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Vitamin D receptor and its immunological role within the human placenta – Analysis of vitamin D metabolism and immunologically important genes at the feto-maternal interface

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Abbreviations

A

AAI	Allergic airway inflammation

B

BAL	Bronchoalveolar lavage

C

Ca	Calcium
cDNA	Copy DNA
CERMEL	Centre de Recherches Médicales de Lambaréné
CMBC	Cord-blood Mononuclear Cells
CRP	C reactive protein
Cyp24a1	24-Hydroxylase
Cyp27b1	25-Hydroxyvitamin D3 1-alpha-hydroxylase

D

DBP	Vitamin D Binding Protein
DEPC	Diethyl pyrocarbonate
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DM1	Type 1 Diabetes mellitus
dNTPs	Deoxynucleoside triphosphate

E

EDTA	Ethylenediaminetetraacetic acid
e.g.	*Exempli gratia*/ for example

F

FACS	Fluorescence-activated cell sorting
FCS	Fetal calf serum

FGR	Fetal growth restriction
Foxp3	Forkhead box P3
H	
HKG	House-keeping gene
Hsd3b1	Hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1
H2O	Water
I	
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
L	
LPS	Lipopolysaccharide
M	
MRI	München, Klinikum rechts der Isar
N	
NK cells	Natural killer cells
P	
PAS	Periodic acid-Schiff
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
Q	
qRT-PCR	Quantitative real-time PCR

R

RNA	Ribonucleic acid
RNase	Ribonuclease
RPMI	Roswell Park Memorial Institute medium
RXR	Retinoid X receptor

S

S. mansoni	Schistosoma mansoni

T

Th1	T helper cell 1
Th2	T helper cell 2
Treg	Regulatory T cells

U

uNK	Uterine natural killer cells

V

VDR	Vitamin D receptor
VDRE	Vitamin D response elements

Others

$1,25(OH)_2D$	1,25-Dihydroxyergocalciferol/Calcitriol
25(OH)D	25-Hydroxycholecalciferol
3βHSD	3β-hydroxysteroid dehydrogenase/isomerase

Abstract

Placental gene expression is known to have an impact on the developing child and can be influenced by many environmental factors. In a murine study focusing on the influence of parasite infections during pregnancy we showed that maternal infection with the tropical helminth *Schistosoma mansoni* indeed is correlated with changed placental gene expression: in placentas from dams mated in the initial Th1 and the chronic regulatory phase of the infection, the placental VDR expression was significantly downregulated. This in turn related with a lower allergy susceptibility of the offspring. Vitamin D through its receptor VDR plays a pivotal role not only in bone metabolism but is also a known modulator of the immune system. Moreover, it has been shown that as reaction to immunological triggers, such as inflammation, VDR expression e.g. within the placenta changes. To investigate whether maternal helminth infection in humans elicit similar effects at the feto-maternal interface we have initiated a pilot study. Here, we analyze placental gene expression in a cohort from Germany and one from helminth-endemic Gabon in central Africa. The first and main aim was to determine whether a helminth-endemic environment during pregnancy would be associated with differences in placental gene expression levels when compared to a healthy German control group. Additionally, differences between the fetal and the maternal side of the placenta in general were investigated. Gene expression levels of placentas were analyzed focusing on genes related to vitamin D metabolism (VDR, Cyp27b1), immunologically important genes (e.g. IFNy, IL-10) and pregnancy related Hsd3b1. In addition, vitamin D, C reactive protein and calcium serum levels from mothers and children at the time of birth were determined. Our preliminary data of 22 German and 13 Gabonese patients elicit distinct differences in placental gene expression and correlation patterns. They partly reflect the findings from the murine study with e.g. significantly lower expression of the VDR in the Gabonese placentas and additionally higher expression levels of the pro-inflammatory IFNy. This supports our hypothesis of a low-level inflammation triggered down-regulation of the placental VDR. Those results constitute preliminary data for a big comparative study in Gabon, where a larger cohort of infected and non-infected mothers will be included. In summary, we hope that this new study will expand our knowledge of the mechanisms underlying the hygiene hypothesis which states that intra-uterine and early-childhood infection can protect from the later development of allergic diseases.

Zusammenfassung

Plazentare Genexpression kann bekanntermaßen einen Einfluss auf das sich entwickelnde Kind haben. In einer Mausstudie, in der wir uns auf den Einfluss von Parasiteninfektionen während der Schwangerschaft konzentrierten, konnten wir folgendes zeigen: In Plazenten von Müttern, die während der Infektion mit dem Helminthen *Schistosoma mansonii* verpaart wurden, war die plazentare Vitamin D Rezeptor (VDR) Expression signifikant herab reguliert. Dies wiederum ging mit einer verringerten Allergieanfälligkeit der Nachkommen einher. Vitamin D und sein Rezeptor spielen eine essentielle Rolle nicht nur im Zusammenhang mit dem Knochenmetabolismus, sondern sind auch ein bekannter Modulator des Immunsystems. So konnte beispielsweise gezeigt werden, dass sich die plazentare VDR-Expression als Reaktion auf immunologische Trigger, wie u.a. Inflammation, verändern kann. Um zu untersuchen, ob maternale Helminthen-Infektionen auch im Menschen zu solch artigen Veränderungen führen, initiierten wir eine Pilotstudie. Hierbei analysierten wir plazentare Genexpressionsmuster von einer Kohorte aus Deutschland und einer aus dem Helminthen-endemischen Land Gabun in Zentralafrika. Eines der Hauptziele hierbei war es zu sehen, ob der Einfluss einer Helminthen-endemischen Umwelt während der Schwangerschaft mit veränderten plazentaren Genexpressions-Levels assoziiert sein würde. Zusätzlich wurden generelle Unterschiede zwischen der fetalen und der maternalen Seite der Plazenta untersucht. Bei den analysierten Genen fokussierten wir uns auf solche des Vitamin D Stoffwechsels (VDR, Cyp27b1), immunologisch wichtige Gene (z.B. IFNy, IL-10) und das mit der Schwangerschaft in Zusammenhang stehende Hsd3b1. Zusätzlich wurden Vitamin D-, CRP- und Calcium-Spiegel zum Geburtszeitpunkt bestimmt. Unsere bisherigen Daten von 22 Deutschen und 13 Gabunischen Patienten zeigen deutliche Unterschiede auf und spiegeln teilweise die Ergebnisse unserer murinen Studie wieder: In den Proben aus Gabun sind im Vergleich zu den deutschen Proben die plazentaren VDR- und Hsd3b1-Expression herunterreguliert bei gleichzeitig höherer Expression des pro-inflammatorischen IFNy. Dies bekräftigt unsere Hypothese einer durch schwachen inflammatorischen Stimulus getriggerten Herabregulation des VDR. Diese Ergebnisse sind die Vorarbeit für eine große vergleichende Studie in Gabun, in die sowohl infizierte, als auch nicht-infizierte Mütter eingeschlossen werden. Zusammenfassend sollen uns die Ergebnisse dabei helfen, die Mechanismen der Hygiene Hypothese besser zu verstehen. Diese konstatiert, dass intra-uterine und frühkindliche Infektionen vor der späteren Entwicklung von Allergien schützen können.

1 Introduction

1.1 The hygiene hypothesis

The hygiene hypothesis states that the incidence of immunological disorders like allergies or autoimmune diseases rises due to the decrease of infectious diseases, especially viral and bacterial infections. The most important basis of this statement is epidemiological data, showing that the population of industrialized and therefore "cleaner" countries is at higher risk for immunological diseases than those from developing countries.

The first one to come up with the hygiene hypothesis was David Strachan in 1989. He found an inverse correlation between the incidence of hay fever and household size, when monitoring more than 17.000 children born in 1958 in Great Britain. These findings suggest that a higher number of older siblings and therefore an increased risk of infections can decrease the risk of developing allergies later in life. [Strachan 1989]

Since then, mainly epidemiological data and animal models have strengthened what was further on named "hygiene hypothesis" and are continuing to do so.

1.1.1 Inverse correlation between viral and bacterial infections and immune disorders

An inverse correlation between the incidence of various infectious diseases, like hepatitis A or tuberculosis, and the incidence of immune disorders like type 1 diabetes mellitus (DM1) or asthma is observed over the last 50 years. In this context, especially the development of antibiotics, vaccines, a better hygiene standard and the rising socio-economic status seem to be of great importance. (summarized in [Bach 2002])

Reviewing the prevalence of allergic diseases, it is conspicuous that its distribution around the world varies tremendously. Taking asthma as an example, the highest prevalence can be found in the UK, Australia, New Zealand and the Republic of Ireland, whereas its prevalence is significantly lower in for example parts of Eastern Europe, Indonesia or China [ISAAC 1998, Masoli et al. 2004].

Especially in those countries with a lower incidence of allergic diseases, infectious diseases like hepatitis A, childhood diarrhoea or parasitic diseases like schistosomiasis are still more often to be found. In general, a north south gradient can be observed in terms of allergic and autoimmune diseases with an obvious decrease from northern to southern parts of the world. [Okada et al. 2010]

Certainly, it has to be taken in account that especially in great parts of Africa, there is no standardized data available.

Apart from a greater standard in hygiene, the development of antibiotics had a great influence on the decreased incidence of infectious diseases: Within the 1990s, a trend towards higher prescription rates of antibiotics could be observed [Sharland and Subgroup 2007]. Additionally, there is great evidence for a positive correlation between the development of immunological disorders like hay fever, eczema or asthma and an early application of antibiotics [Droste et al. 2000].

1.1.2 Influence of environmental factors on the allergy susceptibility

Apart from bacterial and viral infections during childhood, many other determinants seem to influence the allergy susceptibility. One prominent and well investigated factor is the exposure to farming in rural areas. In two cross-sectional studies for example, Ege et al. analysed the prevalence of atopy and asthma in children divided in two groups, one living on farms and the other as control group without farm contact. The children living on farms not only were exposed to a bigger range of environmental microorganisms but were also less prone to develop asthma and atopy. [Ege et al. 2011]

Another striking proof for the impact of ecological factors is the diverging prevalence of e.g. asthma or hay fever in the German population before and after the reunification in 1990. Before 1990, the prevalence of both atopic diseases was distinctly higher in the western German population [von Mutius et al. 1994]. This observation was mainly linked to a different way of lifestyle with e.g. smaller family sizes [von Mutius et al. 1994] and a higher socioeconomic status [Emanuel 1988] in the west. However, after the unification eastern lifestyle adjusted to that in the west and interestingly rising levels of atopy and hay fever could be observed simultaneously in parts of eastern Germany [von Mutius et al. 1998]. This comparison is a prominent example for the influence of environmental factors on allergy develop-

ment since the genetic background of both groups was the same whereas the lifestyle and standard were disparate [von Mutius et al. 1994].

Despite all those illustrations, the importance of genetic predisposition should not be forgotten, since for example monozygotic twins compared to dizygotic twins display a higher concordance in the development of e.g. atopic dermatitis (77% versus 15%) [Bieber 2008].

1.1.3 Expanded hygiene hypothesis: the role of parasite infections

As mentioned above, the hygiene hypothesis originally stems from the assumption that bacterial and viral infections during childhood decrease the risk of developing allergies and other immunological disorders. However, more recent data suggest that also parasite infections have an impact in this context. [Yazdanbakhsh et al. 2002]

Helminths for example are parasitic worms that are extremely common in rural areas of the tropics and subtropics [WHO 2016]. One of the most frequent helminth species are *Schistosomes* that cause an infection named schistosomiasis or bilharzia [WHO 2016]. For instance, in a randomized, controlled exploratory study in chronically helminth infected Gabonese schoolchildren, continuous anti-helminth treatment lead to an increase in developing a skin positive test for house dust mites [van den Biggelaar et al. 2004]. Coherence between parasite infections and allergies is supported by the observation that allergies are particularly low in rural areas of the developing world, where the prevalence of parasitic infections is eminently higher (reviewed in [Amoah et al. 2012]). Still, the mechanisms underlying especially the parasite driven anti-allergic effect remain widely unclear.

The general idea is that infections like those with bacteria or viruses skew the immune system towards the T helper cell 1 (Th1) arm of the immune system in order to eliminate the pathogen. That in turn prevents or counterbalances T helper cell 2 (Th2) driven allergic diseases through among others induced levels of interferon gamma (IFNy). [Oriss et al. 1997, Kuo et al. 2013]

However, parasites do not fit in this line of thought, since helminths for instance stimulate Th2 dominated immunological reactions. In the context of a parasite driven promotion of Th2 responses, regulatory T cells (Treg) seem to play an important role: in a model with *Schistosoma mansoni* it could be demonstrated that functional inactivation of Treg leads to an extended initial Th1 phase of infection [Layland et al. 2007]. One possible explanation of

how parasite infections still prevent its host from allergic diseases is porposed by van den Biggelaar et al. [van den Biggelaar et al. 2000]: In a follow-up study with Gabonese school-children they revealed anti-inflammatory, *Schistosome*-antigen-specific Interleukin-10 (IL-10) to be significantly higher in infected children and this finding was inversely correlated with a positivity of a skin reaction to mites. IL-10 is an anti-inflammatory cytokine that can thus dampen the reactivity of Th1 cells and thereby lead to a lower synthesis and release of pro-inflammatory cytokines like Interleukin-6 (IL-6) or tumor necrosis factor alpha (TNF-α) [Couper et al. 2008]. Important sources of IL-10 are Tregs [Laidlaw et al. 2015]. A lack of IL-10 in turn could be found in alveolar macrophages of patients suffering from asthma [Borish et al. 1996], assorting the observation of van den Biggelaar. Additionally, in mouse models it could be shown that IL-10 indeed dampens inflammation mediated by the Th2 arm of the immune system [Wilson et al. 2007]. The underlying mechanisms of how IL-10 can dampen Th2 driven diseases like asthma or allergies however are complex and remain poorly understood. It is very likely that not only the Th1 and Th2 cell balance influences the development of allergies but also other, yet unknown factors. Nowadays, it is assumed that apart from Th1/2 cells, further T cells (e.g. Treg or Th17) in combination with innate cytokines and cells play a major role in the pathogenesis [Stiemsma et al. 2015].

1.1.4 Animal models and human intervention studies

Epidemiological data plays an important role in the elucidation of the hygiene hypothesis. In this context, especially trials investigating the incidence of allergies and other autoimmune diseases in migrants that moved from areas with lower to those with higher incidences are of interest. For instance, children of Asian origin who moved to the United Kingdom together with their families showed an increase in the risk of developing DM1 [Bodansky et al. 1992]. Nonetheless, those migration studies could only strengthen, but not proof the hygiene hypothesis. Proof of principle could for one thing be reached by the investigation of animal models. Using for example non-obese diabetic mice, it was shown that the incidence of DM1 was particularly dependent on the contact with pathogens: nearly all mice bred in pathogen free facilities developed DM1, whereas the disease rarely occurred in mice bred in normal facility environment [Bach 2002]. A very elegant approach to proof the hygiene hypothesis in a human setting, are prospective intervention studies that mainly focus on the effect of helminth eradication. In the already mentioned study in Gabon for instance, it could be demon-

strated that the risk of chronically infected children developing a skin positive test towards house dust mite antigens rises and therefore directly correlates with repeated anti-helminthic treatment and thereby helminth eradication [van den Biggelaar et al. 2004]. The same effect could be demonstrated in a study performed in Venezuela, where helminths are also endemic: the successful eradication of the latter in children led to an increased reactivity in the skin-test and additionally rising levels of Immunoglobulin (Ig) E antibodies [Lynch et al. 1993]. IgE on the one hand is important for the protection against worm infections and on the other hand is responsible for immediate-type allergies. However, the findings are inconsistent, since in an exemplary big trial in Ecuador, no effect of anti-helminthic treatment on atopy or allergy prevalence could be observed [Cooper et al. 2006].

1.1.5 Influence of prenatal environment on the child's immune system

The environment during childhood shapes the immune system and thereby the allergy susceptibility. Certainly, an important interference takes place a lot earlier, namely already during the pre- and perinatal phase [Gollwitzer and Marsland 2015]. Although this circumstance is known and again strengthened by epidemiological data, the mechanisms that lead to a higher or lower risk of autoimmune disease later in life, are still widely unknown. Many different maternal factors shape the child's immune system (see Figure 1.1, adapted from [Gollwitzer and Marsland 2015]). One of the demonstrated examples that have been investigated intensively is maternal obesity: Apart from the children of obese mothers being at greater risk of obesity themselves [Drake and Reynolds 2010], those subjects were also found to show a higher incidence of immunological disorders like asthma [Lowe et al. 2011]. Further examples for maternal factors and perinatal influencers of the growing child's immune system are demonstrated in Figure 1.1: Interestingly, apart from environmental variables like contact to farm animals or cigarette smoke and continuous physical risk factors like alcohol abuse or obesity, also temporary stimulators like maternal stress reactions or the perinatal usage of antibiotics play a major role [Gollwitzer and Marsland 2015]. We are particularly interested in the influence of maternal helminth infections on the growing child which will be introduced in the following section 1.2.

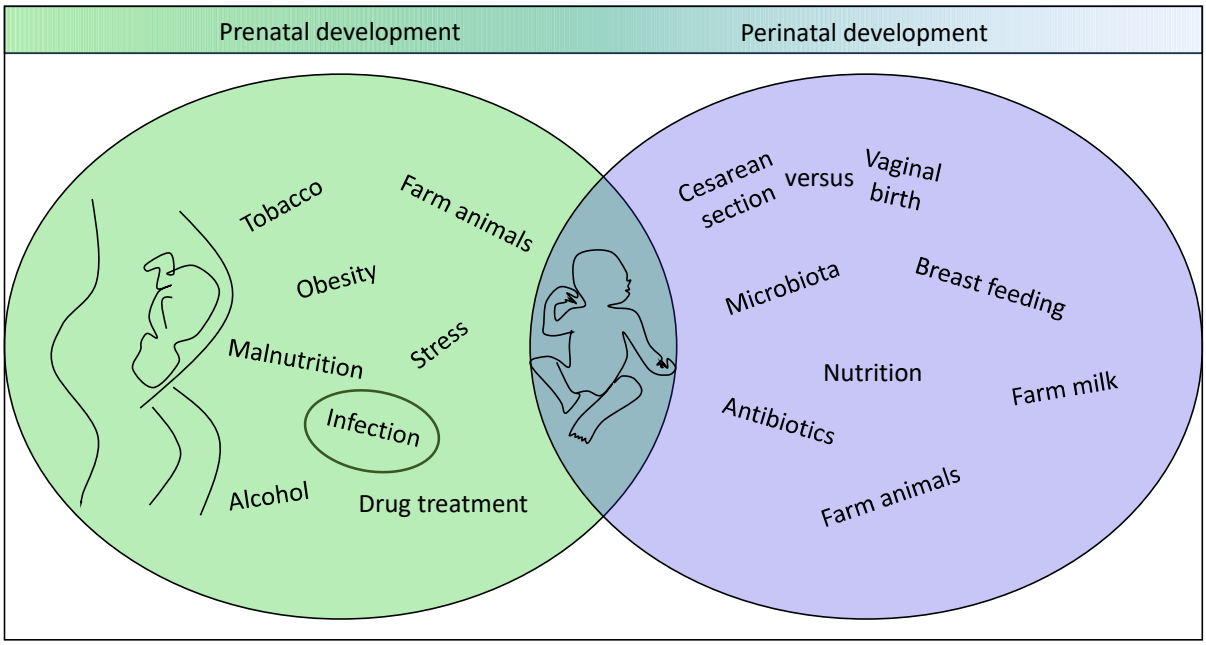

Figure 1.1: Influence factors during the prenatal and perinatal phase

Many influence factors act, directly or indirectly, upon the growing child and its developing immune system. Two distinct phases that are essentially important are the prenatal (green balloon) and perinatal (purple balloon) phase. The term "infection" is encircled since it is the particular influencer in the prenatal phase that we are interested in. *The figure was sketched single-handed and its design and content are adapted from [Gollwitzer and Marsland, "Impact of Early-Life Exposures on Immune Maturation and Susceptibility to Disease", Trends Immunol, p. 687, 2015].*

1.2 Maternal helminth infection and allergy susceptibility of the offspring

In the context of the hygiene hypothesis and especially concerning the role of helminth infections during pregnancy, our group showed that there is a direct link between maternal infections during pregnancy and the offspring's risk for allergic airway inflammation (AAI). The analysis of an experimental model with *Schistosoma mansoni* (*S. mansoni*) showed that in offspring of chronically schistosome infected mothers the development of AAI was severely supressed. [Straubinger et al. 2014]

1.2.1 Immune phases of the schistosome infection

As mentioned above, schistosomes belong to the group of helminthic parasites with approximately 1.5 billion people worldwide being infected, especially in the tropics [WHO 2016].

Most infections are chronic and clinically unapparent or cause mild disease symptoms that are rarely fatal. Instead, they trigger subtle immune modulation of the host's immune system that is presented in Figure 1.2 [Loffredo-Verde 2016, p. 13]. During infection, the host undergoes three different immunological phases that are all characterized by a different and distinctive cytokine composition. Initially, the infection leads to a Th1 prone immune response with the pro-inflammatory IFNy being the most dominate cytokine (acute Th1 phase). [Loffredo-Verde 2016]

Subsequently, the Th1 phase is followed by a Th2 dominated phase and finally a regulatory, immunosuppressive (Reg) phase that allows the parasite to maintain in its host's body [Pearce and MacDonald 2002].

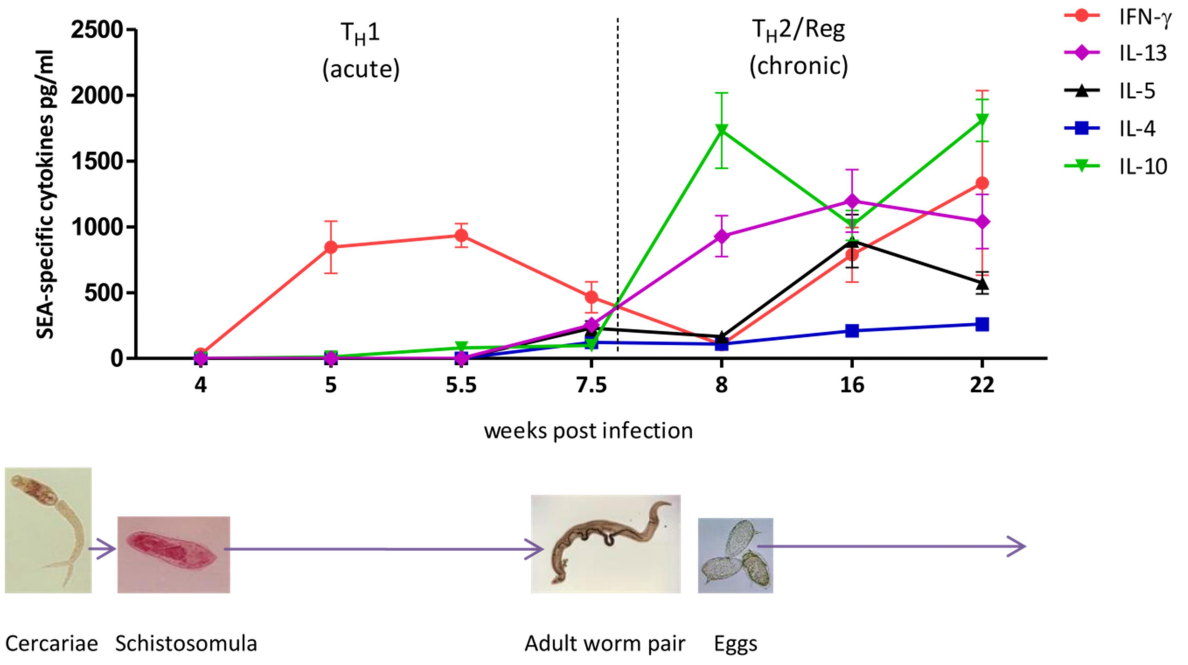

Figure 1.2: Th1 and Th2 immune responses during *S. mansoni* infection in C57/BL/6 mice

Depending on the helminths life-stage, different immune responses are evoked within the host. The acute phase is denoted by a Th1-cell response with high levels of IFNy. As the worms mature with consequencial egg deposition, a stronger Th2-cell response affiliates with high levels of classical Th2 cytokines (e.g. IL-10, IL-5, IL-13) and subsequently a final regulatory immunosuppressive phase 22 weeks post infection (Reg). *Usage with personal permisson of Eva Loffredo-Verde, from [Loffredo-Verde, "The interaction of hepatitis B or C virus infection and schistosomiasis in chronic pathogen-induced liver inflammation", dissertation, p.13, 2016].*

1.2.2 Helminth infections and their influence on pregnancy

Helminth infection affects approximately 1.44 billion people worldwide and is a common event during pregnancy, especially in endemic areas where infection rates range from 10-70% [Straubinger and Prazeres da Costa 2014]. Up to now, maternal schistosomiasis during pregnancy has been postulated to be mainly associated with premature delivery and low birth weight [Siegrist and Siegrist-Obimpeh 1992, Olds 2003, Friedman et al. 2007, Siza 2008]. Studies on schistosome-associated pregnancy performed in Uganda have expanded the knowledge of effects of maternal infection on clinical signs of allergic diseases in infants within the first year of life: they demonstrated that treatment with albendazole (medication used for the treatment of a variety of parasitic worm infestations) during pregnancy leads to an increased incidence of childhood eczema, clearly demonstrating that maternal infection during pregnancy can influence the development of the infant's immune system [Mpairwe et al. 2011].

Even though the parasites do not cross the placental barrier, there is evidence for in utero sensitizing of the child, elucidated through cord blood lymphocyte analysis collected from offspring of infected and uninfected dams. In a study by King et al., it could be elicited that cord blood lymphocytes can produce helminth antigen-specific IgG and polyclonal IgE. These results hint towards an in utero stimulation of B cells and T memory cells of the growing child and support the assumption that maternal host reactions towards helminths can distinctly shape the offspring's developing immune system. [King et al. 1998]

Even though the infection itself is not known to be transferred diaplacentally, schistosome antigens (in form of soluble egg antigen) could be observed in cord blood sera of children born from mothers that were infected at the time of delivery. Thereby, the child's developing immune system might indirectly be shaped or influenced by the maternal infection. [Attallah et al. 2003]

1.2.3 Maternal helminth infections alter offspring's immune responses [Straubinger et al. 2014]

As mentioned above, our group was able to show that in a murine model with *S. mansoni,* maternal infection during pregnancy substantially suppresses the development of AAI in the offspring. All following data were published in *JACI 2014* by Straubinger et al. [Straubinger et al. 2014]. In brief, mothers were mated in the different phases of infection (Th1, Th2, Reg) with uninfected males. Subsequently, their offspring's reaction towards Ovalbumin-induced AAI was compared to that from offspring of non-infected mothers. Ovalbumin is a protein from the egg white of bird eggs that is frequently used to provoke allergic reactions. The severity of AAI was assessed by counting of leucocytes and eosinophils in bronchoalveolar lavage (BAL), lung inflammation score and periodic acid-Schiff (PAS)-stained lung tissue sections. Interestingly, the in a way protective effect of maternal schistosome infection against AAI in the offspring could not be seen in all respective animals, but only in those offspring from mothers that were mated in the Th1- or Reg-phase of the infection. Those from mothers that were mated in the Th2-phase even developed aggravated AAI compared to offspring from non-infected mothers. Assuming a multidimensional system, the placenta as the organ in which most of the feto-maternal crosstalk takes place, was thought to be important for the mediation of the protective effect. Indeed, when performing microarray analysis of the placentas, high amounts of differentially expressed genes were found in each phase (see Figure 1.3, a). In the Th1 and Reg phase those genes were mainly downregulated, whereas in the Th2 phase the differentially expressed genes were principally upregulated. Interestingly, six genes overlapped in the Th1 and Reg phase where the offspring from infected mothers were less susceptible towards AAI. Two of them seemed particularly important: The hydroxy-delta-5-steroid dehydrogenase (Hsd3b1) and the vitamin D receptor (VDR) (see Figure 1.3 b). [Straubinger et al. 2014]

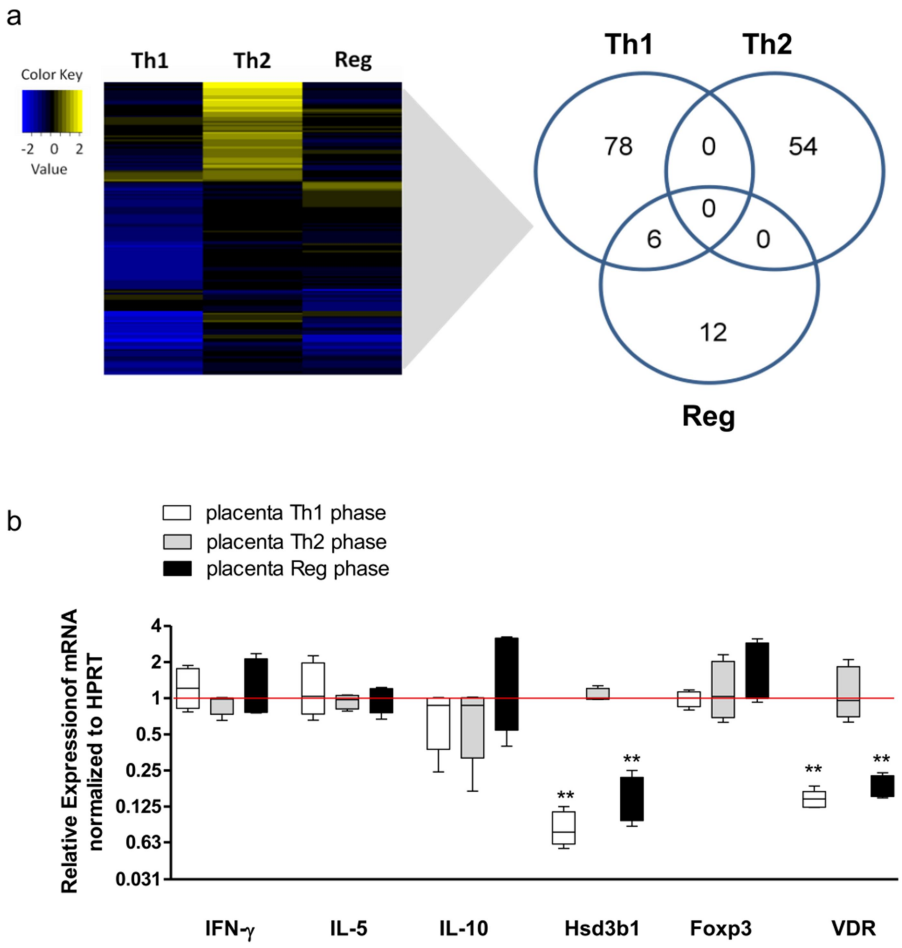

Figure 1.3: Placental gene expression is altered by maternal schistosome infection

Heatmap (a) demonstrates significantly down-regulated (blue) and up-regulated (yellow) genes in placentas from infected vs. uninfected mothers. Number of differentially expressed genes and overlapping genes between the three phases are depicted in Venn diagram. Relative placental target gene mRNA expression of IFNγ, IL-5, IL-10, Hsd3b1, Foxp3, VDR (normalized to hypoxanthine-guanine phosphoribosyltransferase *(HPRT))* (b) of infected in relation to uninfected mothers. *Usage with personal permission of Kathrin Klar, from [Straubinger, "Immunosuppressive and transgenerational effects on the development of allergic airway inflammation during Schistosoma mansoni infection", dissertation, p. 96, 2013], data also published in [Straubinger et al. 2014].*

Hsd3b1 plays an important role in the progesterone production during pregnancy and therefore maintenance of pregnancy itself. Especially the fact that the VDR and therefore an important interface in vitamin D metabolism itself was downregulated appeared to be of particular importance: Since vitamin D metabolism has long been known to not only regulate bone health and calcium homeostasis, but also to have a substantial influence on the immune system and the development of allergies and autoimmune diseases [Prietl et al. 2013, Mirzakhani et al. 2015].

1.3 Vitamin D - effects below bone and calcium metabolism

Vitamin D is known to have various effects with the most investigated and best understood ones being its role in calcium homeostasis and bone health: it increases the calcium resorption from the gut and promotes its incorporation into the bone [Deutzmann 2012]. Apart from those "classical" vitamin D effects, it comprises a series of other impacts on different cells and tissues like modulation of the immune system or regulation of hormone secretion and additionally can have effects on the course of pregnancy [Bikle 2009, Shin et al. 2010, Prietl et al. 2013]. In addition to its increasingly elucidated influence on human health, it is becoming clear that vitamin D deficiency is widely spread among the world's population [Holick 2007]. Especially in pregnant women, vitamin D deficiency seems to be an obvious and nearly epidemic problem [Bodnar et al. 2007, Mulligan et al. 2010]. This might have broad effects not only on maternal but also on the fetal and later in life the child's health. In this context, the placenta as the organ, where most of the feto-maternal crosstalk takes place probably is of pivotal importance. Not only is the placenta able to synthesise active vitamin D itself [Weisman et al. 1979], but it also reacts to higher or lower concentrations of the latter with for instance modulation of local cytokine production or immune response to infection [Shin et al. 2010]: Cultured human decidual natural killer (NK) cells dampen the expression of e.g. TNFα or IL-6 in the presence of calcitriol, the biological active form of vitamin D, whereas the expression of cathelicidin antimicrobial peptide rises [Evans et al. 2006]. Also in trophoblast cells, calcitriol was shown to decrease the expression of TNFα-induced cytokines like IFNy or IL-6 [Diaz et al. 2009]. The role of calcitriol within the human body and especially during pregnancy will be part of the following sections.

1.3.1 Vitamin D synthesis and sustenance within the human body

Vitamin D is a hydrophobic steroid that is not a vitamin in the classical sense, since the human body can synthesize it from non-dietary cholesterol. It further functions as prohormone. The most important representatives are cholecalciferol (vitamin D_3) from animal's origin and ergocalciferol (vitamin D_2) from plant's origin. Its biologic active forms are 1,25-Dihydroxycolecalciferol (Calcitriol, $1,25(OH)_2D$) and 1,25-Dihydroxyergocalciferol. Henceforth, both, vitamin D_2 and D_3 are represented by "vitamin D". As mentioned above, the human body can synthesize Calcitriol from cholesterol or more specifically

7-Dehydrocholesterol, the last intermediate of the cholesterol synthesis. Cholecalciferol is synthesized within the skin by cleavage of the steroid skeleton by ultraviolet radiation. It is then further processed within the liver via hydroxylation which results in 25-Hydroxycholecalciferol (25(OH)D) and is then finally activated by yet another hydroxylation to Calcitriol. The mediation enzyme 1α-hydroxylase (Cyp27b1) is mainly expressed within the proximal tubule of the kidneys. [Deutzmann 2012]

A negative feedback loop is ensured by another hydroxylase, Cyp24a1, which is not only induced by active vitamin D but also degrades 1,25$(OH)_2$D and 25(OH)D via hydroxylation at C24 [Omdahl et al. 2002].

Not only the kidney but also other organs and cells like the placenta or immune cells are known to produce active vitamin D and therefore it is most likely that calcitriol not only acts in an endocrine way but also influences tissues and cells in a para- or even autocrine way [Aranow 2011].

1.3.2 General functions of vitamin D

By now, vitamin D is known to have several effects apart from its “classical” influence on the calcitropic system mentioned above. Among others, it has a broad influence on the innate and adaptive immune system, underlined by the fact that the VDR is expressed in many different types of immune cells like monocytes [Dickie et al. 2010], macrophages [Helming et al. 2005], B cells [Chen et al. 2007] or activated T cells [Daniel et al. 2008]. In general, vitamin D seems to benefit a conversion from a rather pro-inflammatory towards a more tolerogenic state of the immune system (summarized in [Prietl et al. 2013]). Particularly in T cells, vitamin D can lead to a changed cytokine profile [Lemire et al. 1985]. This seems to encourage the development of Th2 cells and thereby facilitate the synthesis and release of rather anti-inflammatory Th2 cytokines (e.g. IL-10) [Boonstra et al. 2001]. In contrast to that, when treated with calcitriol, Th1 cell mediated secretion of pro-inflammatory cytokines, like IFNy, is inhibited [Lemire et al. 1995, Cantorna 2010]. Additionally, vitamin D, through binding to its receptor, directly enhances antimicrobial effects by an upregulation of the transcription of antimicrobial products like defensin β2 or cathelicidin [Wang et al. 2004].

1.3.3 Genomic and non-genomic responses to vitamin D via binding to its receptor VDR

Since vitamin D is strongly hydrophobic and thereby needs to be transported within the bloodstream, the great majority of vitamin D is bound to a serum protein called vitamin D binding protein (DBP) which carries it towards its effector sites [Cooke and Haddad 1989].

The hormonal effects of active vitamin D are diverse and mediated through its binding to the intracellular receptor VDR [Dusso et al. 2005]. The following heterodimerization with the retinoid X receptor (RXR) and nuclear translocation not only influences the calcitropic system but also others like immune responses via the transcription of certain target genes [Guillot et al. 2010]. The sequence that follows after binding and further examples of vitamin D-VDR effects are demonstrated in Figure 1.4: In case of high serum levels of calcium a negative feedback-loop is induced after binding to the VDR. Through reduction of the parathormone secretion from the parathyroid gland less calcium is released from the bone. In Th2 cells on the other hand it upregulates the production of classical Th2 cytokines like IL-10 or IL-13 [Shin et al. 2010]. Additionally, Calcitriol directly influences the body's reaction to infection by increasing the gene expression of cathelicidin antimicrobial peptide [Wang et al. 2004].

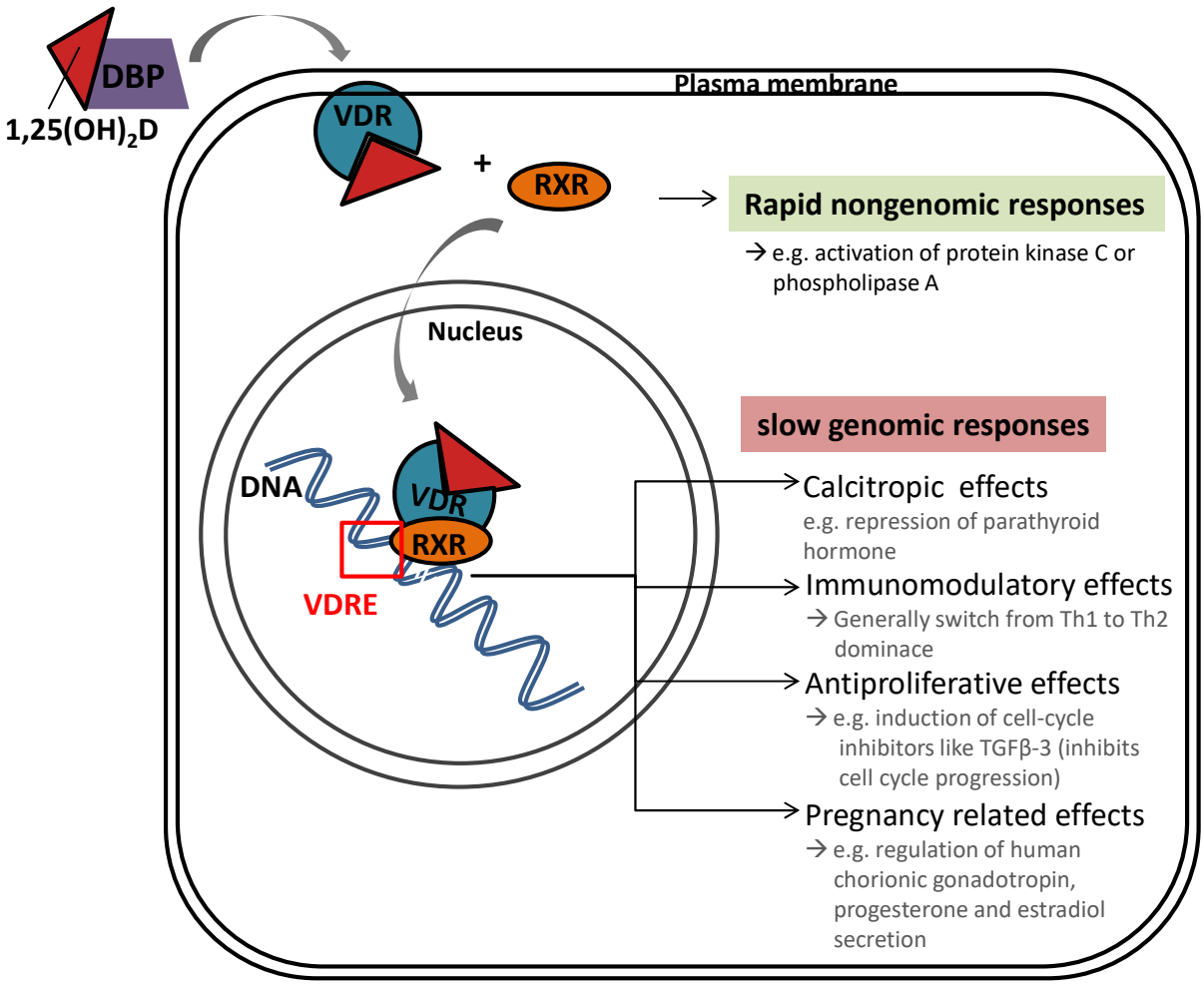

Figure 1.4: Effects of vitamin D by binding to its receptor VDR

$1,25(OH)_2D$ is transported to target cells by DBP. After binding of $1,25(OH)_2D$ to its intracellular receptor VDR heterodimerisation with the retinoid X receptor (RXR) takes place. This complex dislocates into the nucleus and binds to vitamin D response elements (VDRE) of the DNA which in turn leads to different genomic responses. Apart from that, binding of $1,25(OH)_2D$ to its receptor can also lead to nongenomic and therefore faster responses. *The figure was sketched single-handed and its design and content are adapted from [Shin et al., "Vitamin D effects on pregnancy and the placenta", Placenta, Author manuscript p. 18, 2011], additional content from [Dusso et al. 2005, Barrera et al. 2007, Barrera et al. 2008, Nguyen et al. 2015].*

In the context of pregnancy it is interesting to know that the vitamin D-VDR complex plays a role in e.g. estradiol and progesterone secretion [Barrera et al. 2007]. Additionally, a faster non-genomic signalling cascade is provided by plasmatic receptors with for instance activation of protein kinase C or phospholipase A_2 [Mizwicki and Norman 2009]. Protein kinase C generally functions as activator of proteins that play a role in cell growth, differentiation and apoptosis whereas phospholipase A_2 matters in the context of inflammation: it releases ara-

chidonic acid out of glycerophospholipids that is further metabolised to prostaglandins and leucotrienes [Deutzmann 2012].

1.3.4 Pregnancy related effects of vitamin D and its role at the feto-maternal interface

Vitamin D supports important functions of the immune system and therefore influences every individual's health. Accordingly, vitamin D deficiency can lead to adverse health outcomes. Especially the role of vitamin D status during pregnancy seeks more and more attention. Vitamin D deficiency is however not uncommon but rather a wide spread problem around the world: In Europe for example, Cashman et al. showed that around 40% of the population suffers from vitamin D deficiency, regardless of their age, ethnics and latitude of habitation [Cashman et al. 2016]. Vitamin D deficiency, with 25(OH)D as the best surrogate for the bodies sustenance, is classified by the Institute of Medicine (IOM) of the National Academy of Science in the following manner: Sufficiency with levels >30 ng/ml, insufficiency with 25(OH)D levels between 20-30 ng/ml and deficiency with 25(OH)D levels below 20 ng/ml (summarized in [Holick et al. 2011]). Especially in vulnerable groups of our population like pregnant women, vitamin D deficiency is of high prevalence: Bodnar et al. could show that in an American cohort of black and white women, respectively 29.2 % and 54.1 % were not sufficiently supplied with vitamin D at the time of birth [Bodnar et al. 2007]. In another longitudinal study analyzing vitamin D levels in Caucasian women in gestational weeks 12, 20 and 35, depending of the time of measurement 96 %, 96% and 75 % were not sufficiently supplied with vitamin D [Holmes et al. 2009]. The circumstance of a lack of the latter is known to have adverse effects for both the mother and the growing child. The mother is at greater risk for pregnancy diseases like pre-eclampsia [Baker et al. 2010], gestational diabetes mellitus [Zhang et al. 2008] or bacterial vaginosis [Bodnar et al. 2009] whereas children born from vitamin D insufficient mothers are more prone towards chronic diseases later in life with early childhood wheezing [Devereux et al. 2007], asthma [Erkkola et al. 2009] or DM1 [Stene et al. 2000] being prominent and well investigated examples. However, whether there is a direct causal relationship between these diseases and low vitamin D levels is currently unknown. We hypothesize that the placenta as the central organ for the communication between mother and child plays an important role in this context. This assumption is further supported by the knowledge that placental cells contribute decisively to the local vitamin D

metabolism and express all enzymes of the vitamin D metabolism including the VDR itself [Zehnder et al. 2002, Vigano et al. 2006, Pospechova et al. 2009]. In the following section, the placental structure will be further described and its immunological role will be discussed.

1.4 The placenta as central organ in feto-maternal crosstalk

Since the placenta is the main organ for the feto-maternal crosstalk, many investigations focussed on the influence of placental and thereby local vitamin D metabolism [Park et al. 2017, Tamblyn et al. 2017, Workalemahu et al. 2017]. Generally, the placenta forms the barrier between the fetus and the mother and is thereby important for supplying the growing child with nutrients. It also takes care of the gas exchange and is a major site for hormone production of e.g. progesterone and estradiol [Reister 2011], production of cytokines like IL-10 [Roth et al. 1996] and growth factors like the placental growth hormone which regulates gluconeogenesis and lipolysis [Scippo et al. 1993, Zeck et al. 2008]. In addition to those functions, the feto-maternal interface occupies several immunological functions with the protection of the fetus from maternal rejection being one of the most prominent ones.

1.4.1 The placenta: structure of the feto-maternal interface

For the understanding of this organ it is indispensable to have a notion of its general structure (see Figure 1.5). The placenta is built from fetal cells and contains both fetal and maternal blood vessels. The fetal side, meaning the so called chorion and the umbilical cord is covered with epithelium called amnion. The intervillous space, filled with maternal blood, is located between chorion and maternal decidua (see explanation below). Here, the fetal villi, built from umbilical arteries covered with trophoblast cells, dive in. The surrounding trophoblast cells form the placental barrier which separates fetal and maternal blood. The regulated nutrient and gas exchange between mother and child is ensured through diffusion and active transport. Only certain substances like for instance oxygen, electrolytes or glucose can pass the placental barrier.

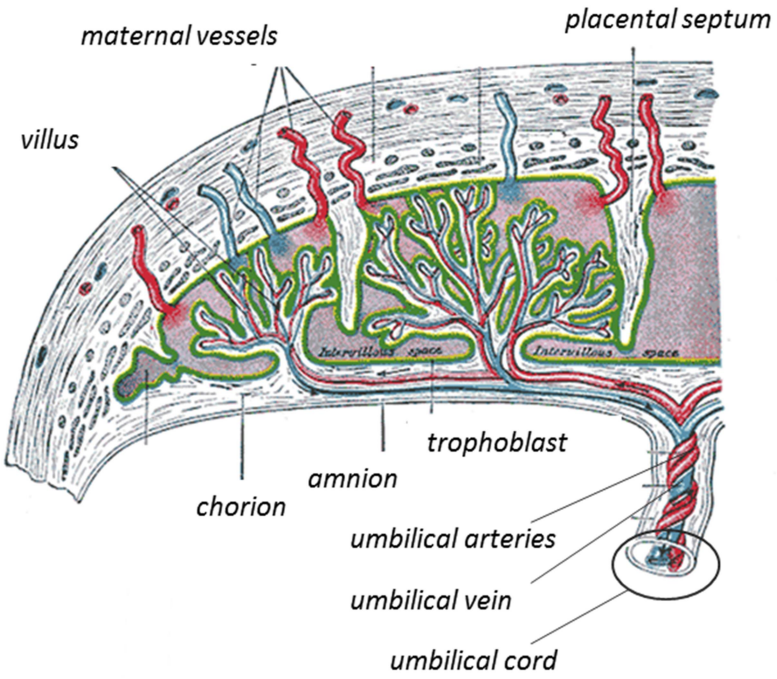

Figure 1.5: Placental structure

In general, the placenta is comprised of a maternal blood lake (intervillous space) into which the fetal villi are diving in, resembling a reversed tree. Villi are built of ramifictions of umbilical arteries. Those are covered by trophoblast cells that form the placental barier. Deoxygenated and nutrient-poor fetal blood is transported to this kind of membrane by the two umbilical arteries and oxygen/nutrients enriched blood is then transported back to the fetus by the umbilical vein. *Figure translated from [Carter and Gray, "Schematischer Aufbau der Plazenta", 1918, from https://commons.wikimedia.org/wiki/File:Plazenta.png, retrieved 28.09.2016].*

The process of the invasion of fetal derived so called trophoblast cells into the maternal uterine endometrium is called placentation (see explanation in Figure 1.6). For this occurrence to be successful, it is important that the endometrium undergoes a functional and structural reorganisation towards an implantation and thereby pregnancy supporting epithelium, the so called decidua. Within the placenta, a close contact between fetal and maternal blood is established to provide the functions mentioned above. That again underlines the importance of the immunological functions of the placenta. [Reister 2011]

Since one placental side is directly connected with the mother and the other directly with the child, it is likely that differences between the fetal and the maternal sides can be found. Thereby, both sides will be investigated within the study at hand, a process that was technically not possibly in the mentioned murine study (see section 1.2.3).

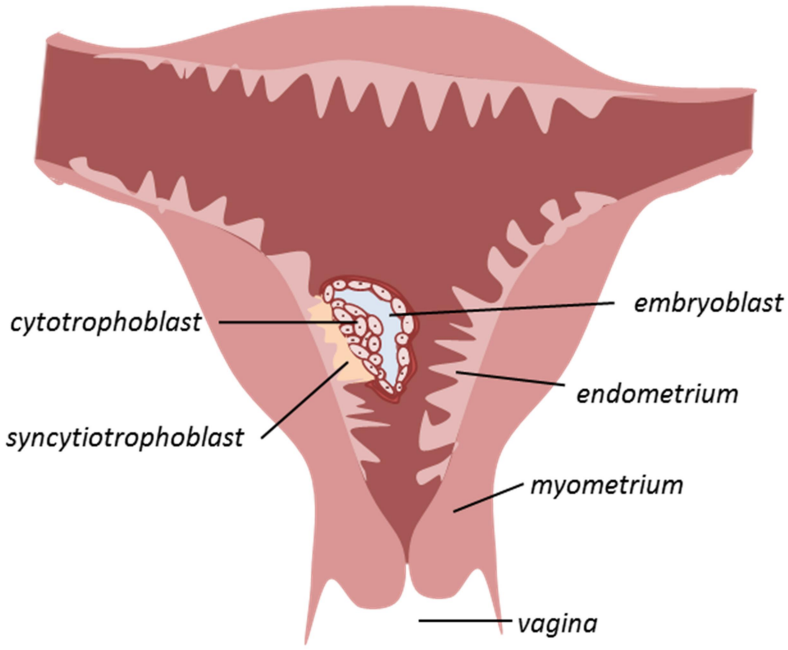

Figure 1.6: Process and structures of placentation after fertilization of the ovum

To enable nidation of the fetal structures into the maternal endometrium (uterine mucosa), structual and functional reorganisation of the latter is needed. After these reorganising processes the endometrium that partly forms the placenta is called decidua. Fetal derived cells, the trophoblast cells, invade into the decidua and form the major cell type of the placenta. More specifically, the trophoblast consists of single cells, the cytotrophoblast and a kind of melded cells that form the syncytiotrophoblast.The latter is the part that invades the decidua. *The figure was sketched single-handed and its design is adapted from [Reister, "Entstehung und Entwicklung einer Schwangerschaft" in Kiechle: "Gynäkologie und Geburtshilfe", p.167, 2011].*

1.4.2 Immunological environment within the placental bed

To perceive its diverse immunological functions, the placental bed composes a unique composition of immunological cells that inhabit the decidua: At least 40% of the decidual stroma cell population is built by leukocytes which belong to the maternal immune system [Bulmer et al. 1991, Erlebacher 2013], including macrophages, T cells (CD4+, CD8+ and Treg), B cells, dendritic cells and a special subpopulation of natural killer cells, the uterine (decidual) natural killer cells (uNK) [Arck and Hecher 2013].

Here, apart from their "normal" role, they are trimmed to reduce the risk of the placenta being attacked in a host versus graft manner that is known from organ transplantation. The two largest leukocyte cell populations are formed by macrophages (≈20%) and uNK cells (≈70%). The latter play a pivotal role in uterine vascular remodelling and are therefore indispensable especially in the first part of pregnancy. Additionally and in line with the assump-

tion that hypertensive pregnancy diseases derive from flawed vascular remodelling during placentation, impairment of uNK cells is known to be linked with pre-eclampsia. [Erlebacher 2013]

Macrophages, as second largest leukocyte population within the feto-maternal interface, are indispensable for decidual tissue remodelling: Accordingly, decidual macrophages are skewed towards the so called M2-state of macrophages which is mainly associated with immunosuppressive effects [Gustafsson et al. 2008]. The role of T cells within the feto-maternal interface currently is not revealed, even though they constitute around 10-20% of the decidual leucocytes and are thereby the third largest population whereas dendritic cells and B-cells are rarely found [Erlebacher 2013]. Alike macrophages, those dendritic cells present within the placental bed are in a rather tolerogenic state and produce for example less IL-12 and more IL-10 compared to dendritic cells outside of the placental bed [Arck and Hecher 2013]. This state in turn can be promoted by different mediators like for instance vitamin D [Ferreira et al. 2012].

However, not only the immune cells within the placental bed implement its immunological tasks but also the placental cells themselves contribute in this regard. The placenta can locally react to a changed immunological environment since its cells (e.g. trophoblast cells) not only express cytokine receptors but also produce pro- and anti-inflammatory cytokines like IFNy, TNF-alpha or IL-10 [Bennett et al. 1996, Jokhi et al. 1997]. Interestingly, the cytokine production could be demonstrated to be influenced by vitamin D within trophoblast cells where it inhibits the production of inflammatory cytokines like IFNy or IL-6 [Diaz et al. 2009].

In turn, various stimuli can affect the placental vitamin D metabolism: Within a murine study, Liu et al. could show that Lipopolysaccharide (LPS), injected intraperitoneally as a very strong inflammatory stimulus, could enhance the expression of placental VDR and Cyp27b1 in the first place [Liu et al. 2011]. That alone emphasises the role of the vitamin D metabolism in the context of inflammation. In a next step, using VDR and Cyp27b1 knockout mice, they showed that an in vitro stimulation of the placentas with LPS led to enhanced inflammation with higher placental expression of IFNy and lower expression of IL-10 [Liu et al. 2011]. This again underlines the generally anti-inflammatory responses that are mediated by vitamin D and emphasizes its importance in the context of inflammation: Vitamin D could be regarded as a protective agent e.g. against massive inflammatory stimuli.

Tavakoli et al. demonstrated that human endometrial cells adapt their cytokine production in vitro when being treated with 1,25$(OH)_2$D. IFNy production was significantly reduced in these cells in the presence of vitamin D. Generally, the cytokine profile of the cells was shaped towards a Th2 phenotype through the treatment with 1,25$(OH)_2$D, similar to the effect of vitamin D on immune cells mentioned above. [Tavakoli et al. 2011]

Taken together, the overall anti-inflammatory effects of vitamin D through its receptor are active locally within the placenta and thereby seem to be crucial for the balance between inflammatory and anti-inflammatory mechanisms or stimuli.

1.5 Aims of the study

In summary, a mechanistic link between VDR/vitamin D and inflammation/infection has been clearly demonstrated. Additionally, a connection between dysregulated vitamin D metabolism during pregnancy and the child's allergy and asthma susceptibility later in life is being discussed. However, to our knowledge, no study so far linked all of those factors with each other and the exact mechanisms of how e.g. parasite infections can protect from the development of allergic diseases remain widely unclear. We assume that placental vitamin D metabolism, particularly the VDR, is one essential component in this context. Consequently, we set up a pilot study to draw first conclusions within a human setting. In more detail we aimed to compare a healthy cohort from Munich, Germany with one from Lambaréné, Gabon. Gabon is a central-African country where helminth infections are endemic. Our specific interest was to get a closer insight of what influence helminth infection or more generally a helminth-endemic environment might have on placental vitamin D metabolism in humans. We hypothesize, with the results of our murine studies in mind (see chapter 1.2.3), that in chronically helminth infected individuals through low-level (systemic and placental) inflammation the placental VDR might be downregulated. Our main aim is to investigate the findings of the murine study as mentioned above in a human setting and to see if differences can be found between the Gabonese group and the German "control group". To address our hypothesis, we implemented a human pilot study in cooperation with the Centre de Recherches Médicales de Lambaréné (CERMEL) in Lambaréné, Gabon, and the Frauenklinik rechts der Isar in Munich, Germany. Within samples of both cohorts from the fetal and ma-

ternal side of the placenta, gene expression levels of VDR, Cyp27b1, IFNy, IL-10, Foxp3 and Hsd3b1 were analysed and compared.

The main questions and aims of this study are enlisted below, divided into 4 different sub-groups.

(1) General aims:
   a) To establish a work flow for the placenta sample collection and processing with the development of a transferable protocol for Gabon.
   b) To establish the quantitative real-time Polymerase Chain Reaction for the measurement of placental gene expression.
   c) To build up a cooperation with both the CERMEL in Lambaréné, Gabon, and the Frauenklinik rechts der Isar in Munich, Germany.

(2) Investigation of placental gene expression:
   a) Are all investigated genes (VDR, Cyp27b1, Hsd3b1, IFNy, IL-10 and Foxp3) constantly expressed on both (maternal and fetal) sides of the placenta?
   b) Do gene expression levels differ when comparing the fetal with the maternal side of the placenta?
   c) Are the investigated genes differentially expressed when comparing a cohort from helminth-endemic Gabon with a control group of Germany?

(3) Investigation of correlation patterns:
   a) Do gene expression levels of the fetal and the maternal side of the placenta correlate?
   b) Do placental cytokine expression levels (pro- and anti-inflammatory) correlate with those of the vitamin D metabolism (VDR, Cyp27b1)?
   c) Do correlation patterns of German and Gabonese cohort differ?

(4) Additional questions addressing the German cohort only:
   a) How good is the vitamin D supplementation in a healthy cohort in general?
   b) Do systemic vitamin D levels correlate with placental VDR expression?

As mentioned above, this study constitutes a pilot study and shall pave the way for a bigger study in Gabon.

2 Material and Methods

2.1 Materials

2.1.1 Equipment

1000Touch™Thermal Cycler	Bio-Rad
Automatic pipettes (2-1000µl)	Gilson®
Centrifuge (Biofuge fresco)	Heraeus
Centrifuge (Eppendorf 5424)	Eppendorf
Centrifuge (Megafuge 3.0R)	Heraeus
Freezer (-20°C)	Bosch
Freezer (-80°C)	Thermo Scientific®
Fridge	Bosch
Glassware	Schott
Microscope (Axiovert)	Zeiss
Multichanel pipettes (Acura® 855; 5-350µl)	Socorex
Multipette® plus	Eppendorf
Mr. Frosty™	Thermo Scientific®
NanoDrop® 1000 Spectrophotometer	Thermo Scientific®
Neubauer counting chamber	Assistent®
Pipetboy (acu)	IBS
Thermocycler (T3000)	Biometra

2.1.2 Software

GraphPad Prism 5	GraphPad Software
Nanodrop® 1000 V 3.7.0	Kisker

2.1.3 Consumables

BD Luer-Lok™ Access Device	BDBioscience
Bood collection tubes (heparin, EDTA)	Sarstedt
Catch all sample collection swabs	Biozym
Cover slips	Roth®
Cryo vials	Alpha Laboratories
Disposable bags	Roth®
Eppendorf tubes (0.5-2ml)	Eppendorf
Falcon tubes (15ml, 50 ml)	Greiner
Forceps (sterile, plastic)	Praxisdienst
Gloves	Meditrade®
Leucosep-Tubes	Greiner
Parafilm M®	Pechiney
PAXGene RNAtubes	BDBioscience
Petri dishes	Greiner
Pipet tips (10-1000µl)	Starlab
Powerlyzer PowerSoil Bead Solution	Dianova
Scalpel No.10	Pfm medical
Scalpels	Feather
Serological pipettes (5-50ml)	Greiner

2.1.4 Reagents

Bicoll, Ficoll separating solution (density 1.077g/mL)	Biochrom AG
Chloroform	Roth®
Deoxynucleoside triphosphate (dNTPs)	Promega
Dimethylsulfoxid (DMSO)	Sigma®
Ethanol 70%-99.8% (v/v)	MRI Pharmacy
Isopropanol	MRI Pharmacy
Light Cycler® 480 Probes Master	Roche
M-MLV RT 5X Reaction Buffer	Promega
M-MLV RT (H-)Point Mutant	Promega
Oligo$(dT)_{15}$	Promega
RNAlater®	Ambion®
Trypan blue solution 0.4% (v/v)	Sigma®

2.1.5 Medium supplements

Fetal calf serum (FCS)	PAA
RPMI 1640	PAA

2.1.6 Kit systems

GenElute™ Mammalian Total RNA Miniprep Kit	Sigma®
Total RNA Kit, peqGOLD	VWR
Tissue homogenizing CKMix - 2mL	Precellys®
QuantiTect Reverse Transcription Kit	Quiagen

2.1.7 Primer sequences

HPRT (reference gene)	
Forward primer	5' tgaccttgatttattttgcatacc 3'
Reverse primer	5' cgagcaagacgttcagtcct 3'
Dual labelled probe	Universal ProbeLibrary Probe #73, Roche
VDR, transcript variant 1	
Forward primer	5’ gaagctgaacttgcatgagga 3’
Reverse primer	5’ gtcctggatggcctcaatc 3’
Dual labelled probe	Universal ProbeLibrary Probe #15, Roche
VDR, transcript variant 2	
Forward primer	5’ tggacggagaaatggactct 3’
Reverse primer	5’ ttcagacccaaaggcttcc 3’
Dual labelled probe	Universal ProbeLibrary Probe #82, Roche
VDR, transcript variant 3	
Forward primer	5' gagcgattggctgtcgat 3'
Reverse primer	5' ttcagacccaaaggcttcc 3'
Dual labelled probe	Universal ProbeLibrary Probe #67, Roche
Cyp27b1	
Forward primer	5' cgcagctgtatggggaga 3'
Reverse primer	5'cacctcaaaatgtgttaggatctg 3'
Dual labelled probe	Universal ProbeLibrary Probe #53, Roche

Foxp3	
Forward primer	5' cttccttgaaccccatgc 3'
Reverse primer	5' gagggtgccaccatgacta 3'
Dual labelled probe	Universal ProbeLibrary Probe #44, Roche
Hsd3b1	
Forward primer	5' tcttcggtgtcactcacagag 3'
Reverse primer	5' ggcacactagcttggacaca 3'
Dual labelled probe	Universal ProbeLibrary Probe #17, Roche
IL-10	
Forward primer	5' gatgccttcagcagagtgaa 3'
Reverse primer	5' gcaacccaggtaacccttaaa 3'
Dual labelled probe	Universal ProbeLibrary Probe #67, Roche
IFNy	
Forward primer	5' ggcattttgaagaattggaaag 3'
Reverse primer	5' tttggatgctctggtcatctt 3'
Dual labelled probe	Universal ProbeLibrary Probe #21, Roche

2.2 Methods

2.2.1 Patient's recruitment

Mothers, patients of the Frauenklinik rechts der Isar in Munich or the Centre de Recherches Médicales de Lambaréné , were recruited to the study within the last hours before birth. The

purpose and aims of the study were explained thoroughly and each patient was enlightened about which sample material was needed. Written informed consent was obtained and not only signed by the mothers but also by the fathers if possible since the study also included their children. Each patient had to fit a certain inclusion criteria and was only included in the study in absence of any exclusion criteria. Those criteria are summarized below. The mothers were followed closely during birth and also the child's birth data and health were monitored and noted directly. Additionally, the patient's history and course of pregnancy were enquired. All obtained information is summarized in chapter 2.2.2 and presented in the results.

Ethical clearance was obtained from the ethical review committee of the faculty of medicine of the *Technische Universität München* (22.11.2013, project number 385/13) and by the *Comité d'Éthique Institutionnel* of the *CERMEL* (18.01.2014, *Numéro CEI-MRU* 13/2013)

2.2.1.1 Inclusion criteria

The patients recruited for the study had to fit the following inclusion criteria:

- Age ≥ 18
- Single pregnancy
- Legally competent and mentally fit to understand and follow the instructions of the study personnel
- Signed patient education and informed consent
- Adequate bone marrow reserve with peripheral granulocyte numbers > 1500/µl and thrombocyte numbers > 40.000/µl
- No status post recurrent thrombosis or lung embolism
- No severe salience during physical examinations
- Documentation of known anaphylaxis
- Spontaneous birth at term (≥ 37 gestational weeks)

2.2.1.2 Exclusion criteria

Patients who met any of the following criteria of exclusion were not recruited for the study:

- Any of the following autoimmune diseases: Anti-Phosphlipid (antibody) syndrome, Goodpasture-Syndrome, rheumatoid arthritis, Lupus erythematodes, Sarcoidosis, ANCA Syndrome, Scleroderma, chronic polychondritis
- Any of the following immunosuppressive diseases: X-chromosomal A-y-Globulinemia, severe combined immunodeficiency (SCID), common variable immunodeficiency (CVID), selective IG A Deficiency
- Known Hepatitis B and/or C, HIV, HSV, CMV, Syphilis, Toxoplasmosis infection
- Other severe and active infections
- Current treatment with Corticosteroids
- Requiring transfusion anaemia
- Any other disease or medical treatment, that according to the principal investigator speak against an inclusion (e.g. pre-eclampsia, HELLP-Syndrome)
- People with dependent or employment relationship to sponsors or investigators
- Known current or former cancer disease

Since only the German but not the Gabonese patients were recruited by ourselves, those patients were questioned and followed throughout birth in more detail than those from Gabon. Therefore, some of the data mentioned in the following is only available from the German cohort. Additionally, it should be mentioned that not all “German” patients originally came from Germany. However, all of them were Caucasian and no originally African patients were included in this cohort.

2.2.2 Ascertained patient’s history

- Maternal age, origin, parity, chronic diseases (e.g. allergies, asthma), supplements and medication during pregnancy
- Course of pregnancy
- Allergy status of the father and older siblings
- Any salience during birth
- Child’s data: sex, birthweight, size, APGAR score, umbilical cord pH value

2.2.3 Sample collection and processing

Of all German patients included in the study, the following sample material was collected. Some samples are italicised since their processing and analysis is not part of this thesis.

- Placenta samples from fetal and maternal sides
- Heparinized maternal blood and cord-blood
- *Pax-gene blood (maternal and cord-blood)*
- *EDTA blood (maternal and cord-blood)*
- *Maternal rectal and vaginal swabs*

The respective samples were stored and further processed as summarized in Table 2.1. Of the included patients from Gabon, we only received placental samples from both the fetal and the maternal side.

Figure 2.1 summarises what data we aimed to gain through the recruitment of our patients and further analysis of the collected samples.

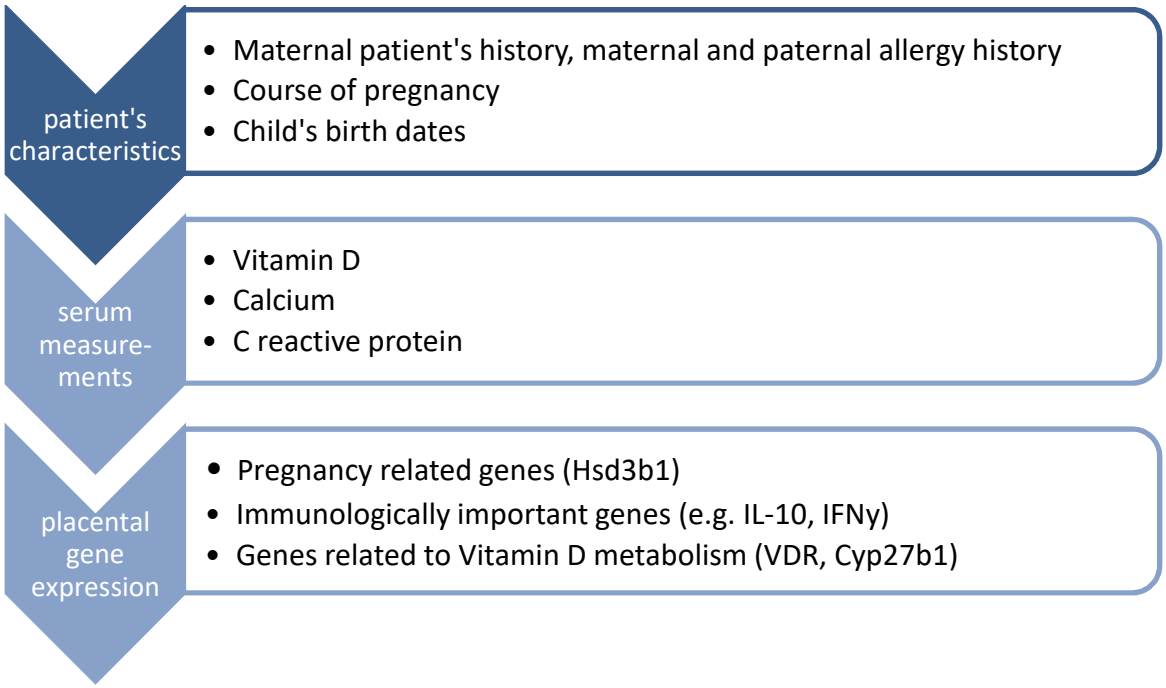

Figure 2.1: Summary of the data and values gained through the patient's characteristics and analysis of the serum and placenta samples

	sampling timeframe	processing/storage
Placenta samples	Directly after the expulsion of the afterbirth	• Stored in 500µl RNA later at 4°C for at least 24 hours up to 5 days • Transferred to freezer (-20°C)
Heparinized maternal blood	Preferably before birth while mother was in the delivery room, if not possible directly after birth	• Stored at room temperature for up to 8 hours • Plasma isolation and PBMC/CBMC isolation/cryopreserve-tion, see chapter 2.2.4
Maternal PAXgene® blood		• Stored at room tempera-ture for min. 2 hours and max. 72 hours • Transferred to freezer (-20°C)
Maternal EDTA blood		• Stored in refrigerator and transferred to freezer (-20°C) as soon as possible
Heparinized cord-blood	Directly after clamping and cutting of the umbilical cord	• Stored at room temperature for up to 8 hours • Plasma isolation and PBMC/CBMC isola-tion/cryopreservation, see chapter 2.2.4
Fetal PAXgene® blood		• Stored at room tempera-ture for min. 2 hours and max. 72 hours • Transferred to freezer (-20°C)
Fetal EDTA blood		• Stored in refrigerator and transferred to freezer (-20°C) as soon as possible
Maternal rectal and vaginal swabs	Preferably before birth while mother was in the delivery room, if not possible directly after birth	• Stored in refrigerator and transferred to freezer (-20°C) as soon as possible

Table 2.1: Summary of sampling process and storage

2.2.4 Isolation and cryopreservation of human peripheral blood mononuclear cells (PBMC)/Cord blood mononuclear cells (CBMC)

For the isolation and following cryopreservation of PBMCs we used a Ficoll gradient technique: For the isolation heparinized maternal blood and cord blood were collected and handled as described in Table 2.1. Before the actual PBMC/CBMC isolation, plasma was preserved after centrifugation of heparinized blood. The following isolation was achieved via Ficoll gradient, graphically demonstrated in Figure 2.2. In brief, blood was added in previously prepared leucosep tubes, filled with Ficoll. After centrifugation, only the upper plasma layer, containing the white blood cells, was further used. After two washing steps with PBS and RPMI complete medium and following centrifugation, the cell pellet was re-suspended in 2 ml of RRMI complete medium and cells were counted in a Neubauer chamber after staining with tryptan blue. After a last centrifugation step the cells were resuspended in 1ml freezing media (10 % DMSO in FCS) per 1x10^7 cells, aliquoted in cryotubes with a maximum of 1x10^7 cells/cryotube and stored in "MrFrosty®" at -80°C for slow cooling down for the next 24 hours. Finally, the cryotubes were transferred to liquid nitrogen until further processing.

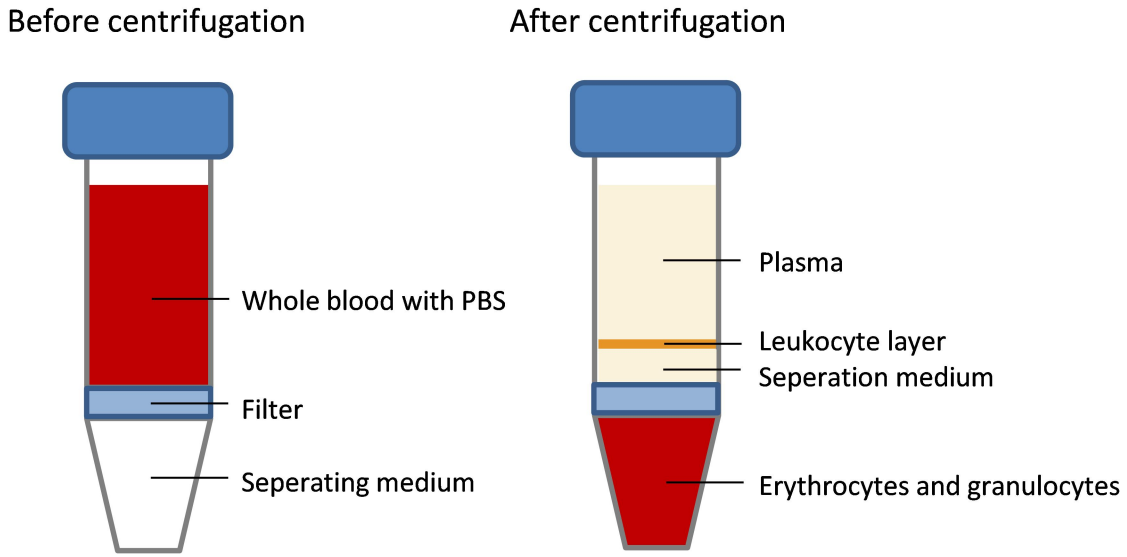

Figure 2.2: PBMC isolation via Ficoll gradient

Through usage of a filter and ficoll as high density medium, Erythrocytes and granulocytes get sucked under the filter after centrifugation whereas the leucocytes as cells of interest form a layer above the filter, inbetween the seperation medium and Plasma.

2.2.5 Quantitative real-time Polymerase Chain Reaction (qRT-PCR)

For the analysis of placental gene expression levels, we performed qRT-PCR. The process from sample collection until qRT-PCR itself will be explained in the following.

2.2.5.1 Placenta sample collection

Placenta samples from healthy German and African mothers were collected from fetal and maternal sides of the placenta right after birth. In the murine study described in section 1.2.3 the whole mouse placenta was used and analysed. Since the human placenta is a lot bigger and could not be analysed in one, we took samples from both the fetal and the maternal side. One placental side is directly connected with the mother and the other directly with the child; thereby it is likely that differences between the fetal and the maternal sides can be found. They were put into labelled cryotubes containing 500µl RNAlater® solution to stabilize placental RNA. Careful attention was payed that each sample was completely surrounded by the RNAlater®, which is pivotal for the integrity and stability of RNA. The samples were stored at 4°C for at least 24 hours and then transferred to -20° for long time storage.

The workflow presented in Figure 2.3 was followed and the listed material was used for the preparation and implementation of the qRT-PCR.

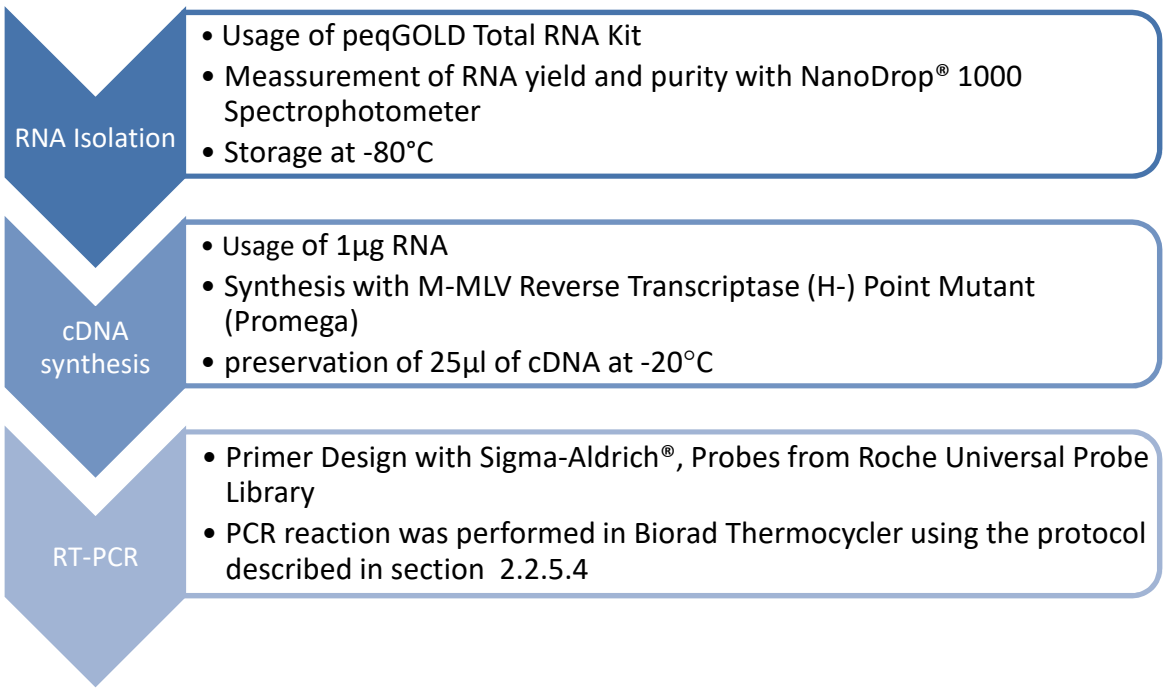

Figure 2.3: Workflow and material for qRT-PCR

2.2.5.2 RNA Isolation

RNA was isolated from the fetal and maternal placenta samples using peqGold total RNA Kit. Before isolation, all tools and surfaces were treated with RNase AWAY to remove RNases and thereby to protect the samples. The method was performed as recommended by the manufacturer. In brief, approximately 40 mg of tissue was cut from the placenta sample and homogenised in 600 µl Lysis buffer using Precellys® Tissue homogenizing CKMix – 2 mL and Precellys®24 tissue homogenizer. The lysate was transferred on a DNA Removing Column. After centrifugation at 12.000*g for 1 minute at room temperature, the flow through was placed in another 1,5 ml Eppendorf tube. After addition of an equal amount of 70% Ethanol and vortexing, the solution was loaded on a PerfectBind RNA Column. Flow-through was discarded after another round of centrifugation. After two washing steps with 500 µl Wash Buffer I and 600 µl Wash Buffer II, the RNA Column was dried by centrifugation for two minutes at 10.000*g. Finally, the RNA was eluted with 50 µl of RNase-free water. RNA yield and purity were measured using a NanoDrop® 1000 Spectrophotometer. The resulting RNA was stored at -80°C until further processing.

2.2.5.3 cDNA synthesis

cDNA was prepared following the Promega usage information for first-strand cDNA synthesis with M-MLV RT (H-) Point Mutant. In short, 1 µg of RNA and 1 µl of Oligo(dT)$_{15}$ were mixed with DEPC-treated water to a total volume of 14 µl. The reaction tubes containing the reaction mix were heated in a thermocycler to 70°C for 5 minutes and then quickly cooled on ice for 5 minutes.

A master mix for the subsequent syntheses was prepared as follows:

Component	Volume
reaction buffer	5 µl
nucleotide mix	1,25 µl
reverse transcriptase	1 µl
water	3,75 µl

After cooling, 11 µl of the master mix were added to each tube, resulting in a total volume of 25 µl. The following protocol was used for the synthesis within a thermocycler: 40°C for 10 minutes, 42°C for 50 minutes (incubation), 70°C for 15 minutes (inactivation of reaction) and subsequent cooling to 4°C. The resulting cDNA was stored at -20°C until further processing.

2.2.5.4 qRT-PCR

qRT-PCR was performed to measure the relative concentrations of certain genes, using Roche Universal Probe Library and HPRT as house-keeping gene. The reaction master mix was prepared as follows:

Component	Volume
LightCycler® Probes Master (Roche)	5 µl
forward primer (10µM)	1 µl
reverse primer (10µM)	1 µl
probe	0,3 µl
water	11,7 µl

The LightCycler® Probes Master (Roche) is a ready-to-use reaction mix containing the Taq Polymerase and appropriate buffers.

After addition of 19µl of the respective reaction master mix in each well of a 384 well plate, 1µl of the particular cDNA was added. The following qRT-PCR was performed in a Thermocycler C 1000 (Bio-Rad) using the following protocol:

	Temperature (°C)	time	
Hot start	95	10 min	
Denaturation	95	10 sec	44 cycles
Annealing	60	30 sec	
Elongation	72	15 sec	
cooling	45	10 sec	
storage	12	∞	

2.2.6 Statistical analysis

All statistical analysis was performed using PRISM® 5.01 (Graph- Pad Software Inc., San Diego, CA, USA). To analyse Gaussian distribution, D'Agostino and Pearson omnibus normality tests were performed. For further analysis of parametrically distributed data, unpaired t-test was performed, whereas nonparametric data was analysed by Mann-Whitney U-test. Statistical dependence was analysed by Spearman's rank correlation coefficient.

Results with a *P* value of <0.05 were considered as significant and *P* values <0.01 as highly significant.

3 Results

All results described in the following are generated to answer the aims of our study already mentioned in chapter 1.5. Figure 3.1 further describes and summarises what data was collected.

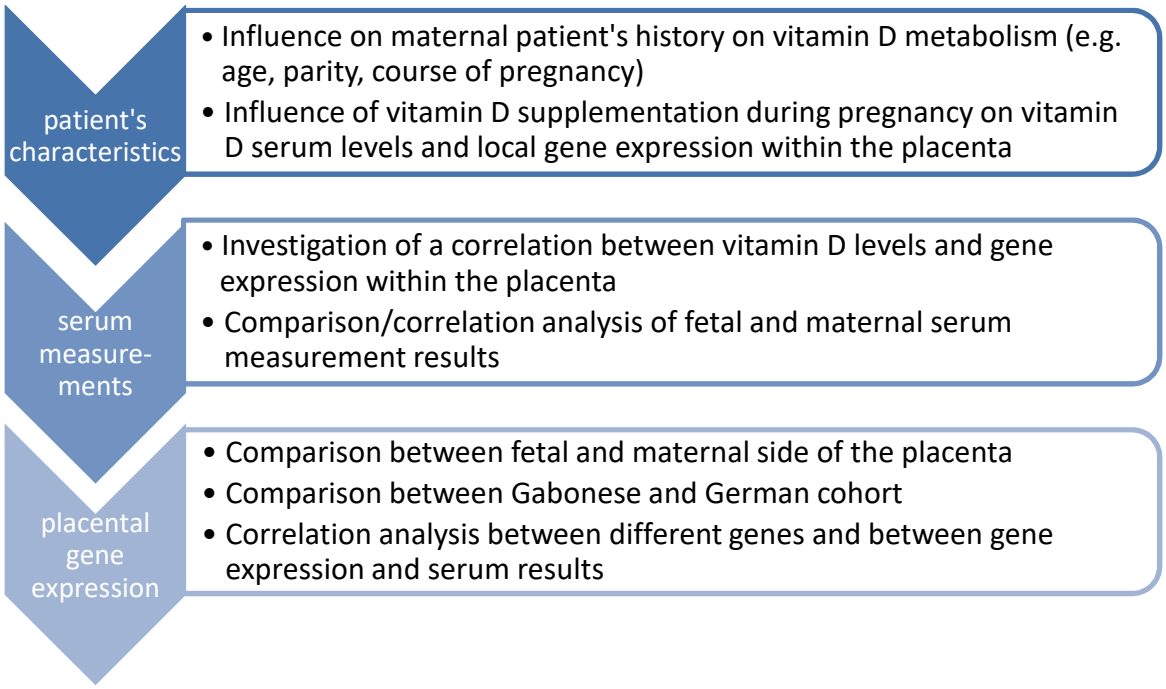

Figure 3.1: Overview of aims addressed with the different results

In the following the results arising from the German-Gabon comparison will initially be described (see chapter 3.1). Subsequently, the additional data gained from the German cohort only will be demonstrated (see chapter 3.2).

3.1 German and Gabonese cohort differ regarding clinical parameters and placental gene expression

As mentioned in chapter 1.5, we implemented a pilot study in cooperation with the CERMEL in Gabon and the Frauenklinik rechts der Isar in Germany comparing a cohort of German and Gabonese mothers and their newly born children. In this comparison we set out to establish the placenta sample collection and processing and in addition the qRT-PCR for the investigation of placental gene expression. The first aim was to see whether all genes of interest were expressed within the feto-maternal interface at all. Secondly, the analysis of differences between the fetal and the maternal side of the placenta on the one hand and between the

German and Gabonese gene expression patterns on the other hand were of our central interest. Up to now, 22 German and 13 patients from Gabon were included in the study. After RNA isolation, two of the fetal Gabonese placenta samples were excluded due to poor RNA quantity and quality.

3.1.1 Clinical details of German and Gabonese patients

Many factors can influence pregnancy and the child's pre- and perinatal developing phase possibly through placental gene expression. Apart from the genetic background and environmental factors, we considered birth mode, maternal age, gravidity, parity and sex of the child as possible confounders. Additionally, we noted birth weight and birth size of all children (see Table 3.1). We only included patients with vaginal deliveries. Differences could be observed in the maternal age with the Gabonese mothers being significantly younger and in the birth size of the Gabonese children who were significantly smaller. However, the birthweight did not differ significantly in the two groups, which poses the question if the technique of measuring the neonate's size might be different. This will have to be clarified in the future.

	Germany	Gabon	
Maternal age, years	33,5 ± 4,9	25,2 ± 7,3	p < 0,001
Median gravidity (min, max)	2 (1, 9)	3 (1, 12)	p = 0,1932
Median parity (min, max)	2 (1, 5)	1 (1, 7)	p = 0,9447
% boys, % girls	59, 41	8, 92	
Birth weight, g	3372 ± 501	3085 ± 323	p = 0,0790
Birth size, cm	52,9 ± 2,5	49,2 ± 1,9	p < 0,001
% vaginal delivery	100	100	

Table 3.1: Comparison of German and Gabonese cohort concerning their clinical details

The two cohorts were compared according to maternal age, gravidity (number of times a women has been pregnant), parity (number of times a women has given birth), numer of boys and girls in the respecting cohorts, birth weight, birth size and mode of birth. *If not labelled differently, data is shown as mean ± standard deviation.*

Taken together, the variation between the two cohorts concerning all listed clinical data was not high. The only two striking exceptions are the maternal age with the Gabonese mothers being significantly younger and the divergence in the sex of the children. In the Gabonese

cohort, the included children were mostly girls. This should be considered as possible confounders when regarding the results.

3.1.2 Placental gene expression levels differ between German and Gabonese cohort

The analysed genes were thematically divided into three groups. The first group comprises VDR and Cyp27b1 as genes related to vitamin D metabolism (see chapter 3.1.2.1). With the second group we aimed to analyse immunologically important genes. IFNy and IL-10 were measured as surrogates for a pro- and anti-inflammatory cytokine for we considered placental inflammation to be important in our trial. Additionally, Foxp3 was measured as marker for regulatory T cells (Treg) that play a tremendous role in helminth infection and eventually allow the parasite to maintain in the human body due to a further tolerogenic state (see section 1.2.1). The last gene representing the third "group" is Hsd3b1 as a pregnancy related gene that is important for the placental progesterone production and thereby maintenance of pregnancy itself.

Apart from comparing the results of the German and Gabonese cohort, we were in particular interested if we could detect differences between the fetal and the maternal side of the placenta. Most studies investigating placental physiology or its role within different diseases, like for instance pre-eclampsia [Enquobahrie et al. 2008] or fetal growth restriction (FGR) [Sitras et al. 2009], to our knowledge only compare placental gene expression in healthy or sick patients in general but do not differentiate between the fetal and maternal side of the placenta. Therefore, little is known about the specific differences between the fetal and maternal side and thereby the possibly preferred localisation of specific genes. However, changes in placental expression of genes related to the vitamin D metabolism are well known and linked to adverse pregnancy outcomes. In placentas that were collected from FGR pregnancies for example, the placental VDR was significantly downregulated [Nguyen et al. 2015]. Also, in pre-ecplamptic patients, placental VDR expression was found to be downregulated whereas Cyp27b1 was upregulated in those placentas [Ma et al. 2012]. Strikingly, none of those studies to our knowledge differ between the fetal and maternal side of the placenta which might but for all that be very important for the results.

Interestingly, a possible connection between first-trimester vitamin D deficiency and placental inflammation could be demonstrated in humans. Zhang et al. could show that extremely low vitamin D levels early in pregnancy were associated with significantly higher occurrence of placental inflammation. Placental inflammation was defined through histologic examination of the placenta after birth, whenever amongst others decidualitis or chorioamnionitis occurred. [Zhang et al. 2018]

Since vitamin D operates through its receptor VDR it could be postulated that also changed VDR expression levels might influence the level of placental inflammation.

3.1.2.1 Expression of placental genes related to vitamin D metabolism

Vitamin D metabolism comprises many different enzymes. Ultimately, 1,25$(OH)_2$D as active form of vitamin D implements its functions through binding to its receptor VDR. In the thesis at hand we measured the expression of VDR and Cyp27b1, the gene encoding for the vitamin D activating enzyme, as two central checkpoints of vitamin D metabolism. Importantly, three different transcript or splice variants of the VDR exist (VDR1, VDR2 and VDR3). To spot which of those would be best for the African-German comparison, we analysed all three variants in the placenta samples from the fetal and maternal side (see Figure 3.2, a-b). Notably on the fetal side, all three transcript variants were significantly lower expressed on the Gabonese compared to the German side of the placenta. In both populations however, VDR1 showed the highest expression on the fetal side of the placenta, the same applies for the maternal side of the German samples. On the maternal side of the Gabonese placenta samples, VDR1 and VDR2 expression levels were comparable. VDR3 was below the detectable threshold in the majority of Gabonese samples. Thus, we decided to use VDR1 as the transcript variant to compare the two groups (see Figure 3.2, c). Regarding both placental sides, significantly lower VDR expression levels were found in the Gabonese compared to the German placentas. Interestingly, also differences between the fetal and maternal side could be seen within the German cohort with VDR expression being significantly higher on the fetal side of the placenta. This was not the case when comparing the fetal and maternal side of the Gabonese placentas.

Regarding Cyp27b1 expression (see Figure 3.2, d), differences between the two cohorts could be measured again: on the maternal side of the placenta, Cyp27b1 expression was

significantly lower in the Gabonese samples. In the latter, differences between the fetal and maternal side were detected with a significantly higher Cyp27b1 expression on the fetal side.

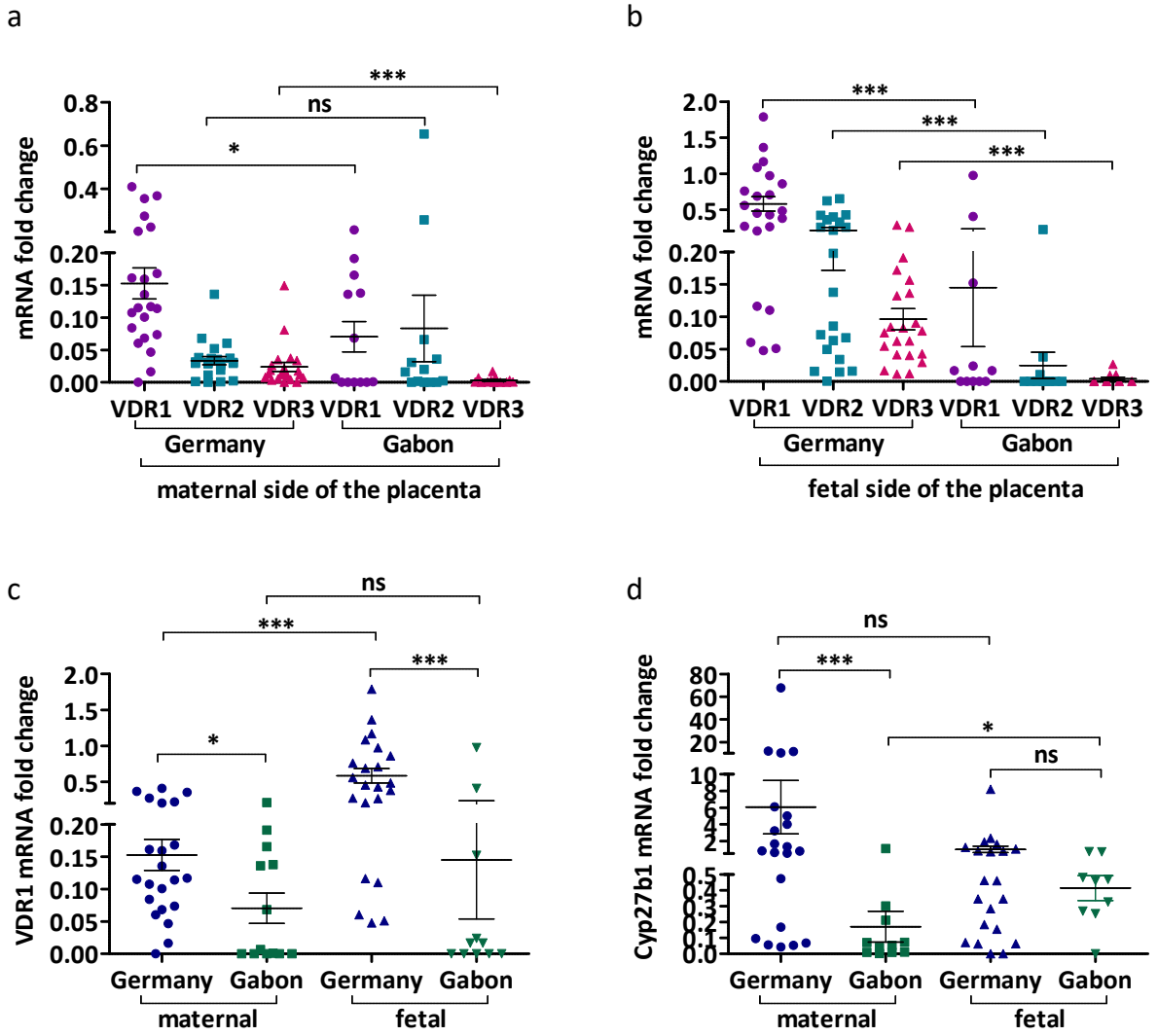

Figure 3.2: Comparison of genes related to vitamin D metabolism

Relative VDR and Cyp27b1 expression, normalized to HPRT as house-keeping gene (HKG), were measured as genes related to vitamin D metabolism. Of the VDR, the three different notable transcript variants were analysed for the maternal (a) and fetal (b) side of the placenta. Gene expression levels of VDR1 (c) and Cyp27b1 (d) were compared between the maternal and fetal sides of the respective placentas and additionally differences between the German and the Gabonese cohort were analysed. *Data is shown as mean ± SEM, asterisks show statistical differences (Mann-Whitney- or t-test) between the groups indicated by the brackets (*p<0,05, **p<0,01, ***p<0,001).*

In summary, both, VDR (respectively on the fetal and the maternal side) and Cyp27b1 (only on the maternal side) were significantly lower expressed in the Gabonese placentas and thereby placentas from the helminth-endemic area. Additionally, the expression levels differ

significantly when comparing the fetal and the maternal side of one cohort. That underlines the importance of looking into both placental sides separately.

3.1.2.2 Placental expression of immunologically relevant genes

To address the inflammatory environment within the placenta, we analysed pro-inflammatory IFNy and anti-inflammatory IL-10 expression (see Figure 3.3, a-b). On the fetal side, IFNy was significantly higher expressed within the Gabonese placentas compared to the German ones. No significant differences were detected regarding the maternal sides. Additionally, the IFNy expression was significantly higher in the fetal compared to the maternal side of Gabonese placentas (see Figure 3.3, a), an observation that could not be seen in the German cohort. Conversely, both, maternal and fetal side IL-10 expression was measured to be significantly lower in the Gabonese compared to the German placenta samples (see Figure 3.3, b). Regarding this cytokine, gene expression was significantly lower on the fetal compared to the maternal side in placentas from Gabonese patients. Again, this could not be observed in the German cohort (see Figure 3.3, b).

Foxp3 expression, as marker for Treg, was also ascertained (see Figure 3.3, c). Treg play an important role in helminth infection (see section 1.1.3). We detected highly significant differences of Foxp3 expression when comparing both the fetal and maternal side of the German with the Gabonese placentas. The Foxp3 expression was significantly lower in the Gabonese samples on both placental sides. Here, no differences could be detected when comparing the maternal with the fetal side within each cohort.

All in all, the immunological milieu within the placental bed of the Gabonese patients of our study was more inflammation prone than the one in German placentas. This is represented by higher IFNy and lower IL-10 and Foxp3 expression and applies especially for the fetal side of the placenta.

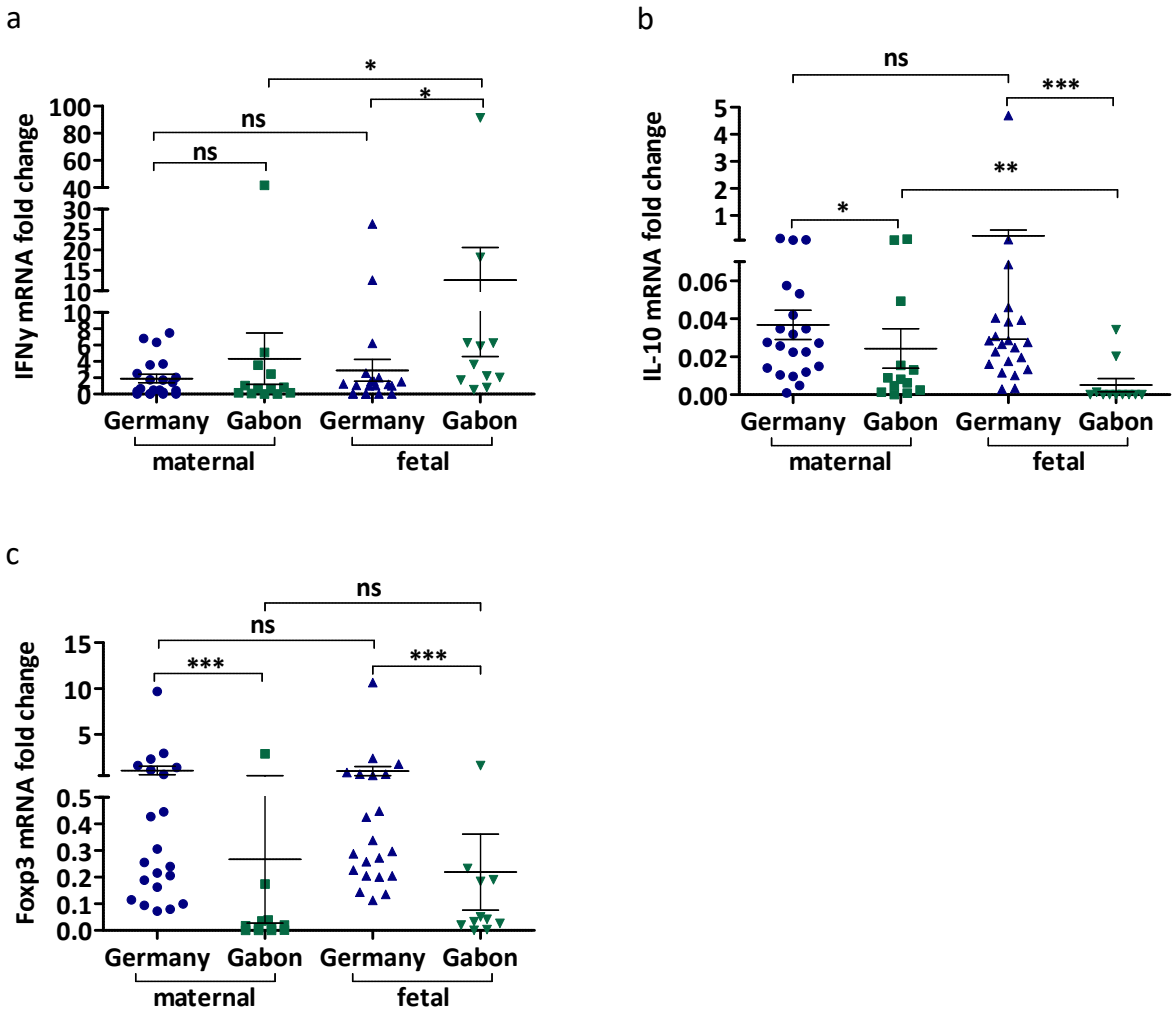

Figure 3.3: Comparison of immunologically important genes

Relative IFNy (a), IL-10 (b) and Foxp3 (c) expression, normalized to HPRT as HKG. Gene expression levels were compared between the maternal and fetal sides of the respective placentas and additionally differences between the German and the Gabonese cohort were analysed. *Data is shown as mean ± SEM, asterisks show statistical differences (Mann-Whitney- or t-test) between the groups indicated by the brackets (*p<0,05, **p<0,01, ***p<0,001).*

3.1.2.3 Hsd3b1 as representative for pregnancy related genes

Hsd3b1 is a gene most important for the placental progesterone production and thereby salvage of pregnancy itself [Peng et al. 2004]. More specifically, it encodes the enzyme type 1 3β-hydroxysteroid dehydrogenase which converts pregnenolone to progesterone (summarized in [Tuckey 2005]). We chose to analyse Hsd3b1 because it belonged to the significantly downregulated genes in chronically infected mice in the murine study that constitutes the basis for this thesis (see chapter 1.2.3). Interestingly, regarding the German cohort, Hsd3b1 expression was significantly higher on the fetal side of the placenta, a difference that

could not be seen in the Gabonese cohort (see Figure 3.4). However, and in context with the murine study, Hsd3b1 expression was significantly lower in the Gabonese placenta samples from the fetal but not the maternal side.

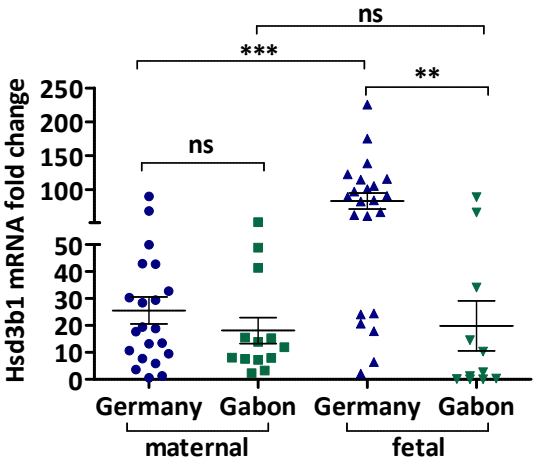

Figure 3.4: Comparison of Hsd3b1 expression

Relative Hsd3b1 expression, normalized to HPRT as HKG. Gene expression levels were compared between the maternal and fetal sides of the respective placentas and additionally differences between the German and the Gabonese cohort were analysed. *Data is shown as mean ± SEM, asterisks show statistical differences (Mann-Whitney- or t-test) between the groups indicated by the brackets (*p<0,05, **p<0,01, ***p<0,001).*

3.1.3 Correlation patterns in comparison between the German and Gabonese cohort

Correlation analysis provides the basis for the hint towards mechanistic links and is essential for generating new hypotheses. For the analysis of a possible connection between the compiled gene expressions of VDR, Cyp27b1, IL-10, IFNy, Foxp3 and Hsd3b1 presented in chapter 3.1.2, Spearman correlation analysis was performed. In this context, we focused on a potential connection between the genes related to vitamin D metabolism and the immunologically important genes. Our specific interest here was to find out whether German and Gabonese correlation patterns were comparable or, as anticipated, if they differed. More specifically, we expected to see a negative correlation between VDR and IFNy in the Gabonese cohort and thereby the patients under influence of a helminth-endemic environment. In patients from Germany and thereby patients without contact to an endemic environment we did not expect any correlation since this cohort constitutes in a way the control group not influenced

by any helminths. Furthermore, the German cohort did not display an inflammatory prone placental milieu in the first place.

Another and rather basic interest of ours was whether gene expression levels between the fetal and the maternal sides of the placenta correlated. This investigation is demonstrated in the following section.

3.1.3.1 Gene expression levels between fetal and maternal side of the placenta do not correlate in large parts

To the best of our knowledge, only view studies exist that investigate differences in gene expression levels between the fetal and maternal side of the placenta: using microarray analysis, Sood et al. found distinct differences in gene expression profiles in samples from fetal, maternal and middle sections of the placenta. Amongst those differentially expressed genes were for instance vasodilator neurokinin B (NKB) and VEGF receptor Fms-like tyrosinase kinase 1. Interestingly, both genes are associated with pre-eclampsia. [Sood et al. 2006]

These findings support the assumption that future studies need to distinguish more closely on where or rather of which placental section the samples are gathered from since this may change the results drastically. Still, it remains unknown if there is a mechanistic link between gene expression levels of the fetal and maternal side of the placenta. To verge on this issue, we performed correlation analysis between the gene expression levels of the fetal and maternal sides of the placenta. The results are shown in Figure 3.5 for the German cohort and in Figure 3.6 for the Gabonese cohort. As demonstrated, no correlation between the fetal and maternal VDR (respectively a), Cyp27b1 (respectively b), Foxp3 (respectively e) or Hsd3b1 (respectively f) expression could be found in both cohorts. However, a strong correlation between fetal and maternal placental IFNy expression was detected in the German cohort (Figure 3.5, c). This was not seen in the placentas of the Gabonese cohort (Figure 3.6, c). In both cohorts then again, a positive correlation between fetal and maternal placental IL-10 (respectively d) could be elucidated.

Concluding, in our set of genes, only cytokine expression levels correlate between the fetal and maternal side of the placenta. The expression levels of the fetal and maternal placental side of genes related to vitamin D metabolism, pregnancy related Hsd3b1 and also Foxp3 did not correlate and are thereby possibly independent from each other.

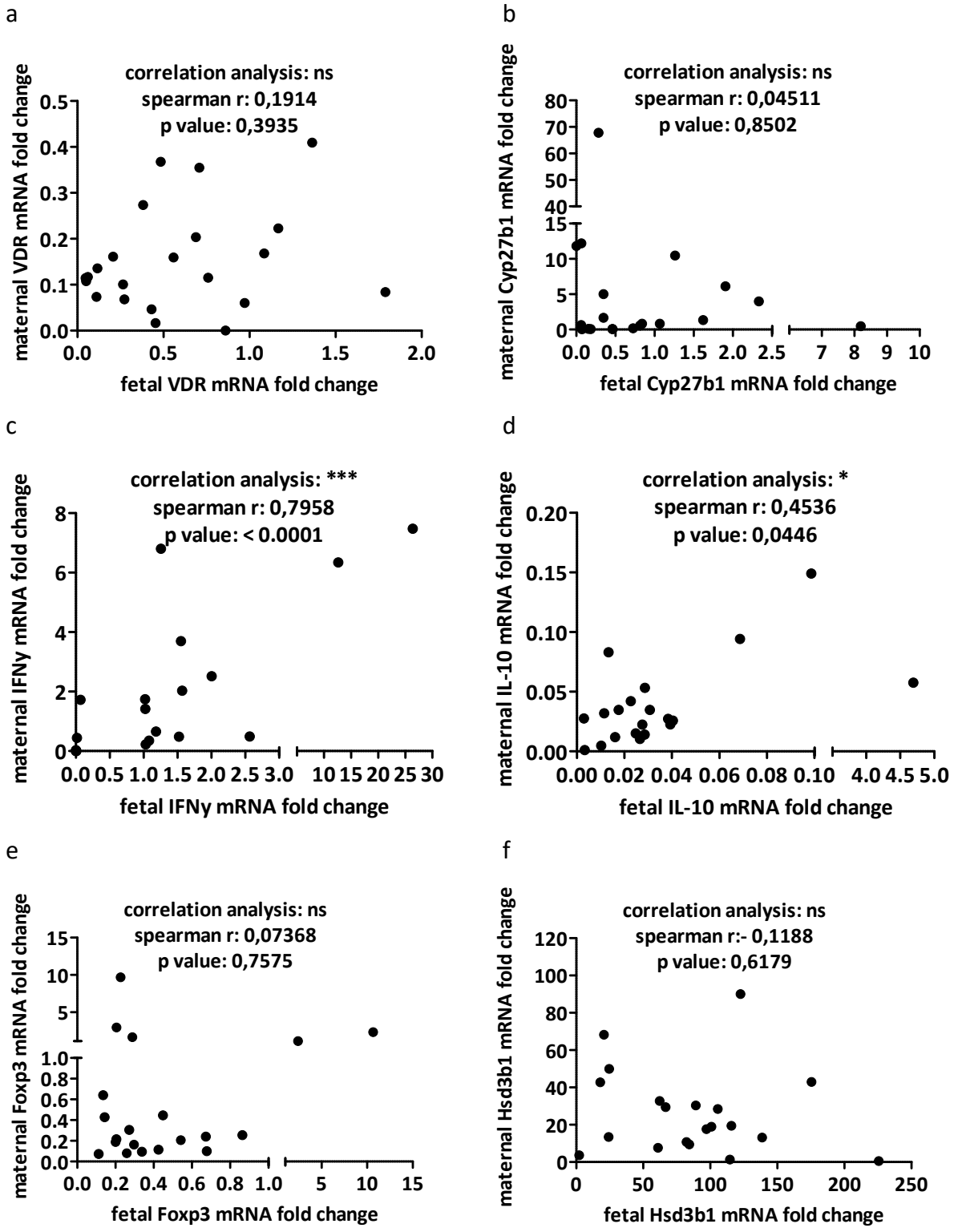

Figure 3.5: Correlation analysis of gene expression levels between fetal and maternal sides of German placentas

Correlation analysis was performed between gene expression levels of the fetal and maternal side of the placenta. No correlation could be found between fetal and maternal expression of VDR (a), Cyp27b1 (b), Foxp3 (e) and Hsd3b1 (f) expression. A positive correlation was detected between fetal and maternal IFNy and IL-10 (f) expression. *Asterisks show statistical significance of correlation (spearman) between the respective genes (*p<0,05, **p<0,01, ***p<0,001).*

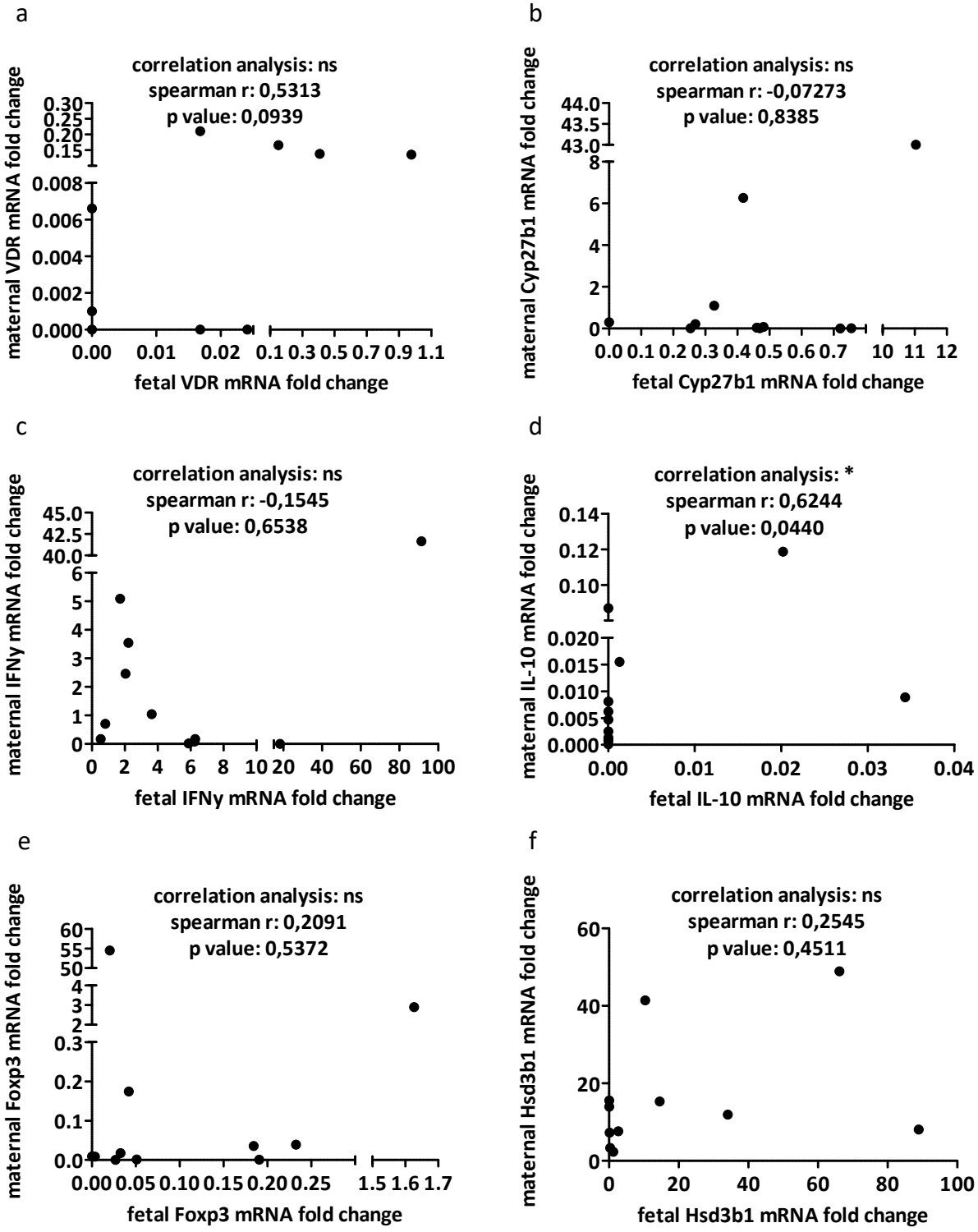

Figure 3.6: Correlation analysis of gene expression levels between fetal and maternal sides of Gabonese placentas

Correlation analysis was performed between gene expression levels of the fetal and maternal side of the placenta. No correlation could be found between fetal and maternal VDR (a), Cyp27b1 (b), IFNy (c), Foxp3 (e) and Hsd3b1 (f) expression. A positive correlation was detected between fetal and maternal IL-10 (f) expression. *Asterisks show statistical significance of correlation (spearman) between the respective genes (*p<0,05, **p<0,01, ***p<0,001).*

3.1.3.2 Correlation between IFNy and VDR expression and additionally between VDR and IL-10 expression only present at fetal side of Gabonese placentas

It is known that active vitamin D through binding to its receptor VDR can promote anti-inflammatory processes. However, the regulation of these effects is complicated, most probably subtly tuned and yet poorly understood. As reviewed in [Cantorna et al. 2015], $1,25(OH)_2D$ can inhibit IFNy production in T cells and additionally can lead to an induction of Treg. Accordingly, within the placenta vitamin D metabolism might operate as protector against high and thereby potentially harmful levels of inflammation. We hypothesize, that in healthy patients (in our case from non-helminth-endemic Germany), no correlation between inflammatory IFNy and vitamin D metabolism can be detected since here no conspicuous inflammation levels are found in the first place. In placentas from helminth-endemic Gabon however, where higher placental IFNy expression was found on the fetal side of the placenta (see section 3.1.2.2) we expected a negative correlation between IFNy and VDR. In this context, we hypothesize a low level inflammation triggered downregulation of the VDR. With regard to the anti-inflammatory role of VDR mentioned above, this potentially negative correlation might turn into a positive correlation in a state with distinctly raised levels of inflammation like for instance acute maternal infection with different pathogens.

Therefore, we aimed to investigate a possible correlation between VDR and the measured cytokines IFNy/IL-10 on the one hand and between Cyp27b1 and IFNy/IL-10 on the other hand (results are demonstrated below).

In the healthy German cohort, with no obvious signs of inflammation or infection (such as helminth infection or others), no correlation between the placental expression of VDR and IFNy (see Figure 3.7, a-b) or of VDR and IL-10 (see Figure 3.8, a-b) was detected. Likewise, no correlation could be seen in the maternal side of the Gabonese placentas (see Figure 3.7/Figure 3.8, c). However, within the fetal side of the Gabonese placentas, a different picture emerged: here, VDR expression negatively correlated with IFNy expression (see Figure 3.7, d) and additionally a positive and even stronger correlation between VDR and IL-10 (see Figure 3.8, d) could be observed.

At this point nevertheless, it should be stated that a correlation only indicates a possible interaction and one can only hypothesize a causal relationship. Additionally, our results only

reflect the situation at the time of birth and do not inform us about the nine months before. Still, it was very interesting to see that specifically the fetal side of the Gabonese placentas differed from all other results not only concerning the correlation between VDR and IFNy but also between VDR and IL-10. To further investigate the immune reactivity at the time of birth, we isolated and cryoconserved PBMCs and CBMCs (see section 2.2.4). Those cells will be further investigated in the future to get an insight of the immunological state of the mothers and children during birth.

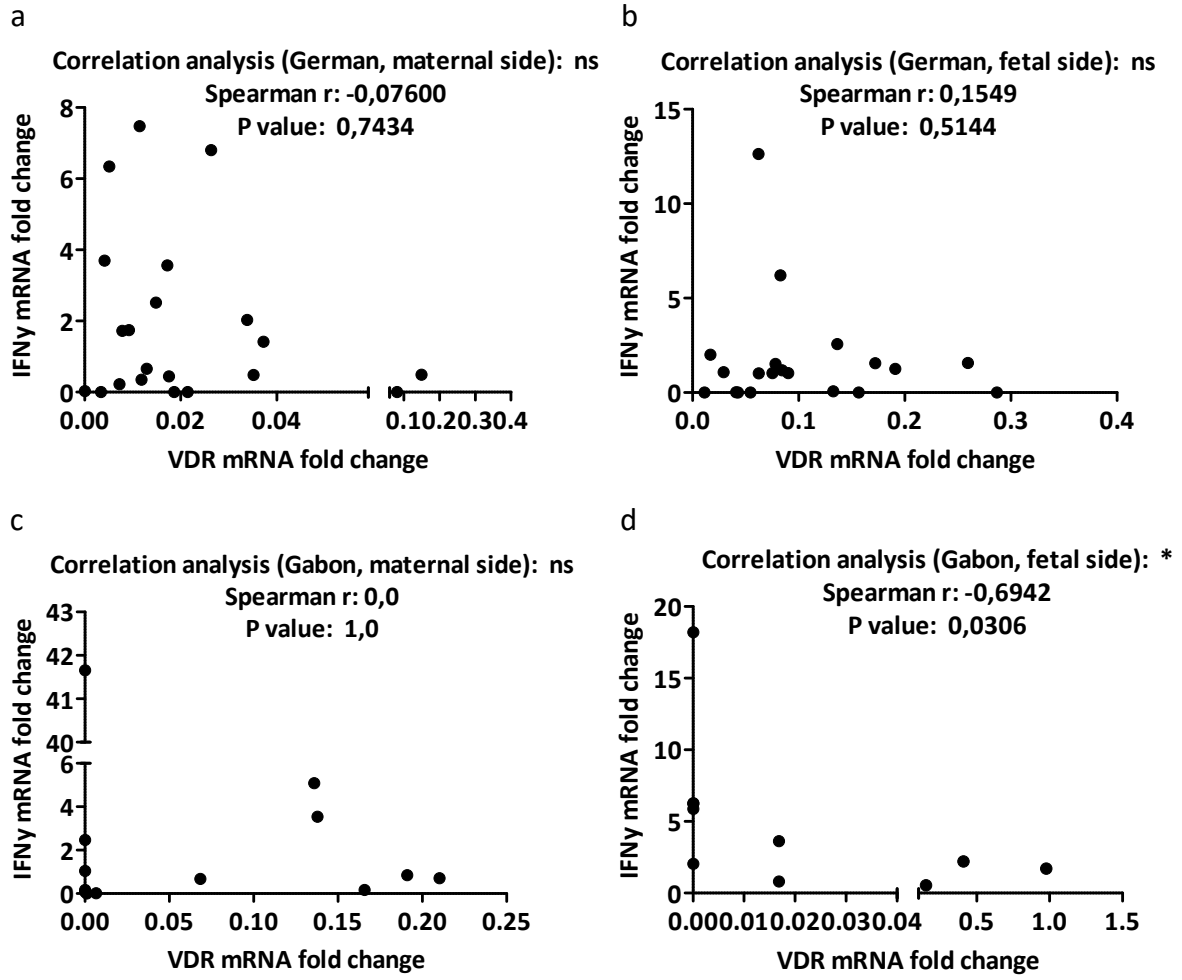

Figure 3.7: Correlation between VDR and IFNy could only be detected on fetal side of Gabonese placenta samples

Correlation analysis was performed between VDR and IFNy expression. Fetal and maternal side were anaylsed seperatly within both cohorts: Maternal side of the German placentas (a), fetal side of the German placentas (b), maternal side of the Gabonese placentas (c) and fetal side of the Gabonese placentas (d). *Asterisks show statistical significance of correlation (spearman) between the respective genes (*p<0,05, **p<0,01, ***p<0,001).*

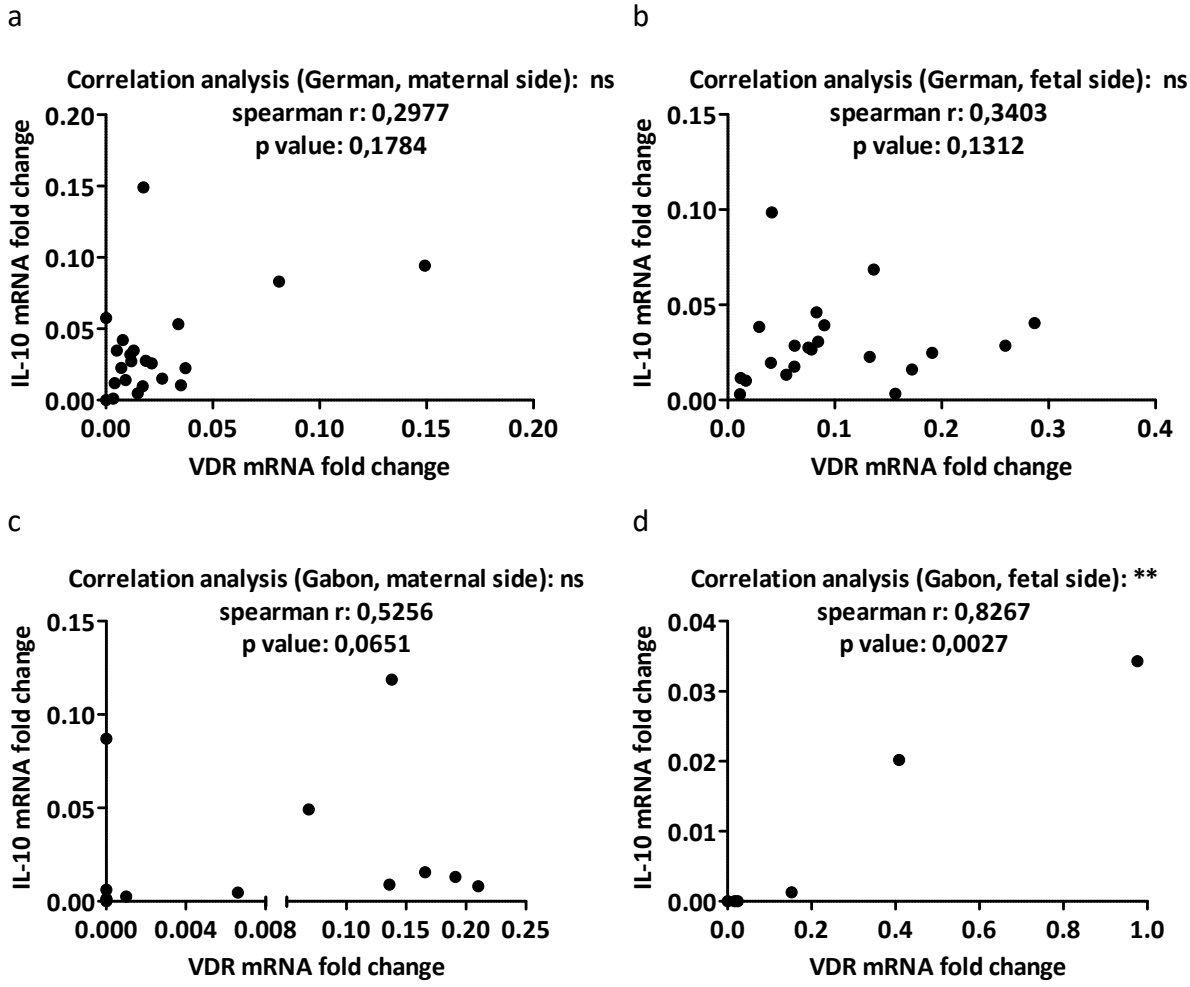

Figure 3.8: Correlation between VDR and IL-10 could only be detected on fetal side of Gabonese placenta samples

Correlation analysis was performed between VDR and IL-10 expression. Fetal and maternal side were anaylsed seperatly within both cohorts: Maternal side of the German placentas (a), fetal side of the German placentas (b), maternal side of the Gabonese placentas (c) and fetal side of the Gabonese placentas (d). *Asterisks show statistical significance of correlation (spearman) between the respective genes (*p<0,05, **p<0,01, ***p<0,001).*

In summary, only within the fetal side of the Gabonese placentas a negative correlation between VDR and IFNy (Figure 3.7, d) and a positive correlation between VDR and IL-10 (Figure 3.8, d) could be observed. This strengthens our hypothesis of a low-level inflammation driven VDR downregulation in placentas from patients of helminth-endemic Gabon.

3.1.3.3 Correlation between Cyp27b1 and IFNy/IL-10 expression is stronger in Gabonese samples

As described in the preceding chapter, active vitamin D can promote anti-inflammatory effects through its receptor VDR. Since Cyp27b1 encodes the gene for the 1-alpha-hydroxylase and thereby the vitamin D activating enzyme, we were further interested in a possible correlation between Cyp27b1 and IFNy or IL-10 expression within the placenta. The feto-maternal interface, namely the decidua and placenta, is known to be an important source for the conversion of 25(OH)D to 1,25$(OH)_2$D in humans [Weisman et al. 1979]. Therefore, it is most likely that the placenta can locally counterbalance higher levels of inflammation, e.g. through altered vitamin D metabolism, and thus protect the growing child, as it is already shown for the murine placenta [Liu et al. 2011]. With this in mind, we analysed the correlations between Cyp27b1 and IFNy expression on the one hand (see Figure 3.9) and between Cyp27b1 and IL-10 on the other hand (see Figure 3.10).

While a positive correlation between the vitamin D activating enzyme and IFNy within the German cohort could only be detected on the maternal side of the placenta (see Figure 3.9, a), a positive correlation was detected on both sides of the placentas from the Gabonese patients (see Figure 3.9, c-d).

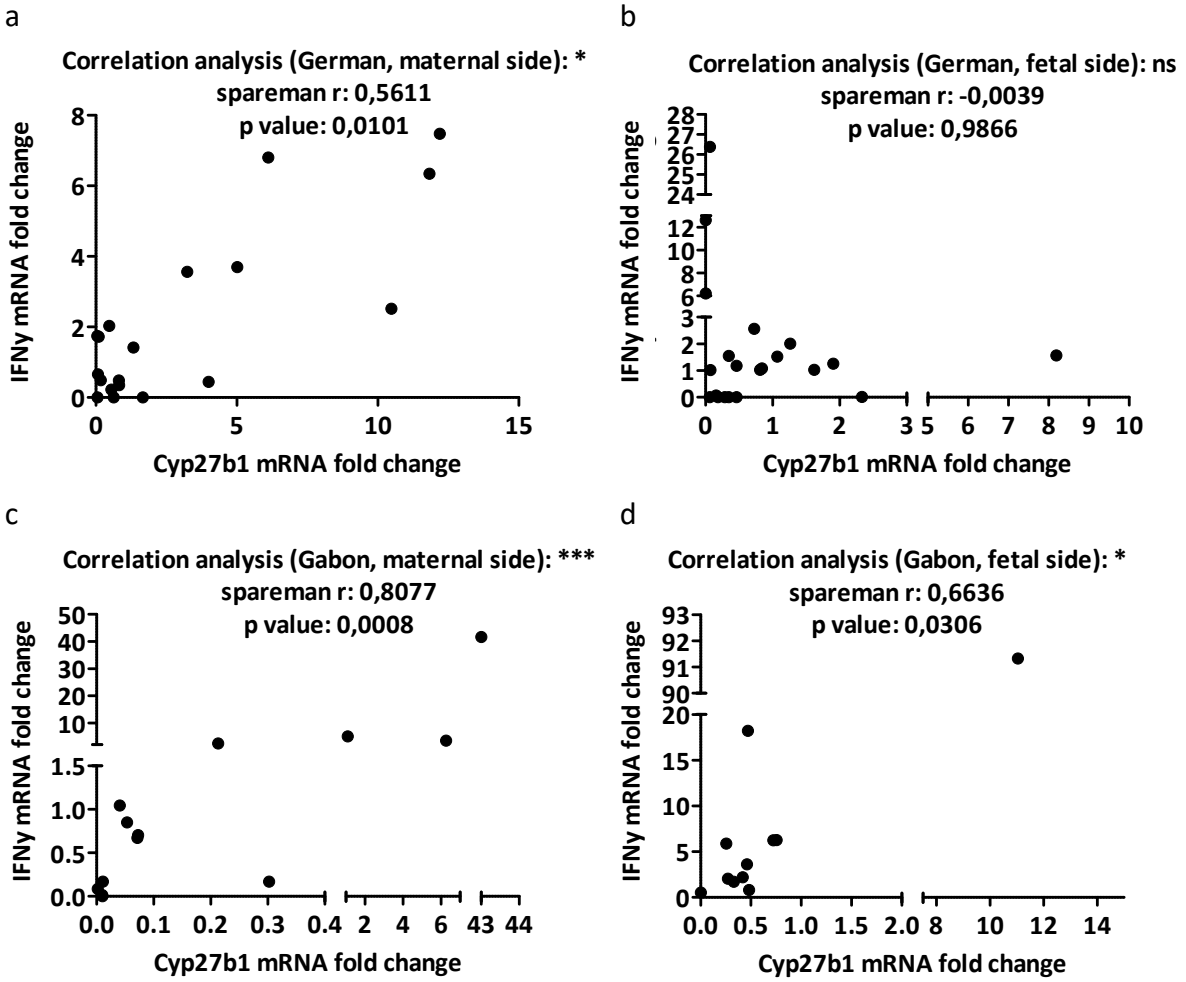

Figure 3.9: Correlation between Cyp27b1 and IFNy expression is strongest in maternal side of Gabonese placentas

Correlation analysis was performed between Cyp27b1 and IFNy expression. Fetal and maternal side were anaylsed seperatly within both cohorts: Maternal side of the German placentas (a), fetal side of the German placentas (b), maternal side of the Gabonese placentas (c) and fetal side of the Gabonese placentas (d). *Asterisks show statistical significance of correlation (spearman) between the respective genes (*p<0,05, **p<0,01, ***p<0,001)*

Additionally, the correlation patterns of the two cohorts were distinctly different concerning Cyp27b1 and IL-10. No correlation could be detected on both sides of the German placentas (Figure 3.10, a-b) and the fetal side of the Gabonese placentas (Figure 3.10, d). However, we did find a highly significant correlation between Cyp27b1 and IL-10 on the maternal side of the Gabonese placentas (Figure 3.10, c).

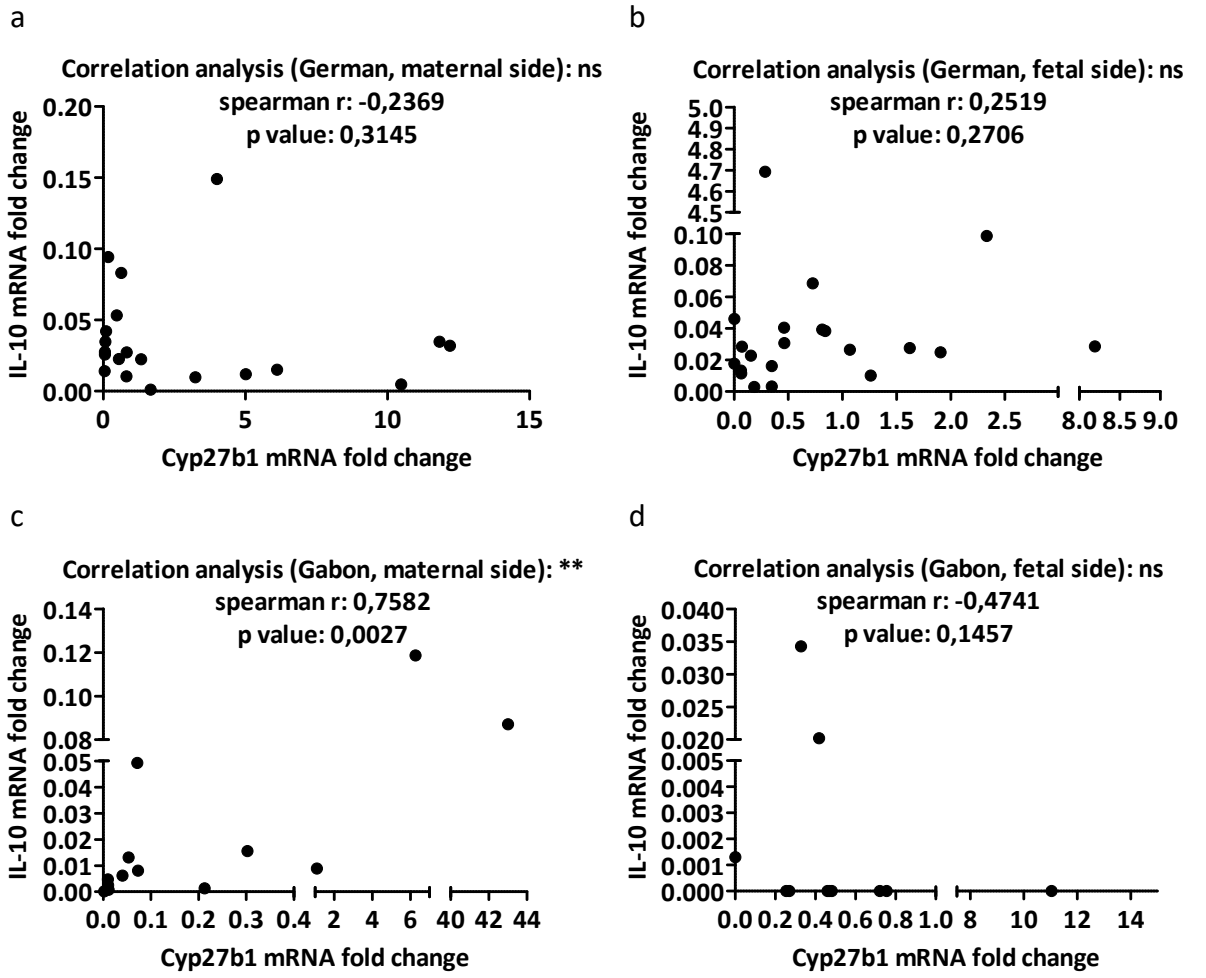

Figure 3.10: Correlation between Cyp27b1 and IL-10 only existent on maternal side of Gabonese placentas

Correlation analysis was performed between Cyp27b1 and IL-10 expression. Fetal and maternal side were anaylsed seperatly within both cohorts: Maternal side of the German placentas (a), fetal side of the German placentas (b), maternal side of the Gabonese placentas (c) and fetal side of the Gabonese placentas (d). *Asterisks show statistical significance of correlation (spearman) between the respective genes (*p<0,05, **p<0,01, ***p<0,001)*

In summary, our results suggest that there are distinct differences when comparing the cohort from helminth-endemic Gabon with the one from non-endemic Germany: Only in the Gabonese placentas a strong correlation between Cyp27b1 and respectively IFNy and IL-10 could be detected that was only vague (Cyp27b1-IFNy) or inexistent (Cyp27b1-IL10) within the German placentas.

The differences detected between the German and the Gabonese cohort concerning their placental gene expression patterns might even be more obvious when regarding a larger study group than ours.

3.2 Further analysis of the German cohort

As mentioned above, we had the possibility to collect more samples and information from the German patients. Thus, in the following the additional results from the German cohort will be described.

3.2.1 Maternal patient's history and clinical details of the mother, father and the child

The mothers were interviewed concerning their patient's history. Table 3.2 summarises the clinical details obtained from the mothers, the allergy status of the father and additionally the child's birth data.

Clinical details			
Parents		Child	
maternal age, years	33,5 ± 4,9	birth weight, g	3372,0 ± 501,5
median parity (min, max)	2 (1 ,5)	size, cm	52,9 ± 2,5
median gravity (min, max)	2 (1, 9)	% girls, % boys	41, 59
% vaginal delivery	100	umbilical artery pH	7,25 ± 0,10
% mothers with allergies	31,8	median 5 min. APGAR (min, max)	9 (7, 10)
% fathers with allergies	50,0		

Table 3.2: Clinical details of the mothers, fathers and their children

Apart from maternal age, parity and gravity, the mothers and fathers were questioned about their allergy history. The children's birth data was collected including birth weight, size, sex, umbilical artery pH and APGAR-Score. *If not labelled differently, data is shown as mean ± standart deviation.*

Five of the mothers stated to have a chronic disease, namely psoriasis, haemochromatosis, prolactinoma, asthma and heterozygous Factor V/II Mutation. Since those were none of the diseases specified in the list of exclusion criteria and also were not considered as confounders by the clinical study investigator, those mothers were included into the analysis.

3.2.1.1 Maternal and paternal allergy history

All mothers and fathers were surveyed concerning their allergy history. 32% of the mothers and 50 % of the fathers stated to suffer from allergies. Amongst those allergies were food-, pollen-, bee-, house dust mite-, animal hair-, drug- and washing powder-allergies. Table 3.3 shows the absolute number of mothers and fathers suffering from those specific allergies, indicating that allergies against pollen and house dust mites are the most common ones in our cohort.

	Affected mothers	Affected fathers
food	2	2
pollen	4	6
bee	0	1
house dust mite	5	4
animal hair	1	2
washing powder	1	0
drugs	1	0

Table 3.3: Distribution of allergies, absolute numbers of effected mothers and fathers are constituted

3.2.2 Course of pregnancy

The course of pregnancy was assessed by noting all medicine that was ingested during the last nine months, including vitamin D supplementation, and by noting any salience during pregnancy.

Half of the mothers ingested magnesium and/or iron if required, due to muscle spasm or anaemia. Three of them supplemented L-thyroxine because of hypothyroidism and another three injected Clexane® as thrombosis prophylaxis. One of the mothers was treated with Utrogest® (progesterone) and Unacid® (Sultamicillin) in the event of cervical insufficiency.

More than half of the mothers ingested dietary supplements containing for example vitamin D or folate. The most common product was Femibion®, which contains iodine, vitamins

B1/2/6/12, vitamin C, vitamin E, Biotin and Niacin besides vitamin D and folate. Since we were especially interested in vitamin D metabolism, we specifically analysed how many of the mothers digested this very vitamin. Figure 3.11 shows the proportion of mothers that stated to have supplemented vitamin D containing products during pregnancy. Most common products that are ingested during pregnancy, like e.g. Femibion®, contain 5-20 µg (=200-800 I.U.) of vitamin D_3 (Cholecalciferol). Regularity and accuracy of the intake was not assessed.

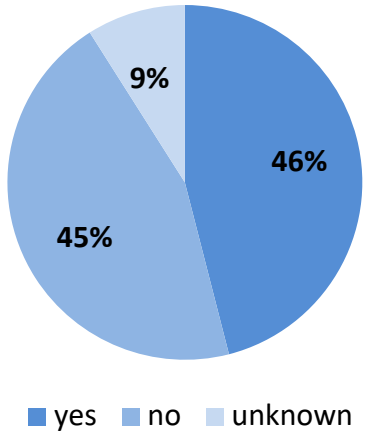

Figure 3.11: Proportion of mothers that supplemented vitamin D containing products during pregnancy

3.2.3 Serum parameters assessed within the German cohort

Heparinized blood was collected from both, the mother and the child (cord blood). As described in chapter 2.2.4, the plasma was aliquoted and stored after centrifugation of the heparinized blood. From those serum samples, levels of vitamin D, calcium and C reactive protein (CRP) were measured in the department of clinical chemistry of the *Klinikum rechts der Isar* (MRI).

3.2.3.1 Vitamin D supply of the mothers and their children is largely insufficient or even deficient

Within the human body, not only biologically active 1,25-hydroxyvitamin D but also its precursors can be measured. To get a closer insight into the body's sustenance with this vitamin, generally 25-hydroxyvitamin D is regarded as the best marker [Holick 2007]. Vitamin D

itself is able to upregulate the expression of its receptor VDR [Pike and Meyer 2010]. However, VDR regulation through vitamin D is tissue specific and differs in tissues that are not linked to calcium homeostasis [Gensure et al. 1998]. Therefore, we measured systemic 25-hydroxyvitamin D levels as a possible influencer or regulator of the placental VDR expression.

The results are shown in Figure 3.12, comparing firstly the maternal with the fetal levels (a). As presented, no significant difference could be evaluated between the two groups. As reviewed by Holick et al. [Holick 2007], the sustenance with vitamin D is generally seen as sufficient when the serum levels are higher than 30 ng/ml, whereas levels between 20-30 ng/ml are assessed as insufficient and levels below 20 ng/ml constitute a deficient vitamin D supply. Clearly, the great majority of mothers was not only insufficiently but even deficiently supplied with 25-hydroxyvitamin D and the same applies for the results from the fetal serum with only slightly higher levels of vitamin D. Also, knowing that 25-hydroxyvitamin D passes the placental barrier [Salle et al. 2000], we expected a connection between the fetal and the maternal levels and could indeed confirm our expectation when performing correlation analysis (Figure 3.12, b). Knowing that nearly half of the mothers stated to have supplemented vitamin D during pregnancy, we analysed whether in those subjects vitamin D levels were higher (Figure 3.12, c-d). When comparing those two groups, on both the fetal and the maternal side, no significant difference could be found. Only a trend towards higher levels could be seen in fetal and maternal vitamin D levels in the particular groups of mothers who stated to have supplemented vitamin D.

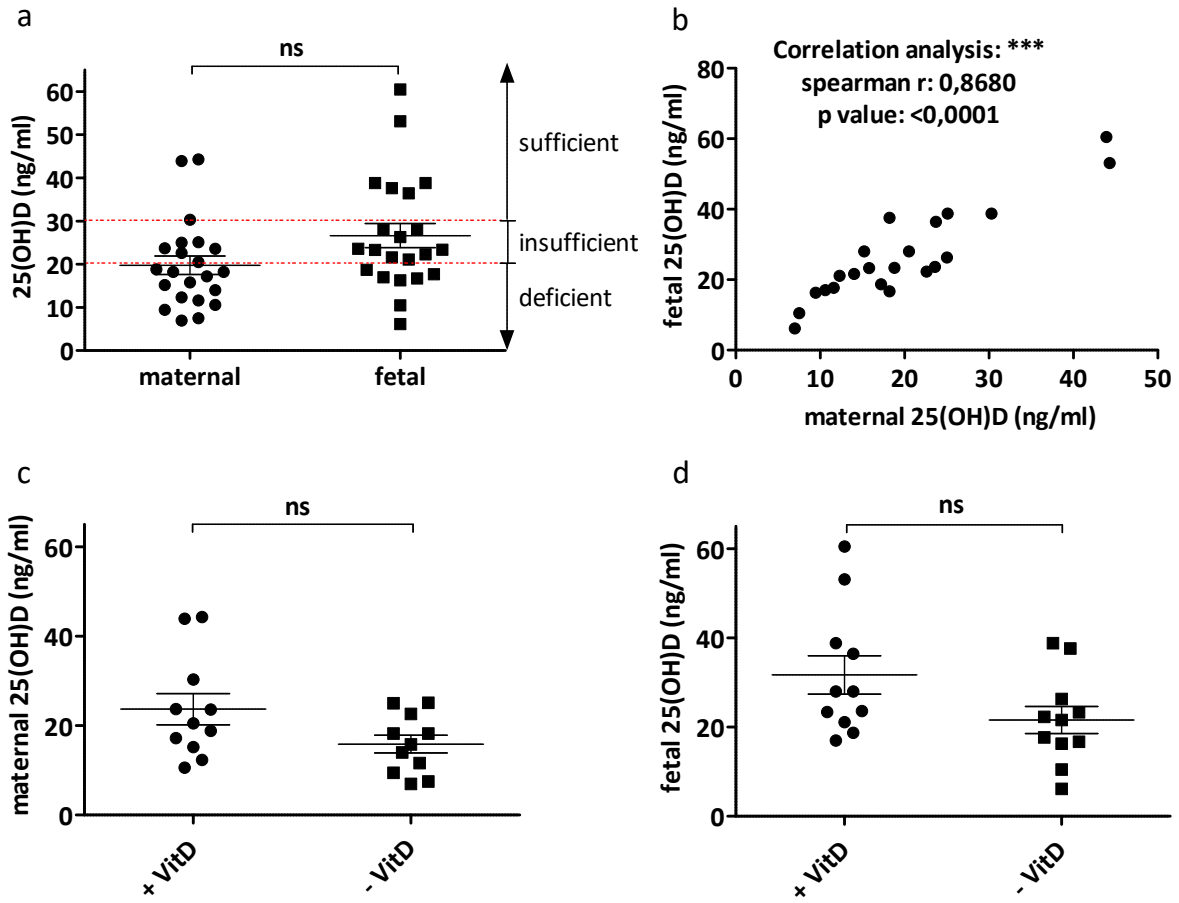

Figure 3.12: Maternal and fetal serum vitamin D levels.

Vitamin D levels were meassured in serum from maternal blood and cord blood. The results (a) were divided in three groups: sufficient (>30 ng/ml), insufficient (20-30 ng/ml) and deficient (<20 ng/ml). Results are shown as mean ± SEM. Correlation of maternal and fetal vitamin D levels (b) was analysed by spearman correlation analysis (Spearman r: 0,8680, p<0,0001). Differences between fetal and maternal vitamin D levels with (+ Vit D) and without (- Vit D) maternal vitamin D supplementaion during pregnancy (c,d) was analysed. *Data is shown as mean ± SEM, asterisks show statistical differences (Mann-Whitney test) between the groups indicated by the brackets (*p<0,05, **p<0,01, ***p<0,001).*

3.2.3.2 Calcium levels significantly higher within fetal compared to maternal serum

Since calcium serum levels are tightly regulated by vitamin D metabolism, we analysed the calcium levels of the mothers and children. Again, fetal and maternal levels were compared and a significant difference was observed. As expected, since the growing fetus has a greater demand of calcium, fetal calcium levels were significantly higher than those from the mothers.

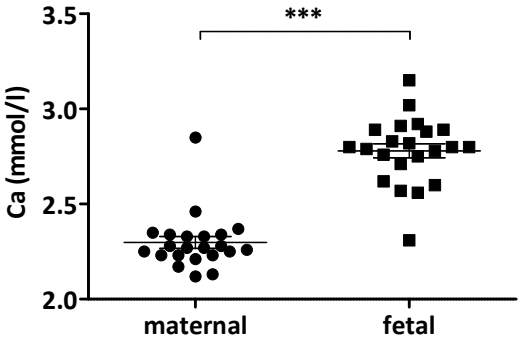

Figure 3.13: Maternal and fetal serum calcium levels

Calcium levels were measured from fetal and maternal serum. *Data is shown as mean ± SEM, asterisks show statistical differences (Mann-Whitney test) between the groups indicated by the brackets (*p<0,05, **p<0,01, ***p<0,001).*

3.2.3.3 Investigation of CRP levels as a marker for systemic inflammation

Since we were interested in local and also systemic inflammatory processes, we analysed CRP levels not only from the mothers but also from their children to get a closer insight of their systemic inflammatory state and so to say as internal control. CRP is produced in the liver and stands for an unspecific surrogate marker of inflammation.

The results from the CRP serum measurements are shown in Figure 3.14, a. Strikingly, CRP levels were lower than the detectable range in all fetal serum samples, which is not surprising because CRP cannot pass the placental barrier [Nielsen et al. 1990]. Thus, we did not expect elevated levels in the fetal serum of subjects born from healthy mothers. Still, already within the fetal liver, CRP can be produced [Pereira et al. 2014] and therefore levels might have been elevated if the offspring were at stress e.g. due to infection. Yet, we wanted to find an explanation for the relatively strong variation of the maternal CRP levels. For practical reasons, some of the maternal blood samples were taken before and some after birth. CRP is known to rise during birth and thus we analysed whether the levels were significantly higher in those samples collected after birth (see Figure 3.14, b). However, no significant difference could be observed between those two subgroups. Still, the assumed positive correlation between the duration of birth and the amount of CRP (see Figure 3.14, c) could be elucidated, demonstrating that it was not the collection point in time but the individual length of birth that led to the variation of maternal CRP levels.

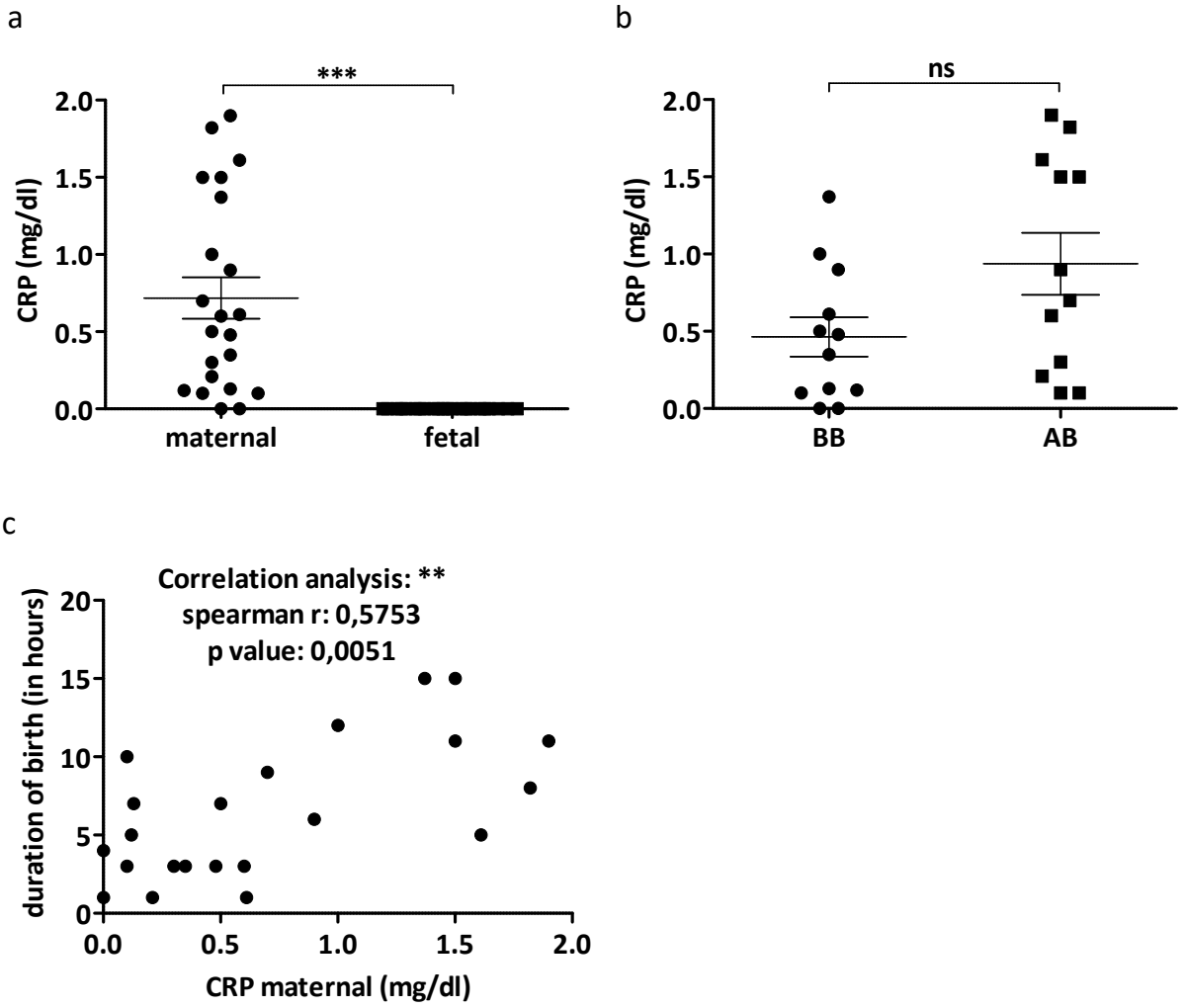

Figure 3.14: Maternal CRP levels are dependent on duration of birth.

Fetal and maternal CRP levels were measured (a). Maternal results were divided in two groups, contingent on whether the blood was taken before (BB) or after (AB) birth (b). Spearman correlation analysis was performed between maternal CRP levels and duration of birth. *Data (a,b) is shown as mean ± SEM, asterisks show statistical differences between the groups indicated by the brackets (*p<0,05, **p<0,01, ***p<0,001).*

3.2.4 Systemic 25(OH)D levels do not correlate with placental VDR expression

As mentioned above, vitamin D contributes to the regulation of its receptor VDR. However, the regulation within different tissues seems to be dependent on the tissue itself and in a trophoblast model with BeWo cells was shown to be dose dependent: Cell culture stimulation with 0,1 and 1 nmol/mL $1,25(OH)_2D$ lead to a reduced, whereas stimulations with 0,01 nmol/ml lead to an increased VDR expression [Knabl et al. 2014]. Therefore, we ana-

lysed a possible correlation between systemic 25(OH)D and placental VDR expression. No correlation could be detected in our samples (see Figure 3.15, a-d). It should be emphasized again that we only measured 25(OH)D and not the bioactive form $1,25(OH)_2D$. However, even if we had measured calcitriol levels, the local concentration within the placenta might have shown a different picture since, as described before (see chapter 3.1.3.3), the placenta itself can synthesize active calcitriol from 25(OH)D.

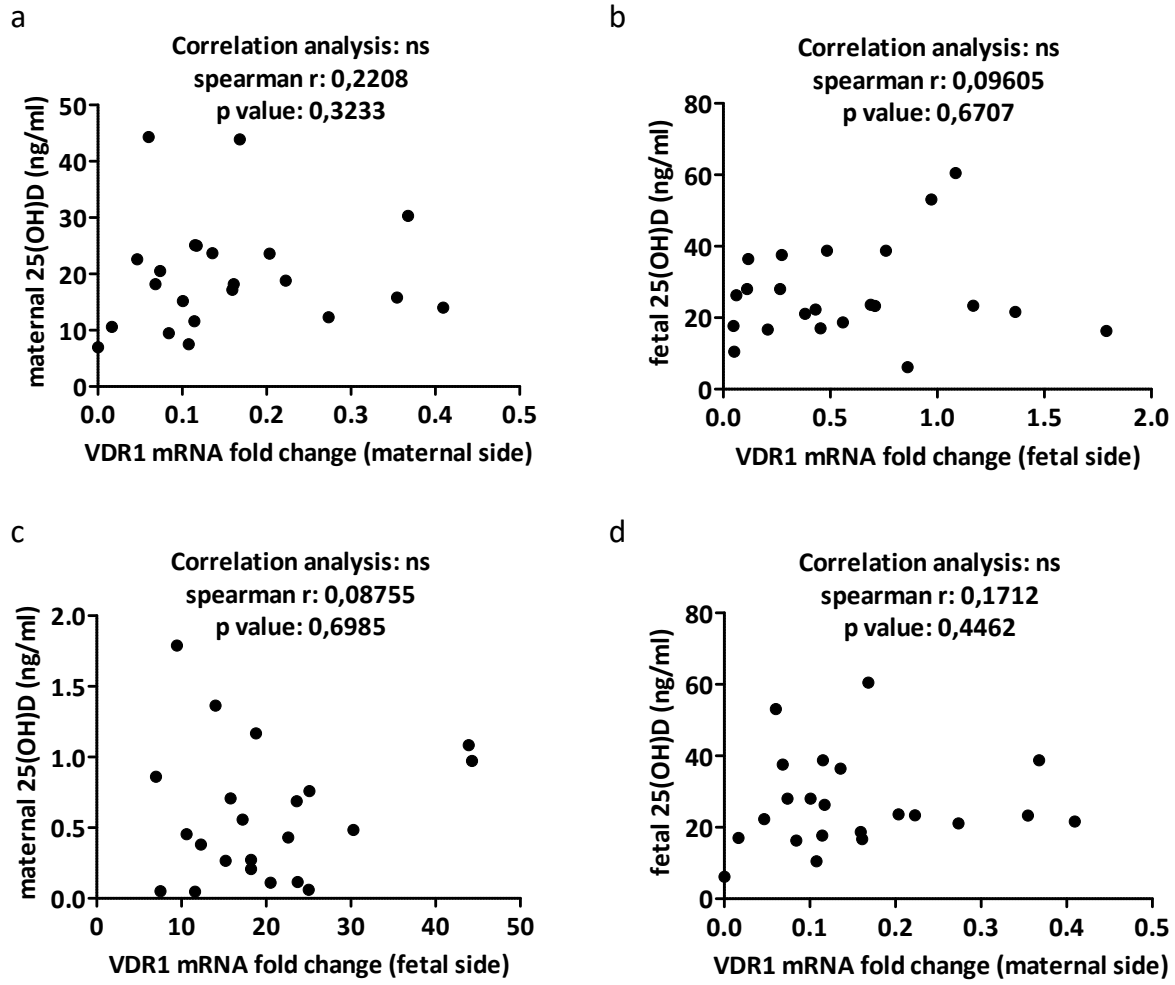

Figure 3.15: Placental VDR expression does not correlate with systemic maternal or fetal serum 25(OH)D levels

Correlation analysis between VDR1 expression and 25(OH)D serum levels was performed. Different groups were seperately analysed: Maternal 25(OH)D and maternal VDR1 (a), fetal 25(OH)D and fetal VDR1 (b), fetal VDR1 and maternal 25(OH)D (c) and maternal VDR1 and fetal 25(OH)D (d). *Asterisks show statistical significance of correlation (spearman) between VDR and 25(OH)D (*p<0,05, **p<0,01, ***p<0,001).*

3.3 Major findings resulting from the pilot study

Taken together we could show the following:

(1) Gene expression levels differ distinctly when comparing a cohort from helminth-endemic Gabon with non-endemic Germany:

a) VDR (respectively on the fetal and the maternal side) and Cyp27b1 (only on the maternal side) were significantly lower expressed in the Gabonese placentas.

b) The immunological milieu within the placental bed of the Gabonese patients is more inflammation prone than the one in German placentas, represented by higher IFNy and lower IL-10 and Foxp3 expression.

c) Placental Hsd3b1 expression was significantly lower in the Gabonese samples from the fetal but not the maternal side compared to the German samples.

(2) Fetal and maternal side of the placenta need to be investigated individually since gene expression levels can differ significantly.

(3) In our set of analysed genes only cytokine expression levels correlate between the fetal and maternal side of the placenta. The expression levels of genes related to vitamin D metabolism, pregnancy related Hsd3b1 and also Foxp3 did not correlate and are therefore possibly independent from each other.

(4) Correlation of gene expression patterns of the two cohorts differ:

a) Only within the fetal side of the Gabonese placentas a negative correlation between VDR and IFNy (Figure 3.7, d) and a positive correlation between VDR and IL-10 (Figure 3.8, d) was observed.

b) Only in the Gabonese placentas a strong positive correlation between Cyp27b1 and IFNy and IL-10 could be detected that was only vague (Cyp27b1-IFNy) or inexistent (Cyp27b1-IL10) within the German placentas.

(5) Additional results from German cohort:

d) Majority of mothers and their children from German cohort is not sufficiently supplied with vitamin D, whether the vitamin was ingested during pregnancy or not.

e) Serum 25(OH)D levels do not correlate with placental VDR expression.

f) Maternal CRP levels increase with duration of birth.

4 Discussion

4.1 Placental gene expression levels differ when comparing a cohort from helminth-endemic Gabon with non-endemic Germany

The placenta plays a pivotal role during pregnancy and functions as interface between the mother and the child. Accordingly, it seems almost self-explanatory that genes expressed in the placenta play a decisive role in placental physiology. The growing fetus, as it has been known for many years now, can suffer not only from short- but even further long-term consequences arising from impaired placental gene expression and thereby function [Gheorghe et al. 2010]. Fetal growth and development for instance was shown to be influenced by changed placental gene expression in malaria infected women, where upregulated placental TNF-alpha and IL-8 expression was correlated with intrauterine growth retardation [Moormann et al. 1999]. Another example is the impact of changed placental vitamin D metabolism. As summarized in [Knabl et al. 2017], altered placental VDR expression is associated with adverse pregnancy outcomes, e.g. the development of pre-eclampsia, gestational diabetes or preterm birth.

Within our cohort from helminth-endemic Gabon, the overall placental gene expression levels of VDR, Cyp27b1, IL-10, Hsd3b1 and Foxp3 where lower and of IFNy were higher when compared to the German cohort. These general differences in gene expression levels might be due to various reasons and will be discussed in this section.

It is likely that environmental or genetic factors can influence placental gene expression. This might partly explain the diverging gene expression patterns comparing our German and Gabonese cohort (see section 3.1.2). For instance, a lack of nutrients, as it could have been the case in the Gabonese patients, was indicated in several studies to lead to instant health-damaging effects both in the placenta and the fetus (reviewed in [Gheorghe et al. 2010]). Still, most studies highlight altered gene expression in pathological conditions. In turn, comparisons of gene expression patterns of generally healthy cohorts, like in our study, are missing.

Another factor that might intensify the observed differences in gene expression levels between the German and Gabonese cohort however could be the technique of sampling. Wyatt et al described a dependency of gene expression levels on the specific patch of sampling within the placental disk, suggesting a reflection of local villous perfusion [Wyatt et al. 2005]. With this in mind, the samples were only collected from placental parts that were not macroscopically calcified and thereby probably less perfused. Possible influencers of the gene expression levels on the fetal side of the placenta might additionally have been the amniotic membranes. However, they were dissected carefully to avoid falsification of the results. Still, there may have been differences in sampling in the Gabonese and German groups, but these were minimized as much as possible through training.

Gabon was chosen for the comparison in our pilot study since helminth infections are highly endemic within this country's population. Still, only three of the included patients were tested helminth positive at the time of birth. Looking at the results of the different gene expression patterns in isolation and with the results of our murine studies in mind, the Gabonese results yet reflect the gene expression pattern that we would expect in placentas from chronically helminth infected subjects. Accordingly, just like in the placentas from the infected mice (see chapter 1.2.3), VDR and Hsd3b1 were downregulated compared to the German placentas. Additionally, higher levels of inflammation, reflected by upregulated IFNy and downregulated IL-10 compared to the German placentas, could be detected. The possibility that our Gabonese mothers had had contact to helminths before or even during pregnancy but before birth is high by all means. This raises the question whether not even erstwhile and elapsed or treated infections can have an impact on placental gene expression and thereby the child's developing immune system.

Additionally, it has to be mentioned that at least 9 of the 13 Gabonese patients received anti-parasitic treatment at the time of birth in form of maloxine, mebendazole, helminthox or albendazole. Medication of two of the patients was not known and remains unclear. Another two did not receive anti-parasitic medication. According to our collaboration partners in Gabon, anti-parasitic treatment during pregnancy or birth belongs to the routinely and precautionary prescribed medication. That alone, the difference in medication ingested in the two cohorts, might also have contributed to the measured differences in placental gene expression. As mentioned in chapter 1.2.2, studies performed in Uganda strengthen the assumption that anti-parasitic treatment during pregnancy might directly, and not only indi-

rectly through eradication of the parasite itself, affect placental gene expression [Mpairwe et al. 2011].

However, especially in our study, these assumptions remain rather speculative for three reasons: Firstly, our Gabonese cohort with only 13 patients is rather small. Therefore, additional trials with bigger participant numbers will be needed. Secondly, we do not have any information about the allergy and eczema status of the children and thereby cannot correlate our results with the susceptibility to allergies in those subjects. Thirdly and maybe most importantly, the differences we see between the German and Gabonese cohort might indeed in a large part be mediated through genetic or environmental factors additional to helminth infection or better said a helminth-endemic environment.

4.1.1 Gene expression levels differ between fetal and maternal sides of the placenta and the extend of the difference depends on the regarded cohort

As stated above, levels of placental gene expression most likely play an important role in pregnancy outcome and potentially later in the child's life. Still, specific localisations of certain genes or gene families within the placenta remain unclear. To our knowledge, most studies only compare placental gene expression between placentas from healthy and non-healthy patients that suffer for example from pre-eclampsia [Brew et al. 2016] or other pregnancy-related diseases. In doing so, they for instance only use samples from one side of the placenta. Still, Wyatt et al. reported that the specific placental site where samples were taken from the placental disk influence gene expression levels [Wyatt et al. 2005]. Likewise, regarding the results from the gene expression levels of the German cohort in our study, differences in gene expression between the fetal and maternal side of the placenta could be detected (see section 3.1.2). Both, VDR and Hsd3b1 were simultaneously higher expressed on the fetal side of the German placentas. This difference was not measured in the Gabonese cohort. This is a novel finding since none of the previously mentioned studies has addressed such "side-specific" differences. That is particularly interesting when taking into consideration that a majority of studies does not even describe from where exactly the analysed placenta samples were taken. Analysing samples from all different parts of the placenta might thereby change the results from many trials. It should even be considered that from each placenta multiple samples should be taken from both the fetal and the maternal side. It

might not be enough to take just one sample from each side and compare them to each other, since gene expression from central placental parts were shown to differ from those of more peripheral regions [Burton et al. 2014]. It would also be interesting to see how high the gene expression levels of the investigated genes are in the middle sections of the placenta. Thus, the physiological role of a higher expression of those genes on the fetal side of the placenta yet needs to be elucidated. The detected differences might be evoked through a different cell composition on the two sides: the maternal side with direct contact to the decidua is probably more populated with maternal immune cells like uNK cells or macrophages [Mori et al. 2016]. It would be particularly interesting to analyse specific cell markers (e.g. through qRT-PCR) of exemplary immune cells or also trophoblast cells on both placental sides in future investigations. This might give a hint towards what specific cells could be responsible for the detected differences.

To the best of our knowledge, only one other study investigated placental gene expression patterns from healthy patients in general and likewise reported systematic differences in placental gene expressions depending of the sampling site, namely fetal, maternal and middle sections of the placenta. Here, microarray analysis was performed to investigate differences in gene expression levels. Two examples of those over 200 differentially expressed genes are known to be involved in pre-eclampsia: neurokinin B and the VEGF receptor Fms-like tyrosine kinase 1. [Sood et al. 2006]

This highlights the importance of a separated analysis of the fetal and the maternal side of the placenta, especially when comparing gene expression levels in placentas from healthy and non-healthy (e.g. pre-eclampsia) patients.

Specifically, the VDR plays an important role within the feto-maternal interface that will be further discussed in the following section 4.1.2.

4.1.2 Specific role of VDR within the human placenta

The VDR plays a crucial role in the mediation of vitamin D signalling. Especially during pregnancy, locally produced 1,25$(OH)_2$D supports anti-inflammatory responses in maternal decidua [Evans et al. 2006] and fetal trophoblast cells: It dampens the expression of inflammatory cytokines like TNF-alpha or IFNy and upregulates the transcription of antimicrobial products like defensin β2 or cathelicidin [Wang et al. 2004]. Within the German cohort of

our study, the VDR was significantly higher expressed on the fetal side of the placenta (see section 3.1.2.1). With the mentioned functions of the vitamin D - VDR signalling in mind, a higher VDR expression on the fetal side of the placenta might play a role in building up a kind of additional “barrier” that could protect the growing child from infection or more generally inflammation. Still, this significant difference in VDR expression between the fetal and maternal side of the placenta could not be detected in the Gabonese cohort. Moreover, VDR expression was significantly lower on both sides of the Gabonese placentas compared to those from the German patients. Thereby, it could be stated that helminth infection or more carefully expressed life in a helminth-endemic region might lead to a dysregulation of the placental VDR. Apart from potential negative effects of a lower VDR expression in the Gabonese placentas, VDR downregulation might also promote beneficial effects for the growing fetus like the hypothesized lower allergy susceptibility later in life.

The balance between Th1 and Th2 cytokines might be shifted towards a pro-inflammatory Th1 milieu through VDR downregulation. The growing fetus or more specifically its developing immune system might in this way be biased towards a Th1 prone state and that again could decrease Th2 driven diseases like allergies or asthma during childhood. [Straubinger 2013, p. 122]

This assumption is strengthened by the known fact that vitamin D through its receptor VDR does contribute to the ratio of Th1/Th2 by promoting the development Th2 cells and suppressing Th1 cells [Sahebnasagh et al. 2017]. Therefore, through downregulation of the latter the Th1/Th2 balance might be shifted in favour of Th1 cells with hence long-term effects like lower predisposition to various immunological disorders as mentioned above.

4.1.3 Hsd3b1 as placental specific gene and the role of progesterone in pregnancy

The second gene which was significantly higher expressed in the German placentas was Hsd3b1 which is indispensable for the placental progesterone production [Simard et al. 1996]. The major role of this hormone during pregnancy is to maintain pregnancy; accordingly treatment with an anti-progesterone agent during early pregnancy induces abortion. During later stages of pregnancy, placental progesterone acts as guardian against preterm birth by maintaining myometrial quiescence [Mendelson et al. 2016]. Additionally, proges-

terone, through binding to its receptor, promotes important anti-inflammatory responses and is generally seen as an anti-inflammatory hormone [Hardy et al. 2006, Robinson and Klein 2012].

Apart from binding to the progesterone receptor, progesterone also has a high binding affinity to glucocorticoid receptors. On immune cells for example these are expressed to a greater extent than the progesterone receptor. Thereby, for instance in dendritic cells, progesterone can inhibit cytokine production induced by toll-like receptors. [Jones et al. 2010]

Within our German cohort, placental Hsd3b1 expression was detected to be significantly higher on the fetal side of the placenta. Notably, of all investigated genes Hsd3b1 expression was by far the highest, again underlining its indispensability during pregnancy. Equally to the VDR expression pattern, higher levels of Hsd3b1 on the fetal side of the placenta might play a role in the protection of the fetus. The latter might accordingly be shielded against inflammation or infection by higher expression of Hsd3b1 on the fetal side of the placenta and thus higher local progesterone levels. However, and also similarly to the VDR, Hsd3b1 expression was found to be significantly lower in the Gabonese placentas and in the latter no significant difference between the fetal and maternal side could be detected. Emphasizing again that we only consulted gene expression levels at the time of birth, the situation might then again possibly be different in early or mid-pregnancy. Regardless of all progesterone-related functions of Hsd3b1, it should be stated that this gene belonged to the six overlapping genes that were found to be downregulated in the placentas from offspring which were less susceptible to AAI in our murine study (see chapter 1.2.3). Thus, and maybe in connection with the VDR, Hsd3b1 might take an important mechanistic role in the context of the hygiene hypothesis. The influence of Hsd3b1 here could be seen in parallel to the one stated above for the VDR , namely through a shift of the Th1/Th2 balance: During pregnancy, higher levels of progesterone dampen the induction of Th1 responses, consequently leading to a Th2 bias with exemplary higher amounts of for example IL-4 or IL-5 [Piccinni et al. 1995]. In turn, lower placental Hsd3b1 expression with thereby potentially lower progesterone synthesis, could promote a Th1 prone state in the child's developing immune system with eventually lower predisposition for Th2 mediated diseases later in life. In this regard, potentially low-level inflammatory responses within the placenta might be an important intermediate link. This assumption is strengthened by the fact that progesterone is indeed known to sup-

port Th2 responses and does dampen Th1 responses [Piccinni et al. 1995, Piccinni et al. 2000].

4.1.4 Placental milieu is more inflammation prone in samples from Gabonese mothers

Regarding pro-inflammatory IFNy and anti-inflammatory IL-10, placental inflammation at the time of birth was higher in the samples from the Gabonese cohort, particularly on the fetal side (see section 3.1.2.2). Here, IFNy was higher expressed and IL-10 was lower expressed compared to the German placentas. Additionally, Foxp3 as marker for regulatory T cells was significantly lower expressed. Those results were highly significant.

Cytokines, and in particular IFNy, play an important role during pregnancy. Apart from the production in natural killer cells and a pregnancy-related subset the uterine natural killer cells, production of this cytokine is known in placental trophoblast cells (reviewed in [Murphy et al. 2009]).

In this context, Straubinger et al. revealed IFNy to be vital for the offspring's lower susceptibility against AAI by use of IFNy-deficient, schistosome infected dams: here, in the lung tissue of the respective offspring higher levels of lung inflammation were found. These findings came along with elevated white blood cells within the lung tissue. The experiment was implemented with animals in the Th1-phase of schistosome infection, where beforehand a protective effect of maternal infection could be seen concerning the offspring's susceptibility towards allergic airway infection. This underlines the assumption that the pro-inflammatory IFNy could be pivotal in the mechanistic link between infection and protection against allergies. [Straubinger et al. 2014]

We hypothesised that low-level placental inflammation takes a pivotal role in influencing the offspring's developing immune system. Additionally, we proposed that this effect was mediated or supported at least by a downregulation of the placental VDR in placentas from helminth-endemic Gabon. Indeed, both lower expression of VDR and higher IFNy expression were detected in the Gabonese placentas. A link between placental vitamin D metabolism, most likely through its receptor VDR, and IFNy is already known and demonstrated in different studies [Diaz et al. 2009, Liu et al. 2011], yet we showed significant differences in placen-

tal gene expression when comparing a cohort from helminth-endemic Gabon with one from Germany.

One could assume that not only acute helminth infection during pregnancy but also former contact to helminths or at the minimum live in a helminth-endemic area can impact on placental gene expression. This might lead to beneficial long-term effects in the child, e.g. lower allergy susceptibility. The results of our presented human pilot study at least partly reflect the results from our study of Straubinger at al. [Straubinger et al. 2014], where maternal helminth infection in mice led to a downregulation of VDR and Hsd3b1 which was associated with low-level placental inflammation; a corresponding mechanism can thereby be assumed in humans. As mentioned before, further work and studies comparing infected and non-infected mothers with similar genetic background will be needed to be able to draw definite conclusions.

4.2 Correlation analysis as basis for further hypotheses

To investigate theoretical connections between different collected data, correlation analysis is suitable. Even though no causal link can be drawn from a statistically significant positive or negative correlation, it can be perfectly used to draw theoretical conclusions and for the development of new hypotheses. In the following sections, all performed correlation analysis will be discussed.

4.2.1 Gene expression levels of fetal and maternal side of the placenta do mostly not correlate

As mentioned above, only little is known about differences between the fetal and the maternal side of the placenta regarding especially gene expression levels. We could show that gene expression levels can indeed differ significantly between the fetal and the maternal side when regarding certain genes like VDR or IFNy (see section 3.1.2). Next, we wanted to find out whether the expression levels of the fetal and the maternal side correlated with each other. Generally, a correlation in this term could for example mean that the cells from one side directly regulate or influence the cells from the other side of the placenta. No correlation in contrast could suggest rather separated “systems” with different prior functions of each placental side.

In both of our study cohorts, no correlation could be found between the expression levels of fetal and maternal VDR, Cyp27b1, Foxp3 and Hsd3b1. Interestingly, only the cytokine expressions within the fetal and the maternal side (IFNy and IL-10) correlated positively in the German cohort, whereas in the Gabonese cohort a positive correlation could only be found when regarding IL-10.

One placental side might "communicate" with the other by for instance biochemical messengers like hormones or by immunological cells of the placental bed through up- or down-regulation of cytokine synthesis. This might explain the positive correlation between the expression of IFNy/IL-10 of the fetal and the maternal side of the German cohort: Theoretically, higher cytokine expression on one of the placental sides could, through transportation with the blood stream, influence the cytokine expression on the other side. That in turn could lead to changed gene expression levels of other gene classes like VDR or Cyp27b1 in our case. However, this connection between the cytokine levels of the fetal and the maternal side might be interrupted when a certain threshold is overstepped: IFNy expression levels were significantly higher in the fetal side of the Gabonese compared to the German placentas (see section 3.1.2.2) and here, no correlation between the IFNy expression of the fetal and the maternal placental side was found. This could be interpreted as a protective decoupling when placental inflammation is comparatively high (in our case compared to the German cohort), in order to protect the fetus from "too much" inflammatory stimulus. However, it should be emphasized that higher RNA expression levels like measured in this study not necessarily lead to higher levels of the actual protein. Since all of these assumptions remain theoretical and only base on the results of our pilot study, further investigations will be needed to explore the crosstalk between the fetal and the maternal side of the placenta.

4.2.2 German and Gabonese placentas show different correlation pattern

Not only the gene expression levels varied between the German and the Gabonese cohort but also the correlation patterns were found to be dissimilar (see section 3.1.3). In brief, the correlation between vitamin D metabolism and inflammation, with IFNy and IL-10 as representatives, was stronger within the placentas from Gabonese subjects. Whereas no correlation could be found between VDR and IL-10 or IFNy within the German samples, a correlation could be found on the fetal side of the Gabonese placentas: VDR and IFNy correlated negatively whereas a positive correlation was found between the VDR and IL-10. This sup-

ports our assumption that infection or close contact to pathogens can upregulate placental inflammation. IFNy seems to be crucial in this regard (see section 4.1.4). Higher levels of this cytokine might directly downregulate the placental VDR with potential long-term effects on the developing immune system (see section 4.1.2).

Resembling results were revealed regarding the correlation between Cyp27b1 and the cytokines: Within the German cohort, a positive correlation could only be measured between IFNy and Cyp27b1 on the maternal side of the placenta. In the Gabonese samples Cyp27b1 and IFNy correlated on both placental sides. Additionally, on the maternal side a strong correlation between IL-10 and Cyp27b1 was detected. It could be concluded that in Gabonese or even more generally in central-African patients, where infections are a lot more likely than in Germany, local vitamin D metabolism is more tightly connected to placental inflammation. However, the causality remains unclear, although a connection between placental vitamin D metabolism and distinct pregnancy outcomes is known [Diaz et al. 2002, Fischer et al. 2007, Cho et al. 2013, Nguyen et al. 2015]. In pregnant women suffering from preeclampsia for example, a changed placental expression pattern of vitamin D metabolizing enzymes (e.g. lower Cyp27b1 expression) is just as well-known as simultaneously higher levels of inflammatory cytokines deriving from the feto-maternal interface [Barrera et al. 2015]. Also infectious diseases, including parasite infections like schistosomiasis, are known to increase placental cytokine composition that probably cause immune cells to aggregate within the placenta: In placentas from mothers with *Plasmodium falciparum* infection, higher levels of exemplary IFNy and TNF-alpha were measured in plasma from the intervillous space [Suguitan et al. 2003]. Accordingly, placental blood levels of TNF-alpha and IL-6 were found to be increased in patients with Schistosoma japonicum infection [Kurtis et al. 2011]. With this in mind, our assumption is that prenatal influencing factors, for instance maternal infection [Mpairwe et al. 2014] or completely different ones like farm exposure [von Mutius 2012], can influence childhood health mechanistically through changed placental gene expression and thereby physiology. The specific role of vitamin D status during pregnancy on the child's health later in life will be further discussed in chapter 4.3.1.

We found a by far stronger and clearer correlation between Cyp27b1 and IFNy in the Gabonese placentas compared to the German samples. This observation might be linked to the generally higher expression levels of pro-inflammatory IFNy within the placenta. Additionally to the VDR, it has been known for long that the placenta expresses all necessary compo-

nents of the vitamin D signalling, including the vitamin D activating enzyme Cyp27b1 and the katabolic enzyme Cyp24a1 [Gray et al. 1979, Weisman et al. 1979]. Thereby, the placenta can synthesize active $1,25(OH)_2D$ out of 25(OH)D and additionally can inactivate it again through hydroxylation at C24 [Avila et al. 2007]. By an upregulation of Cyp27b1 and consequently increased $1,25(OH)_2D$ synthesis, the placental trophoblast cells themselves can react to an adverse immunological milieu by repression of pro-inflammatory cytokines that are for instance induced by TNF-alpha [Diaz et al. 2009]. This might explain the stronger correlation between Cyp27b1 and IFNy in the Gabonese placentas, where inflammation was generally higher. Similar to macrophages, in trophoblast cells production of the antimicrobial protein cathelicidin can be enhanced through intracrine $1,25(OH)_2D$ synthesis [Liu et al. 2009]. Thus, also within the placenta, bioactive vitamin D can remarkably shape immune responses [White 2008].

Taken together, the positive correlation between Cyp27b1 and IFNy in the placentas from our Gabonese cohort could be interpreted as follows: Cyp27b1 might be upregulated in case of exceeding a certain threshold of inflammation, in our case surrogated by IFNy expression. Cyp27b1 and an enhanced production of bioactive vitamin D might therefore act as counterbalance for inflammation.

4.3 German cohort

4.3.1 Serum 25(OH)D levels in the majority insufficient or deficient

Within the German cohort, beyond investigating placental gene expression levels, we analysed vitamin D serum levels. 25(OH)D or calcidiol is generally used to assess human vitamin D status which is considered sufficient when levels are higher than 30 ng/ml [Holick and Chen 2008]. The majority of the German mothers were inadequately supplied with this vitamin and the biggest group showed not only insufficient but even deficient vitamin D levels less than 20 ng/ml of 25(OH)D (see section 3.2.3.1). This supports the general opinion that a high proportion of the German population is undersupplied with vitamin D [Hintzpeter et al. 2008]. Even though more of the babies were sufficiently supplied, the majority also showed insufficient or deficient levels. Generally, since exposure to sunlight is essential for the vitamin D synthesis [Holick 2003], seasonal differences in vitamin D sustenance are to be ex-

pected. However, all births and thereby sample collection took place in July and August 2015 where solar radiation was high. Still, the results were not surprising since vitamin D deficiency is a known and common global problem and some even term vitamin D deficiency to be pandemic [Holick 2008, Cashman et al. 2016]. A subpopulation vulnerable for and endangered by vitamin D deficiency is pregnant women [Schroth et al. 2005].

However, also in this subgroup, insufficient supply with vitamin D is no rarity. Here, it is associated with higher rates of gestational diabetes, pre-eclampsia or preterm birth. Still, treatment of vitamin D deficiency during pregnancy is currently not recommended routinely, since evidence for direct health benefits for the mother and child are missing. [WHO 2012]

Furthermore, apart from adverse pregnancy outcomes, a role of vitamin D status during pregnancy for the child's health later in life has been proposed (summarized in [Erkkola 2011]). Maternal lack of vitamin D during pregnancy for example is associated with the child being at higher risk for autoimmune diseases like multiple sclerosis [Munger et al. 2016] or childhood eczema [Wei et al. 2016]. However, excessive maternal vitamin D levels can probably adversely affect the child's susceptibility to eczema or asthma, too [Gale et al. 2008]. Cord-blood 25(OH)D levels significantly correlated with those from the mothers in our cohort. Thereby, maternal vitamin D status can be seen as predictive for the child's vitamin D status at birth, a predictive cohesion that was already stated in the 1980s [Hollis and Pittard 1984]. Low cord-blood vitamin D levels were then again shown to be inversely associated with childhood wheezing and respiratory infections [Belderbos et al. 2011, Camargo et al. 2011]. With all those potential adverse health-consequences in mind, it is alarming that the majority of our study population was not sufficiently supplied with vitamin D.

It will be interesting to see how high the vitamin D serum levels are in the Gabonese mothers and children in future studies. Three variants will matter in this context: Firstly, Gabon as central African country lies close to the equator, thereby solar radiation is a lot higher than in Germany. This might be the most important factor that could lead to higher vitamin D levels in Gabonese people (compared to Germany). However and secondly, dark-skinned humans are known to have generally lower 25(OH)D levels compared to lighter skinned people. In a study performed in Switzerland for instance, vitamin D levels were measured in pregnant women and simultaneously their skin colour was investigated, showing significantly higher rates of vitamin D deficiency in darker skinned women [Richard et al. 2017].

Similar results were found by Powe et al., investigating Vitamin D levels white and black skinned Americans: the dark-skinned cohort had lower levels of the investigated vitamin. The same group however highlights the third and probably very important factor: Apart from Vitamin D levels being lower in darker skinned people, simultaneously lower levels of DPB were measured, consequently leading to comparable levels of bioavailable vitamin D. The hydrophobic vitamin D is in a large part bound to its carrier protein DBP which thereby distinctly influences its bioavailability. Consequently, it seems to be too easy to only compare 25(OH)D levels but instead the DBP should always be analysed in parallel. [Powe et al. 2013]

Intervention trials are ethically difficult, especially in pregnant women. Nevertheless, it seems necessary that future studies are implemented to build uniform and safe supplementation guidance for pregnant women. Most mothers of our cohort that supplemented vitamin D did this in form of combination tablets that contain several different vitamins like for instance Femibion®. Femibion® contains 800 I.U. of vitamin D, an amount that is found in most of the products recommended for pregnancy. In light of the fact that we could not find significant differences in the vitamin D serum levels of mothers that did or did not supplement vitamin D (see Figure 3.12, c-d), the question should be stated how effective such supplementation is. Apart from the assumption that the amount of daily vitamin D intake is simply too low in the most commonly recommended products for pregnancy, also the intake itself might be flawed in many cases: It is recommended in the instruction leaflets to take it together with a big amount of fluids and in relation with food to be able to resorb this fat-soluble vitamin at all.

4.3.2 Systemic 25(OH)D levels do not correlate with placental VDR expression

The VDR is known to be internally regulated through vitamin D itself. To investigate whether we could find a connection between placental VDR expression and systemic levels of 25(OH)D, we performed correlation analysis between the two measured variables. No correlation could be found, not when correlating the results from either fetal or maternal side with each other nor when correlating fetal with maternal results (see section 3.2.4). Bioactive $1,25(OH)_2D$ interestingly regulates the VDR itself [Santiso-Mere et al. 1993]. This autoregulation happens directly through enhancers within the VDR gene [Zella et al. 2007]. An autologous upregulation of the VDR by $1,25(OH)_2D$ has for long been known and was

demonstrated in different cell lines [Costa et al. 1985]. Most studies only focus on the VDR regulation by 1,25$(OH)_2$D and not its precursor 25(OH)D. That might explain why we could not reveal a correlation between vitamin D and placental VDR, since we only measured 25(OH)D as surrogate for the individual's sustenance with this vitamin. Additionally, not systemic but potentially local vitamin D levels within the placenta might be primarily responsible for placental VDR regulation. Moreover, an important notion might be that the VDR autoregulation is tissue specific and might thereby especially differ between tissues that are involved in calcium homeostasis, like bone or kidney, or others like the placenta [Gensure et al. 1998, Knabl et al. 2014].

In BeWo cells that are often used as trophoblast model, Knabl et al. could demonstrate a concentration dependant VDR regulation by calcitriol. They were the first to show that specific concentrations of 1,25$(OH)_2$D can not only up- but also downregulate VDR in trophoblast cells. [Knabl et al. 2014]

Since 25(OH)D is the direct precursor of 1,25$(OH)_2$D, it stands to reason that 1,25$(OH)_2$D depends on 25(OH)D levels, at least in patients without kidney diseases as the main 1,25$(OH)_2$D production takes place in the kidneys [Deutzmann 2012]. Thereby, one could conclude that also systemic 1,25$(OH)_2$D levels do not correlate with placental VDR, since in our study its precursor does not show correlation with placental VDR, too. This assumption however remains speculative and needs to be verified in the future. Nevertheless, as mentioned above, it might not be the systemic vitamin D levels but the locally within the placenta produced ones that regulate placental VDR expression. This assumption is strengthened by the observation that placental cells can synthesize 1,25$(OH)_2$D and produce tissue concentrations that can even exceed serum concentrations [Zerwekh and Breslau 1986]. In summary, it might be interesting to measure placental vitamin D levels in future studies.

Then again, it should be added that to definitely analyse the amount of bioavailable vitamin D, also its transporter DBP should be taken in account (see section 4.3.1 and [Powe et al. 2013]).

5 Résumé and Outlook

It has been proposed for a long time that an increase of allergic and autoimmune diseases can at least partly be attributed to a decreasing incidence of infectious diseases [Strachan 1989]. The original idea which focused on bacterial and viral infections has been expanded towards parasites and particularly helminths as “balancing” factors counteracting the incidence and course of allergies and autoimmune diseases [Versini et al. 2015]. Apart from immune regulatory processes that are induced by such chronic helminth infections and which counterbalance allergies in infected hosts, such “protective” effects have recently been observed in the next (uninfected) generation: Here, offspring from chronically infected mothers showed strongly suppressed development of allergic airway inflammation when triggered with a strong allergen [Straubinger et al. 2014]. These observations and its underlying mechanism have important implications other than only the occurrence of allergic diseases. It is likely that for instance vaccine efficacy, the development of autoimmune diseases or other non-communicable diseases might be affected. The underlying mechanisms of this feto-maternal crosstalk however remain largely unclear. In this context, we assume the placenta as the connecting organ and simultaneously important barrier between mother and child to play a pivotal role. Many different gene classes are expressed here. Thereby, it is hypothesized that altered local (placental) gene expression patterns play a major role in the context of how environmental factors shape the health of the developing child. In a murine study, we could reveal that placental VDR and Hsd3b1 expression might take a leading role in this regard: Downregulation of both genes was detected in placentas from helminth infected mothers and this correlated significantly with a lower allergy susceptibility of the offspring [Straubinger et al. 2014]. These findings are particularly interesting in terms of the immunomodulatory functions of vitamin D through its receptor VDR. Generally, the VDR is known to promote anti-inflammatory effects by enhancing the development of Tregs and damping the release of pro-inflammatory cytokines like IFNy [Mora et al. 2008]. Additionally, several studies revealed that adequate maternal vitamin D levels can promote a protective effect in the child against the later development of for example wheezing [Camargo et al. 2007] or asthma [Litonjua and Weiss 2007].

Since allergies are much less common in helminth-endemic areas like the African country Gabon, in contrast to high-income countries like Germany, we performed a pilot study and compared gene expression levels between Gabonese and German placental samples focus-

ing on VDR, Cyp27b1, IFNy, IL-10, Hsd3b1 and Foxp3. The samples were taken from both the fetal and the maternal side of the organ. Thus, we could not only investigate differences in expression levels between the two cohorts, but were also able to analyse local differences inside the organ. Additionally, correlation patterns between the different analysed genes of one placental side and supplementary between the fetal and the maternal placental side were gathered. Population-based differences were detected both in gene expression levels as well as in correlation patterns. For example, VDR and Hsd3b1 expression were found to be significantly lower in the samples from helminth-endemic Gabon which resembled findings from our previous experimental murine studies using the maternal helminth infection model. Additionally, the placental milieu in the Gabonese cohort was more inflammation-prone represented by upregulated IFNy and simultaneously downregulated IL-10 expression. In addition, we could demonstrate that the fetal and the maternal side should be analysed separately when regarding the human placenta, since gene expression levels can differ significantly from one side to the other. Interestingly, most gene expression levels did not correlate between the fetal and the maternal side of the placenta in both cohorts, apart from the cytokine expression levels which did correlate in large part.

As summarized above, the two cohorts diverged not only in gene expression levels, but also concerning the analysed correlation patterns. In more detail, VDR expression for instance did not correlate with either IFNy or IL-10 within the German samples. However, in the Gabonese samples, expression levels of fetal VDR positively correlated with those of IL-10, and negative correlation was observed between VDR and IFNy. We assume the generally higher levels of pro-inflammatory IFNy to be accountable for this finding. Thus, considering that this delicate feto-maternal border is constantly in contact with environmental challenges such as microbes, interdependent gene regulation only seems to occur once a certain threshold of inflammation (represented by IFNy) is reached. Nevertheless, one should be careful when interpreting correlation analyses, since a positive or negative correlation does not necessarily prove underlying causality.

Another major finding within this thesis was distinct differences in gene expression levels between the fetal and the maternal side of the placenta such as the expression of the VDR which was significantly higher on the fetal compared to the maternal side of the German placentas. Interestingly, gene expression levels of the fetal side did mostly not correlate with those from the maternal side. These differences suggest that gene expression within these

two anatomical sites is regulated via distinct mechanisms and might thus even fulfil different functions. Nevertheless, the expression levels per se might be influenced for instance through soluble cytokines like maternal IFNy or IL-10 which however was not investigated in this study since we only analysed gene expression levels. Interestingly though, the only gene classes that correlated between the fetal and the maternal side were those of the analysed cytokines. One exception was the Gabonese IFNy expression, which did not correlate between the fetal and the maternal side. In theory, assuming that higher gene expression levels lead to higher levels of soluble cytokines, it might come to a deregulation when overstepping a specific threshold of inflammation (again represented through IFNy) as kind of protecting mechanism for the growing child.

To the best of our knowledge, this is the first study which investigates the influence of a helminth-endemic environment during pregnancy on placental gene regulation as a possible mechanistic link explaining transgenerational effects of environmental factors on the offspring's immune system. Nevertheless, our results stem from two very small cohorts that can be distinguished in their ethnic and geographic background. This as well as further limitations such as differences in nutrition, general hygiene standard and ethnical background were discussed in detail in the discussion section above. Thus, further studies to investigate possible mechanistic links between helminth infection, placental vitamin D metabolism and the child's health later in life are needed and have been initiated by our group within a new study (HELMVIT).

Presentations

- "Lunch seminar" presentation at the *Institut für Medizinische Mikrobiologie, Immunologie und Hygiene* (Klinikum rechts der Isar der Technischen Universität München, April 2016)
- Poster presentation at the "13th Congress of the International Society for Immunology of Reproduction and the European Society for Reproductive Immunology" (Erfurt, July 2016)

  poster titel: "Vitamin D receptor (VDR) and its immunological role within the placenta - Analysis of vitamin D metabolism and immunologically important genes at the feto-maternal interface"

  authors: Jutta Harder, Sonakshi Bhattacharjee, Eva Loffredo-Verde and Clarissa Prazeres da Costa

  abstract published in the "Journal of reproductive immunology", Vol. 115, June 2016
- "Mittagsteach" presentation at the *Frauenklinik rechts der Isar* (TUM, October 2016)
- ePoster at the "10th European Congress on Tropical Medicine and International Health" (Antwerpen, Oktober 2017)

  poster titel: "Vitamin D related placental gene expression in women living in helminth endemic Gabon"

  authors: Harder J., Esen M., Adegnika A.A., Yazdanbakhsh M., Prazeres da Costa C.

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Bibliography

Amoah et al. 2012

Amoah, A. S., Forson, A. G. and Boakye, D. A. (2012). "A review of epidemiological studies of asthma in Ghana." Ghana Med J 46(2 Suppl): p. 23-28.

Aranow 2011

Aranow, C. (2011). "Vitamin D and the immune system." J Investig Med 59(6): p. 881-886. doi: 10.2310/JIM.0b013e31821b8755.

Arck and Hecher 2013

Arck, P. C. and Hecher, K. (2013). "Fetomaternal immune cross-talk and its consequences for maternal and offspring's health." Nat Med 19(5): p. 548-556. doi: 10.1038/nm.3160.

Attallah et al. 2003

Attallah, A. M., Ghanem, G. E., Ismail, H. and El Waseef, A. M. (2003). "Placental and oral delivery of Schistosoma mansoni antigen from infected mothers to their newborns and children." Am J Trop Med Hyg 68(6): p. 647-651.

Avila et al. 2007

Avila, E., Diaz, L., Barrera, D., Halhali, A., Mendez, I., Gonzalez, L., Zuegel, U., Steinmeyer, A. and Larrea, F. (2007). "Regulation of Vitamin D hydroxylases gene expression by 1,25-dihydroxyvitamin D3 and cyclic AMP in cultured human syncytiotrophoblasts." J Steroid Biochem Mol Biol 103(1): p. 90-96. doi: 10.1016/j.jsbmb.2006.07.010.

Bach 2002

Bach, J. F. (2002). "The effect of infections on susceptibility to autoimmune and allergic diseases." N Engl J Med 347(12): p. 911-920. doi: 10.1056/NEJMra020100.

Baker et al. 2010

Baker, A. M., Haeri, S., Camargo, C. A., Jr., Espinola, J. A. and Stuebe, A. M. (2010). "A nested case-control study of midgestation vitamin D deficiency and risk of severe preeclampsia." J Clin Endocrinol Metab 95(11): p. 5105-5109. doi: 10.1210/jc.2010-0996.

Barrera et al. 2007

Barrera, D., Avila, E., Hernandez, G., Halhali, A., Biruete, B., Larrea, F. and Diaz, L. (2007). "Estradiol and progesterone synthesis in human placenta is stimulated by calcitriol." J Steroid Biochem Mol Biol 103(3-5): p. 529-532. doi: 10.1016/j.jsbmb.2006.12.097.

Barrera et al. 2008

Barrera, D., Avila, E., Hernandez, G., Mendez, I., Gonzalez, L., Halhali, A., Larrea, F., Morales, A. and Diaz, L. (2008). "Calcitriol affects hCG gene transcription in cultured human syncytiotrophoblasts." Reprod Biol Endocrinol 6: p. 3. doi: 10.1186/1477-7827-6-3.

Barrera et al. 2015

Barrera, D., Diaz, L., Noyola-Martinez, N. and Halhali, A. (2015). "Vitamin D and Inflammatory Cytokines in Healthy and Preeclamptic Pregnancies." Nutrients 7(8): p. 6465-6490. doi: 10.3390/nu7085293.

Belderbos et al. 2011

Belderbos, M. E., Houben, M. L., Wilbrink, B., Lentjes, E., Bloemen, E. M., Kimpen, J. L., Rovers, M. and Bont, L. (2011). "Cord blood vitamin D deficiency is associated with respiratory syncytial virus bronchiolitis." Pediatrics 127(6): p. e1513-1520. doi: 10.1542/peds.2010-3054.

Bennett et al. 1996

Bennett, W. A., Lagoo-Deenadayalan, S., Brackin, M. N., Hale, E. and Cowan, B. D. (1996). "Cytokine expression by models of human trophoblast as assessed by a semiquantitative reverse transcription-polymerase chain reaction technique." Am J Reprod Immunol 36(5): p. 285-294.

Bieber 2008

Bieber, T. (2008). "Atopic dermatitis." N Engl J Med 358(14): p. 1483-1494. doi: 10.1056/NEJMra074081.

Bikle 2009

Bikle, D. (2009). "Nonclassic actions of vitamin D." J Clin Endocrinol Metab 94(1): p. 26-34. doi: 10.1210/jc.2008-1454.

Bodansky et al. 1992

Bodansky, H. J., Staines, A., Stephenson, C., Haigh, D. and Cartwright, R. (1992). "Evidence for an environmental effect in the aetiology of insulin dependent diabetes in a transmigratory population." BMJ 304(6833): p. 1020-1022.

Bodnar et al. 2009

Bodnar, L. M., Krohn, M. A. and Simhan, H. N. (2009). "Maternal vitamin D deficiency is associated with bacterial vaginosis in the first trimester of pregnancy." J Nutr 139(6): p. 1157-1161. doi: 10.3945/jn.108.103168.

Bodnar et al. 2007

Bodnar, L. M., Simhan, H. N., Powers, R. W., Frank, M. P., Cooperstein, E. and Roberts, J. M. (2007). "High prevalence of vitamin D insufficiency in black and white pregnant women residing in the northern United States and their neonates." J Nutr 137(2): p. 447-452.

Boonstra et al. 2001

Boonstra, A., Barrat, F. J., Crain, C., Heath, V. L., Savelkoul, H. F. and O'Garra, A. (2001). "1alpha,25-Dihydroxyvitamin d3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells." J Immunol 167(9): p. 4974-4980.

Borish et al. 1996

Borish, L., Aarons, A., Rumbyrt, J., Cvietusa, P., Negri, J. and Wenzel, S. (1996). "Interleukin-10 regulation in normal subjects and patients with asthma." J Allergy Clin Immunol 97(6): p. 1288-1296.

Brew et al. 2016

Brew, O., Sullivan, M. H. and Woodman, A. (2016). "Comparison of Normal and Pre-Eclamptic Placental Gene Expression: A Systematic Review with Meta-Analysis." PLoS One 11(8): p. e0161504. doi: 10.1371/journal.pone.0161504.

Bulmer et al. 1991

Bulmer, J. N., Longfellow, M. and Ritson, A. (1991). "Leukocytes and resident blood cells in endometrium." Ann N Y Acad Sci 622: p. 57-68.

Burton et al. 2014

Burton, G. J., Sebire, N. J., Myatt, L., Tannetta, D., Wang, Y. L., Sadovsky, Y., Staff, A. C. and Redman, C. W. (2014). "Optimising sample collection for placental research." Placenta 35(1): p. 9-22. doi: 10.1016/j.placenta.2013.11.005.

Camargo et al. 2011

Camargo, C. A., Jr., Ingham, T., Wickens, K., Thadhani, R., Silvers, K. M., Epton, M. J., Town, G. I., Pattemore, P. K., Espinola, J. A., Crane, J., New Zealand, A. and Allergy Cohort Study, G. (2011). "Cord-blood 25-hydroxyvitamin D levels and risk of respiratory infection, wheezing, and asthma." Pediatrics 127(1): p. e180-187. doi: 10.1542/peds.2010-0442.

Camargo et al. 2007

Camargo, C. A., Jr., Rifas-Shiman, S. L., Litonjua, A. A., Rich-Edwards, J. W., Weiss, S. T., Gold, D. R., Kleinman, K. and Gillman, M. W. (2007). "Maternal intake of vitamin D during pregnancy and risk of recurrent wheeze in children at 3 y of age." Am J Clin Nutr 85(3): p. 788-795.

Cantorna 2010

Cantorna, M. T. (2010). "Mechanisms underlying the effect of vitamin D on the immune system." Proc Nutr Soc 69(3): p. 286-289. doi: 10.1017/S0029665110001722.

Cantorna et al. 2015

Cantorna, M. T., Snyder, L., Lin, Y. D. and Yang, L. (2015). "Vitamin D and 1,25(OH)2D regulation of T cells." Nutrients 7(4): p. 3011-3021. doi: 10.3390/nu7043011.

Cashman et al. 2016

Cashman, K. D., Dowling, K. G., Skrabakova, Z., Gonzalez-Gross, M., Valtuena, J., De Henauw, S., Moreno, L., Damsgaard, C. T., Michaelsen, K. F., Molgaard, C., Jorde, R., Grimnes, G., Moschonis, G., Mavrogianni, C., Manios, Y., Thamm, M., Mensink, G. B., Rabenberg, M., Busch, M. A., Cox, L., Meadows, S., Goldberg, G., Prentice, A., Dekker, J. M., Nijpels, G., Pilz, S., Swart, K. M., van Schoor, N. M., Lips, P., Eiriksdottir, G., Gudnason, V., Cotch, M. F., Koskinen, S., Lamberg-Allardt, C., Durazo-Arvizu, R. A., Sempos, C. T. and Kiely, M. (2016). "Vitamin D deficiency in Europe: pandemic?" Am J Clin Nutr 103(4): p. 1033-1044. doi: 10.3945/ajcn.115.120873.

Chen et al. 2007

Chen, S., Sims, G. P., Chen, X. X., Gu, Y. Y., Chen, S. and Lipsky, P. E. (2007). "Modulatory effects of 1,25-dihydroxyvitamin D-3 on human B cell differentiation." Journal of Immunology 179(3): p. 1634-1647.

Cho et al. 2013

Cho, G. J., Hong, S. C., Oh, M. J. and Kim, H. J. (2013). "Vitamin D deficiency in gestational diabetes mellitus and the role of the placenta." Am J Obstet Gynecol 209(6): p. 560 e561-568. doi: 10.1016/j.ajog.2013.08.015.

Cooke and Haddad 1989

Cooke, N. E. and Haddad, J. G. (1989). "Vitamin D binding protein (Gc-globulin)." Endocr Rev 10(3): p. 294-307. doi: 10.1210/edrv-10-3-294.

Cooper et al. 2006

Cooper, P. J., Chico, M. E., Vaca, M. G., Moncayo, A. L., Bland, J. M., Mafla, E., Sanchez, F., Rodrigues, L. C., Strachan, D. P. and Griffin, G. E. (2006). "Effect of albendazole treatments on the prevalence of atopy in children living in communities endemic for geohelminth parasites: a cluster-randomised trial." Lancet 367(9522): p. 1598-1603. doi: 10.1016/S0140-6736(06)68697-2.

Costa et al. 1985

Costa, E. M., Hirst, M. A. and Feldman, D. (1985). "Regulation of 1,25-dihydroxyvitamin D3 receptors by vitamin D analogs in cultured mammalian cells." Endocrinology 117(5): p. 2203-2210. doi: 10.1210/endo-117-5-2203.

Couper et al. 2008

Couper, K. N., Blount, D. G. and Riley, E. M. (2008). "IL-10: the master regulator of immunity to infection." J Immunol 180(9): p. 5771-5777.

Daniel et al. 2008

Daniel, C., Sartory, N. A., Zahn, N., Radeke, H. H. and Stein, J. M. (2008). "Immune modulatory treatment of trinitrobenzene sulfonic acid colitis with calcitriol is associated with a change of a T helper (Th) 1/Th17 to a Th2 and regulatory T cell profile." Journal of Pharmacology and Experimental Therapeutics 324(1): p. 23-33. doi: 10.1124/jpet.107.127209.

Devereux et al. 2007

Devereux, G., Litonjua, A. A., Turner, S. W., Craig, L. C., McNeill, G., Martindale, S., Helms, P. J., Seaton, A. and Weiss, S. T. (2007). "Maternal vitamin D intake during pregnancy and early childhood wheezing." Am J Clin Nutr 85(3): p. 853-859.

Deutzmann 2012

Deutzmann, R., 2012, Zelluläre Kommunikation. In: Rassow, J.H., et al. (Ed.), Duale Reihe Biochemie (p. 527-650). Thieme: Stuttgart.

Diaz et al. 2002

Diaz, L., Arranz, C., Avila, E., Halhali, A., Vilchis, F. and Larrea, F. (2002). "Expression and activity of 25-hydroxyvitamin D-1 alpha-hydroxylase are restricted in cultures of human syncytiotrophoblast cells from preeclamptic pregnancies." J Clin Endocrinol Metab 87(8): p. 3876-3882. doi: 10.1210/jcem.87.8.8730.

Diaz et al. 2009

Diaz, L., Noyola-Martinez, N., Barrera, D., Hernandez, G., Avila, E., Halhali, A. and Larrea, F. (2009). "Calcitriol inhibits TNF-alpha-induced inflammatory cytokines in human trophoblasts." J Reprod Immunol 81(1): p. 17-24. doi: 10.1016/j.jri.2009.02.005.

Dickie et al. 2010

Dickie, L. J., Church, L. D., Coulthard, L. R., Mathews, R. J., Emery, P. and McDermott, M. F. (2010). "Vitamin D-3 down-regulates intracellular Toll-like receptor 9 expression and Toll-like receptor 9-induced IL-6 production in human monocytes." Rheumatology 49(8): p. 1466-1471. doi: DOI 10.1093/rheumatology/keq124.

Drake and Reynolds 2010

Drake, A. J. and Reynolds, R. M. (2010). "Impact of maternal obesity on offspring obesity and cardiometabolic disease risk." Reproduction 140(3): p. 387-398. doi: 10.1530/REP-10-0077.

Droste et al. 2000

Droste, J. H., Wieringa, M. H., Weyler, J. J., Nelen, V. J., Vermeire, P. A. and Van Bever, H. P. (2000). "Does the use of antibiotics in early childhood increase the risk of asthma and allergic disease?" Clin Exp Allergy 30(11): p. 1547-1553.

Dusso et al. 2005

Dusso, A. S., Brown, A. J. and Slatopolsky, E. (2005). "Vitamin D." Am J Physiol Renal Physiol 289(1): p. F8-28. doi: 10.1152/ajprenal.00336.2004.

Ege et al. 2011

Ege, M. J., Mayer, M., Normand, A. C., Genuneit, J., Cookson, W. O., Braun-Fahrlander, C., Heederik, D., Piarroux, R., von Mutius, E. and Group, G. T. S. (2011). "Exposure to environmental microorganisms and childhood asthma." N Engl J Med 364(8): p. 701-709. doi: 10.1056/NEJMoa1007302.

Emanuel 1988

Emanuel, M. B. (1988). "Hay fever, a post industrial revolution epidemic: a history of its growth during the 19th century." Clin Allergy 18(3): p. 295-304.

Enquobahrie et al. 2008

Enquobahrie, D. A., Meller, M., Rice, K., Psaty, B. M., Siscovick, D. S. and Williams, M. A. (2008). "Differential placental gene expression in preeclampsia." Am J Obstet Gynecol 199(5): p. 566 e561-511. doi: 10.1016/j.ajog.2008.04.020.

Erkkola et al. 2009

Erkkola, M., Kaila, M., Nwaru, B. I., Kronberg-Kippila, C., Ahonen, S., Nevalainen, J., Veijola, R., Pekkanen, J., Ilonen, J., Simell, O., Knip, M. and Virtanen, S. M. (2009). "Maternal vitamin D intake during pregnancy is inversely associated with asthma and allergic rhinitis in 5-year-old children." Clin Exp Allergy 39(6): p. 875-882. doi: 10.1111/j.1365-2222.2009.03234.x.

Erkkola 2011

Erkkola, M. N., B.I.; Viljakainen, H.T. (2011). Maternal Vitamin D During Pregnancy and Its Role To Immune-mediated Diseases In The offspring. In: Litwack, G. (Ed.), Vitamins and the Immune System (pp. 240-254). Oxford: Elsevier.

Erlebacher 2013

Erlebacher, A. (2013). "Immunology of the maternal-fetal interface." Annu Rev Immunol 31: p. 387-411. doi: 10.1146/annurev-immunol-032712-100003.

Evans et al. 2006

Evans, K. N., Nguyen, L., Chan, J., Innes, B. A., Bulmer, J. N., Kilby, M. D. and Hewison, M. (2006). "Effects of 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 on cytokine production by human decidual cells." Biol Reprod 75(6): p. 816-822. doi: 10.1095/biolreprod.106.054056.

Ferreira et al. 2012

Ferreira, G. B., Kleijwegt, F. S., Waelkens, E., Lage, K., Nikolic, T., Hansen, D. A., Workman, C. T., Roep, B. O., Overbergh, L. and Mathieu, C. (2012). "Differential protein pathways in 1,25-dihydroxyvitamin d(3) and dexamethasone modulated tolerogenic human dendritic cells." J Proteome Res 11(2): p. 941-971. doi: 10.1021/pr200724e.

Fischer et al. 2007

Fischer, D., Schroer, A., Ludders, D., Cordes, T., Bucker, B., Reichrath, J. and Friedrich, M. (2007). "Metabolism of vitamin D3 in the placental tissue of normal and preeclampsia complicated pregnancies and premature births." Clin Exp Obstet Gynecol 34(2): p. 80-84.

Friedman et al. 2007

Friedman, J. F., Mital, P., Kanzaria, H. K., Olds, G. R. and Kurtis, J. D. (2007). "Schistosomiasis and pregnancy." Trends Parasitol 23(4): p. 159-164. doi: 10.1016/j.pt.2007.02.006.

Gale et al. 2008

Gale, C. R., Robinson, S. M., Harvey, N. C., Javaid, M. K., Jiang, B., Martyn, C. N., Godfrey, K. M., Cooper, C. and Princess Anne Hospital Study, G. (2008). "Maternal vitamin D status during pregnancy and child outcomes." Eur J Clin Nutr 62(1): p. 68-77. doi: 10.1038/sj.ejcn.1602680.

Gensure et al. 1998

Gensure, R. C., Antrobus, S. D., Fox, J., Okwueze, M., Talton, S. Y. and Walters, M. R. (1998). "Homologous up-regulation of vitamin D receptors is tissue specific in the rat." J Bone Miner Res 13(3): p. 454-463. doi: 10.1359/jbmr.1998.13.3.454.

Gheorghe et al. 2010

Gheorghe, C. P., Goyal, R., Mittal, A. and Longo, L. D. (2010). "Gene expression in the placenta: maternal stress and epigenetic responses." Int J Dev Biol 54(2-3): p. 507-523. doi: 10.1387/ijdb.082770cg.

Gollwitzer and Marsland 2015

Gollwitzer, E. S. and Marsland, B. J. (2015). "Impact of Early-Life Exposures on Immune Maturation and Susceptibility to Disease." Trends Immunol 36(11): p. 684-696. doi: 10.1016/j.it.2015.09.009.

Gray et al. 1979

Gray, T. K., Lester, G. E. and Lorenc, R. S. (1979). "Evidence for extra-renal 1 alpha-hydroxylation of 25-hydroxyvitamin D3 in pregnancy." Science 204(4399): p. 1311-1313.

Guillot et al. 2010

Guillot, X., Semerano, L., Saidenberg-Kermanac'h, N., Falgarone, G. and Boissier, M. C. (2010). "Vitamin D and inflammation." Joint Bone Spine 77(6): p. 552-557. doi: 10.1016/j.jbspin.2010.09.018.

Gustafsson et al. 2008

Gustafsson, C., Mjosberg, J., Matussek, A., Geffers, R., Matthiesen, L., Berg, G., Sharma, S., Buer, J. and Ernerudh, J. (2008). "Gene expression profiling of human decidual macrophages: evidence for immunosuppressive phenotype." PLoS One 3(4): p. e2078. doi: 10.1371/journal.pone.0002078.

Hardy et al. 2006

Hardy, D. B., Janowski, B. A., Corey, D. R. and Mendelson, C. R. (2006). "Progesterone receptor plays a major antiinflammatory role in human myometrial cells by antagonism of nuclear factor-kappaB activation of cyclooxygenase 2 expression." Mol Endocrinol 20(11): p. 2724-2733. doi: 10.1210/me.2006-0112.

Helming et al. 2005

Helming, L., Bose, J., Ehrchen, J., Schiebe, S., Frahm, T., Geffers, R., Probst-Kepper, M., Balling, R. and Lengeling, A. (2005). "1 alpha,25-dihydroxyvitamin D-3 is a potent suppressor of interferon gamma-mediated macrophage activation." Blood 106(13): p. 4351-4358. doi: 10.1182/blood-2005-03-1029.

Hintzpeter et al. 2008

Hintzpeter, B., Mensink, G. B., Thierfelder, W., Muller, M. J. and Scheidt-Nave, C. (2008). "Vitamin D status and health correlates among German adults." Eur J Clin Nutr 62(9): p. 1079-1089. doi: 10.1038/sj.ejcn.1602825.

Holick 2003

Holick, M. F. (2003). "Vitamin D: A millenium perspective." J Cell Biochem 88(2): p. 296-307. doi: 10.1002/jcb.10338.

Holick 2007

Holick, M. F. (2007). "Vitamin D deficiency." N Engl J Med 357(3): p. 266-281. doi: 10.1056/NEJMra070553.

Holick 2008

Holick, M. F. (2008). "The vitamin D deficiency pandemic and consequences for nonskeletal health: mechanisms of action." Mol Aspects Med 29(6): p. 361-368. doi: 10.1016/j.mam.2008.08.008.

Holick et al. 2011

Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., Gordon, C. M., Hanley, D. A., Heaney, R. P., Murad, M. H., Weaver, C. M. and Endocrine, S. (2011). "Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline." J Clin Endocrinol Metab 96(7): p. 1911-1930. doi: 10.1210/jc.2011-0385.

Holick and Chen 2008

Holick, M. F. and Chen, T. C. (2008). "Vitamin D deficiency: a worldwide problem with health consequences." Am J Clin Nutr 87(4): p. 1080S-1086S.

Hollis and Pittard 1984

Hollis, B. W. and Pittard, W. B., 3rd (1984). "Evaluation of the total fetomaternal vitamin D relationships at term: evidence for racial differences." J Clin Endocrinol Metab 59(4): p. 652-657. doi: 10.1210/jcem-59-4-652.

Holmes et al. 2009

Holmes, V. A., Barnes, M. S., Alexander, H. D., McFaul, P. and Wallace, J. M. (2009). "Vitamin D deficiency and insufficiency in pregnant women: a longitudinal study." Br J Nutr 102(6): p. 876-881. doi: 10.1017/S0007114509297236.

ISAAC 1998

ISAAC (1998). "Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee." Lancet 351(9111): p. 1225-1232.

Jokhi et al. 1997

Jokhi, P. P., King, A. and Loke, Y. W. (1997). "Cytokine production and cytokine receptor expression by cells of the human first trimester placental-uterine interface." Cytokine 9(2): p. 126-137. doi: 10.1006/cyto.1996.0146.

Jones et al. 2010

Jones, L. A., Kreem, S., Shweash, M., Paul, A., Alexander, J. and Roberts, C. W. (2010). "Differential modulation of TLR3- and TLR4-mediated dendritic cell maturation and function by progesterone." J Immunol 185(8): p. 4525-4534. doi: 10.4049/jimmunol.0901155.

King et al. 1998

King, C. L., Malhotra, I., Mungai, P., Wamachi, A., Kioko, J., Ouma, J. H. and Kazura, J. W. (1998). "B cell sensitization to helminthic infection develops in utero in humans." J Immunol 160(7): p. 173-176.

Knabl et al. 2014

Knabl, J., Huttenbrenner, R., Hutter, S., Gunthner-Biller, M., Riedel, C., Hiden, U., Kainer, F., Desoye, G. and Jeschke, U. (2014). "Gestational Diabetes Mellitus Upregulates Vitamin D Receptor in Extravillous Trophoblasts and Fetoplacental Endothelial Cells." Reprod Sci: 22(3): p. 358-6 doi: 10.1177/1933719114542020.

Knabl et al. 2017

Knabl, J., Vattai, A., Ye, Y., Jueckstock, J., Hutter, S., Kainer, F., Mahner, S. and Jeschke, U. (2017). "Role of Placental VDR Expression and Function in Common Late Pregnancy Disorders." Int J Mol Sci 18(11): p. 2340 doi: 10.3390/ijms18112340.

Kuo et al. 2013

Kuo, C. H., Kuo, H. F., Huang, C. H., Yang, S. N., Lee, M. S. and Hung, C. H. (2013). "Early life exposure to antibiotics and the risk of childhood allergic diseases: an update from the perspective of the hygiene hypothesis." J Microbiol Immunol Infect 46(5): p. 320-329. doi: 10.1016/j.jmii.2013.04.005.

Kurtis et al. 2011

Kurtis, J. D., Higashi, A., Wu, H. W., Gundogan, F., McDonald, E. A., Sharma, S., PondTor, S., Jarilla, B., Sagliba, M. J., Gonzal, A., Olveda, R., Acosta, L. and Friedman, J. F. (2011). "Maternal Schistosomiasis japonica is associated with maternal, placental, and fetal inflammation." Infect Immun 79(3): p. 1254-1261. doi: 10.1128/IAI.01072-10.

Laidlaw et al. 2015

Laidlaw, B. J., Cui, W., Amezquita, R. A., Gray, S. M., Guan, T., Lu, Y., Kobayashi, Y., Flavell, R. A., Kleinstein, S. H., Craft, J. and Kaech, S. M. (2015). "Production of IL-10 by CD4(+) regulatory T cells during the resolution of infection promotes the maturation of memory CD8(+) T cells." Nat Immunol 16(8): p. 871-879. doi: 10.1038/ni.3224.

Layland et al. 2007

Layland, L. E., Rad, R., Wagner, H. and da Costa, C. U. (2007). "Immunopathology in schistosomiasis is controlled by antigen-specific regulatory T cells primed in the presence of TLR2." Eur J Immunol 37(8): p. 2174-2184. doi: 10.1002/eji.200737063.

Lemire et al. 1985

Lemire, J. M., Adams, J. S., Kermani-Arab, V., Bakke, A. C., Sakai, R. and Jordan, S. C. (1985). "1,25-Dihydroxyvitamin D3 suppresses human T helper/inducer lymphocyte activity in vitro." J Immunol 134(5): p. 3032-3035. doi:

Lemire et al. 1995

Lemire, J. M., Archer, D. C., Beck, L. and Spiegelberg, H. L. (1995). "Immunosuppressive actions of 1,25-dihydroxyvitamin D3: preferential inhibition of Th1 functions." J Nutr 125(6 Suppl): p. 1704S-1708S.

Litonjua and Weiss 2007

Litonjua, A. A. and Weiss, S. T. (2007). "Is vitamin D deficiency to blame for the asthma epidemic?" J Allergy Clin Immunol 120(5): p. 1031-1035. doi: 10.1016/j.jaci.2007.08.028.

Liu et al. 2009

Liu, N., Kaplan, A. T., Low, J., Nguyen, L., Liu, G. Y., Equils, O. and Hewison, M. (2009). "Vitamin D induces innate antibacterial responses in human trophoblasts via an intracrine pathway." Biol Reprod 80(3): p. 398-406. doi: 10.1095/biolreprod.108.073577.

Liu et al. 2011

Liu, N. Q., Kaplan, A. T., Lagishetty, V., Ouyang, Y. B., Ouyang, Y., Simmons, C. F., Equils, O. and Hewison, M. (2011). "Vitamin D and the regulation of placental inflammation." J Immunol 186(10): p. 5968-5974. doi: 10.4049/jimmunol.1003332.

Loffredo-Verde 2016

Loffredo-Verde, E. (2016). The interaction of hepatitis B or C virus infection and schistosomiasis in chronic pathogen-induced liver inflammation. Dissertation. Technische Universität München.

Lowe et al. 2011

Lowe, A., Braback, L., Ekeus, C., Hjern, A. and Forsberg, B. (2011). "Maternal obesity during pregnancy as a risk for early-life asthma." J Allergy Clin Immunol 128(5): p. 1107-1109 e1101-1102. doi: 10.1016/j.jaci.2011.08.025.

Lynch et al. 1993

Lynch, N. R., Hagel, I., Perez, M., Di Prisco, M. C., Lopez, R. and Alvarez, N. (1993). "Effect of anthelmintic treatment on the allergic reactivity of children in a tropical slum." J Allergy Clin Immunol 92(3): p. 404-411.

Ma et al. 2012

Ma, R., Gu, Y., Zhao, S., Sun, J., Groome, L. J. and Wang, Y. (2012). "Expressions of vitamin D metabolic components VDBP, CYP2R1, CYP27B1, CYP24A1, and VDR in placentas from normal and preeclamptic pregnancies." Am J Physiol Endocrinol Metab 303(7): p. E928-935. doi: 10.1152/ajpendo.00279.2012.

Masoli et al. 2004

Masoli, M., Fabian, D., Holt, S., Beasley, R. and Global Initiative for Asthma, P. (2004). "The global burden of asthma: executive summary of the GINA Dissemination Committee report." Allergy 59(5): p. 469-478. doi: 10.1111/j.1398-9995.2004.00526.x.

Mendelson et al. 2016

Mendelson, C. R., Montalbano, A. P. and Gao, L. (2016). "Fetal-to-Maternal Signaling in the Timing of Birth." J Steroid Biochem Mol Biol 170: p. 19-27 doi: 10.1016/j.jsbmb.2016.09.006.

Mirzakhani et al. 2015

Mirzakhani, H., Al-Garawi, A., Weiss, S. T. and Litonjua, A. A. (2015). "Vitamin D and the development of allergic disease: how important is it?" Clin Exp Allergy 45(1): p. 114-125. doi: 10.1111/cea.12430.

Mizwicki and Norman 2009

Mizwicki, M. T. and Norman, A. W. (2009). "The vitamin D sterol-vitamin D receptor ensemble model offers unique insights into both genomic and rapid-response signaling." Sci Signal 2(75): p. re4. doi: 10.1126/scisignal.275re4.

Moormann et al. 1999

Moormann, A. M., Sullivan, A. D., Rochford, R. A., Chensue, S. W., Bock, P. J., Nyirenda, T. and Meshnick, S. R. (1999). "Malaria and pregnancy: placental cytokine expression and its relationship to intrauterine growth retardation." J Infect Dis 180(6): p. 1987-1993. doi: 10.1086/315135.

Mora et al. 2008

Mora, J. R., Iwata, M. and von Andrian, U. H. (2008). "Vitamin effects on the immune system: vitamins A and D take centre stage." Nat Rev Immunol 8(9): p. 685-698. doi: 10.1038/nri2378.

Mori et al. 2016

Mori, M., Bogdan, A., Balassa, T., Csabai, T. and Szekeres-Bartho, J. (2016). "The decidua-the maternal bed embracing the embryo-maintains the pregnancy." Semin Immunopathol 38(6): p. 635-649. doi: 10.1007/s00281-016-0574-0.

Mpairwe et al. 2014

Mpairwe, H., Ndibazza, J., Webb, E. L., Nampijja, M., Muhangi, L., Apule, B., Lule, S., Akurut, H., Kizito, D., Kakande, M., Jones, F. M., Fitzsimmons, C. M., Muwanga, M., Rodrigues, L. C., Dunne, D. W. and Elliott, A. M. (2014). "Maternal hookworm modifies risk factors for childhood eczema: results from a birth cohort in Uganda." Pediatr Allergy Immunol 25(5): p. 481-488. doi: 10.1111/pai.12251.

Mpairwe et al. 2011

Mpairwe, H., Webb, E. L., Muhangi, L., Ndibazza, J., Akishule, D., Nampijja, M., Ngom-wegi, S., Tumusime, J., Jones, F. M., Fitzsimmons, C., Dunne, D. W., Muwanga, M., Rodrigues, L. C. and Elliott, A. M. (2011). "Anthelminthic treatment during pregnancy is associated with increased risk of infantile eczema: randomised-controlled trial results." Pediatr Allergy Immunol 22(3): p. 305-312. doi: 10.1111/j.1399-3038.2010.01122.x.

Mulligan et al. 2010

Mulligan, M. L., Felton, S. K., Riek, A. E. and Bernal-Mizrachi, C. (2010). "Implications of vitamin D deficiency in pregnancy and lactation." Am J Obstet Gynecol 202(5): p. 429 e421-429. doi: 10.1016/j.ajog.2009.09.002.

Munger et al. 2016

Munger, K. L., Aivo, J., Hongell, K., Soilu-Hanninen, M., Surcel, H. M. and Ascherio, A. (2016). "Vitamin D Status During Pregnancy and Risk of Multiple Sclerosis in Offspring of Women in the Finnish Maternity Cohort." JAMA Neurol 73(5): p. 515-519. doi: 10.1001/jamaneurol.2015.4800.

Murphy et al. 2009

Murphy, S. P., Tayade, C., Ashkar, A. A., Hatta, K., Zhang, J. and Croy, B. A. (2009). "Interferon gamma in successful pregnancies." Biol Reprod 80(5): p. 848-859. doi: 10.1095/biolreprod.108.073353.

Nguyen et al. 2015

Nguyen, T., Yong, H., Borg, A., Brennecke, S. and Murthi, P. (2015). "Decreased placental vitamin D receptor contributes to aberrant cell-cycle gene regulation in human idiopathic fetal growth restriction." Placenta 36(9): p. A44. doi: 10.1016/j.placenta.2015.07.318.

Nguyen et al. 2015

Nguyen, T. P., Yong, H. E., Chollangi, T., Borg, A. J., Brennecke, S. P. and Murthi, P. (2015). "Placental vitamin D receptor expression is decreased in human idiopathic fetal growth restriction." J Mol Med (Berl) 93(7): p. 795-805. doi: 10.1007/s00109-015-1267-1.

Nielsen et al. 1990

Nielsen, F. R., Bek, K. M., Rasmussen, P. E., Qvist, I. and Tobiassen, M. (1990). "C-reactive protein during normal pregnancy." Eur J Obstet Gynecol Reprod Biol 35(1): p. 23-27.

Okada et al. 2010

Okada, H., Kuhn, C., Feillet, H. and Bach, J. F. (2010). "The 'hygiene hypothesis' for autoimmune and allergic diseases: an update." Clin Exp Immunol 160(1): p. 1-9. doi: 10.1111/j.1365-2249.2010.04139.x.

Olds 2003

Olds, G. R. (2003). "Administration of praziquantel to pregnant and lactating women." Acta Trop 86(2-3): p. 185-195.

Omdahl et al. 2002

Omdahl, J. L., Morris, H. A. and May, B. K. (2002). "Hydroxylase enzymes of the vitamin D pathway: expression, function, and regulation." Annu Rev Nutr 22: p. 139-166. doi: 10.1146/annurev.nutr.22.120501.150216.

Oriss et al. 1997

Oriss, T. B., McCarthy, S. A., Morel, B. F., Campana, M. A. and Morel, P. A. (1997). "Cross-regulation between T helper cell (Th)1 and Th2: inhibition of Th2 proliferation by IFN-gamma involves interference with IL-1." J Immunol 158(8): p. 3666-3672.

Park et al. 2017

Park, H., Wood, M. R., Malysheva, O. V., Jones, S., Mehta, S., Brannon, P. M. and Caudill, M. A. (2017). "Placental vitamin D metabolism and its associations with circulating vitamin D metabolites in pregnant women." Am J Clin Nutr 106(6): p. 1439-1448. doi: 10.3945/ajcn.117.153429.

Pearce and MacDonald 2002

Pearce, E. J. and MacDonald, A. S. (2002). "The immunobiology of schistosomiasis." Nat Rev Immunol 2(7): p. 499-511. doi: 10.1038/nri843.

Peng et al. 2004

Peng, L., Huang, Y., Jin, F., Jiang, S. W. and Payne, A. H. (2004). "Transcription enhancer factor-5 and a GATA-like protein determine placental-specific expression of the Type I human 3beta-hydroxysteroid dehydrogenase gene, HSD3B1." Mol Endocrinol 18(8): p. 2049-2060. doi: 10.1210/me.2004-0028.

Pereira et al. 2014

Pereira, L. H., Machado, J. R., Olegario, J. G., Rocha, L. P., Silva, M. V., Guimaraes, C. S., Reis, M. A., Castellano, L. R., Ramalho, F. S. and Correa, R. R. (2014). "Interleukin-6 and C-reactive protein are overexpressed in the liver of perinatal deaths diagnosed with fetal inflammatory response syndrome." Dis Markers 2014: p. 252780. doi: 10.1155/2014/252780.

Piccinni et al. 1995

Piccinni, M. P., Giudizi, M. G., Biagiotti, R., Beloni, L., Giannarini, L., Sampognaro, S., Parronchi, P., Manetti, R., Annunziato, F., Livi, C. and et al. (1995). "Progesterone favors the development of human T helper cells producing Th2-type cytokines and promotes both IL-4 production and membrane CD30 expression in established Th1 cell clones." J Immunol 155(1): p. 128-133.

Piccinni et al. 2000

Piccinni, M. P., Scaletti, C., Maggi, E. and Romagnani, S. (2000). "Role of hormone-controlled Th1- and Th2-type cytokines in successful pregnancy." J Neuroimmunol 109(1): p. 30-33.

Pike and Meyer 2010

Pike, J. W. and Meyer, M. B. (2010). "The vitamin D receptor: new paradigms for the regulation of gene expression by 1,25-dihydroxyvitamin D(3)." Endocrinol Metab Clin North Am 39(2): p. 255-269, table of contents. doi: 10.1016/j.ecl.2010.02.007.

Pospechova et al. 2009

Pospechova, K., Rozehnal, V., Stejskalova, L., Vrzal, R., Pospisilova, N., Jamborova, G., May, K., Siegmund, W., Dvorak, Z., Nachtigal, P., Semecky, V. and Pavek, P. (2009). "Expression and activity of vitamin D receptor in the human placenta and in choriocarcinoma BeWo and JEG-3 cell lines." Mol Cell Endocrinol 299(2): p. 178-187. doi: 10.1016/j.mce.2008.12.003.

Powe et al. 2013

Powe, C. E., Evans, M. K., Wenger, J., Zonderman, A. B., Berg, A. H., Nalls, M., Tamez, H., Zhang, D., Bhan, I., Karumanchi, S. A., Powe, N. R. and Thadhani, R. (2013). "Vitamin D-binding protein and vitamin D status of black Americans and white Americans." N Engl J Med 369(21): p. 1991-2000. doi: 10.1056/NEJMoa1306357.

Prietl et al. 2013

Prietl, B., Treiber, G., Pieber, T. R. and Amrein, K. (2013). "Vitamin D and immune function." Nutrients 5(7): p. 2502-2521. doi: 10.3390/nu5072502.

Reister 2011

Reister, F. (2011). Entstehung und Entwicklung einer Schwangerschaft. In: Kiechle, M. (Ed.), Gynäkologie und Geburtshilfe (pp. 165-186). München: Elsevier.

Richard et al. 2017

Richard, A., Rohrmann, S. and Quack Lotscher, K. C. (2017). "Prevalence of Vitamin D Deficiency and Its Associations with Skin Color in Pregnant Women in the First Trimester in a Sample from Switzerland." Nutrients 9(3): p. pii: E260. doi: 10.3390/nu9030260.

Robinson and Klein 2012

Robinson, D. P. and Klein, S. L. (2012). "Pregnancy and pregnancy-associated hormones alter immune responses and disease pathogenesis." Horm Behav 62(3): p. 263-271. doi: 10.1016/j.yhbeh.2012.02.023.

Roth et al. 1996

Roth, I., Corry, D. B., Locksley, R. M., Abrams, J. S., Litton, M. J. and Fisher, S. J. (1996). "Human placental cytotrophoblasts produce the immunosuppressive cytokine interleukin 10." J Exp Med 184(2): p. 539-548. doi:

Sahebnasagh et al. 2017

Sahebnasagh, B., Hosseinzadeh-Attar, M. J. and Sharifi, A. (2017). "Risks associated with preeclampsia: possible mechanisms." Reviews in Clinical Medicine 4(1): p. 39-44. doi: 10.22038/rcm.2017.13735.1156.

Salle et al. 2000

Salle, B. L., Delvin, E. E., Lapillonne, A., Bishop, N. J. and Glorieux, F. H. (2000). "Perinatal metabolism of vitamin D." Am J Clin Nutr 71(5 Suppl): p. 1317S-1324S.

Santiso-Mere et al. 1993

Santiso-Mere, D., Sone, T., Hilliard, G. M. t., Pike, J. W. and McDonnell, D. P. (1993). "Positive regulation of the vitamin D receptor by its cognate ligand in heterologous expression systems." Mol Endocrinol 7(7): p. 833-839. doi: 10.1210/mend.7.7.8413308.

Schroth et al. 2005

Schroth, R. J., Lavelle, C. L. and Moffatt, M. E. (2005). "Review of vitamin D deficiency during pregnancy: who is affected?" Int J Circumpolar Health 64(2): p. 112-120.

Scippo et al. 1993

Scippo, M. L., Frankenne, F., Hooghe-Peters, E. L., Igout, A., Velkeniers, B. and Hennen, G. (1993). "Syncytiotrophoblastic localization of the human growth hormone variant mRNA in the placenta." Mol Cell Endocrinol 92(2): p. R7-13.

Sharland and Subgroup 2007

Sharland, M. and Subgroup, S. P. (2007). "The use of antibacterials in children: a report of the Specialist Advisory Committee on Antimicrobial Resistance (SACAR) Paediatric Sub-group." J Antimicrob Chemother 60 Suppl 1: p. i15-26. doi: 10.1093/jac/dkm153.

Shin et al. 2010

Shin, J. S., Choi, M. Y., Longtine, M. S. and Nelson, D. M. (2010). "Vitamin D effects on pregnancy and the placenta." Placenta 31(12): p. 1027-1034. doi: 10.1016/j.placenta.2010.08.015.

Siegrist and Siegrist-Obimpeh 1992

Siegrist, D. and Siegrist-Obimpeh, P. (1992). "Schistosoma haematobium infection in pregnancy." Acta Trop 50(4): p. 317-321.

Simard et al. 1996

Simard, J., Durocher, F., Mebarki, F., Turgeon, C., Sanchez, R., Labrie, Y., Couet, J., Trudel, C., Rheaume, E., Morel, Y., Luu-The, V. and Labrie, F. (1996). "Molecular biology and genetics of the 3 beta-hydroxysteroid dehydrogenase/delta5-delta4 isomerase gene family." J Endocrinol 150 Suppl: p. S189-207.

Sitras et al. 2009

Sitras, V., Paulssen, R., Leirvik, J., Vartun, A. and Acharya, G. (2009). "Placental gene expression profile in intrauterine growth restriction due to placental insufficiency." Reprod Sci 16(7): p. 701-711. doi: 10.1177/1933719109334256.

Siza 2008

Siza, J. E. (2008). "Risk factors associated with low birth weight of neonates among pregnant women attending a referral hospital in northern Tanzania." Tanzan J Health Res 10(1): p. 1-8.

Sood et al. 2006

Sood, R., Zehnder, J. L., Druzin, M. L. and Brown, P. O. (2006). "Gene expression patterns in human placenta." Proc Natl Acad Sci U S A 103(14): p. 5478-5483. doi: 10.1073/pnas.0508035103.

Stene et al. 2000

Stene, L. C., Ulriksen, J., Magnus, P. and Joner, G. (2000). "Use of cod liver oil during pregnancy associated with lower risk of Type I diabetes in the offspring." Diabetologia 43(9): p. 1093-1098. doi: 10.1007/s001250051499.

Stiemsma et al. 2015

Stiemsma, L. T., Reynolds, L. A., Turvey, S. E. and Finlay, B. B. (2015). "The hygiene hypothesis: current perspectives and future therapies." Immunotargets Ther 4: p. 143-157. doi: 10.2147/ITT.S61528.

Strachan 1989

Strachan, D. P. (1989). "Hay fever, hygiene, and household size." BMJ 299(6710): p. 1259-1260.

Straubinger 2013

Straubinger, K. (2013). Immunosuppressive and transgenerational effects on the development of allergic airway inflammation during Schistosoma mansoni infection. Dissertation. Technische Universität München.

Straubinger et al. 2014

Straubinger, K., Paul, S., Prazeres da Costa, O., Ritter, M., Buch, T., Busch, D. H., Layland, L. E. and Prazeres da Costa, C. U. (2014). "Maternal immune response to helminth infection during pregnancy determines offspring susceptibility to allergic airway inflammation." J Allergy Clin Immunol: 134(6): p. 1271-1279. doi: 10.1016/j.jaci.2014.05.034.

Straubinger and Prazeres da Costa 2014

Straubinger, K. and Prazeres da Costa, C. (2014). Maternal helminth infections. In: Horsnell, W. (Ed.), How Helminths Alter immunity to Infection (pp. 27-48). New York: Spinger.

Suguitan et al. 2003

Suguitan, A. L., Jr., Leke, R. G., Fouda, G., Zhou, A., Thuita, L., Metenou, S., Fogako, J., Megnekou, R. and Taylor, D. W. (2003). "Changes in the levels of chemokines and cytokines in the placentas of women with Plasmodium falciparum malaria." J Infect Dis 188(7): p. 1074-1082. doi: 10.1086/378500.

Tamblyn et al. 2017

Tamblyn, J. A., Susarla, R., Jenkinson, C., Jeffery, L. E., Ohizua, O., Chun, R. F., Chan, S. Y., Kilby, M. D. and Hewison, M. (2017). "Dysregulation of maternal and placental vitamin D metabolism in preeclampsia." Placenta 50: p. 70-77. doi: 10.1016/j.placenta.2016.12.019.

Tavakoli et al. 2011

Tavakoli, M., Jeddi-Tehrani, M., Salek-Moghaddam, A., Rajaei, S., Mohammadzadeh, A., Sheikhhasani, S., Kazemi-Sefat, G. E. and Zarnani, A. H. (2011). "Effects of 1,25(OH)2 vitamin D3 on cytokine production by endometrial cells of women with recurrent spontaneous abortion." Fertil Steril 96(3): p. 751-757. doi: 10.1016/j.fertnstert.2011.06.075.

Tuckey 2005

Tuckey, R. C. (2005). "Progesterone synthesis by the human placenta." Placenta 26(4): p. 273-281. doi: 10.1016/j.placenta.2004.06.012.

van den Biggelaar et al. 2004

van den Biggelaar, A. H., Rodrigues, L. C., van Ree, R., van der Zee, J. S., Hoeksma-Kruize, Y. C., Souverijn, J. H., Missinou, M. A., Borrmann, S., Kremsner, P. G. and Yazdanbakhsh, M. (2004). "Long-term treatment of intestinal helminths increases mite skin-test reactivity in Gabonese schoolchildren." J Infect Dis 189(5): p. 892-900. doi: 10.1086/381767.

van den Biggelaar et al. 2000

van den Biggelaar, A. H., van Ree, R., Rodrigues, L. C., Lell, B., Deelder, A. M., Kremsner, P. G. and Yazdanbakhsh, M. (2000). "Decreased atopy in children infected with Schistosoma haematobium: a role for parasite-induced interleukin-10." Lancet 356(9243): p. 1723-1727. doi: 10.1016/S0140-6736(00)03206-2.

Versini et al. 2015

Versini, M., Jeandel, P. Y., Bashi, T., Bizzaro, G., Blank, M. and Shoenfeld, Y. (2015). "Unraveling the Hygiene Hypothesis of helminthes and autoimmunity: origins, pathophysiology, and clinical applications." BMC Med 13: p. 81. doi: 10.1186/s12916-015-0306-7.

Vigano et al. 2006

Vigano, P., Lattuada, D., Mangioni, S., Ermellino, L., Vignali, M., Caporizzo, E., Panina-Bordignon, P., Besozzi, M. and Di Blasio, A. M. (2006). "Cycling and early pregnant endometrium as a site of regulated expression of the vitamin D system." J Mol Endocrinol 36(3): p. 415-424. doi: 10.1677/jme.1.01946.

von Mutius 2012

von Mutius, E. (2012). "Maternal farm exposure/ingestion of unpasteurized cow's milk and allergic disease." Curr Opin Gastroenterol 28(6): p. 570-576. doi: 10.1097/MOG.0b013e32835955d3.

von Mutius et al. 1994

von Mutius, E., Martinez, F. D., Fritzsch, C., Nicolai, T., Reitmeir, P. and Thiemann, H. H. (1994). "Skin test reactivity and number of siblings." BMJ 308(6930): p. 692-695.

von Mutius et al. 1994

von Mutius, E., Martinez, F. D., Fritzsch, C., Nicolai, T., Roell, G. and Thiemann, H. H. (1994). "Prevalence of asthma and atopy in two areas of West and East Germany." Am J Respir Crit Care Med 149(2 Pt 1): p. 358-364. doi: 10.1164/ajrccm.149.2.8306030.

von Mutius et al. 1998

von Mutius, E., Weiland, S. K., Fritzsch, C., Duhme, H. and Keil, U. (1998). "Increasing prevalence of hay fever and atopy among children in Leipzig, East Germany." Lancet 351(9106): p. 862-866. doi: 10.1016/S0140-6736(97)10100-3.

Wang et al. 2004

Wang, T. T., Nestel, F. P., Bourdeau, V., Nagai, Y., Wang, Q., Liao, J., Tavera-Mendoza, L., Lin, R., Hanrahan, J. W., Mader, S. and White, J. H. (2004). "Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression." J Immunol 173(5): p. 2909-2912.

Wei et al. 2016

Wei, Z., Zhang, J. and Yu, X. (2016). "Maternal vitamin D status and childhood asthma, wheeze, and eczema: A systematic review and meta-analysis." Pediatr Allergy Immunol 27(6): p. 612-619. doi: 10.1111/pai.12593.

Weisman et al. 1979

Weisman, Y., Harell, A., Edelstein, S., David, M., Spirer, Z. and Golander, A. (1979). "1 alpha, 25-Dihydroxyvitamin D3 and 24,25-dihydroxyvitamin D3 in vitro synthesis by human decidua and placenta." Nature 281(5729): p. 317-319.

White 2008

White, J. H. (2008). "Vitamin D signaling, infectious diseases, and regulation of innate immunity." Infect Immun 76(9): p. 3837-3843. doi: 10.1128/IAI.00353-08.

WHO 2012

WHO. Guideline: Vitamin D supplementation in pregnant women. 2012 [cited 11.09.2018]; Available from: http://www.who.int/nutrition/publications/micronutrients/guidelines/vit_d_supp_pregnant_women/en/.

WHO 2016

WHO. Schistosomiasis. (fact sheet). 2018 [cited 11.09.2018]; Available from: http://www.who.int/mediacentre/factsheets/fs115/en/.

WHO 2016

WHO. Soil-transmitted helminth infections. (fact sheet) 2018 [cited 11.09.2018]; Available from: http://www.who.int/mediacentre/factsheets/fs366/en/.

Wilson et al. 2007

Wilson, M. S., Elnekave, E., Mentink-Kane, M. M., Hodges, M. G., Pesce, J. T., Ramalingam, T. R., Thompson, R. W., Kamanaka, M., Flavell, R. A., Keane-Myers, A., Cheever, A. W. and Wynn, T. A. (2007). "IL-13Ralpha2 and IL-10 coordinately suppress airway inflammation, airway-hyperreactivity, and fibrosis in mice." J Clin Invest 117(10): p. 2941-2951. doi: 10.1172/JCI31546.

Workalemahu et al. 2017

Workalemahu, T., Badon, S. E., Dishi-Galitzky, M., Qiu, C., Williams, M. A., Sorensen, T. and Enquobahrie, D. A. (2017). "Placental genetic variations in vitamin D metabolism and birthweight." Placenta 50: p. 78-83. doi: 10.1016/j.placenta.2016.12.028.

Wyatt et al. 2005

Wyatt, S. M., Kraus, F. T., Roh, C. R., Elchalal, U., Nelson, D. M. and Sadovsky, Y. (2005). "The correlation between sampling site and gene expression in the term human placenta." Placenta 26(5): p. 372-379. doi: 10.1016/j.placenta.2004.07.003.

Yazdanbakhsh et al. 2002

Yazdanbakhsh, M., Kremsner, P. G. and van Ree, R. (2002). "Allergy, parasites, and the hygiene hypothesis." Science 296(5567): p. 490-494. doi: 10.1126/science.296.5567.490.

Zeck et al. 2008

Zeck, W., Widberg, C., Maylin, E., Desoye, G., Lang, U., McIntyre, D., Prins, J. and Russell, A. (2008). "Regulation of placental growth hormone secretion in a human trophoblast model--the effects of hormones and adipokines." Pediatr Res 63(4): p. 353-357. doi: 10.1203/01.pdr.0000304935.19183.07.

Zehnder et al. 2002

Zehnder, D., Evans, K. N., Kilby, M. D., Bulmer, J. N., Innes, B. A., Stewart, P. M. and Hewison, M. (2002). "The ontogeny of 25-hydroxyvitamin D(3) 1alpha-hydroxylase expression in human placenta and decidua." Am J Pathol 161(1): p. 105-114.

Zella et al. 2007

Zella, L. A., Kim, S., Shevde, N. K. and Pike, J. W. (2007). "Enhancers located in the vitamin D receptor gene mediate transcriptional autoregulation by 1,25-dihydroxyvitamin D3." J Steroid Biochem Mol Biol 103(3-5): p. 435-439. doi: 10.1016/j.jsbmb.2006.12.019.

Zerwekh and Breslau 1986

Zerwekh, J. E. and Breslau, N. A. (1986). "Human placental production of 1 alpha,25-dihydroxyvitamin D3: biochemical characterization and production in normal subjects and patients with pseudohypoparathyroidism." J Clin Endocrinol Metab 62(1): p. 192-196. doi: 10.1210/jcem-62-1-192.

Zhang et al. 2008

Zhang, C., Qiu, C., Hu, F. B., David, R. M., van Dam, R. M., Bralley, A. and Williams, M. A. (2008). "Maternal plasma 25-hydroxyvitamin D concentrations and the risk for gestational diabetes mellitus." PLoS One 3(11): p. e3753. doi: 10.1371/journal.pone.0003753.

Zhang et al. 2018

Zhang, Q., Chen, H., Wang, Y., Zhang, C., Tang, Z., Li, H., Huang, X., Ouyang, F., Huang, H. and Liu, Z. (2018). "Severe vitamin D deficiency in the first trimester is associated with placental inflammation in high-risk singleton pregnancy." Clin Nutr: p. doi: 10.1016/j.clnu.2018.06.978.

[Amoah et al. 2012]

Amoah, A. S., Forson, A. G. and Boakye, D. A. (2012). "A review of epidemiological studies of asthma in Ghana." Ghana Med J **46**(2 Suppl): p. 23-28. doi:

[Aranow 2011]

Aranow, C. (2011). "Vitamin D and the immune system." J Investig Med **59**(6): p. 881-886. doi: 10.2310/JIM.0b013e31821b8755.

[Arck and Hecher 2013]

Arck, P. C. and Hecher, K. (2013). "Fetomaternal immune cross-talk and its consequences for maternal and offspring's health." Nat Med **19**(5): p. 548-556. doi: 10.1038/nm.3160.

[Attallah et al. 2003]

Attallah, A. M., Ghanem, G. E., Ismail, H. and El Waseef, A. M. (2003). "Placental and oral delivery of Schistosoma mansoni antigen from infected mothers to their newborns and children." Am J Trop Med Hyg **68**(6): p. 647-651. doi:

[Avila et al. 2007]

Avila, E., Diaz, L., Barrera, D., Halhali, A., Mendez, I., Gonzalez, L., Zuegel, U., Steinmeyer, A. and Larrea, F. (2007). "Regulation of Vitamin D hydroxylases gene expression by 1,25-dihydroxyvitamin D3 and cyclic AMP in cultured human syncytiotrophoblasts." J Steroid Biochem Mol Biol **103**(1): p. 90-96. doi: 10.1016/j.jsbmb.2006.07.010.

[Bach 2002]

Bach, J. F. (2002). "The effect of infections on susceptibility to autoimmune and allergic diseases." N Engl J Med **347**(12): p. 911-920. doi: 10.1056/NEJMra020100.

[Baker et al. 2010]

Baker, A. M., Haeri, S., Camargo, C. A., Jr., Espinola, J. A. and Stuebe, A. M. (2010). "A nested case-control study of midgestation vitamin D deficiency and risk of severe preeclampsia." J Clin Endocrinol Metab **95**(11): p. 5105-5109. doi: 10.1210/jc.2010-0996.

[Barrera et al. 2007]

Barrera, D., Avila, E., Hernandez, G., Halhali, A., Biruete, B., Larrea, F. and Diaz, L. (2007). "Estradiol and progesterone synthesis in human placenta is stimulated by calcitriol." J Steroid Biochem Mol Biol **103**(3-5): p. 529-532. doi: 10.1016/j.jsbmb.2006.12.097.

[Barrera et al. 2008]

Barrera, D., Avila, E., Hernandez, G., Mendez, I., Gonzalez, L., Halhali, A., Larrea, F., Morales, A. and Diaz, L. (2008). "Calcitriol affects hCG gene transcription in cultured human syncytiotrophoblasts." Reprod Biol Endocrinol **6**: p. 3. doi: 10.1186/1477-7827-6-3.

[Barrera et al. 2015]

Barrera, D., Diaz, L., Noyola-Martinez, N. and Halhali, A. (2015). "Vitamin D and Inflammatory Cytokines in Healthy and Preeclamptic Pregnancies." Nutrients **7**(8): p. 6465-6490. doi: 10.3390/nu7085293.

[Belderbos et al. 2011]

Belderbos, M. E., Houben, M. L., Wilbrink, B., Lentjes, E., Bloemen, E. M., Kimpen, J. L., Rovers, M. and Bont, L. (2011). "Cord blood vitamin D deficiency is associated with respiratory syncytial virus bronchiolitis." Pediatrics **127**(6): p. e1513-1520. doi: 10.1542/peds.2010-3054.

[Bennett et al. 1996]

Bennett, W. A., Lagoo-Deenadayalan, S., Brackin, M. N., Hale, E. and Cowan, B. D. (1996). "Cytokine expression by models of human trophoblast as assessed by a semiquantitative reverse transcription-polymerase chain reaction technique." Am J Reprod Immunol **36**(5): p. 285-294. doi:

[Bieber 2008]

Bieber, T. (2008). "Atopic dermatitis." N Engl J Med **358**(14): p. 1483-1494. doi: 10.1056/NEJMra074081.

[Bikle 2009]

Bikle, D. (2009). "Nonclassic actions of vitamin D." J Clin Endocrinol Metab **94**(1): p. 26-34. doi: 10.1210/jc.2008-1454.

[Bodansky et al. 1992]

Bodansky, H. J., Staines, A., Stephenson, C., Haigh, D. and Cartwright, R. (1992). "Evidence for an environmental effect in the aetiology of insulin

dependent diabetes in a transmigratory population." BMJ **304**(6833): p. 1020-1022. doi:

[Bodnar et al. 2009]

Bodnar, L. M., Krohn, M. A. and Simhan, H. N. (2009). "Maternal vitamin D deficiency is associated with bacterial vaginosis in the first trimester of pregnancy." J Nutr **139**(6): p. 1157-1161. doi: 10.3945/jn.108.103168.

[Bodnar et al. 2007]

Bodnar, L. M., Simhan, H. N., Powers, R. W., Frank, M. P., Cooperstein, E. and Roberts, J. M. (2007). "High prevalence of vitamin D insufficiency in black and white pregnant women residing in the northern United States and their neonates." J Nutr **137**(2): p. 447-452. doi:

[Boonstra et al. 2001]

Boonstra, A., Barrat, F. J., Crain, C., Heath, V. L., Savelkoul, H. F. and O'Garra, A. (2001). "1alpha,25-Dihydroxyvitamin d3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells." J Immunol **167**(9): p. 4974-4980. doi:

[Borish et al. 1996]

Borish, L., Aarons, A., Rumbyrt, J., Cvietusa, P., Negri, J. and Wenzel, S. (1996). "Interleukin-10 regulation in normal subjects and patients with asthma." J Allergy Clin Immunol **97**(6): p. 1288-1296. doi:

[Brew et al. 2016]

Brew, O., Sullivan, M. H. and Woodman, A. (2016). "Comparison of Normal and Pre-Eclamptic Placental Gene Expression: A Systematic Review with Meta-Analysis." PLoS One **11**(8): p. e0161504. doi: 10.1371/journal.pone.0161504.

[Bulmer et al. 1991]

Bulmer, J. N., Longfellow, M. and Ritson, A. (1991). "Leukocytes and resident blood cells in endometrium." Ann N Y Acad Sci **622**: p. 57-68. doi:

[Burton et al. 2014]

Burton, G. J., Sebire, N. J., Myatt, L., Tannetta, D., Wang, Y. L., Sadovsky, Y., Staff, A. C. and Redman, C. W. (2014). "Optimising sample collection for placental research." Placenta **35**(1): p. 9-22. doi: 10.1016/j.placenta.2013.11.005.

[Camargo et al. 2011]

Camargo, C. A., Jr., Ingham, T., Wickens, K., Thadhani, R., Silvers, K. M., Epton, M. J., Town, G. I., Pattemore, P. K., Espinola, J. A., Crane, J., New Zealand, A. and Allergy Cohort Study, G. (2011). "Cord-blood 25-hydroxyvitamin D levels and risk of respiratory infection, wheezing, and asthma." Pediatrics **127**(1): p. e180-187. doi: 10.1542/peds.2010-0442.

[Camargo et al. 2007]

Camargo, C. A., Jr., Rifas-Shiman, S. L., Litonjua, A. A., Rich-Edwards, J. W., Weiss, S. T., Gold, D. R., Kleinman, K. and Gillman, M. W. (2007). "Maternal intake of vitamin D during pregnancy and risk of recurrent wheeze in children at 3 y of age." Am J Clin Nutr **85**(3): p. 788-795. doi:

[Cantorna 2010]

Cantorna, M. T. (2010). "Mechanisms underlying the effect of vitamin D on the immune system." Proc Nutr Soc **69**(3): p. 286-289. doi: 10.1017/S0029665110001722.

[Cantorna et al. 2015]

Cantorna, M. T., Snyder, L., Lin, Y. D. and Yang, L. (2015). "Vitamin D and 1,25(OH)2D regulation of T cells." Nutrients **7**(4): p. 3011-3021. doi: 10.3390/nu7043011.

[Cashman et al. 2016]

Cashman, K. D., Dowling, K. G., Skrabakova, Z., Gonzalez-Gross, M., Valtuena, J., De Henauw, S., Moreno, L., Damsgaard, C. T., Michaelsen, K. F., Molgaard, C., Jorde, R., Grimnes, G., Moschonis, G., Mavrogianni, C., Manios, Y., Thamm, M., Mensink, G. B., Rabenberg, M., Busch, M. A., Cox, L., Meadows, S., Goldberg, G., Prentice, A., Dekker, J. M., Nijpels, G., Pilz, S., Swart, K. M., van Schoor, N. M., Lips, P., Eiriksdottir, G., Gudnason, V., Cotch, M. F., Koskinen, S., Lamberg-Allardt, C., Durazo-Arvizu, R. A., Sempos, C. T. and Kiely, M. (2016). "Vitamin D deficiency in Europe: pandemic?" Am J Clin Nutr **103**(4): p. 1033-1044. doi: 10.3945/ajcn.115.120873.

[Chen et al. 2007]

Chen, S., Sims, G. P., Chen, X. X., Gu, Y. Y., Chen, S. and Lipsky, P. E. (2007). "Modulatory effects of 1,25-dihydroxyvitamin D-3 on human B cell differentiation." Journal of Immunology **179**(3): p. 1634-1647. doi:

[Cho et al. 2013]

Cho, G. J., Hong, S. C., Oh, M. J. and Kim, H. J. (2013). "Vitamin D deficiency in gestational diabetes mellitus and the role of the placenta." Am J Obstet Gynecol **209**(6): p. 560 e561-568. doi: 10.1016/j.ajog.2013.08.015.

[Cooke and Haddad 1989]

Cooke, N. E. and Haddad, J. G. (1989). "Vitamin D binding protein (Gc-globulin)." Endocr Rev **10**(3): p. 294-307. doi: 10.1210/edrv-10-3-294.

[Cooper et al. 2006]

Cooper, P. J., Chico, M. E., Vaca, M. G., Moncayo, A. L., Bland, J. M., Mafla, E., Sanchez, F., Rodrigues, L. C., Strachan, D. P. and Griffin, G. E. (2006). "Effect of albendazole treatments on the prevalence of atopy in children living in communities endemic for geohelminth parasites: a cluster-randomised trial." Lancet **367**(9522): p. 1598-1603. doi: 10.1016/S0140-6736(06)68697-2.

[Costa et al. 1985]

Costa, E. M., Hirst, M. A. and Feldman, D. (1985). "Regulation of 1,25-dihydroxyvitamin D3 receptors by vitamin D analogs in cultured mammalian cells." Endocrinology **117**(5): p. 2203-2210. doi: 10.1210/endo-117-5-2203.

[Couper et al. 2008]

Couper, K. N., Blount, D. G. and Riley, E. M. (2008). "IL-10: the master regulator of immunity to infection." J Immunol **180**(9): p. 5771-5777. doi:

[Daniel et al. 2008]

Daniel, C., Sartory, N. A., Zahn, N., Radeke, H. H. and Stein, J. M. (2008). "Immune modulatory treatment of trinitrobenzene sulfonic acid colitis with calcitriol is associated with a change of a T helper (Th) 1/Th17 to a Th2 and regulatory T cell profile." Journal of Pharmacology and Experimental Therapeutics **324**(1): p. 23-33. doi: DOI 10.1124/jpet.107.127209.

[Devereux et al. 2007]

Devereux, G., Litonjua, A. A., Turner, S. W., Craig, L. C., McNeill, G., Martindale, S., Helms, P. J., Seaton, A. and Weiss, S. T. (2007). "Maternal vitamin D intake during pregnancy and early childhood wheezing." Am J Clin Nutr **85**(3): p. 853-859. doi:

[Diaz et al. 2002]

Diaz, L., Arranz, C., Avila, E., Halhali, A., Vilchis, F. and Larrea, F. (2002). "Expression and activity of 25-hydroxyvitamin D-1 alpha-hydroxylase are

restricted in cultures of human syncytiotrophoblast cells from preeclamptic pregnancies." J Clin Endocrinol Metab **87**(8): p. 3876-3882. doi: 10.1210/jcem.87.8.8730.

[Diaz et al. 2009]

Diaz, L., Noyola-Martinez, N., Barrera, D., Hernandez, G., Avila, E., Halhali, A. and Larrea, F. (2009). "Calcitriol inhibits TNF-alpha-induced inflammatory cytokines in human trophoblasts." J Reprod Immunol **81**(1): p. 17-24. doi: 10.1016/j.jri.2009.02.005.

[Dickie et al. 2010]

Dickie, L. J., Church, L. D., Coulthard, L. R., Mathews, R. J., Emery, P. and McDermott, M. F. (2010). "Vitamin D-3 down-regulates intracellular Toll-like receptor 9 expression and Toll-like receptor 9-induced IL-6 production in human monocytes." Rheumatology **49**(8): p. 1466-1471. doi: DOI 10.1093/rheumatology/keq124.

[Drake and Reynolds 2010]

Drake, A. J. and Reynolds, R. M. (2010). "Impact of maternal obesity on offspring obesity and cardiometabolic disease risk." Reproduction **140**(3): p. 387-398. doi: 10.1530/REP-10-0077.

[Droste et al. 2000]

Droste, J. H., Wieringa, M. H., Weyler, J. J., Nelen, V. J., Vermeire, P. A. and Van Bever, H. P. (2000). "Does the use of antibiotics in early childhood increase the risk of asthma and allergic disease?" Clin Exp Allergy **30**(11): p. 1547-1553. doi:

[Dusso et al. 2005]

Dusso, A. S., Brown, A. J. and Slatopolsky, E. (2005). "Vitamin D." Am J Physiol Renal Physiol **289**(1): p. F8-28. doi: 10.1152/ajprenal.00336.2004.

[Ege et al. 2011]

Ege, M. J., Mayer, M., Normand, A. C., Genuneit, J., Cookson, W. O., Braun-Fahrlander, C., Heederik, D., Piarroux, R., von Mutius, E. and Group, G. T. S. (2011). "Exposure to environmental microorganisms and childhood asthma." N Engl J Med **364**(8): p. 701-709. doi: 10.1056/NEJMoa1007302.

[Emanuel 1988]

Emanuel, M. B. (1988). "Hay fever, a post industrial revolution epidemic: a history of its growth during the 19th century." Clin Allergy **18**(3): p. 295-304. doi:

[Enquobahrie et al. 2008]

Enquobahrie, D. A., Meller, M., Rice, K., Psaty, B. M., Siscovick, D. S. and Williams, M. A. (2008). "Differential placental gene expression in preeclampsia." Am J Obstet Gynecol **199**(5): p. 566 e561-511. doi: 10.1016/j.ajog.2008.04.020.

[Erkkola et al. 2009]

Erkkola, M., Kaila, M., Nwaru, B. I., Kronberg-Kippila, C., Ahonen, S., Nevalainen, J., Veijola, R., Pekkanen, J., Ilonen, J., Simell, O., Knip, M. and Virtanen, S. M. (2009). "Maternal vitamin D intake during pregnancy is inversely associated with asthma and allergic rhinitis in 5-year-old children." Clin Exp Allergy **39**(6): p. 875-882. doi: 10.1111/j.1365-2222.2009.03234.x.

[Erkkola 2011]

Erkkola, M. N., B.I.; Viljakainen, H.T. (2011). Maternal Vitamin D During Pregnancy and Its Role To Immune-mediated Diseases In The offspring. In Litwack, G. (Ed.), Vitamins and the Immune System (pp. 240-254). Oxford: Elsevier

[Erlebacher 2013]

Erlebacher, A. (2013). "Immunology of the maternal-fetal interface." Annu Rev Immunol **31**: p. 387-411. doi: 10.1146/annurev-immunol-032712-100003.

[Evans et al. 2006]

Evans, K. N., Nguyen, L., Chan, J., Innes, B. A., Bulmer, J. N., Kilby, M. D. and Hewison, M. (2006). "Effects of 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 on cytokine production by human decidual cells." Biol Reprod **75**(6): p. 816-822. doi: 10.1095/biolreprod.106.054056.

[Ferreira et al. 2012]

Ferreira, G. B., Kleijwegt, F. S., Waelkens, E., Lage, K., Nikolic, T., Hansen, D. A., Workman, C. T., Roep, B. O., Overbergh, L. and Mathieu, C. (2012). "Differential protein pathways in 1,25-dihydroxyvitamin d(3) and dexamethasone modulated tolerogenic human dendritic cells." J Proteome Res **11**(2): p. 941-971. doi: 10.1021/pr200724e.

[Fischer et al. 2007]

Fischer, D., Schroer, A., Ludders, D., Cordes, T., Bucker, B., Reichrath, J. and Friedrich, M. (2007). "Metabolism of vitamin D3 in the placental tissue of normal and preeclampsia complicated pregnancies and premature births." Clin Exp Obstet Gynecol **34**(2): p. 80-84. doi:

[Friedman et al. 2007]

Friedman, J. F., Mital, P., Kanzaria, H. K., Olds, G. R. and Kurtis, J. D. (2007). "Schistosomiasis and pregnancy." Trends Parasitol **23**(4): p. 159-164. doi: 10.1016/j.pt.2007.02.006.

[Gale et al. 2008]

Gale, C. R., Robinson, S. M., Harvey, N. C., Javaid, M. K., Jiang, B., Martyn, C. N., Godfrey, K. M., Cooper, C. and Princess Anne Hospital Study, G. (2008). "Maternal vitamin D status during pregnancy and child outcomes." Eur J Clin Nutr **62**(1): p. 68-77. doi: 10.1038/sj.ejcn.1602680.

[Gensure et al. 1998]

Gensure, R. C., Antrobus, S. D., Fox, J., Okwueze, M., Talton, S. Y. and Walters, M. R. (1998). "Homologous up-regulation of vitamin D receptors is tissue specific in the rat." J Bone Miner Res **13**(3): p. 454-463. doi: 10.1359/jbmr.1998.13.3.454.

[Gheorghe et al. 2010]

Gheorghe, C. P., Goyal, R., Mittal, A. and Longo, L. D. (2010). "Gene expression in the placenta: maternal stress and epigenetic responses." Int J Dev Biol **54**(2-3): p. 507-523. doi: 10.1387/ijdb.082770cg.

[Gollwitzer and Marsland 2015]

Gollwitzer, E. S. and Marsland, B. J. (2015). "Impact of Early-Life Exposures on Immune Maturation and Susceptibility to Disease." Trends Immunol **36**(11): p. 684-696. doi: 10.1016/j.it.2015.09.009.

[Gray et al. 1979]

Gray, T. K., Lester, G. E. and Lorenc, R. S. (1979). "Evidence for extra-renal 1 alpha-hydroxylation of 25-hydroxyvitamin D3 in pregnancy." Science **204**(4399): p. 1311-1313. doi:

[Guillot et al. 2010]

Guillot, X., Semerano, L., Saidenberg-Kermanac'h, N., Falgarone, G. and Boissier, M. C. (2010). "Vitamin D and inflammation." Joint Bone Spine **77**(6): p. 552-557. doi: 10.1016/j.jbspin.2010.09.018.

[Gustafsson et al. 2008]

Gustafsson, C., Mjosberg, J., Matussek, A., Geffers, R., Matthiesen, L., Berg, G., Sharma, S., Buer, J. and Ernerudh, J. (2008). "Gene expression profiling of human decidual macrophages: evidence for immunosuppressive phenotype." PLoS One **3**(4): p. e2078. doi: 10.1371/journal.pone.0002078.

[Hardy et al. 2006]

Hardy, D. B., Janowski, B. A., Corey, D. R. and Mendelson, C. R. (2006). "Progesterone receptor plays a major antiinflammatory role in human myometrial cells by antagonism of nuclear factor-kappaB activation of cyclooxygenase 2 expression." Mol Endocrinol **20**(11): p. 2724-2733. doi: 10.1210/me.2006-0112.

[Helming et al. 2005]

Helming, L., Bose, J., Ehrchen, J., Schiebe, S., Frahm, T., Geffers, R., Probst-Kepper, M., Balling, R. and Lengeling, A. (2005). "1 alpha,25-dihydroxyvitamin D-3 is a potent suppressor of interferon gamma-mediated macrophage activation." Blood **106**(13): p. 4351-4358. doi: DOI 10.1182/blood-2005-03-1029.

[Hintzpeter et al. 2008]

Hintzpeter, B., Mensink, G. B., Thierfelder, W., Muller, M. J. and Scheidt-Nave, C. (2008). "Vitamin D status and health correlates among German adults." Eur J Clin Nutr **62**(9): p. 1079-1089. doi: 10.1038/sj.ejcn.1602825.

[Holick 2003]

Holick, M. F. (2003). "Vitamin D: A millenium perspective." J Cell Biochem **88**(2): p. 296-307. doi: 10.1002/jcb.10338.

[Holick 2007]

Holick, M. F. (2007). "Vitamin D deficiency." N Engl J Med **357**(3): p. 266-281. doi: 10.1056/NEJMra070553.

[Holick 2008]

Holick, M. F. (2008). "The vitamin D deficiency pandemic and consequences for nonskeletal health: mechanisms of action." Mol Aspects Med **29**(6): p. 361-368. doi: 10.1016/j.mam.2008.08.008.

[Holick et al. 2011]

Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., Gordon, C. M., Hanley, D. A., Heaney, R. P., Murad, M. H., Weaver, C. M. and Endocrine, S. (2011). "Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline." J Clin Endocrinol Metab **96**(7): p. 1911-1930. doi: 10.1210/jc.2011-0385.

[Holick and Chen 2008]

Holick, M. F. and Chen, T. C. (2008). "Vitamin D deficiency: a worldwide problem with health consequences." Am J Clin Nutr **87**(4): p. 1080S-1086S. doi:

[Hollis and Pittard 1984]

Hollis, B. W. and Pittard, W. B., 3rd (1984). "Evaluation of the total fetomaternal vitamin D relationships at term: evidence for racial differences." J Clin Endocrinol Metab **59**(4): p. 652-657. doi: 10.1210/jcem-59-4-652.

[Holmes et al. 2009]

Holmes, V. A., Barnes, M. S., Alexander, H. D., McFaul, P. and Wallace, J. M. (2009). "Vitamin D deficiency and insufficiency in pregnant women: a longitudinal study." Br J Nutr **102**(6): p. 876-881. doi: 10.1017/S0007114509297236.

[ISAAC 1998]

ISAAC (1998). "Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee." Lancet **351**(9111): p. 1225-1232. doi:

[Jokhi et al. 1997]

Jokhi, P. P., King, A. and Loke, Y. W. (1997). "Cytokine production and cytokine receptor expression by cells of the human first trimester placental-uterine interface." Cytokine **9**(2): p. 126-137. doi: 10.1006/cyto.1996.0146.

[Jones et al. 2010]

Jones, L. A., Kreem, S., Shweash, M., Paul, A., Alexander, J. and Roberts, C. W. (2010). "Differential modulation of TLR3- and TLR4-mediated dendritic cell maturation and function by progesterone." J Immunol **185**(8): p. 4525-4534. doi: 10.4049/jimmunol.0901155.

[King et al. 1998]

King, C. L., Malhotra, I., Mungai, P., Wamachi, A., Kioko, J., Ouma, J. H. and Kazura, J. W. (1998). "B cell sensitization to helminthic infection develops in utero in humans." J Immunol **160**(7): p. 173-176. doi:

[Knabl et al. 2014]

Knabl, J., Huttenbrenner, R., Hutter, S., Gunthner-Biller, M., Riedel, C., Hiden, U., Kainer, F., Desoye, G. and Jeschke, U. (2014). "Gestational Diabetes Mellitus Upregulates Vitamin D Receptor in Extravillous Trophoblasts and Fetoplacental Endothelial Cells." Reprod Sci: p. doi: 10.1177/1933719114542020.

[Knabl et al. 2017]

Knabl, J., Vattai, A., Ye, Y., Jueckstock, J., Hutter, S., Kainer, F., Mahner, S. and Jeschke, U. (2017). "Role of Placental VDR Expression and Function in Common Late Pregnancy Disorders." Int J Mol Sci **18**(11): p. doi: 10.3390/ijms18112340.

[Kuo et al. 2013]

Kuo, C. H., Kuo, H. F., Huang, C. H., Yang, S. N., Lee, M. S. and Hung, C. H. (2013). "Early life exposure to antibiotics and the risk of childhood allergic diseases: an update from the perspective of the hygiene hypothesis." J Microbiol Immunol Infect **46**(5): p. 320-329. doi: 10.1016/j.jmii.2013.04.005.

[Kurtis et al. 2011]

Kurtis, J. D., Higashi, A., Wu, H. W., Gundogan, F., McDonald, E. A., Sharma, S., PondTor, S., Jarilla, B., Sagliba, M. J., Gonzal, A., Olveda, R., Acosta, L. and Friedman, J. F. (2011). "Maternal Schistosomiasis japonica is associated with maternal, placental, and fetal inflammation." Infect Immun **79**(3): p. 1254-1261. doi: 10.1128/IAI.01072-10.

[Laidlaw et al. 2015]

Laidlaw, B. J., Cui, W., Amezquita, R. A., Gray, S. M., Guan, T., Lu, Y., Kobayashi, Y., Flavell, R. A., Kleinstein, S. H., Craft, J. and Kaech, S. M. (2015). "Production of IL-10 by CD4(+) regulatory T cells during the resolution

of infection promotes the maturation of memory CD8(+) T cells." Nat Immunol **16**(8): p. 871-879. doi: 10.1038/ni.3224.

[Layland et al. 2007]

Layland, L. E., Rad, R., Wagner, H. and da Costa, C. U. (2007). "Immunopathology in schistosomiasis is controlled by antigen-specific regulatory T cells primed in the presence of TLR2." Eur J Immunol **37**(8): p. 2174-2184. doi: 10.1002/eji.200737063.

[Lemire et al. 1985]

Lemire, J. M., Adams, J. S., Kermani-Arab, V., Bakke, A. C., Sakai, R. and Jordan, S. C. (1985). "1,25-Dihydroxyvitamin D3 suppresses human T helper/inducer lymphocyte activity in vitro." J Immunol **134**(5): p. 3032-3035. doi:

[Lemire et al. 1995]

Lemire, J. M., Archer, D. C., Beck, L. and Spiegelberg, H. L. (1995). "Immunosuppressive actions of 1,25-dihydroxyvitamin D3: preferential inhibition of Th1 functions." J Nutr **125**(6 Suppl): p. 1704S-1708S. doi:

[Litonjua and Weiss 2007]

Litonjua, A. A. and Weiss, S. T. (2007). "Is vitamin D deficiency to blame for the asthma epidemic?" J Allergy Clin Immunol **120**(5): p. 1031-1035. doi: 10.1016/j.jaci.2007.08.028.

[Liu et al. 2009]

Liu, N., Kaplan, A. T., Low, J., Nguyen, L., Liu, G. Y., Equils, O. and Hewison, M. (2009). "Vitamin D induces innate antibacterial responses in human trophoblasts via an intracrine pathway." Biol Reprod **80**(3): p. 398-406. doi: 10.1095/biolreprod.108.073577.

[Liu et al. 2011]

Liu, N. Q., Kaplan, A. T., Lagishetty, V., Ouyang, Y. B., Ouyang, Y., Simmons, C. F., Equils, O. and Hewison, M. (2011). "Vitamin D and the regulation of placental inflammation." J Immunol **186**(10): p. 5968-5974. doi: 10.4049/jimmunol.1003332.

[Loffredo-Verde 2016]

Loffredo-Verde, E. (2016). The interaction of hepatitis B or C virus infection and schistosomiasis in chronic pathogen-induced liver inflammation. Dissertation. Technische Universität München.

[Lowe et al. 2011]

Lowe, A., Braback, L., Ekeus, C., Hjern, A. and Forsberg, B. (2011). "Maternal obesity during pregnancy as a risk for early-life asthma." J Allergy Clin Immunol **128**(5): p. 1107-1109 e1101-1102. doi: 10.1016/j.jaci.2011.08.025.

[Lynch et al. 1993]

Lynch, N. R., Hagel, I., Perez, M., Di Prisco, M. C., Lopez, R. and Alvarez, N. (1993). "Effect of anthelmintic treatment on the allergic reactivity of children in a tropical slum." J Allergy Clin Immunol **92**(3): p. 404-411. doi:

[Ma et al. 2012]

Ma, R., Gu, Y., Zhao, S., Sun, J., Groome, L. J. and Wang, Y. (2012). "Expressions of vitamin D metabolic components VDBP, CYP2R1, CYP27B1, CYP24A1, and VDR in placentas from normal and preeclamptic pregnancies." Am J Physiol Endocrinol Metab **303**(7): p. E928-935. doi: 10.1152/ajpendo.00279.2012.

[Masoli et al. 2004]

Masoli, M., Fabian, D., Holt, S., Beasley, R. and Global Initiative for Asthma, P. (2004). "The global burden of asthma: executive summary of the GINA Dissemination Committee report." Allergy **59**(5): p. 469-478. doi: 10.1111/j.1398-9995.2004.00526.x.

[Mendelson et al. 2016]

Mendelson, C. R., Montalbano, A. P. and Gao, L. (2016). "Fetal-to-Maternal Signaling in the Timing of Birth." J Steroid Biochem Mol Biol: p. doi: 10.1016/j.jsbmb.2016.09.006.

[Mirzakhani et al. 2015]

Mirzakhani, H., Al-Garawi, A., Weiss, S. T. and Litonjua, A. A. (2015). "Vitamin D and the development of allergic disease: how important is it?" Clin Exp Allergy **45**(1): p. 114-125. doi: 10.1111/cea.12430.

[Mizwicki and Norman 2009]

Mizwicki, M. T. and Norman, A. W. (2009). "The vitamin D sterol-vitamin D receptor ensemble model offers unique insights into both genomic and rapid-response signaling." Sci Signal **2**(75): p. re4. doi: 10.1126/scisignal.275re4.

[Moormann et al. 1999]

Moormann, A. M., Sullivan, A. D., Rochford, R. A., Chensue, S. W., Bock, P. J., Nyirenda, T. and Meshnick, S. R. (1999). "Malaria and pregnancy: placental cytokine expression and its relationship to intrauterine growth retardation." J Infect Dis **180**(6): p. 1987-1993. doi: 10.1086/315135.

[Mora et al. 2008]

Mora, J. R., Iwata, M. and von Andrian, U. H. (2008). "Vitamin effects on the immune system: vitamins A and D take centre stage." Nat Rev Immunol **8**(9): p. 685-698. doi: 10.1038/nri2378.

[Mori et al. 2016]

Mori, M., Bogdan, A., Balassa, T., Csabai, T. and Szekeres-Bartho, J. (2016). "The decidua-the maternal bed embracing the embryo-maintains the pregnancy." Semin Immunopathol **38**(6): p. 635-649. doi: 10.1007/s00281-016-0574-0.

[Mpairwe et al. 2014]

Mpairwe, H., Ndibazza, J., Webb, E. L., Nampijja, M., Muhangi, L., Apule, B., Lule, S., Akurut, H., Kizito, D., Kakande, M., Jones, F. M., Fitzsimmons, C. M., Muwanga, M., Rodrigues, L. C., Dunne, D. W. and Elliott, A. M. (2014). "Maternal hookworm modifies risk factors for childhood eczema: results from a birth cohort in Uganda." Pediatr Allergy Immunol **25**(5): p. 481-488. doi: 10.1111/pai.12251.

[Mpairwe et al. 2011]

Mpairwe, H., Webb, E. L., Muhangi, L., Ndibazza, J., Akishule, D., Nampijja, M., Ngom-wegi, S., Tumusime, J., Jones, F. M., Fitzsimmons, C., Dunne, D. W., Muwanga, M., Rodrigues, L. C. and Elliott, A. M. (2011). "Anthelminthic treatment during pregnancy is associated with increased risk of infantile eczema: randomised-controlled trial results." Pediatr Allergy Immunol **22**(3): p. 305-312. doi: 10.1111/j.1399-3038.2010.01122.x.

[Mulligan et al. 2010]

Mulligan, M. L., Felton, S. K., Riek, A. E. and Bernal-Mizrachi, C. (2010). "Implications of vitamin D deficiency in pregnancy and lactation." Am J Obstet Gynecol **202**(5): p. 429 e421-429. doi: 10.1016/j.ajog.2009.09.002.

[Munger et al. 2016]

Munger, K. L., Aivo, J., Hongell, K., Soilu-Hanninen, M., Surcel, H. M. and Ascherio, A. (2016). "Vitamin D Status During Pregnancy and Risk of Multiple Sclerosis in Offspring of Women in the Finnish Maternity Cohort." JAMA Neurol **73**(5): p. 515-519. doi: 10.1001/jamaneurol.2015.4800.

[Murphy et al. 2009]

Murphy, S. P., Tayade, C., Ashkar, A. A., Hatta, K., Zhang, J. and Croy, B. A. (2009). "Interferon gamma in successful pregnancies." Biol Reprod **80**(5): p. 848-859. doi: 10.1095/biolreprod.108.073353.

[Nguyen et al. 2015]

Nguyen, T., Yong, H., Borg, A., Brennecke, S. and Murthi, P. (2015). "Decreased placental vitamin D receptor contributes to aberrant cell-cycle gene regulation in human idiopathic fetal growth restriction." Placenta **36**(9): p. A44. doi: 10.1016/j.placenta.2015.07.318.

[Nguyen et al. 2015]

Nguyen, T. P., Yong, H. E., Chollangi, T., Borg, A. J., Brennecke, S. P. and Murthi, P. (2015). "Placental vitamin D receptor expression is decreased in human idiopathic fetal growth restriction." J Mol Med (Berl) **93**(7): p. 795-805. doi: 10.1007/s00109-015-1267-1.

[Nielsen et al. 1990]

Nielsen, F. R., Bek, K. M., Rasmussen, P. E., Qvist, I. and Tobiassen, M. (1990). "C-reactive protein during normal pregnancy." Eur J Obstet Gynecol Reprod Biol **35**(1): p. 23-27. doi:

[Okada et al. 2010]

Okada, H., Kuhn, C., Feillet, H. and Bach, J. F. (2010). "The 'hygiene hypothesis' for autoimmune and allergic diseases: an update." Clin Exp Immunol **160**(1): p. 1-9. doi: 10.1111/j.1365-2249.2010.04139.x.

[Olds 2003]

Olds, G. R. (2003). "Administration of praziquantel to pregnant and lactating women." Acta Trop **86**(2-3): p. 185-195. doi:

[Omdahl et al. 2002]

Omdahl, J. L., Morris, H. A. and May, B. K. (2002). "Hydroxylase enzymes of the vitamin D pathway: expression, function, and regulation." Annu Rev Nutr **22**: p. 139-166. doi: 10.1146/annurev.nutr.22.120501.150216.

[Oriss et al. 1997]

Oriss, T. B., McCarthy, S. A., Morel, B. F., Campana, M. A. and Morel, P. A. (1997). "Crossregulation between T helper cell (Th)1 and Th2: inhibition of Th2 proliferation by IFN-gamma involves interference with IL-1." J Immunol **158**(8): p. 3666-3672. doi:

[Park et al. 2017]

Park, H., Wood, M. R., Malysheva, O. V., Jones, S., Mehta, S., Brannon, P. M. and Caudill, M. A. (2017). "Placental vitamin D metabolism and its associations with circulating vitamin D metabolites in pregnant women." Am J Clin Nutr **106**(6): p. 1439-1448. doi: 10.3945/ajcn.117.153429.

[Pearce and MacDonald 2002]

Pearce, E. J. and MacDonald, A. S. (2002). "The immunobiology of schistosomiasis." Nat Rev Immunol **2**(7): p. 499-511. doi: 10.1038/nri843.

[Peng et al. 2004]

Peng, L., Huang, Y., Jin, F., Jiang, S. W. and Payne, A. H. (2004). "Transcription enhancer factor-5 and a GATA-like protein determine placental-specific expression of the Type I human 3beta-hydroxysteroid dehydrogenase gene, HSD3B1." Mol Endocrinol **18**(8): p. 2049-2060. doi: 10.1210/me.2004-0028.

[Pereira et al. 2014]

Pereira, L. H., Machado, J. R., Olegario, J. G., Rocha, L. P., Silva, M. V., Guimaraes, C. S., Reis, M. A., Castellano, L. R., Ramalho, F. S. and Correa, R. R. (2014). "Interleukin-6 and C-reactive protein are overexpressed in the liver of perinatal deaths diagnosed with fetal inflammatory response syndrome." Dis Markers **2014**: p. 252780. doi: 10.1155/2014/252780.

[Piccinni et al. 1995]

Piccinni, M. P., Giudizi, M. G., Biagiotti, R., Beloni, L., Giannarini, L., Sampognaro, S., Parronchi, P., Manetti, R., Annunziato, F., Livi, C. and et al. (1995). "Progesterone favors the development of human T helper cells producing Th2-type cytokines and promotes both IL-4 production and membrane CD30 expression in established Th1 cell clones." J Immunol **155**(1): p. 128-133. doi:

[Piccinni et al. 2000]

Piccinni, M. P., Scaletti, C., Maggi, E. and Romagnani, S. (2000). "Role of hormone-controlled Th1- and Th2-type cytokines in successful pregnancy." J Neuroimmunol **109**(1): p. 30-33. doi:

[Pike and Meyer 2010]

Pike, J. W. and Meyer, M. B. (2010). "The vitamin D receptor: new paradigms for the regulation of gene expression by 1,25-dihydroxyvitamin D(3)." Endocrinol Metab Clin North Am **39**(2): p. 255-269, table of contents. doi: 10.1016/j.ecl.2010.02.007.

[Pospechova et al. 2009]

Pospechova, K., Rozehnal, V., Stejskalova, L., Vrzal, R., Pospisilova, N., Jamborova, G., May, K., Siegmund, W., Dvorak, Z., Nachtigal, P., Semecky, V. and Pavek, P. (2009). "Expression and activity of vitamin D receptor in the human placenta and in choriocarcinoma BeWo and JEG-3 cell lines." Mol Cell Endocrinol **299**(2): p. 178-187. doi: 10.1016/j.mce.2008.12.003.

[Powe et al. 2013]

Powe, C. E., Evans, M. K., Wenger, J., Zonderman, A. B., Berg, A. H., Nalls, M., Tamez, H., Zhang, D., Bhan, I., Karumanchi, S. A., Powe, N. R. and Thadhani, R. (2013). "Vitamin D-binding protein and vitamin D status of black Americans and white Americans." N Engl J Med **369**(21): p. 1991-2000. doi: 10.1056/NEJMoa1306357.

[Prietl et al. 2013]

Prietl, B., Treiber, G., Pieber, T. R. and Amrein, K. (2013). "Vitamin D and immune function." Nutrients **5**(7): p. 2502-2521. doi: 10.3390/nu5072502.

[Reister 2011]

Reister, F. (2011). Entstehung und entwicklung einer Schwangerchaft. (Ed.), Gynäkologie und Geburtshilfe (pp. 165-186). München: Elsevier

[Richard et al. 2017]

Richard, A., Rohrmann, S. and Quack Lotscher, K. C. (2017). "Prevalence of Vitamin D Deficiency and Its Associations with Skin Color in Pregnant Women in the First Trimester in a Sample from Switzerland." Nutrients **9**(3): p. doi: 10.3390/nu9030260.

[Robinson and Klein 2012]

Robinson, D. P. and Klein, S. L. (2012). "Pregnancy and pregnancy-associated hormones alter immune responses and disease pathogenesis." Horm Behav **62**(3): p. 263-271. doi: 10.1016/j.yhbeh.2012.02.023.

[Roth et al. 1996]

Roth, I., Corry, D. B., Locksley, R. M., Abrams, J. S., Litton, M. J. and Fisher, S. J. (1996). "Human placental cytotrophoblasts produce the immunosuppressive cytokine interleukin 10." J Exp Med **184**(2): p. 539-548. doi:

[Sahebnasagh et al. 2017]

Sahebnasagh, B., Hosseinzadeh-Attar, M. J. and Sharifi, A. (2017). "Risks associated with preeclampsia: possible mechanisms." Reviews in Clinical Medicine **4**(1): p. 39-44. doi: 10.22038/rcm.2017.13735.1156.

[Salle et al. 2000]

Salle, B. L., Delvin, E. E., Lapillonne, A., Bishop, N. J. and Glorieux, F. H. (2000). "Perinatal metabolism of vitamin D." Am J Clin Nutr **71**(5 Suppl): p. 1317S-1324S. doi:

[Santiso-Mere et al. 1993]

Santiso-Mere, D., Sone, T., Hilliard, G. M. t., Pike, J. W. and McDonnell, D. P. (1993). "Positive regulation of the vitamin D receptor by its cognate ligand in heterologous expression systems." Mol Endocrinol **7**(7): p. 833-839. doi: 10.1210/mend.7.7.8413308.

[Schroth et al. 2005]

Schroth, R. J., Lavelle, C. L. and Moffatt, M. E. (2005). "Review of vitamin D deficiency during pregnancy: who is affected?" Int J Circumpolar Health **64**(2): p. 112-120. doi:

[Scippo et al. 1993]

Scippo, M. L., Frankenne, F., Hooghe-Peters, E. L., Igout, A., Velkeniers, B. and Hennen, G. (1993). "Syncytiotrophoblastic localization of the human growth hormone variant mRNA in the placenta." Mol Cell Endocrinol **92**(2): p. R7-13. doi:

[Sharland and Subgroup 2007]

Sharland, M. and Subgroup, S. P. (2007). "The use of antibacterials in children: a report of the Specialist Advisory Committee on Antimicrobial Resistance (SACAR) Paediatric Subgroup." J Antimicrob Chemother **60 Suppl 1**: p. i15-26. doi: 10.1093/jac/dkm153.

[Shin et al. 2010]

Shin, J. S., Choi, M. Y., Longtine, M. S. and Nelson, D. M. (2010). "Vitamin D effects on pregnancy and the placenta." Placenta **31**(12): p. 1027-1034. doi: 10.1016/j.placenta.2010.08.015.

[Siegrist and Siegrist-Obimpeh 1992]

Siegrist, D. and Siegrist-Obimpeh, P. (1992). "Schistosoma haematobium infection in pregnancy." Acta Trop **50**(4): p. 317-321. doi:

[Simard et al. 1996]

Simard, J., Durocher, F., Mebarki, F., Turgeon, C., Sanchez, R., Labrie, Y., Couet, J., Trudel, C., Rheaume, E., Morel, Y., Luu-The, V. and Labrie, F. (1996). "Molecular biology and genetics of the 3 beta-hydroxysteroid dehydrogenase/delta5-delta4 isomerase gene family." J Endocrinol **150 Suppl**: p. S189-207. doi:

[Sitras et al. 2009]

Sitras, V., Paulssen, R., Leirvik, J., Vartun, A. and Acharya, G. (2009). "Placental gene expression profile in intrauterine growth restriction due to placental insufficiency." Reprod Sci **16**(7): p. 701-711. doi: 10.1177/1933719109334256.

[Siza 2008]

Siza, J. E. (2008). "Risk factors associated with low birth weight of neonates among pregnant women attending a referral hospital in northern Tanzania." Tanzan J Health Res **10**(1): p. 1-8. doi:

[Sood et al. 2006]

Sood, R., Zehnder, J. L., Druzin, M. L. and Brown, P. O. (2006). "Gene expression patterns in human placenta." Proc Natl Acad Sci U S A **103**(14): p. 5478-5483. doi: 10.1073/pnas.0508035103.

[Stene et al. 2000]

Stene, L. C., Ulriksen, J., Magnus, P. and Joner, G. (2000). "Use of cod liver oil during pregnancy associated with lower risk of Type I diabetes in the offspring." Diabetologia **43**(9): p. 1093-1098. doi: 10.1007/s001250051499.

[Stiemsma et al. 2015]

Stiemsma, L. T., Reynolds, L. A., Turvey, S. E. and Finlay, B. B. (2015). "The hygiene hypothesis: current perspectives and future therapies." Immunotargets Ther **4**: p. 143-157. doi: 10.2147/ITT.S61528.

[Strachan 1989]

Strachan, D. P. (1989). "Hay fever, hygiene, and household size." BMJ **299**(6710): p. 1259-1260. doi:

[Straubinger 2013]

Straubinger, K. (2013). Immunosuppressive and transgenerational effects on the development of allergic airway inflammation during Schistosoma mansoni infection. Dissertation. Technische Universität München.

[Straubinger et al. 2014]

Straubinger, K., Paul, S., Prazeres da Costa, O., Ritter, M., Buch, T., Busch, D. H., Layland, L. E. and Prazeres da Costa, C. U. (2014). "Maternal immune response to helminth infection during pregnancy determines offspring susceptibility to allergic airway inflammation." J Allergy Clin Immunol: p. doi: 10.1016/j.jaci.2014.05.034.

[Straubinger and Prazeres da Costa 2014]

Straubinger, K. and Prazeres da Costa, C. (2014). Maternal helminth infections. In Horsnell, W. (Ed.), How Helminths Alter immunity to Infection (pp. 27-48). New York: Spinger

[Suguitan et al. 2003]

Suguitan, A. L., Jr., Leke, R. G., Fouda, G., Zhou, A., Thuita, L., Metenou, S., Fogako, J., Megnekou, R. and Taylor, D. W. (2003). "Changes in the levels of chemokines and cytokines in the placentas of women with Plasmodium falciparum malaria." J Infect Dis **188**(7): p. 1074-1082. doi: 10.1086/378500.

[Tamblyn et al. 2017]

Tamblyn, J. A., Susarla, R., Jenkinson, C., Jeffery, L. E., Ohizua, O., Chun, R. F., Chan, S. Y., Kilby, M. D. and Hewison, M. (2017). "Dysregulation of

maternal and placental vitamin D metabolism in preeclampsia." Placenta **50**: p. 70-77. doi: 10.1016/j.placenta.2016.12.019.

[Tavakoli et al. 2011]

Tavakoli, M., Jeddi-Tehrani, M., Salek-Moghaddam, A., Rajaei, S., Mohammadzadeh, A., Sheikhhasani, S., Kazemi-Sefat, G. E. and Zarnani, A. H. (2011). "Effects of 1,25(OH)2 vitamin D3 on cytokine production by endometrial cells of women with recurrent spontaneous abortion." Fertil Steril **96**(3): p. 751-757. doi: 10.1016/j.fertnstert.2011.06.075.

[Tuckey 2005]

Tuckey, R. C. (2005). "Progesterone synthesis by the human placenta." Placenta **26**(4): p. 273-281. doi: 10.1016/j.placenta.2004.06.012.

[van den Biggelaar et al. 2004]

van den Biggelaar, A. H., Rodrigues, L. C., van Ree, R., van der Zee, J. S., Hoeksma-Kruize, Y. C., Souverijn, J. H., Missinou, M. A., Borrmann, S., Kremsner, P. G. and Yazdanbakhsh, M. (2004). "Long-term treatment of intestinal helminths increases mite skin-test reactivity in Gabonese schoolchildren." J Infect Dis **189**(5): p. 892-900. doi: 10.1086/381767.

[van den Biggelaar et al. 2000]

van den Biggelaar, A. H., van Ree, R., Rodrigues, L. C., Lell, B., Deelder, A. M., Kremsner, P. G. and Yazdanbakhsh, M. (2000). "Decreased atopy in children infected with Schistosoma haematobium: a role for parasite-induced interleukin-10." Lancet **356**(9243): p. 1723-1727. doi: 10.1016/S0140-6736(00)03206-2.

[Versini et al. 2015]

Versini, M., Jeandel, P. Y., Bashi, T., Bizzaro, G., Blank, M. and Shoenfeld, Y. (2015). "Unraveling the Hygiene Hypothesis of helminthes and autoimmunity: origins, pathophysiology, and clinical applications." BMC Med **13**: p. 81. doi: 10.1186/s12916-015-0306-7.

[Vigano et al. 2006]

Vigano, P., Lattuada, D., Mangioni, S., Ermellino, L., Vignali, M., Caporizzo, E., Panina-Bordignon, P., Besozzi, M. and Di Blasio, A. M. (2006). "Cycling and early pregnant endometrium as a site of regulated expression of the vitamin D system." J Mol Endocrinol **36**(3): p. 415-424. doi: 10.1677/jme.1.01946.

[von Mutius 2012]

von Mutius, E. (2012). "Maternal farm exposure/ingestion of unpasteurized cow's milk and allergic disease." Curr Opin Gastroenterol **28**(6): p. 570-576. doi: 10.1097/MOG.0b013e32835955d3.

[von Mutius et al. 1994]

von Mutius, E., Martinez, F. D., Fritzsch, C., Nicolai, T., Reitmeir, P. and Thiemann, H. H. (1994). "Skin test reactivity and number of siblings." BMJ **308**(6930): p. 692-695. doi:

[von Mutius et al. 1994]

von Mutius, E., Martinez, F. D., Fritzsch, C., Nicolai, T., Roell, G. and Thiemann, H. H. (1994). "Prevalence of asthma and atopy in two areas of West and East Germany." Am J Respir Crit Care Med **149**(2 Pt 1): p. 358-364. doi: 10.1164/ajrccm.149.2.8306030.

[von Mutius et al. 1998]

von Mutius, E., Weiland, S. K., Fritzsch, C., Duhme, H. and Keil, U. (1998). "Increasing prevalence of hay fever and atopy among children in Leipzig, East Germany." Lancet **351**(9106): p. 862-866. doi: 10.1016/S0140-6736(97)10100-3.

[Wang et al. 2004]

Wang, T. T., Nestel, F. P., Bourdeau, V., Nagai, Y., Wang, Q., Liao, J., Tavera-Mendoza, L., Lin, R., Hanrahan, J. W., Mader, S. and White, J. H. (2004). "Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression." J Immunol **173**(5): p. 2909-2912. doi:

[Wei et al. 2016]

Wei, Z., Zhang, J. and Yu, X. (2016). "Maternal vitamin D status and childhood asthma, wheeze, and eczema: A systematic review and meta-analysis." Pediatr Allergy Immunol **27**(6): p. 612-619. doi: 10.1111/pai.12593.

[Weisman et al. 1979]

Weisman, Y., Harell, A., Edelstein, S., David, M., Spirer, Z. and Golander, A. (1979). "1 alpha, 25-Dihydroxyvitamin D3 and 24,25-dihydroxyvitamin D3 in vitro synthesis by human decidua and placenta." Nature **281**(5729): p. 317-319. doi:

[White 2008]

White, J. H. (2008). "Vitamin D signaling, infectious diseases, and regulation of innate immunity." Infect Immun **76**(9): p. 3837-3843. doi: 10.1128/IAI.00353-08.

[WHO 2012]

WHO. (2012, 26.09.2016). " Guideline: Vitamin D supplementation in pregnant women." from http://www.who.int/nutrition/publications/micronutrients/guidelines/vit_d_supp_pregnant_women/en/.

[WHO 2016]

WHO. (2016, February 2016). "Schistosomiasis." from http://www.who.int/mediacentre/factsheets/fs115/en/.

[WHO 2016]

WHO. (2016, March 2016). "Soil-transmitted helminth infections." Retrieved 09.09.2016, 2016, from http://www.who.int/mediacentre/factsheets/fs366/en/.

[Wilson et al. 2007]

Wilson, M. S., Elnekave, E., Mentink-Kane, M. M., Hodges, M. G., Pesce, J. T., Ramalingam, T. R., Thompson, R. W., Kamanaka, M., Flavell, R. A., Keane-Myers, A., Cheever, A. W. and Wynn, T. A. (2007). "IL-13Ralpha2 and IL-10 coordinately suppress airway inflammation, airway-hyperreactivity, and fibrosis in mice." J Clin Invest **117**(10): p. 2941-2951. doi: 10.1172/JCI31546.

[Workalemahu et al. 2017]

Workalemahu, T., Badon, S. E., Dishi-Galitzky, M., Qiu, C., Williams, M. A., Sorensen, T. and Enquobahrie, D. A. (2017). "Placental genetic variations in vitamin D metabolism and birthweight." Placenta **50**: p. 78-83. doi: 10.1016/j.placenta.2016.12.028.

[Wyatt et al. 2005]

Wyatt, S. M., Kraus, F. T., Roh, C. R., Elchalal, U., Nelson, D. M. and Sadovsky, Y. (2005). "The correlation between sampling site and gene expression in the term human placenta." Placenta **26**(5): p. 372-379. doi: 10.1016/j.placenta.2004.07.003.

[Yazdanbakhsh et al. 2002]

Yazdanbakhsh, M., Kremsner, P. G. and van Ree, R. (2002). "Allergy, parasites, and the hygiene hypothesis." Science **296**(5567): p. 490-494. doi: 10.1126/science.296.5567.490.

[Zeck et al. 2008]

Zeck, W., Widberg, C., Maylin, E., Desoye, G., Lang, U., McIntyre, D., Prins, J. and Russell, A. (2008). "Regulation of placental growth hormone secretion in a human trophoblast model--the effects of hormones and adipokines." Pediatr Res **63**(4): p. 353-357. doi: 10.1203/01.pdr.0000304935.19183.07.

[Zehnder et al. 2002]

Zehnder, D., Evans, K. N., Kilby, M. D., Bulmer, J. N., Innes, B. A., Stewart, P. M. and Hewison, M. (2002). "The ontogeny of 25-hydroxyvitamin D(3) 1alpha-hydroxylase expression in human placenta and decidua." Am J Pathol **161**(1): p. 105-114. doi:

[Zella et al. 2007]

Zella, L. A., Kim, S., Shevde, N. K. and Pike, J. W. (2007). "Enhancers located in the vitamin D receptor gene mediate transcriptional autoregulation by 1,25-dihydroxyvitamin D3." J Steroid Biochem Mol Biol **103**(3-5): p. 435-439. doi: 10.1016/j.jsbmb.2006.12.019.

[Zerwekh and Breslau 1986]

Zerwekh, J. E. and Breslau, N. A. (1986). "Human placental production of 1 alpha,25-dihydroxyvitamin D3: biochemical characterization and production in normal subjects and patients with pseudohypoparathyroidism." J Clin Endocrinol Metab **62**(1): p. 192-196. doi: 10.1210/jcem-62-1-192.

[Zhang et al. 2008]

Zhang, C., Qiu, C., Hu, F. B., David, R. M., van Dam, R. M., Bralley, A. and Williams, M. A. (2008). "Maternal plasma 25-hydroxyvitamin D concentrations and the risk for gestational diabetes mellitus." PLoS One **3**(11): p. e3753. doi: 10.1371/journal.pone.0003753.

[Zhang et al. 2018]

Zhang, Q., Chen, H., Wang, Y., Zhang, C., Tang, Z., Li, H., Huang, X., Ouyang, F., Huang, H. and Liu, Z. (2018). "Severe vitamin D deficiency in the first trimester is associated with placental inflammation in high-risk singleton pregnancy." Clin Nutr: p. doi: 10.1016/j.clnu.2018.06.978.