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# The Influence of Pregnancy-Associated Hormones on Immune Responses and the Contribution of Immuno-Endocrine Crosstalk to Successful Pregnancy

Bachelor's Thesis Biomedical Sciences – Biology

ISABEL VAN DER KLAAUW (S4940830)

SUPERVISED BY DR. M.M. FAAS

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## Abstract

Pregnancy is a fascinating phenomenon when it comes to tolerance of the fetus by the maternal immune system. A fetus consists partially of foreign material, namely the fathers, making it semiallogeneic. However, instead of attacking, the immune system starts to switch from a pro- to anti-inflammatory environment after early pregnancy. This is achieved together with a variety of hormones, of which estrogens, progesterone, hCG and relaxin are discussed due to their prominent roles in gestation and recurrence in studies. The immunological shift can be divided into two physical locations: the maternal periphery and maternal-fetal interface. Pregnancy-associated hormones can alter a variety of immune cells, including macrophages, T helper cells, regulatory T cells and natural killer cells. Several cytokines such as IL-10, TGF-β and IFN-γ have shown to be affected as well. The present thesis focuses on the key hormonal changes during pregnancy, how these hormones regulate immunological modulation and how immune-endocrine crosstalk contributes to successful pregnancy.



Key words: Pregnancy, immune-endocrine crosstalk, fetal tolerance, immunological shift, maternal-fetal interface, maternal periphery, estrogens, progesterone, hCG, relaxin.

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# Introduction

How the maternal immune system is able to tolerate the fetus during pregnancy remains a fascinating concept on both an endocrinological and immunological level. The fetus is semiallogeneic (Svensson-Arvelund et al., 2015), meaning that it is partially non-self. Successful pregnancy requires effective innate immunity to protect both mother and fetus against infection, but tolerance against paternal antigens of the fetus must be maintained as well (Wan et al., 2007). This poses quite a challenge for the maternal immune system. Even though much research has already been done concerning the underlying mechanisms, the exact details are only partially understood.

Pregnancy involves both physical and chemical alterations regulated by the increase of several steroid and peptide hormones. The most well-known steroid (sex) hormones are progesterone and estrogens, including estrone (E1), estradiol (E2) and estriol (E3) (Thomas & Potter, 2013). E2 is the most potent form of estrogen in non-pregnant females (Thomas & Potter, 2013) while E3 is the major estrogen in pregnancy; it is produced in high concentrations by the placenta (Darne et al., 1987). Estrogens and progesterone normally regulate the menstrual cycle, while during pregnancy both are significantly increased in maternal peripheral blood and in placental tissue to ensure establishment and maintenance of the fetus (Stewart et al., 1993). E2 triggers luteinizing hormone (LH) mid-menstrual cycle, resulting in the LH surge to initiate ovulation (Monroe et al., 1972). Additionally, E2 and progesterone prepare the endometrium for implantation in the luteal phase after ovulation by inducing decidualization, a process in which stromal cells surrounding the implanting embryo differentiate into decidual cells (Ramathal et al., 2010).

Peptide hormones that increase during pregnancy include human chorionic gonadotropin hormone (hCG) and relaxin. HCG is the first hormone to interact between mother and fetus since it is already released during the first step of implantation (Reshef et al., 1990) making it the hormone that is detected by a pregnancy test. After its peak in the first trimester, it keeps on being released during gestation (Braunstein et al., 1976). Relaxin promotes the relaxation of muscles and arteries (Longo et al., 2003).

Altogether, numerous hormones act a part in the regulation of pregnancy and while their direct effects are of key importance, the immune system is indirectly modified as well. Immunity undergoes several changes as a response to complex immune-endocrine interactions. These immunological adaptations are essential for tolerating the semiallogeneic fetus (Schumacher et al., 2013). During implantation, an injury-induced inflammatory response promotes the transition of a nonreceptive uterus into a receptive uterus (Gnainsky et al., 2010). Implantation and pregnancy success is dependent on the immune system and endometrial receptivity (Pourmoghadam et al., 2020). As gestation progresses, the mother's immune system undergoes an immunological shift: immunity is altered to upregulate anti-inflammatory and innate responses and thus downregulates an adaptive and proinflammatory environment (Kraus et al., 2011). An influx of pro-inflammatory immune cells and cytokines into the myometrium during delivery induces renewed inflammation (Shynlova et al., 2012; Romero et al., 1989). The shift can be divided into two parts; modifications that occur at the maternal periphery and at the maternal-fetal interface.

Although both the endocrine and immune system majorly contribute to the gestation process, their precise interaction remains under investigation. Therefore, this thesis aims to discuss the key altered hormonal levels during pregnancy and how these changes influence immune responses. The crosstalk between the endocrine and immune system will be discussed to review how they contribute to successful pregnancy.

## Key hormonal changes during pregnancy

Pregnancy is characterized by temporal physiological changes that can be attributed to altered hormonal levels. In pregnancy, hormonal fluctuations play a crucial role in fetal establishment and maintenance to ensure successful development (Stewart et al., 1993). A variety of hormones are affected during pregnancy, of which estrogens, progesterone and human chorionic gonadotropin are the most fundamental (Stewart et al., 1993; Challis et al., 2009).

Steroid hormones start to gradually rise after conception to establish and safeguard pregnancy (Darne et al., 1987; Piccinni et al., 1995). The increased secretion of ovarian steroid hormones might be the result of the preimplantation embryo giving a gonadotropic stimulus (Stewart et al., 1993). Estrogens have broad reproductive and non-reproductive functions. During placentation, E2 is a crucial factor in trophoblast invasion and remodeling of decidual spiral arteries in the uterus (Hsieh et al., 2023). E2 levels rise as the placenta grows during pregnancy; maternal plasma estrogen levels increase gradually as gestation progresses (O'Leary et al., 1991). E3 is the major estrogen with an exponential rise after 34 weeks of pregnancy (Darne et al., 1987). In early stages, estrogens are mainly produced by the corpus luteum, until the luteal-placental shift, which signifies the moment pregnancy maintenance shifts from control by the corpus luteum to placental control (Ziegler et al., 2023). Estrogens are then synthesized in large amounts by the placenta (Rosenfeld & Rivera, 1978b).

Additionally, estrogens such as E1 and E2 modulate vascular endothelium function and can act as vasodilators on reproductive tissue (Rosenfeld & Rivera, 1978b). Moreover, human endometrial cells show enhanced cell proliferation as a response to estrogens (Pavlik & Katzenellenbogen, 1978) via the expression of estrogen receptors (ER) (Bryś et al., 2009), suggesting their crucial function in maintaining the lining of the uterus and preparing it for gestation and birth. A thick endometrium optimizes implantation and nutrition. Estradiol combined with progesterone upregulates mucin-1 at the receptive endometrium, which promotes adhesion and implantation to a proper site (Meseguer et al., 2001). Progesterone is a central regulator of pregnancy maintenance. Its main functions are the stimulation of endometrial decidualization (Ramathal et al., 2010) and inhibition of smooth muscle contractility to maintain myometrial quiescence (Mesiano et al., 2002). The latter ensures that early labor does not take place and thus, progesterone withdrawal is linked to labor induction (Mesiano et al., 2002). Progesterone helps the endometrium thicken via progesterone receptors (PR) (Bryś et al., 2009).

After fertilization, progesterone levels are regulated by hCG. This hormone induces the production of progesterone by the corpus luteum of the ovary (Ziegler et al., 2023). The placenta takes over progesterone (and estrogen) production after the luteal-placental shift in the first trimester, as there are sufficient syncytiotrophoblast cells present (Handschuh et al., 2007). Syncytiotrophoblast cells cover the outer layer of the placental villous trees and invade the wall of the uterus during implantation. This establishes transport of nutrients, gases, and waste between mother and fetus (Handschuh et al., 2007).

HCG is produced and secreted by trophoblast cells of the blastocyst, which is the earliest cell stage of an embryo (Reshef et al., 1990). Via growth and maturation of the endometrium, hCG can prepare the uterus for implantation and thus support the blastocyst (Burton et al., 2002). During implantation of the early placenta, hCG facilitates trophoblast invasion and migration. Moreover, hCG stimulates endometrial glands (Burton et al., 2002) and angiogenesis in the endothelium of the uterus (Licht et al., 1998) to ensure optimal blood supply and nutrition for the placenta and fetus. Relaxin is not mentioned in literature as much as the aforementioned hormones but is still of importance in gestation. It is also derived from the corpus luteum (Piccinni & Romagnani, 1996; Danielson et al., 1999). Relaxin plays a role in implantation, placentation, and the maintenance of pregnancy in general by for example enhancing trophoblast survival and proliferation due to its anti-apoptotic effects (Lodhi et al., 2013). The contractility of the myometrium is affected by relaxin, since it inhibits spontaneous uterine activity that could result in preterm labor. (Longo et al., 2003).

Moreover, relaxin is a potent vasodilator, thus it is thought to influence pregnancy blood pressure (Longo et al., 2003). These effects were shown in a study by Danielson et al. (1999), since relaxin administration to rats revealed renal and osmoregulatory effects resembling those in pregnancy, such as reduced plasma osmolality (Danielson et al., 1999).

While estrogens and progesterone steadily increase during pregnancy (Johansson & Jonasson, 1971; O'Leary et al., 1991), hCG and relaxin already peak in the first trimester and then slowly decline, but remain measurable (Braunstein et al., 1976; Eddie et al., 1986). The four discussed key hormonal fluctuations during all three trimesters are depicted in figure 1.

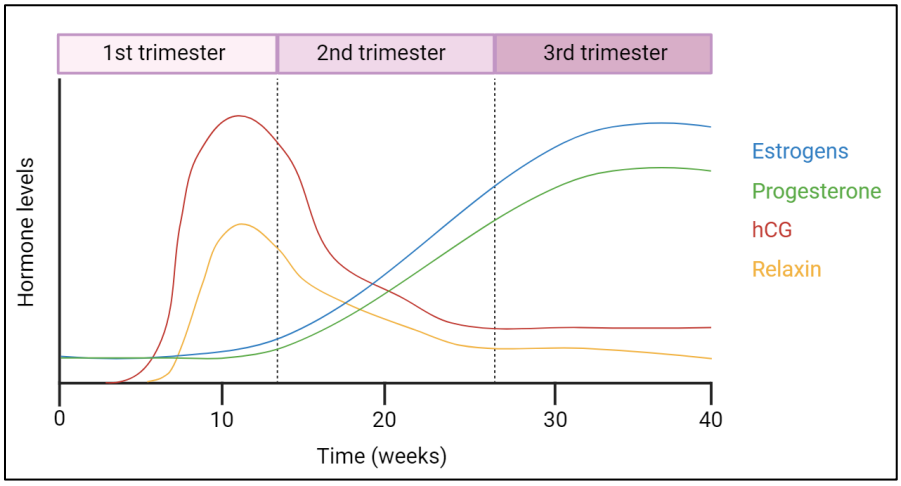


*Figure 1: Key hormonal fluctuations during pregnancy. Estrogens and progesterone continue to increase throughout pregnancy while hCG and relaxin peak in the first trimester and then decline but remain detectable (based on figure 1a by Fuhler (2020)).*

# Immunological adaptations in pregnancy

Hormonal changes play a key role in pregnancy progression to adapt the maternal body to the fetus. However, the endocrine system contributes significantly to the immunological shift as well, as crosstalk between hormonal and immunological factors contribute to fetal tolerance (Schumacher et al., 2013). Interaction between mother and fetus takes place at the placental attachment site in the uterus, which is where the maternal decidua develops: the maternal-fetal interface (figure 2). It is known that maternal-derived immune cells reside here, thus local regulation is necessary to prevent a harmful immune response to the fetus (Loewendorf et al., 2014).

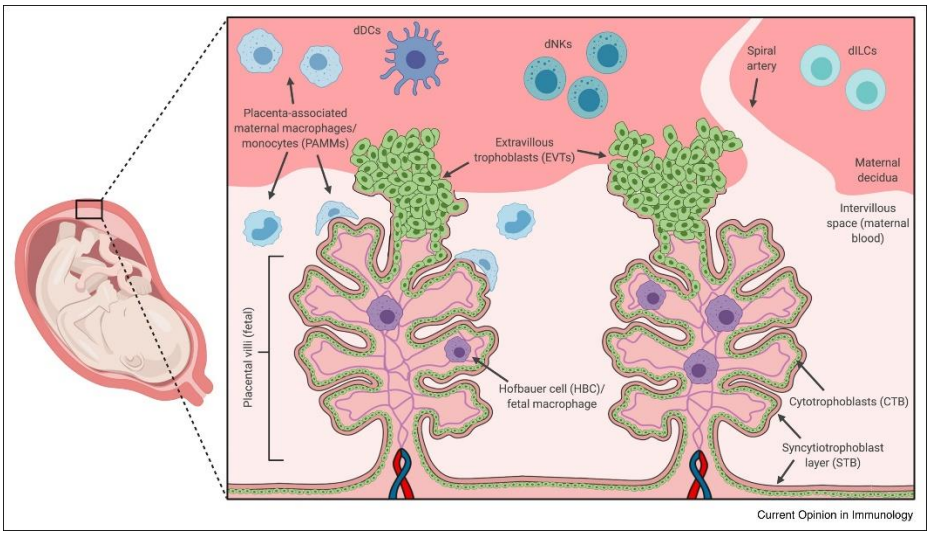


*Figure 2: The maternal-fetal interface composed of the maternal-derived decidua and fetal-derived placenta. Trophoblast cells invade the endometrium, resulting in the influx of maternal-derived immune cells including natural killer cells, dendritic cells, macrophages, monocytes and innate lymphoid cells (Semmes & Coyne, 2022).*

## Pregnancy stages and immunity

### Stage I

The first stage of pregnancy, including implantation, placentation and the first trimester, triggers an inflammatory response. The process of implantation and placentation involves damage of the endometrium by the invasion of trophoblast cells and influx of a variety of immune cells, such as dendritic cells (DCs), macrophages and proinflammatory cytokines at the maternal-fetal interface (Gnainsky et al., 2010). It is suggested that these inflammatory immune cells are involved in the development of a receptive endometrium, due to the expression of MIP-1B (a macrophage inflammatory protein) and its mediation by TNF-α (Gnainsky et al., 2010). The influx of uterine DCs (uDCs) are required for successful embryo implantation and decidualization in mice by finetuning decidual angiogenesis, promoting blood vessel maturation and preventing embryo resorption (Plaks et al., 2008). Infertile women that underwent endometrial scratching showed increased pregnancy rate and implantation chance (Gibreel et al., 2012), indicating that implantation success is partially dependent on an injury-induced inflammatory reaction for proper repair and development of early pregnancy.

Two major subtypes of macrophages can be distinguished: M1 cells and M2 cells. M1 cells are linked to strong immune response induction while M2 cells appear to have a suppressive function to immunity and increase neovascularization (Zajac et al., 2013). The inflammatory environment during implantation would implicate the prominent presence of M1 cells.

### Stage II

During the second stage, a pregnant female's immune system is shifted from proinflammatory to anti-inflammatory responses. This does not imply immunosuppression, but rather an alteration in immune priorities including strengthened innate immune barriers and a reduction in adaptive and

inflammatory responses (Kraus et al., 2011). There is a switch to a M2 phenotype to tolerate foreign fetal antigens after implantation. This switch is partly regulated by hCG at the maternal-fetal interface to maintain immunological homeostasis (Furcron et al., 2016; Rami et al., 2014).

Furthermore, pregnancy represents a higher type 2 T helper cell to type 1 T helper cell ratio in the maternal peripheral blood to prevent damage to the pregnancy (Raghupathy et al., 2000). The associated cytokines are likely mediated by pregnancy hormones (Piccinni & Romagnani, 1996). CD4+ T lymphocytes are essential in steering immune responses by producing cytokines. Th1 cytokines include IL-2, TNF-α and IFN-γ and are responsible for proinflammatory responses, while Th2 cytokines IL-4, IL-5, IL-10 and IL-13 are involved in IgE allergic reactions and anti-inflammatory responses (Lee et al., 2019).
The Th1 to Th2 immune shift occurs both peripherally and locally at the maternal-fetal interface (Lin et al., 1993; Krishnan et al., 1996). A Th1/Th2 disbalance can result in preterm delivery due to a proinflammatory bias (El-Shazly et al., 2004), thus it is crucial for healthy gestation to maintain proper balance towards the Th2 type.

Regulatory T cells (Tregs or CD4+CD25+) are a subset of CD4+ T cells and express the transcription factor FoxP3, which is important in the immunosuppressive function of Tregs and their development. Maternal FoxP3+ greatly increases during pregnancy in the maternal bloodstream and locally at the maternal-fetal interface. (Sasaki et al., 2007; Loewendorf et al., 2014). Fetal-specific Tregs are recruited at the maternal-fetal interface where they help maintain tolerance to fetal antigens and boost a homeostatic environment for fetal survival (Rowe et al., 2012). The fetal placenta itself creates a tolerant uterine environment by producing regulatory factors, such as macrophage colony-stimulating factor (M-CSF), IL-10 and TGF-β, to induce homeostatic macrophages and Tregs and maintain excessive Th activation (Svensson-Arvelund et al., 2015).

### Stage III

The third and last stage of pregnancy implies completed development of the fetus, thus parturition can be initiated. Decidual M2 cells are abundant at term and have an immunoregulatory role prior to term pregnancy (Xu et al., 2016). Spontaneous delivery involves renewed inflammation due to an influx of leukocytes into the myometrium (Shynlova et al., 2012) and increased production of pro-inflammatory cytokines such as IL-1 (Romero et al., 1989). The recruitment of immune cells before and during labor helps in the process of tissue repair and regeneration after birth (Shynlova et al., 2012). Tissue macrophages, monocytes and neutrophils showed to infiltrate the myometrium around the time of parturition, indicating a role in labor activation. These alterations were followed by increased proinflammatory cytokine genes in a mouse model (Shynlova et al., 2012). Furthermore, Osman (2003) showed that neutrophils and macrophages infiltrate the cervix during labor, indicating an inflammatory process (Osman, 2003). Moreover, there is a bias back towards Th1 responses, as Th1 proinflammatory cytokine TNF-α peaked in early labor implying labor to be a proinflammatory state (Buonocore et al., 1995).

During parturition, M2 cells undergo M1 polarization (Furcron et al., 2016; Xu et al., 2016). Via the stimulation of these proinflammatory conditions, estrogen activation and a drop in progesterone (Mesiano et al., 2002), uterine contractions take place to deliver the baby and placenta. Altered myometrial responsiveness to progesterone and estrogens resulting in delivery is likely due to changes in receptor expression (Mesiano et al., 2002). A global illustration of the immunological shift during pregnancy is depicted in figure 3.

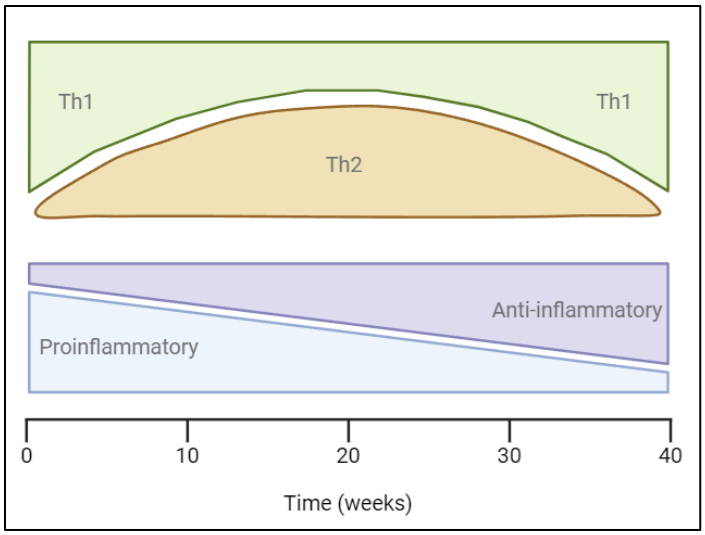


*Figure 3: Altered immune responses during pregnancy. There is a switch from a proinflammatory to anti-inflammatory environment (based on figure 1 by Robinson & Klein, 2012). Furthermore, Th1 responses are dominant during early pregnancy, which is taken over by Th2 as gestation progresses and near the end Th1 is dominant again (based on figure 1a by Fuhler (2020)).*

### Receptors

Receptors for pregnancy hormones are present on most immune cells, connecting immune adaptations to reproduction and pregnancy (Littauer et al., 2017; Salem, 2004). Modulation of immunity is thought to be achieved by direct binding to a specific receptor expressed by immune cells (Salem, 2004). For instance, pregnancy-associated hormones can modulate the recruitment of monocytes, their differentiation intro macrophages and their function in the reproductive tract (Tonello & Poli, 2007).

ERs are expressed in the endometrium and vagina and their gene expression is significantly increased after E3 treatment (Bryś et al., 2009). Estrogens can also mediate immune responses by binding to ERs on several lymphoid tissue cells, such as lymphocytes, macrophages, and DCs (Phiel et al., 2005). The expression of subtypes ERα and ERβ can vary; ERα is highly expressed in T cells while ERβ is mostly present in B cells (Phiel et al., 2005). Treatment with E3 resulted in higher endometrial PR gene levels as well, indicating progesterone can act on the endometrium (Bryś et al., 2009). In the immune system, progesterone mediates responses through PR-A and PR-B. These receptors are expressed on various immune cells, including natural killer cells (NKs), macrophages, DCs and T cells (Teilmann et al., 2006). Membrane-associated PRs are detectable on CD4+ and CD8+ T cells, indicating that progesterone can act on these cells (Lissauer et al., 2015). Uterine NKs express both nucleus ERs and PRs through which their expression is modulated (Kuang et al., 2010).

HCG can interact with the hCG/LH receptor, which is the same receptor LH can bind to. It is expressed by uterine spiral arteries and binding of hCG to this receptor induces syncytiotrophoblast differentiation and angiogenesis (Shi, 1993). In a murine system, the CG/LH receptor is expressed by DCs (Schumacher et al., 2013) and Tregs (Schumacher et al., 2009b; Schumacher et al., 2013). High hCG levels can promote Treg migration to the maternal-fetal interface to facilitate immune tolerance toward the fetus (Schumacher et al., 2009b). Relaxin can bind to its receptor LGR7, which has an increased expression in fetal membrane in early pregnancy stages compared to that at term (Lowndes et al., 2006). Through a LGR7-dependent mechanism, relaxin can stimulate leukocyte migration and activity (Figueiredo et al., 2006).

## Immunological shift at the maternal-fetal interface and relation with pregnancy-associated hormones

Maternal tolerance allows a mother to continue pregnancy to term, even though the fetus presents foreign fetal antigens. The maternal-fetal interface consists of specialized tissue: the maternal decidua and the fetal placenta, and a variety of infiltrated decidual immune cells (Loewendorf et al., 2014; Furcron et al., 2016). Crosstalk between this tissue ensures nourishment of the fetus and prevents rejection by the mother's immune system (Svensson-Arvelund et al., 2015).

The maternal-fetal interface involves immune cells, such as maternal peripheral blood mononuclear cells (PBMC), making direct contact with cytotrophoblasts, which are specialized epithelial cells of the placenta (Drake et al., 2001; Nakayama et al., 2002). PBMCs include T cells, B cells, NKs and monocytes (Pourmoghadam et al., 2020).
The invasion of the uterine wall by extravillous cytotrophoblasts is crucial for the establishment of pregnancy (Handschuh et al., 2007). Invasive cytotrophoblasts come across specialized maternal NK cells, macrophages, and T cells that accumulate within the uterine wall during pregnancy, which they can attract by producing MIP-1α (Drake et al., 2001). The fetal placenta induces homeostatic M2 macrophages and Tregs mainly through trophoblast cells (Svensson-Arvelund et al., 2015). This increase was coupled with induced IL-10 production and reduced Th cell activation.

In the fetal part of the placenta, the maternal and fetal tissue is largely separated by adjoining multinucleated syncytiotrophoblasts (SYNs), which are involved in the exchange of gases and nutrients between the mother and the fetus (Handschuh et al., 2007). Cytotrophoblast stem cells lie close to macrophages called Hofbauer cells in the fetal part as well (Drake et al., 2001). SYNs can produce progesterone and hCG, which is particularly important in early pregnancy due to hCG being required for progesterone production and pregnancy maintenance (Handschuh et al., 2007). Moreover, SYNs lack class I and II MHC antigens which prevents recognition by the immune system, so trophoblast cells do not provoke a T cell response from contacting maternal tissue (Hanna et al., 2006).

The increased recruitment of immune cells, such as NKs, macrophages, mast cells (MC) and Tregs at the maternal-fetal interface is facilitated by pregnancy-associated hormones (Jensen et al., 2010; Kuang et at., 2010; Furcron et al., 2016). Uterine NKs (uNK) accumulate in the maternal decidua and make direct contact with fetal trophoblasts during pregnancy (Hanna et al., 2006). They are also referred to as decidual NKs in literature. Recruitment of uNK precursor cells from the maternal blood is induced by rising plasma estrogens and limited by rising progesterone (Van Den Heuvel et al., 2005; Furcron et al., 2016). Trophoblast invasion is regulated by uNKs by production of the IL-8 and IFN-inducible protein-10 chemokines (Hanna et al., 2006). Immune tolerance and successful pregnancy are promoted by uNKs through downregulating proinflammatory Th17 cells via secretion of IFN-γ (Fu et al., 2012).

Th2-specific cytokines IL-4, IL-5, and IL-10 were detectable in fetal-placental cells in all three trimesters of gestation and at term, indicating their production at the maternal-fetal interface (Lin et al., 1993; El-Shazly et al., 2004). Furthermore, placentas from preterm delivery contained increased levels of Th1-associated cytokines IL-2, IFN-γ and IL-12 (El-Shazly et al., 2004), supporting a proinflammatory bias. Th17 cells are highly proinflammatory, likely contributing to recurrent spontaneous abortion (RSA) (Wang et al., 2010). Increased numbers of Th17 cells together with decreased Tregs in both the periphery and decidua from RSA patients suggest an immunological disbalance (Wang et al., 2010). Thus, a proper balance between Th17 and Treg is crucial for healthy pregnancy outcomes.

### Human chorionic gonadotropin hormone

When it comes to suppressing the maternal immune system and improving immune tolerance, hCG is key. Administration of hCG results in a significant inhibition of M-CSF and IGF-BP-1, which is a marker of decidualization. Growth factors such as VEGF were significantly stimulated. These measurements indicate the important endometrial role of hCG in decidualization, angiogenesis and tissue remodeling (Licht et al., 1998).

The proportion of macrophages and neutrophils decreased after hCG administration (Furcron et al., 2016). Additionally, hCG increased the number of M2 cells in late pregnancy, promoting an anti-inflammatory environment that participates in maternal-fetal tolerance during late pregnancy and the process of labor at term (Furcron et al., 2016). This implies that hCG administration reduces the proportion of innate immune cells with a proinflammatory role at the maternal-fetal interface in mid and late pregnancy (Furcron et al., 2016).
According to Wan et al. (2008), hCG can modify DCs to a tolerogenic phenotype at the maternal-fetal interface. A combination of hCG and LPS increased the IL-10:IL-12 ratio and decreased TNF-α and antigen-specific T cell proliferation by DCs (Wan et al., 2008).

The production of hCG has shown to enhance frequency, recruitment, and suppressive function of Tregs (Schumacher et al., 2013). A mouse study by Furcron et al. (2016) showed that hCG administration increased Tregs and Th17 cells at the maternal-fetal interface (Furcron et al., 2016). Another study by Schumacher et al. (2013) found comparable results, as their research showed an increased number of Tregs after hCG application as well, both peripherally and locally. Tregs also showed to elevate the secretion of IL-10 and TGF-β, and hCG restored Treg levels back to normal after disturbed tolerance, which prevented abortion (Schumacher et al., 2013). There is a possible preference for recruiting fetus-specific Tregs from the maternal peripheral blood to the maternal-fetal interface to support local maintenance of immune responses (Tilburgs et al., 2008). Thus, prominent levels of hCG during early pregnancy ensure the migration of Tregs to the maternal-fetal interface, contributing to tolerance towards the fetus. Altogether, hCG appears to function as one of several factors to prevent rejection of the fetal-placental tissue.

### Estrogens and progesterone

Estrogens and progesterone have distinctive and overlapping effects on immunity. A mouse study by Kuang et al. (2010) revealed that estrogens or progesterone are required for uterine expression of uNKs through binding to their receptors; ER and PR. It is suggested that hormonal regulation is necessary for homing of uNK precursors already before embryo implantation (Kuang et al., 2010). UNKs are not cytotoxic, and they are likely to play an important role in the regulation of trophoblast invasion and angiogenesis to contribute to successful placentation and immunity (Kuang et al., 2010). UNKs are dominant lymphocytes at the implantation site and a source of VEGF (Wang et al., 2000b) and IFN-γ (Ashkar et al., 2000). The expression of VEGF may help in the process of uterine neovascularization and is regulated by granulated metrial glands, which are components belonging to a NK lineage (Wang et al., 2000b). Furthermore, IFN-γ derived from uNKs can modify the expression of genes that initiate the instability of vessels in the uterine vasculature and stroma. This implies a regulatory role in the facilitation of pregnancy-induced remodeling of decidual arteries (Ashkar et al., 2000).

MCs express steroid receptors, suggesting that they can be influenced by steroid hormones (Jensen et al., 2010). E2 and progesterone upregulate chemokine receptor expression on MCs, together with inducing their degranulation and migration from the periphery into the maternal-fetal interface (Jensen et al., 2010). This may prepare the uterus for implantation through production of histamine and VEGF (Jensen et al., 2010). Histamine from the uterus has been suggested to be a key regulator in implantation because of its ability to change uterine vascular permeability and increase stromal decidualization (Johnson & Dey, 1980).

In a human cell study by Lee et al. (2011), the regulatory role of progesterone on fetal T cell differentiation was investigated. Progesterone plays a role in the differentiation of naïve cord blood fetal T cells into Tregs, and the majority of these cells expressed FoxP3 (Lee et al., 2011). They also discovered that progesterone suppressed the differentiation of cord blood CD4+ T cells into Th17 and the expression of the IL-6 receptor, which both have a proinflammatory effect. The upregulation of Tregs and downregulation of Th17 by progesterone imply a role in inducing tolerance (Lee et al., 2011) and reduce immunological disbalance that could lead to RSA (Wang et al., 2010). Furthermore, RSA patients have lost their NK-mediated regulatory response, leading to local inflammation caused by Th17 cells at the maternal-fetal interface (Fu et al., 2012).

Effector or activated maternal T cells can lead to harmful inflammation and preterm labor (Arenas-Hernandez et al., 2019). CD4+ and CD8+ T cells alter their cytokine production as a response to progesterone depending on the dose. Alteration is subtle when administrating progesterone concentrations comparable to those in maternal blood, while the effects are more profound at maternal-fetal interface concentrations (Lissauer et al., 2015). Maternal T cells from women with spontaneous preterm labor expressed more granzyme B and perforin at the maternal-fetal interface, which are pro-inflammatory molecules. In vivo T cell activation also increased inflammatory responses at the maternal-fetal interface, such as the release of B cell cytokines and proinflammatory macrophage polarization (Arenas-Hernandez et al., 2019). Progesterone reduces the production of IFN-γ, TNF-α, IL-5, and IL-10 and increases IL-4 by both CD4+ and CD8+ T cells. This enhanced IL-4 production is regulated via increased IL-4-expressing maternal CD8+ T cells and their cytokine production (Lissauer et al., 2015).

The modulation of T cell function by steroid hormones helps to understand normal pregnancy biology and supports possible therapy opportunities in pregnancies more prone to fetal loss (Lissauer et al., 2015). Treatment with steroid hormones might prevent preterm labor and counteract proinflammatory responses by activating T cells at the maternal-fetal interface (Arenas-Hernandez et al., 2019).

### Relaxin

A study by Horton et al. (2011) examined the effects of relaxin on cytokine secretion from primary decidual macrophages at the end of pregnancy. Relaxin caused a decrease in proinflammatory cytokines CSF-2 and IL-8 at 4 hours of treatment, while longer treatment was not significant. However, the longer treatment increased anti-inflammatory IL-6 secretion, but shorter treatment did not show significance (Horton et al., 2011). This could indicate relaxin has a significant role in an anti-inflammatory response.
However, relaxin increases IL-6 and IL-8 secretion, suggesting that induced decidual relaxin expression can cause proinflammatory cytokine production to rise from fetal membranes (Bryant-Greenwood et al., 2007). Additionally, pregnancy levels of relaxin favored the development of Th cells producing Th1 type cytokines, and these cell lines had increased production of TNF-β and IFN-γ (Piccinni & Romagnani, 1996). These findings correspond with the proinflammatory effects of hCG, which would match with their similar levels throughout pregnancy (figure 1).

## Immunological shift at the maternal periphery and relation with pregnancy-associated hormones

Throughout pregnancy, the maternal immune system undergoes significant changes to protect both mother and fetus. The affected immune cells can differ depending on where they are measured. The immunological shift at the maternal periphery consists of the mother's circulating immune cells in the blood and organs.

A study by Sasaki et al. (2007) showed that peripheral blood Tregs increased in normal late pregnancy, and that the majority of these CD4+ CD25bright Tregs, expressed FoxP3+ (Sasaki et al., 2007). A similar study measured the number of Tregs and IL-17 producing cells within circulating CD4+ T cells in late pregnancy and found that cells producing IL-17 decreased in normal pregnancy (Santner-Nanan et al., 2009). IL-17 is a proinflammatory cytokine, thus it being low at the end of pregnancy is in line with the immunological shift (figure 2). CD4+Foxp3+ Treg numbers were enhanced as well (Santner-Nanan et al., 2009). Both studies compared cell numbers to women with preeclampsia.

There is a bias towards Th2 cytokine expression during pregnancy, resulting in an adjustment of the innate and adaptive immune system. These Th2 cytokines can downregulate Th1 responses against parasite infections in the periphery (Krishnan et al., 1996) and clinically improve Th1 autoimmune diseases (Piccinni & Romagnani, 1996). Infection in pregnant mice came with reduced IFN-γ and increased IL-4, IL-5, and IL-10 production by the spleen and popliteal lymph nodes (Krishnan et al., 1996).

Normal pregnancy has a general activation of circulating granulocytes and monocytes (Luppi et al., 2002). This was demonstrated by the increased expression of adhesion molecules, which mediate leukocyte responses to inflammatory stimuli. Increased monocyte numbers induced the regulation of phagocytosis, IL-12 and IL-1β production and T cell activity, while granulocytes could synthesize more IL-8 (Luppi et al., 2002). The stimulation of these immune cells in the maternal blood supports the idea that innate immunity is elevated while adaptive responses are suppressed.

### Human chorionic gonadotropin hormone

The peripheral impact of the hormone hCG during pregnancy is diverse and it can promote healthy pregnancy outcomes. Intrauterine administration of hCG-activated PBMCs increased pregnancy rate and live birth in patients with recurrent implantation failure (Pourmoghadam et al., 2020). A similar study found that hCG-enhanced PBMCs from pregnant women increased murine embryo and trophoblastic cell invasion (Nakayama et al., 2002). Human monocytes respond to high hCG doses by increased IL-8 production (Kosaka et al., 2002), which can stimulate cell proliferation and the migration of endothelial cells (Koch et al., 1992). HCG can also directly enhance macrophage function through receptor mediation and therefore increase innate immunity (Wan et al., 2007). Proinflammatory IL-6 and IL-12 were increased as well, as was phagocytosis of apoptotic cells (Wan et al., 2007). This possibly contributes to early pregnancy consisting of an inflammatory environment. Thus, hCG affects PBMC function during pregnancy.

It is hypothesized by Khan et al. (2001) that hCG inhibits Th1 mediated autoimmune diseases (Khan et al., 2001). In this study, hCG administration lowered diabetes symptoms, reversed inflammatory infiltration in the pancreas and downregulated the activity of Th1 cells, such as IFN-γ production. As mentioned previously, a study by Schumacher et al. (2013) supports the idea that the temporal tolerance during pregnancy is partially achieved due to the activity of Tregs. They found a peripheral and local increased number of Tregs after hCG application, coupled with an elevated secretion of IL-10 and TGF-β. These cytokines induce the suppressive activity of Tregs (Schumacher et al., 2013).

B cells produced more IL-10 when cultured with serum from normal pregnancy than that from patients with spontaneous abortion or non-pregnancy (Rolle et al., 2013). These activated B cells (CD19+) strongly suppressed Th1 cytokine TNF-α production from cocultured T cells in this study, indicating the important role of Bregs in suppressing harmful immune responses and promoting tolerance. HCG can regulate the number and function of Bregs and is therefore a critical factor (Rolle et al., 2013).

Looking at these studies, it can be concluded that hCG has a duplex function because of its effect on both pro- and anti-inflammatory factors. This could however depend on the location of measurement. Systemically, hCG has a proinflammatory effect, but it is likely to have a different, anti-inflammatory function at the maternal-fetal interface (Furcron et al., 2016).

### Estrogens

Steroid hormones can have both a pro- and anti-inflammatory function, depending on the type of immune cell and the environment. Estrogens can influence macrophage function, since these immune cells express ERs (Phiel et al., 2005). Acting on ERα regulates the anti-inflammatory role of macrophages (Vegeto et al., 2004). Locally increased estrogens showed to have possible activating effects on macrophage and fibroblast cell proliferation, contributing to autoimmunity (Cutolo et al., 2004). When treating influenza A virus with E2, neutrophil numbers significantly increased compared to the placebo group, also causing virus-specific CD8 T cells to be induced (Robinson et al., 2014). This enhanced the production of IFN-γ and TNF-α as well, improving infection outcome.

Because of the immunological shift, the number of certain cytokines changes as gestation continues. Estrogens can modify immune function by stimulating anti-inflammatory cytokines (IL-10, IL-4, and TGF-β) and inhibiting those that promote inflammation (IL-12, TNF-α and IFN-γ) (Salem, 2004). The amount of IFN-γ is high at early stages, but its levels change due to the shift (Salem, 2004).
E2 treatment had multiple effects on the DC population in a study by Liu et al. (2002), including reduced production of inflammatory cytokines TNF-α, IFN-γ and IL-12 by mature DCs. This could contribute to a downregulation of Th1 cells (Liu et al., 2002).
Nakaya et al. (2006) performed a mouse study in which the effect of E2 on cytokine production was examined. However, IFN-γ production was enhanced by E2 stimulation and the number of NKs also increased. This stimulating effect is thought to be mediated via ERs (Nakaya et al., 2006). The fact that estrogens induce either pro- or anti-inflammatory effects could be due to the level of estrogen used in experiments. Early pregnancy supports an inflammatory environment (Gnainsky et al., 2010) and has low estrogen levels compared to late pregnancy (O'Leary et al., 1991) (figure 1), which is after the immunological shift.

Estrogens show to have an influence on Tregs already before pregnancy, since their rise in serum levels just before ovulation matches a peak in Tregs at the same time (Arruvito et al., 2007). Estrogens induce the expression of Foxp3, and estrogen treatment increases CD4+CD25+ Tregs, in an animal model (Loewendorf et al., 2014).

### Progesterone

The immunological effects of progesterone are mediated through progesterone-induced blocking factor (PIBF), which is a protein that promotes Th2 cytokine production (Szekeres-Bartho, 2008; Szekeres-Bartho & Polgar, 2010). Activated pregnancy lymphocytes express PRs, which enables progesterone to activate PIBF. Its production is a feature of normal pregnancy (Szekeres-Bartho & Polgar, 2010).

Progesterone induces the recruitment of NKs to the uterus by inducing the secretion of CXCR3 ligands Mig and IP-10 from cultured endometrial stromal cells (Kitaya et al., 2004). NKs are attracted to CXCR3 ligands. Mig and IP-10 were found in several places in the uterus, including the surface epithelia, glandular epithelia, and stroma (Kitaya et al., 2004). Stromal cells respond to progesterone

by increasing IL-15 trans-presentation to uNKs, inducing their expansion and differentiation (Wilkens et al., 2013).

The DC system is adapted in pregnancy to promote Th2 type responses (Bachy et al., 2008). In a study by Bachy et al (2008) monocytes differentiated into less phenotypically mature DC, which had less co-stimulatory and antigen-presenting molecules. Inflammatory stimuli made monocyte-derived DCs secrete more IL-10 and less IL-12, which enhances Th2 cells and Tregs to improve tolerance (Bachy et al., 2008). Progesterone induces an immature phenotype in bone marrow derived DCs so it may be involved in the maternal immune response through affecting DC differentiation, maturation and function (Liang et al., 2006). Similar results were found by Butts et al. (2007); progesterone inhibits the proinflammatory function of bone marrow derived DCs by suppressing their activity, resulting in decreased production of cytokines IL-1β and TNF when compared to untreated cells. Stimulation of progesterone inhibited proliferation of T cells as well (Butts et al., 2007).

Progesterone can promote CD4+ T cells to shift from a Th1 to Th2 type via high concentrations of PIBF, which is characterized by increased levels of IL-4, IL-5, and IL-10 (Szekeres-Bartho et al., 1996; Piccinni et al., 1995). B cell proliferation is promoted and Th1 cytokines are inhibited by IL-10 (Szekeres-Bartho, 2008). This implies that progesterone production at the placenta may be partially responsible for increased production of Th2 type cytokines, which is crucial for the progression of a successful pregnancy (Piccinni et al., 1995).
In vivo T cell activation, prior to inducing preterm labor, caused maternal hypothermia, bradycardia, systemic inflammation, cervical dilation, intra-amniotic inflammation, and fetal growth restriction in a murine model. This indicates that increased T cell activation causes pathological inflammation and might even result in preterm labor (Arenas-Hernandez et al., 2019).

### Relaxin

A rhesus monkey study revealed that pregnancy levels of relaxin treatment resulted in multiple significant effects on the uterus, including the endometrium showing a more decidualized morphology and a greater number of arterioles and lymphocytes (Goldsmith & Weiss, 2009). Relaxin stimulates the recruitment, migration and adhesion of leukocytes by binding to its receptor LGR7, coupled with proportional cAMP accumulation for intracellular messaging (Figueiredo et al., 2006). Incubation of rat coronary endothelial cells with LPS and relaxin caused a significant reduction of adherent neutrophils and endothelial expression of adhesion molecules, suggesting an anti-inflammatory role (Nistri et al., 2003). Asthmatic guinea pigs that received relaxin showed reduced respiratory abnormalities, decreased mast cell degranulation and leukocyte infiltration. Increased alveolar capillary dilation and reduction of the air-blood barrier were observed as well (Bani et al., 1997). Thus, the effect of relaxin on immune cells can be conflicting in research.

## Discussion and conclusion

In the present thesis, several key hormones that change during pregnancy are discussed, which include estrogens (estrone, estradiol and estriol), progesterone, human chorionic gonadotropin hormone and relaxin. These hormones are crucial for the regulation of healthy gestation, as they induce both endocrinological and immunological modifications to successfully endure gestation. The functional activity of these hormones on the immune system can be divided into those occurring at the maternal periphery and at the maternal-fetal interface. During pregnancy, the immune system of the mother shifts from a pro- to anti-inflammatory state, to ensure tolerance against the semoallogeneic fetus (Schumacher et al., 2013; Svensson-Arvelund et al., 2015). It is reviewed how pregnancy-associated hormones regulate the changing maternal body and recruit, promote, and inhibit certain immune cells.

While a fair number of literature can be found concerning the influence of certain pregnancy-related hormones on immune responses, there still are knowledge gaps and conflicting topics. The type of immune cells present is highly dependent on the stage of pregnancy and physical location. The start of gestation consists of inflammation which is necessary for implantation (Gnainsky et al., 2010). Because of the immunological shift, immune cell levels are changed. It is only until delivery that an inflammatory environment reoccurs (Shynlova et al., 2012; Romero et al., 1989). To accurately measure immune cell levels, it must be determined whether the shift has already taken place or not. Arruvito et al (2007) explains that menstrual cycle stages should be considered when investigating Tregs in non-pregnant women, since fluctuating hormones alter their numbers (Arruvito et al., 2007), indicating how important hormone levels are when investigating immunity.

It was observed that a significant amount of research on immunological sex differences with respect to hormones could be found, especially how estrogens and progesterone affect immune cells. Yet, research on how certain hormones act on individual immune cell types in pregnancy is still lacking. Conflicting results in studies could be due to the immunological shift. Thus, the effect of pregnancy-associated hormones on immunity is unfortunately still somewhat contradictory and unclear.

Further research on the regulatory functions of pregnancy-associated hormones on immunity can improve the understanding of immune-endocrine crosstalk. Insight into the exact underlying mechanisms can help in treating pregnancy complications concerning abnormal immune responses and fetal intolerance. Progesterone, for instance, might prevent preterm labor when applied in a clinically approved treatment (Arenas-Hernandez et al., 2019). Knowing the exact expression of ERs and PRs on immune cells may provide a basis for analyzing the mechanisms of immune modulation by estrogens and progesterone, which in turn could help identify therapeutic targets for treating immunologic disorders (Phiel et al., 2005). Crosstalk between PRs and ERs in the myometrium can provide further understanding how estrogens and progesterone affect the onset of delivery (Mesiano et al., 2002). Moreover, therapy with hCG-activated PBMCs may be an effective approach for women with recurrent IVF failure, since it has shown to promote healthy gestation (Pourmoghadam et al., 2020). Relaxin can be a possible therapeutic for treating asthma, possibly reducing the chance of further complications such as high blood pressure (Bani et al., 1997).

To conclude, pregnancy is a unique condition with a modulated, not suppressed, immune system, resulting in altered responses against immunological challenges. The exact nature of immune-endocrine crosstalk in pregnancy remains to be discovered.

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# Afterword

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