plasma CBC is similar at 54-74% of gestation, but at term, fetal plasma CBC can be assumed to decrease, causing increasing free fetal plasma cortisol levels.*

### 3.4.7 Summary baboon fetal cortisol

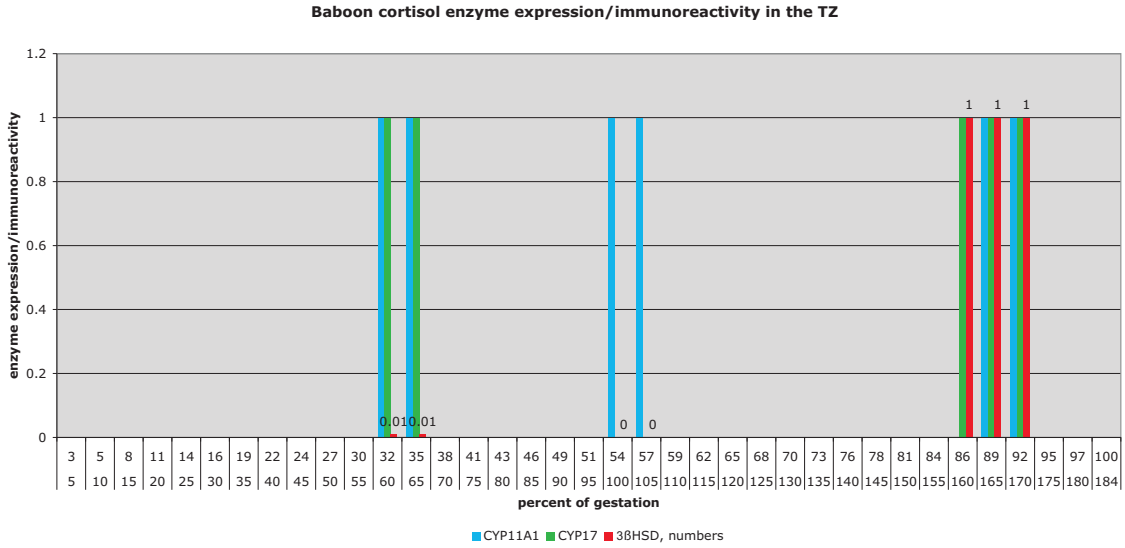


Figure 3.40. Baboon cortisol enzyme expression in the TZ

*At 32-35% of gestation, fetal adrenal 3βHSD expression is low and the presence of CYP11A1 and CYP17 expression could indicate sufficient cortisol synthesis. 3βHSD immunoreactivity has disappeared in the TZ by 54-57% of gestation. CYP17 protein in the TZ ceases similarly between 32-35% and 54-57% of gestation, assuming decreasing or disappearing fetal adrenal cortisol synthesis during at period. Cortisol synthesis due to enzyme expression can be assumed to have reappeared at least by 86%, and cortisol production between 86-92% of gestation is assumingly markedly stronger than before.*

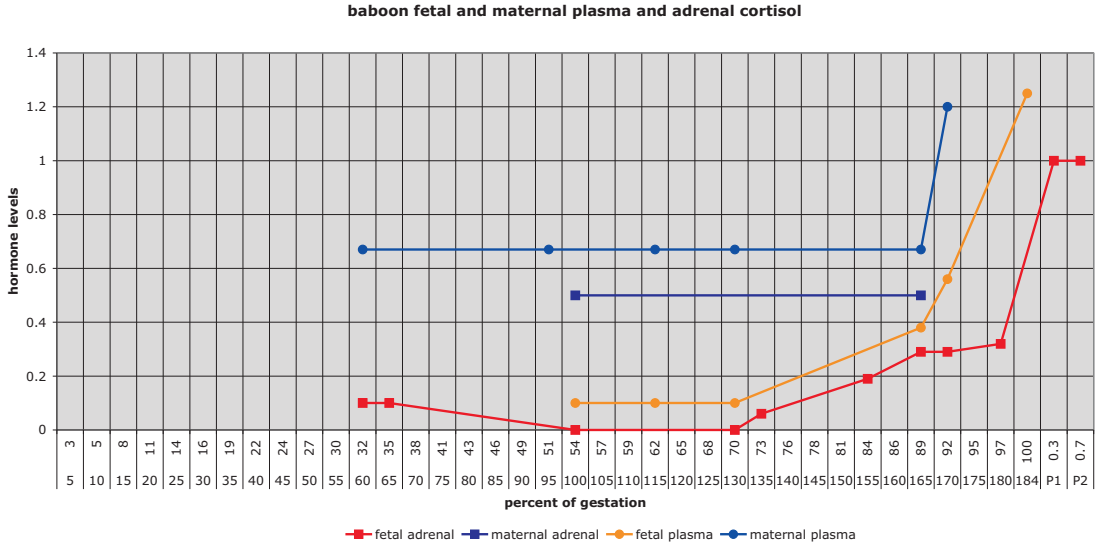


Figure 3.41. Baboon - summary adrenal and plasma cortisol

*Enzyme expression indicates cortisol synthesis in the fetal baboon adrenal between 32-35% of gestation, at the beginning of the fetal period, at a time when adrenal ACTH receptors are already expressed. Maternal plasma cortisol concentrations are moderately high at 32% and 51% of gestation. Between 54-70% of gestation, fetal plasma levels remain low and fetal adrenal cortisol synthesis is negligible. The placental barrier might be relatively tight during that period, as it seems that only low amounts of maternal plasma cortisol transfer into the fetus. Maternal free cortisol fraction remains constant from 54-90% of gestation. From 70% until 89% of gestation, fetal plasma and adrenal cortisol concentrations increase in parallel to moderate respectively moderately low levels, while maternal plasma cortisol concentrations are constant between 70-89% and only increase dramatically between 89-92% of gestation to high levels. This increment could be caused by an increasing maternal adrenal cortisol synthesis. The sudden increase in maternal plasma cortisol concentrations and the slightly delayed increase in fetal plasma cortisol between 89-92% of gestation could negative feedback on fetal ACTH and subsequently fetal adrenal cortisol synthesis, which stops increasing during that period. Fetal free plasma cortisol fraction increases approximately between 74-90% of gestation in parallel with fetal plasma cortisol concentrations.*

*Fetal adrenal cortisol synthesis remains roughly constant between 92-97% of gestation. Between 97% and 0.3% of weaning, fetal adrenal cortisol surges to very high levels.*

*Whether maternal cortisol production decreases between 97-100% of gestation or further increases is unknown. Fetal plasma cortisol concentrations reach high levels at term.*

### 3.4.8 Sheep cortisol synthesis

#### 3.4.8.1 Sheep fetal adrenal cortisol

The sheep fetal adrenals were assumed to show steroidogenesis early as well as late in gestation, with a period of quiescence in between [57]. Already at 25% (day38), sinusoidal blood vessel were present in the fetal cortex and by 25-29% (day38-43), the zF exhibited steroidogenic organelles, their amount increasing to high levels at 37% (day56) of gestation [386]. By 69% (day104), low levels of steroidogenic organelles indicated low cortisol synthesis, but between 89-98% (day134-147) of gestation, levels increased to a maximum and remained very high by 2% (PND2) of weaning [57, 58]. Cortisol content in the fetal adrenals at 55% (day82) was moderate, but significantly ($P<0.05$) decreased by 6.5 fold to the limit of detection at 68% (day102) of gestation. At 81% (day122) and 88% (day132), cortisol content was low but had significantly ($P<0.05$) increased again by 6 fold at 98% (day147) of gestation. At term, adrenal cortisol content is 2.1 fold higher ($P<0.05$) compared to 55% (day82) of gestation. During labor and in neonates at 1% (PND1) and 8% (PND7) of weaning, cortisol content did not significantly differ from 98% (day147) of gestation [233].

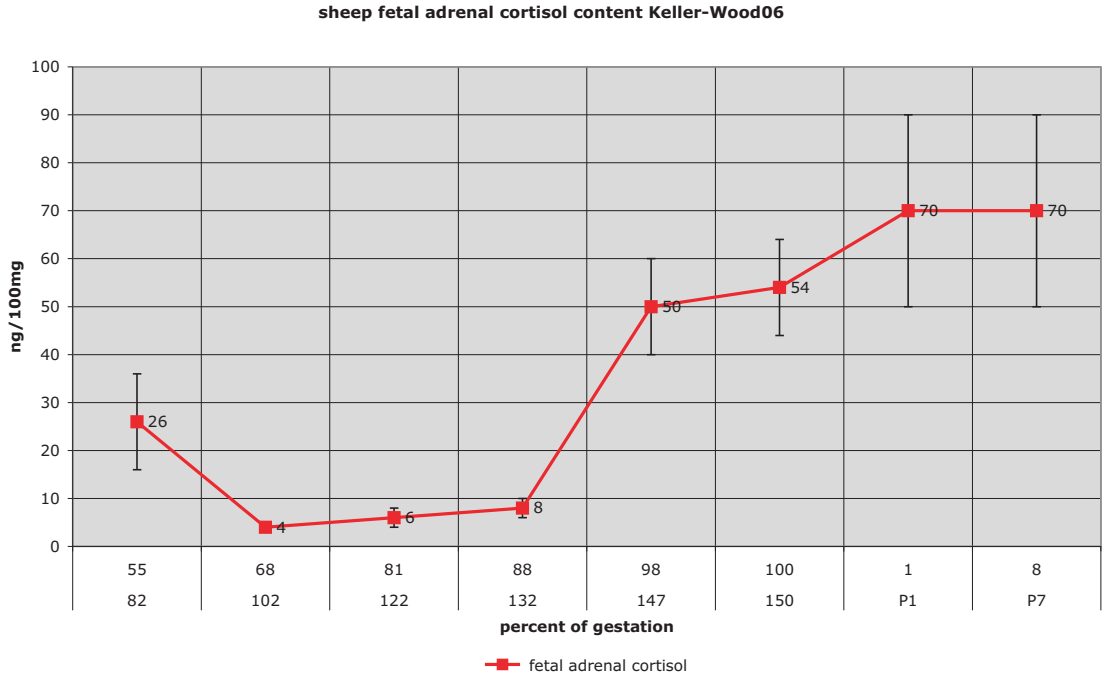


Figure 3.42. Sheep fetal adrenal cortisol content [233]

Glickman et Challis in 1980 showed that fetal adrenal cells were able to secrete cortisol already at 36% (day54), while Wintour et al. 1975 detected fetal cortisol secretion even at 32% (day48) of gestation [163, 499]. Cortisol output, in the absence and presence of ACTH, showed similar development. At 36% (day54), 89% (day134) and 100% (day150), fetal adrenal cortisol output was significantly higher ($P<0.001$) in the presence of ACTH, but adrenal cortisol output was unresponsive to ACTH at 69% (day104) of gestation. Between 36-69% (day54-105) of gestation, cortisol output significantly ($P<0.001$) decreased by 9 fold in the absence and by 5.4 fold in the presence of ACTH. Cortisol output remained constantly low at 89% (day134), but had significantly ($P<0.001$)

increased at 100% (day150) of gestation by 2.7 fold without ACTH and by 11.5 fold with ACTH. At 36% (day54) and at 100% (day150) of gestation, the cortisol output showed similar values in the absence of ACTH, but a 4.3 fold higher cortisol output at term in the presence of ACTH [163].

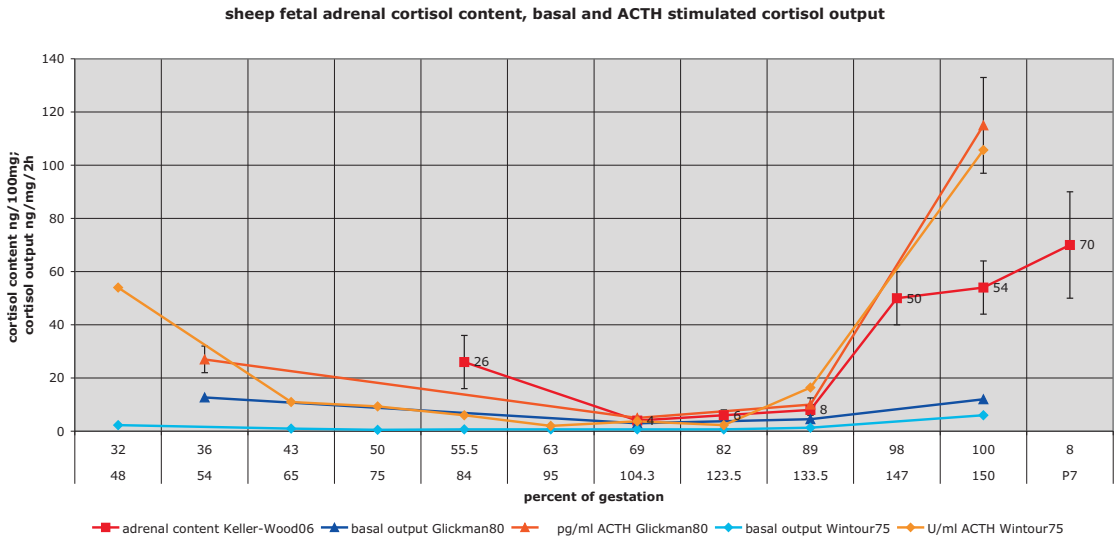


Figure 3.43. Sheep fetal adrenal cortisol content, basal and ACTH stimulated cortisol output

Wintour et al. 1975 refined the picture of adrenal responsiveness to ACTH even further. Fetal adrenal cortisol production rate in response to ACTH was moderate (2 times lower than during labor) at 32% (day48), but decreased already at 42-48% (day63-72) by 5 fold and reached a nadir between 65-85% (day98-128) of gestation [499] The development of responsiveness correlates with ACTH receptor mRNA expression in the fetal sheep adrenals in late gestation. The relative abundance of ACTH receptor mRNA increased significantly ($P<0.05$) between 85-94% (day127-140.5) of gestation and further ($P<0.05$) with the onset of labor [156].

*Cortisol synthesis in the fetal sheep is already detectable at 32% of gestation. Cortisol content in the fetal adrenal gland is moderate at 55%, subsequently decreases at 68% and remains low until 88% of gestation. By 98% of gestation, fetal cortisol content has increased dramatically to 2 fold higher levels than at 55% of gestation. Cortisol content does not significantly change during labor or in neonates at 1-8% of weaning. Cortisol production rate in response to ACTH shows a similar trinomial pattern with moderate responsiveness at 32% and 36%, lower response at 43-56%, and very low responsiveness at 63-82% of gestation. Responsiveness increases from 90% of gestation to high levels at term. Cortisol content and cortisol responsiveness early in gestation is 50% lower than at term. Integrating the data, it can be assumed that the fetal adrenal cortex is able to produce moderate levels of cortisol during 32-56% of gestation. Between 63-82% of gestation, cortisol output in the presence or absence of ACTH is minimal. Cortisol synthesis dramatically increases between 90% of gestation and term, but does not change further during parturition or until 8% of weaning.*

### 3.4.8.2 Sheep fetal plasma cortisol

Fetal plasma cortisol (from fetal umbilical artery) decreased significantly ($P<0.025$) between 52-72% (day78-108.5) by 2.5 fold and increased by 3 fold at 89% (day133.5) of gestation. Between 89-100% (day133.5-150) of gestation, fetal plasma cortisol increased significantly ($P<0.001$) by

another 8.2 fold [499]. This pattern of fetal plasma cortisol is similar to the changes in cortisol synthesis in fetal adrenal cortex. Braun et al. 2009 was able to show gender differences. Between 33-67% (day50-100) of gestation, fetal plasma cortisol (from cardiac blood and umbilical artery) showed a significant ($P<0.05$) decrease by 5.1 fold in females and by 3.4 fold in males. By 93% (day140) of gestation, fetal plasma cortisol had significantly increased again ($P<0.05$) by 4.6 fold in females and by 3.8 fold in males [62]. Plasma cortisol (from vascular catheter) concentration in the fetus rose at least from 89% (day133) on, while maternal plasma levels began to rise only at 99% (day148) of gestation. Fetal plasma cortisol concentration increased slowly between 89-93% (day 133-140), then more dramatically until 97% (day146) of gestation by 4.5 fold. From 97% (day147) till 100% (day150) of gestation, fetal plasma cortisol increased by 2 fold [260, 516].

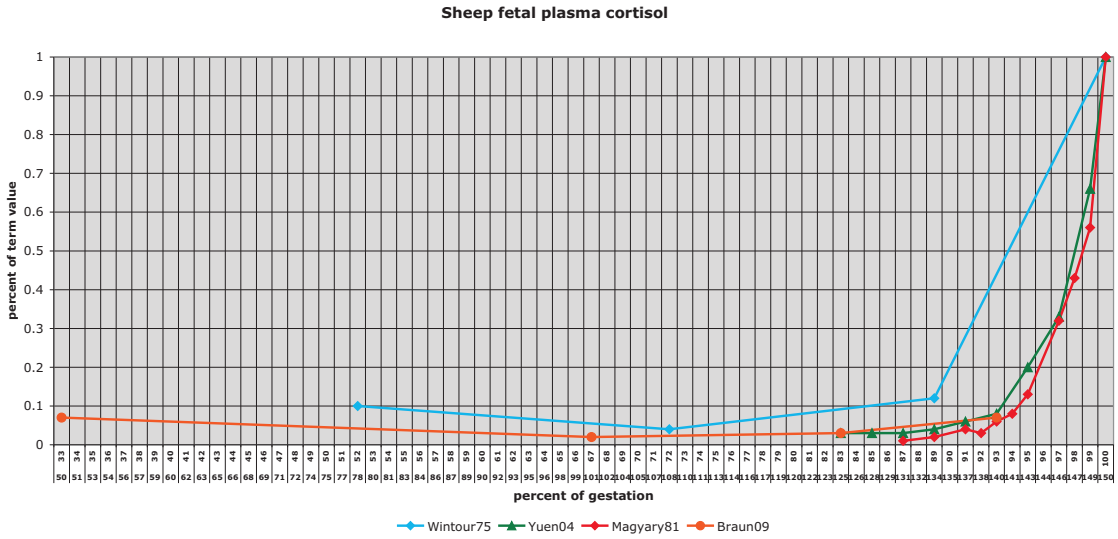


Figure 3.44. Sheep fetal plasma cortisol

*Fetal plasma cortisol levels change in parallel with fetal adrenal cortisol synthesis. Fetal plasma cortisol concentration decreases from 33-52% to 67-72% of gestation. By 89-93% of gestation, cortisol in fetal circulation has increased again. These decrease and increase are stronger in female fetuses than in male fetuses. From 93% of gestation until term, fetal plasma cortisol increases dramatically to very high levels.*

#### 3.4.8.3 Sheep maternal plasma cortisol

No significant change in maternal plasma cortisol was detectable from non-pregnant values to 10% (day9) or until 65% (day98) of gestation [50]. Nor did McMullen et al. 2004 measured a significant change over the period between 20-90% (day30-135) of gestation [290]. While the decrease of maternal plasma cortisol by 2.1 fold between 30-55% (day45-83) of gestation failed to reach significance, maternal plasma ACTH decreased significantly ($P=0.011$) between 18-56% (day27-84) by 1.5 fold and was constant at least until 65% (day98) of gestation [46, 50]. Cortisol concentration in maternal circulation significantly ($P=0.017$) increased between 55-69% (day83-103) of gestation by 2.4 fold. Subsequently, it decreased significantly ($P<0.05$) again by 1.7 fold until 95% (day143) of gestation [46]. Maternal plasma cortisol was constant between 89-99% (day133-148), when it increased by more than 14 fold until 100% (day150) of gestation [260].

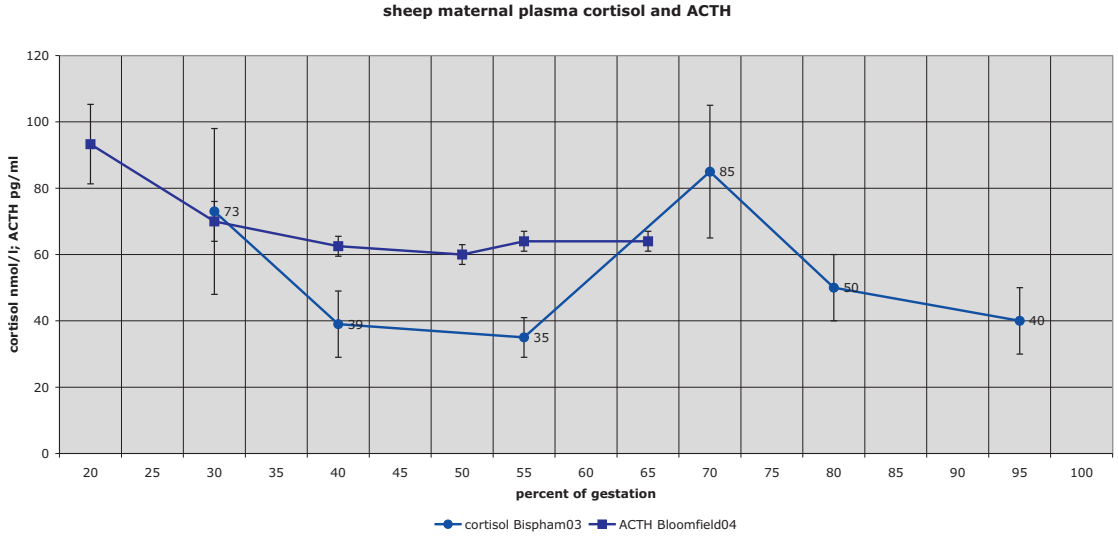


Figure 3.45. Sheep maternal plasma cortisol and ACTH

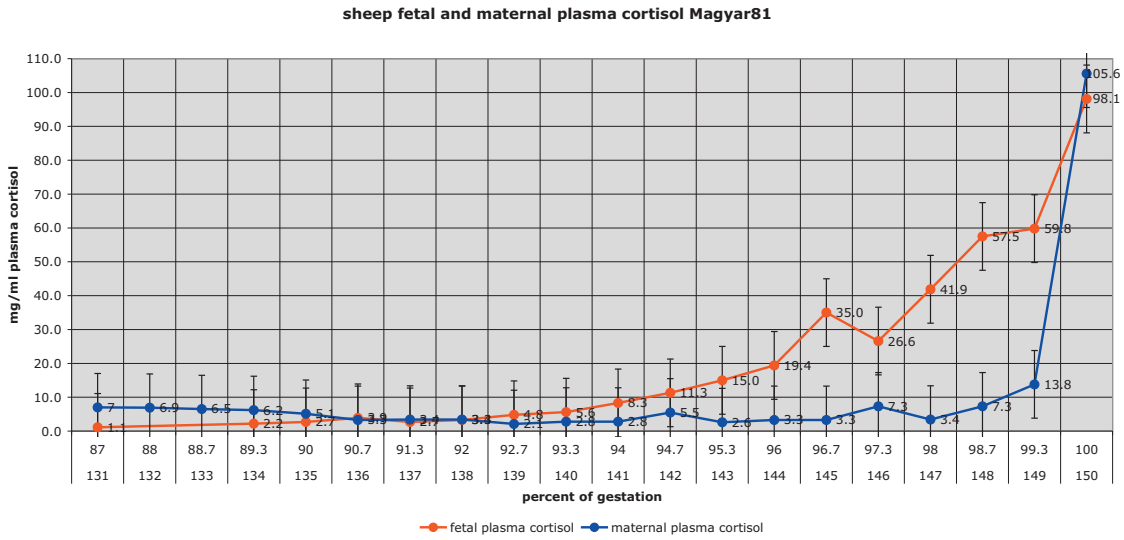


Figure 3.46. Sheep fetal and maternal plasma cortisol Magyar81

*The cortisol concentration in maternal circulation fails to show a significant decrease between 30-55%, even when maternal plasma ACTH levels decrease significantly between 18-56% of gestation. Between 55-69%, maternal plasma cortisol significantly increases and decreases again by 95% of gestation. Maternal plasma cortisol concentration seems to remain low between 89-99% and then dramatically increases between 99-100%, which is much later than the increment in fetal plasma*

*cortisol levels from 89% of gestation onward.*

3.4.8.4 Sheep fetal-maternal plasma cortisol ratio

Around 80% (day120) of gestation, approximately 40% of fetal plasma cortisol was of maternal origin. Earlier in gestation, almost all cortisol in fetal circulation originated from fetal adrenal cortisol production [36]. Between 57-74% (day86-111) of gestation, fetal to maternal plasma cortisol ratio is only 0.04. By 88% (day132.5), the ratio is already 0.73, but increases further to 2.5 by 96% (day143.5) of gestation (data from [435, 499]). Mean concentrations of fetal plasma cortisol are clearly higher than maternal plasma cortisol concentrations between 94-99% (day141-149) of gestation. At 100% (day150) of gestation, maternal and fetal values are comparable. The ratio of fetal to maternal plasma cortisol increases from 0.16 at 87% (day131) to 0.85 at 90-91% (day135-137) of gestation. Then the ratio increases slowly to 2.5 by 94% (day141) and to 10.1 by 98% (day147) of gestation. Between 98-99% (day147-149) the ratio decreased to 4.3 and between 99-100% (day149-150) of gestation to 0.9 (data from [260]).

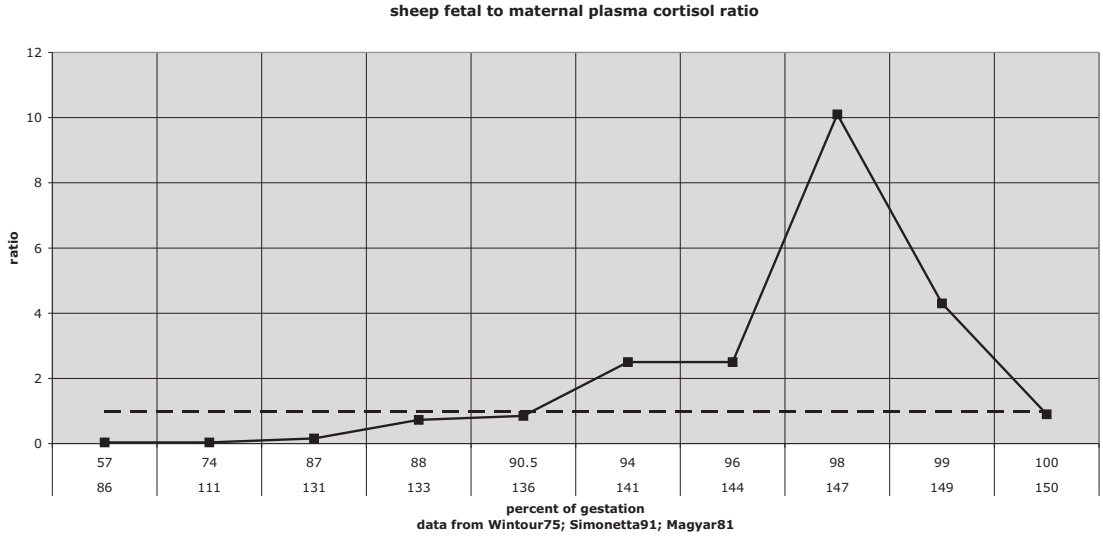


Figure 3.47. Sheep fetal to maternal plasma cortisol ratio

*Between 57% and 74% of gestation, fetal to maternal plasma cortisol ratio is very low, as maternal plasma cortisol concentration increases and fetal plasma cortisol levels decrease over that period. Maternal plasma cortisol concentration decreases from 70-95%, while fetal cortisol levels increase, assumingly causing the increment in the ratio. Maternal plasma cortisol levels remain very low until 99%, but already fetal plasma cortisol concentration dramatically increases to very high levels, resulting in the great increase in the ratio. While cortisol in fetal circulation continues to increase, maternal plasma cortisol shows the most dramatic increment from very low levels at 99% to a similar concentration than in fetal circulation at 100% of gestation. As a result, the fetal to maternal plasma cortisol ratio dramatic falls from 10 to 1.*

### 3.4.9 Summary sheep fetal cortisol

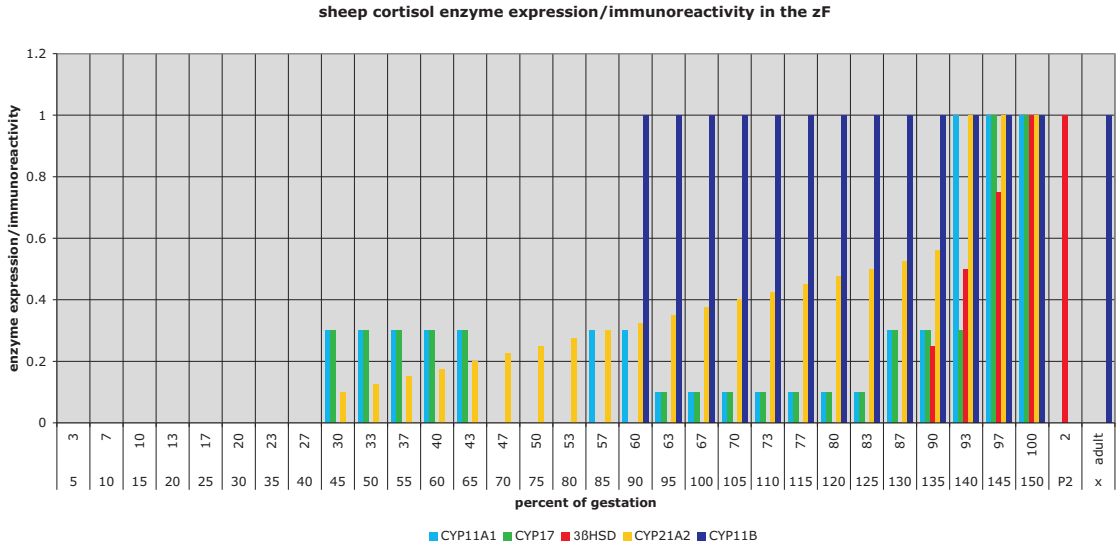


Figure 3.48. Sheep cortisol enzyme expression in the zF

*Cortisol enzyme expression presents a picture of early as well as late steroid synthesis but a period of low or absent synthesis in between. During 30-43% of gestation, both CYP11A1 and CYP17 are strongly expressed and moderate expression of CYP11A1 seems to be additionally apparent at 57-60% of gestation. The expression of both enzymes is very low at 63-83% of gestation. At 90% of gestation, expression of CYP17 and CYP11A1 has moderately increased and 3βHSD and CYP21A1 are present in moderate levels. CYP17 expression remains moderate around 93%, and reach together with 3βHSD expression very high levels by 100% of gestation. At term all required enzymes for cortisol synthesis are strongly expressed.*

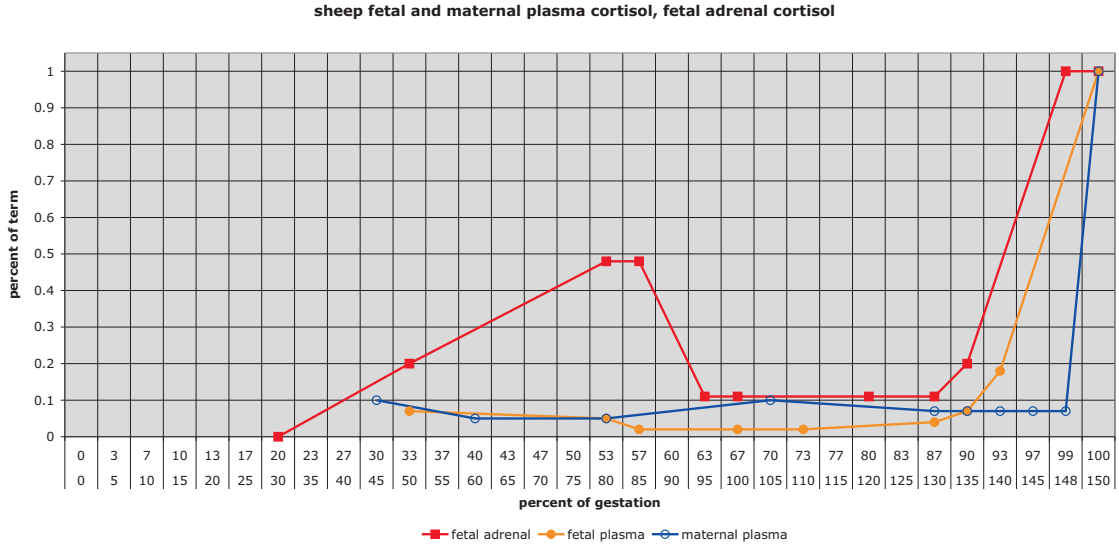


Figure 3.49. Sheep - summary adrenal and plasma cortisol

*Looking at fetal and maternal plasma cortisol concentrations, as well as fetal adrenal cortisol synthesis, the following picture could occur.*

*Between 30-60% of gestation, enzyme expression in the fetal zF indicates cortisol synthesis. Already between 32-36% of gestation, sufficient fetal adrenal cortisol synthesis is verified, and the response to ACTH is high during that time. Between 33-57% of gestation, fetal cortisol synthesis might increase, as by 55% of gestation, the fetal adrenal cortex was able to produce moderate levels of cortisol. Between 18-56% of gestation, maternal plasma ACTH levels significantly decrease and maternal cortisol concentration can be assumed to follow, which might dis-inhibit the negative feedback on the fetal HPA axis. Between 33-52% and 67-72% of gestation, fetal plasma cortisol decreases and fetal cortisol synthesis nearly ceases between 55% and 68%, also verified by enzyme expression. The decrease in fetal cortisol synthesis is inversely correlated with increasing maternal cortisol levels between 55-69% of gestation. During that period, the ratio of fetal to maternal plasma cortisol is only very low. Fetal cortisol production and fetal cortisol enzyme expression remain very low between 63-83% of gestation and cortisol, produced in response to ACTH, reaches its nadir. From 69%, maternal plasma cortisol concentration decreases again until 87%, while fetal plasma cortisol levels increase very slowly from 73% of gestation onward, reaching a fetal to maternal plasma ratio of roughly 1 at 90% of gestation. No sufficient net cortisol transfer across the placenta is assumed at this point. By 90% of gestation, enzyme expression and cortisol synthesis in response to ACTH have slowly increased again. It can be assumed that between roughly 90% and 99% of gestation, the strong increment in fetal plasma cortisol originates from the increasing fetal adrenal cortisol production, during a time when maternal plasma cortisol remains low and the fetal to maternal ratio constantly increases from approximately 1 to 10. From 99% of gestation on, maternal plasma cortisol increases strongly, and cortisol in fetal and maternal circulation reach similar levels at term.*

### 3.4.10 Guinea pig cortisol synthesis

#### 3.4.10.1 Guinea pig fetal adrenal cortisol

At 32% (day22) of gestation, steroidogenic organelles were detected in the cells of the cortical blastema, and its amount increased strongly in the inner zone cells by 38% (day26) of gestation, at which point the adrenals encompassed an inner zF and an outer zG [47, 48, 333]. Between 44-74% (day30-50) of gestation, the amount of steroidogenic organelles increased dramatically in the zF [47]. At 37-51% (day25-35) of gestation, the fetal guinea pig adrenal gland was able to convert [3H]-progesterone into [3H]-corticosterone and [3H]-cortisol [342]. The fetal adrenal cortisol concentrations in both genders did not change significantly between 91-99% (day62-67) of gestation. Between 99-100% (day67-68) of gestation, adrenal cortisol concentrations increased significantly ($P<0.001$) by 2.8 fold in males and by 1.9 fold in females. From birth until 5% (PND1) of weaning, adrenal cortisol concentrations did not significantly change in female newborn but increased in male newborn ($P=0.006$) by 1.3 fold [105].

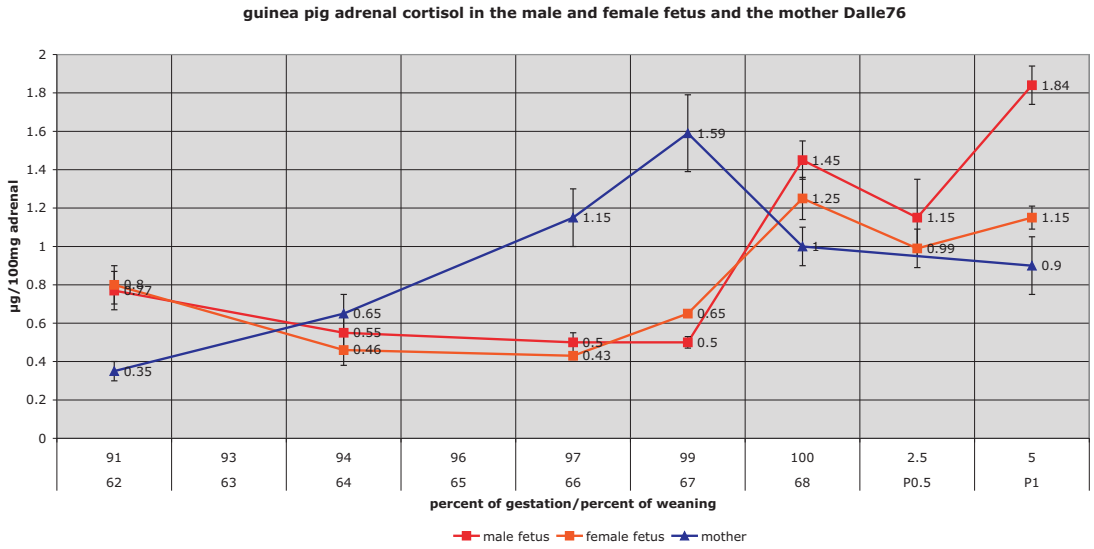


Figure 3.50. Guinea pig adrenal cortisol in fetus and mother [105]

Between 50-82% (day34-56) of gestation, the presence or absence of physiological plasma ACTH concentrations elicited nearly no difference in fetal adrenal cortisol synthesis. The fetal adrenals synthesized cortisol in very low levels between 50-63% (day34-43) and in twice higher but still very low levels at 63-82% (day43-56) of gestation. From here on, the fetal adrenal cortex was responsive to ACTH and cortisol synthesis increased from 63-82% (day43-56) until 82-100% (day56-68) of gestation by 7.5 fold in the absence and by 25 fold in the presence of physiological ACTH levels. Physiological ACTH levels increased cortisol release between 82-100% (day56-68) of gestation by 5.1 fold and at 5% (PND1) of weaning by 3 fold [395]. At 87-94% (day59-64), the same ACTH levels elicited roughly 2-3 times higher cortisol production than at 71-82% (day48-56) of gestation. Between 87-94% (day59-64) of gestation, cortisol production did not reach a plateau, but monotonically increased [218].


Figure 3.51. Guinea pig fetal adrenal cortisol secretion in response to ACTH [395]

*Already between 37-51% of gestation, the fetal guinea pig adrenal gland is able to synthesize cortisol from progesterone. Between 50-82% of gestation, cortisol synthesis is not responsive to ACTH and fetal cortisol synthesis is very low. From now on, the fetal adrenal cortisol synthesis is responsive to ACTH. Physiological ACTH levels increase cortisol synthesis until 87-94% of gestation by another 2.5 fold. Between 91-99% of gestation, adrenal cortisol production does not change in the fetal guinea pig, but between 99% and 100% of gestation, an increase of approximately 3 fold in males and 2 fold in females takes place.*

#### 3.4.10.2 Guinea pig maternal adrenal cortisol, adrenal cortisol ratio

Maternal adrenal cortisol production increased significantly (P<0.001) between 91-99% (day62-67) of gestation by nearly 5-fold, during a time when fetal cortisol synthesis was constant. Between 99% (day67) of gestation and birth maternal cortisol synthesis decreased significantly (P=0.008) by 1.6 fold, anti-parallel to the increase in fetal cortisol production. Fetal to maternal adrenal cortisol production ratio is 1.2 at 91% (day62), subsequently decreases to 0.7 at 94% (day64) and 0.4 at 99% (day67) of gestation. At 100% (day68) of gestation, adrenal cortisol concentrations of the fetus are 1.3 fold higher than the ones of the mother (data from [105]).

*Maternal adrenal cortisol production increases strongly from 91-99% of gestation. At birth, maternal cortisol synthesis has decreased again. The ratio of fetal to maternal cortisol synthesis decreases from 1.2 to 0.4 between 91-99% of gestation, due to dramatically increasing maternal adrenal cortisol synthesis during constant fetal cortisol production. By term, the ratio of fetal to maternal cortisol production has increased again to 1.3, caused by the late surge in fetal cortisol synthesis and the decrement in maternal cortisol production.*

#### 3.4.10.3 Guinea pig fetal and neonatal cortisol, free plasma cortisol

Owen et al. 2003 and Matthews 1998 detected very low fetal plasma cortisol levels (trunk blood) at 59% (day40) and 73-81% (day50-55), but a significant (P<0.05) increase by 10-15 fold at 94% (day64) of gestation [277, 335]. Fetal plasma cortisol (trunk blood) significantly (P<0.001)

increased from 91% to 99% (day62-67) of gestation by 9.6 fold, but remained unchanged until by 5% (PND1) of weaning [105]. Between 91-99% (day62-67) of gestation, 90% of the cortisol found in fetal plasma was assumingly of maternal origin and adrenal content of the fetus only increased over the last day [108]. Malinowska et al. 1974 investigated postnatal plasma cortisol in the newborn until adulthood. From 2.5 hours after birth until 19% (PND4), plasma cortisol concentrations did not change significantly, while between 19-95% (PND4-20) of weaning, there was a significant ($P<0.01$) 4-fold decrease to adult like levels [270]. The percentage of free cortisol in fetal circulation remained roughly constant between 88-94% (day60-64) of gestation. The fraction of free cortisol increased between 94-100% (day64-68) of gestation from 13% to 27%, due to a decrease in CBG bound cortisol from 80% to 57%. Directly after delivery, the percentage of free plasma cortisol in the neonate had decreased again to 16%. At 100% (day68) of gestation, the ratio of fetal to maternal free plasma cortisol is 0.54, indicating twice higher free cortisol levels in the mother than in the fetus (data from [108]).


Figure 3.52. Guinea pig fetal plasma cortisol

*Fetal plasma cortisol concentrations seem to remain very low between roughly 60-81% or most likely even until 91%, but levels increase sufficiently by 94% of gestation and further to high levels until 99% of gestation. Subsequently, plasma cortisol levels remain constant until at least 19% of weaning but neonatal cortisol in circulation decreases dramatically to assumingly adult like levels by 95% of weaning. As fetal adrenal cortisol synthesis remains unchanged until 99% of gestation, the increment in fetal plasma cortisol concentrations is assumingly due to placental transfer of maternal cortisol, as maternal adrenal cortisol strongly increases over that period. It is assumed that between 91-99% of gestation, 90% of fetal plasma cortisol is of maternal origin.*

*From 88% till 91% of gestation, the percentage of free cortisol in fetal plasma stays constant, but increases by 1.8 fold until term in parallel with decreasing percentage of cortisol bound to CBG. At 100% of gestation, free cortisol of the fetus is roughly half of the free cortisol fraction in the mother. Already during delivery the percentage of free plasma cortisol in the fetus decreases again.*

3.4.10.4 Guinea pig maternal plasma cortisol, plasma cortisol ratio

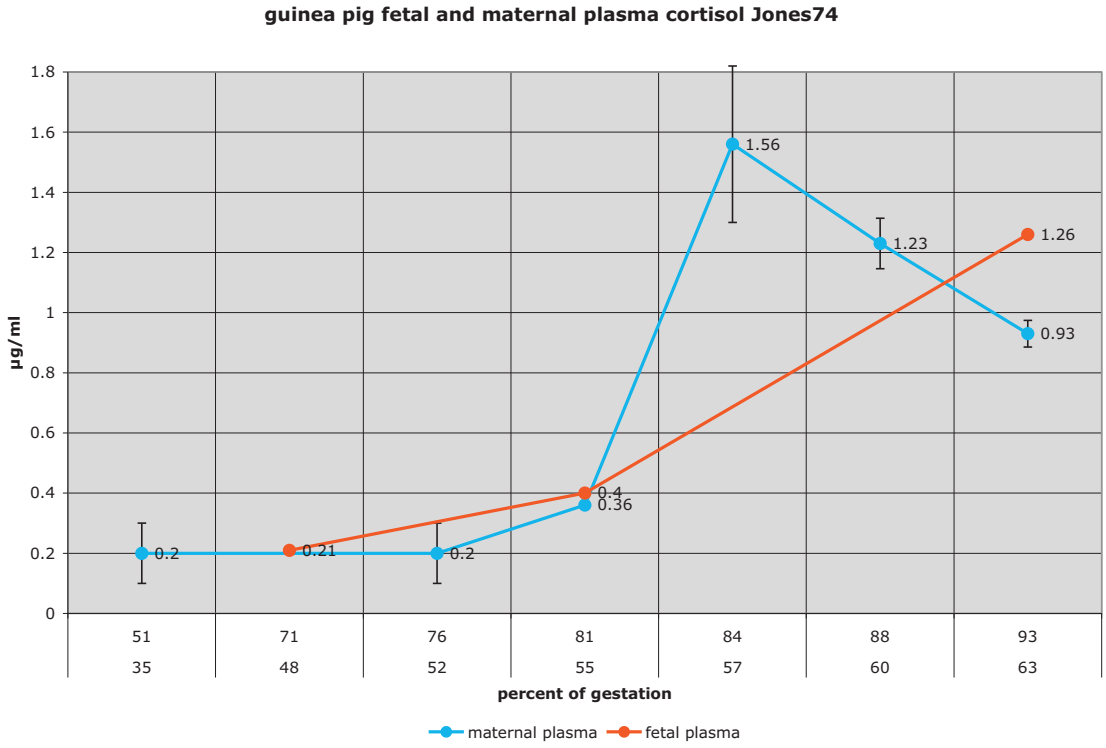


Figure 3.53. Guinea pig fetal and maternal plasma cortisol [217]

Between 51-76% (day35-52) of gestation, the concentration of maternal plasma corticosteroid, which contained predominantly cortisol, was very low. Maternal plasma cortisol increased significantly (P<0.001) between 81-88% (day 55-60) by 3.4 fold, but decreased (P=0.002) again between 88-93% (day60-63) of gestation by 1.3 fold [217]. At 91% (day62), cortisol concentrations in maternal circulation were already very high and significantly (P<0.01) increased until 99% (day67) of gestation by 1.8 fold [105]. During parturition maternal plasma cortisol significantly (P<0.001) decreased by 1.5 fold. The percentage of free cortisol in maternal circulation increased from 2% to 14% between 88-100% (day60-68) and did not change further during delivery. The percentage of cortisol bound to CBG in maternal circulation decreased over the same time from 96% to 63% [108]. At 81% (day55), the cortisol concentrations in fetal and maternal circulation are not significantly different, while at 93% (day63) of gestation, fetal to maternal plasma cortisol ratio is 1.4 (P=0.001) (data from [217]). Surprisingly, by using data from Dalle et al. 1976, we receive by far lower ratios. The ratio of fetal to maternal plasma cortisol increases was from 0.05 to 0.25 between 91-100% (day62-68) of gestation (data from [105]).

*Maternal plasma cortisol is very low at 51-76%, then increases strongly until it peaks at 84% and decreases again at 93% of gestation. Maternal plasma cortisol increases by 2 fold between 91-100% of gestation but decreases already during labor again. Over the last week, the percentage of free cortisol in maternal plasma increases by 7 fold. Still at term, the absolute concentrations of free plasma cortisol are only two times higher in maternal then in fetal circulation. Similar to the situation in the fetus, percentage of maternal plasma cortisol bound to CBG decreases in late*

*gestation. Beside dramatic differences in the absolute values of fetal to maternal plasma cortisol ratios between the two authors, over the period between 81-100% of gestation, the ratio increases.*

### 3.4.11 Summary guinea pig fetal cortisol

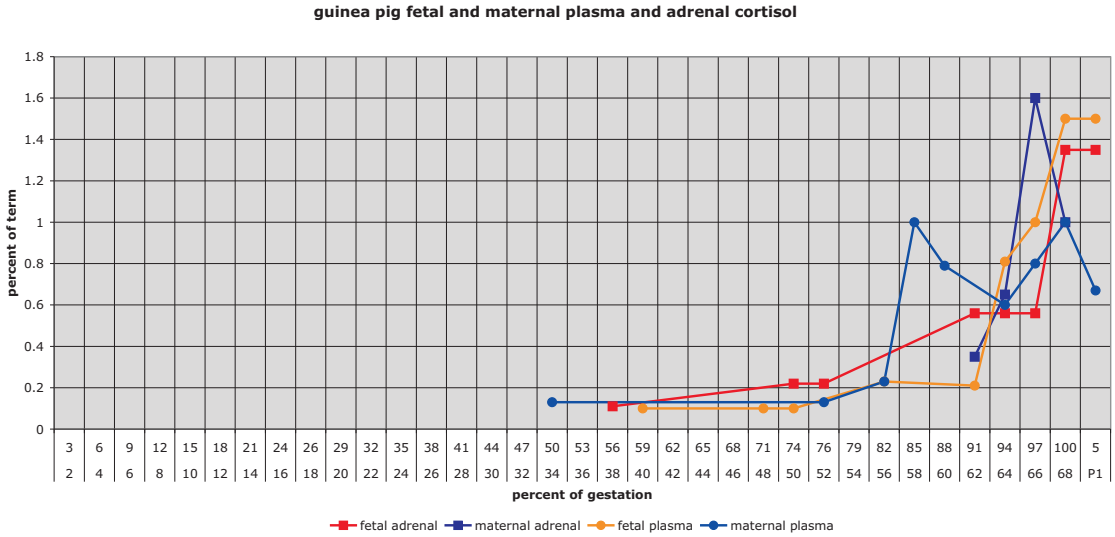


Figure 3.54. Guinea pig fetal and maternal plasma and adrenal cortisol

- *Investigating the cortisol production in the fetal and neonatal guinea pig is quite difficult, due to the rare data especially early in gestation. Enzyme expression during gestation could indicate cortisol synthesis at least between 41-46% as well as 66-73% of gestation. After birth data of enzyme expression indicate additionally to the zF, cortisol synthesis in the zR. Enzyme expression in the zR, at least in young adults, changes in parallel with adrenal cortisol secretion. The fetal adrenal gland is able to produce cortisol at least between 37-51% of gestation. Over that period, the increase in steroidogenic organelles in the zF indicates an increase in adrenal cortisol synthesis. Between 50-82% of gestation, presence or absence of ACTH only elicit very low cortisol production. In the period between 87% of gestation and term, the fetal adrenal cortisol synthesis is by far more responsive to ACTH. Cortisol synthesis assumingly remains very low roughly between 50-87% and reaches a plateau at moderate levels at 91-99% of gestation. Between 99-100% of gestation, adrenal cortisol synthesis dramatically increases to high levels and remains constant in females but further increases in males until 5% of weaning.*

- *Maternal adrenal cortisol synthesis increases by 5 fold between 91% and 99%, but has decreased already between 99% and 100% of gestation by 1.6 fold. The ratio of fetal to maternal cortisol production is roughly 1.2 at 91% of gestation, but gradually decreases to 0.4 at 99% of gestation, due to increasing maternal synthesis. At 100% of gestation, the ratio is again 1.3, as a result of suddenly dramatic increment of fetal adrenal cortisol synthesis.*

- *Fetal plasma cortisol levels assumingly remain low after 59%, increase slightly by 82%, remain roughly constant until 91%, before a dramatic increase takes place. Between 91-94% of gestation, fetal plasma cortisol increases by 4 fold and again by 1.2 fold by 97% of gestation. Between 97% and term, cortisol in fetal circulation increases strongly by 1.5 fold. From birth*

*until 19%, neonatal plasma cortisol remains unchanged but by 95% of weaning, neonatal cortisol in circulation decreases dramatically to assumingly adult like levels. This decrease could rather reflect decreasing cortisol synthesis in the zR than in the zF as it is indicated by enzyme expression later in young adults.*

- *Maternal plasma cortisol concentrations remain, analog to fetal plasma cortisol concentrations, low between 50-76%, increase slightly in parallel with fetal plasma cortisol to similar levels at 82%, subsequently peak by 85% of gestation, increasing by over 4 fold. By 94%, maternal plasma cortisol decreases to moderate and slightly lower levels than in fetal circulation, just to increase again to a second peak at term. Subsequently the concentration decreases already during parturition.*

- *Fetal cortisol synthesis seems to be present by 37-51% of gestation. From 56% till roughly 87% of gestation, fetal adrenal cortisol synthesis remains very low and largely unresponsive to ACTH, but can be assumed to increase to moderately low levels by 91-99% and then dramatically increase until term. Fetal plasma cortisol concentrations remain very low roughly between 59-91%, but have strongly increased by 93% of gestation and further until birth. The first peak of maternal plasma cortisol concentrations at 85% of gestation could remain unnoticed in fetal circulation, in case of high placenta 11$\beta$HSD2 expression. Between 94-97% of gestation, fetal plasma cortisol concentrations increase together with maternal plasma cortisol levels. This happened regardless of the low fetal adrenal cortisol synthesis, indicating low 11$\beta$HSD2 expression around term and strong contribution of maternal cortisol to fetal circulation. Between 99-100%, fetal cortisol synthesis peaks, while maternal cortisol production climaxes at 99% and decreases already by 100% of gestation, together with the decreasing maternal plasma cortisol concentrations during parturition. Fetal plasma cortisol, now mostly of fetal origin, remains constantly high together with fetal adrenal cortisol from birth to 5% of weaning.*

### 3.4.12 Rat glucocorticoid synthesis

#### 3.4.12.1 Rat fetal adrenal cortisol

At 70% (e15.5) of gestation, medulla cells started to immigrate into the cortex [53]. Cells of the zF were present in the fetal adrenal cortex as early as 73% (e16) of gestation when the fetal period began [304, 470]. While proliferation was high throughout the adrenal cortex from 73-82% (e16-18), levels were decreasing at 86% (e19) of gestation and were much lower in the zF than in the zG [303, 304, 452]. The adult rat was unable to synthesize cortisol in the adrenals, due to the lack of the CYP17 expression [96]. Surprisingly, Decker et al. 1981 were able to detect cortisol content in the fetal rat adrenal gland. Adrenal cortisol was measurable from 73% (e16) until 96% (e21) of gestation and in newborn rats. Cortisol content increased continuously (P=0.032) between 73-86% (e16-19) of gestation by 3.6 fold. Between 86-91% (e19-20), cortisol content decreased significantly (P=0.02) by 11 fold to very low levels and remained constantly low between 91-96% (e20-21) of gestation and in newborns [123]. Unfortunately, cortisol content at 100% (e22) of gestation is not investigated. Kalavsky et al. 1971 also detected cortisol in the adrenal gland of the fetal rat. Between 82-86% (e18-19) of gestation, cortisol content decreased significantly (P=0.001) by 7.6 fold. No data were available for 91% (e20) of gestation. At 96% (e21) and in newborns, fetal adrenal cortisol had decreased (P<0.001) to undetectable values compared to 86% (e19) of gestation [226].

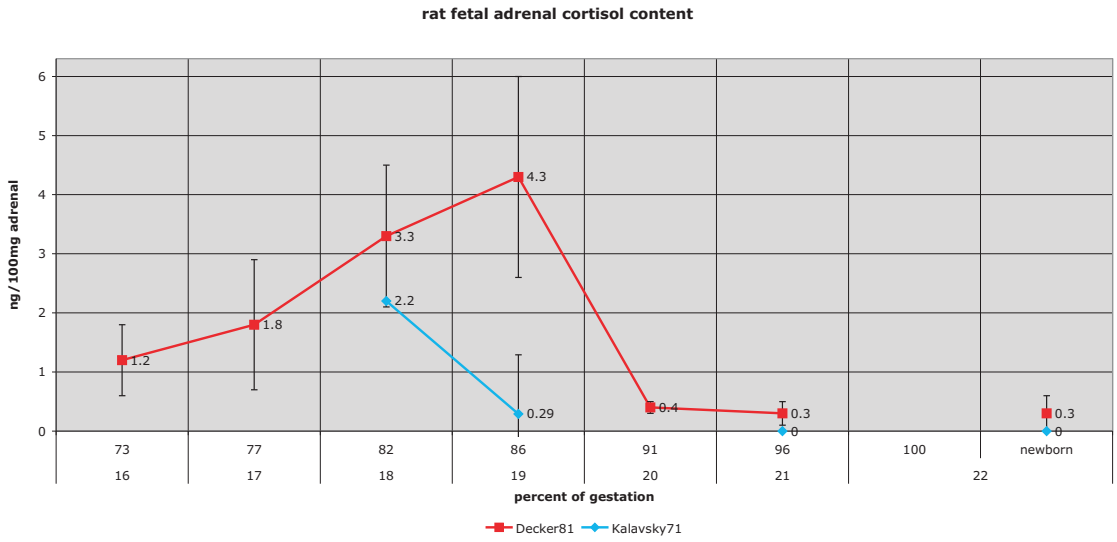


Figure 3.55. Rat fetal adrenal cortisol content

*It is very surprising to find cortisol synthesis transiently in the fetal rat, as it is known that adult rats lack the enzyme CYP17 and cannot synthesize cortisol. In late gestation between 73% and 86%, fetal adrenal cortisol concentrations increase by 3.6 fold to maximal levels, but have already decreased dramatically by 8-11 fold to very low levels at 91% of gestation. Between 91-100% of gestation as well as in newborns, cortisol in the adrenal gland is very low or absent.*

#### 3.4.12.2 Rat fetal adrenal corticosterone

Adrenal corticosterone production was detected as early as 61-73% (e13.5-16) of gestation [123, 390]. Corticosterone content in the adrenals increased significantly (P<0.001) from very low levels at 73% (e16) to a peak at 86% (e19) of gestation by 39 fold. At 86% (e19) of gestation, fetal

corticosterone content was slightly greater than the average concentrations in the adrenals of adult rats. In Kalavsky et al. 1971, corticosterone content between 86-96% (e19-21) of gestation had significantly (P=0.001) decreased by 7.1 fold and was constantly low in newborns. Decker et al. 1981 presented decreasing (P=0.004) corticosterone content between 86-96% (e19-21) of gestation by 2.5 fold and a further significant (P<0.001) decrease in newborn by another 3 fold [123, 226]. There was no significant change in adrenal corticosterone content directly before delivery, during delivery or after the newborn was expelled [93]. The fetal adrenal corticosterone production was unresponsive to physiological ACTH levels between 89-94% (e19.5-20.5) of gestation. Between 94-98% (e20.5-21.5) of gestation and in newborn, the presence of ACTH elicited a significant (P<0.05) increase [395].

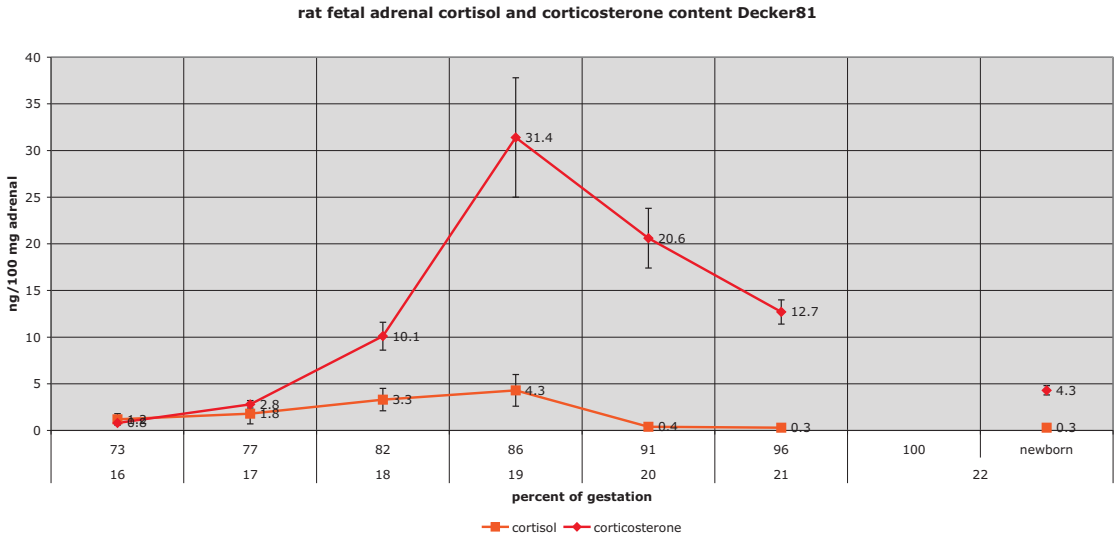


Figure 3.56. Rat fetal adrenal cortisol and corticosterone content [123]

The fetal adrenal cortisol levels are much lower than corticosterone levels. The ratio of adrenal cortisol to corticosterone at 73% (e16) is 1.5, but decreases already by 77% (e17) to 0.64, to 0.14 at 86% (e19) and further to 0.02 by 91% (e20) of gestation. In newborn rats, the ratio has slightly increased again to 0.07 (data from [123]).

*In summary, corticosterone is synthesized as early as 61-73% of gestation. Corticosterone content increases slowly until 82%, then the increment is dramatic until 86% of gestation, when maximal values are reached. Between 86% and 96%, adrenal corticosterone content sufficiently decreases again, and further decreases between 96% and 100% of gestation. Fetal adrenal corticosterone levels are very low respectively undetectable in neonates. The ratio of adrenal cortisol to corticosterone is 1.5 at 73%, but drops to very low levels by 91% of gestation and remains very low in newborn. The fetal adrenal corticosterone production is unresponsive to ACTH between 89-94% but fetal adrenal response to ACTH increases markedly between 94% and 98% of gestation.*

#### 3.4.12.3 Rat maternal adrenal corticosterone

At 91-96% (e20-21), maternal adrenal corticosterone content was moderate and constant, but significantly increased (P=0.003) at 100% (e22) of gestation by 4.4 fold. Maternal adrenal corticosterone level was elevated during parturition. The ratio of fetal to maternal adrenal corticosterone content is 2.5 at 91%, 2.1 at 96% and with higher maternal than fetal adrenal corticosterone content

at 100% of gestation, decreased to 0.4 (data from [93]).

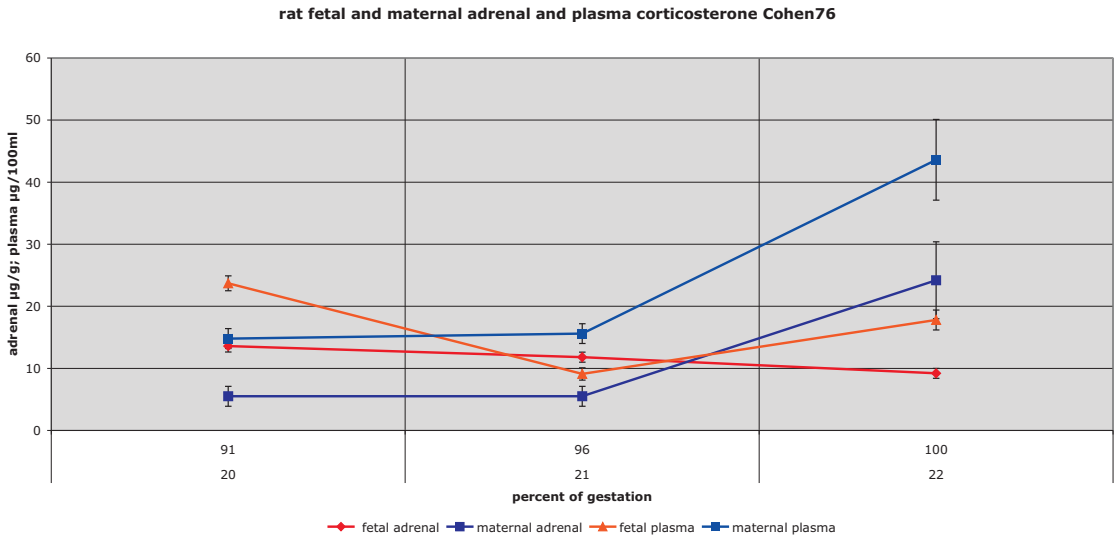


Figure 3.57. Rat fetal and maternal adrenal and plasma corticosterone [93]

*Corticosterone content in the maternal adrenals is constant from 91% till 96%, subsequently increases to very high levels by 100% of gestation. The ratio of fetal to maternal adrenal corticosterone is 2.1-2.5 at 91-96% of gestation but decreases to 0.4 by 100% of gestation.*

#### 3.4.12.4 Rat fetal and neonatal plasma corticosterone

Corticosterone in fetal plasma was non-detectable at 55% (e12) and very low at 64% (e14) of gestation. By 77% (e17) of gestation, levels had increased by 54-fold [127]. Samtani et al. 2006 discovered a significant ($P<0.02$) increase between 77-82% (e17-18) of gestation by 3.4 fold. Levels remained high by 86% (e19) and then significantly ($P=0.03$) decreased until 96% (e21) of gestation by 2.1 fold [401]. Cohen et al. 1976 presented between 91-96% (e20-21), a similar significant ($P<0.001$) decrease of 2.6 fold and a significant ($P<0.05$) increase of 2 fold by 100% (e22) of gestation. In Holt et al. 1968, levels remained constant between 96-100% of gestation. There was no change during parturition [93, 200].

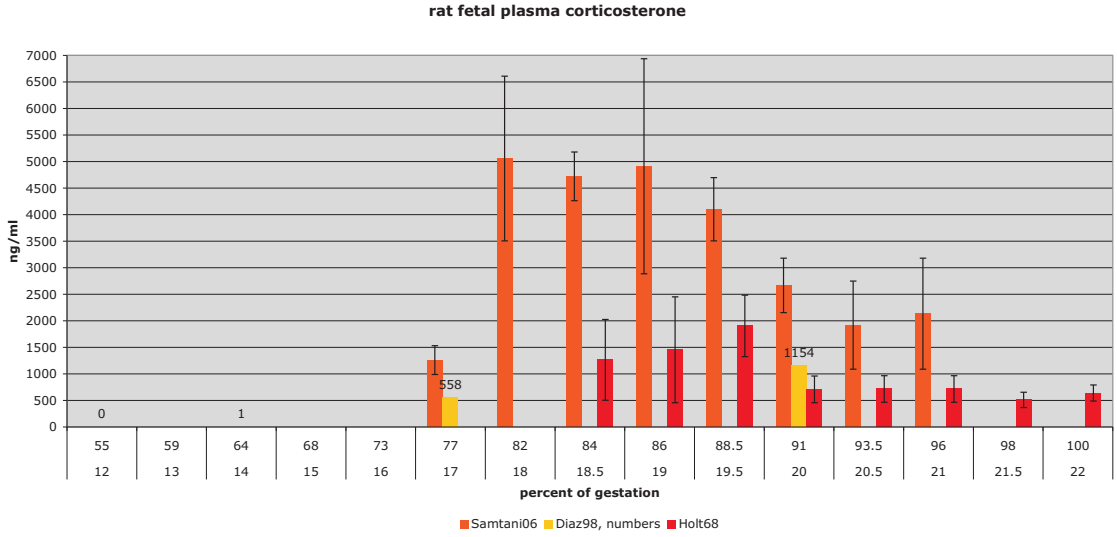


Figure 3.58. Rat fetal plasma corticosterone

Neonatal plasma corticosterone concentrations increased strongly (P<0.001) in the first 5 hours after birth by 3.5 fold [200]. Due to Levine 2001, plasma corticosterone in the neonate decreased dramatically by 10% (PND2) and stayed low until roughly 67% (PND14) of weaning [247]. Total plasma corticosterone levels were extremely low between 29-57% (PND6-12) of weaning. By 67% (PND14), plasma corticosterone concentrations had significantly (P<0.001) increased by 4.7 fold and further increased by 4.5 fold (P<0.001) until 100% (PND21) of weaning. Levels peaked at PND24, reaching concentrations significantly (P=0.001) higher than at 100% (PND21) of weaning. Subsequently, levels significantly (P<0.001) decreased by PND27 and were still roughly two times lower than peak values in adult males [21, 191]. Plasma corticosterone values seem to be extremely low in adulthood and low after birth compared to maximal prenatal values. Peak values between 82-88% of gestation are around 4-24 times higher than in adults (data from [20, 21, 401]).

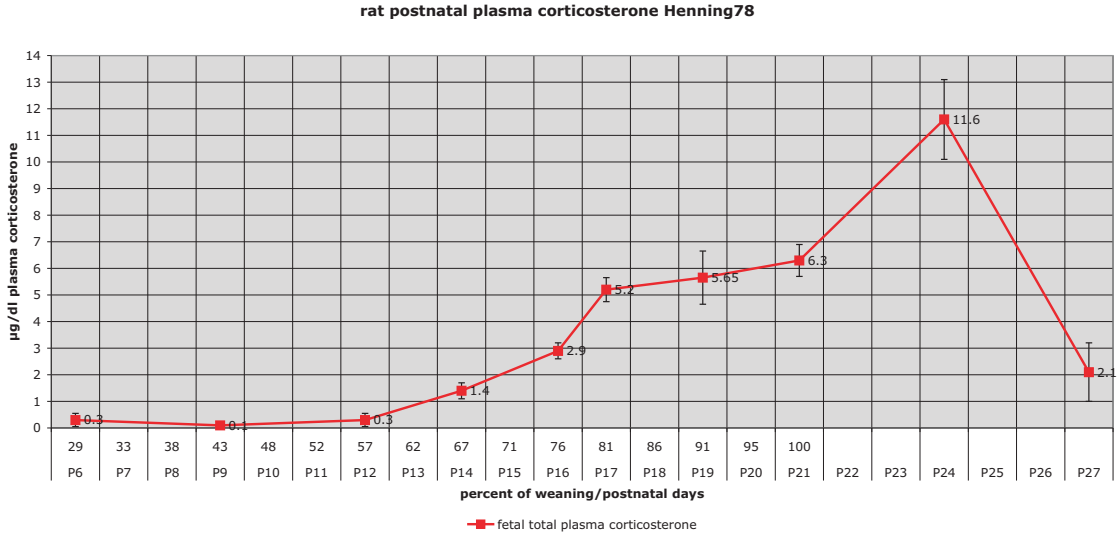


Figure 3.59. Rat postnatal plasma corticosterone [191]

*Fetal plasma corticosterone is undetectable at 55% and very low at 64% of gestation. Levels strongly increase by 77% and further to maximal concentrations at 82% of gestation. Levels remain high at 86% and between 91-96% of gestation, fetal plasma corticosterone decreases to moderately low levels, and subsequently remains constant during parturition. In the first 5h after birth, there is a dramatic increase in neonatal plasma corticosterone concentrations. Plasma corticosterone decreases to very low concentrations by 10% and remains low at least until 57% of weaning. By 67% of weaning, the concentrations increase again and increase further to peak at PND24 above adult levels. Until PND27, plasma corticosterone decreases to adult values.*

#### 3.4.12.5 Rat maternal plasma corticosterone, plasma corticosterone ratio

Between non-pregnant status and 4.5% (e1) of gestation, maternal plasma corticosterone concentrations significantly ($P<0.05$) decreased by 1.5 fold. Levels stayed constantly low until 41% (e9), then progressively increased ($P<0.001$) between 41-96% (e9-21) of gestation by 2.3 fold [20]. Sinha et al. 1997 discovered a significant ($P<0.01$) and markedly decrease in maternal plasma corticosterone concentrations between 82-86% (e18-19) by 3.2 fold and a significant ($P<0.01$) recovery at 96% (e21), to similar levels than at 82% of gestation [437]. Maternal plasma corticosterone concentrations were constant at 91-96% (e20-21) but significantly ($P<0.001$) increased between 96-100% (e21-22) of gestation by 2.8 fold. During parturition, maternal plasma corticosterone was elevated [93].

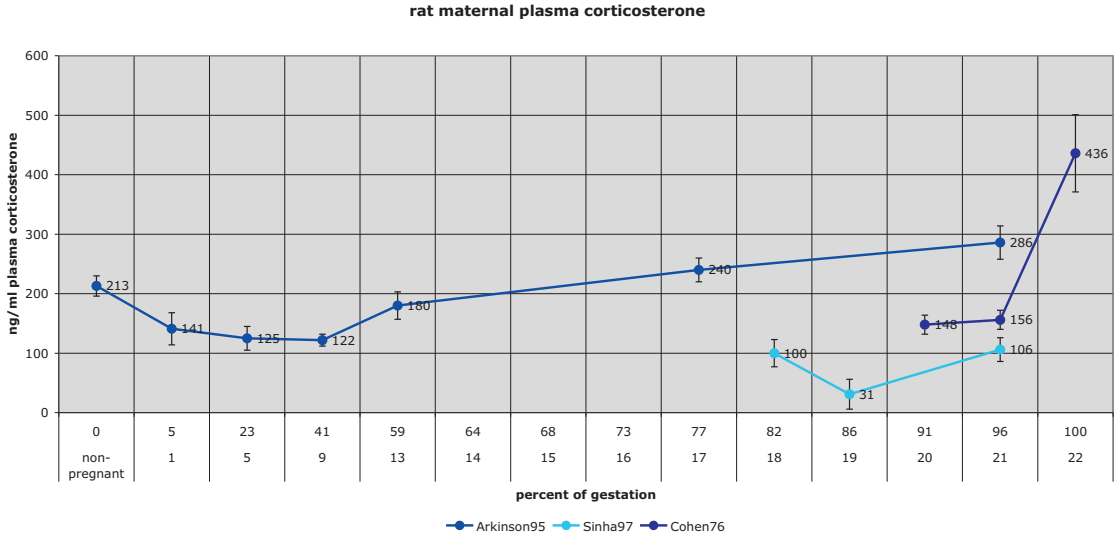


Figure 3.60. Rat maternal plasma corticosterone

Holt et al. 1968 presented 6 times higher plasma corticosterone concentrations in the fetus than in the mother at 86% (e19) of gestation [200]. At 86% (e19) of gestation, fetal to maternal plasma corticosterone ratio peaks at 2.1 for male fetuses and at 3.6 for female fetuses, but prior to that at 82% (e18), the ratio is 0.8 and afterwards at 96% (e21) of gestation is again 0.9-1 (data from [437]). By using the data from Cohen et al. 1976, at 91% (e20) of gestation the fetal to maternal plasma corticosterone ratio is 1.6, but decreases to 0.6 at 96% (e21) and to 0.4 at 100% (e22) of gestation (data from [93]).

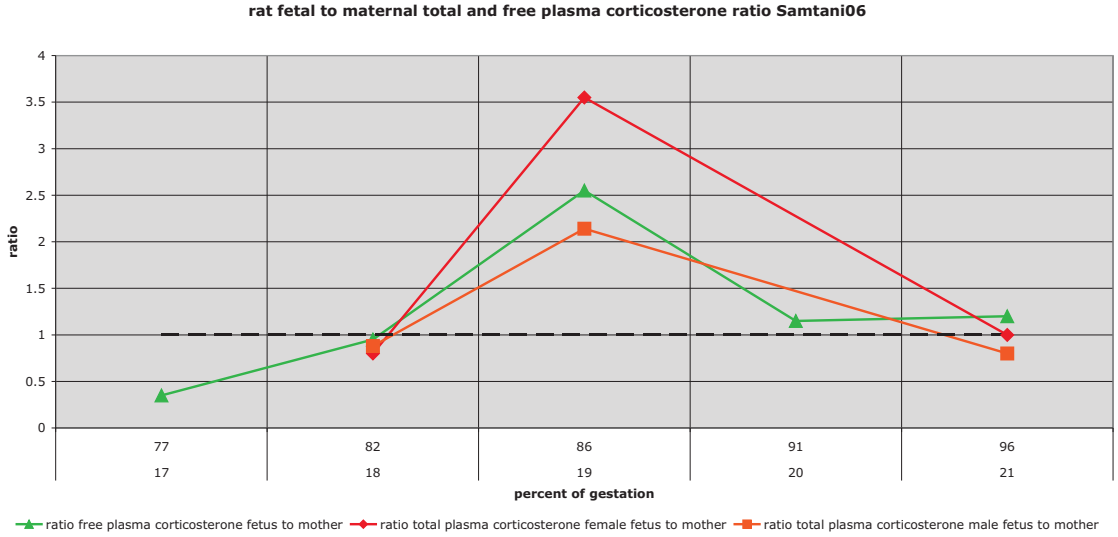


Figure 3.61. Rat fetal to maternal ratio of total and free plasma corticosterone [401]

*Maternal plasma corticosterone concentrations between 5-41% of gestation are significantly lower than pre-pregnancy levels. By 77% of gestation, maternal plasma corticosterone concentrations have recovered to similar levels compared to before pregnancy. Between 82-86% of gestation, levels suddenly and markedly decrease by more than 3 fold, to recover again by 91% of gestation. Between 91-96%, maternal plasma corticosterone remains constant but dramatically increases until 100% of gestation. At 82% of gestation, the maternal plasma corticosterone concentrations are slightly higher than corticosterone levels in fetal circulation. By 86% of gestation, fetal plasma corticosterone concentrations are markedly higher than the ones of the mother, especially in the female fetus, due to extremely low maternal plasma values. Subsequently, maternal plasma corticosterone concentrations increase, while corticosterone levels in fetal circulation decrease again, causing a ratio of 1.6 at 91% of gestation. The ratio further decreases to roughly 1 at 96%, before the dramatic increase in maternal plasma levels between 96-100% of gestation causes a decrease in the ratio down to 0.4.*

#### 3.4.12.6 Rat plasma CBG and free plasma corticosterone

Fetal free plasma corticosterone concentrations rose to a maximum at 77% (e17) and remained constant until at least 96% (e21) of gestation [401]. The fetal plasma CBG concentrations significantly (P=0.03) increased by 1.4 fold between 73-77% (e16-17) of gestation. Levels remained high between 77-86% (e17-19), then continuously decreased (P=0.003) by 3.2-3.5 fold until 96% (e21) of gestation. CBG in fetal circulation remained low at 100% of gestation [401, 472]. Maternal plasma CBG concentrations decreased significantly (P=0.038) by 1.6 fold in parallel with fetal plasma CBG levels from a plateau at 73-86% (e16-20) to non-pregnant values at 100% (e22) of gestation [472].

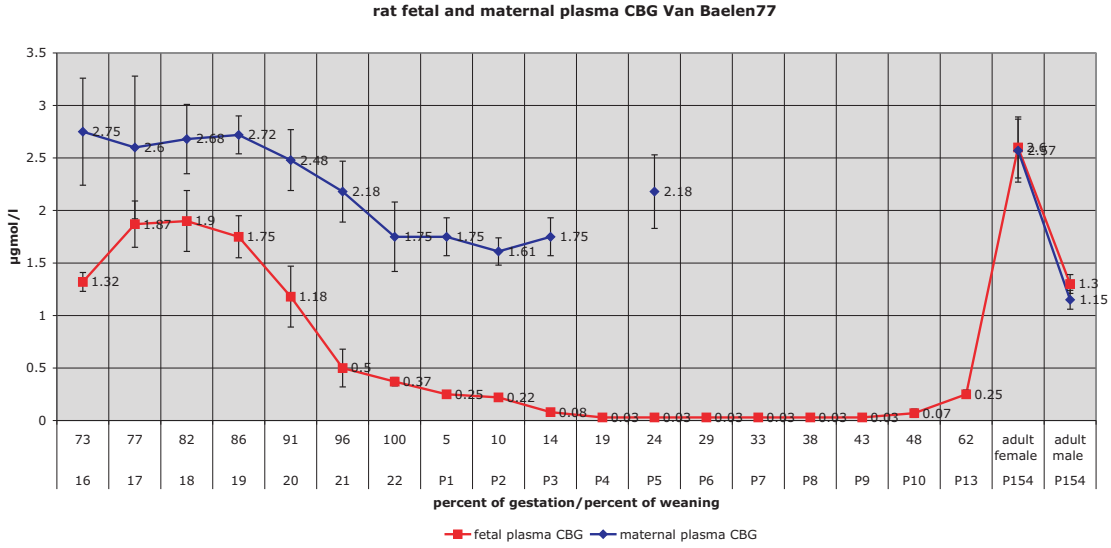


Figure 3.62. Rat fetal and maternal plasma CBG [472]

The ratio of fetal to maternal free plasma corticosterone concentrations was roughly distributed around 1 [401]. More precisely, the ratio was 0.4 at 77% (e17) and 1 at 82% (e18) of gestation. At 86% (e19), the ratio was 2.6 and decreased to 1.2 at 91-96% (e20-21) of gestation (data from [401] see Figure 3.67). Fetal and maternal plasma CBG levels were not significantly different at 77-82% (e17-18), but fetal plasma CBG concentrations were significantly (P=0.006) lower than maternal plasma CBG levels at 73% (e16) and between 86-100% (e19-22) of gestation. Fetal to maternal plasma CBG ratio decreased from 0.6 at 86% (e19) to 0.2 at 100% (e22) of gestation (data from [472]).

Postnatal, the free plasma corticosterone fraction remained low at 29% (PND6) and at 43% (PND9) of weaning. Other than total plasma corticosterone, free corticosterone levels had significantly (P=0.044) increased already at 57% (PND12) of weaning by 2.5 fold. Free plasma corticosterone fraction continuously increased until it peaked at 76-91% (PND16-19) of weaning (P<0.001 compared to PND14), while total corticosterone concentrations in circulation only peaked at PND24. Between 91% (PND19) of weaning and PND27, free corticosterone levels remained unchanged [191]. From low levels at birth, neonatal plasma CBG concentrations slowly and continuously decreased to very low levels until 19% (PND4) of weaning. Neonatal plasma CBG remained very low until 43% (PND9), increased slightly by 48% (PND10) and significantly (P=0.011) increased further by 62% (PND13) of weaning. At 62% (PND13) of weaning, the plasma CBG concentrations were roughly 8 fold lower than in adulthood [472].

*The free plasma corticosterone concentrations of the fetus rise to a maximum at 77% and remain constant until 96% of gestation. Fetal plasma CBG increases to high levels between 73-77% and remains high until 86% of gestation. Subsequently, CBG in fetal circulation dramatically decreases to low levels at 96% and remains low by 100% of gestation, maybe exhibiting a similar pattern than the assumed total plasma corticosterone concentrations. This assumption would result in very high fetal free plasma corticosterone percentage of total corticosterone around 96% of gestation. Maternal plasma CBG is already high at 73% of gestation, remains high until 86%, in the following gradually decreases to moderate levels by 100% of gestation, which might increase maternal free plasma corticosterone at term as well. Similar to the total plasma corticosterone ratio, the ratio of fetal to maternal free plasma corticosterone concentrations is very high at 86% of gestation. Before*

*at 77% of gestation, maternal free corticosterone concentrations are markedly higher than the ones of the fetus. At 82% and again at 91-96% of gestation, fetal free plasma corticosterone levels are similar to free corticosterone concentrations in maternal circulation. After birth, the plasma CBG concentrations continuously decrease to very low levels by 14-19%, subsequently remain unchanged until 43% of weaning. Neonatal free plasma corticosterone remains low at 29-43% of weaning, during a time, when the plasma CBG and total corticosterone concentrations are very low. Neonatal free corticosterone increases from 57% to a peak at 76-91% of weaning, then remains high by PND27. Plasma CBG levels slowly increase at 48%, and dramatically increase between 62% of weaning and adulthood.*

### 3.4.13 Summary rat fetal glucocorticoid

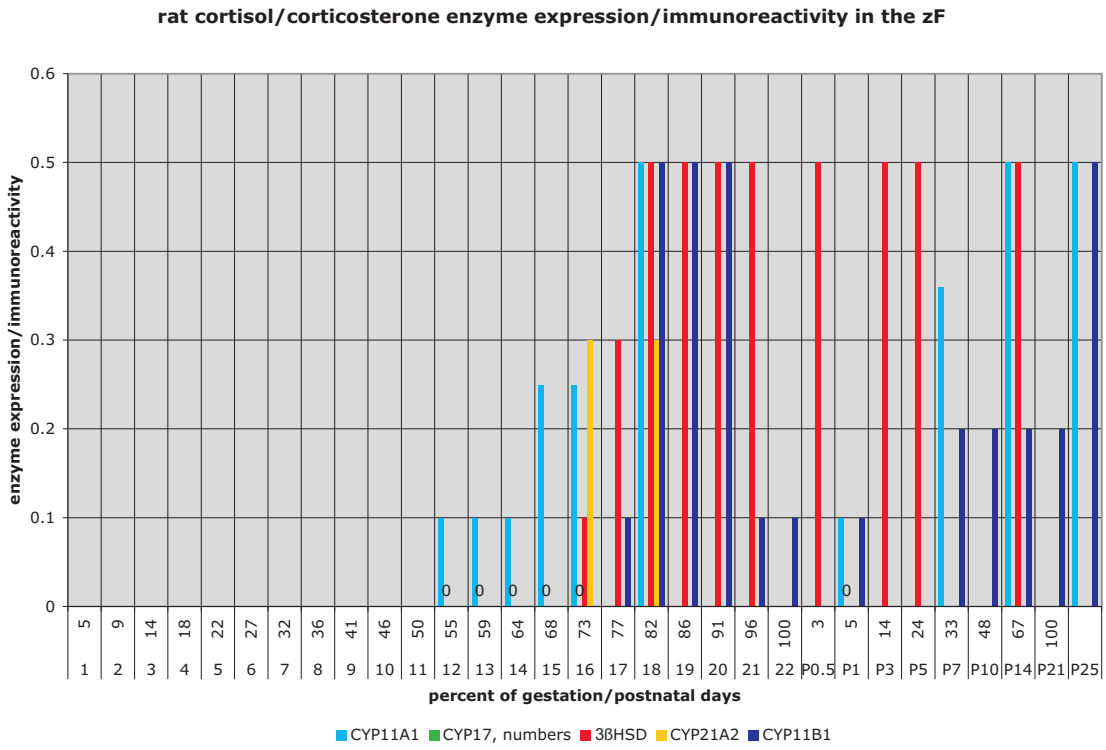


Figure 3.63. Rat cortisol/corticosterone enzyme expression in the zF

*The following assumption about corticosterone and cortisol synthesis in the fetal adrenal cortex of the rat can be made according to enzyme expression. Corticosterone synthesis can be assumed to start between 55-73% at low levels, increases at least until 82% and remains high until 91%, then decreases by 96% and remains low at 100% of gestation and at 5% of weaning. Cortisol synthesis is absent between 55-73% of gestation and at 5% of weaning. Enzyme expression indicates low corticosterone synthesis until 33% and assumingly an increase at 67% of weaning with high synthesis at PND25.*

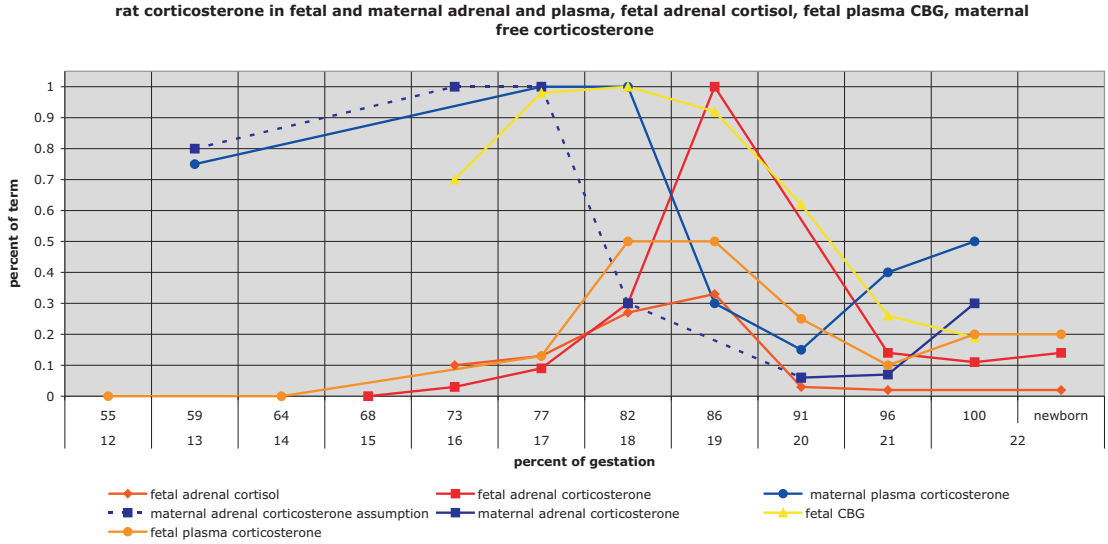


Figure 3.64. Rat - summary adrenal and plasma cortisol and corticosterone

*At 68% of gestation, fetal adrenal corticosterone production is absent and fetal plasma corticosterone concentrations are very low, assumingly because high placental 11βHSD2 activity prevents the high maternal plasma corticosterone levels from being transferred into the fetus. At 73-77% of gestation, the fetus produces low amounts of corticosterone in agreement with enzyme expression. Surprisingly, adrenal cortisol content is present in the fetus at 73% of gestation, even when no CYP17 expression could be detected at this point. Cortisol content is very low at 73% of gestation, but still higher than corticosterone production. Between 73-77% of gestation, fetal CBG significantly increases to very high levels, but instead of the assumed decrease in free glucocorticoid, fetal free plasma corticosterone increases to maximal levels at 77% and remains high until at least 96% of gestation. Adrenal cortisol production increases in parallel with corticosterone production to maximum values at 86% of gestation. The increase in fetal corticosterone synthesis is much more dramatic and reaches much higher levels than fetal cortisol synthesis. Fetal plasma corticosterone concentrations increase to maximal levels around 82-86% of gestation, while maternal plasma corticosterone concentrations decrease dramatically to low levels over that period. At 86% of gestation, the free plasma corticosterone ratio in fetus to mother reaches maximal values and fetal plasma CBG concentrations remain very high. Fetal adrenal cortisol production decreases from 86% on abruptly to very low or absent values at 91% of gestation, while corticosterone synthesis decreases to moderate levels. Fetal plasma corticosterone decreases from 86-91% of gestation. Decreasing fetal plasma CBG levels might increase fetal free corticosterone concentrations. By 96% of gestation, fetal adrenal corticosterone reaches very low levels and while maternal plasma corticosterone increases moderately, maternal adrenal corticosterone values remain low. Fetal plasma CBG concentrations are very low, assuming a high fraction of free corticosterone in fetal circulation. Where the increase in maternal plasma corticosterone concentrations at 96% of gestation originates from is not clear, as fetal and maternal glucocorticoid syntheses are low. In case of low placental 11βHSD levels, the decreasing fetal corticosterone concentrations might be still sufficient enough to transfer across the placenta into the mother. After 96% of gestation, maternal adrenal corticosterone increases. By 100% of gestation, the increasing maternal adrenal and plasma corticosterone levels have caused a small but significant increase in fetal plasma corticosterone concentrations, while fetal corticosterone synthesis remains very low in newborn.*

### 3.4.14 Mouse glucocorticoid synthesis

#### 3.4.14.1 Mouse fetal adrenal cortisol

Similar to the rat, the adult mouse adrenal cortex is unable to synthesize androgens and cortisol, due the absence of CYP17 [96, 229]. The zG and zF were distinguished by 82% (e16) of gestation. Between 82-92% (e16-18) of gestation, proliferation decreased in both zF and zG, but was still much higher in the zG than in the zF. Over the same period, steroidogenic organelles showed much higher levels in the zF [483, 519]. Cortisol content in the adrenal gland of the fetal mouse was not detectable between 74-95% (e14.5-18.5) of gestation, nor was it detectable in newborn mice [123]. Still, the expression of the enzyme CYP17 indicated a possible cortisol synthesis in the female fetus between 69-100% (e13.5-19.5) and in newborn and in male fetus from 69% (e13.5) until 79% (e15.5) or 85% (e16.5) of gestation [185, 232] (see also Chapter 3.3.8.2). An assumed higher and longer cortisol synthesis in the female compared to the male fetus could counteract a possible inhibition through maternal corticosterone transfer into the fetus, as transfer into the female fetus has been shown to be stronger than into the male fetus [309] (see Chapter 2.1.3.3 g)).

*While enzyme expression indicates cortisol synthesis the adrenal cortex of the fetal mouse, cortisol content is not detected between 74-95% of gestation or in newborn. A possible cortisol synthesis could start around 69% of gestation and is still present in female neonate, while a synthesis might cease in the male fetus between 79-90% of gestation.*

#### 3.4.14.2 Mouse fetal adrenal corticosterone

Decker et al. 1981 detected very low adrenal corticosterone levels at 74% (e14.5) of gestation [123]. From 72-77% (e14-15) of gestation, the mouse fetal adrenal was reactive to ACTH 1-24 administration [519]. In adrenalectomized dams at 74% (e14.5) of gestation, plasma corticosterone concentration was only 3% of the concentration at that time in intact dams. By 80% (e15.5), plasma corticosterone concentration in the adrenalectomized dam had increased and by 85% (e16.5) of gestation, the concentration was not different from plasma corticosterone concentration in intact dams [309]. This indicates that between 74-80% (e14.5-15.5) of gestation, the fetal adrenal starts to secrete sufficient amounts of corticosterone, and the amount increases by 85% (e16.5), at which point transfer across the placenta results in normal concentration of corticosterone in the serum of adrenalectomized dams. Corticosterone content increased significantly ($P=0.032$) by 2.4 fold from very low levels at 74% (e14.5) until 80% (e15.5), increased ($P<0.001$) further by another 2.8 fold until 85% (e16.5) and by 1.4 fold ($P=0.014$) until a peak was reached at 90% (e17.5) of gestation. By 95% (e18.5) of gestation, fetal adrenal corticosterone content had significantly ($P=0.004$) decreased by 1.6 fold. Data at 100% (e19.5) of gestation were not available. From 95% (e18.5) of gestation until 5% (PND1) of weaning, corticosterone content had significantly ($P=0.001$) decreased by 3.5 fold. Corticosterone content in the newborn mouse was not significantly different from the very low levels at 74% (e14.5) of gestation [123].

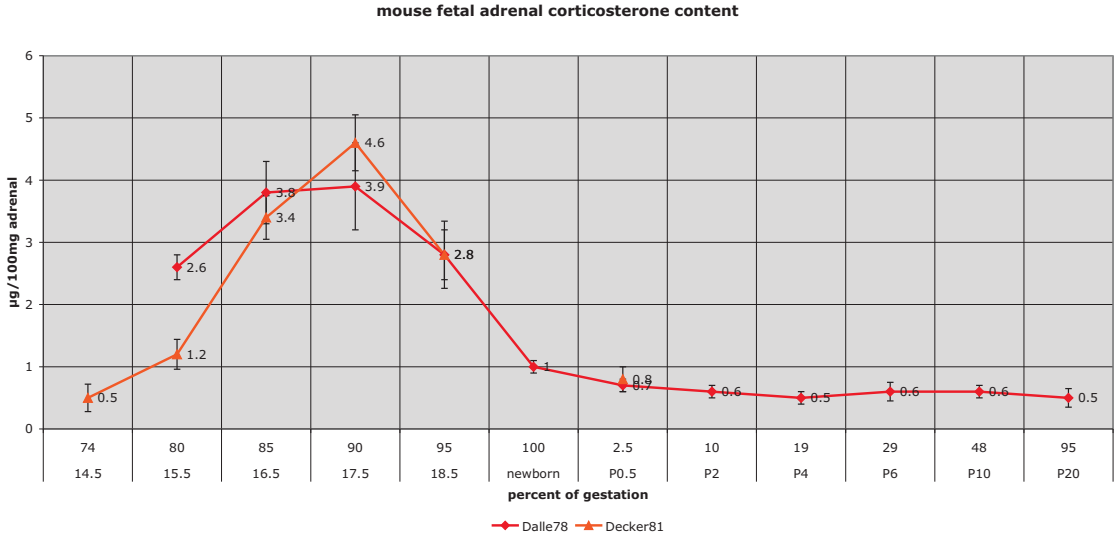


Figure 3.65. Mouse fetal adrenal corticosterone content

Dalle et al. 1978 showed that fetal adrenal corticosterone content increased significantly (P=0.026) by 1.5 fold between 80-85% (e15.5-16.5) and was maximal between 85-90% (e16.5-17.5) of gestation. From 90% (e17.5), adrenal corticosterone content significantly (P<0.001) decreased by 3.9 fold until 100% of gestation after vaginal delivery [107]. In premature newborns, adrenal corticosterone content decreased significantly (P<0.001) by 1.8 fold between birth and 6 hours after birth and by 3.3 fold between 6 hours and 10% (PND2) of weaning. Adrenal corticosterone content was unchanged at 29% (PND6) of weaning [256]. Dalle et al. 1978 detected a decrease of 1.4 fold (P=0.034) between delivery and 2.5% (PND0.5) of weaning. Adrenal corticosterone content remained very low and did not significantly change between 5-19% (PND1-4), nor at 29% (PND6) , 48% (PND10) or 95% (PND20) of weaning [107].

*Fetal adrenal corticosterone synthesis begins around 72-74%, possibly in response to ACTH, and progressively increases until it peaks at 85-90% of gestation. Between 90-100% of gestation, corticosterone synthesis in the fetal adrenal gland continuously decreases to low levels at birth. From birth until 2.5%, adrenal corticosterone content decreases to very low levels and remains very low until at least 95% of weaning.*

#### 3.4.14.3 Mouse fetal plasma corticosterone

Fetal plasma corticosterone concentration remained very low between 64-74% (e12.5-14.5) and increased by 7.3 fold at 80-85% (e15.5-16.5) of gestation [398]. Plasma corticosterone concentration significantly (P=0.009) decreased between 80-90% (e15.5-17.5) of gestation by 1.6 fold [107]. Levels decreased further (P=0.004) between 90-95% (e17.5-18.5) of gestation by 1.5 fold [475]. In total, fetal plasma corticosterone remained constant between 80-85% (e15.5-16.5) and decreased significantly (P<0.001) between 85% (e16.5) of gestation and newborn (e19.5) by 2.5 fold [107]. Plasma corticosterone was present in newborn mice of both genders, while plasma cortisol was below detection level [185]. At 90-95% of gestation, adrenalectomy in the dam led to significantly higher fetal plasma corticosterone concentrations [309].

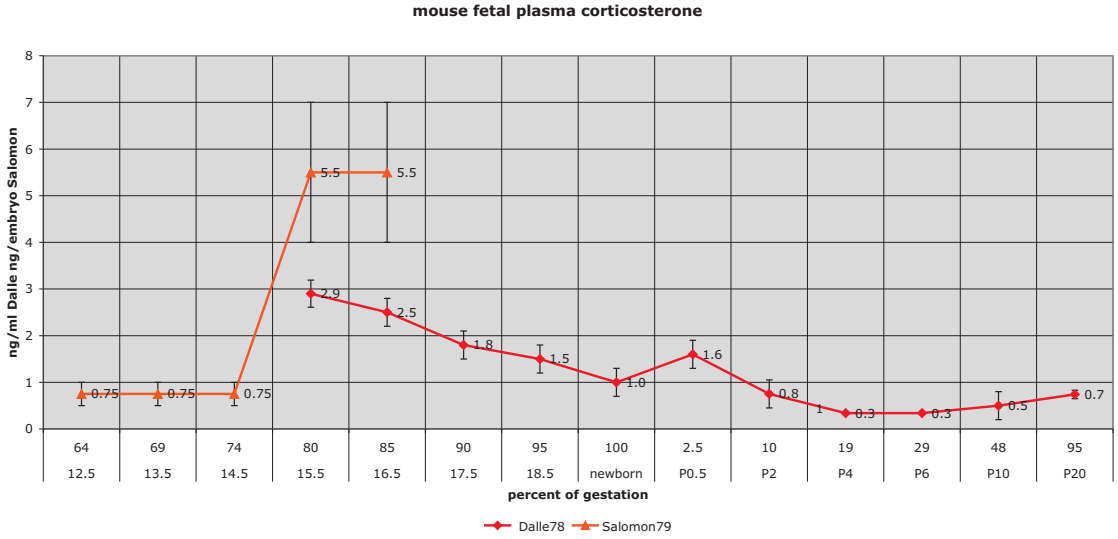


Figure 3.66. Mouse fetal plasma corticosterone

The increase in plasma corticosterone from birth until 2.5% (PND0.5) of weaning was not significant. Between birth and 19% (PND4) of weaning, neonatal plasma corticosterone concentration significantly (P=0.02) decreased by 3.3 fold to very low levels. Neonatal plasma corticosterone remained unchanged between 19-29% (PND4-6) or even 48% (PND10) of weaning [107]. Basal plasma corticosterone concentration stayed very low between 5-57% (PND1-12) and was significantly (P<0.001) elevated at 67% (PND14) and 76% (PND16) of weaning. By 86% (PND18), basal plasma corticosterone concentration significantly (P<0.0001) increased compared to 76% (PND16) of weaning and was similar to adult levels [410, 411]. By 95% (PND20), the plasma corticosterone concentration had significantly increased (P<0.001) by 2.2 fold compared to 29% (PND6) of weaning [107].

*Fetal plasma corticosterone concentration is extremely low from 64% till 74% of gestation. By 80% of gestation, the plasma corticosterone concentration has dramatically increased. From 80-85% of gestation until term, fetal plasma corticosterone decreases to low levels. Adrenalectomy of the dam significantly increased fetal plasma corticosterone concentrations at 90-95% of gestation, possibly due to the annulled inhibition of maternal plasma corticosterone on fetal adrenal corticosterone synthesis during that time. In the first hours after birth, neonatal plasma corticosterone concentration might increase, while adrenal corticosterone levels already decrease. Adrenal and plasma corticosterone concentration decrease until 10% respectively 19% and stay low in the SHRP until approximately 57% of weaning. At least by 95% of weaning, plasma corticosterone has increased again.*

#### 3.4.14.4 Mouse maternal adrenal corticosterone, adrenal corticosterone ratio

Maternal adrenal corticosterone content did not significantly change between 80% (e15.5) and 100% (e19.5) of gestation. Maternal adrenal corticosterone levels were sufficiently lower than fetal adrenal corticosterone concentrations over that period [107]. The fetal to maternal adrenal corticosterone ratio increases between 80-85% (e15.5-16.5) from 3.3 to 6.9, remains high until 95% (e18.5) of gestation and decreases to 1.9 at 100% of gestation after delivery (data from [107]).

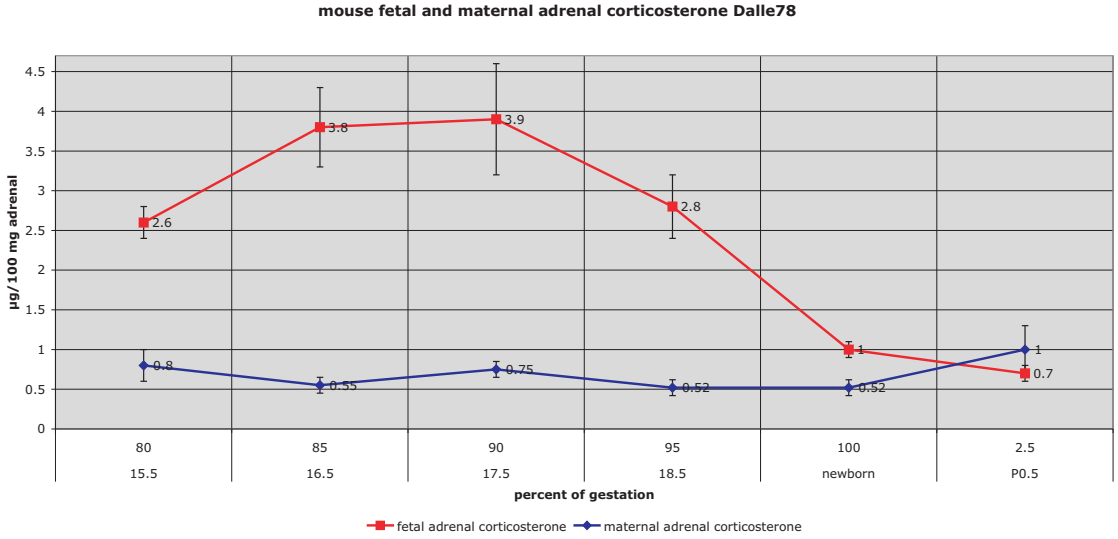


Figure 3.67. Mouse fetal and maternal adrenal corticosterone [107]

*Maternal adrenal corticosterone content does not change from 80% of gestation until after delivery. Compared to fetal adrenal corticosterone, maternal adrenal corticosterone levels are very low. The ratio of fetal to maternal adrenal corticosterone increases to very high levels between 80-85% due to increasing fetal corticosterone synthesis, and decreases again from 90% of gestation until after delivery to a ratio of roughly 2.*

#### 3.4.14.5 Mouse maternal plasma corticosterone, plasma corticosterone ratio

Dalle et al. 1978 showed a significant (P<0.001) increase from non-pregnant values until 80% (e15.5) of gestation by 38.8 fold [107]. Corticosterone concentration in maternal plasma increased significantly (P<0.001) by 6.6 fold from non-pregnant values until 54% (e10.5) of gestation. Between 54-64% (e10.5-12.5), maternal plasma corticosterone increased significantly (P<0.001) by 3.2 fold and again until 85% (e16.5) of gestation (P=0.004) by another 2.9 fold. Between 80-85% (e16.5) of gestation, the level did not change [30]. Maternal plasma corticosterone decreased significantly (P<0.001) between 85-95% of gestation by 1.7 fold or between 80% until 1 hour after delivery (P<0.001) by 10.3 fold [107, 475].

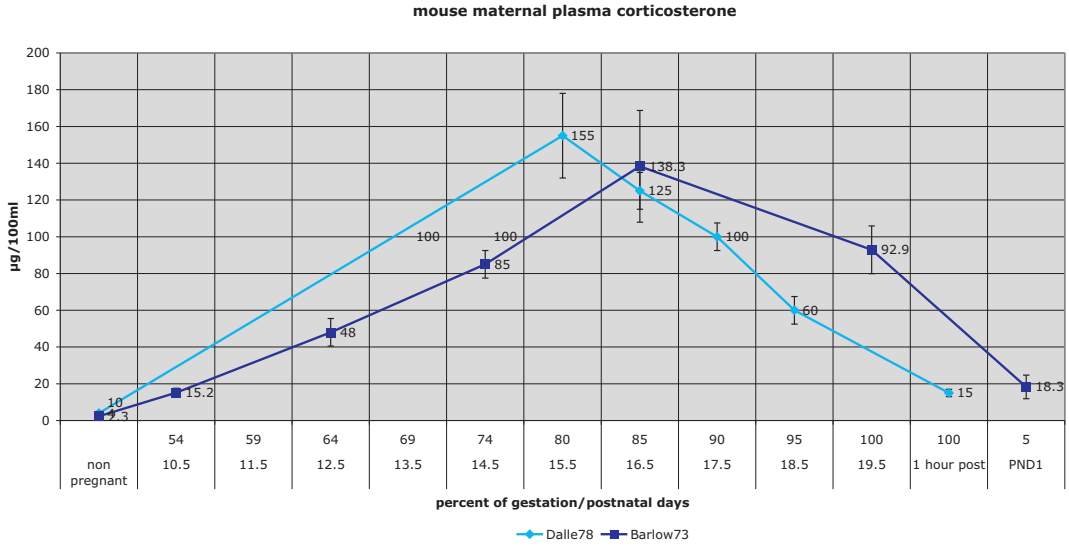


Figure 3.68. Mouse maternal plasma corticosterone

The maternal plasma corticosterone concentration was much higher than the one of the fetus during late gestation [107]. The fetal to maternal plasma corticosterone ratio is extremely low between 69-74% (e13.5-14.5) and shows values of 0.18-0.25 between 80-95% (e15.5-18.5) of gestation. After vaginal delivery, fetal and maternal plasma corticosterone levels are roughly similar with a ratio of 0.7 (data from [107, 398]).

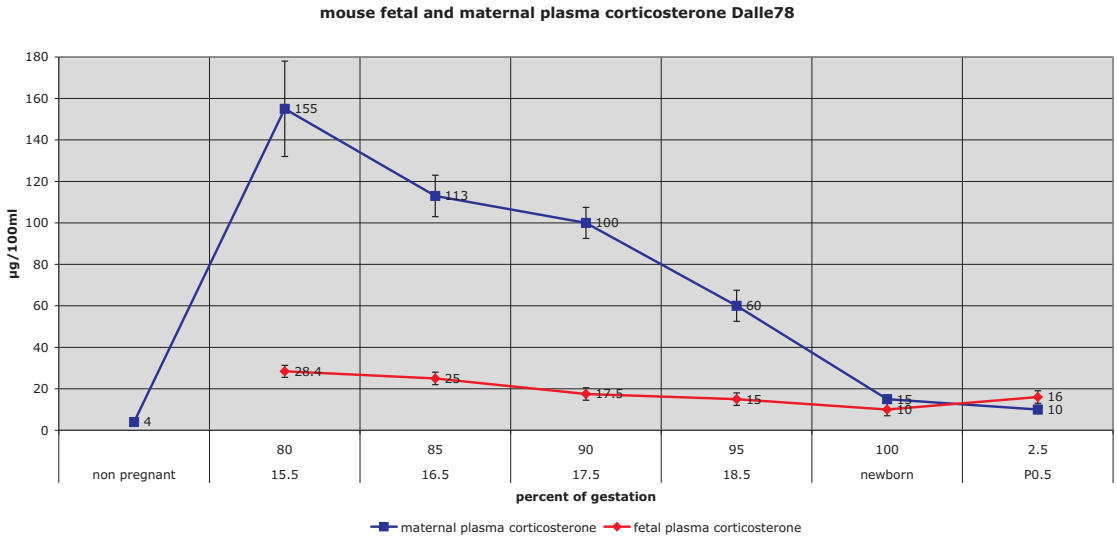


Figure 3.69. Mouse fetal and maternal plasma corticosterone [107]

*The maternal plasma corticosterone concentration increases slowly from non-pregnant values until 54% of gestation and continuously increases until a peak at 80-85% of gestation. Subsequently, maternal plasma corticosterone concentration decreases to low levels until after delivery. While between 80% of gestation and delivery, the adrenal corticosterone content is much higher in the fetus than in the mother and varies between 1.9-6.9, maternal plasma corticosterone levels are much higher than corticosterone in fetal circulation and only increase from 0.18 to 0.7 over that period. This could be caused by a very high metabolic clearance rate in fetal circulation during that time.*

#### 3.4.14.6 Mouse CBG expression

As no information is available about fetal free plasma corticosterone or CBG in the mouse, it will be referred to CBG expression in liver and kidney. Hepatic IR-CBG and CBG mRNA were absent in the fetal mouse liver between 28-54% (e5.5-10.5) and mRNA was first detected in low levels at 59% (e11.5) of gestation. At 80/85% (e15.5/16.5) of gestation, the abundance of hepatic CBG mRNA was maximal. By 90% (e17.5), the amount had already decreased again, and further decreased at 95% (e18.5) until the signals for hepatic IR-CBG and CBG mRNA were both undetectable at 100% (e19.5) of gestation [419]. Postnatal, CBG mRNA was absent in the neonatal liver from birth to 33% (PND7) of weaning, when a faint signal was detectable. Levels increased from 48% (PND10) of weaning until PND28 to adult levels, at which point sexual dimorphism was detectable with higher levels of hepatic CBG expression in the female mouse. Plasma CBC developed in a similar way, with low levels at 33-48% (PND7-10), a dramatic increase at 67% (PND14) and further increment until 100% (PND21) of weaning, with again higher levels in the female than in the male at that point [420]. During gestation, CBG mRNA was undetectable in the fetal kidney, but was present at birth and increased between 33-100% (PND7-21) of weaning to maximal levels. By PND28, CBG mRNA expression in the kidney had dramatically decreased and was absent by PND42 and in adult [420].

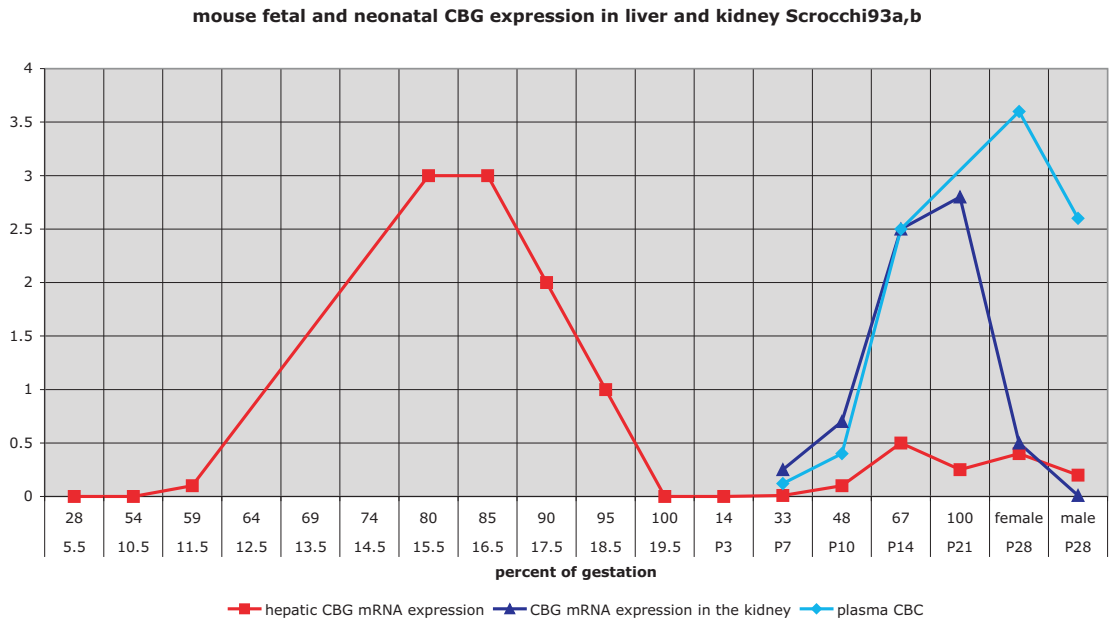


Figure 3.70. Mouse fetal and neonatal CBG expression in liver and kidney [419, 420]

*Lacking any information about CBG or free corticosterone concentration in the circulation of the fetal mouse, fetal hepatic CBG expression will be taken into account as an indicator for the development of free corticosterone/cortisol concentration in fetal plasma. Between 59% and 80/85% of gestation, fetal hepatic CBG mRNA expression increases to maximal levels and decreases at 90% and further at 95% to low levels at 100% of gestation. Plasma CBG can be assumed to follow the development of mRNA expression in a shortly delayed but similar pattern. As plasma corticosterone concentration peaks at 80-85% and decreases thereafter until 100% of gestation in a surprisingly analog way, free corticosterone levels might stay roughly constant. At 100% of gestation and until approximately 33%, with the absence of CBG expression in the liver and low CBC plasma concentration at 33% of weaning, a high percentage of free corticosterone can be assumed. After 33% of weaning, CBG expression in the liver and the kidney both increase in parallel with plasma CBC. CBG expression in the kidney is maximal at 100% of weaning, subsequently decreases dramatically toward PND28, while plasma CBC further increases in females and remains constant in males at PND28. So we assume after birth, high free corticosterone levels until at least 33% of weaning.*

### 3.4.15 Summary mouse fetal glucocorticoid

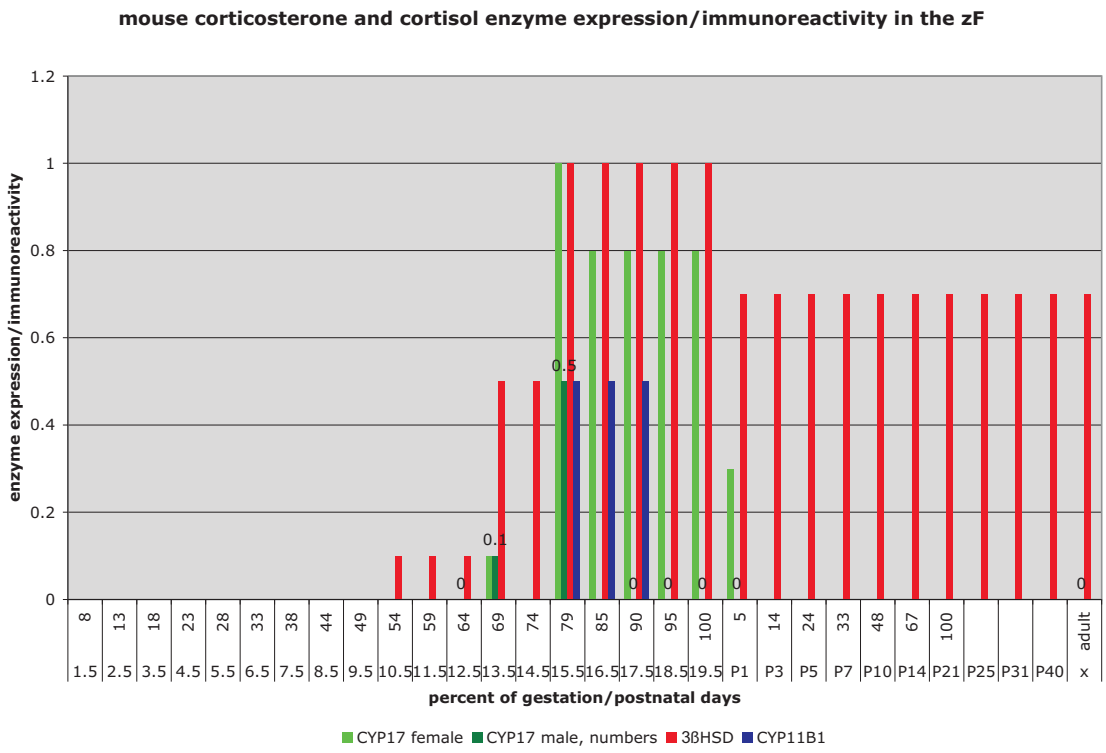


Figure 3.71. Mouse corticosterone and cortisol enzyme expression in the zF

*Cortisol synthesis, due to the presence of CYP17 expression, might be absent at 64% and start at 69% of gestation in both genders. CYP17 expression is maximal at 79% of gestation, but stronger in female fetuses than in males. The assumed cortisol synthesis seems to remain high in the female until birth, but after the peak at 79% might decrease in the male fetus, to be absent by approximately 90% of gestation until birth. This stronger and longer predicted cortisol production in the*

*female fetus compared to the male fetus is surprising, but could counteract the stronger corticosterone transfer from the mother into the female and its inhibition on the fetal adrenal glucocorticoid synthesis. A possible cortisol synthesis with a peak at 79% of gestation could support the increasing corticosterone synthesis during that period. Low corticosterone synthesis is assumed at 54-59% of gestation, due to low expression of 3βHSD. By 64% of gestation, the additional expression of CYP11A1 and CYP21A1 indicates corticosterone synthesis. It can be assumed that corticosterone synthesis increases from 64-79% and is sufficient during 79-90% of gestation, when CYP11B1 expression is additionally present. At 5-24% of weaning, lower enzyme expression suggests decreased corticosterone synthesis. CYP21A1 and CYP11A1 expression are lower postnatal between 24% of weaning until PND40 than in adults, indicating less corticosterone synthesis over that period.*

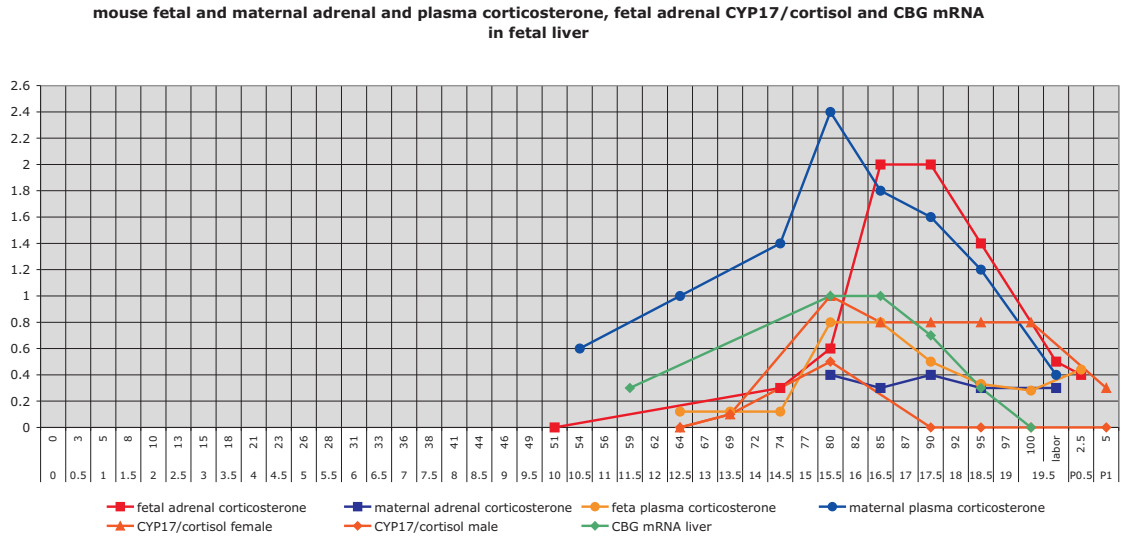


Figure 3.72. Mouse - summary adrenal and plasma cortisol and corticosterone

*Investigating the development of fetal and maternal plasma and adrenal corticosterone, fetal CBG levels as well as the assumed fetal cortisol synthesis, the following picture could appear. The maternal plasma corticosterone concentration starts to increase early from 54% until 74%, without affecting the constantly low corticosterone levels in fetal circulation between 64-74% of gestation, assuming high placental 11βHSD2 expression over that period. A parallel increase in maternal adrenal corticosterone synthesis can be assumed between 54-75% of gestation. Due to enzyme expression, low fetal adrenal corticosterone synthesis could start at 54-64%, followed by a moderate increase until 74% of gestation. Cortisol synthesis can be assumed to be absent at 64% of gestation, according to lack of CYP17 expression. From 69% onward, increasing cortisol synthesis might to be present until a peak at 80% of gestation. At this time, fetal corticosterone synthesis increases as well, in parallel with increasing fetal plasma corticosterone concentration between 74-80% of gestation. Fetal CBG expression in the liver increases from low to maximal levels between 59-80% of gestation. At 80% of gestation, a possible peak in fetal cortisol synthesis and increasing corticosterone production as well as maximal fetal plasma corticosterone concentration occur. The high CBG expression between 80-85% of gestation could dampen free glucocorticoids in fetal circulation. Compared to the peaking maternal plasma corticosterone concentration at 80% of gestation, even the maximal fetal plasma corticosterone concentration at the same time is relatively low. The high fetal CBG expression could be necessary to prevent maternal rather than fetal glucocorticoids in fetal plasma to negatively influence fetal organ maturation or maternal inhibition on fetal glucocorticoid synthesis. By 80% of*

*gestation, maternal adrenal corticosterone content assumingly is already at low levels again and corticosterone in maternal circulation peaks and subsequently begins to decrease. From 80% of gestation onward, fetal corticosterone synthesis dramatically increases. Cortisol synthesis in the female fetus might remain relatively strong, while it could cease in the male fetus by 90% of gestation. Maternal plasma corticosterone continues to decrease and maternal adrenal corticosterone synthesis remains constantly low. Fetal adrenal corticosterone production peaks around 85-90% of gestation, but the fetal plasma corticosterone concentration has already decreased again in parallel with corticosterone concentration in maternal circulation. On the other hand, decreasing fetal CBG expression might increase the free fraction of glucocorticoids in fetal circulation. By term, cortisol synthesis in the female fetus could be still relatively strong, while fetal adrenal corticosterone content has decreased to low levels. Cortisol synthesis in the female fetus could decrease to low concentrations at 2.5-5% of weaning. Maternal and fetal plasma corticosterone concentrations have reached similar low levels after delivery and 2.5% of weaning.*

### 3.4.16 Adrenal 11$\beta$HSD

*In the fetal human, sheep and mouse, 11$\beta$HSD is detected in the adrenal gland. The presence of 11$\beta$HSD2 in this location could possibly inactivate maternal glucocorticoids before they might inhibit fetal glucocorticoid synthesis or prevent fetal glucocorticoids from being released into fetal circulation. Adrenal 11$\beta$HSD1, on the other hand, could enhance the fetal output of active glucocorticoids.*

#### 3.4.16.1 Human adrenal 11$\beta$HSD2

In the human fetal adrenal cortex, 11$\beta$HSD1 mRNA was lacking and 11$\beta$HSD2 mRNA was present, while in the adult adrenal gland, 11$\beta$HSD2 expression was absent. In the fetal adrenals at 15% (wk6), 11$\beta$HSD2 was still absent, but was moderately expressed between 23-30% (wk8-10) and IR-11$\beta$HSD2 was apparent between 25-60% (wk 10-24) of gestation [95, 101, 428, 451]. In agreement, Murphy et al. 1981 presented in the fetal adrenals moderately low conversion of cortisol to cortisone (11$\beta$HSD2) between 25-50% (wk10-20) of gestation, but the absence from birth to 4% (PND14) of weaning [313]. The exact location of this 11$\beta$HSD isoform in the fetal adrenal gland was further investigated. Between 30-40% (wk12-16) as well as between 25-60% (wk10-24) of gestation, IR-11$\beta$HSD2 was detected in the FZ, but not in the DZ or the medulla [95, 101]. No information about detection in the TZ is available. Surprisingly at 30% (wk12) of gestation, 11$\beta$HSD2 mRNA was only apparent in the outer DZ. There seemed to be a discrepancy between the site of 11$\beta$HSD2 expression and protein synthesis, with 11$\beta$HSD2 mRNA being transcribed in the DZ, but 11$\beta$HSD2 protein being present in the inner FZ [95]. At 15% (PND56) of weaning and in adults, IR-11$\beta$HSD2 was absent in cortex and medulla [101].

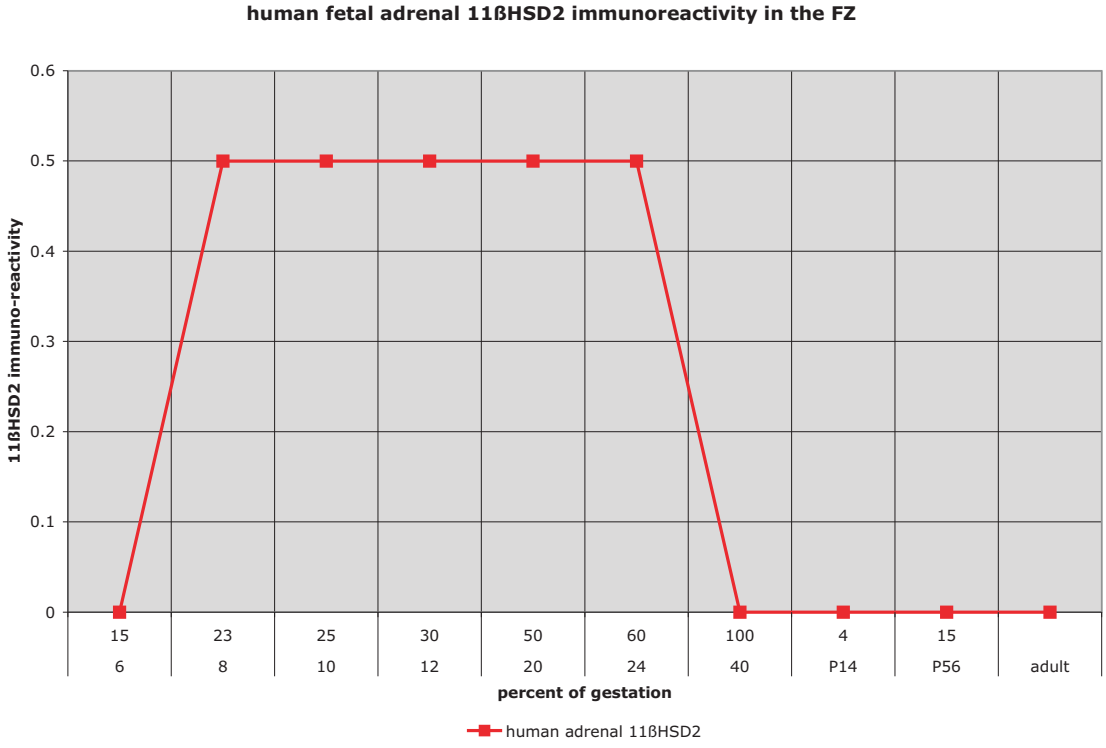


Figure 3.73. Human fetal adrenal 11$\beta$HSD2 immunoreactivity in the FZ

*The human fetal adrenal seems only to express 11βHSD2 during gestation. The enzyme is absent at 15%, but appears between 23-30% and is present at least until 60% of gestation. No information verifies or excluded expression or protein in the TZ. At birth and postnatal as well as in adults, the adrenals lack 11βHSD2 immunoreactivity. During gestation, 11βHSD2 mRNA only appears in the DZ, while immunoreactivity is solemnly present in the FZ. The exclusive presence of IR-11βHSD2 in the FZ might protect this zone from disturbing influences of cortisol during androgen synthesis.*

#### 3.4.16.2 Sheep adrenal 11βHSD2

In fetal and adult sheep adrenal gland, 11βHSD1 expression was absent, while 11βHSD2 mRNA was highly expressed [392, 507]. In the adrenal cortex of the adult sheep, 11βHSD2 mRNA was highly expressed in the zR and the zF, but only low in the zG [507]. In fetal adrenals, 11βHSD2 mRNA was abundantly expressed between 62-85% (day93-128), but significantly ($P<0.05$) decreased by 1.5 fold between 85-97% (day128-146) of gestation [289]. At 90% (day135) of gestation and at term, 11βHSD2 mRNA expression was absent [509]. A not physiological premature rise in cortisol at 77% (day116) of gestation significantly ($P<0.05$) suppressed fetal adrenal 11βHSD2 expression by 2.2 fold, which could explain the decrease in 11βHSD2 expression late in gestation, during high levels of ACTH and cortisol [392].

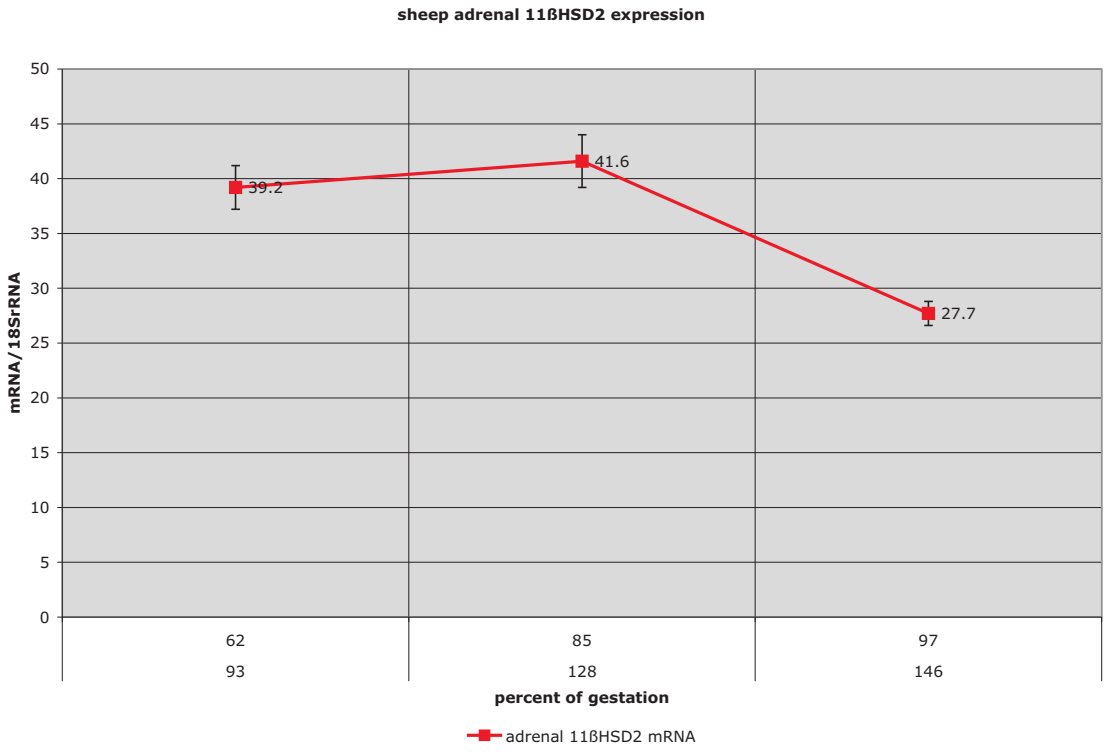


Figure 3.74. Sheep adrenal 11βHSD2 mRNA

*In the fetal sheep adrenals, only 11βHSD2 is expressed. The abundant expression at 62-85% significantly decreases at 97% of gestation. At term, adrenal 11βHSD2 mRNA is undetectable. Interestingly, cortisol administration decreases 11βHSD2 expression, which might explain decreasing adrenal 11βHSD2 levels in late gestation.*

#### 3.4.16.3 Mouse adrenal 11$\beta$HSD

In mouse fetal adrenals, both isoform of 11$\beta$HSD were present [464]. 11$\beta$HSD2 mRNA was abundantly expressed at 72% (e14) but by 77% (e15) of gestation, the expression ceased [65]. 11$\beta$HSD1 mRNA was absent at 67-87% (e13-17) but increased to low levels at 97% (e19) of gestation. By 2.5% (PND0.5) of weaning, adrenal 11$\beta$HSD1 expression was still present in low levels [464]. Adrenal activity of 11$\beta$HSD (assumingly 11$\beta$HSD1) was detected in the zF between 5-67% (PND1-14), but was nearly absent from 100% (PND21) of weaning until PND70. In zG and x zone, only traces of 11$\beta$HSD1 (?) activity were detected at birth [180].

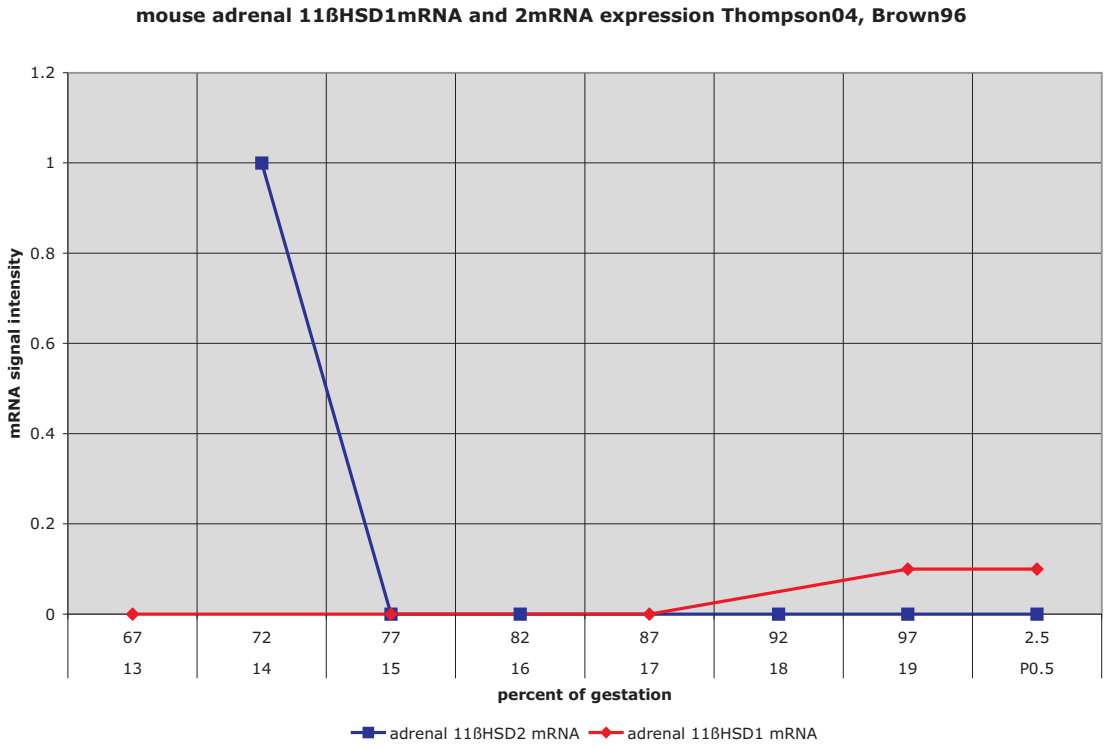


Figure 3.75. Mouse adrenal 11$\beta$HSD1,2 expression [65, 464]

*At 72% of gestation, the expression of 11$\beta$HSD2 in the mouse fetal adrenal cortex is high, inactivating present cortisol/corticosterone, possibly to protect the fetal organism from too early glucocorticoid exposure. By 77% of gestation, a dramatic decrease in 11$\beta$HSD2 expression ceases the enzyme from the fetal adrenal gland, allowing an increase of active glucocorticoids. Between 87-97% of gestation, not only the absence of the glucocorticoid inactivating 11$\beta$HSD2, but also the presence of the activating 11$\beta$HSD1 increase the residing active glucocorticoids.*

*In all three species, fetal adrenal 11$\beta$HSD2 expression decreases toward the end of gestation. While in the human adrenals, expression decreases at some point between 60-100%, in the sheep, the decrement takes place between 85-97% and in the fetal mouse adrenals already between 72-77% of gestation. In the fetal mouse adrenals, additionally the expression of 11$\beta$HSD1 is apparent in between 87-100% of gestation.*

### 3.5 Androgen effects on glucocorticoid synthesis

Due to its strong interaction with the synthesis of glucocorticoids in the fetal adrenal, the production of androgens will be here shortly reviewed. As mentioned before (see Chapter 3.3), adrenal glucocorticoid and androgen synthesis share common precursors and enzymes. They are produced in close proximity and might be synthesized in more than one adrenal zone. At least in young adults, it is assumed that cortisol is synthesized together with androgen in the zF and the zR of the guinea pig. Adrenal cortisol but also androgen synthesis is stimulated by ACTH. Primates and sheep synthesize the androgen DHEAS, while the guinea pig produces androstenedione in the adrenal cortex. In the fetal rat and mouse, adrenal androgen synthesis is absent. Surprisingly, androgens of the rat's dam still display an important regulatory function in fetal glucocorticoid synthesis. The DHEAS production in the fetal adrenals of primates is an indispensable substrate for placental estrogen synthesis. Large amounts of DHEAS are synthesized in the FZ, which occupies the bulk of the fetal adrenal cortex and interacts with fetal glucocorticoid synthesis. The androgen production in the adrenal cortex of the fetal sheep and guinea pig is less important as a precursor for estrogen synthesis. Other than the large FZ in the primate fetus, the analogical zR was absent in the fetal sheep during gestation and was only detectable in the guinea pig late in gestation. While in the fetal primate, the primary steroid produced in the adrenal cortex was DHEAS, in the sheep fetus, the primary steroid was cortisol, which then stimulated DHEAS production in the ovine placenta. Essential during sexual differentiation, the interaction between adrenal glucocorticoid and androgen seems to be decisive in preventing virilization of the female fetus [43, 85, 94, 96, 170, 221, 299, 299, 380, 417, 437, 465, 492].

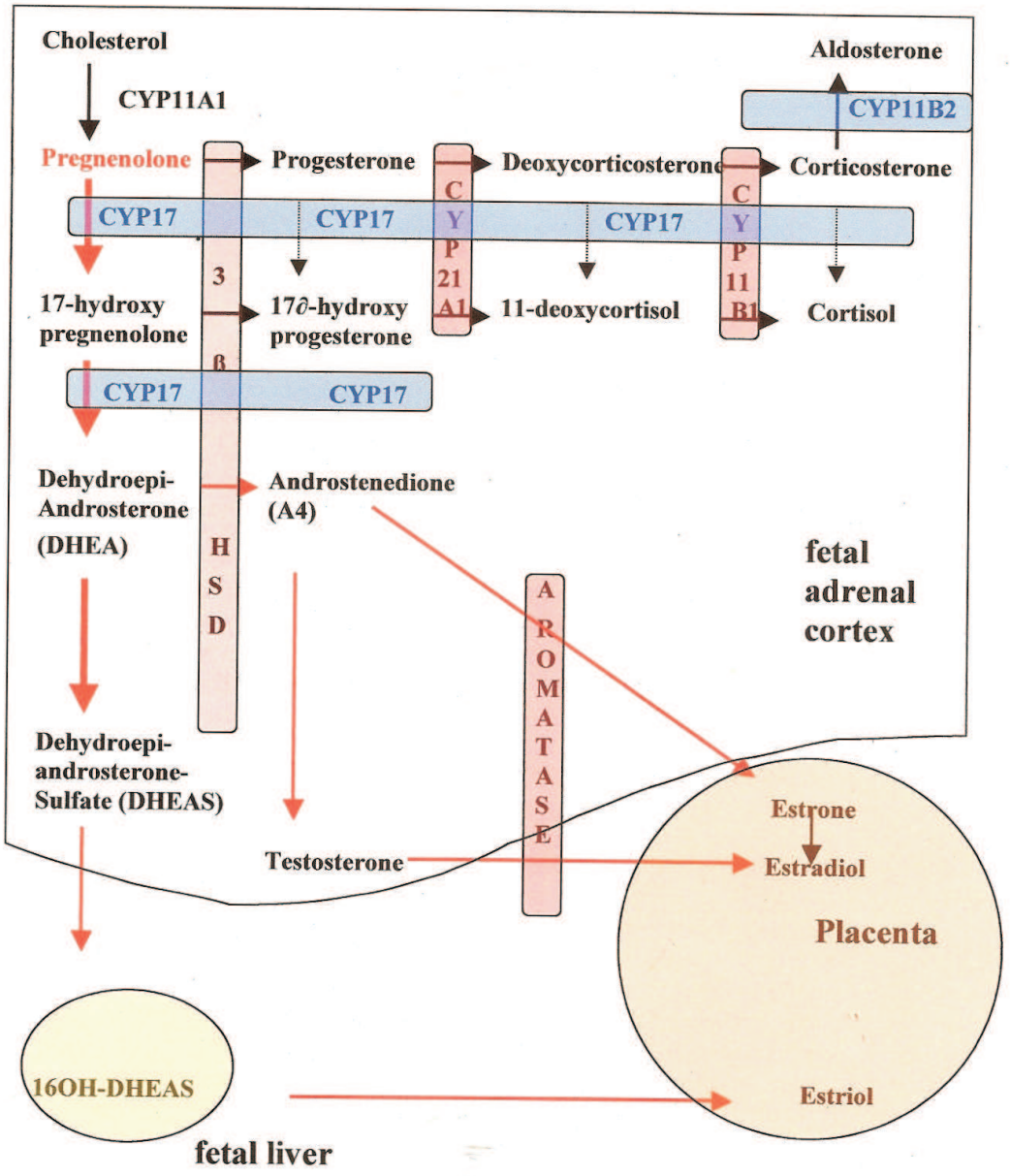


Figure 3.76. Human adrenal androgen synthesis

### 3.5.1 Human androgen effects

The androgen synthesizing FZ appeared first in the human adrenal cortex. At 20% (wk8), the human FZ exhibited organelles indicating active steroidogenesis and at 23% (wk9) of gestation, the FZ was highly vascularized for hormone transport to and from the FZ. Additionally, enzyme expression indicates a beginn of sufficient DHEAS synthesis at 20% of gestation, when cortisol synthesis

is still absent or very low [38, 142, 153, 179, 211]. By 25% (wk10) of gestation, it was shown that ACTH stimulated fetal adrenal cortisol but also androgen synthesis. The early but short boost of cortisol biosynthesis around 25% of gestation is thought to negative feedback on the pituitary ACTH production, to inhibit adrenal androgen synthesis and by that safeguard sexual differentiation of the female fetus between 25-35% (wk10-14) of gestation. During sexual differentiation, female fetuses can virilize when their adrenal cortexes secrete high levels of androgens. Placental aromatase, which metabolizes androgens to estrogens and can be assumed to safely remove androgens from the circulation, was still absent in humans at 25% (wk10) of gestation. Cortisol production assumingly competes with DHEAS synthesis for the common precursor pregnenolone, leading to inversely proportional fetal DHEAS and cortisol syntheses and the necessary low androgen levels during sexual differentiation. Nearly all estriol in human maternal circulation derived from the fetal adrenal androgen production and the very low maternal plasma estriol concentrations at 15-30% (wk6-12) of gestation indicate the low fetal adrenal DHEAS synthesis during that critical time. Additionally, between 23-38% of gestation, 3HSD expression, an enzyme necessary for cortisol but not for DHEAS synthesis, seems to present in the FZ, and could indicate cortisol synthesis rather than androgen synthesis in the FZ during sexual differentiation [169, 170, 235, 360, 492]. Around 30% (wk12) of gestation, the cortisol/DHEAS ratio drastically decreased due to decreasing cortisol biosynthesis. Fetal cortisol synthesis was low or absent between 40-55% (wk16-22) of gestation. During that period, fetal androgen (especially DHEAS) production seems to be sufficient, which was indicated by an increment in DHEAS enzymes after 33% (wk13) of gestation, a significant increase in maternal estradiol concentration between 30-48% (wk12-19) and an increasing fetal DHEAS plasma pool after 45% (wk18) of gestation. During increasing fetal adrenal androgen synthesis, placental aromatase was now present and prevented virilization of the female fetus and its mother by removing fetal adrenal androgens from the circulation. Maternal estradiol concentration increased further until 58% (wk23), remained constant between 58-78% (wk23-31) then progressively increased until 98% (wk39) of gestation, assuming a similar development in fetal adrenal DHEAS synthesis. Other than plasma cortisol, fetal plasma DHEAS concentrations did not significantly change with active labor in the umbilical cord [235, 320, 332, 340, 466]. White et al. 2006 summarized the following development of fetal adrenal DHEAS synthesis. Fetal adrenal DHEAS content decreased during genital differentiation and the transient cortisol production, but dramatically increased at midgestation, during the time of low fetal cortisol secretion. DHEAS secretion was high and increased slowly further in late gestation in parallel with an increment in cortisol secretion [492].

*We can assume the following interplay in fetal DHEAS and cortisol synthesis:*

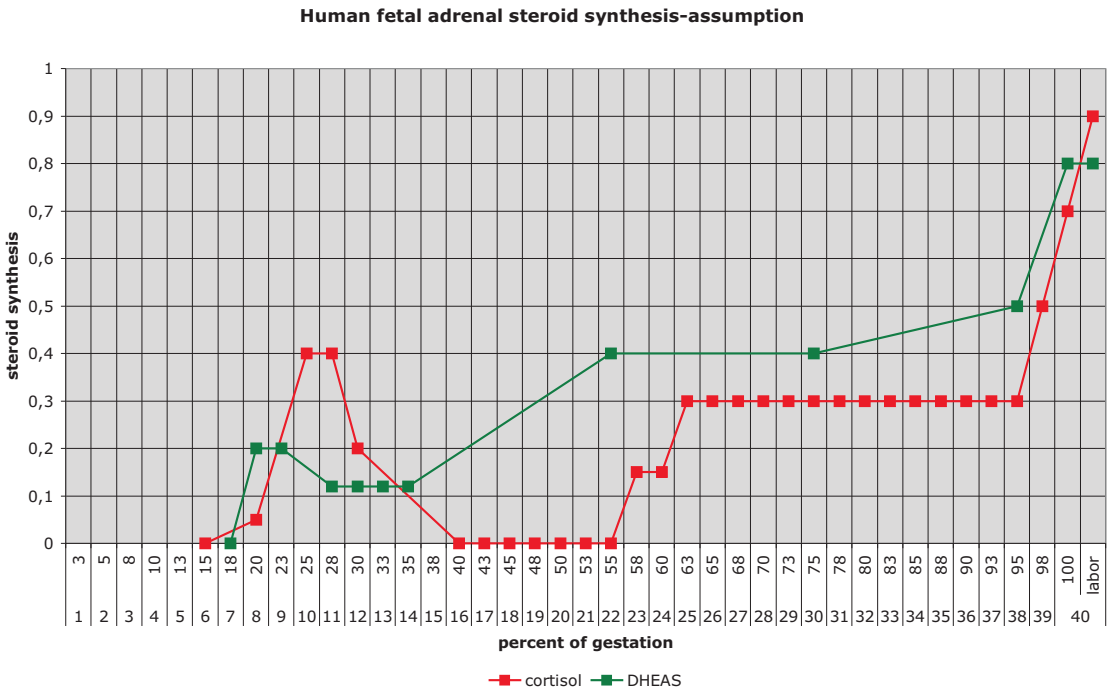


Figure 3.77. Human - summary estimated DHEAS and cortisol synthesis

*In the human fetus, adrenal DHEAS production seems to be sufficiently present at 20% of gestation due to enzyme expression and steroidogenic organelles in the FZ. Fetal cortisol synthesis is absent or very low at this point. DHEAS and cortisol synthesis in the human fetal adrenal cortex exhibit an inversely proportional relationship during long periods of gestation. The early peak in cortisol synthesis by 25% of gestation is assumed to negative feedback on ACTH synthesis, a stimulator for adrenal androgen synthesis. Fetal DHEAS synthesis is low during sexual differentiation between 25-35% of gestation, when androgens could virilize the female fetus and placental aromatase is not yet expressed. Also, the possibility exists that cortisol rather than DHEAS is synthesized during this critical time in the FZ. Fetal cortisol synthesis decreases after 25% and is absent between 40-55% of gestation, which most likely reduces glucocorticoid negative feedback on ACTH stimulated DHEAS synthesis. A strong increase in DHEAS synthesis during low or absent fetal cortisol synthesis is present. DHEAS production is assumed to reach a moderately high plateau between 55-75% of gestation during the period of reappearance of cortisol synthesis. Cortisol and DHEAS synthesis seems to increase subsequently in parallel until late gestation. Being highly speculative here, it might be that cortisol and DHEAS synthesis gain independence from each other after 55% of gestation, possibly due to fully dissociation of the FZ and the TZ or due to sufficient pregnenolone supply as a precursor for both cortisol and DHEAS synthesis. Fetal plasma DHEAS concentration remains constant during labor, while fetal cortisol further increases.*

### 3.5.2 Rhesus monkey androgen effects

Fetal sexual differentiation of the gonads already occurred in the fetal rhesus monkey between 22-26% of gestation. During that time, low fetal adrenal androgen synthesis, as well as low fetal and maternal plasma DHEAS concentrations are required to prevent virilization of the female fetus. It was possible to show that the androgen concentration in maternal circulation decreased significantly from 8-17% (day13-28) to 26% (day43) of gestation [381, 449]. By 27-30% (day45-50), the FZ cells of the rhesus monkey adrenal cortex exhibited signs of active steroidogenesis and enzyme expression suggests sufficient fetal DHEAS synthesis, before fetal cortisol synthesis is assumed at 30% (day50) of gestation. Also the placenta acquired substantial amounts of aromatase activity, which safely removes fetal DHEAS from the circulation. Subsequently, enzyme expression in the FZ indicates very low or absent androgen synthesis at 49% (day80), at a point were sufficient fetal adrenal cortisol synthesis is detected. This presents an inverse correlation between fetal adrenal cortisol and DHEAS synthesis around midgestation. Fetal plasma DHEAS concentration was very low at least between 62-75% (day103-123) of gestation. In late gestation, adrenal cortisol and DHEAS synthesis increased toward term. An inverse interaction was present again in the newborn, where plasma cortisol decreased by 1% of weaning and plasma DHEAS concentrations reached high levels [144, 238, 271, 291, 423, 425].

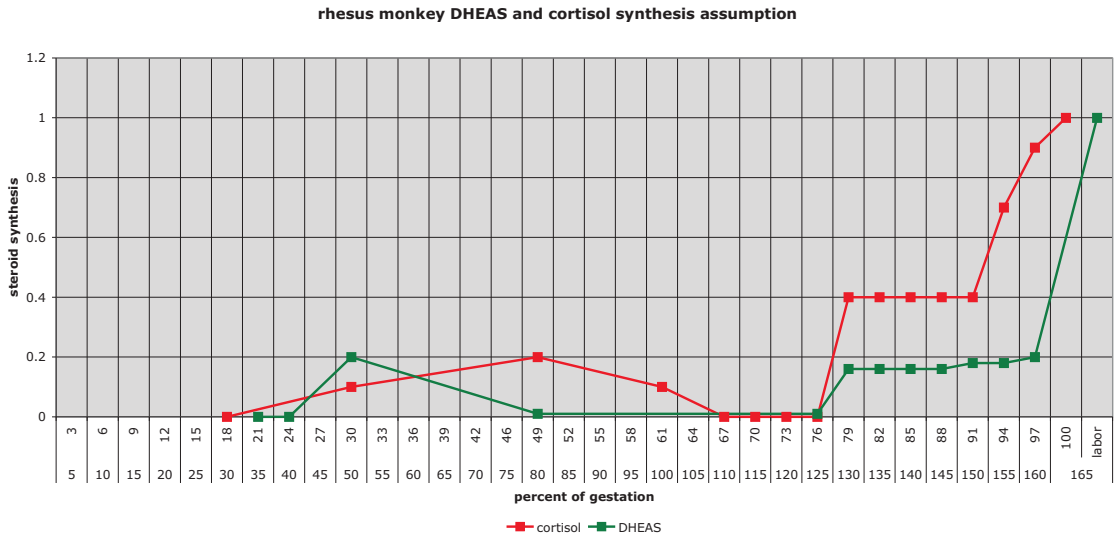


Figure 3.78. Rhesus monkey - summary estimated DHEAS and cortisol synthesis

*With sexual differentiation taking place at 22-26% of gestation, we assume low fetal adrenal DHEAS synthesis during that time, as well as low fetal and maternal plasma DHEAS concentrations. Sufficient DHEAS synthesis might appear slightly earlier than cortisol synthesis in the fetal adrenal cortex. During midgestation, fetal DHEAS synthesis is very low or absent, when sufficient fetal adrenal cortisol synthesis is present. This inverse correlation between adrenal cortisol and DHEAS synthesis is presentable again in the newborn rhesus monkey.*

### 3.5.3 Baboon androgen effects

Like in humans and rhesus monkeys during fetal gonadal differentiation, low fetal and maternal androgen levels are required to protect the female fetus from virilization. Estradiol is synthesized by the placenta from fetal and maternal DHEAS and is then released back into the circulation of mother and unborn. Estradiol was detected in fetal and maternal blood at least at 33% (day60) of gestation, but the maternal plasma concentration was low during that time, suggesting low fetal and maternal DHEAS synthesis maybe in connection with sexual differentiation [135]. DHEAS and cortisol syntheses in the fetal adrenal cortex of the baboon show a very distinctive and ongoing inverse correlation. Around 30% of gestation, enzyme expression suggested sufficient fetal cortisol synthesis during this period of assumingly low fetal DHEAS production. At 54-57% of gestation, the stress hyporesponsive period started in baboons, and fetal adrenal cortisol synthesis was negligible and unresponsive to ACTH. Fetal DHEAS synthesis was sufficient and very responsive to ACTH. ACTH receptor expression in the fetal adrenal had dramatically increased between 33-55% (day61-101) of gestation in parallel with increasing fetal DHEAS production. Over the period of low or negligible fetal cortisol synthesis, fetal DHEAS concentration in the umbilical artery remained unchanged, but was significantly higher than in maternal circulation, assuming sufficient fetal adrenal DHEAS synthesis during that time. By 84% of (day155) of gestation, cortisol synthesis had slowly increased and the fetal adrenal cortex produced similar amounts of cortisol and DHEAS. Still DHEAS synthesis was more responsive to ACTH than fetal cortisol production. The fetal plasma DHEAS concentration remained unchanged until 92% (day170) of gestation. At 98% (day180) of gestation, the fetal adrenal still produced 10 times more DHEAS than cortisol. The surge in fetal cortisol synthesis took place between 97% of gestation and 0.3% of weaning. At 3% (PND10) of weaning, the neonatal adrenal cortex again produced similar amounts of cortisol and DHEAS and syntheses of both were unresponsive to ACTH, while at 10% (PND30) of weaning, responsivity to ACTH was present in both cases but more cortisol than DHEAS was synthesized [1, 4, 134, 135, 348].

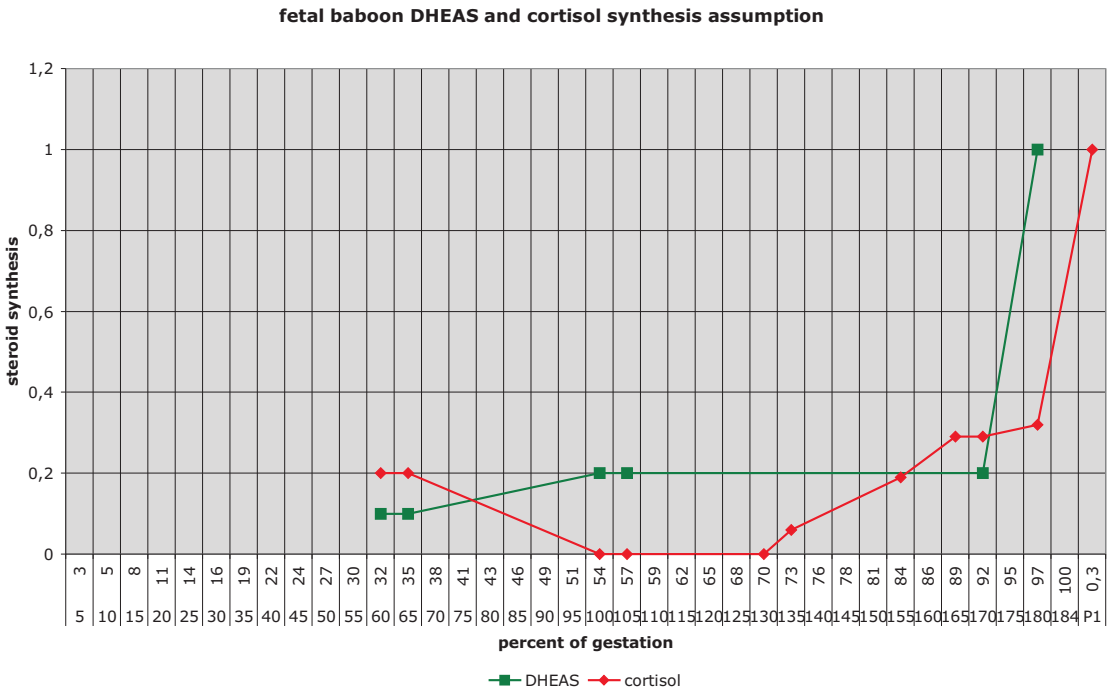


Figure 3.79. Baboon - summary estimated DHEAS and cortisol synthesis

*The following interactions between cortisol and DHEAS syntheses in the fetal baboon adrenals could be possible. Around 30% of gestation, sufficient cortisol production in the fetal baboon adrenal gland is assumed, which could inhibit the fetal androgen synthesis to prevent possible gonadal differentiation. At midgestation, during the stress hyporesponsive period, fetal cortisol synthesis is negligible but DHEAS production in the fetal adrenal has increased and is responsive to ACTH, other than cortisol synthesis. This demonstrates the inverse correlation in the fetal adrenal synthesis of both hormones. DHEAS synthesis strongly increases in late gestation, preliminary to the cortisol surge. Responsivity to ACTH and the expression of ACTH receptors in the adrenal can be assumed to play a decisive role in the interaction of cortisol and DHEAS syntheses.*

### 3.5.4 Guinea pig androgen effects

During gestation, the necessary estrogen derives in the guinea pig from maternal androgen synthesis in the ovaries, supported by fetal androstenedione synthesis in the ovaries and the adrenals [334, 373]. Fetal adrenal androstenedione production seems to be of minor importance in this species and a zR can only be distinct in late gestation. The function of the zR in guinea pigs is not well understood. While in humans, androgens are produced in the appropriate location (FZ), in the young adult guinea pig the zG and zF produce androgen, and the zR is less important. On the other hand, the guinea pig zR produces sufficient amounts of cortisol additionally to the zF after birth, and cortisol synthesis decreases in the former location in young adults [47, 94, 141, 514]. Already at 32-34% (day22-23), androgen synthesis in the fetal adrenals was detectable and androgenic organelles were present in the cortical blastema cells, before cortisol synthesis was verified at 37-51% (day30-50) of gestation [47, 333, 342]. During sexual differentiation, stronger fetal cortisol synthesis could assure low fetal adrenal androgen synthesis, to prevent masculinization of the female fetus. During the period of low fetal cortisol synthesis, which is assumed between 56-81% of gestation, the fetal adrenal cortex is more androgenic than later in gestation [333, 334, 517].

*The adrenal cortex is less important for androgen synthesis in the fetal guinea pig compared to the primates. On the other hand, the zR seems to be significant for cortisol synthesis at least after birth. The fetal adrenal cortex seems to start androgen production before cortisol synthesis is present and sufficient fetal androgen synthesis is assumed during low fetal cortisol production.*

### 3.5.5 Rat and mouse androgen effects

Adult rats and mice are unable to produce androgens in the adrenal cortex, due to the lack of the enzyme CYP17. Instead they synthesize androgens in the gonads [24, 96]. During pregnancy, the fetal rat was able to synthesize cortisol in the adrenals. Cortisol production requires CYP17 expression. The transient expression of CYP17 was verified in the adrenal cortex of the fetal mouse. The presence of CYP17 for cortisol synthesis in the adrenals of fetal rat and mouse could also allow androgen synthesis in this location. In the female newborn mouse, adrenal CYP17 expression was present and DHEA but not cortisol was detectable in the circulation. It cannot be excluded that adrenal androgen production was responsible for the plasma DHEA levels in the newborn female mouse [123, 185, 232]. An interesting regulatory mechanism between androgens and glucocorticoids occurred in the fetal and maternal rat. Before 50% (<e11) of gestation, the dam's ovary was the principal source of androgen production. Later in gestation, the maternal ovaries lost this ability. From 50% (>e11) gestation on, the placenta was able to produce androgens and its androgen secretion peaked at 82% (e18) of gestation and declined before birth. Placental androgens were transported to the maternal gonads for aromatization [484]. Assumingly as a cause of placental androgen secretion, the maternal plasma DHEA concentration increased significantly ($P<0.05$) between 82-86% (e18-19) and decreased ($P<0.05$) again between 86-96% (e19-21) of gestation. This development was inversely proportional to maternal plasma corticosterone levels, which significantly ($P<0.01$) decreased between 82-86% (e18-19) and significantly increased between 86-96% (e19-21) of gestation [437] (see also Chapter 3.4.12.5).

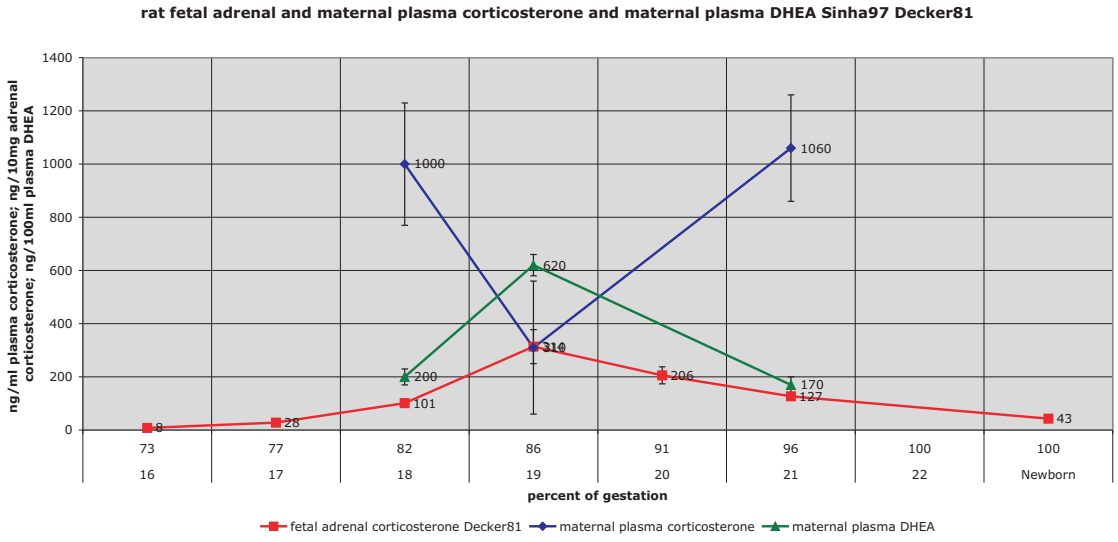


Figure 3.80. Rat fetal adrenal and maternal plasma corticosterone and DHEA [123, 437]

On the other hand in Decker et al. 1981 it was shown that fetal adrenal corticosterone content increased significantly ($P=0.001$) from 82% (e18) to peak values at 86% (e19) and had significantly ($P=0.004$) decreased again by 96% (e21) of gestation [123] (see also Chapter 3.4.12.2).

*The adult rat and mouse are unable to synthesize androgens in the adrenals. It was shown that the adrenals produce cortisol in fetal rats and most likely in fetal mice. Cortisol like androgen synthesis requires the expression of CYP17, a hormone verified in the fetal mouse adrenal. With the presence of this enzyme fetal adrenal androgen synthesis in both species cannot be excluded. In the second half of gestation, placental androgens are transported into the mother and are aromatized in*

*the maternal ovary to estrogens. We assume that the peak in placental androgen synthesis at 82% of gestation causes the subsequent increment in maternal plasma DHEA concentration at 86% of gestation. This could cause the sudden and transient decrease in maternal plasma corticosterone concentration, which in turn could release the fetal adrenal gland from inhibition and be responsible for the strong fetal adrenal corticosterone synthesis at 86% of gestation. The subsequent decline in placenta androgen synthesis is followed by decreasing maternal plasma DHEA levels and an increasing maternal plasma corticosterone concentration. The latter again causes inhibition of fetal adrenal corticosterone synthesis, exhibited by decreasing fetal corticosterone production before term. Even while the adrenal glands of the mother and maybe of the fetus are unable to synthesize androgens, maternal plasma DHEA seems to play a crucial role in the transient liberation of fetal glucocorticoid synthesis and an inverse correlation between androgen and glucocorticoid similar to the relationships in the other species occurs.*

## 3.6 Summary fetal and neonatal adrenal glucocorticoid synthesis

### 3.6.1 Human fetal and neonatal adrenal cortisol synthesis

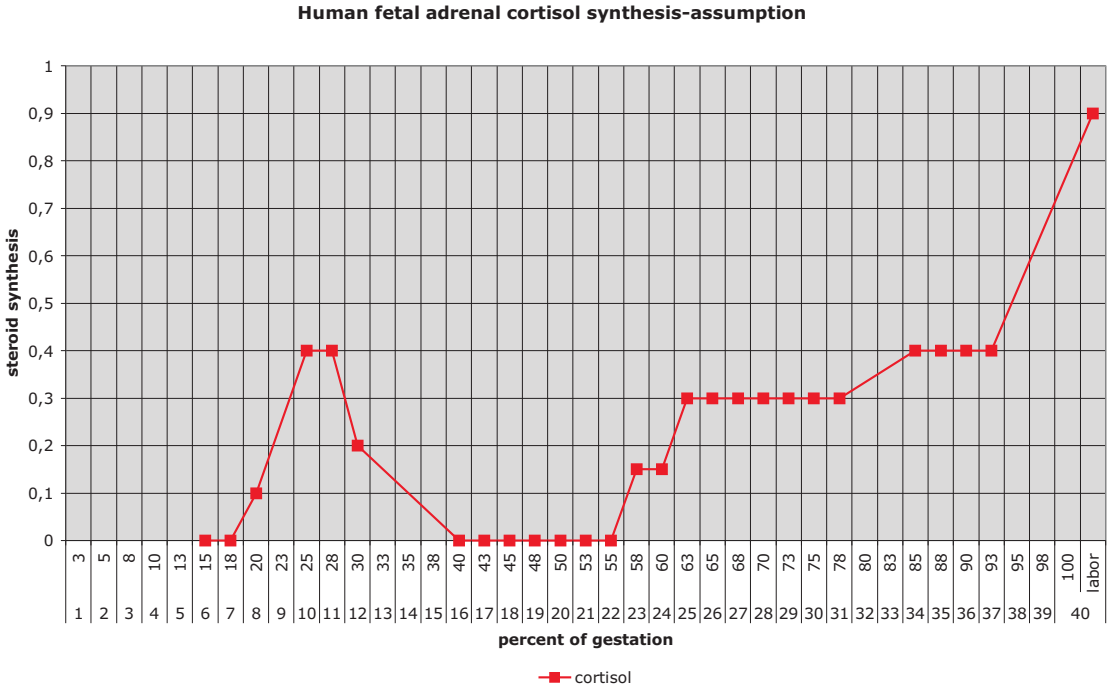


Figure 3.81. Human estimated prenatal adrenal steroid synthesis

*In summary, it is shown that the human fetal adrenal cortex is able to produce cortisol already early in gestation. Fetal adrenal glucocorticoid synthesis is detectable at 21% and cortisol production is moderate at 25% of gestation. Sufficient human fetal DHEAS synthesis is apparent even by 20% of gestation.*

*Fetal adrenal cortisol synthesis is moderately high by 25% and has decreased by 30% of gestation. The short and transient peak in fetal cortisol production in early gestation is thought to decrease fetal adrenal DHEAS synthesis during gonad differentiation to prevent virilization of the female fetus. Fetal adrenal cortisol and DHEAS syntheses exhibit an inversely proportional development until midgestation. Fetal cortisol synthesis decreases to very low or absent levels by 40% to remain unchanged until 55% of gestation, presenting a stress hyporesponsive period of the human fetus during midgestation.*

*By 58%, fetal adrenal cortisol synthesis is assumed to increased again, reach moderate levels by 63%, remain constant until 78% and increase again by 85% of gestation. A late gestational surge in fetal cortisol synthesis between 93% and labor is assumed.*

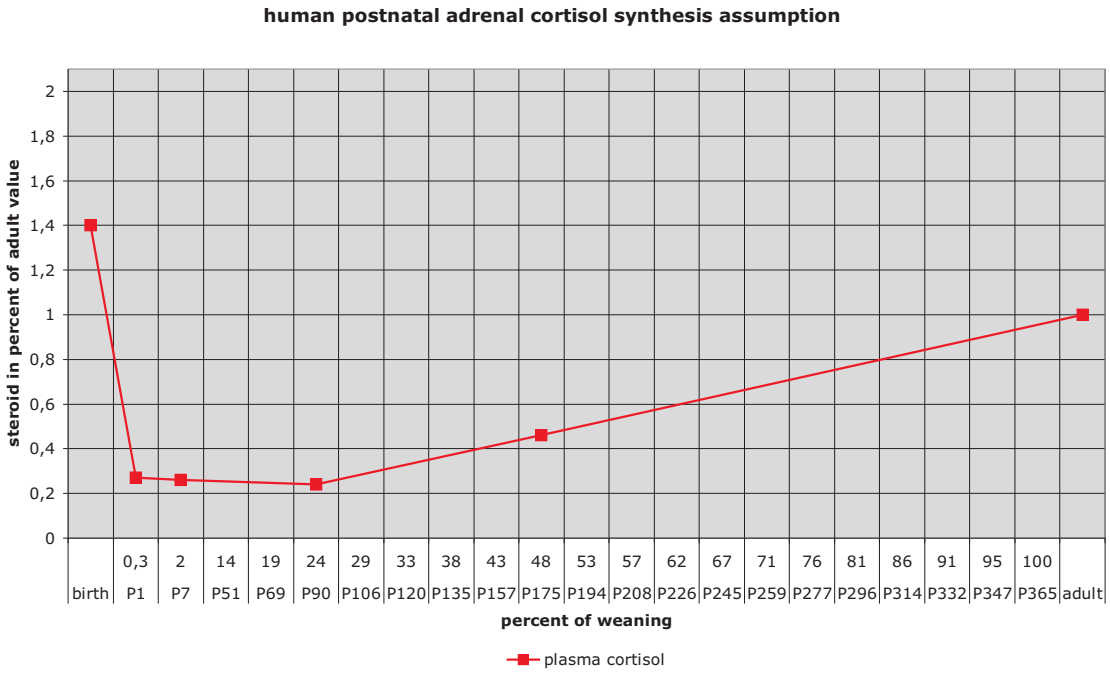


Figure 3.82. Human estimated postnatal adrenal steroid synthesis

*After birth by 0.3% of weaning, the plasma cortisol concentration decreases strongly. By 2% of weaning, the plasma cortisol concentration has not changed. Until 24% of weaning, plasma cortisol levels remain constant. Plasma cortisol levels have increased by 48% of weaning. Postnatal values for cortisol concentration in circulation are in the range of adult values.*

### 3.6.2 Rhesus monkey fetal and neonatal adrenal cortisol synthesis

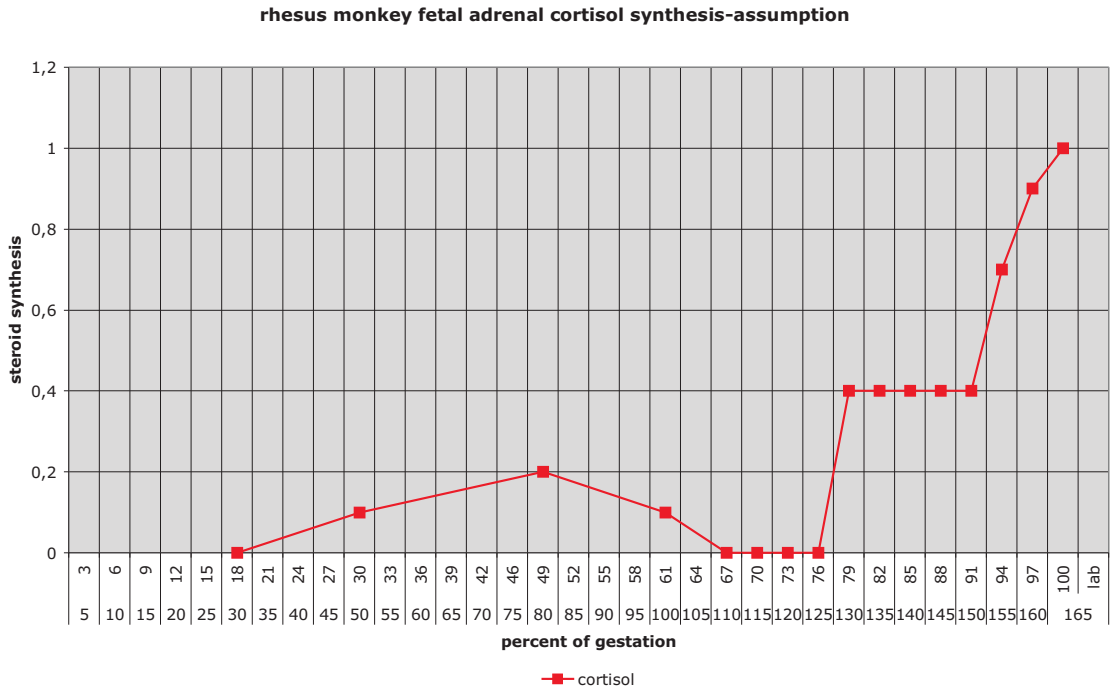


Figure 3.83. Rhesus monkey estimated prenatal adrenal steroid synthesis

*By 27-30% of gestation, adequate fetal adrenal DHEAS synthesis is already present, before fetal cortisol production is suggested by 30% of gestation. At 44% of gestation, sufficient fetal adrenal cortisol synthesis is detectable and enzyme expression suggests fetal adrenal cortisol synthesis at 49% of gestation, during the time when DHEAS synthesis is low or absent. Between 30-49% of gestation, free cortisol concentration in maternal circulation assumingly decreases, which could be responsible for increasing fetal cortisol synthesis.*

*Cortisol production in the fetal rhesus monkey could decrease at 61% and seems to be very low or absent between 67-76% of gestation. At 79% of gestation, cortisol synthesis seems to reappear and due to enzyme expression, synthesis could be moderate between 79-91% and increase strongly between 91% and 97-100% of gestation.*

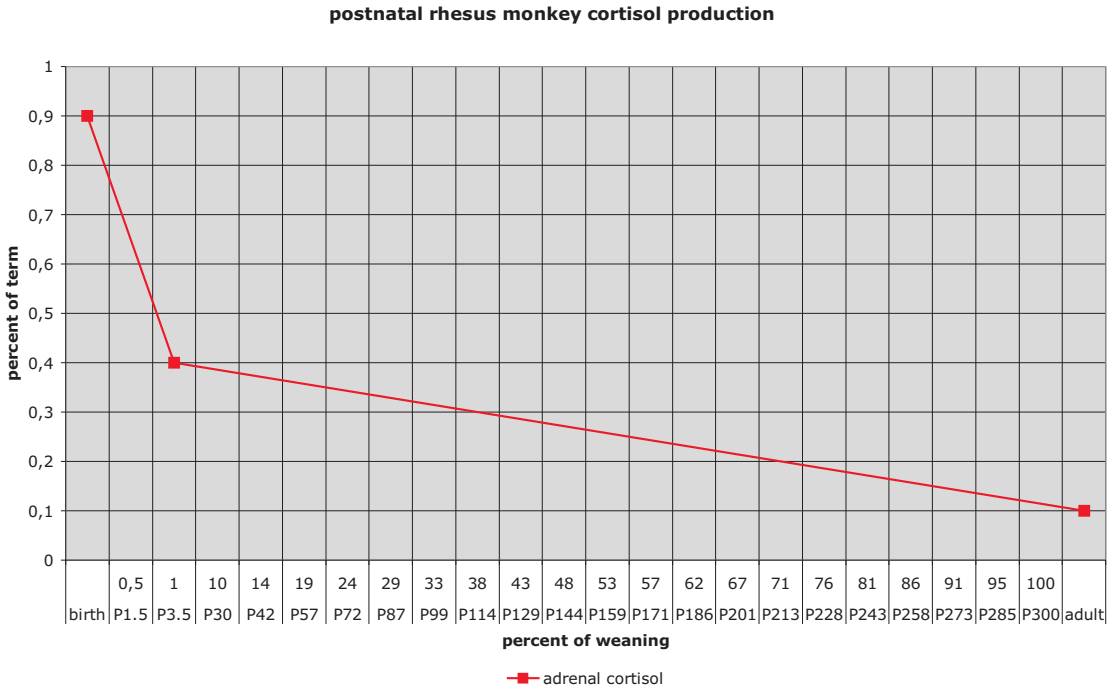


Figure 3.84. Rhesus monkey estimated postnatal adrenal steroid synthesis

*The cortisol production rate is comparable between 80% of gestation and 1% of weaning but decreases strongly in adults. The plasma cortisol concentration is lower in the fetus at 80% of gestation due to a high metabolic clearance rate compared to the newborn. The plasma DHEAS concentration increases during labor and reaches high levels by 1% of weaning. Between labor and 1% of weaning, adrenal cortisol synthesis can be assumed to decrease strongly. Until adulthood, cortisol production rate further decreases.*

### 3.6.3 Baboon fetal and neonatal adrenal cortisol synthesis

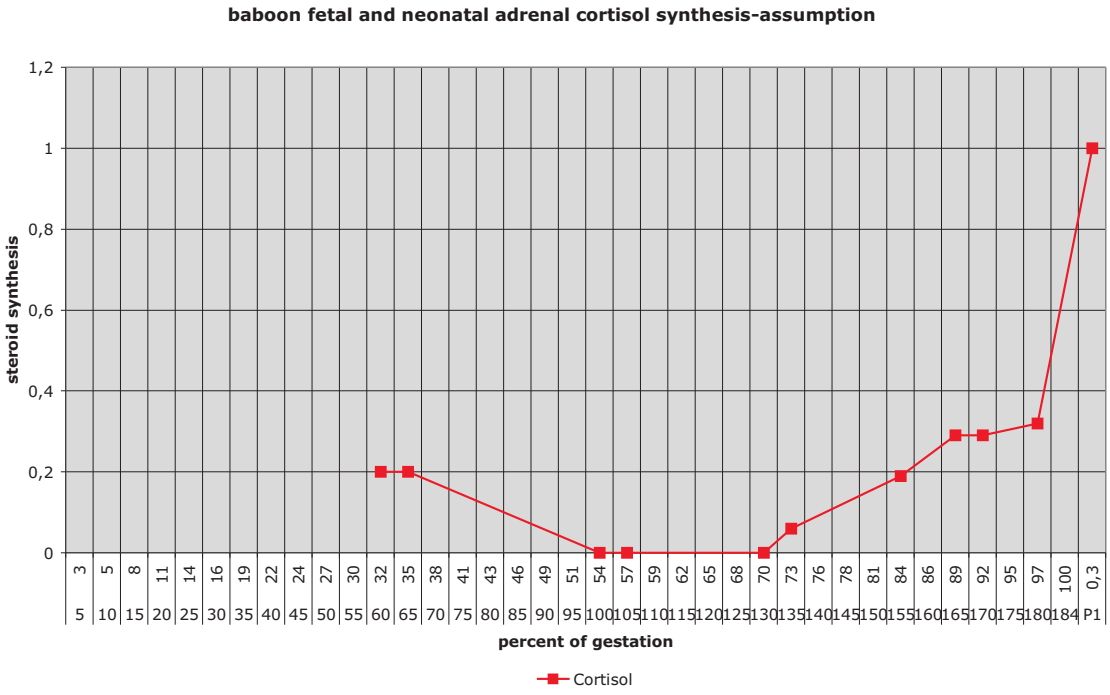


Figure 3.85. Baboon estimated prenatal adrenal steroid synthesis

*Cortisol synthesis can be assumed in the fetal baboon adrenal in early gestation. By 32-35% of gestation cortisol production seems to be present in the adrenals of baboon fetus. Fetal cortisol synthesis is absent at 54% until possibly 70% of gestation. DHEAS synthesis might be low at 32-35% of gestation, but can be assumed to have increased during the stress hyporesponsive period. Fetal cortisol synthesis slowly increases between 70-89%, subsequently seems to remain roughly constant until 97% of gestation, at a point where fetal adrenal DHEAS synthesis has dramatically increased, causing 10 times more DHEAS synthesis in the fetal adrenal cortex than cortisol production. Cortisol synthesis surges subsequently between 97% of gestation and 0.3% of weaning.*

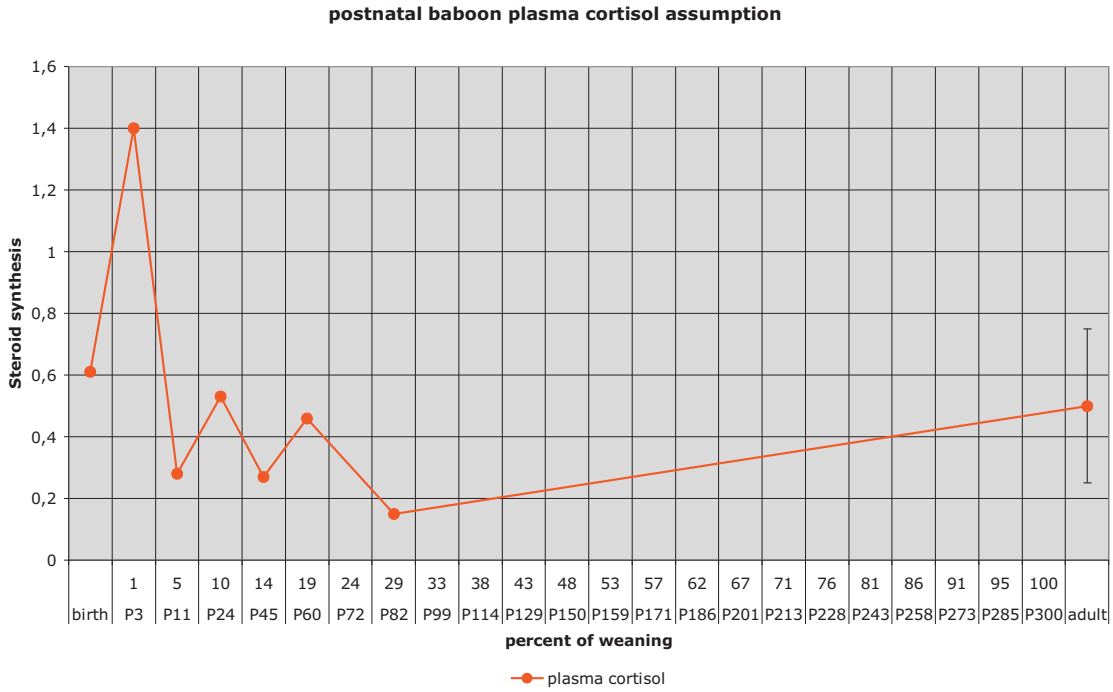


Figure 3.86. Baboon estimated postnatal adrenal steroid synthesis

*The plasma cortisol concentration increases strongly after birth and peaks at 1% of weaning. Plasma cortisol levels decrease already dramatically by 2%, and further decrease by 4% of weaning. At 3% of weaning, the adrenals synthesize similar amounts of DHEAS and cortisol. By 10% of weaning, adrenal cortisol synthesis is higher than DHEAS production. The plasma cortisol concentration shows strong fluctuation until at least 29% of weaning. Toward adulthood, the plasma cortisol concentration has slightly increased.*

### 3.6.4 Sheep fetal and neonatal adrenal cortisol synthesis

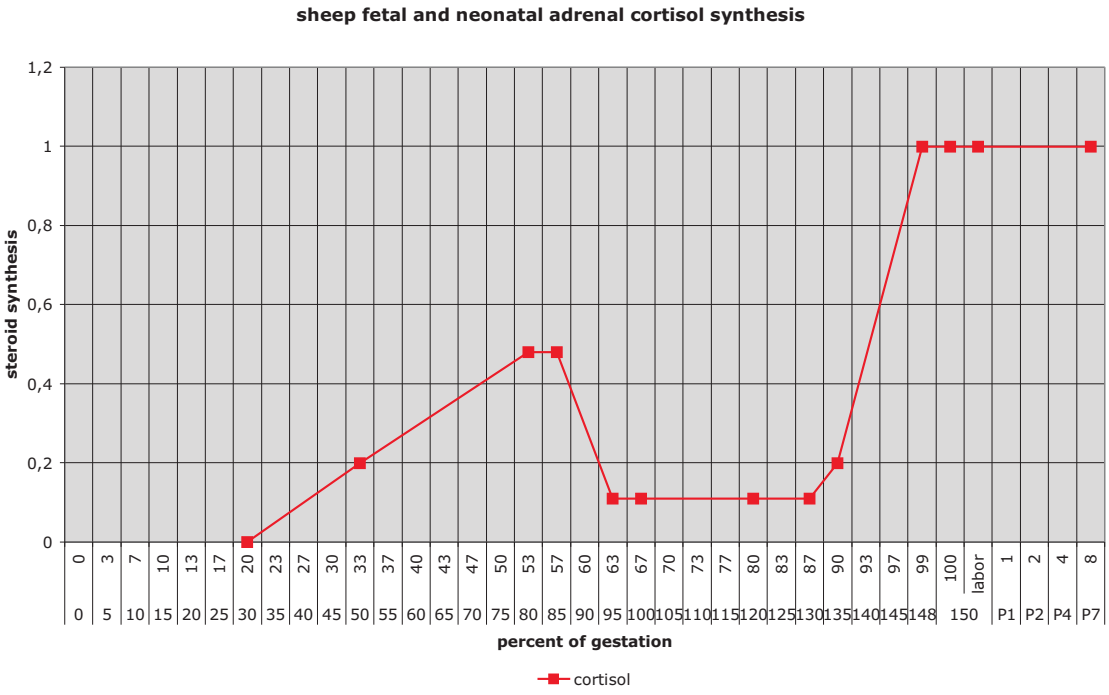


Figure 3.87. Sheep estimated pre- and postnatal adrenal steroid synthesis

*Androgen synthesis in the fetal adrenal cortex can be assumed to be very low during gestation and no distinctive zR can be identified before birth. Fetal adrenal cortisol synthesis is already present by 32% of gestation and might have increased at 37% of gestation. Cortisol synthesis is assumed to remain moderate until 55% but has decreased to the limit of detection by 63-68% of gestation. Fetal cortisol production remains very low until 88% of gestation. Between 90-98% of gestation, fetal cortisol production increases dramatically to high levels and remains high during labor.*

*After birth, adrenal cortisol content is similar between labor and 8%, and 3βHSD expression remains unchanged between term and 2% of weaning, assuming constant cortisol synthesis at least until 8% of weaning. No further information until weaning is available.*

### 3.6.5 Guinea pig fetal and neonatal adrenal cortisol synthesis

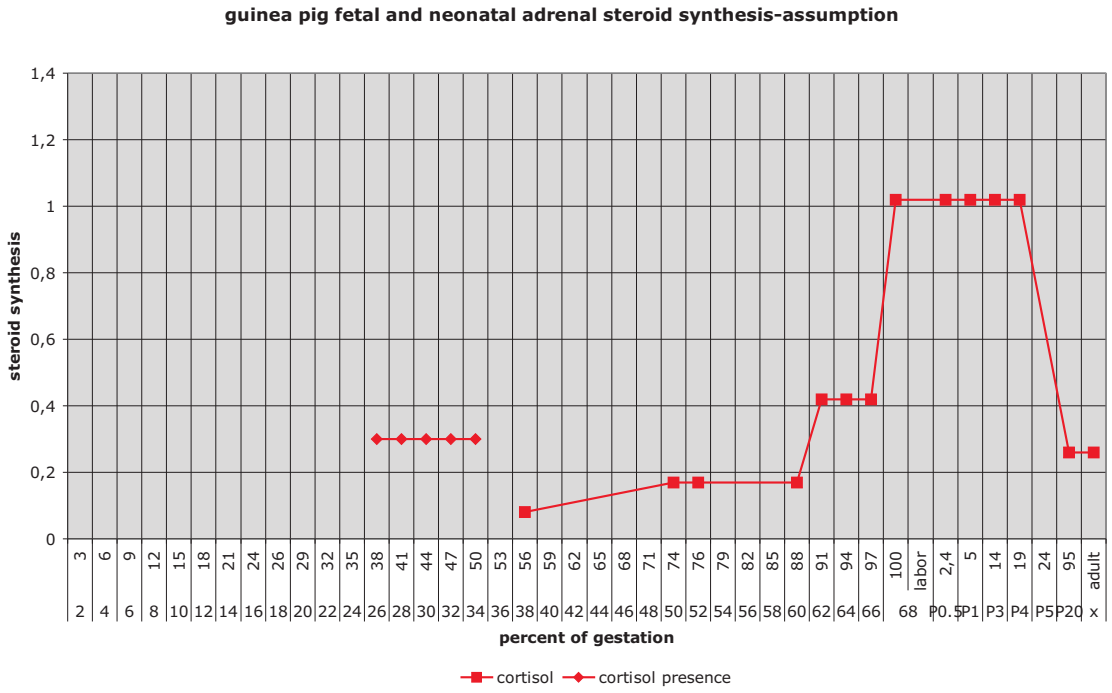


Figure 3.88. Guinea pig estimated pre- and postnatal adrenal steroid synthesis

*Already at 32-34% of gestation, the fetal adrenals synthesize androgens, assumingly before cortisol synthesis is present. During gonad differentiation at 37-40% of gestation, decreasing fetal androgen synthesis in parallel with increasing cortisol synthesis might be apparent. At 41% of gestation, 3βHSD activity in the fetal adrenals could indicate cortisol synthesis. The adrenal cortex of the guinea pig fetus is able to synthesize cortisol at least between 37-51% of gestation. Between 50-63% of gestation, cortisol production is unresponsive to ACTH and very low. Low cortisol synthesis is assumed until 87% of gestation. By 91% of gestation, cortisol synthesis seems to have reached moderate levels and remains constant until 99% of gestation, when cortisol production in the fetal adrenals increases dramatically to high levels.*

*After birth, the plasma cortisol concentration remains constant until 19% but decreases to very low adult-like levels by 95% of weaning.*

### 3.6.6 Rat fetal and neonatal adrenal glucocorticoid synthesis

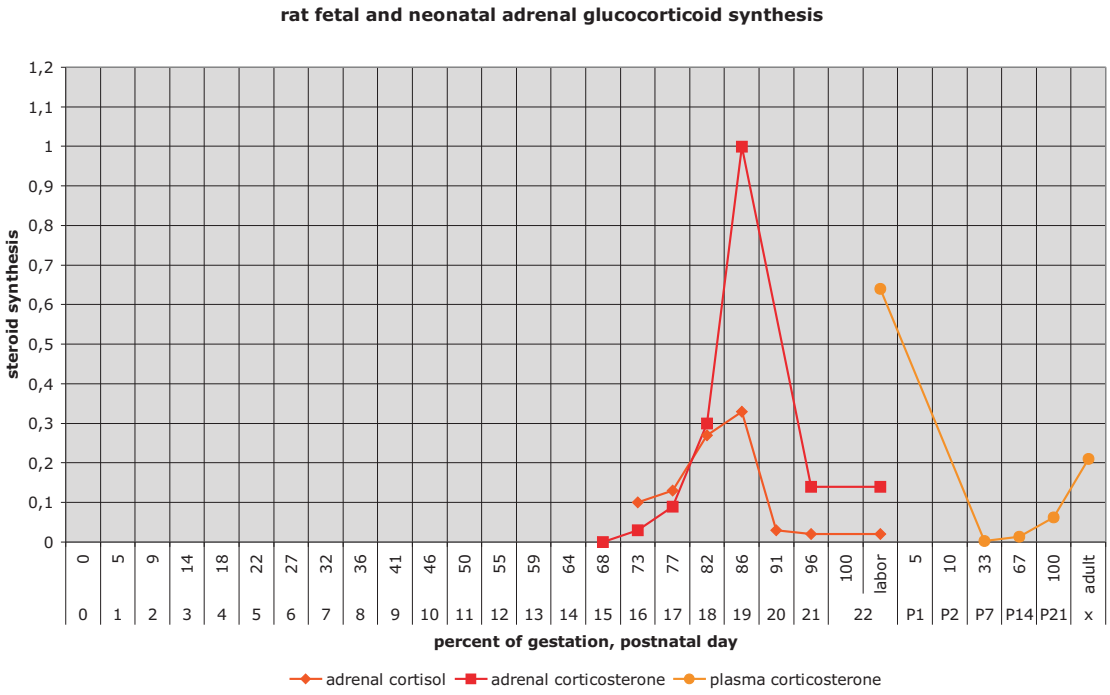


Figure 3.89. Rat estimated pre- and postnatal adrenal steroid synthesis

*The adult rat is unable to synthesize androgens in the adrenal cortex. Due to the presence of CYP17 expression in the fetal rat (a necessary enzyme for cortisol synthesis as well), transient fetal androgen synthesis in the adrenals cannot be excluded. Adrenal corticosterone synthesis is detected as early at 61-73% of gestation. Fetal adrenal cortisol content is apparent at 73% of gestation. Only at 73% of gestation is fetal adrenal cortisol synthesis higher than corticosterone production. The syntheses of both glucocorticoids increase between 73-82% of gestation. At 86% of gestation, adrenal cortisol and corticosterone levels reach their maxima, but adrenal corticosterone content is much higher than cortisol content. At 91% of gestation, the synthesis of corticosterone decreases to moderate and cortisol production decreases to very low levels. While cortisol synthesis remains very low or absent until term and in newborn, adrenal corticosterone concentration is low at 96% of gestation and either remains constant or even further decreases in newborn.*

*Corticosterone levels in circulation remain low during labor. Five hours after birth, the plasma corticosterone concentration shows a moderate peak but decreases to very low levels by assumingly already 10% to remain very low until 57% of weaning. Subsequently the plasma corticosterone concentration increases by 67% and further by 100% of weaning to peak at PND24, only to decrease dramatically by PND27 and to increase again moderately in adulthood.*

### 3.6.7 Mouse fetal and neonatal adrenal glucocorticoid synthesis

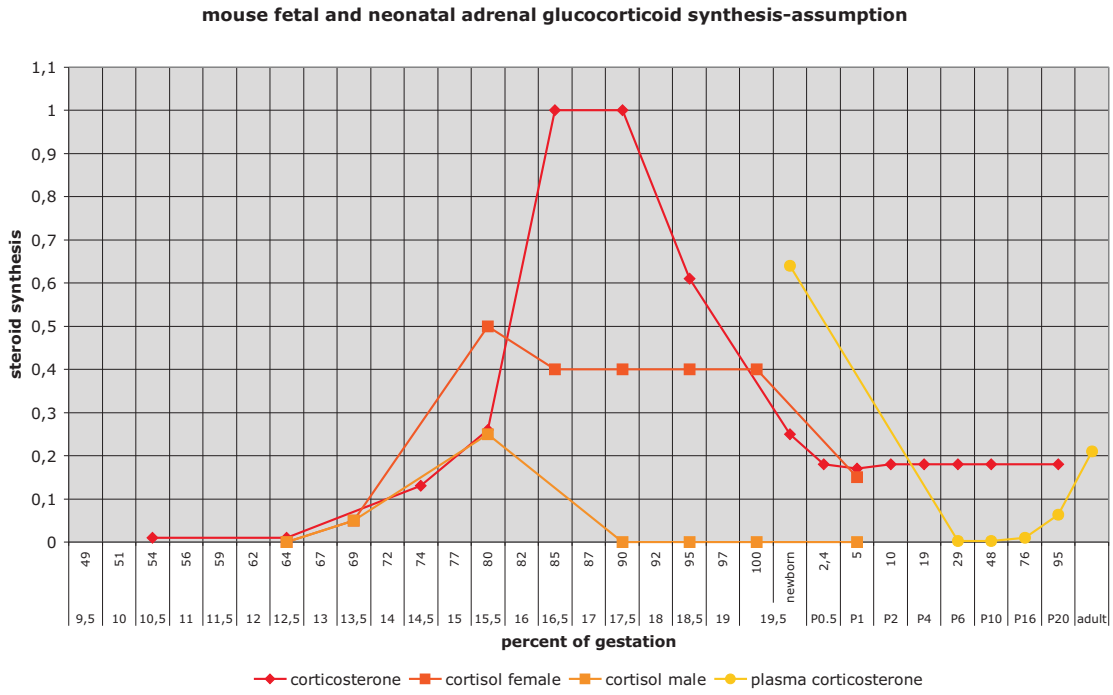


Figure 3.90. Mouse estimated pre- and postnatal adrenal steroid synthesis

*Fetal adrenal corticosterone synthesis starts in the mouse between 54-74% of gestation. Cortisol synthesis, due to the expression of CYP17, can be assumed to be absent at 64%, and could begin in both genders at 69% of gestation. As no further information about fetal adrenal cortisol synthesis beside CYP17 enzyme expression is present, it is not possible to compare the quantity of corticosterone synthesis and the possible cortisol synthesis; the presented amounts of cortisol in relation to corticosterone are not verified. In the following, cortisol synthesis is an assumption and derives solemnly from data of CYP17 expression. Cortisol synthesis could be maximal at 79% of gestation and would be stronger in females than in males. Adrenal corticosterone production progressively increases until it peaks at 85-90% of gestation. From 90% till 100% of gestation, corticosterone synthesis in the fetal adrenals continuously decreases to low levels at birth. Cortisol synthesis might remain moderately high in females until the end of gestation and might be still present in neonates. In the male fetus, cortisol synthesis could decrease after 79% of gestation and might be absent between approximately 90% of gestation and parturition. A potential higher and longer cortisol synthesis in the female compared to the male fetus could counteract an assumed inhibition by the transferred corticosterone from mother to fetus, as transport into the female fetus was shown to be stronger than into the male fetus. Maybe a united fetal synthesis of cortisol and corticosterone around 80% of gestation could induce a decrease in maternal corticosterone levels.*

*From birth until 2.4%, adrenal corticosterone content decreases and remains low until at least 95% of weaning. The plasma corticosterone concentration decreases strongly from birth until 19%, remains very low until 57% and starts to increase by 67% to reach adult like levels at 86% of weaning.*

## 3.7 Comparison of fetal adrenal glucocorticoid synthesis between species

We will here review, in regard of the beginning and the development of fetal steroidogenesis, adrenal steroid detection including morphological indicators and steroid enzyme expression.

On the basis of morphological features, the following time points for adrenal maturation, concerning hormone syntheses, are found:

TABLE 3.6

Adrenal maturation for hormone synthesis

| | Maturation for steroidogenesis |
|---|---|
| Human | 20-23 |
| Rhesus monkey | 27-36 |
| Baboon | (27) |
| Sheep | 25-31 |
| Guinea pig | 32-38 |
| Rat | 70-73 |
| Mouse | 62-72 |

The subsequent results show the starting time for adrenal glucocorticoid (and androgen) synthesis between the different species. The numbers show the verified detection of the steroid in the fetal adrenals, the numbers in the brackets are the estimated starting times based on all additional information, especially morphological and enzymatic features.

TABLE 3.7

Start of steroid synthesis

| | Cortisol | Corticosterone | DHEAS/Androstenedione |
|---|---|---|---|
| Human | 21-25 | | 25 (20) |
| Rhesus monkey | 44 (30) | | (27-30) |
| Baboon | 54-71 (32-35) | | 54-58 (32-35) |
| Sheep | 32 (25-29) | | |
| Guinea pig | 37-51 (32-38) | | 32-34 |
| Rat | 73 | 61-73 | |
| Mouse | (69) | 74 (54-74) | |

Based on data from the three different sources (morphological indicators, enzyme expression and adrenal steroid detection) the following conclusions can be drawn:

The human adrenals assumingly are able to synthesize DHEAS by 20% and cortisol by 21-25% of gestation. The rhesus monkey could develop the ability to synthesize DHEAS already between 27-30% and cortisol at 30% of gestation. The fetal baboon adrenal produces cortisol and DHEAS most likely already between 32-35% of gestation. Cortisol synthesis might start in the fetal sheep as early as 25-29% of gestation. The adrenals of the fetal guinea pig can synthesize androgens by 32-34% of gestation and cortisol synthesis is assumingly present at 32-38% of gestation. Corticosterone synthesis appears in the fetal rat between 61-73% and cortisol synthesis is verified by 73% of gestation. In the fetal mouse, corticosterone synthesis might be already present between 54-74% of gestation and cortisol synthesis is assumed. This would be coherent with the slightly earlier adrenal maturation in the mouse between 62-72% and in the rat between 70-73% of gestation.

Data strongly support the presence of a period of fetal respectively neonatal stress hyporesponsivity, with low or absent fetal/neonatal adrenal glucocorticoid production transiently between times of stronger synthesis, in all species.

TABLE 3.8

Period of low or absent steroid synthesis

| | Cortisol | Corticosterone |
|---|---|---|
| Human | 40-55 | |
| Rhesus monkey | 67-76 | |
| Baboon | 54-70 | |
| Sheep | 63-87 | |
| Guinea pig | 56-81 | |
| Rat | | 96-w57 |
| Mouse | | 100-w57 |

A period of low or absent cortisol synthesis, following stronger cortisol production, is assumed in all three primate species as well as in the sheep and in the guinea pig during gestation. Our data indicate the beginning of this period earliest in gestation in the human adrenals, subsequently in the guinea pig and the baboon, then in the sheep and the rhesus monkey. The period seems to encompass only 9% of the gestational time in the rhesus monkey and assumingly is longest in the sheep and guinea pig with 24-25% of gestational time. Human and baboon most likely have a period of low or absent cortisol synthesis encompassing 15-16% of gestational time. Rat and mouse are exceptions in this respect, as theirs glucocorticoid syntheses decrease to low levels before respectively at term. The transient periods of low corticosterone synthesis encompass in the rat and in the mouse the time from 96%, respectively 100% of gestation, until roughly 57% of weaning. The transient low or absent glucocorticoid synthesis could be beneficial during organ maturation. It was indicated earlier which impact prenatal glucocorticoid exposure have on different maturation processes. Little or absent fetal glucocorticoid synthesis would be only beneficial, during low maternal glucocorticoid transfer (see Chapter 2.1.3, 2.1.4).

# CHAPTER 4

## Summary pre- and postnatal HPA axis

In the following, we will combine the results of *Chapter 2* and *Chapter 3*, to give a comprehensive review of the fetal and neonatal HPA axis. Fetal and maternal adrenal and plasma glucocorticoid concentrations will be investigated, under the influence of androgen transfer and of placental transfer, controlled by expression of 11$\beta$HSD and P-gp. The free glucocorticoid levels in circulation or alternatively CBG levels elicit the active fraction of the total glucocorticoid concentration. 11$\beta$HSD expression in fetal adrenal, depending on the type of 11$\beta$HSD, assumingly inhibits or enhances fetal glucocorticoid secretion. The plasma ACTH concentration, as a direct secretagogue for fetal glucocorticoid synthesis, and the expression of the ACTH precursor POMC in the pituitary will be included. ACTH synthesis and secretion is stimulated by hypothalamic CRH and AVP. The GR expression in the pituitary and the PVN permit inhibition of POMC and CRH expression through glucocorticoid negative feedback.

### 4.1 Human prenatal HPA axis

In terms of fetal adrenal cortisol synthesis, we will again investigate fetal plasma and adrenal as well as maternal plasma cortisol, in respect of the data for placental 11$\beta$HSD2 and P-gp expression, to test the correctness of our assumptions about placental cortisol transfer. Further we will include the available data about fetal pituitary ACTH synthesis and plasma ACTH concentration, GR expression in the pituitary, and fetal adrenal 11$\beta$HSD2 expression, the latter as a possible cortisol inhibitor inside the FZ.

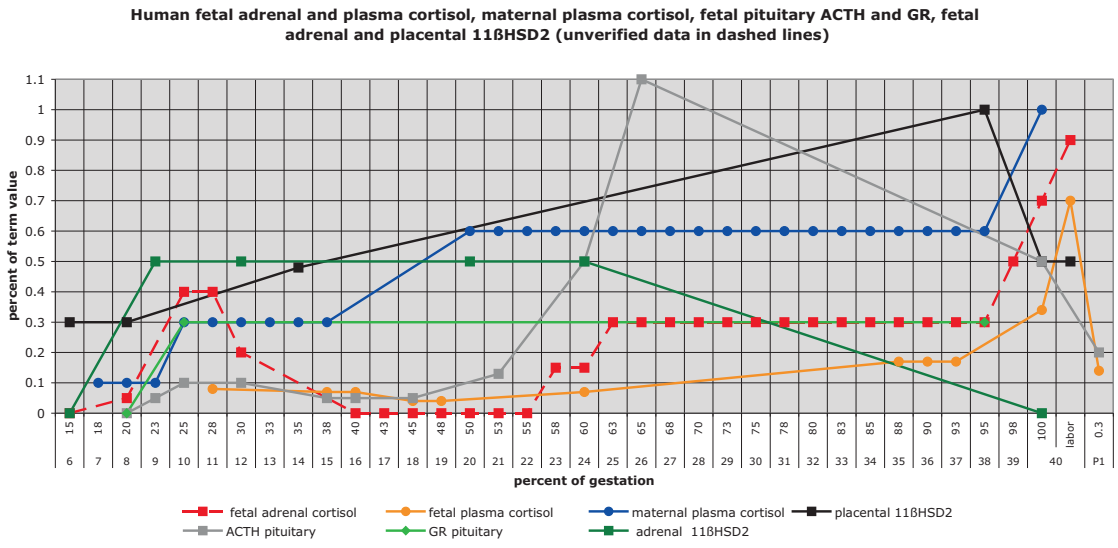


Figure 4.1. Human prenatal HPA axis

Fetal and maternal plasma cortisol concentrations show a significant correlation during gestation and maternal cortisol transfer into the fetus is assumed to negative feedback on fetal adrenal

cortisol synthesis by inhibiting fetal pituitary ACTH release. Our data support the assumption that glucocorticoids develop anti-parallel to androgens and that both groups might inhibit each other especially during early gestation.

Adrenal DHEAS synthesis starts in human fetus around 20% of gestation, when cortisol synthesis is still absent or low. Cortisol inactivating 11$\beta$HSD2 appears in the FZ already between 15-23% of gestation, possibly as a protective mechanism inside the FZ, against cortisol transferred from the TZ. Alternatively, the presence of 11$\beta$HSD2 could inhibit a possible cortisol synthesis in the FZ early in gestation.

It is shown that POMC and IR-ACTH are detectable in the human fetal pituitary at 23-25% of gestation. At this time, ACTH responsive adrenal cortisol synthesis as well as glucocorticoid negative feedback in the fetal pituitary are apparent, the latter due to the appearance of pituitary GR expression between 20-25% of gestation. Data indicate an early peak in pituitary ACTH levels and assumingly ACTH concentration in fetal circulation, approximately at the time of the first elevation in fetal adrenal cortisol content around 25% of gestation. GR generated glucocorticoid negative feedback in the pituitary might be responsible for the decrease in pituitary ACTH levels between 31-39% of gestation. It is assumed that along with cortisol negative feedback on ACTH synthesis, the latter a potent stimulator of DHEAS synthesis as well, fetal DHEAS synthesis decreases and is low during the sexual differentiation between 25-35% of gestation, to prevent the female fetus from virilization.

After assumingly 25-28% of gestation, fetal adrenal cortisol synthesis decreases around 30% and ceases or is very low by 40% of gestation. The maternal plasma cortisol concentration increases between 23-25% of gestation. An increment in placental 11$\beta$HSD2 protein between 20-36% of gestation decreases the transfer of maternal cortisol into the fetus. This could protect the early burst of fetal adrenal cortisol synthesis against inhibition from maternal cortisol. 11$\beta$HSD2 expression in the placenta is assumed to increase with ongoing gestation. Around 40-45% of gestation, the fetal plasma cortisol concentration and fetal pituitary ACTH levels can be assumed to decrease. Adrenal 3$\beta$HSD expression indicates low or absent fetal adrenal cortisol synthesis from 40% until 55-58% of gestation. During this stress hyporesponsive time of negligible fetal adrenal cortisol synthesis, fetal adrenal DHEAS synthesis increases. By 66% of gestation, fetal pituitary ACTH content has increased to high levels. IR-3$\beta$HSD in the TZ is present again around 55-60% of gestation, which indicates the reappearance of fetal adrenal cortisol synthesis. Adrenal cortisol synthesis could have reached moderate values and might remain roughly constant between 63-93% of gestation. We assume an increase in the fetal plasma cortisol concentration after 60% and moderately low, roughly constant levels until 93% of gestation, indicated by measurements in umbilical blood. By 90% of gestation, the plasma cortisol concentration in the umbilical artery is markedly higher than in umbilical vein, revealing a stronger transfer from fetus to mother than vice versa. After 86% of gestation, placental P-gp expression at the side of feto-maternal exchange decreases, which could allow this stronger placental transfer of cortisol. After 93% of gestation, fetal plasma cortisol is assumed to increase strongly, protected by most likely very high placental 11$\beta$HSD2 levels. A dramatic surge in fetal cortisol synthesis is expected from 95% of gestation until term or further during labor as fetal plasma cortisol concentration continuously increases during labor. After 95% of gestation, placental 11$\beta$HSD2 activity decreases, increasing placental cortisol transfer, and the maternal plasma cortisol concentration increases strongly, which decreases the ratio of plasma cortisol in umbilical artery to vein further toward term. Still at term, the ratio of cortisol in umbilical artery to vein is slightly higher than 1, assuming higher transfer from the fetus to the mother than vice versa. At term, adrenal 11$\beta$HSD2 seems to be absent. Pituitary and plasma ACTH concentrations decrease to low levels at term, possibly as a consequence of glucocorticoid negative feedback on the pituitary. No information is available about GR expression in the pituitary or PVN and neither about CRH expression in the PVN during this late surge of fetal cortisol synthesis. But high levels of GR in the pituitary and maybe also in the PVN can be assumed to elicit the strong feedback inhibition at term, to restrain and decrease cortisol production after labor. Negative feedback might be strong at birth or in general during gestation, since much lower levels of plasma CBG are present in the fetus compared to the adult, which could indicate a

high fraction of active cortisol in fetal circulation.

## 4.2 Human postnatal HPA axis

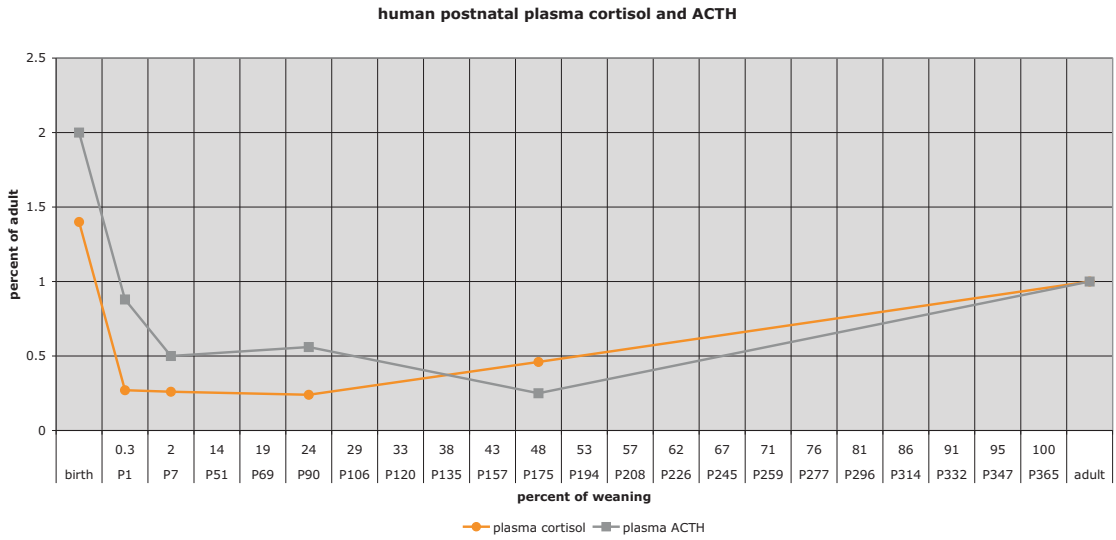


Figure 4.2. Human postnatal HPA axis

The only information available in the human neonate regards the plasma cortisol and ACTH concentrations. From birth until 0.3% of weaning, plasma cortisol and ACTH concentrations decrease dramatically in parallel. The decrease in ACTH levels might be still caused by glucocorticoid negative feedback on the pituitary. Plasma cortisol does not significantly change from 0.3% to 2% of weaning. Levels remain low for both hormones from roughly 2% to 25-48% of weaning, but seems to increase to moderate levels in adulthood.

## 4.3 Rhesus monkey pre- and postnatal HPA axis

Additionally to cortisol levels in adrenal and plasma of the fetus as well as maternal circulation, we will include the fetal plasma ACTH concentration, pituitary POMC and ACTH levels, the maternal estrogen concentration and the assumption concerning placental 11$\beta$HSD2.

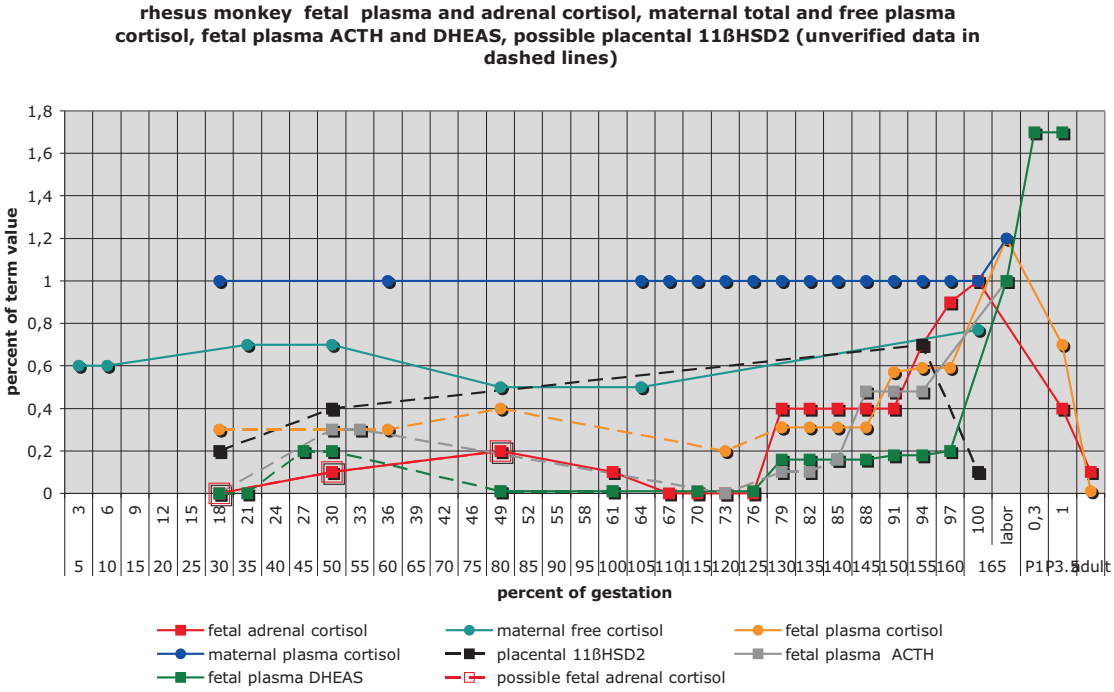


Figure 4.3. Rhesus monkey pre- and postnatal HPA axis

The following development could be possible. Between 22-26% of gestation, fetal gonad sexual differentiation occurs and low fetal adrenal DHEAS synthesis as well as low fetal and maternal plasma DHEAS concentrations are required, which was confirmed by maternal plasma androgen levels. By 27% of gestation, the placenta shows sufficient aromatase activity to metabolize DHEAS to estrogen and removes fetal DHEAS from the circulation. By that time, sufficient fetal adrenal DHEAS was indicated, most likely before cortisol synthesis appears in the adrenal cortex. Pituitary POMC cells are apparent by 30% and IR-ACTH in the pituitary can be detected between 30-33% of gestation. ACTH is a potent stimulator for adrenal DHEAS as well as for cortisol synthesis. At 30%, fetal adrenal cortisol synthesis might be present and is verified between 44% and 53% of gestation. Enzyme expression indicates at best very low fetal adrenal DHEAS synthesis from 49% of gestation on, assuming an inverse correlation between fetal adrenal cortisol and DHEAS synthesis. Enzyme expression might indicate fetal adrenal cortisol synthesis between 30-64% of gestation. The maternal plasma cortisol concentration seems to remain constant between 36-64% of gestation. On the other hand, the CBG concentration in maternal plasma increases to maximal levels by 56% of gestation, assuming a strong decrease in the fraction of free cortisol during that time. Decreasing maternal free cortisol levels could allow the fetal adrenal cortex to be dis-inhibited and have an early peak in cortisol synthesis around 50% of gestation. A parallel development in fetal plasma cortisol concentration might be possible. Due to a maximal CBG concentration between 48-64% of gestation, maternal free cortisol levels might remain low. Subsequently, the maternal plasma

CBG concentration decreases and could increase maternal free cortisol levels again. This increment might cause decreasing fetal cortisol synthesis. The fetal adrenal cortisol synthesis might cease, due to enzyme expression, by 67% and seems to remain absent until 79% of gestation. Between 78-82% of gestation, cortisol synthesis from progesterone is minimal. A very low or absent fetal plasma ACTH concentration can be assumed already around 62-67% and is verified by 79% of gestation. Due to enzyme expression, reappearance of fetal adrenal cortisol synthesis can be assumed between 76-79% of gestation. During that time of increment in fetal adrenal cortisol synthesis, only approximately 40% of fetal plasma cortisol derives from the mother and most of maternal cortisol is inactivated during placental transfer, assuming high placental 11$\beta$HSD2 levels, which could protect the fetal cortisol production. Around 79-88% of gestation, the fetal plasma cortisol concentration is moderately low and fetal plasma ACTH levels remain low. By 91% of gestation, the fetal plasma cortisol concentration increases moderately, and the fetal-maternal plasma cortisol concentration ratio increases strongly. From here on, enzyme expression indicates a stronger increment in fetal adrenal cortisol synthesis in parallel with increasing fetal plasma ACTH concentration. The fetal plasma cortisol concentration surges dramatically from 97% of gestation until labor and maternal plasma cortisol as well as fetal plasma ACTH concentrations increase in parallel during parturition. Between labor and 1% of weaning, the fetal plasma cortisol concentration decreases, and decreasing neonatal adrenal cortisol synthesis is assumed. The plasma DHEAS concentration reaches very high levels at 0.3-1% of weaning. By adulthood, adrenal and plasma cortisol levels have decreased dramatically compared to neonates. No information concerning GR expression in the PVN or the pituitary is available for the rhesus monkey. Additionally, data for CRH expression in the PVN are missing, making it impossible to conclude about glucocorticoid negative feedback during the fetal cortisol surge.

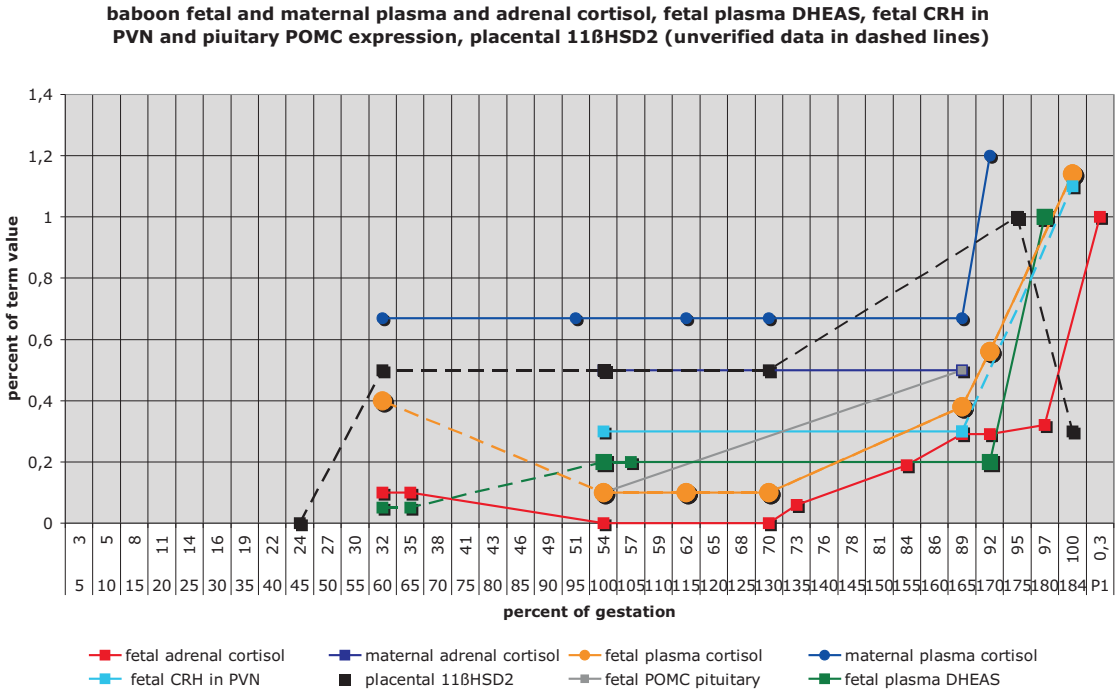


Figure 4.4. Baboon pre- and postnatal HPA axis

In the fetal adrenal cortex of the baboon, ACTH receptor expression is present at least by 33% of gestation and increases until 55% of gestation. Enzyme expression could indicate adrenal cortisol synthesis between 32-35% of gestation. The estradiol concentration in maternal circulation is low at 33% gestation, which could be an indicator for low fetal adrenal androgen synthesis. In maternal plasma, the cortisol concentration is moderately high at 32% and 54% of gestation. By 54% of gestation, fetal adrenal cortisol synthesis is absent and ACTH fails to elicit an increment in cortisol production, but DHEAS synthesis is highly responsive to ACTH. Between 54-70% of gestation, the fetal plasma cortisol concentration remains unchanged. Over that period, the fetal plasma DHEAS concentration is significantly higher than in maternal circulation. This suggests low fetal cortisol levels, when fetal DHEAS levels are higher and vice versa, demonstrating the inverse correlation of adrenal androgen and cortisol synthesis. Between 54-89% of gestation, the fetal CRH expression in the PVN is moderate and the placental 11$\beta$HSD2 expression increases to high levels in that period. Between 70-89% of gestation, fetal adrenal and plasma cortisol concentrations increase from very low to moderately low levels. The maternal plasma cortisol concentration is similar at 70% and 89% and increases dramatically between 89-92% of gestation to high levels. Fetal pituitary POMC expression increases strongly sometime between 54-89%, possibly in parallel with the increasing fetal adrenal cortisol synthesis between 70-89% of gestation. Fetal adrenal cortisol remains constant between 89-97% of gestation, while fetal plasma DHEAS and cortisol concentrations increase strongly from 92% until 97% respectively 100% of gestation. This increment in fetal plasma cortisol concentration is most likely a result of increasing maternal plasma cortisol concentrations. Between 97% and 0.3% of weaning, fetal adrenal cortisol surges to a very high level. These already sufficient data can be completed by the following assumptions. Very low plasma estradiol levels in maternal circulation at

32% of gestation suggest low fetal and maternal DHEAS synthesis during this period, possibly because gonadal differentiation takes places. During the assumed early peak in fetal cortisol synthesis around 32-35% of gestation, POMC expression in the pituitary and plasma ACTH concentration could be moderate, possibly together with the CRH expression in the PVN. Over that period, maternal adrenal and plasma cortisol levels could show a diminution or strong placental 11$\beta$HSD2 expression could protect the fetal cortisol synthesis. Moderately low fetal POMC expression and plasma ACTH concentration at 54% of gestation together with increasing adrenal ACTH receptor expression might be responsible for increasing fetal DHEAS synthesis, which is highly responsive to ACTH at this point, when fetal cortisol synthesis ceases by 54% of gestation. Between 54-70% of gestation, fetal adrenal cortisol synthesis is very low or absent, and the fetal plasma cortisol concentration remains low. The DHEAS concentration in fetal plasma is higher than in maternal circulation, indicating sufficient fetal DHEAS synthesis. As the maternal plasma cortisol concentration is moderately high at that point, assumingly the maternal adrenals produce sufficient amounts of cortisol. The placental barrier must be relatively tight against maternal cortisol transfer into the fetus. Between 70-89% of gestation, the fetal adrenal cortisol synthesis slowly increases to moderate levels in parallel with the fetal plasma cortisol concentration, the pituitary POMC expression and assumingly the plasma ACTH concentration. This suggests low cortisol negative feedback on the pituitary at this period. Placental 11$\beta$HSD1/2 protein ratio decreases and placental inactivation of cortisol increases strongly by 98% of gestation. As the maternal plasma cortisol concentration increases between 89-92% of gestation to high levels, increasing maternal cortisol production could be apparent. Fetal plasma cortisol concentration increases further after 89% of gestation. Following high levels of cortisol inactivation at 98% of gestation, placental 11$\beta$HSD expression might change to lower inactivation, and a strong maternal cortisol transfer could explain the dramatic increase in fetal plasma cortisol concentration during the period when fetal adrenal cortisol synthesis remains constant. During that period, fetal plasma and adrenal DHEAS levels increase strongly, causing 10 times higher DHEAS production than cortisol synthesis in the fetal adrenals at 98% of gestation. The very high ACTH responsive DHEAS synthesis takes place during assumingly an increment of plasma ACTH concentration and of pituitary POMC expression. Possibly CRH or parvocellular AVP expression in the PVN increase in parallel, and low GR expression in the pituitary and the PVN could elicit low cortisol negative feedback during that time. Fetal cortisol synthesis increases to high levels from 97% of gestation till 0.3% of weaning. Whether maternal cortisol production decreases between 97-100% of gestation or further increases is unknown. The fetal plasma cortisol concentration reaches high levels at term and similarly high cortisol levels in maternal circulation can be assumed due to parturition stress and the expected decrease in placental 11$\beta$HSD2 level to low levels. By 0.3% of weaning, neonatal plasma cortisol concentration is high but has decreased again by 5% of weaning.

## 4.5 Sheep prenatal HPA axis

In the following we will complete the already sufficient data about fetal adrenal and plasma cortisol levels with the information about influencing factors from higher brain centers and the placenta.

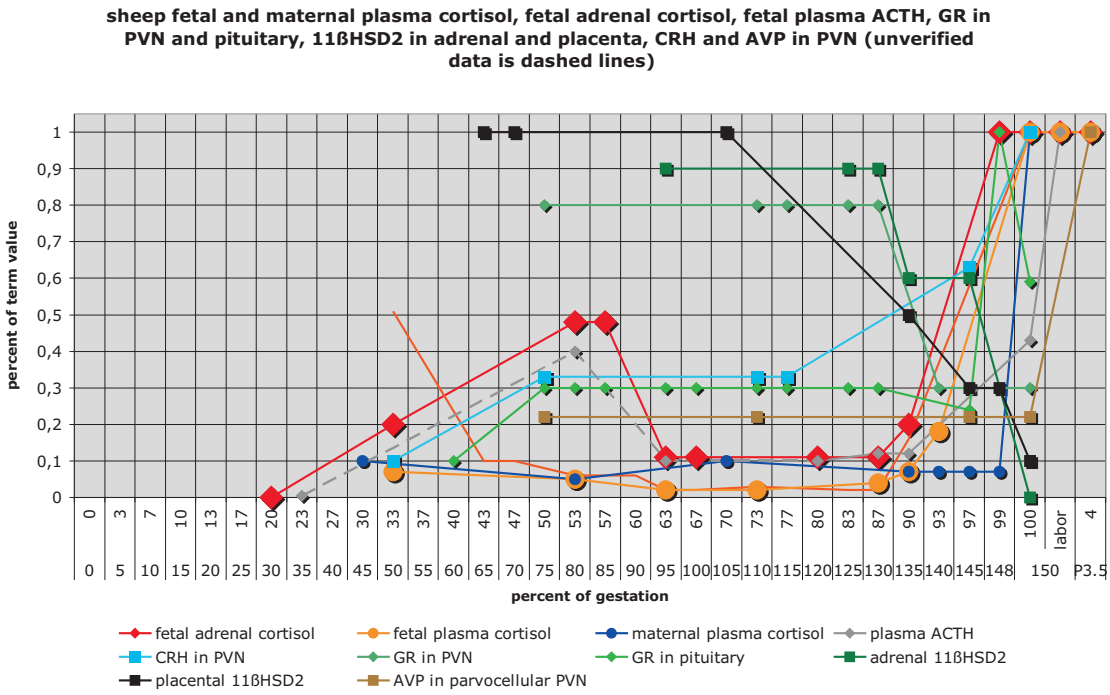


Figure 4.5. Sheep prenatal HPA axis

Fetal adrenal cortisol synthesis is absent at 20%, but is detectable by 32% of gestation. Already at this point, fetal sheep adrenal cortisol production is responsive to ACTH. Fetal POMC expression in the pituitary peaks at 30% to remain low between 33-50% of gestation. The fetal plasma ACTH concentration is very low at 23%, but no further information is available until 80% of gestation. Cortisol enzyme expression indicates sufficient fetal cortisol synthesis between 30-60% of gestation and adrenal cortisol content exhibits already half the amount of the term value by 55% of gestation. It is assumed that fetal plasma ACTH concentration shows also an increment during the period of sufficient fetal cortisol synthesis. The moderate responsivity of cortisol synthesis to ACTH at 32% decreases by 42-45% and further reaches a nadir at 65% of gestation. Around 55% of gestation, during moderately high fetal adrenal cortisol content, the maternal plasma cortisol and maternal ACTH concentration show lower levels and the fetal plasma cortisol concentration is higher around 33-53% than at 67-73% of gestation. At 43-47% and at 70% of gestation, placental 11$\beta$HSD dehydrogenase activity is very high, decreasing placental cortisol transfer into the fetus and possibly protecting the peak in fetal adrenal cortisol synthesis from maternal cortisol inhibition. No information is available about placental P-gp expression in the sheep. The CRH expression in the PVN increases between 33-50% of gestation. AVP expression is detectable at very low levels in the whole PVN already at 30% of gestation. In the parvocellular PVN, the location for stress regulation, AVP expression is low at 50% of gestation. At 50% of gestation, GR expression in the PVN is very high. A strong negative feedback on hypothalamic CRH synthesis might prevent a further increment

of CRH expression in the PVN, as levels remain constant until 75% of gestation. GR expression in the pituitary increases between 40-50% and remains constant until 87% of gestation. This additional increase in glucocorticoid feedback on the pituitary might be responsible for an assumed decrease in the plasma ACTH concentration by 63% of gestation, causing a decrement in the stimulation of fetal adrenal cortisol production. Between 57-63%, fetal cortisol synthesis decreases to low levels and fetal plasma cortisol concentration decreases by 63-73% of gestation. The fetal plasma ACTH concentration is low at 80% and high placental 11$\beta$HSD2 dehydrogenase activity could protect the fetus against maternal cortisol transfer, although the maternal plasma cortisol concentration is low during that period as well. The adrenal 11$\beta$HSD2 expression is very high at 63-87% of gestation, possibly to prevent the residual fetal adrenal cortisol from secreting into the circulation. Around 73% of gestation, the AVP expression in the parvocellular PVN remains low but pituitaric POMC expression has reached moderately high levels. The fetal adrenal cortisol synthesis increases slowly at 87-90% of gestation, together with the fetal plasma cortisol concentration. Over the same period, the expression of 11$\beta$HSD2 in the adrenals decreases which might allow more cortisol secretion into the circulation. After 87% of gestation, GR expression in the pituitary decreases slightly, which might be sufficient to inhibit glucocorticoid negative feedback on the pituitary and causes the increase in the fetal plasma ACTH concentration. The GR expression in the PVN strongly decreases between 87-90% of gestation, resulting in decreasing negative feedback on CRH expression. At least by 97% of gestation, CRH expression in the PVN has strongly increased in this context. Placental 11$\beta$HSD2 expression has decreased to moderate levels by 89% of gestation. From 90%, respectively 93% of gestation onward, fetal adrenal and plasma cortisol levels increase. By 97% of gestation, CRH expression in the PVN has increased while AVP expression in parvocellular PVN remains still low and unchanged. The placental 11$\beta$HSD1/2 dehydrogenase has further decreased by 97% and assumingly remains moderately low until 99% of gestation. Placental 11$\beta$HSD1/2 dehydrogenase activity might be still sufficient enough to inhibit cortisol transfer into the mother, which would explain the persistence of maternal plasma cortisol concentration at very low levels until 99% of gestation. At 99% of gestation, the GR expression in the pituitary exhibits a sudden peak. This temporarily very high glucocorticoid feedback on the pituitary could prevent the plasma ACTH concentration from increasing too early. Finally, the maternal plasma cortisol concentration also increases dramatically to high levels between 99-100% of gestation, which could be accompanied by a further decrease in placental 11$\beta$HSD2 expression to very low levels. Fetal POMC and CRH expression, as well as fetal plasma and adrenal cortisol levels are maximal at 100% of gestation and fetal plasma ACTH concentration has increased in concert with a decrement in pituitary GR expression. During labor, the fetal plasma ACTH concentration reaches maximal levels in parallel with low GR expression in the PVN, assuming very low glucocorticoid negative feedback on CRH expression in the PVN and on ACTH release from the pituitary at that point. While AVP expression in the parvocellular PVN still remains low at term, it has reached very high levels at least at 4% of weaning. It cannot be excluded that AVP regulation on ACTH release has reached high level already during labor to assist CRH during parturition. This very sophisticated interplay between cortisol surge and cortisol negative feedback could be demonstrated due to the amount of data available for the fetal sheep.

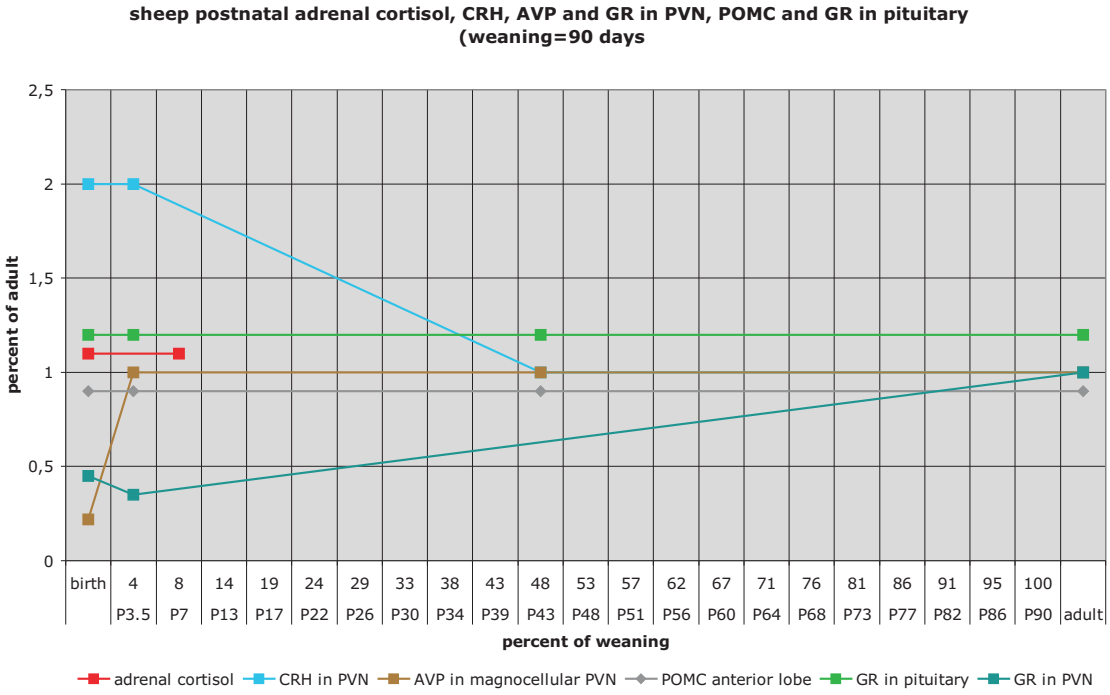


Figure 4.6. Sheep postnatal HPA axis

The neonatal adrenal cortisol content does not change between birth and 8% of weaning. POMC and GR expression in the pituitary remain constant from birth to 4%, and further until 50% of weaning and in adults. No change in glucocorticoid negative feedback regulation on pituitary ACTH levels is assumed. The expression of CRH in the PVN is very high at birth and at 4%, but has decreased to moderate adult like levels at least by 50% of weaning. Between birth and 4%, GR expression in the PVN decreases slightly, but subsequently increases to moderate levels by adulthood or possibly already by 50% of weaning, in line with decreasing CRH expression. AVP expression in the parvocellular PVN increases strongly between birth and 4% and subsequently remains constantly high by 50% of weaning and in adulthood. In the neonatal sheep, CRH expression decreases and AVP expression increases, assuming a more important influence of AVP on ACTH synthesis at this time. The decrement in CRH expression seems to be caused by increasing glucocorticoid negative feedback due to increasing GR expression in the PVN.

## 4.7 Guinea pig prenatal HPA axis

At least in the second half of gestation, the amount of data for the HPA axis in the fetal guinea pig is quite sufficient. Beside fetal and maternal adrenal and plasma cortisol, information is available about placental 11$\beta$HSD and P-gp expression, conducting maternal and fetal cortisol interplay. Fetal cortisol synthesis regulated by ACTH and the latter influenced through CRH and AVP can be investigated, as well as glucocorticoid negative feedback on PVN and pituitary through GR expression.

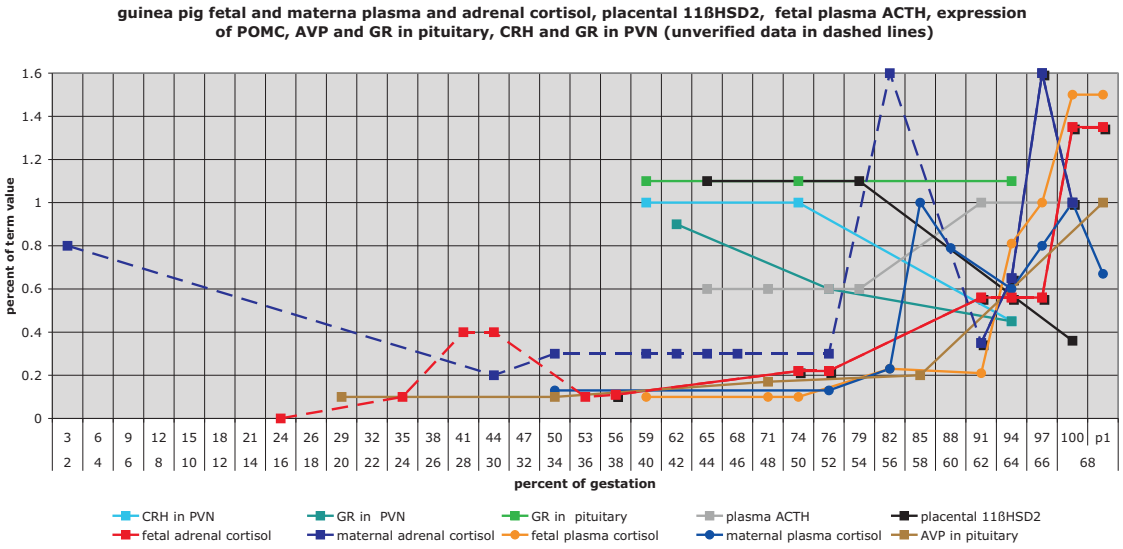


Figure 4.7. Guinea pig prenatal HPA axis

For the first half of gestation, data are very rare but the following assumptions can be drawn.

- The fetal adrenal cortex produces androgens already at 32% of gestation, before cortisol synthesis is apparent. It is possible that the fetal adrenal cortex exhibits a lower peak of cortisol synthesis already around 26-50% of gestation and fetal adrenal cortisol synthesis is verified between 37-51% of gestation. Sexual differentiation in the fetal guinea pig takes place between 37-40% of gestation and the necessity of stronger fetal cortisol synthesis is assumed during the sexual differentiation, to decrease fetal androgen exposure and by that preventing masculinization of the female fetus. The later might be less important in the guinea pig because adrenal androgen synthesis is assumingly less significant in this species compared to primates.

- The maternal plasma cortisol concentration is very low at 51% of gestation and most likely low maternal cortisol synthesis is present during the assumed elevation in fetal cortisol synthesis. The placenta might also synthesize high amounts of 11$\beta$HSD2, to prevent maternal cortisol transfer and possibly avoiding inhibition of the fetal adrenal cortex. The elevation in fetal plasma cortisol synthesis could have been induced by possibly higher fetal plasma ACTH concentration and CRH expression in the PVN, maybe together with low GR expression in pituitary and PVN, to prevent negative feedback on the higher control centers. Data only exist of AVP content in the whole pituitary. This is a limitation, as AVP in the whole pituitary reflects AVP content in the posterior pituitary rather than in the anterior pituitary, and only

AVP in the latter is involved in stress regulation and elicits ACTH release. Still the possibility exists that AVP transfers from the posterior to the anterior pituitary through blood vessels and that a major increment in AVP in the posterior pituitary could induce an increment in the anterior pituitary as well. Pituitary AVP is known to be very low between 29-85% of gestation, and in case this is indicative for the content in the anterior pituitary as well, it would suggest no important role of AVP in pituitary ACTH stimulation during that time. Fetal plasma cortisol might transiently increase over the period of possibly higher fetal adrenal cortisol synthesis.

- By 53% of gestation, fetal adrenal cortisol synthesis is unresponsive to ACTH and is very low. The fetal plasma cortisol concentration remains low at 59-81% of gestation. The maternal plasma cortisol concentration stays low until 74% of gestation. Between 60-74% of gestation, the fetal adrenal cortex is more androgenic that later in gestation, which might explain the low cortisol synthesis. Additionally, fetal adrenal cortisol synthesis is still unresponsive to ACTH at this point and remains unresponsive and assumingly low until 87% of gestation. GR expression in the pituitary is very high at least between 59-94% of gestation and indicates a functional glucocorticoid negative feedback on ACTH release over that period.

- The maternal plasma cortisol concentration shows a first peak at 84% of gestation, which assumingly is preceded by a similar peak in maternal cortisol synthesis. The fetal plasma cortisol concentration increases slightly in parallel with maternal plasma cortisol levels between at least 74-82% of gestation, which could be due to the decreasing P-gp expression in the placenta over that period. Is can be assumed that maternal cortisol similar to dexamethasone is able to decrease placental P-gp expression. But while the maternal plasma cortisol concentration increases further, fetal plasma cortisol levels remain constantly low until 91% of gestation, possibly due to still high 11$\beta$HSD2 expression in the placenta and low fetal cortisol synthesis. By 91% of gestation, the cortisol synthesis in the fetal adrenal cortex increases to moderately low levels. Maternal adrenal cortisol synthesis reaches a nadir at the same time, which leads to a transiently higher cortisol production in the fetus than in the mother. At 91% of gestation, the fetal plasma ACTH concentration is moderately high and fetal cortisol response to ACTH is very strong. Still at this point, sufficient functional glucocorticoid negative feedback on ACTH release can be assumed and might prevent a too strong increment in fetal cortisol synthesis. The maternal plasma cortisol concentration reaches its low at 94% of gestation but by now the fetal plasma cortisol concentration has increased to similar low cortisol levels as in maternal circulation. The AVP expression at least in the whole pituitary has strongly increased. CRH expression has reached relatively low levels, surprisingly in parallel with GR expression in the PVN. An expected increment in CRH expression due to decreasing negative feedback might have remained undetected in consequence of the chosen sampling times. Fetal and maternal plasma cortisol concentrations together increase at 94% of gestation, most likely due to the low 11$\beta$HSD2 expression. At 99% of gestation, the maternal cortisol production is maximal. Fetal adrenal cortisol synthesis remains unchanged on moderately low levels until 99% of gestation, but now strongly increases. Low negative feedback on the CRH expression in the PVN at the end of gestation, indicated by low GR expression in the PVN, could allow an increment in CRH expression after 94% of gestation. The latter could be a possible explanation for the surge in fetal cortisol synthesis at the end of gestation. Alternatively, CRH expression could remain low around birth and a high AVP expression in the anterior pituitary could be present and could regulate ACTH synthesis and the surge in fetal cortisol synthesis at this point. The fetal plasma ACTH concentration might further increase during labor in reaction to a possible decrease in GR pituitary expression at the end of gestation.

- The fetal adrenals produce high amounts of cortisol between 99-100% gestation, when maternal cortisol synthesis has already decreased again. The fetal plasma cortisol concentration reaches high levels at term.

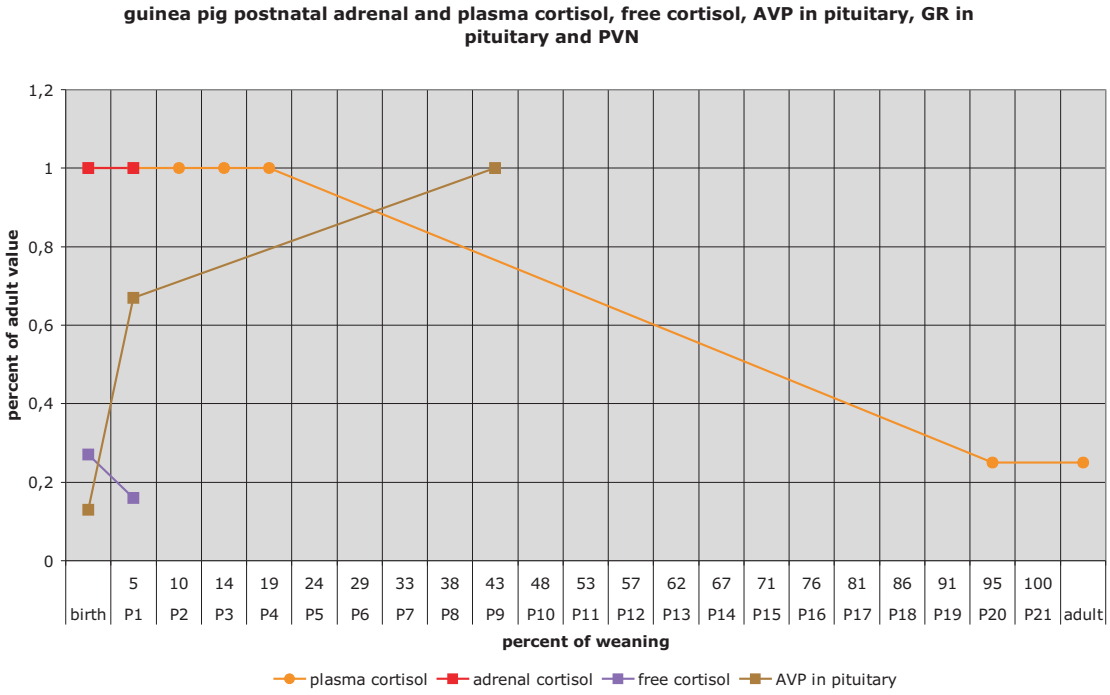


Figure 4.8. Guinea pig postnatal HPA axis

In the newborn guinea pig, adrenal and plasma cortisol levels remain very high from birth to 5% of weaning, while the plasma concentration of free cortisol decreases over that period, which could indicate a decrease in cortisol negative feedback. The AVP content in the pituitary increases strongly from birth to 5% of weaning with the possibility that a major increment in AVP in the posterior pituitary might increase AVP levels in the anterior pituitary as well. The plasma cortisol concentration remains very high until 17%, but strongly decreases to low adult like values by 95% of weaning. GR expression in the pituitary at 33% of weaning is unchanged compared to the expression in late gestation and could indicate functional glucocorticoid negative feedback on ACTH concentrations, which might explain decreasing plasma cortisol levels at the end of weaning. Unfortunately, no information about ACTH synthesis after birth is available. CRH expression in the PVN is similarly low at 33% of weaning as in late gestation. The AVP content in the pituitary further increases from 5% to high levels at 43% of weaning. In case these data reflect in any form AVP content in the anterior pituitary, it might be possible that around 33-43% of weaning, AVP rather than the low CRH levels are responsible for ACTH release. At 33% of weaning, GR and CRH expression are as low as during late gestation in the PVN. This either indicates an immature CRH system at this point or the expression of GR in the PVN is still sufficient enough to cause negative feedback on the PVN and dampen CRH expression. In case GR expression in the PVN recovers later again, functional glucocorticoid negative feedback on CRH expression could be present again. A shift from AVP-responsible ACTH release to CRH-responsible ACTH release might occur.

## 4.9 Rat prenatal HPA axis

The amount of data for fetal HPA axis in the rat is very comprehensive. For a better overview, we will divide the factors. The first part will show fetal adrenal and plasma corticosterone, maternal plasma corticosterone, placental 11$\beta$HSD2 expression and plasma ACTH levels. The second part will focus on the involvement of pituitary and hypothalamus.

As the fetal adrenal HPA axis is only sufficiently mature to express the investigated factors from 55% of gestation on, we will merely start with displaying data from the second half of gestation.

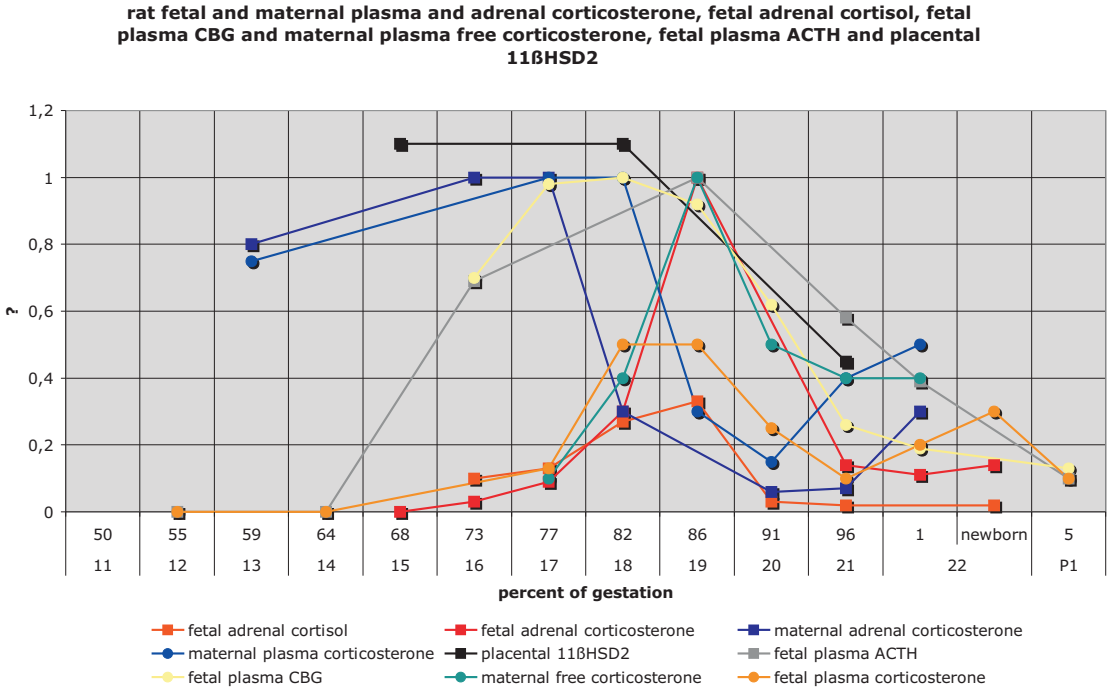


Figure 4.9. Rat prenatal HPA axis I

At 68-82% of gestation, the high placental 11$\beta$HSD2 expression, as suggested earlier, could protect the fetus from the high maternal plasma corticosterone levels. This could explain the very low fetal plasma corticosterone concentration, until fetal adrenal glucocorticoid synthesis starts at 61-73% of gestation. Fetal adrenal corticosterone synthesis is detectable from 61-73% and low fetal adrenal cortisol content is first presented at 73% of gestation. Analog to the guinea pig, we would expect a decrease in placental P-gp expression directly at the start of fetal adrenal glucocorticoid surge. The investigated time point at 68% of gestation might have been already too late in gestation to detect a decrease of P-gp in the rat placenta. At 73% of gestation, fetal adrenal cortisol synthesis is higher than corticosterone production. Between 73-77% of gestation, the fetal plasma corticosterone concentration, as well as adrenal cortisol and corticosterone contents, increase slowly, but fetal free plasma corticosterone concentration increases strongly to maximal levels. At 77% of gestation, the maternal free corticosterone concentration is less than half as high as the one in the fetus, beside a relatively high maternal plasma corticosterone concentration. High 11$\beta$HSD2 expression seems to sufficiently protect the fetal glucocorticoid production. At 82% of gestation, the fetal adrenal cortisol synthesis reaches high levels and fetal adrenal corticosterone production is still moderately low. The fetal plasma corticosterone concentration has gotten to high levels

around 82% of gestation and is similarly high as the maternal plasma levels, together with comparable concentrations of free plasma corticosterone in fetus and mother. Still placental 11$\beta$HSD2 expression remains very high. An interesting correlation between androgen and glucocorticoids occurs in the rat. The placenta is in the second half of gestation the main source of androgen synthesis and secrets androgens in the maternal circulation for the subsequent aromatization of androgens to estrogens in the dam's ovary. Placental androgen synthesis peaks at 82% and is assumed to be pivotal for the following dramatic increase in maternal plasma DHEA concentration between 82-86% of gestation. Inversely proportional to the maternal plasma DHEA concentration, the corticosterone concentration in maternal circulation dramatically decreases over that period. This decrease is assumed to dis-inhibit the corticosterone synthesis in the fetal adrenal and as a result, the fetal corticosterone production increases to peak values between 82-86% of gestation. By 86% of gestation, fetal corticosterone and cortisol syntheses are maximal, together with the fetal plasma ACTH and corticosterone concentrations at most. Fetal total and free plasma corticosterone concentrations are 3-4 times higher compared to the levels in maternal circulation. During this fetal glucocorticoid surge, GR expression in the pituitary is moderately high. By 91% of gestation, fetal adrenal cortisol content has decreased to very low levels. The maternal adrenal corticosterone synthesis and maternal plasma corticosterone concentration are still lower than in the fetus, but free corticosterone concentrations in the circulation of both mother and fetus are comparable. In parallel with decreasing placental androgen secretion, maternal plasma DHEA levels have dramatically decreased by 96% of gestation, accompanied by increasing maternal plasma corticosterone concentration. The latter could have inhibited the fetal corticosterone production, which reached low levels by 96% of gestation. Still, the fetal adrenal corticosterone synthesis is two times higher than the very low maternal production. Maternal and fetal plasma corticosterone concentrations are similar at 96% of gestation. The plasma ACTH concentration decreases to moderate levels. At term, fetal glucocorticoid synthesis is very low and maternal corticosterone synthesis is more than twice higher compared to the fetus. The plasma ACTH concentration has also reached low levels. The fetal plasma corticosterone concentration has slightly increased with increasing maternal plasma corticosterone levels and assumingly low 11$\beta$HSD2 expression. During parturition, fetal adrenal and plasma corticosterone levels are not elevated. The neonatal plasma corticosterone concentration transiently increases at 5 hours after birth, subsequently decreases strongly in the first two days after birth. By 5% of weaning, fetal total and free plasma corticosterone as well as plasma ACTH concentrations are very low.

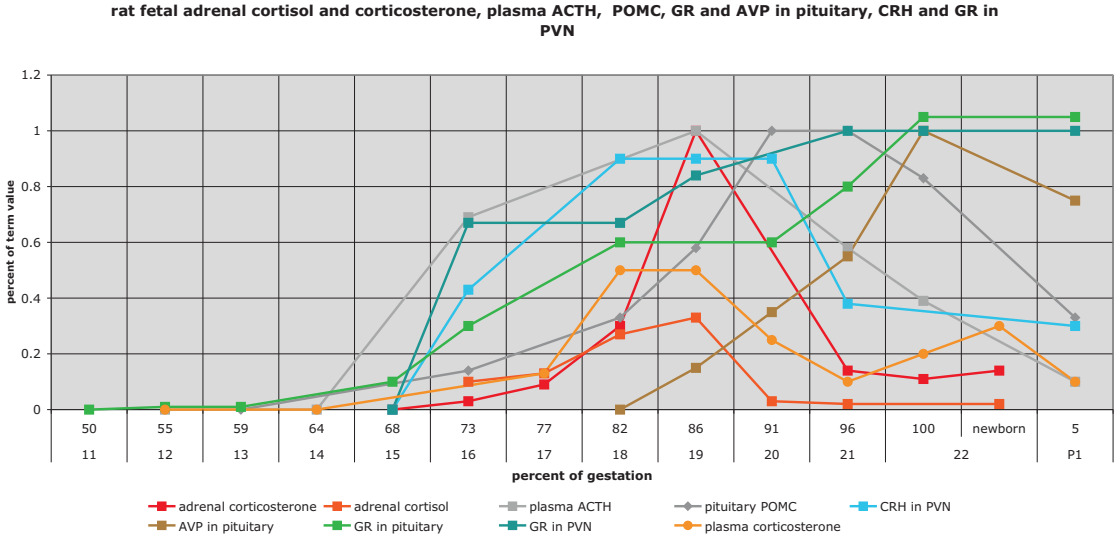


Figure 4.10. Rat prenatal HPA axis II

In the following, we will mainly look at glucocorticoid negative feedback on pituitary and PVN. At 73% of gestation, the fetal plasma corticosterone concentration is very low. A high plasma ACTH concentration appears already at 73% of gestation, while POMC expression in the pituitary is still low. Pituitary AVP content is absent, but the CRH expression in PVN reaches already moderately low levels, assuming exclusive control of CRH over pituitary ACTH/POMC synthesis at this time. GR expression in the pituitary is moderately low. In the PVN, GR expression has reached already high levels. As a result, glucocorticoid negative feedback on CRH expression in the PVN would be possible at 73% of gestation, in case the low fetal plasma corticosterone levels are able to elicit negative feedback. Inhibition on pituitary POMC and ACTH concentrations due to GR expression in this location assumingly increases between 59-68% and reaches moderately low levels by 73% of gestation. While POMC levels in the pituitary remain low assumingly due to this feedback inhibition, high plasma ACTH levels are present. The fetal adrenal corticosterone and cortisol syntheses are still very low at this point.

At 82% of gestation, the fetal plasma corticosterone concentration is at most. The CRH expression in the PVN has reached maximal levels and GR expression in the PVN has remained constantly high, while GR expression in the pituitary has reached moderate levels. POMC expression in the pituitary is still moderately low during that time, beside a very high plasma ACTH concentration. Until 86% of gestation, the fetal plasma ACTH concentration has reached maximal levels together with adrenal corticosterone and cortisol synthesis at most. CRH expression remains maximal and AVP appears in the PVN and reaches moderately low levels at least in the pituitary. AVP level in the pituitary reflects rather the content of the posterior pituitary than the interior pituitary important here, and these data are of limited significance. During this surge in fetal glucocorticoid synthesis, beside assumingly strong glucocorticoid negative feedback in the PVN and at least moderate negative feedback in the pituitary, CRH expression is at most and the plasma ACTH concentration as well as POMC expression is maximal respectively moderately high. This arises the question why the functional negative feedback in both locations fails to prevent this peak in adrenal glucocorticoid production. A possibility answer could lay in the maximal CBG concentration in fetal plasma at 86% of gestation and an expected low fetal free plasma corticosterone concentration during that time. By 91% of gestation, fetal adrenal cortisol and corticosterone have decreased to

very low respectively moderate levels and the fetal plasma corticosterone concentration decreases. The GR expression in the pituitary remains moderately high and POMC expression is maximal. The plasma ACTH concentration decreases from 91% of gestation on. At 96% of gestation, GR expression in the PVN is very high and the CRH expression decreases. At this point, fetal plasma CBG has reached low levels. At least in the pituitary, AVP values have reached moderate levels. POMC expression is still maximal. The still increasing, strong GR expression in the pituitary assumingly causes the decreasing plasma ACTH levels. The very high GR expression in the PVN elicits feedback inhibition on CRH expression, maybe due to again higher fetal free plasma levels caused by the decreasing CBG concentration. Fetal corticosterone synthesis now has also decreased to low levels together with a low fetal plasma corticosterone concentration. By 100% of gestation, fetal plasma corticosterone concentration has increased slightly assumingly as a consequence of the increment in maternal plasma corticosterone levels. Fetal glucocorticoid synthesis is very low at this point. The plasma ACTH concentration and the CRH expression in PVN are relatively low, most likely due to maximal feedback caused by the very high pituitary and PVN GR expression. AVP content in the pituitary has reached its maximum and might reach also the anterior pituitary through connecting vessel between the two lobes.

## 4.10 Rat postnatal HPA axis

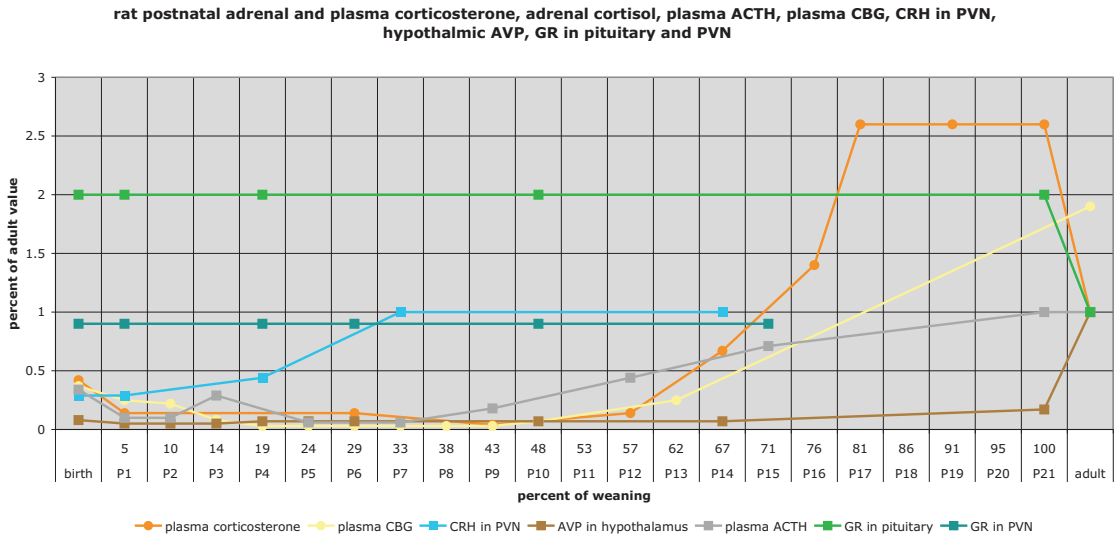


Figure 4.11. Rat postnatal HPA axis

In the newborn rat, the plasma corticosterone concentration has increased further by 5 hours of life, but by 5% of weaning, it has decreased again. This short neonatal increment in the plasma corticosterone concentration could have increased negative feedback even further, during a time were GR expression in both PVN and pituitary remain high. This assumption is supported by low POMC expression and plasma ACTH concentration as well as low CRH expression directly after birth. As AVP levels in the whole hypothalamus are very low, low AVP content in the here important parvocellular PVN could be assumed. Pituitary GR expression is strong and constant from birth until 48% of weaning. The plasma ACTH concentration shows a small peak between 10-24% of weaning, but due to the scarce data during that period, it is unknown whether the plasma corticosterone concentration follows this development. Over the period of increasing plasma

ACTH levels between 5-14% of weaning, CRH increases in parallel and might be responsible for the stimulation of ACTH release, as AVP levels remain low. The plasma CBG concentration is already low at birth, but decreases further to very low levels between 5-19% and remains constant until 43-48% of weaning. During this decrement, plasma corticosterone concentration seems to remain constant, which would result in increasing free plasma corticosterone levels. The later might elicit a strong negative feedback on the pituitary, and as a result plasma ACTH levels decrease between 14-24% of weaning. GR expression in the PVN can be assumed to remain constant from birth until at least 71% of weaning. CRH expression in the PVN remains low from birth to 5% then slowly increases until 19% of weaning. Subsequently it shows a stronger increment by 33% to remain constant until at least 67% of weaning. The strong increment of CRH expression during decreasing plasma ACTH levels indicates functional negative feedback in the pituitary but makes the function of negative feedback questionable in the PVN. The plasma concentrations of ACTH and corticosterone display extremely low levels at 24-33%, respectively low but constant level roughly between 5-57% of weaning. AVP expression in the hypothalamus is very low from birth to 67-100% of weaning but increases strongly in adults. Whether this reflects the events in the very specific parvocellular portion of the PVN is unknown. The plasma ACTH concentration gradually increases from 43% to adult-like levels at 95% of weaning. The plasma corticosterone concentration increases strongly from 57% to very high levels by 81%, remains high until at least 100% of weaning, but has decreased to moderate levels in adults. The plasma CBG concentration increases slowly between 48-62% of weaning. Until adulthood, plasma CBG levels have dramatically increased. Between 100% of weaning and adulthood, GR expression in the pituitary has strongly decreased, decreasing functional negative feedback on the pituitary in adults. The possibility exists that the increment in the plasma ACTH concentration after 67% until the end of weaning might be caused by CRH rather than AVP, due to the low levels of the latter. In summary, negative feedback on ACTH assumingly remains strong until at least the end of weaning, while negative feedback on CRH expression does not appear to function until assumingly 33% of weaning.

## 4.11 Mouse prenatal HPA axis

As in the rat, the amount of data for the mouse fetal HPA axis initiates the distribution of the information into two parts. The first part is again focusing on fetal and maternal glucocorticoids, their placental transfer by 11$\beta$HSD and P-gp expression, and the secretagogue ACTH. The second part will focus on the regulation of the adrenal by higher brain centers and glucocorticoid negative feedback. We will start with our evaluation at 49% of gestation, as the adrenal cortex of the fetal mouse is not steroidogenic before this time.

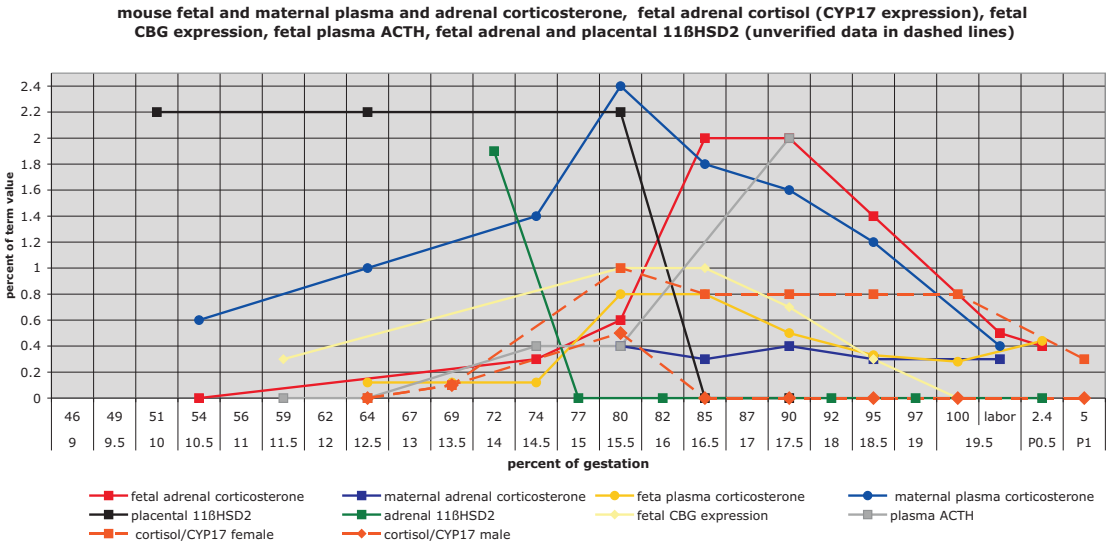


Figure 4.12. Mouse prenatal HPA axis I

Fetal enzyme expression indicates very low corticosterone synthesis by 54-64% and the possible advent of cortisol synthesis at 69% of gestation. By 74% of gestation, adrenal corticosterone synthesis is verified. Already between 54-74%, the maternal plasma corticosterone concentration increases strongly. Regardless, the fetal plasma corticosterone concentration remains constantly low between 64-74% of gestation, most likely due to the high placental 11$\beta$HSD2 expression. POMC expression in the anterior pituitary is first detected at 64% and the plasma ACTH concentration assumingly remains very low at 59-64% of gestation. The very high expression of adrenal 11$\beta$HSD2 at 72% of gestation could inactivate the newly produced fetal glucocorticoids, protecting the fetal organism from too early exposure by glucocorticoids. Adrenal 11$\beta$HSD2 expression has ceased already by 77% of gestation. The latter could imply that inactivation of glucocorticoids in the fetal adrenal is now not needed. The fetal plasma ACTH concentration has increased to moderately low levels around 74-80% of gestation. By 80% of gestation, the fetal and maternal plasma corticosterone concentrations and possibly fetal adrenal cortisol synthesis have reached maximal levels. Maternal adrenal corticosterone synthesis remains constant between 80-100% of gestation but at much lower levels than the fetal production. The maternal plasma concentration is higher compared to fetal plasma levels. The difference between maternal and fetal plasma corticosterone concentrations decreases toward the end of gestation. Fetal CBG expression is at most between 80-85% of gestation. Fetal corticosterone synthesis only peaks at 85-90% of gestation. Most likely due to the transfer of maternal corticosterone into the fetus, the fetal plasma corticosterone concentration roughly follows corticosterone levels in maternal circulation and reaches its maximum already at 80% of gestation, before fetal corticosterone synthesis is at most. It has to be consid-

ered that placental P-gp (Abcb1b) expression and ABCB1 protein levels have decreased by 80% of gestation at a time when placental 11$\beta$HSD2 expression is still high. The decrease in placental P-pg expression might be rather responsible for the placental transfer of maternal corticosterone into the fetus at the advent of increasing fetal corticosterone synthesis. The fetal plasma ACTH concentration has strongly increased at least by 92% of gestation. Between 80-90% of gestation, the placental 11$\beta$HSD2 expression suddenly ceases and the 11$\beta$HSD1 expression is strong, at which point the placenta switches from mainly glucocorticoid inactivation to glucocorticoid activation. Fetal adrenal cortisol synthesis might disappear in the male fetus between 80-90% and decrease in the female fetus after 80% of gestation. Fetal CBG expression decreases in parallel with the fetal and maternal plasma corticosterone concentrations after 85% of gestation. We expect the fetal plasma ACTH concentration to decrease in parallel with decreasing fetal plasma corticosterone levels. At birth, the fetal and maternal plasma corticosterone concentrations are nearly equally low. Fetal adrenal corticosterone synthesis and maybe female cortisol synthesis have reached low levels. At this point, fetal adrenal corticosterone synthesis is still roughly twice higher compared to maternal production. Fetal CBG expression is undetectable at 100% of gestation and high free glucocorticoid levels in circulation might be possible. The neonatal plasma corticosterone concentration shows (at least in premature animals) a small increase between term and the first 6 hours after birth, but has decreased dramatically in parallel with the plasma ACTH concentration by 5% of weaning.

Focusing now on factors from higher brain areas, which influence fetal glucocorticoid synthesis, as well as on glucocorticoid negative feedback, the following interactions occur:

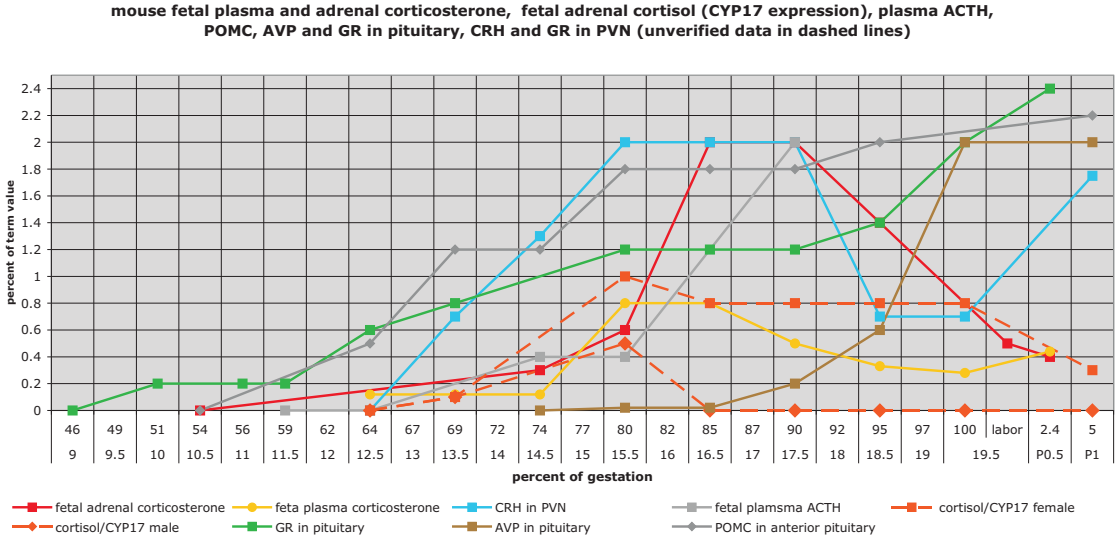


Figure 4.13. Mouse prenatal HPA axis II

The POMC expression in the pituitary only has sufficiently increased by 69% while the fetal plasma ACTH concentration seems to remain very low at 59-64% of gestation. GR expression in the pituitary is detected already at 51% but remains very low until 59% of gestation. The pituitaric GR expression has increased by 64% of gestation. This could explain the low plasma ACTH concentration at 64% of gestation due to glucocorticoid negative feedback, in case the still low fetal plasma corticosterone concentration, more precisely its free fraction, is effectual. POMC expression strongly increases until a plateau is reached at 69-74%, while the plasma ACTH concentration has increased to low levels between 74-80% of gestation. At 80% of gestation, GR expression

in the pituitary has reached moderately high levels at a time when fetal plasma corticosterone concentration is at its most. Appropriate glucocorticoid negative feedback on the pituitary would be expected. GR expression in the hypothalamus is apparent at 64% of gestation, but no further information is available. The CRH expression in the PVN increases dramatically between 64-80% and remains maximal until 90% of gestation. At this point, glucocorticoid negative feedback on CRH expression in the PVN has been verified. AVP protein is only present in the PVN by 85% of gestation, and AVP content remains in the whole pituitary very low between 74-85% and only slowly increases after 90% of gestation. On the contrary, POMC expression reaches very high levels already at 80% and remains constant until 90% of gestation, similar to CRH expression. The plasma ACTH concentration has strongly increased by 90% of gestation. It can be assumed that this is caused in response to the CRH rather than AVP. Between 80-85% of gestation, the fetal corticosterone concentration in the circulation and in the adrenal, as well as probably adrenal cortisol synthesis, are at most and subsequently decrease in parallel to low levels at birth. From 90% of gestation on, CRH expression strongly decreases to moderately low levels at 95% of gestation, maybe due to glucocorticoid negative feedback. An increase in fetal glucocorticoid negative feedback supposedly takes place between 95-100% of gestation due to strongly decreasing fetal plasma CBG during nearly constant corticosterone concentrations and the assumed increase in the free corticosterone fraction. AVP expression at least in the whole pituitary increases exponentially between 95% of gestation and birth to very high levels, which might reach also the anterior pituitary. From 90% of gestation until after birth, POMC expression slightly further increases, despite the strong increment in the pituitary GR expression. No information about the plasma ACTH concentration at this point is available, but levels might decrease in parallel with fetal corticosterone, due to strong negative feedback. The strong increment in POMC expression might have been caused by AVP, as CRH expression is reduced at birth. POMC is not only the precursor for ACTH, but also for other hormones, which could explain the assumed diverging development in plasma ACTH concentration and POMC expression. The low fetal plasma corticosterone concentration at term is accompanied by a moderately low CRH expression.

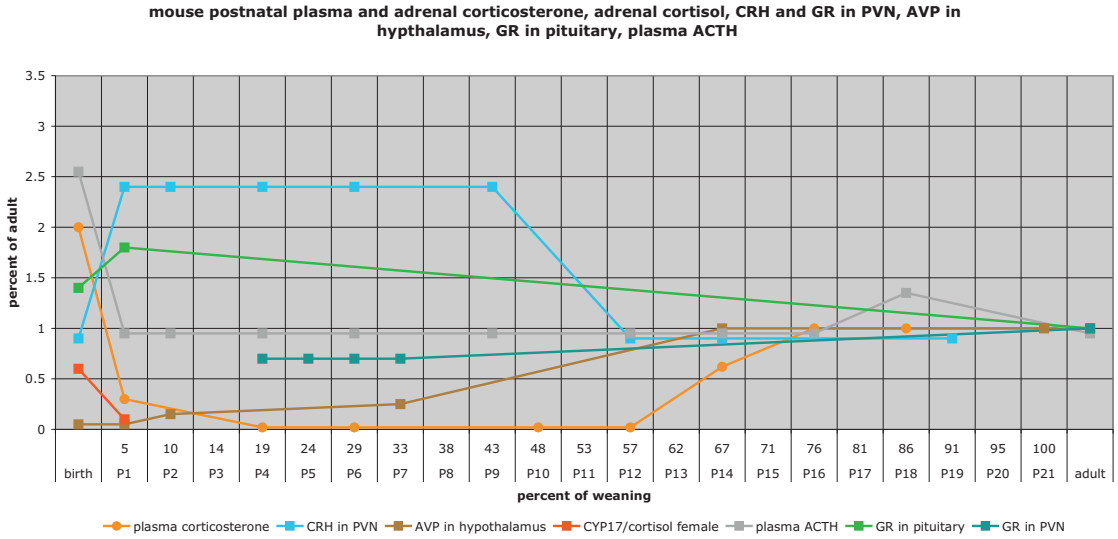


Figure 4.14. Mouse postnatal postnatal HPA axis

The following development in the neonatal mouse HPA axis occurs. Adrenal corticosterone content is low at birth and has decreased further by 2.5% of weaning. Neonatal plasma corticosterone concentration might peak marginally at 12 hours after birth but has reached very low levels by 19% of weaning. At 5% of weaning, a possible adrenal cortisol synthesis in the female neonate could have reached low levels, as no cortisol was detectable in the circulation of newborns of both genders. The neonatal plasma ACTH concentration seems to decrease after birth, maybe in consequence of negative feedback. The latter could be caused by the corticosterone peak at 12 hours after birth and a high fraction of free corticosterone due to very low hepatic CBG expression from term to 33% of weaning. The already strong GR expression in the pituitary at birth has slightly further increased by 5% of weaning, and could have induced the assumed decrease in plasma ACTH concentration. CRH expression in the PVN increases strongly from term to 5% but no information about GR expression in the PVN is available before 19% of weaning. Hypothalamic AVP levels have to be regarded with care as the content in the whole hypothalamus most likely mask the levels in the here important parvocellular PVN. Between term and 5% of weaning, hypothalamic AVP levels remain very high in comparison to the AVP content in the fetus. Still compared to levels in adulthood, the hypothalamic AVP content is very low at 5% of weaning, while the expression of CRH is high in relation to fetal and adult values. The plasma corticosterone concentration remains very low roughly between 19-57% and increases to adult-like levels around 76% of weaning. The plasma ACTH concentration stays constant between 5-76% but has increased by 86% of weaning. CRH expression remains at very high levels between 5-43%, subsequently decreases to assumingly adult values by 57% of weaning. GR expression in the PVN is constant between 19-33% of weaning. Hypothalamic AVP content stays very low until 33% but has strongly increased to possibly adult levels by 67% of weaning. This roughly mirrors the development of fetal plasma corticosterone levels. It could be possible that AVP rather than CRH controls ACTH synthesis from birth to at least 57% of gestation. The latter assumption occurs, as CRH expression in the PVN increases at 5% of weaning when plasma corticosterone decreases, remains high during very low corticosterone levels and decreases again around the time of increasing corticosterone concentration. This development

of CRH expression could be the result of immature negative feedback on the PVN. After 57% of weaning, CRH and corticosterone development converge and functional feedback on CRH expression could be restored.

# CHAPTER 5

## Summary and general discussion

### 5.1 Summary

In the previous chapters, we analyzed data about the species-specific development of the HPA axis. Our comparative analysis focused on the influence of the placenta and mother, and resulted in a comprehensive documentation. It has been investigated, at what time each single factor of the HPA axis first appears, and how the factors develop and interact during gestation and early life in commonly used research animals and humans. These questions are of great importance, since these species are routinely used as models for the developing human pre- and postnatal stress system in a broad number of research areas. Early (pre- and postnatal) adverse experience is capable of affecting components of the stress system and can determine stress vulnerability and stress-related disorders later in life. The ability to compare directly time points or periods of pre- and postnatal HPA development between the commonly used animal models and humans allows to determine more precisely, which animal model at which time during development may best represent the human situation.

In *Chapter 2*, an overview of placental, as well as fetal hypothalamic and pituitaric factors regulating steroid synthesis in the adrenal cortex, is presented. Especially placental 11$\beta$HSD, as a key player in the exposure of the fetal organism to maternal glucocorticoids, showed distinct species-specific differences, with regard to the presence of its two isoforms at the side of feto-maternal exchange and their expression over time. While syncytioblast cells of humans and guinea pigs only expressed the glucocorticoid inactivating isoform 11$\beta$HSD2, both isoforms were expressed in baboons, sheep, rats and mice, with the specialty in the sheep that 11$\beta$HSD1 had mainly 11$\beta$HSD2 activity. Humans, sheep, guinea pigs, rats and mice all showed a late gestational decrease in the ability of the placenta to inactivate glucocorticoid transfer. This inability was amplified in rats and mice by the increasing expression of glucocorticoid activating 11$\beta$HSD1. Assumingly, an increment in placental transfer was assisted by a decreasing expression of placental P-gp in humans, guinea pigs and mice in the second half of gestation. At the end of gestation, this exposure of the fetus to high amounts of maternal or fetal glucocorticoids is even sufficient enough that the fetus can supply the mother. Profound species-specific discrepancies in the effects of placental factors on fetal and maternal HPA axis regulation could be revealed. Placental CRH, a stimulator of maternal and fetal pituitary ACTH secretion, and a supporter of delivery at least in humans, was verified in rhesus monkeys and was assumed in baboons, sheep, rats and possibly guinea pigs. CRH-BP, the binding protein for CRH in circulation, was present in human and rhesus monkey plasma, but not in the blood system of baboons and sheep. In humans, the decrease of the latter at the end of gestation increased the fraction of free CRH and was assumed to trigger parturition. The influence of CRH on delivery suggests strong species-specific differences in the regulation of birth.

Hypothalamic CRH and AVP both stimulate pituitary ACTH release. AVP synthesis, other than CRH expression, seems to be less sensitive to glucocorticoid negative feedback and might counteract the inhibited CRH expression. A concurrent appearance of hypothalamic CRH as well as AVP expression could be observed in the human and sheep fetus. First CRH and (apart from the guinea pig) AVP expression appeared in fetal human, sheep and guinea pig approximately after one trimester, and in rat and mouse, after two trimesters. While in the fetal sheep, CRH expression increased towards term, in rat, mouse and guinea pig, expression decreased toward the end of gestation. After birth, the CRH expression in the hypothalamus suggests immaturity during the SHRP of rat and mouse and, possibly, in the postnatal sheep and guinea pig. A late gestational increase in AVP content might be present in guinea pig, rat and mouse, but the data are of limited informative value as the hormone was measured in the whole pituitary. In the sheep,

parvocellular AVP in the PVN did not change during gestation, but increased very strongly in the newborn lamb. A species-specific interplay of CRH and AVP in the release of pituitary ACTH was revealed. Functional glucocorticoid negative feedback on CRH synthesis, due to hypothalamic GR expression, was suspected in sheep, mouse and rat immediately from the beginning of hypothalamic CRH expression. GR expression in the hypothalamic PVN decreased in sheep and guinea pig toward term, and increased again after birth in the sheep, in congruence with increasing CRH expression in the sheep in late gestation and decreasing expression after birth. The rat exhibited a strong preterm increment in hypothalamic GR expression. The expected strong negative feedback was verified by decreasing CRH expression before birth. Pituitary expression of ACTH and its precursor POMC was first verified in humans, rhesus monkeys and sheep after 23-30% of gestation, and, again, only after approximately 2/3 of gestation in rat and mouse. POMC expression appeared earlier in the pituitary anterior lobe than in the intermediate lobe in both rat and mouse. A divergent expression pattern was presented during gestation in the two pituitaric lobes of the guinea pig. Functional glucocorticoid negative feedback, by the appearance of pituitary GR, was verifiable right from the advent of pituitary ACTH expression in humans, rats, mice, and most likely in sheep. The plasma ACTH concentrations increased in rhesus monkeys toward term and in sheep during labor, but decreased in the rat and possibly in humans toward the end of gestation. During labor, the increase of plasma ACTH in the sheep is congruent with the sudden dramatic decrease in pituitary GR expression. After birth, the plasma ACTH concentrations strongly decreased in humans, rats and mice over the first 10% of weaning, and high pituitary GR expression (indicating strong negative feedback on pituitary ACTH synthesis) was detectable in rats and mice.

In *Chapter 3*, we focused on steroidogenesis in the fetal and postnatal adrenal cortex, with the emphasis on glucocorticoid synthesis and its relation to maternal regulation. We first addressed the question of the degree of maturity of the stress system at birth. Typical for nidicolous (remaining in the nest after birth) species, rats and mice exhibited the highest dependency on their mother after delivery. A less mature/more programmable stress system was expected in these species. Sheep and guinea pigs were on the other end of the spectrum of independency, which was thought to require a less programmable but rather mature stress system from birth onwards. Humans, rhesus monkeys and baboons exhibit a position between the two other groups regarding independency and are expected to possess some degree of maturity in their stress response. With respect to our results in *Chapter 3*, the previous assumptions could be confirmed, as neonatal cortisol/corticosterone synthesis remained sufficient at least until 24%, respectively 8% of weaning in guinea pigs and sheep. In humans, as well as in rats and mice, the plasma cortisol/corticosterone concentrations strongly decreased directly after birth and only increased again around 50% of weaning and then further towards adulthood. In rhesus monkeys, plasma cortisol concentrations decreased only moderately after birth but were still elevated compared to adults. The neonatal baboon showed a strong peak in the plasma cortisol concentration after birth, and then undulating lower but adult-like levels until at least 30% of weaning. Aware of the partially very incomplete data, we conclude that rats and mice show the expected reduced glucocorticoid synthesis for stress hypo-responsivity after birth. The ability of the human to synthesize cortisol seems to be lower in comparison to the rhesus monkey and the baboon, with the sheep and the guinea pig exhibiting sufficient neonatal cortisol synthesis to respond to stress. Prenatal, it was shown that the human fetus started to synthesize cortisol already at 21-25% of gestation. Our data suggested the presence of glucocorticoid synthesis in rhesus monkeys, baboons, and sheep at least at 30-35%, in guinea pigs not later than 37-51%, in the rat fetus between 61-73%, and in the fetal mouse between 54-69% of gestation. In both humans and sheep, data verified a transient early period of sufficient cortisol synthesis. In humans, this period encompassed the time between 21-40% and in the fetal sheep between 33-63% of gestation. The data suggested the existence of transiently stronger cortisol synthesis in rhesus monkeys, baboons and guinea pigs as well. Evidence was presented that a period of low or absent fetal cortisol synthesis followed the early peak in humans and sheep. A period of low or absent cortisol synthesis was verified additionally in fetal rhesus monkeys and baboons. The presented data suggested the existence of transiently low or absent cortisol synthesis in the fetal guinea pig as well. In fetal rats and mice, glucocorticoid synthesis started only in the second half of

gestation, increased to peak values by 80-86% of gestation, and reached low levels again at birth. Transiently low glucocorticoid synthesis encompassed the SHRP after birth, and, at least in the rat, there might be a second peak in corticosterone synthesis around PND24. In the other species, fetal cortisol synthesis dramatically increased at the end of gestation. This surge took place roughly over the last 10% of gestation and was observed in the baboon between 97-100% and in the guinea pig as late as 99-100% of gestation. Androgen synthesis in the fetal adrenals was exhibited even earlier than cortisol synthesis in humans and guinea pigs, and a similar development was proposed in the rhesus monkey. During sexual differentiation, fetal glucocorticoid synthesis inhibited fetal androgen synthesis to prevent virilization of the female fetus. Glucorticoid and androgen synthesis showed an inversely proportional development in most of the species and strong regulatory interactions were assumed between both groups of adrenal hormones. Possible explanations for the presented findings will be considered in the following discussion.

## 5.2 General discussion

This closing section illustrates the findings in a broad relation to the existing knowledge of fetal programming, discusses the strength and weaknesses of the chosen methods, and provides an outlook for further research questions and for designing studies in this field.

### 5.2.1 Findings in the context of fetal programming

#### 5.2.1.1 a) concerning fetal glucocorticoid synthesis

In *Chapter 3* of this review, we presented the development of fetal glucocorticoid synthesis in different species and the human over time.

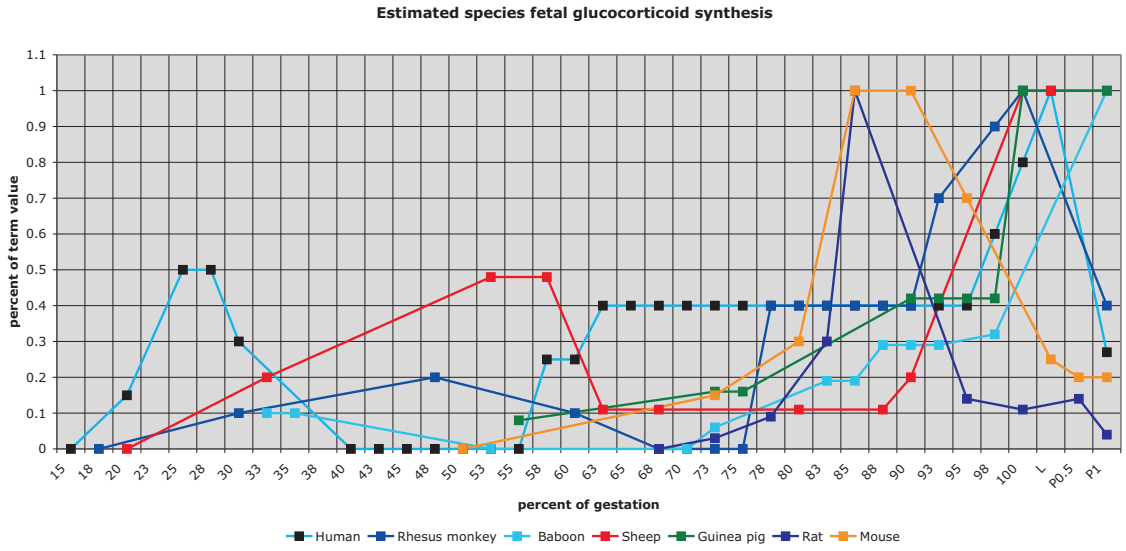


Figure 5.1. Estimated species fetal glucocorticoid synthesis

In the seventies, Levine and his colleagues discovered a period of reduced stress reactivity in the postnatal rat [248]. We were able to reveal a transient period of low or absent glucocorticoid synthesis already before birth in the developing human, rhesus monkey and baboon, as well as in the sheep and assumingly in the guinea pig. In the postnatal rat and mouse, this period was present until approximately 50% of weaning. A phase of low exposure to glucocorticoids present in the fetus was verified by the detection of high placental 11$\beta$HSD2 expression (inhibiting placental cortisol transfer from the mother into the fetus) and/or relatively low fetal respectively maternal plasma glucocorticoid concentrations in most of these species. In this stress hyporesponsive period, the developing organism is most vulnerable to high levels of glucocorticoids and there seems to be a common attempt of the fetus and the mother to avoid the latter. A transient negligibility of glucocorticoids in fetus or neonate suggests maturational processes inside the brain, vulnerable to the inhibiting effects of glucocorticoids [109, 280].

Glucocorticoids bind to their intracellular receptors and the complexes translocate into the nucleus. Here the glucocorticoid-receptor complex operates as a transcription factor by binding to the glucocorticoid responsive elements (GRE), and promoting the activation or inactivation of gene expression. In general, glucocorticoids are assumed to induce cell differentiation but diminish tissue growth, by on one hand activating gene expression involved in the differentiated phenotype

and on the other hand by inhibiting cell division directly. Stress and glucocorticoids can inhibit the growth of tissue in different organs. Inside the brain, it was shown that maternal glucocorticoid administration delayed neuronal myelination in the fetal sheep. Additionally, the birth of neurons was suppressed and deleterious effects like degeneration and depletion of neurons were detected in the offspring of rhesus monkeys. In the human cortex, gyrification, the folding of brain surface, was decreased and in the neurons of rats, glucocorticoids prevented the maturation of synaptic function. [44, 127, 136, 240, 250, 307, 323, 393, 402, 469].

Remarkably, in rhesus monkey, sheep and guinea pig, the period of lowness/absence of fetal cortisol synthesis coincides approximately with the estimated brain growth spurt (transient period of rapid growth) in each species [128].

TABLE 5.1

Growth spurt in species

| In percent of gestation | Rhesus monkey | Sheep | Guinea pig |
|---|---|---|---|
| Low glucocorticoid synthesis | 67-76% | 63-87% | 58-81% |
| Brain growth spurt | 58-81% | 50-79% | 63-85% |

Programming of the brain strongly depends on the timing of maximal brain growth. With the brain growth spurt occurring during the fetal stress hyporesponsive period, where the fetal adrenal cortex glucocorticoid synthesis is negligible, we can assume very vulnerable maturational processes inside the CNS during this period of accelerated brain growth.

The start of the stress hyporesponsive period coincides closely in humans, guinea pigs, rats and mice (and at least broadly in rhesus monkeys) with the end of the neurogenesis in the visual cortex and with the beginning of neural development in the somatosensory cortex [91]. The somatosensory cortex is located in the postcentral gyrus of the cerebral cortex, and processes sensory experiences involved in touch, which also include the sensitivity to pain and the proprioception of the body in space [22]. According to Cascio 2010, somatosensory input is critical for the development of social and communication skills, e.g. for the establishment of bonding and secure attachment of the offspring to its mother. The tactile perception is the first sense to develop in uteri. Somatosensory processing is associated with the recall of emotions and is explored in connection with diseases such as ADHD, autism or cerebral palsy. Cerebral palsy describes a group of disorders with affected motor maturation due to adverse experience during pre- and perinatal development [79].

By examining programming studies, regarding the maximal vulnerability of the developing brain during the stress hyporesponsive period to early adverse experience, such as maternal synthetic glucocorticoid administration, a series of interesting results occur.

Our data suggest a very low corticosterone synthesis in the rat after birth till roughly 57% of weaning. The well-known epigenetic experiments by Meaneys group from McGill University discovered the impact of reduced maternal care (in form of tactile stimuli) on hippocampal GR expression, resulting in increased HPA axis activity with anxiety and learning disabilities in the offspring. More precisely, offspring of low licking and grooming mothers were only prone to this programming during a very short time, during the first (till 33% of weaning), but not during the second week of life (till 67% of weaning) [487]. The former time period coincides with the period of low neonatal glucocorticoid synthesis and neurogenesis in the somatosensory cortex, the latter with increasing neonatal glucocorticoid production. In other words, fetal programming by tactile stimuli during the neurogenesis of the somatosensory cortex in rats only seems possible in the face of low fetal glucocorticoid exposure.

In humans, it was shown that an enhancement of the maternal cortisol concentration at 38% and 49% of gestation (period of low/absent fetal cortisol synthesis: 40-55% of gestation) significantly decreased the physical and neuromuscular maturation of newborns [145]. The motor deficits in cerebral palsy were not only associated with differences in somatosensory perception but were caused by insult or injury during pre- and perinatal development [79]. Again, the requirement of low fetal cortisol exposure can be expected during the maturation of the somatosensory cortex, in order to prevent deleterious programming effects on the brain.

Programming of the HPA axis has been shown to involve hippocampal GR expression, an area responsible for restraining HPA activity [294]. In sheep and guinea pigs, synthetic glucocorticoid exposure of the fetus, during the discovered species-specific period of low or absent fetal cortisol synthesis, altered MR/GR expression in hippocampus [122, 255, 335, 442]. In the mouse, hippocampal MR expression is affected by synthetic glucocorticoids at the advent of fetal glucocorticoid synthesis, but not during high fetal glucocorticoid synthesis. On the other hand, synthetic glucocorticoid administration to the human mother between 60-83% of gestation failed to show an effect on fetal hippocampal GR/MR expression [329], possibly because the exposure was outside the vulnerable period of low or absent fetal cortisol synthesis between 40-55% of gestation.

Synthetic glucocorticoid administration during the vulnerable period can be assumed to affect not only the hippocampus GR/MR expression, but also other factors of the developing LHPA axis. In guinea pigs, it was shown that synthetic glucocorticoid exposure in the period of 59-90% of gestation (period of low/absent fetal cortisol synthesis: 58-81% of gestation) inhibited the fetal HPA axis function at the level of hypothalamic CRH and plasma cortisol [285]. In a follow-up study, the same research group by Matthews from the University of Toronto could demonstrate that fetal exposure to adversity, induced during this vulnerable period of gestation, programmed the HPA axis in a long-lasting and gender-dependent manner, resulting in reduced basal and activated plasma cortisol levels (hypocortisolism) in the adult male offspring [255]. Hypocortisolism is associated with a long list of stress-related diseases such as the earlier mentioned fibromyalgia, as well as chronic fatigue or PTSD [89, 186]. Uno et al. 1994 verified changes in volumetric measures of the cerebellum and irreversible deficiencies in hippocampal neurons of the rhesus monkey by maternal dexamethasone treatment at 82% of gestation, which is past the period of low/absent fetal cortisol synthesis between 67-76% of gestation [469].

We expect specific effects of glucocorticoid exposure on fetal brain programming according to the timing of the impact, in relation to the maturation of different brain areas, and in relation to low or high fetal glucocorticoid synthesis. One in eight pregnancies in the US ends in a preterm delivery [367] and NIH supports the use of antenatal corticosteroids between 60-85% of gestation for pregnant women at risk of preterm birth [327]. This period covers exactly the cerebellar neurogenesis in the human fetus [34]. By 63% of gestation, cortisol synthesis in the human fetus just recovers from the period of absence, which we predicted as especially vulnerable for maturational processes. The concept of a period of physiologically low/absent glucocorticoid exposure of the fetus needs further investigation, and could question the synthetic glucocorticoid administration close to the vulnerable period, and the assumed highly vulnerable maturational processes.

#### 5.2.1.2 b) concerning glucocorticoid negative feedback

Maternal adversity (undernourishment, stress) can lead to a low birth weight/preterm delivery, with the risk of metabolic disorders in adulthood. In this respect, increased fetal glucocorticoid exposure can reduce fetal growth, trigger placental CRH-induced labor, increase fetal HPA activity (hypercortisolism), and change gene expression in favor of increased energy production and enhanced cardiovascular reactivity. The exposure of the fetus to maternal glucocorticoids depends on the placental 11$\beta$HSD2 activity. Hypercortisolism can be inflicted by decreased hippocampal GR expression, which then fails to negative feedback on CRH expression in the hypothalamus and subsequently results in increased pituitary ACTH and adrenal cortisol release [294, 520].

The risk for obesity and metabolic syndrome is related to alterations in the POMC gene expression and impaired glucocorticoid feedback. Plagemann et al. 2009 showed that neonatal overfeeding

of rats led to hypermethylation of the gene promoter of the main anti-appetite stimulant POMC, causing a failure to up-regulate POMC expression in the face of high insulin and peptin plasma concentrations. As a result, overfeeding classifies as a risk factor for obesity, diabetes, and cardiovascular diseases in correlation with the metabolic syndrome [368].

Buhl et al. 2010 were able to reverse chronic HPA axis hyperactivity and insulin resistance (risk factor for metabolic syndrome) in low-birth-weight rats by restoring glucocorticoid negative feedback on the level of the hypothalamus and insulin sensitivity through SSRI treatment [66]. As presented in *Chapter 2*, physiologically, glucocorticoids self-regulate theirs own secretion by negative feedback on hippocampus, hypothalamus and pituitary. GR activation in the hippocampus inhibits hypothalamic CRH expression. Glucocorticoid negative feedback through GR in hypothalamus and pituitary dampens CRH expression in the hypothalamic PVN and ACTH synthesis/POMC expression in the anterior pituitary [338, 471]. AVP is less sensitive to negative feedback than CRH, and can be assumed to counteract the inhibition of CRH-stimulated ACTH release [71, 183]. In this review, it was possible to show the strong dependence of glucocorticoid negative feedback on the investigated species and the time of gestation. Functional glucocorticoid negative feedback on hypothalamic CRH expression and pituitary ACTH synthesis (based on local GR expression) could be verified as soon as both sites were mature enough to synthesize these hormones.

The question arises how glucocorticoid negative feedback on CRH and ACTH syntheses has to change in order to allow the dramatic increase of fetal glucocorticoid production late in gestation. The surge in cortisol synthesis of the fetal sheep was accompanied by a very low negative feedback on CRH expression until term, and a decreasing negative feedback on ACTH synthesis during labor. In the rat, fetal glucocorticoid synthesis peaked around 86% of gestation, and subsequently decreased toward the end of gestation. Here the strong increment in fetal glucocorticoid synthesis took place during moderate negative feedback on both hypothalamus and pituitary, while the subsequent decreased corticosterone production toward term correlated with maximal negative feedback in both locations. Data concerning feedback regulation during the surge of fetal glucocorticoid synthesis are less complete in the remaining species. In late gestation, when cortisol synthesis in the guinea pig had increased to moderate levels, negative feedback on CRH synthesis decreased, but instead of the expected increase in CRH expression, the latter had also declined. It is assumed that an increment might have remained undetected due to the chosen sampling times. The plasma ACTH concentration increased in parallel with cortisol levels during that period, while GR expression in the pituitary seems to remain constant. No further information about GR expression at the sides of feedback regulation is available during the subsequent strong increment in fetal cortisol synthesis. The mouse exhibited increasing glucocorticoid negative feedback in the pituitary when the plasma ACTH concentration remained low, and constant GR expression when ACTH strongly increased. The subsequent rise of glucocorticoid negative feedback in the pituitary is assumingly accomplished by decreasing ACTH levels in parallel with the decrement in fetal corticosterone synthesis. A similar development is assumed for negative feedback regulation on CRH expression.

The interaction and interference of the single players in the HPA axis is very complex. During increasing fetal glucocorticoid synthesis in the presented species, either constant or decreasing negative feedback on the PVN and the pituitary are revealed. Already in 2002, Wadhwa et al. conceptualized that the effects of maternal stress on the fetal developmental outcome are modulated by the nature, timing and duration of occurrence of stress during gestation [479]. In this work, we extend this list, and add as modulating factors species-specific differences concerning placental 11$\beta$HSD2 expression and the fetal HPA axis development under the influence of glucocorticoid negative feedback regulation.

This review gives the possibility to see the HPA axis entirely, and that might help to decide which factors of the stress system are worth investigating.

#### 5.2.1.3 c) concerning interaction of androgen and glucocorticoid synthesis

Sexual differentiation during gestation is a very vulnerable period, and seemed to be programmed epigenetically. One of the best-known factors in epigenetics is DNA methylation, where methyl

groups are added to the promoter region, and repress gene expression. During early embryonic development of female mammals, one of the two X chromosomes is permanently inactivated through DNA methylation [9, 343]. Sexual differentiation seems to be programmed in the fetal female spotted hyena by androgen exposure, leading to physiological male-like genitalia and dominance over males. Due to very low placental aromatase activity in this species, the placenta seems to convert androstenedione to testosterone rather than estrogen, resulting in virilization of the female external genitalia [130, 504].

The review of White in 2006 illustrated a similar danger of virilization in the human female fetus by androgen exposure during genital differentiation. Congenital adrenal hyperplasia, a condition going along with virilization, can be caused by a deficit of enzymes needed for cortisol synthesis. As a result, high ACTH levels, due to missing glucocorticoid negative feedback, amplify adrenal androgen synthesis and cause virilization. Synthetic glucocorticoid administration to the mother can prevent virilization [492]. Preceding the earlier discussed period of low/absent fetal production (see *Chapter 3*), our analysis showed a transient period of sufficient fetal cortisol synthesis in human and sheep, and assumable in rhesus monkey, baboon and guinea pig. Androgen synthesis in the fetal adrenal was detected even earlier than cortisol synthesis in human and guinea pig, and a similar situation is proposed in the rhesus monkey. The early boost of human fetal cortisol synthesis around 25% of gestation is thought to negatively feedback on ACTH induced adrenal androgen synthesis and safeguard sexual differentiation (between 25-35% of gestation). An intact glucocorticoid negative feedback on ACTH production was verified during the early burst of human fetal cortisol synthesis (see *Chapter 2*). In most reviewed species, a divergent development of androgens and glucocorticoids was affirmable at some point during gestation in fetus and/or mother, assuming an inner control system, which assures the inhibition of androgen and glucocorticoid syntheses during vulnerable periods. An interesting regulation between androgens and glucocorticoids was revealed in rats, a species seemingly unable to synthesize androgens in the adrenal. During late gestation, placental androgens are aromatized in the maternal ovary. Peaking placental androgen synthesis late in gestation is assumed to increase maternal plasma DHEA level shortly after, which seems to cause the simultaneous sudden dip in the maternal plasma corticosterone concentration. Without inhibition by the maternal corticosterone, the fetal adrenal corticosterone synthesis apparently peaks at this time. Conversely, decreasing placental androgen synthesis directly before birth seems to decline the maternal plasma DHEA concentration, to disinhibit the maternal plasma corticosterone concentration, resulting in a reduction of corticosterone synthesis in fetal adrenals.

### 5.2.2 Strength and weaknesses of the methodical approach

This study reviewed the very comprehensive literature, published on behalf of the selected research animals and the human, concerning physiological development of the fetal and neonatal HPA axis under the influence of the mother and the placenta. In this study, a new method was developed and applied, which permits a direct comparison of a time point or time period (in percent of gestation; percent of weaning) of an event across different species or of multiple events in one species. When looking at a single event in a given species, the amplitude of the event can be expressed as percent of term or adult value. This technique can detect correlations and differences of events or developments from procreation to birth as well as from birth to weaning in and among species. The strength of this method is to present, with regard to time and trends in amplitude, the first appearance and the development of interacting hormones, enzymes and receptors, from fetal/neonatal hypothalamus, pituitary, adrenal, as well as the placenta and the maternal HPA axis, and to combine all relevant information together in a comprehensive and significant picture. This permits conclusions about advent and progress of HPA function, and the discovery of patterns, models, biological circuits, regulations, positive and negative feedback control, inhibiting and activating influences and chain of events concerning the developing stress system. As an example, the required enzymes for adrenal steroid synthesis could be presented in an extensive time- and magnitude-related picture in order to establish the beginning and the periods of presence, absence, and high or low fetal/neonatal steroid synthesis. By comparing the events between the different

animal species and the human, similar patterns across the species were discovered. In addition, missing data became obvious, and similarities and differences among the species allowed the evaluation of the species for different research questions as the most appropriate animal model for the human.

A weakness of this work is that when comparing a single event across species or multiple events in a species, only a trend in an event's amplitude over time can be deduced. Another limitation of this review is the strong dependency on early studies. Much of the basic research concerning the physiology of different HPA axis factors was done in the seventies and eighties. Research methods change and refine over time. The more recent research is focused mostly on altered HPA axis function, influenced by drug administration or other causal factors for adverse pregnancy conditions. Here, data from the unaffected control group were integrated in this review, but these data had to be carefully evaluated and compared with regard to the time during pre- and postnatal development, method of measurement, environment of mother and fetus in the absence of adverse stimuli. Each species had to be investigated strictly individually, by overcoming the temptation of being influenced by the knowledge about phylogenetic close related species (e.g. among rodent species, among primate species), before analogies could be evaluated. Missing or inaccurate data points over time had to be carefully interpreted, adding the risk of incorrect conclusions.

### 5.2.3 Outlook

On the basis of the missing data for individual factors of the HPA axis in different species, we will expose gaps in our knowledge about the development of the fetal/neonatal stress system. Furthermore, an attempt will be made to discuss the most appropriate animal species as a model for the pre- and postnatal human HPA axis, based on different research areas, under the current state of knowledge, economy of research and public concerns, in order to prevent unnecessary future research. Finally, an outlook concerning research in this field on the basis of findings of this review will be provided.

#### 5.2.3.1 a) missing data

By summarizing all investigated factors from *Chapter 2 and Chapter 3*, concerning the fetal and neonatal HPA axis for each species, we are able to form a ranking for completeness among the species and define important gaps for future research.

The most comprehensive research in this field was done on the rat. It would be helpful to know more about the placental 11$\beta$HSD synthesis before 70% and between 95-100%, as well as maternal adrenal corticosterone synthesis before 91% of gestation, to further understand the interaction of fetal and maternal glucocorticoid synthesis. To better understand the interplay of CRH and AVP in ACTH regulation, the investigation of AVP expression specifically in the parvocellular PVN is necessary in all species except the sheep. The mouse HPA axis has also been extensively studied. The problem to detect fetal adrenal cortisol synthesis might be solvable by using a more sensitive assay and a gender-specific time frame, encompassing 64-90% of gestation in the male fetus and from 64% of gestation to 5% of weaning in the female fetus. GR expression in the PVN needs to be further investigated in this species to understand feedback regulations.

The data concerning the sheep's fetal HPA axis in the prenatal period are quite comprehensive as well. In general, the investigation of the fetal sheep HPA axis before 50% of gestation and in the neonatal period is necessary. Placental CRH expression should be re-investigated in all non-primate species with its impact on fetal glucocorticoid synthesis and initiation of parturition.

The guinea pig HPA axis is well studied between 59-94% of gestation, but data before and after this period until birth are very limited. To understand the fetal cortisol surge at term in spite of glucocorticoid negative feedback on CRH and ACTH synthesis, GR expression in the PVN and the anterior pituitary have to be further investigated in the guinea pig, as such data are entirely missing in all three primate species. Also, the HPA axis of the neonatal guinea pig needs to be better understood.

In the rhesus monkey fetus, the investigation of placental 11$\beta$HSD expression is completely missing and in the baboon, the available data are too scarce to be conclusive. Fetal adrenal cortisol and DHEAS synthesis need further investigation in both species. Data for GR expression in the pituitary and PVN are not available and information about CRH, AVP, POMC expression as well as plasma ACTH in the pre- and postnatal periods is still missing. For the human fetus and neonate, data are mostly unavailable for adrenal steroid synthesis (beside steroid enzyme expression), GR expression in the pituitary and PVN, CRH, AVP and POMC expression. Placental 11$\beta$HSD2 expression should be further investigated.

In general, gender differences are hardly taken into account in nearly all of these studies and may reveal interesting mechanisms.

#### 5.2.3.2 b) animal model for human pre- and postnatal HPA axis

Restrained by ethic and legitimate bounds, direct research on the human fetus is mainly confined to very short periods during pregnancy, in connection with treatment of embryonic or maternal diseases, abortion or fetal death, preterm and term birth.

An appropriate animal model needs to be found to substitute for the missing data concerning the human fetal and neonatal HPA axis. In general, it is not possible to designate the most adequate animal model for the investigation of human pre- and postnatal HPA axis as it is dependent on the research question at hand, the state of knowledge concerning the single species, economic aspects and even public concerns.

The most comprehensive data about the fetal HPA axis are available for the rat, as the 'classical' animal used in stress research. Lately, studies in this field were performed to an increasing extent in the mouse, with the advantage, that mutation and gene knockout studies are well established in this species. Additionally, the husbandry of both species, with regard to space and time (short reproduction cycle), as well as limited public ethic concern, when compared e.g. to primate research, is beneficial for the quantity and quality of a study. One major difference between rats and mice on one hand, and the human on the other, is the fetal adrenal steroid synthesis. In these rodents, the fetal adrenals do not synthesize androgens, and glucocorticoid (here primarily corticosterone) synthesis start only late in gestation. Unlike the human fetus, where adrenal cortisol synthesis shows an early peak and a second peak at birth, the adrenal glucocorticoid synthesis in the fetal rat and mouse peaks late in gestation and then decreases to low levels in both species before birth.

For the guinea pig, with respect of husbandry/financial expenses (a three times longer reproduction cycle) and ethical concerns, advantages are roughly comparable to those of rats and mice. Unfortunately, the extent of knowledge concerning the guinea pigs' fetal/neonatal HPA axis is not comparable to rat and mouse. The temporal pattern of fetal glucocorticoid synthesis in the guinea pig is consistent with that of the human fetus in contrast to the former two species. As in the human fetus, an early peak in fetal adrenal cortisol synthesis appears to be present in the fetal guinea pig, followed by a stress hyporesponsive period, and a second peak at term is verified. The late gestational surge in cortisol allows the investigation of changes in negative feedback. On the other hand, fetal adrenal androgen synthesis does not have a comparable order of magnitude and importance as in the primate fetus and the interactions of fetal adrenal cortisol and androgen syntheses are less obvious.

Concerning the sheep, the state of knowledge about the fetal and maternal HPA axis is nearly exhaustive. As a disadvantage, the sheep has a two times longer reproduction cycle compared to the guinea pig and much higher financial and spatial demands for husbandry, as well as ethical concerns. In the fetal sheep, a similar picture to that in the human of fetal adrenal cortisol synthesis is present, and the stress hyporesponsive period of negligible fetal cortisol production between the two peaks of stronger cortisol synthesis is confirmed during gestation. Keeping in mind that up to 80% of the human fetal adrenal cortex is assigned to androgen synthesis, as a precursor for placental estrogen production, the less important androgen synthesis in fetal adrenal of the sheep, similar to the guinea pig, implicates differences in fetal adrenal androgen-cortisol interactions.

Rhesus monkey and baboon are expensive to keep and ethical objections are substantial. Their

reproduction cycles are long, but still comparable between rhesus monkey and sheep. In both primate species, similar to the human, early sufficient fetal adrenal cortisol synthesis, a following decrement, and a second peak at term, can be assumed. The synthesis of fetal androgen, as a precursor for placental estrogen synthesis, is necessary and extensive in all three species, and while similar fetal-placental interactions seem to be present, variations occur in the magnitude and development of fetal androgen synthesis among the two primate species and the human. The latter is reflected in the interactions of fetal adrenal cortisol and androgen syntheses.

Unfortunately, data from both primate species are less comprehensive (especially postnatal) compared to data from the non-primate species. As mentioned before, depending on the research question, every species has its advantages and disadvantages as a model for the human. Concerning regulations between fetal adrenal androgen and cortisol and interactions between the fetal adrenal and the placenta, rhesus monkey and baboon could be the species of choice.

With references to prenatal influences between hypothalamus, pituitary and adrenal, regarding fetal and maternal cortisol synthesis and the interplay of glucocorticoid negative feedback during the fetal cortisol surge at term, both sheep and guinea pig are very valuable, especially because of their similar temporal HPA axis development to primates.

From the immense research done especially in rats and, more and more, in mice, unique insight can be gained with regard to fetal/neonatal and maternal HPA axis function. However, the broad temporal differences in HPA axis development between these species and the human have to be taken into account.

#### 5.2.3.3 c) future research

In future, animal studies investigating the programming effects of early adversity (in particular stress/synthetic glucocorticoid administration) on the developing stress system during pre- and postnatal period should include the vulnerable time of low/absent fetal/neonatal glucocorticoid synthesis in the different species. This vulnerable period has to be further verified. The effect of glucocorticoid exposure on the single factors of the developing HPA axis as well as on the organ (especially brain and neuron) maturation should be investigated during this special period, and compared against results before and after this period. This research has to be extended, as this review was focusing only on the glandotropic stress system (HPA axis). Even inside this system, important regulatory variables such as the GR/MR expression in the hippocampus could not yet be included. The next step would be a follow up study investigating the developing ergotropic system (norepinephrine from the locus coeruleus and epinephrine from the sympathetic nervous system) and the trophotropic system (serotonin from the dorsal raphe nucleus, partly acetylcholine from the parasympathetic nervous system), as all three systems interact in the most sophisticated way during stress, and play their specific role in stress vulnerability and stress-related disorders.

We need more basic research investigating the fetal stress system in promising animal models in order to verify their advantages and disadvantages as a model for the human.

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Erklärung

Ich erkläre hiermit, dass die Dissertationsschrift mit dem Titel

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von mir selbstständig erstellt wurde. Ich habe keine au$\beta$er den angegebenen Quellen und Hilfsmitteln verwendet und die aus fremden Quellen direkt oder indirekt übernommenen Gedanken als solche gekennzeichnet. Die vorliegende Arbeit wurde in gleicher oder ähnlicher Form bisher weder veröffentlicht noch einer anderen Prüfungskommission vorgelegt.

Trier, den 1. Januar 2011

(Marja K. Gerginov)