# Chapter 1 - Introduction

The establishment and maintenance of pregnancy relies on successful fetal implantation and placental development in a receptive uterine environment. At the epicenter of these processes is the maternal-fetal interface, where cells comprising the maternal uterine lining, or decidua, encounter and interact with invading fetal extravillous trophoblast cells (EVT)[1]. The invasion of EVT is crucial in forming the placenta and facilitating proper nutrient and gas exchange between the fetus and the mother. Additionally, maternal immune cells residing in the decidua must adapt to tolerate the semi-allogeneic fetal cells, which express paternal antigens, and support their growth and coexistence over the duration of pregnancy[2]. In this chapter, I will first describe the biology and immunology of the maternal-fetal interface during early pregnancy, providing an overview of the characterized cell types and functions. Next, I will describe existing questions with regard to the maternal-fetal interface and how these motivate the aims and design of the study described in this thesis.

## 1.1 Formation of the maternal-fetal interface during early pregnancy

### *1.1.1 Decidualization is critical to establishing a favorable uterine environment for pregnancy*

The human endometrium, or inner epithelial layer of the uterus, progresses through extensive growth, remodeling, shedding, and regeneration with each menstrual cycle[3]. During the postovulatory (secretory) phase of the cycle, rising levels of uterine progesterone and elevation of intracellular cyclic adenosine monophosphate (cAMP)[4,5] activate signaling pathways targeting HOXA10, FOXO1, HAND2, and other transcription factors to initiate the process of decidualization[6,7]. Decidualization is marked by an extensive series of structural and functional changes to the endometrium, including the differentiation of endometrial stromal cells (ESCs) from a fibroblastic to epithelial morphology. These epithelioid, decidualized ESCs secrete an extensive repertoire of hormones, cytokines, and growth factors, including prolactin (PRL)[8], IL-15[9], and IGFBP-1[10]; extracellular matrix proteins, such as fibronectin and collagen[11]; and angiogenic regulators, such as vascular endothelial growth factor (VEGF)[12]. Meanwhile, uterine glands embedded throughout the endometrium also exhibit increased secretory activity[13], and specialized immune cells, primarily tissue-resident decidual NK cells and macrophages[14,15], begin to increasingly infiltrate the endometrium. These radical changes collectively transform the endometrium into a dense, vascularized tissue known as the decidua, which in the event of a pregnancy is capable

of mediating embryo homing and attachment to the uterus[16], undergoing extensive vascular and tissue remodeling[17,18], regulating placental growth and nourishing the embryo during placenta formation[13,19], and modulating local immune responses to the fetus and placenta[14,15], among other functions. Ultimately, the decidua is uniquely primed to receive an implanting embryo, facilitate the successful establishment of a pregnancy, and undergo further adaptations to support a pregnancy.

### *1.1.2 Fetal implantation and placentation*

After fertilization, the zygote develops into a blastocyst consisting of an inner cell mass, which evolves into the embryo, and a trophectoderm, which gives rise to the placenta and other extraembryonic tissues[20]. Following initial contact and stable adhesion of the blastocyst to the endometrium, various trophoblast lineages emerge to guide embryo implantation and placentation. In the villous pathway, cytotrophoblasts fuse into multinucleated syncytiotrophoblast cells (SCT), which make up the outermost layer of the trophectoderm[21]. SCT ultimately come into contact with the maternal blood, enabling the maternal-fetal exchange of gases and nutrients which is essential to the growth and development of the fetus[20].

As the blastocyst is anchored in the uterus, cytotrophoblasts continue proliferating, forming cell columns that penetrate past the syncytiotrophoblast and eventually give rise to the chorionic villi of the placenta[22]. Cytotrophoblasts within these extensions differentiate along the extravillious pathway into interstitial and endovascular EVT, which play complementary roles in vascular remodeling and immunomodulation[1,21]. The interstitial EVT invade further into the decidualized endometrium and the myometrium, promoting the recruitment of uterine arterioles and ultimately fusing into multinucleated placental giant bed cells at the end of their migration[21]. In the process, they come into direct contact with maternal immune cells and decidual stromal cells, and are therefore involved in modulating immune responses at the maternal-fetal interface[23]. Meanwhile, endovascular EVT invade directly into the uterine vasculature, replacing vascular endothelial and smooth muscle cells lining the maternal spiral arteries[22]. This transforms the arteries into dilated, low resistance vessels that circulate maternal blood into the trophoblast-lined intervillous space of the placenta[21]. In the intervillous space, maternal blood bathes the placental chorionic villi and establishes direct contact with the SCT to permit nutrient and gas exchange.

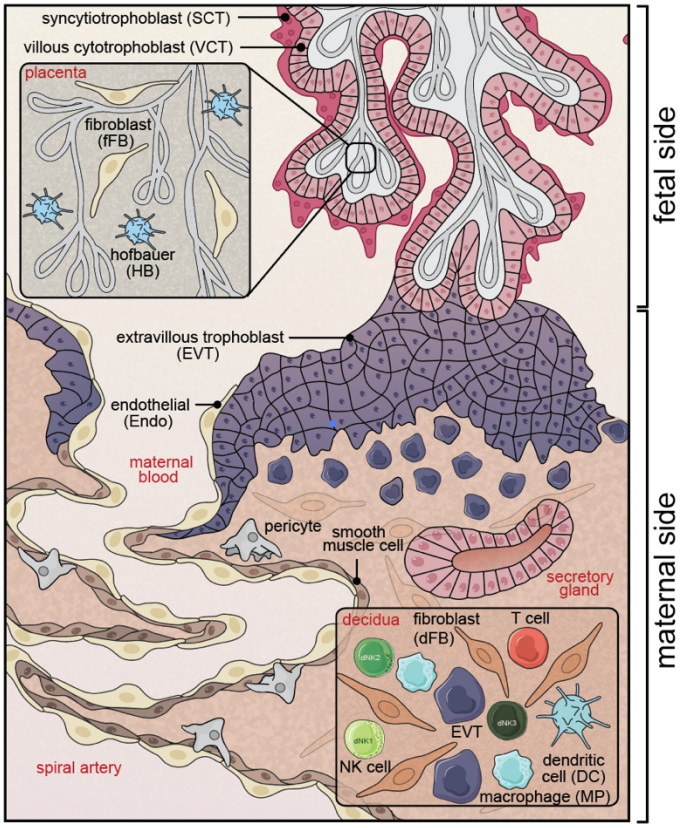


**Figure 1. Diagram of the structure and primary cell populations of the maternal-fetal interface.** (from Vento-Tormo R, Efremova M, et al., under submission)

Precise control of trophoblast invasion and differentiation is crucial for pregnancy success, and a multitude of factors govern this process. The coordinated production of matrix metalloproteinases and tissue inhibitors of metalloproteinases by both trophoblast cells and decidual cells is critical to fine-tuning the timing and extent to which trophoblasts degrade the uterine extracellular matrix and migrate through the endometrium[24]. Additionally, as trophoblasts in the oxygen-poor decidual milieu invade towards the maternal spiral arteries and encounter an increasingly oxygen-rich environment, they shift from a proliferative to differentiating state[25]. Meanwhile, exposure to various cytokines, growth factors, and other molecules of the decidual secretome prompts trophoblast cells to undergo integrin switching and to upregulate expression of angiogenic factors, such as VEGF and VEGFR[26,27], and cell adhesion ligands, such as vascular cell adhesion molecule 1 (VCAM-1) and platelet endothelial cell adhesion molecule (PECAM-1; CD31)[26,28]. In addition to further modulating trophoblast invasiveness, these mechanisms also alter the adhesive properties of trophoblasts to resemble those of endothelial cells, thus enhancing their capacity to localize to and integrate into the maternal spiral arteries[26,29].

Overall, successful establishment of the maternal-fetal interface relies on the spatiotemporal synchronization of numerous molecular and cellular interactions between the decidual and fetal cells[20,22]. This complex interplay is highly consequential in configuring the maternal and fetal vascular systems to facilitate proper gas exchange, nutrient and waste transfer, and adequate delivery of immunoglobulins and other molecules critical to embryonic development throughout the duration of pregnancy[30]. Impairments in many processes involved in placentation, such as defective trophoblast invasion or incomplete remodeling of the maternal spiral arteries, have been shown to be strongly predisposing factors for preeclampsia[20,25,31], fetal growth restriction[22,28,32], and other serious complications of pregnancy.

Additionally, over the course of placentation and pregnancy, fetal trophoblasts come into direct contact with the immune cell-rich uterine tissues and the maternal blood[21,33]. While this hemochorial placenta is highly efficient, it also poses an immunological challenge in which maternal leukocytes must continuously encounter invading, semi-allogeneic fetal cells. Given the considerable levels of maternal immune cell recruitment to the maternal-fetal interface and the radical morphological changes and functional adaptations that maternal and fetal tissues undergo during early pregnancy[2,20], the maternal immune system must develop tailored responses at the maternal-fetal interface that at once facilitate local tissue disruption and repair, tolerate maternal-fetal coexistence, and promote successful fetal growth and development.

The trophoblasts appear to be equipped with their own mechanisms of escaping immune elimination. Most notably, they do not express the classical MHC I molecules HLA-A and HLA-B, which are the primary targets of traditional T cell-mediated allogeneic responses, instead preferentially expressing HLA-G as well as HLA-E and HLA-C[20,34,35]. This unique surface repertoire enables them to regulate immune responses at the maternal-fetal interface by binding receptors expressed by decidual NK and myeloid cells[22,23,35].

## 1.2 Immune cell constituents of the maternal-fetal interface

### *1.2.1 Decidual natural killer cells*

Decidual NK cells (dNK) begin to accumulate in the secretory endometrium prior to blastocyst implantation and comprise around 70% of the immune cell population at the maternal-fetal interface during early pregnancy[36]. In contrast to their peripheral blood counterparts, they exhibit a $CD56^{+}CD16^{-}$

$CD9^+$ surface phenotype[37] and produce a distinct set of cytokines, growth factors, and chemokines[38,39]. These characteristics impart unique properties to dNK, including their ability to regulate EVT migration into the decidua[39]. While conventional NK cells tend to release cytolytic granzyme- and perforin-containing granules to destruct target cells, dNK demonstrate poor killing upon activation, a mechanism potentially contributing to maternal immune tolerance towards semi-allogeneic fetal cells[2,31].

NK cell function is generally regulated by the interaction of various NK receptors with MHC class I molecules on target cells. The functional outcome of NK receptor-MHC engagement depends on whether the receptor is inhibitory or activating, leading to either dampening or stimulation of NK cell response[40]. In particular, the family of killer cell immunoglobulin-like receptors (KIRs) expressed by NK cells consists of both inhibitory and activating receptors that are often variably or stochastically expressed between one NK cell and another[41].

HLA-G, which is expressed by EVT, binds to NK cell receptor LIR-1, impairing dNK cytotoxicity and possibly inducing the secretion of cytokines and angiogenic factors to further promote endometrial vascularization[42]. HLA-E binds the NK inhibitory receptor NKG2A and also engages the activating receptor NKG2C at lower affinity[35,43]. Meanwhile, HLA-C molecules are capable of binding both inhibitory and activating KIRs, with relative affinity to specific KIRs dictated by HLA-C allotype[44]. The *HLA-C* gene is highly polymorphic, with more than 2000 documented variant alleles[45], which broadly give rise to either HLA-C1-type or HLA-C2-type molecules. The genes encoding KIRs are multiallelic as well and can be inherited in variable copy number between generations in addition to being expressed in varied combinations on individual NK cells[41]. Because the genetic diversity of KIR and HLA genes is germline-encoded, KIR and HLA genes on different chromosomes are inherited independently[41,45]. As a result, KIRs on maternal NK cells may be able to bind fetal HLA-C allotypes that the mother herself does not possess (i.e. paternal in origin), and similarly, fetal HLA-C molecules may be ligands to maternal KIRs that the fetus does not possess. Thus, different KIR/HLA-C interactions can conceivably produce different degrees of NK activation and inhibition, and in turn, alter the extent to which dNKs and trophoblasts collectively promote tolerogenicity and inflammation at the maternal-fetal interface[46]. Indeed, particular maternal KIR and fetal HLA-C pairings have been shown to influence risk of developing pregnancy complications. Combinations of fetal HLA-C2 allotypes with maternal KIR haplotypes containing inhibitory NK receptor KIR2DL1 are associated with poor placentation and greater incidence of preeclampsia[44] and fetal growth restriction[47]. On the other hand, combinations of

fetal HLA-C2 with maternal haplotypes containing activating NK receptor KIR2DS1 are associated with fetal overgrowth[48]. NK receptor-mediated crosstalk therefore appears to substantially regulate dNK function and heavily influence the successful establishment and progression of pregnancy. The range of cell types and mechanisms by which occurs and their respective consequences have not been fully elucidated yet will be critical to understanding the balance between remodeling, immunity, and tolerance maintained by dNK cells.

### *1.2.2 Decidual macrophages*

Mononuclear phagocytes, including macrophages, account for ~20-25% of decidual leukocytes[2,49] and collectively appear to assume multifaceted roles during early pregnancy. As the second most abundant immune cell population in the decidua, they serve as the predominant antigen processing and presenting cells at the maternal-fetal interface and are hypothesized to help promote maternal immune tolerance through moderation of local NK and T cell responses. Among other mechanisms, decidual macrophages have been shown to produce high levels of the immunosuppressive cytokine IL-10[50]; dampen T cell activation and cytokine production through signaling via inhibitory PD-1 and B7-family molecules[51]; express low levels of B7-1 and B7-2, which are activating ligands for the CD28 co-receptor on T cells[50]; induce regulatory T cells via IDO and TGF-β production[52]; and reduce cytolytic activity in dNK cells through TGF-β[53].

At the same time, during blastocyst implantation, parturition, uterine infection, and other events in pregnancy characterized by a proinflammatory state, macrophages have been observed to significantly accumulate at the maternal-fetal interface[2,49]. Indeed, decidual macrophages exhibit high expression of pattern recognition receptors, including CD163, CD206 (mannose receptor), and CD209 (DC-SIGN)[54,55], and facilitate self-recruitment through the production of monocyte chemoattractants and mitogens[2,56], indicating their ability to detect pathogens and damaged cells and to initiate inflammatory responses. Furthermore, upon stimulation by LPS, decidual macrophages have been shown to secrete the inflammatory cytokines IL-1β and TNF; however, other classical proinflammatory cytokines, such as IL-12, are not produced, and IL-10 production is still constitutively elevated[56,57]. This suggests that macrophages are capable of orchestrating a state of controlled inflammation at the maternal-fetal interface in which pathogen clearance and response to damaged tissue are balanced with the maintenance of a tolerogenic environment.

Additionally, macrophages have been directly linked to vascular and tissue remodeling and trophoblast differentiation, as they localize to the decidual arteries alongside dNK cells prior to the replacement of vascular endothelial and smooth muscle cells by extravillous trophoblasts[2,58]. They express elevated levels of the extracellular matrix proteins fibronectin and collagen[54], matrix metalloproteinases MMP7 and MMP9[54,58], complement protein C1q[54], and angiogenic factor VEGF and its receptor VEGFR-1/FLT-1[56,59]. Fibronectin and C1q may specifically be involved in enhancing macrophage phagocytosis of apoptotic vascular cells before their persistence in the decidua triggers acute inflammation[60,61]. In addition, decidual macrophages have been shown to produce IL-33, which stimulates cytotrophoblast proliferation and extravillous trophoblast migration[62]. Reciprocally, the activation of decidual macrophages has been linked to trophoblast and potentially endometrial stromal cell-mediated production of M-CSF, GM-CSF, and IL-10[57,63].

Existing knowledge of decidual macrophages has primarily been described in relation to the M1/M2 model of macrophage polarization, developed to parallel the concept of Th1/Th2 differentiation in T helper cells[64]. M1 macrophages are associated with Th1 response and are referred to as “classically activated” macrophages[65]. They express pattern recognition receptors and upon stimulation by factors such as IFN-γ, TNF, LPS, or GM-CSF, secrete pro-inflammatory cytokines, upregulate antigen-presenting MHC class II molecules, antigen-processing peptidases, and costimulatory molecules, and initiate other responses congruent with Type 1 inflammation and pathogen elimination[66]. Meanwhile, stimuli of M2 macrophages include “alternative activation” by IL-4/IL-13, immune complexes in combination with TLR engagement, and IL-10 and glucocorticoids[65,67]. Overall, M2 macrophages exhibit an umbrella of phenotypes including wound healing and tissue remodeling, resolution of inflammation, and Th2-type immune responses[68]. Specifically, IL-4 induces macrophage fusion and decreases phagocytosis, and IL-10/glucocorticoid-stimulated macrophages exhibit induction of complement (C1Q), IL-10, CD163, and mannose receptor (MRC1), among other molecules[69].

There are varying hypotheses for how macrophages polarize within the decidua. One model posits that decidual macrophages are M2 at baseline and induced towards M1 upon pathogen stimulation or during the implantation and parturition stages of pregnancy[49]. Another proposes the existence of two distinct decidual macrophage subsets which are distinguished primarily by high and low expression of the integrin adhesion receptor CD11C but do not fall completely along the M1/M2 polarization axis in terms of their phenotypes or their cytokine profiles[70,71].

An emerging consensus, however, is that the M1/M2 model should be revised[72–74], as it is overly rigid and fails to account for the phenotypic plasticity and functional diversity of macrophages. In particular, tissue-resident macrophages tend to exhibit adaptations heavily tailored to their local environments and the unique functional demands of their tissues[73], with notable examples being splenic red pulp and liver macrophages, which are specialized for heme group processing[75,76], and osteoclasts, which are critical for bone remodeling[77]. Such observations are inadequately accounted for by the general M1/M2 macrophage model. Thus, further characterization of decidual macrophages may prove insightful in further understanding the potential spectrum of functions performed by tissue-resident macrophages as well as further clarify the overall biology of the maternal-fetal interface.

### *1.2.3 Decidual dendritic cells*

Dendritic cells (DCs) are present in low proportions at the maternal-fetal interface, comprising less than 2% of the immune compartment[78]. Various surface phenotypes have been described for decidual DCs, with mature cells expressing CD83[79] and subsets distinguishable by $BDCA1^+$ and $BDCA3^+$ expression[80] or by $HLADR^+$ and $HLADR^-$ expression[78].

In general, DCs either reside in the lymphoid organs or are situated within nonlymphoid peripheral tissues. Although both classes of DCs engage in antigen uptake and presentation, nonlymphoid tissue-resident DCs have shown to be the main mediators of T cell priming and response to peripheral tissue antigens, as upon activation, they are able to migrate through the lymphatic vessels to the draining lymph nodes[81,82]. However, the antigen-presenting potential of decidua-resident DCs must be reconciled with the observation that during pregnancy, the fetus is tolerated and there is no apparent fetal allorecognition and rejection. Studies in mice, for example, have shown that fetal antigens are still presented to T cells in draining lymph nodes and other secondary lymphoid organs, but there is no resulting accumulation of T cells at embryo implantation sites, suggesting that decidual DCs and other maternal antigen-presenting cells fail to initiate allogeneic T-cell responses to fetal and placental antigens[2,82].

In mice, decidual DC densities have been found to steadily decrease during early pregnancy, and in humans, decidual stromal cells have been demonstrated to inhibit DC differentiation and T cell activation via prostaglandin E2 and IDO production[83]. Moreover, even upon LPS stimulation, activated and mature murine decidual DCs are unable to migrate to regional draining lymph nodes[84]. This

phenomenon, termed DC entrapment, appears to be due in part to the absence of lymphatic vessels near the decidual spiral arterioles, thus preventing trophoblast-activated DCs from homing to uterine lymph nodes[84]. Rather, the only lymphatic vessels in the mouse uterus expressing CCL21, the chemokine ligand directing DC homing[85], are restricted to the myometrium[82]. In humans, similar mechanical and biochemical barriers may apply to limit DC activation, migration, and recruitment of peripheral T cells[83].

On the other hand, complete absence of decidual DCs also appears to be detrimental to pregnancy, as DC-depleted mice exhibited impaired spiral artery remodeling[86]. This points to a potential role for DCs in ensuring vascular restructuring and interacting with other cell populations localized to the uterine vasculature[87,88]. Moreover, mature $CD83^+$ DCs appear to still be capable of stimulating Th2 responses in T cells, although their ability to initiate Th1 responses may be impaired[89]. Thus, decidual DCs, despite being functionally suppressed as a potential threat to the fetoplacental unit, may still serve as potential antigen-presenting cells to decidual T cells, perhaps to direct contained, localized immune responses to pathogens.

### *1.2.4 Decidual T cells*

T cells represent around 10-20% of the decidual leukocyte population during early pregnancy[90], consisting of roughly similar proportions of $CD4^+$ and $CD8^+$ T cells, along with small numbers of gamma-delta T cells[91] and $CD4^-CD8^-$ T cells[90]. Among $CD4^+$ T cells in first trimester human decidua, around 5% are Tregs[92], and overall, most T cells are antigen-experienced rather than naive ($CD45RO^+/CD45RA^-$)[93]. However, the precise proportions of these subsets and their enrichment in decidua relative to peripheral blood remain unclear, as some populations were analyzed in non-perfused decidua samples and therefore may in fact be intravascular[90]. The proportion of decidual T cells actually exhibiting specificity towards fetal antigens remains unknown as well[2]. Many T cells, including regulatory T cells, may traffic through peripheral tissues even in the absence of local inflammation or infection, and thus their presence in a tissue does not imply antigen-specific homing. Additionally, many T cells may have populated the decidua before or at the time of blastocyst implantation[90].

Most existing studies view effector T cells as antagonistic towards fetal tolerance and pregnancy success. Indeed, effector T cell accumulation at the maternal-fetal interface is associated with

complications such as chronic deciduitis[94], villitis of unknown etiology[95], and chronic chorioamnionitis[94,96], although the antigen specificities of these T cells are unknown. There is also no apparent path of direct antigen presentation to T cells residing in the secondary lymphoid organs via migrating DCs, which are trapped in the decidua[84]. Instead, fetal antigens reaching the lymph nodes appear to be disseminated systemically through the blood in a cell-free manner. Rather than promoting massive effector T cell activation and expansion, this less acute mode of antigen exposure results in the deletion of many responding T cells or their conversion into regulatory T cells (Tregs)[23]. Meanwhile, based on mice studies, even in the event of peripheral T cell expansion into activated Th1 and CTLs, their ability to actually extravasate into the decidua appears to be impaired due to epigenetic silencing of the genes producing Th1 and cytotoxic T cell chemokines CXCL9, CXCL10, CXCL11, and CCL5 in decidual stromal cells[97]. Given the low proportion of naive T cells in the decidua, however, it is possible that rather than initially encountering antigens at the maternal-fetal interface, the T cells first encountered antigen elsewhere before migrating to the uterus, where local antigen presentation by decidual DCs may further reinforce their activation[90]. One possible role for the persistence of effector T cells in the decidua and the potential for intradecidual DC antigen presentation may be to retain the ability to combat decidual infections and pathogens yet avoid compromising the overall tolerogenicity of the maternal-fetal interface[78,90].

A similar concept may apply to decidua-resident Treg subsets. In mice, Tregs were induced in lymph nodes upon DC presentation of seminal fluid antigens and exposure to TGF-β, and shown to home to the endometrium during the peri-implantation period[90,98]. Following blastocyst implantation and throughout the progression of early pregnancy, decidual Tregs may further expand upon local, possibly DC-mediated[78], exposure to fetal antigens, in order to dampen the activity of effector T cells at the maternal-fetal interface, or to counter general decidual inflammation through antigen nonspecific mechanisms such as secretion of IL-10 and TGF-β[90].

## 1.3 Recapitulating questions regarding the maternal-fetal interface

Cellular functions at the maternal-fetal interface appear to center around balancing immunological tolerance to the antigenically foreign fetus, immunity towards pathogens and generation of controlled inflammatory responses, and important nonimmune processes such as placentation and tissue remodeling. However, complete knowledge of the biology of the human maternal-fetal interface remains

limited by several factors. First, much existing knowledge of the initial events in embryonic development and the biology of implantation and placentation have been inferred from animal models of pregnancy[2,99]. While these share some key parallels with human pregnancy[99], they also differ in important aspects. For example, mice possess a dissimilar placental anatomy and exhibit significantly more superficial trophoblast invasion into the decidua[2]. This means that many findings in animal models may be species-specific and of limited relevance to humans. Meanwhile, due to ethical restrictions and limited availability, human fetal and decidual samples from the initial stages of pregnancy or during early pregnancy are unable to be widely obtained and studied[20]. Furthermore, many previous studies of the immunological state of human pregnancy have treated maternal-fetal coexistence as analogous to host-graft interactions, in which a constant state of immunosuppression is necessary to promote the growth of the fetus or maintenance of the transplant[23,100,101]. However, this model has since been challenged by accumulating evidence of the many dynamic adaptations exhibited by the maternal immune system during the establishment and early stages of pregnancy[23,102,103]. As a result, knowledge of the biology of early pregnancy in the human context remains largely incomplete. While many targeted studies of specific decidual and placental cell populations have proven insightful, the full range of cell types at the maternal-fetal interface and their potential functions and interactions remain uncharacterized.

Additionally, it is clear from existing studies that decidual and placental biology, and fetal implantation and development, reciprocally shape each other while heavily influencing pregnancy outcomes and the pathological mechanisms underlying infertility, preeclampsia, recurrent miscarriage, aberrant fetal growth, premature birth, and other reproductive and developmental disorders[2]. A more complete understanding of the maternal-fetal interface would therefore also advance efforts to better identify the cellular basis for these conditions. Moreover, the *in utero* environment has been increasingly linked to long-term health impacts[104], suggesting that the biology of the maternal-fetal interface may become consequential for general human health as well.

## 1.4 Introduction to the project

### *1.4.1 Studying the maternal-fetal interface at single-cell resolution*

In this project, single cell RNA-sequencing (scRNA-seq) was applied to study cell types and cell functions in the human maternal-fetal interface during early pregnancy. Since the first assay to sequence all mRNAs in an unbiased fashion from single cells[105], a number of technological advances,

including improvements in single-cell isolation, cell capture, and methods for sample multiplexing (combining and parallel processing of samples), have facilitated both the large-scale generation and sensitive profiling of single cell transcriptomes[106–108]. These developments have made it possible to leverage scRNA-seq to analyze whole tissues, such as the decidua and placenta, at single-cell resolution. Meanwhile, advances in computation and in methods to meaningfully analyze single-cell data have enabled scRNA-seq to resolve distinct cell types and cell states on the basis of gene expression differences, as well as capture processes such as cellular differentiation and cell-cell communication[107], both of which would greatly contribute to dissecting the maternal-fetal interface. Thus, utilizing scRNA-seq to comprehensively identify and characterize cell populations comprising the decidua and placenta represents a promising avenue for furthering our understanding of the biology of early human pregnancy and of the maternal-fetal interface.

However, there are still remaining challenges in analyzing and interpreting scRNA-seq data. One challenge is confidently distinguishing cells in samples where the constituent cells may be derived from genetically distinct donors or from multiple sources (e.g. unwanted contamination). In the context of the maternal-fetal interface, this is particularly relevant as the decidual and placental tissues contain both cells from the mother and cells from the fetus. Robustly determining the genetic origin of each will be critical for annotating cellular functions and studying maternal-fetal crosstalk. Additionally, because scRNA-seq experiments are still accompanied by many practical limitations and potential sources of technical artifacts[106], such as the confounding influence of cellular dissociation protocols on gene expression[109], these must be detected and corrected for in the single-cell data of the maternal-fetal interface. Finally, a necessary task of every single-cell transcriptome-based study is placing findings in the context of existing knowledge of the cell types or tissues under study. Thus, consideration of existing gene expression data of the healthy and diseased decidua and placenta, as well as assays of gene expression at the protein level, would serve as important enhancements for an scRNA-seq-based analysis of the maternal-fetal interface.

### *1.4.2 Project aims*

We sought to obtain a comprehensive overview of the maternal-fetal interface at the single-cell level through analyses integrating single cell transcriptomics of the decidua and placenta with orthogonal experimental approaches and datasets. First, we aimed to robustly annotate all cell populations

comprising the maternal-fetal interface by combining scRNA-seq gene expression analysis with inference of the maternal or fetal genetic origin of each cell, and by identifying and excluding cells exhibiting scRNA-seq-induced perturbations in observed gene expression. Next, we aimed to further study the local adaptation of the maternal immune system during early pregnancy by performing protein-level validation of marker genes distinguishing novel maternal immune populations, and by identifying potential functions assumed by these cells that are tailored to the unique functional demands of maternal-fetal interface. Finally, we aimed to better understand the cellular basis for various complications of pregnancy and other fertility-related traits, by mapping genes previously implicated in these conditions to specific cell types at the maternal-fetal interface.

### *1.4.3 Project design*

Four maternal decidua samples, two fetal placenta samples, and matched maternal peripheral blood samples from early pregnancies were sequenced via droplet-based scRNA-seq (10x Chromium)[110]. An additional five decidua and two peripheral blood samples, enriching for specific cell subsets of interest, were also sorted via FACS and sequenced using the plate-based scRNA-seq protocol Smart-seq2[111]. Both sets of single-cell transcriptomic data were used to identify preliminary cell types and cell states at the maternal-fetal interface.

In order to better annotate cell populations, we sought to determine the fetal or maternal origin of all decidual and placental cells with the use of corresponding maternal and fetal whole genome sequencing data. We also aimed to ensure the robustness of our scRNA-seq-based analyses by identifying and excluding putative cell subpopulations exhibiting aberrantly high levels of tissue dissociation-induced genes[109].

For selected cell populations at the maternal-fetal interface, we sought to improve their functional annotation by leveraging Gene Ontology and Reactome term enrichment analysis, gene expression signatures of known cell types, and public placental and endometrial gene expression datasets. We also used FACS and CyTOF to validate the characterization of these cell populations at the protein level. Finally, in order to examine all identified cell populations in terms of their relevance to diseases and conditions affecting the maternal-fetal interface, we examined the cell type-level expression patterns of genes previously linked with complications of pregnancy or fertility-related conditions via genome-wide association studies (GWAS) or functional genomics studies.

### *1.4.4 Overview of thesis*

This thesis is divided into six chapters. Following this introduction (Chapter 1), Chapter 2 provides a description of the experiments and data analyses performed in this study. In Chapter 3, I present the results of combining scRNA-seq with whole genome sequencing to infer the maternal or fetal genetic identity of each cell, and of utilizing bulk RNA-sequencing to determine the presence of potential technical artifacts in scRNA-seq data. Next, in Chapter 4, I discuss our protein-level validation of novel immune cell subsets at the maternal-fetal interface, along with our additional functional characterization of maternal mononuclear phagocyte populations in the context of established gene signatures and tissue-level gene expression data. Chapter 5 describes our single cell transcriptomics-level analyses of genes previously linked to complications of pregnancy or fertility-related conditions, and our insights into the relevance of specific cell types to these diseases or conditions. Finally, in Chapter 6, I summarize the overall conclusions from this study and potential future work to be undertaken.