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Single-Cell Immunobiology of the Maternal-Fetal Interface

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Abstract

Pregnancy success requires constant dialogue between the mother and developing conceptus. Such crosstalk is facilitated through complex interactions between maternal and fetal cells at distinct tissue sites, collectively termed the maternal-fetal interface. The emergence of single-cell technologies has enabled a deeper understanding of the distinct processes taking place at the maternal-fetal interface as well as the discovery of novel pathways and immune and non-immune cell types. Single-cell approaches have also been applied to decipher the cellular dynamics throughout pregnancy, in parturition, and in obstetrical syndromes such as recurrent spontaneous abortion, preeclampsia, and preterm labor. Furthermore, single-cell technologies have been utilized during the recent COVID-19 pandemic to evaluate placental viral cell entry and the impact of SARS-CoV-2 infection on maternal and fetal immunity. In this review, we summarize the current knowledge of cellular immunobiology in pregnancy and its complications that has been generated through single-cell investigations of the maternal-fetal interface.

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Introduction

Pregnancy is a dynamic period of growth and development that is initiated at fecundation and culminates in parturition, resulting in the delivery of the conceptus (1). To successfully complete this journey, a coordinated series of complex events is required, which starts with the establishment of dialogue between the mother and embryo (2–9). Such communication is facilitated through multiple sites of maternal-fetal interaction, which have been collectively termed the maternal-fetal interface (10–15). Such sites represent anatomically distinct areas of immunological contact; namely, the decidua basalis, where maternal immune cells and decidual stromal cells interact with fetal extravillous trophoblast; the placental intervillous space, in which circulating maternal immune cells interact with the fetal syncytiotrophoblast; and the boundary where the decidua parietalis meets the chorion laeve in the chorioamniotic membranes (11, 13, 14, 16). Therefore, the maternal-fetal interface comprises numerous complex immune and non-immune cellular interactions that support implantation (17–21), promote fetal and placental development (22, 23), maintain homeostasis (17, 19, 24–37), and participate in the inflammatory milieu implicated in parturition (38–47). Accordingly, the disruption of maternal-fetal homeostasis at different stages of pregnancy has been implicated in multiple pathologies ranging from pregnancy loss to preterm delivery (26, 27, 30–32, 36, 37, 44, 45, 48–51). Hence, deciphering the mechanisms of maternal-fetal crosstalk that lead to a normal term delivery as well as those implicated in obstetrical disease is paramount.

Recent investigations have leveraged the use of single-cell technologies to evaluate the transcriptome, proteome, and epigenome of the maternal-fetal interface at single-cell resolution. Among these, single-cell RNA-sequencing (scRNA-seq) was the first to be described (52–60) and has seen the most widespread use across reproductive science (61–65). Indeed, scRNA-seq was utilized to provide the first insights into the cellular heterogeneity and interaction networks of the maternal-fetal interface in early (66–77) and late (78–80) gestation, and the resulting datasets of these pioneer studies have since been integrated into a number of subsequent investigations. Furthermore, single-cell technologies have been highly effective at uncovering the unique biological processes taking place at the maternal-fetal unit during mid-gestation (66, 81–85) as well as those inflammatory pathways implicated in the processes of term and preterm parturition (80, 86–90). Pertinent to the current pandemic, scRNA-seq has also provided important knowledge about the mechanisms of cell entry for SARS-CoV-2 as well as its deleterious effects on the maternal-fetal unit (91–99). Herein, we aim to provide an overview of such studies in a succinct and comprehensive manner to foster future research utilizing single-cell technologies to decipher the cellular mechanisms of disease for pregnancy complications.

The first trimester

The first trimester represents an important developmental window for the fetus, which relies heavily on proper placentation and spiral artery remodeling (100–104). To aid in these processes, a carefully orchestrated series of interactions is required that involves maternal pro-angiogenic or tolerogenic immune cells, mesenchymal, endothelial, and decidual stromal cells, and the invading fetal trophoblast. The first trimester has therefore been an

attractive target period for investigating the cellular states, functions, and interactions that drive pregnancy establishment (66–77). Moreover, due to the importance of a healthy first trimester for continued pregnancy, multiple single-cell studies have targeted the maternal-fetal interface during this time to help unravel the mechanisms underlying pathologies such as recurrent spontaneous abortion (71, 73–75, 77) (Figure 1). Next, we summarize the insights into the maternal-fetal immunobiology of the first trimester obtained using single-cell technologies.

Placental cells in the first trimester

Some of the first studies of the early placenta utilized scRNA-seq to characterize the major cell types and their subsets present in this organ (66–68). One survey utilized two scRNA-seq platforms (commercial 10x Genomics platform and a custom Drop-seq platform) to characterize the placental villi, and found good correlation between these technologies (67). Two other investigations of the first trimester maternal-fetal interface utilized either Smart-seq2 alone (66) or an integrated dataset obtained using Smart-seq2 and 10x Genomics (68). Placental cell types were largely consistent between studies, with villous cytotrophoblasts (VCTs), syncytiotrophoblasts (SCTs, also referred to as STBs), extravillous trophoblasts (EVTs), fibroblasts, endothelial cells, erythroblasts, and Hofbauer cells all being identified by their expression profiles (66–69). Comparison of relative abundance indicated that, after trophoblast subsets, the most represented placental cell type was fibroblasts, followed by endothelial cells, erythroblasts, and Hofbauer cells (67), highlighting the capacity of scRNA-seq to map the cellular heterogeneity of the placenta.

In addition to their identification, the further characterization of trophoblast cell types revealed that CTBs and EVTs include multiple subsets that displayed differing fusion potential or proliferative capacity, respectively (66). One of these CTB subsets displayed comparatively higher Syncytin-2 expression (66), suggesting fusion competence that would allow SCT formation (105). Pseudotime analysis indicated a transition from CTB to EVT subtypes (66), consistent with other reports that proposed two trophoblast differentiation pathways by which VCT could transition to the EVT or SCT subsets (68). Such endpoint EVTs represented the most invasive trophoblast subset that is found in the maternal decidua (68) and characterized by expression of specific factors such as *SOX4* (70). A recent investigation utilized scRNA-seq of the first trimester placenta together with a prior dataset (68) to assess the trophoblast cell states implicated in the establishment, renewal, and differentiation of these cells (76). Four CTB states (CTB1–4), a SCT precursor-like CTB state (SCTp), two column trophoblast (EVT precursor (106)) states, and an EVT state were identified based on gene expression (76). While the relative frequencies of the four CTB states increased throughout the first trimester, the column trophoblast and EVT states decreased from early to late first trimester and SCT progenitors remained constant (76), highlighting the fluctuation of trophoblast cell states during this period. Combined RNA velocity and single-cell trajectory analyses were used to map primitive trophoblasts to the CTB2/CTB3 clusters, from which two putative differentiation pathways emerged: one directed toward EVT and the other toward SCTp (76). To validate this finding, Monocle 3 and Slingshot were applied and agreement between these two pseudotime analyses was demonstrated, indicating CTB2 as the closest point of origin (76). Thus, scRNA-seq allows

for the dissection of the origins and differentiation trajectories of the heterogeneous cell types that form the placenta.

It is worth mentioning the tools that have been developed for trajectory inference by which single cells are ordered based on similarities in gene expression (107), as was performed in the studies described above. A large number of trajectory inference methods have been developed, with more being generated often; yet, the largest difference among them is whether the topology is fixed and, if not, what kind of topology can be detected (107). A thorough comparison of current trajectory inference techniques indicated high variability between methods, and thus it is clear that investigators should test multiple methods with their data, and more so when *a priori* knowledge of topology is lacking (107). Importantly, the effort required to identify and utilize such tools has been greatly diminished by the availability of resources and databases that can guide users to the best trajectory inference approaches (107–109).

The characterization of other major placental cell types revealed that, in addition to trophoblasts, the fibroblast population includes multiple subsets, with two of these displaying expression profiles reminiscent of myofibroblasts (67), which could support the contractile properties of anchoring villi (110). Hofbauer cells were also found to include two subsets, of which one was found to express an activated, MHC-II-expressing state that could be important for debris clearance in the developing placenta (66). Other cell types identified in the placenta include two distinct subsets of mesenchymal stromal cell (MSC), which displayed gene expression profiles indicative of participation in cell adhesion/migration or angiogenic processes, respectively (66).

The abovementioned reports show that scRNA-seq represents a useful technology for uncovering the composition, differentiation pathways, and expression profiles of cell types in the placenta, a unique organ that carries out critical functions for fetal growth and development (111, 112). Importantly, such knowledge can be leveraged to unravel the molecular underpinnings of placental diseases.

Decidual cells in the first trimester

The first trimester decidua represents a more heterogeneous cellular compartment compared to the placenta, given the increased abundance of immune cell types in addition to tissue cells (67, 68, 113). Natural killer (NK) cells, antigen-presenting cells (APCs) such as dendritic cells (DCs), macrophages, innate lymphoid cells (ILCs), and T cells represent major subsets in the decidua, in addition to decidual stromal cells (DSCs), fibroblasts, smooth muscle cells, endometrial epithelial cells, lymphatic endothelial cells, and vascular endothelial cells (67, 68, 113). The DSC compartment comprises multiple subsets characterized by their spatial location as well as their expression profiles (68, 71). Pseudotime analysis of DSCs and fibroblasts indicated that the fibroblast 1 subset can either differentiate to DSCs or fibroblast 2 (67). The differentiation of DSCs was further explored in a recent study of the murine uterus, in which the single-cell profiles of this tissue were compared between implantation sites (decidualized uterus) and inter-implantation sites (non-decidualized uterus) (72). In this study, the decidual cellular compartment was subdivided into deep stromal, proliferating deep stromal, superficial stromal, intermediate decidual, and

decidual cells, each with expression profiles indicative of distinct functionality (72). Two potential pseudotime differentiation pathways were determined: the first was a deep stromal > superficial stromal transition, and the second was a deep stromal > proliferative deep stromal > intermediate decidual/decidual transition, which was considered as corresponding to decidualization (72). Consistent with prior receptor-ligand interaction analyses in the decidua (67, 68), interaction networks between decidual cells and different major subsets such as immune cells, endothelial cells, and trophoblast were also demonstrated (72). Interactions between decidual and immune cells included immunological pathways such as cytokine-cytokine receptor interaction, phagosome, natural killer cell-mediated cytotoxicity, and NOD-like receptor signaling pathway, among others (72). Interestingly, interactions between decidual and endothelial cells showed substantial overlap with those between decidual and immune cells, as did the interactions between decidual cells and trophoblasts; notable terms shared among all three interactions included the TGF-beta signaling pathway and the MAPK signaling pathway (72). Thus, decidualization involves a core set of signaling pathways that are shared among multiple cell types in this compartment, suggesting that this is a cooperative process. Expanding on this approach, a separate investigation utilized scRNA-seq data of the murine day 7.5 uterus to deconvolute spatial transcriptomics data derived from the same tissue, which allows for the assignment of single cells to specific uterine microenvironments (114). The integration of these datasets revealed the distinct distribution of different stromal subsets within the uterus as well as their clustering in relation to other cell types (114). Thus, the spatial localization of cell subsets at the maternal-fetal interface may provide insight into their functionality and potential interactions with neighboring cells.

The inference of cell-cell communications from scRNA-seq data has been an area of ongoing research. The first iteration of tools developed for this purpose was based on the expression of single receptor-ligand pairs (115–117), which did not account for receptors that function as multi-unit complexes. To overcome such a limitation, the CellPhoneDB v2.0 tool was developed, which could predict enriched cell-cell signaling based on the minimum average expression of all members of multi-unit complexes (118). More recently, this concept was further built upon by considering signaling cofactors such as soluble agonists/antagonists and membrane-bound co-receptors, which resulted in the creation of the cell-cell communication analysis tool CellChat (119). While such databases provide a powerful new means of exploring scRNA-seq datasets, it should be considered that the resulting inferences are based solely on gene expression and thus require careful interpretation.

Other key players during early human pregnancy are decidual NK cells, which can be classified into different subsets based on single-cell clustering (67, 68, 73) or imaging mass cytometry (IMC) (113). Among these, a study reported that one NK subsets (dNK1) is the most responsive to HLA-C/HLA-G and expresses more cytoplasmic granules compared to other subsets (68); thus, these decidual immune cells cells may be central for modulating EVT invasion during the first trimester. Pseudotime analysis of decidual NK cells indicated a potential differentiation pathway wherein the endpoint subsets showed enrichment of similar immune-related processes (73). However, alternative pathways wherein endpoint NK cells can return to an intermediate state have also been described (74). Decidual NK cells represent an important cellular contributor to pregnancy maintenance, given that a

disruption in their functions (such as by poor maternal-fetal KIR-HLA matching) has been linked to complications such as spontaneous abortion or preeclampsia (120–123). A recent IMC-based investigation of the maternal-fetal interface identified six distinct clusters of NK cells (113). Consistent with a prior demonstration (68), a subset of CD69-expressing decidual NK cells (termed dNK1) was the most prominent in the first trimester (113). In addition to NK cells, a subset of T cells with a cytotoxic expression profile is also found in the first-trimester decidua (67), which may participate in the process of EVT invasion and remodeling. Other notable immune cells subsets at the early maternal-fetal interface include macrophages, which are reported to display single-cell gene expression profiles that are distinct from the conventional M1/M2 paradigm (75). The latter observation is consistent with the recently proposed role of decidual macrophages as homeostatic sentinels that promote fetal growth and sustain pregnancy in early (32) and late (37) gestation. Indeed, analysis of cell clustering based on IMC revealed that myeloid cells are most prominently localized to the trophoblast microenvironment as well as the microenvironment of other immune cells at the maternal-fetal interface (113). Moreover, among the six identified myeloid clusters, two were specifically enriched in the first trimester and lacked HLA-DR expression (113). These observations lend further support to the role of decidual macrophages as homeostatic mediators, even in the first trimester.

Taken together, these data provide deep insight into the cellular processes that characterize the first trimester decidua. In particular, single-cell transcriptomic analysis is useful for uncovering the interactions between critical immune cells (such as NK cells) and stromal cells, which can inform the overall cellular dynamics of the maternal-fetal interface.

Cellular interactions at the maternal-fetal interface during the first trimester

A key strength of scRNA-seq is the capacity to infer cellular interactions within the target tissue using analytical methods such as CellChat, which is based on the expression of receptors and ligands by cell type pairs (119). Mapping of the mostly highly expressed receptor-ligand pairs among placental and decidual cells revealed prevalent cell-cell interactions taking place in these compartments (67, 68). Notable cell-cell interactions at the maternal-fetal interface include those between EVTs and maternal immune cells, which comprise signaling related to immunomodulation, growth, angiogenesis, and cell adhesion/recruitment (68). Yet, complex interaction networks also occur among the DSCs, fibroblasts, endothelial cells, NK cells, and macrophages to support cellular differentiation and homeostasis (67, 68). Moreover, cell-cell interaction analyses are not confined to a single tissue, as studies incorporating both the decidua and placental villi have provided an overview of the strongest intercellular interactions between these compartments (67). A novel study mined bulk RNA-seq data from the first trimester decidua and placental villi to identify sexually dimorphic receptor-ligand pairs that could influence cellular interactions in these compartments (69). Subsequent scRNA-seq was utilized to identify the top sexually dimorphic genes within major cell type clusters (e.g., trophoblast, stromal, Hofbauer, APC, and endothelial) as well as placental cell ligands that interact with decidual cell receptors (69). Through this method, 91 sexually dimorphic receptor-ligand pairs were identified as being expressed by cell types at the maternal-fetal interface, which corresponded to processes such as immunomodulation, hormonal regulation, and metabolism (69). Thus,

scRNA-seq can also provide evidence of sex-specific differences in cell-cell interactions that are characteristic of healthy pregnancies.

Single-cell landscape of recurrent spontaneous abortion

Comparative studies have applied scRNA-seq technologies to investigate the cellular repertoire and interactions in obstetric disease during early pregnancy, namely recurrent spontaneous abortion (RSA) (71, 73–75, 77). The single-cell investigation of decidual leukocyte subsets indicated an increased presence of T cells in RSA cases (73, 74), which was accompanied by diminished frequencies of specific macrophage (73, 74) and NK subsets (73–75). For example, a $CSF1^{+}CD59^{+}$ NK cell subset that was prevalent in normal pregnancy was found to be reduced in RSA (75). Moreover, expression and interaction analyses indicated more active pro-inflammatory signaling for decidual leukocytes such as T cells, macrophages, and NK cells in RSA cases compared to controls (73–75). Macrophage interactions with T cells and NK cells are also altered in RSA, as one macrophage subset showed enrichment of processes related to NK cell chemotaxis in healthy pregnancies but shifted towards processes related to T cell chemotaxis in RSA (74). The latter finding was verified using immunofluorescence staining, which revealed more prevalent co-localization of macrophages with NK cells in healthy pregnancy and more co-localization of macrophages with T cells in RSA (74). Thus, altered macrophage interactions with NK cells may result in aberrant promotion of T-cell responses associated with disease in early pregnancy (Figure 1).

In addition to a general state of decidual immune activation, evaluation of the DSC compartment indicated a disease-driven shift in these cells, with several subsets diminishing in RSA patients (71). Notably, a new DSC subset emerged only in RSA cases, which displayed gene expression enriched for cell apoptosis and senescence, potentially indicating abnormal DSC differentiation (71). Consistent with these observations, the developmental trajectories of DSCs were altered in RSA, with one potential differentiation pathway being almost completely absent (71). Moreover, overall decidual cell-cell communications were increased in RSA cases (71). Cellular interaction analyses suggested that DSCs function as a central hub that communicates with other cell types in this compartment (71), and thus defective DSC development or function could trigger a chain of adverse events implicated in RSA. This concept is further supported by the reported reduced expression of the MYC-associated factor X (*MAX*) in DSCs from RSA patients, which is implicated in proper decidualization and may indicate that impairment of this process is a contributing factor to disease development (77).

The above investigations demonstrate the capacity for single-cell technologies to provide novel insights into pregnancy disease such as RSA, which remains a challenge for obstetrics.

The second trimester

The second trimester represents a period of continued growth and development, characterized by a homeostatic environment at the maternal-fetal interface that is maintained by the cells present in these compartments (124–127). One exploratory study of placental cell types included second trimester samples as a means of comparing changes in single-

cell composition throughout pregnancy (66). In this study, EVTs derived from the second-trimester placental villi formed two distinct clusters, one with enrichment of wound and cell adhesion processes and the other with enrichment of growth, response to stimulus, and hormone responses (66). Moreover, such EVTs represented the endpoint of the predicted differentiation pathway when combined with pseudotime trajectory analysis of first-trimester trophoblast subsets (66). These findings confirms that trophoblast differentiation continues throughout the second trimester. More recently, the developmental trajectories of CTBs were specifically explored in the human second trimester placenta to avoid the inflammation and apoptosis associated with parturition in late pregnancy (128). In this study, both the smooth chorion (i.e., the chorion layer of the chorioamniotic membranes) and the villous chorion (within the placenta) were compared using scRNA-seq (128). Consistent with other reports, major cell types included CTB, EVT, immune cells, stromal cells, and a small subset of epithelial cells (128). Notably, a subset of CTBs was identified as being unique to the smooth chorion, termed SC-CTBs, and showed high expression of cytokeratins required for tissue integrity, as verified by immunofluorescence imaging (128). Trajectory analyses revealed that SC-CTBs in the smooth chorion and SCTs in the villous chorion shared a common progenitor (128). Notably, smooth chorion CTBs were found to exhibit greater inhibitory effects on EVTs compared to villous chorion CTBs in an *ex vivo* setting, and this affect was attributed to the SC-CTBs present in this compartment (128). Taken together, these findings point to distinct trophoblast cell types present in each compartment of the maternal-fetal interface that exhibit differential functionality despite originating from a shared progenitor.

Another recent investigation of the human maternal-fetal interface during the second trimester performed mass cytometry (CyTOF) to survey the leukocyte population present in this compartment (84). This technique was coupled with IMC and immunofluorescence staining to demonstrate the spatial distribution of specific cell types within the placental tissue as well as validate tissue residence by localizing such cells outside of the fetal vasculature (84). $CD4^+$ T cells and NK cells were observed to be more abundant in the decidua, with macrophages being more highly present in the placental villi (84). Specifically, the placental villi were enriched for subsets of $CCR7^-$ macrophages and DCs as well as $CD8^+CD69^-$ T cells, suggesting a relatively inactive status of these cells (84). Deeper evaluation of expressed markers indicated subsets of $CD163^{hi}$ and $CD163^{lo}$ macrophages in the placental villi, with the former likely to be a classical Hofbauer subset, and evaluation of chemokine receptors on placental cells showed reduced expression of several of these markers, which was confirmed by the bulk RNA-seq data (84). The majority of placental T cells were found to be $CD8^+$ memory T cells, with marker expression suggestive of Th2 differentiation, while $CD4^+$ Tregs were more abundant in the decidua (84). A later IMC-based study demonstrated similar findings and further indicated that the immune cell compartment undergoes changes in mid-pregnancy, such as a gradual increase in myeloid cells coupled with declining NK cell abundance (113). As noted above, such a decline in decidual NK cells included a substantial decrease in the abundance of the CD69+ dNK1 subset (113). Similarly, specific myeloid subsets showed distinct trends throughout gestation, supporting the concept that the overall changes in total NK cell or myeloid populations may not reflect the changes in individual subsets (113). Together, these

observations provide an overview of the diverse immune cell landscape in the placental villi, and provide further evidence of a largely homeostatic microenvironment at the maternal-fetal interface during mid-gestation.

Several investigations have also explored the murine placental tissues during mid-gestation to unravel the cellular differentiation taking place at this time (81–83, 85). One study performed single-nuclei RNA-seq (snRNA-seq) to ensure adequate representation of SCTs, and evaluated the murine placenta at days 9.5, 10.5, 12.5, and 14.5 of gestation (82). In this study, which was focused on placental labyrinth development, the trophoblast population was dissected to identify sinusoidal trophoblast giant cells, SCT subsets including precursors, glycogen cells, spongiotrophoblasts and their precursors, and junctional zone precursors (82). Such findings expand on an earlier report that utilized scRNA-seq of the day 9.5 placenta to identify major clusters of progenitor trophoblasts and trophoblast giant cells in addition to DSCs, NK cells, and endothelial cells (83). Pseudotime trajectory analysis was implemented to describe several distinct differentiation pathways that included precursor, intermediate, and endpoint states as well as the key genes participating in each pathway (82). Moreover, based on the multiple sampled time points, additional temporal input was used to generate a map of placental development during mid-gestation and infer the functional roles of each identified cell type in this process (82). In a separate investigation of the day 10.5 placenta, trajectory analysis were extended to also include Hofbauer cell subsets, demonstrating a clear progression of Monocyte > Hofbauer cluster 1 > Hofbauer cluster 2 (85). Consistent with the homeostatic microenvironment in early (32) and late (37) gestation, the most abundant placental cell-cell interactions at day 10.5 were between endothelial cell, pericyte, decidual cell, and labyrinth SCT pairs (85). Finally, another investigation focused on the fetal cells present in the placenta in mid-gestation by using a mating strategy wherein transgenic GFP^+ male mice were mated with wild type females (81). This study showed that hematopoietic precursors exist in the placenta that can give rise to a subset of fetal macrophages (81), shedding new light on cellular ontogeny in this organ.

Collectively, these studies elucidate the placental developmental processes taking place during mid-pregnancy, and highlight this homeostatic period as an area for future investigations.

The third trimester and delivery

Placental cellular immunobiology

Single-cell technologies have allowed for an unprecedented level of insight into the cellular populations and networks at the maternal-fetal interface in the third trimester, both in the presence and absence of labor. Indeed, two pioneering studies focused on the cellular dynamics of the human placenta derived from term cesarean section deliveries to provide a single-cell atlas of this organ (78, 79). The first study focused on placental trophoblast cell types as well as a small subset of maternal immune cells, and incorporated these data together with sequencing of SCTs collected by laser microdissection, primary undifferentiated endometrial stromal fibroblast cells, and primary decidual cells to obtain a combined dataset that is representative of placental cellular composition (78). The trophoblast clusters were assigned identities as VCT subsets and EVTs, with EVTs

showing gene expression signatures associated with modulation of extracellular matrix, vascularization, and immune pathways (78). Interestingly, it was noted that SCTs displayed low-level expression of MHC-II, which is in contrast to other trophoblast subsets (78). The small maternal immune cell cluster was identified as DCs, which could be contaminating cells derived from the uterus (78). In a subsequent study, placental cell type clusters were identified that corresponded to populations of vascular endothelial cells, vascular smooth muscle cells, villous stromal cells, macrophages, trophoblasts, DSCs, DCs, T cells, and an erythrocytic subset, with the trophoblast cluster being further divided into EVTs, CTBs, and SCTs (79). By including paired biopsies sampled proximally and distally to the umbilical cord insertion point, it was shown that there is spatial heterogeneity that is reflected in the relative abundance of cell types such as DSCs and endothelial cells (79), which may be due to the convergence of chorionic arteries and veins at the umbilical cord (129). Analysis of the placental cell-cell communications network indicated likely interactions between adjacent maternal and placental cell types, with DSCs showing a high amount of signaling to and from SCT and EVT (78). Inter-trophoblast communication was also highlighted, as each CTB subset displayed putative interactions with SCT and EVT (78). Notably, by contrasting cell-cell communications using the undifferentiated endometrial stromal fibroblast cells and DSCs, it was found that decidualization enhances signaling potential between DSCs and the fetal trophoblast (78). Together, these two pioneer studies provided important new insights into placental cellular interactions as well as valuable single-cell datasets that have been leveraged by later investigations (80, 130–132).

To enhance the translational value of single-cell placental dynamics, Tsang *et al.* extrapolated the cellular signatures to the maternal circulating cell-free RNA (79). This novel method was based on studies showing that cell-free DNA and RNA derived from the fetus/placenta are found in the maternal circulation (133–140). Using this approach, it was found that expression profiles corresponding to DSCs, endothelial cells, smooth muscle cells, stromal cells, and monocytes steadily increased in the maternal circulation throughout pregnancy (79). By contrast, EVT, SCT, and B-cell profiles tended to decrease, particularly towards the end of gestation (79). Finally, the overall T-cell population signature decreased in mid-pregnancy and then increased in the third trimester and post-partum period (79), which is in line with the concept that T-cell activation is implicated in the inflammatory milieu that accompanies term parturition at the maternal-fetal interface (38–40, 43, 45, 50, 141).

A recent report focused on *in vitro*-expanded placental mesenchymal stem/stromal cells demonstrated multiple subsets contained within this population, including some with immunomodulatory gene expression signatures (142). Moreover, these cells expressed cytokines such as *CCL2*, immunomodulatory factors such as IFITMs, and the regulatory factor *PRDM1* (BLIMP-1), and scATAC-seq indicated high chromatin accessibility of immune regions (142), further supporting the involvement of placental mesenchymal stem/stromal cells in immune processes. Moreover, the findings generated in this study support the potential application of placental mesenchymal stem/stromal cells for regenerative or immunomodulatory cell-based therapies.

To date, only one report has provided comparative scRNA-seq analysis of the human placental villi in the presence and absence of spontaneous labor at term (80). In particular, relative differences in the proportions of CTB subsets, activated T cells, monocytes, and macrophages are observed between the labor and non-labor placental villi, with such immune subsets including cells of maternal and fetal origin (80). Moreover, term labor was associated with substantial changes in gene expression across multiple placental cell types including macrophages, monocytes, stromal cells, EVTs, and CTB (80). Such expression changes were enriched for labor-associated terms, such as vascular smooth muscle contraction in the fibroblast subset and cell cycle/metabolism in EVTs (80). Moreover, by applying an approach similar to that reported in (79), gene signatures corresponding to placental cell types could be monitored in the maternal circulation throughout pregnancy, and signatures of NK cells and T cells were found to be enhanced with term labor (80). Thus, such analyses not only provide insight into the labor-specific placental changes that occur at the single-cell level, but provide a potential means of monitoring pregnancy and labor in the maternal circulation.

Decidual cellular immunology

The cellular composition of the decidua undergoes modification throughout gestation in preparation for the inflammatory process of labor (45, 47, 141, 143). Single-cell surveys of the decidua obtained from term cesarean deliveries characterized the overall proportions of cell types in this compartment, including a substantial fraction of T cells (both resting and activated), NK cells, DSCs, endothelial cells, and fibroblasts as well as invasive EVTs, and subsets such as macrophages showed substantial labor-associated changes in gene expression (80, 87) (Figure 2). These data are in line with prior cytological surveys of the decidua showing that leukocytes are attracted to this compartment prior to the onset of labor (40, 43, 49, 141, 144–147). Moreover, a new subset of lymphatic endothelial decidual (LED) cell was described in the chorioamniotic membranes that displayed an expression profile enriched for cellular interactions and adhesion (80). This observation suggested that LEDs present in the chorioamniotic membranes may be functionally mediating the influx of immune cells into this compartment during the process of labor (80). Indeed, immunofluorescence staining of the chorioamniotic membranes revealed the co-expression of the lymphatic marker LYVE1 and the endothelial marker CD31, demonstrating the presence of lymphatic vessels in the decidua parietalis (80). Consistently, a single-cell study focused on decidual endothelial subsets in term non-labor deliveries identified five distinct cell clusters with differing expression profiles, two of which were enriched for cell adhesion processes (86). Together, these studies suggested that decidual endothelial cells, including the novel LED cell type, can contribute to the accumulation of infiltrating lymphocytes into the maternal-fetal interface in preparation for and during term parturition.

Mucosal-associated invariant T (MAIT) cells, an innate-like T-cell subset expressing a restricted TCR (148), have also been detected at the maternal-fetal interface (149–151); yet, the expression profiles and functionality of these cells has been underexplored. A targeted investigation utilized combined flow cytometry, CITE-seq, and scRNA-seq approaches to characterize MAIT cells in the decidua basalis and parietalis, demonstrating that the majority of these cells displayed an effector memory phenotype (152). Decidual MAIT

cells exhibited gene expression profiles distinct from those found in the periphery, including genes involved in immune suppression and cell migration (152). Key differentiation factors upregulated in MAIT cells compared to conventional T cells included *PLZF* and *EOMES*, and *in vitro* stimulation resulted in secretion of IFNγ and TNF together with granzyme B, indicating that these cells are functional and can participate in maternal-fetal immune responses (152).

Recent investigations have also compared the changes in cellular composition and cell-cell signaling between decidual tissues derived from labor and non-labor deliveries to evaluate the participation of individual subsets in the inflammatory process of parturition. Spontaneous term labor was shown to result in the increased prevalence of activated T cells, monocytes, macrophages, and DSCs in the decidua basalis compared to term deliveries without labor (80). Moreover, IMC analysis of the maternal-fetal interface at term indicated increased abundance of myeloid cells and T cells compared to second trimester samples (113). Such changes are consistent with prior studies indicating an influx of immune cells to the maternal-fetal interface during parturition (40, 43, 47, 141, 144, 146).

More prominent than changes in cell type proportions are the transcriptomic changes that occur to facilitate labor. Macrophages, stromal cells, monocytes, T cells, and EVTs, among others, showed drastic differential gene expression during term labor compared to their non-labor counterparts, and such genes were enriched for inflammatory and immunomodulatory pathways (80). Similarly, decidual endothelial cell clusters each showed labor-specific enrichment of inflammatory pathways such as IL-17 signaling, leukocyte differentiation, myeloid differentiation, and cytokine-related terms (86), as did DSC and decidual EVT subsets (87), suggesting that decidual immune and tissue cell subsets propagate inflammation to drive parturition. Such changes in the transcriptomic profiles of decidual cells manifest in altered cell-cell communications, as indicated by numerous enhanced signaling pathways such as IL-1β – IL-1R1, IL-6 – IL-6R, TNFSF14 – LTBR, and multiple chemokine – chemokine receptor pathways (88). Rather than solely regulating new pathways, labor also seems to involve the continued upregulation or downregulation of already-modulated signaling pathways (88), suggesting that transcriptomic changes implicated in labor may be initiated earlier in gestation. In particular, T cells undergo significant shifts in intercellular communications with other decidual cells, involving pathways such as Notch, NF-κB, MAPK, Jak-STAT, and chemokine signaling, among others (88). However, these underlying labor-associated transcriptomic changes did not seem to impact the TCR clonality and diversity within the decidua (88), consistent with the concept that the decidual T-cell compartment is primarily composed of memory T cells (43, 84, 152). Together, these data point to a decidual inflammatory response implicated in labor that involves transcriptomic changes in specific cell types from the innate and adaptive limbs of the immune system.

The use of scRNA-seq to decipher the mechanisms leading to preterm labor

Spontaneous preterm labor is not simply the premature activation of the common pathway of parturition, but rather represents a pathological process that is distinct from normal labor at term (153). Unravelling the molecular mechanisms underlying spontaneous preterm

labor is therefore an ongoing endeavor within our group. Thus, we have leveraged scRNA-seq to compare and contrast the cellular composition and transcriptomic profiles of the decidua, placenta, and chorioamniotic membranes in term and preterm labor to improve the understanding and potential prediction of this obstetrical syndrome (80) (Figure 2). Compared to term labor, preterm labor involves substantial changes in gene expression within the EVT and CTB populations, potentially indicating a distinct response in these cell types (80). Moreover, within preterm labor-affected subsets such as EVTs and CTBs, specific genes were identified that showed opposite direction of change compared to term labor, indicating that distinct cellular mechanisms are involved in these processes (80). To extend the clinical relevance of our findings, we applied our scRNA-seq-derived signatures to the cellular transcriptome of the maternal circulation and showed that signatures of maternal macrophages, monocytes, and activated T cells were elevated in women who underwent spontaneous preterm labor compared to gestational age-matched controls, supporting a role for intravascular immune activation in the pathophysiology of preterm labor (80). Thus, the maternal circulation may provide a useful window that can be used to monitor preterm labor-specific events at the maternal-fetal interface.

More recently, we have undertaken the investigation of the cellular interactions implicated in pathological labor using a murine model of preterm birth induced by the intra-amniotic inoculation of *E. coli* (90). We evaluated the key tissues implicated in the common pathway of labor (i.e., the uterus, decidua, and cervix) at single-cell resolution and demonstrated that preterm labor affects the cellularity of the uterus, decidua, and cervix through immune cell infiltration and altered transcriptomic profiles of non-immune cell types in a tissue-specific manner (90). In the uterus, both innate and adaptive immune cell subsets (e.g., neutrophils, macrophages, DCs, NK cells, and T cells) contributed to pathways implicated in preterm labor, such as cytokine and chemokine signaling, as did non-immune subsets (e.g., fibroblasts, stromal cells, epithelial cells, smooth muscle cells, and endothelial cells), and the interaction strength among cell types was altered with preterm labor (90). Similar changes in cell-cell signaling were observed in the decidua; yet, unique pathways such as IL-17 were also revealed in this tissue (90). The cellularity of the cervix predominantly involved epithelial cells, which showed increased incoming interactions from other tissue cell subsets during preterm labor (90). Importantly, although specific cell types displayed consistent signaling across tissues, each also exhibited tissue-specific processes with preterm labor, indicating unique functions tailored to the tissue microenvironment (90). Together, these findings provide novel insight into the cellular changes taking place in the murine reproductive tissues during preterm labor and lead to premature delivery.

Placental scRNA-seq sheds light into the pathophysiology of preeclampsia

Preeclampsia is primarily a placental and intravascular disease (154), and thus this tissue has been the target of single-cell investigations aimed at uncovering the molecular mechanisms underlying the development of this obstetrical syndrome (79, 155–157). One of the first comprehensive scRNA-seq studies integrated placenta-derived single-cell signatures with bulk transcriptomic data derived from the plasma cell-free RNA of women with early-onset preeclampsia and healthy pregnancies (79). Signatures corresponding to decidual cells, endothelial cells, and EVTs were upregulated in the maternal plasma with preeclampsia,

whereas the SCT signature was reduced (79). Analysis of the transcriptional heterogeneity of genes involved in cell migration, cell proliferation and apoptosis were more variable in preeclamptic patients compared to normal term deliveries; moreover, genes annotated to cell death had overall higher expression in preeclampsia (79), which is consistent with prior associations between this disease and trophoblast apoptosis (158–162) (Figure 2). Consistently, the direct scRNA-seq comparison of preeclamptic and healthy placentas indicated disease-driven enrichment of genes annotated to oxidative stress and inflammation in EVTs (155, 157), and those annotated to cell cycle and protein folding in SCTs (157). Moreover, clustering of differentially regulated genes revealed a module with reduced activity in preeclampsia that was related to cytokines, regulation of cell death, and differentiation (157). Two transcription factors, *CEBPB* and *GTF2B*, were identified as being greatly reduced in the disease state, and thus could be implicated in the trophoblast dysfunction characteristic of preeclampsia (157).

Preeclampsia has been traditionally classified into two subsets, with each being characterized by distinct pathophysiology: early-onset and late-onset (154, 163–165). To unravel cellular changes that may distinguish early- and late-onset preeclampsia, an *in silico* investigation utilized placental single-cell signatures derived from the study of Vento-Tormo *et al.* (68) to deconvolute bulk transcriptomic data of placentas from women with either form of the disease (156). The number of genes differentially regulated in early-onset preeclampsia compared to its control group was substantially greater than in late-onset (156), which is consistent with early-onset being largely a placental disease (154, 166–168). Moreover, the direct comparison of early- and late-onset preeclampsia also resulted in a large set of differentially expressed genes, further indicating distinct underlying pathophysiology (156). Deeper investigation of changes at single-cell resolution indicated that EVTs and fibroblasts play a key role in early-onset preeclampsia, displaying dysregulated signaling associated with angiogenesis and fibrosis (156). Importantly, novel trophoblast-derived markers were identified as being upregulated in early-onset preeclampsia, such as Epstein-Barr virus induced 3 (*EBI3*), and such proteins could be detected as being elevated in the maternal circulation (156). Therefore, the combination of placental single-cell transcriptomics with soluble biomarker discovery can represent a viable approach for the prediction and diagnosis of preeclampsia.

Myometrial cell types and their contributions to spontaneous term labor

The uterine myometrium and the cervix are both key organs in the common pathway of parturition (169–171). Thus, investigation of the cellular processes taking place in these tissues prior to the initiation of labor can provide a baseline measurement that is useful for the study of physiological or pathological labor. A recent study obtained myometrial and cervical biopsies during planned cesarean hysterectomy without evidence of labor in the third trimester and performed scRNA-seq (172). The resulting data were cross-referenced with the Human Protein Atlas to identify major cell type clusters, which included endothelial, epithelial, and stromal cells, smooth muscle cells, and leukocytes (172). Expression of CD74 (HLA class II histocompatibility antigen gamma chain) could distinguish the epithelial, endothelial, and leukocyte clusters from the stromal and smooth muscle cells (172). As CD74 regulates the function of MHC class II molecules (173) and is

thought to be mainly restricted to APCs under homeostatic conditions, a potential underlying state of tissue inflammation was proposed that could drive CD74 expression in epithelial/endothelial cells (174, 175). This finding was consistent with the observed upregulation of genes associated with inflammation (172); yet, given that biopsies were taken prior to active labor, such inflammation could represent preparation for the onset of parturition.

We recently undertook a single-cell survey of the human uterine tissue (primarily myometrium) to further investigate the molecular mechanisms underlying the transition from a quiescent to a contractile state prior to term labor (89). Myometrial biopsies were obtained from term deliveries either with or without spontaneous labor for scRNA-seq, from which a total of 24 immune and non-immune cell types were identified, including multiple subsets of smooth muscle cells and macrophages (89). Specifically, we classified three subsets of smooth muscle cell according to their transcriptomic profiles, which were then validated by protein expression (89). We showed that the first smooth muscle subset displayed an expression profile involving smooth muscle contraction, the second included processes related to neutrophil biology (e.g., neutrophil elastase expression), and the third exhibited increased interferon (IFN)-γ signaling, demonstrating the differing roles of these novel subsets in the process of labor (89). Transcriptomic characterization of macrophage subsets and stromal cell types was also reported (89). Comparison of overall changes in cell abundance indicated a substantial labor-specific increase in the presence of stromal cells, endothelial cells, monocytes, decidual cells, and myofibroblasts, which was accompanied by reductions in macrophage and lymphocyte subsets (89). Consistently, the most dramatic labor-driven changes in gene expression were found in stromal cells, endothelial cells, monocytes, and macrophages, which included an overall enrichment of multiple muscle- and contraction-related processes (89). We also evaluated intercellular communications and identified major signaling pathways implicated in labor such as complement, contraction, IL-1, TGF-β, and THY1 (89). In particular, we show that specific myometrial cell types such as decidual cells, EVTs, myofibroblasts, smooth muscle cells, and stromal cells act as receivers of IL-1 (89), which is a considered a master regulator of human parturition (176–179). By comparing scRNA-seq data with bulk transcriptomics of the human myometrium, we demonstrated a high degree of agreement between these datasets, with single-cell technology providing better coverage of differential gene expression (89). Consistent with prior analyses, gene expression signatures corresponding to scRNA-seq-derived myometrial cell types could be monitored in the maternal circulation, allowing for the evaluation of cellular dynamics throughout pregnancy (89). Importantly, such comparison indicated agreement in the labor-specific enrichment of specific cell type signatures, namely monocytes, thereby providing potential biomarkers that could be indicative of labor progression (89).

In addition to providing insight into the cellular changes and interactions associated with term labor, single-cell technology has also been applied to evaluate resident regulatory T cells (Tregs) present in the placental bed (uterine tissues) biopsied after term cesarean delivery (180). Uterine Tregs (uTregs) expressed a core transcriptomic signature consistent with classical Tregs, which was even more pronounced than peripheral Tregs and included expression of *FOXP3*, *CTLA4*, and *IL2RA* (180). *In vitro* assays confirmed the suppressive functionality of these cells, which was consistent with the observed gene expression/

pathway enrichment indicative of activated, effector uTregs (180). By comparing uTregs with publically available Treg datasets from other tissues, it was shown that such cells displayed transcriptomic overlap with tissue- and tumor-infiltrating Tregs (180). These data provide invaluable insight into uTregs that participate in the modulation of local effector T cells to prevent aberrant immune activation during pregnancy.

Together, the studies outlined above point to the uterine myometrium as an underexplored site of cellular interactions and signaling that are necessary for physiological labor at term. Future single-cell investigations may further explore such pathways in the context of pathological labor leading to preterm birth as well as other pregnancy complications.

Single-cell profile of the maternal-fetal interface in patients with COVID-19

Since the emergence of the SARS-CoV-2 virus in late 2019, investigators around the globe have shifted research efforts to uncovering the molecular mechanisms that dictate maternal infection, disease severity, and risk of vertical transmission during pregnancy. Initially, to further this goal, multiple reports undertook *in silico* analysis of previously generated single-cell data (66, 68, 78) to evaluate the expression of the canonical SARS-CoV-2 cell entry mediators angiotensin converting enzyme 2 (*ACE2*) (181–183) and transmembrane protease serine 2 (*TMPRSS2*) (183) at the maternal-fetal interface (92, 94–96, 99, 184, 185). Each of these studies observed independent expression of *ACE2* and *TMPRSS2* in decidual cells such as DSCs and perivascular cells (92, 96, 184) and/or in placental trophoblast subsets (92, 95, 96, 185). However, viral cell entry requires the co-expression of both *ACE2* and *TMPRSS2* within the same cell (183), and the evidence for co-expression of such cell entry mediators by placental cells was unclear, given that some studies indicated detectable co-expression (95, 185) while others suggested this was rare (94, 96, 99). Therefore, to provide further clarity in this regard, we undertook a combined approach that utilized: 1) previous single-cell data of the first-trimester maternal-fetal interface (Vento-Tormo *et al.* 2018 (68)); 2) new single-cell data of the decidua and placenta from an indicated second-trimester hysterectomy; and 3) our previous single-cell dataset of the third-trimester decidua basalis, placental villi, and chorioamniotic membranes (Pique-Regi *et al.* 2019 (80)) (91). We found that a minimal number of cells co-express *ACE2* and *TMPRSS2* at the maternal-fetal interface throughout gestation, even with an extremely permissive expression threshold (91). To overcome the limitation of low SCT representation in our single-cell dataset (due to their multinucleated morphology), snRNA-seq of placental tissues was performed and confirmed that co-expression of *ACE2* and *TMPRSS2* is minimal among these cells (91). Thus, our data support the absence of meaningful co-expression of classical cell entry mediators for SARS-CoV-2 at the maternal-fetal interface throughout pregnancy, a concept that has since been further confirmed (186).

Regardless of the expression patterns of viral cell entry mediators, a central question amidst the COVID-19 pandemic has been whether maternal infection results in a fetal/placental immune response that can lead to adverse pregnancy outcomes and, more importantly, whether the fetus itself is impacted. A study utilized bulk and single-cell transcriptomic analyses of the placental tissues from women diagnosed with COVID-19 to demonstrate upregulation of genes associated with immune response compared to uninfected controls

(93). At single-cell resolution, such COVID-19-driven changes included enrichment of cytotoxic molecules in NK cells and signs of T-cell activation, and endothelial cells similarly displayed signs of activation and immune response (93). This finding is consistent with a case report in which scRNA-seq was performed using placental tissues from a pregnant COVID-19 patient who was delivered at 28 weeks of gestation, showing that $CD8^+$ T cells were activated (98). Moreover, cell-cell communication analysis indicated increased interactions between immune cells at the maternal-fetal interface in COVID-19 (93, 98), including between T cells and monocytes/NK cells (93). In light of this evidence pointing to a maternal immune response induced by COVID-19 during pregnancy, we undertook a comprehensive multi-disciplinary investigation of maternal-fetal immunity in SARS-CoV-2-infected pregnant women, most of whom were asymptomatic, and showed using scRNA-seq that maternal T cells and macrophages in the chorioamniotic membranes display substantial changes in gene expression compared to cells from uninfected pregnant women (97). We then compared our T-cell signatures with previously generated single-cell signatures of peripheral T cells from hospitalized COVID-19 patients, and observed a positive correlation between these datasets (97), suggesting that T cells derived from the maternal-fetal interface of pregnant COVID-19 patients display similar characteristics to those found in the circulation of non-pregnant patients with severe disease. Shared genes were enriched for protein translation processes; yet, some differentially expressed genes were unique to maternal-fetal interface-derived T cells (97). The combined differentially expressed genes from the maternal-fetal interface of women with COVID-19 showed enrichment of multiple interferon signaling pathways, indicating that SARS-CoV-2 infection drives an anti-viral immune response even when the virus itself is not present in this compartment (97). Overall, these novel findings support the value of single-cell datasets that can be leveraged to investigate relevant cellular and molecular targets for diseases such as COVID-19, which in turn can inform translational research directed at therapeutic interventions.

Limitations of single-cell technology

The emergence and popularization of single-cell techniques has opened new avenues of scientific discovery across multiple disciplines. Yet, the widespread use of such methods has also highlighted their limitations in comparison to other cellular and molecular tools, such as the inherent discovery-based nature of current single-cell approaches. Moreover, the analysis of scRNA-seq datasets requires advanced computational approaches that rely on algorithmic clustering and associations to determine cell identities and profiles, which can differ based on the platforms and analysis pipelines utilized (as has been extensively reviewed in (187–190) and elsewhere). For this reason, researchers utilizing single-cell approaches have often chosen to validate key findings (e.g., identification of novel cell subsets) by using alternative approaches such as flow cytometry, imaging methods, or animal models. Indeed, we have utilized immunofluorescence imaging to confirm our identification of a potentially novel cell type, lymphoid endothelial decidual (LED) cells, in the human chorioamniotic membranes (80). Similarly, we used the same approach to validate our identification of distinct subsets of smooth muscle cell in the human myometrium based on expression of oxytocin receptor, elastase, or IFNγ (89). For studies in which the identification of novel cell types represents a primary outcome, we consider that additional functional and phenotypic analyses are essential to ensure that such populations do not represent artifacts resulting from data

processing and analysis. Overall, we consider that the application of validation techniques is essential for providing confirmatory analysis of discoveries made using exploratory single-cell approaches.

An important consideration for scRNA-seq and other single-cell technologies is the analytical approach that will be utilized to dissect the generated data. A complete discussion of single-cell analysis pipelines and tools is outside of the scope and expertise of this review, and such topics have been covered more extensively elsewhere (187, 188, 190). Yet, we have successfully applied several of the tools described in this review, such as CellChat cell-cell communication analysis (119) and Slingshot trajectory analysis (191), to our single-cell investigations of the human maternal-fetal interface (Figure 3). In particular, an important consideration when studying the placental and decidual tissues at single-cell resolution is the maternal or fetal origin of each cell. To overcome this potential limitation and aid in the interpretation of our scRNA-seq data, we have successfully incorporated maternal and fetal genotyping data into our analysis pipeline (80, 89, 97, 192). Other limitations of scRNA-seq in the context of maternal-fetal immunology include the difficulty of obtaining biopsies for research, for which we have proposed and utilized two different translational solutions: first, we have shown that specific cellular signaling pathways implicated in labor are shared between the human and murine myometrium (90). While validation remains necessary when comparing humans and mice, such findings can lay the groundwork for future single-cell investigations of pregnancy. In parallel, the evaluation of placenta-derived single-cell signatures in the maternal circulation has been shown to represent a viable approach for monitoring pregnancy and its complications in a non-invasive manner, as has been described in this review (79, 80, 89, 131). Taken together, these findings indicate that scRNA-seq represents a translationally-relevant approach for investigation of the maternal-fetal interface; yet, we consider that the careful application of this technology and its analysis is required to ensure the generation of useful and reproducible results.

Conclusions

Collectively, the reports summarized here emphasize the value of single-cell technology for characterizing the cellular dynamics and interactions at the maternal-fetal interface. Given the cellular heterogeneity of the decidua, placenta, and reproductive tissues, “omics” techniques that provide information at single-cell resolution are rapidly becoming the new standard for investigations of the molecular mechanisms underlying developmental processes, physiological and pathological labor, and other obstetrical diseases. The findings reviewed herein provide a rich framework of single-cell data, generated at different gestational time points and under various conditions, which can be a starting point for future studies. Importantly, we recognize that there are often disparities between studies that can result from differences in technologies, individual platforms, and cell-type classification methods, among other variables. Thus, given the abundance of single-cell datasets that have been generated from the maternal-fetal unit, there is potential for unified analyses that integrate such data under consistent cell-type annotations and analysis pipelines to represent single-cell dynamics at different time points throughout pregnancy in a comparable manner. In summary, this review demonstrates the power of single-cell technologies to uncover new cellular immunobiological pathways and interactions that may have translational relevance

for pregnancy disease, and it is our hope that this summary can act as a catalyst for future investigations of the maternal-fetal interface.

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Abbreviations

APC	antigen-presenting cell
ATAC-seq	assay for transposase-accessible chromatin with high-throughput sequencing
CITE-seq	cellular indexing of transcriptomes and epitopes by sequencing
CTB	cytotrophoblast
CyTOF	cytometry by time-of-flight
DC	dendritic cell
DSC	decidual stromal cell
EVT	extravillous trophoblast
HLA	human leukocyte antigen
IMC	imaging mass cytometry
IL	interleukin
ILC	innate lymphoid cell
KIR	killer immunoglobulin-like receptor
LED	lymphoid endothelial decidual cell
MAIT cell	mucosal-associated invariant T cell
MSC	mesenchymal stromal cell
MHC	major histocompatibility complex
NK cell	natural killer cell
RSA	recurrent spontaneous abortion
scRNA-seq	single-cell RNA-sequencing

SCT	syncytiotrophoblast (termed “STB” in some studies)
snRNA-seq	single-nucleus RNA-sequencing
TCR	T-cell receptor
Treg	regulatory T cell
UMAP	uniform manifold approximation and projection
VCT	villous cytotrophoblast

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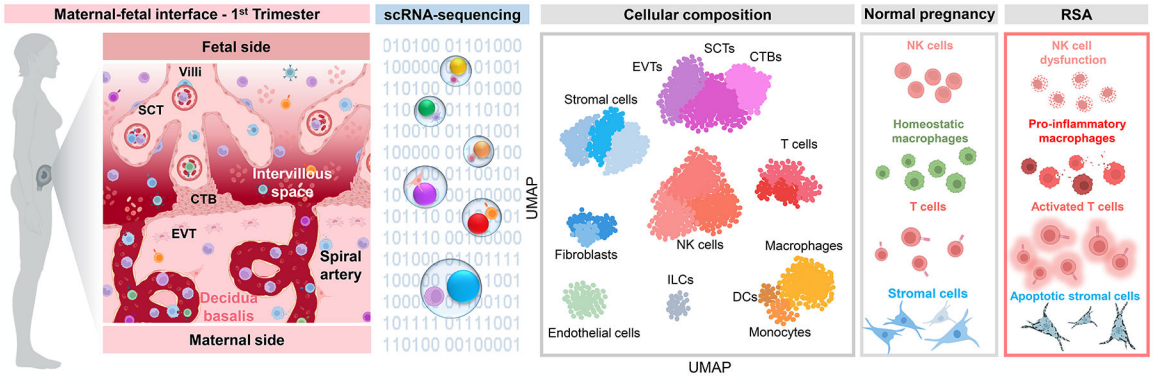

Figure 1. The single-cell immunobiology of the maternal-fetal interface in early pregnancy.

The first-trimester maternal-fetal interface is marked by placental growth, trophoblast invasion and angiogenesis, and the establishment of maternal-fetal dialogue to promote homeostasis for the remainder of pregnancy. During this period, the placental compartment primarily comprises trophoblast cell types, fibroblasts, stromal cells, and Hofbauer cells (fetal macrophages), as represented in the UMAP plot based on data reported by Vento-Tormo *et al.* 2018. By contrast, the decidua displays more heterogeneous cellularity, characterized by major populations of decidual stromal cells, NK cells, macrophages, and endothelial cells. Other leukocyte subsets are also represented in the early decidua; namely, T cells, innate lymphoid cells, and dendritic cells. Single-cell investigations of recurrent spontaneous abortion (RSA) have suggested that this disease includes the altered composition of decidual NK cells and generalized T-cell infiltration and activation, together with the acquisition of a pro-inflammatory phenotype by NK cells and macrophages. Indeed, it was proposed that a shift away from macrophage-NK cell interactions toward macrophage-T cell interactions may contribute to RSA. The decidual stromal compartment may also be affected, as enrichment of cellular processes related to apoptosis and senescence implicates impaired stromal cell differentiation in RSA pathophysiology. Abbreviations used: SCT, syncytiotrophoblast; CTB, cytotrophoblast; EVT, extravillous trophoblast; NK cell, natural killer cell; DC, dendritic cell; ILC, innate lymphoid cell; UMAP, uniform manifold approximation and projection. Figure created using BioRender.

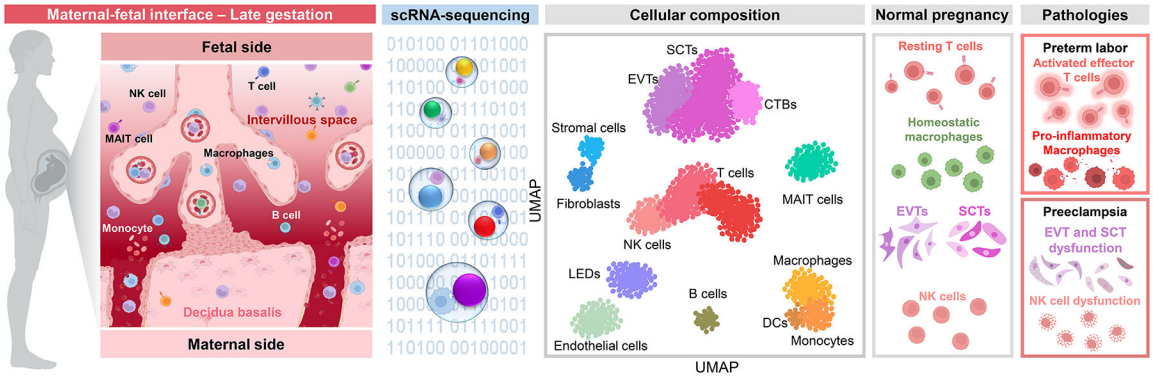

Figure 2. The single-cell immunobiology of the maternal-fetal interface in late pregnancy, preterm parturition, and preeclampsia.

As the end of pregnancy nears, the maternal-fetal interface undergoes changes in cellular composition and gene expression profiles in preparation of labor onset. The decidual lymphocyte population is predominantly composed of T cells, which include a subset of mucosal-associated invariant T cells (as visualized in the UMAP plot based on data reported by Pique-Regi *et al.* 2019). Decidual stromal and endothelial cells, including the recently described lymphoid endothelial decidual cells, undergo shifts in their expression profiles to display cellular processes and interaction networks that support leukocyte infiltration and activation in the maternal-fetal interface to promote labor. Placental trophoblast cell types also display labor-specific alterations in their signaling profiles, and cell-cell communication between placental and decidual cell types is shown to be enhanced with labor, indicative of an overall increase in intercellular signaling across tissue compartments at the maternal-fetal interface. While preterm labor also involves altered trophoblast signaling, many impacted genes are regulated in the opposite direction compared to normal labor at term, suggesting the distinct activation of placental cell types. Importantly, preterm labor is characterized by the pathological aberrant activation of effector T cells and monocytes/macrophages at the maternal-fetal interface. In preeclampsia, placental trophoblast and fibroblast cell types show enrichment of genes associated with inflammation, oxidative stress, and angiogenesis, indicative of dysregulated signaling that could contribute to placental dysfunction. Such single-cell transcriptomic changes are much more apparent in placentas from cases of early-onset preeclampsia, which is consistent with this disease subset being largely placenta-driven. A number of reports have also implicated dysfunction of decidual NK cells in the pathogenesis of preeclampsia, which could result from functional variations driven by maternal killer cell immunoglobulin-like receptor (KIR) and fetal HLA-C interactions. Abbreviations used: SCT, syncytiotrophoblast; CTB, cytotrophoblast; EVT, extravillous trophoblast; MAIT cell, mucosal-associated invariant T cell, NK cell, natural killer cell; DC, dendritic cell; LED, lymphoid endothelial decidual cell; UMAP, uniform manifold approximation and projection. Figure created using BioRender.

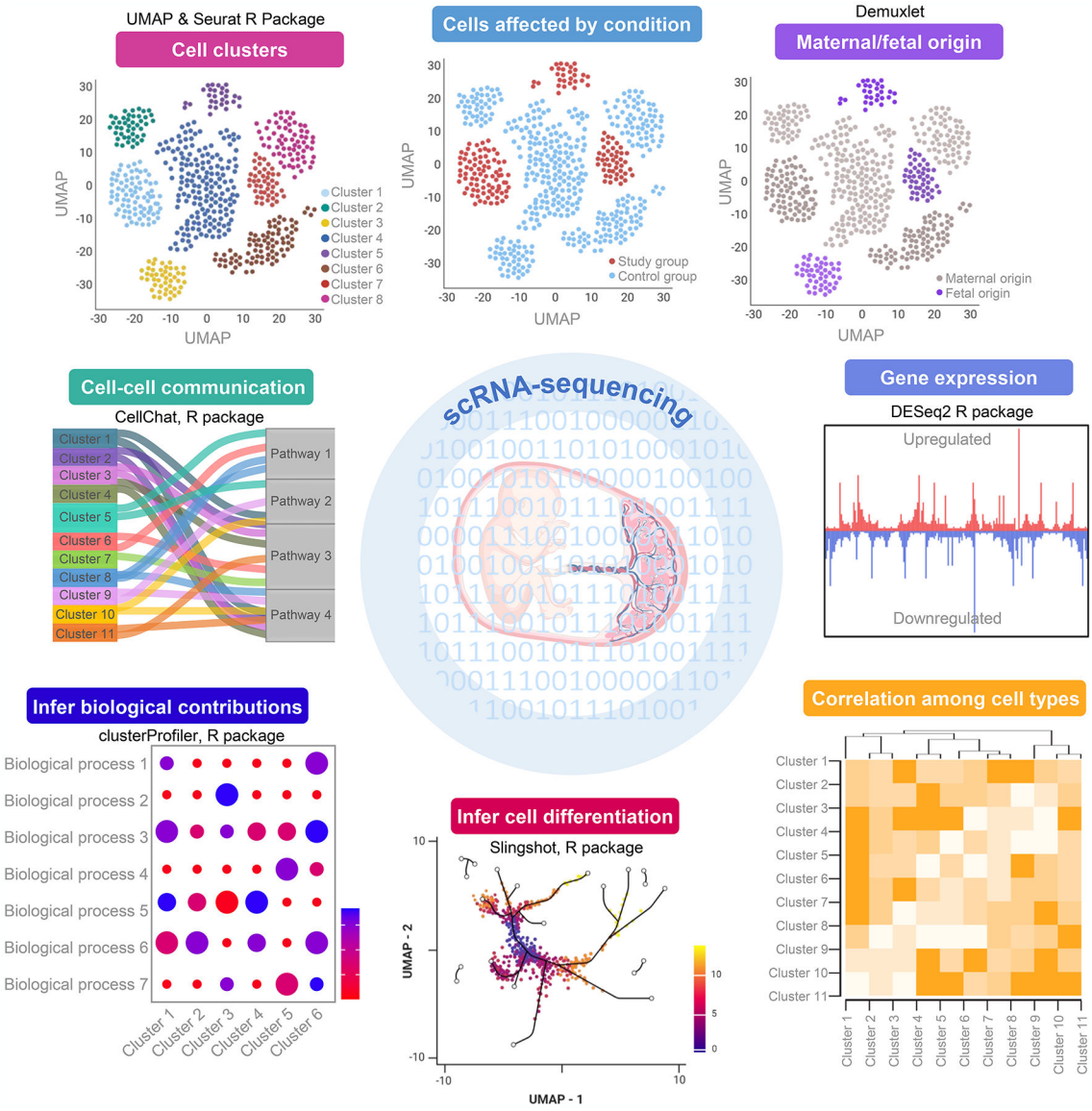

Figure 3. Overview of applied single-cell analytics tools.

Diagram providing representative images of single-cell data visualization and analysis together with the methods used for their creation. Clockwise direction starting from the upper left pane: Seurat (193, 194) is used to normalize and combine cell count data matrices for subsequent visualization using uniform manifold approximation and projection (UMAP) plots. The demultiplexing of barcoded single-cell libraries allows further visualization according to sample metadata, such as control and study groups. We have further expanded this concept specifically for studies of the maternal-fetal interface to incorporate maternal and fetal genotype data using demuxlet (195), thereby allowing for the identification of maternal and fetal cells as outlined in (Pique-Regi *et al.* (89)) and (Garcia-Flores *et al.* (192)). The differential expression of specific genes can be evaluated using DESeq2 (196), which allows for additional downstream analyses such as correlation among cell types, visualization of cell differentiation trajectories using Slingshot (191), or inference

of cell type contributions to biological processes using clusterProfiler (197). We have also implemented the recently-developed CellChat (119) to unravel cell-cell communication networks based on the expression of ligands, receptor subunits, cofactors, and other related molecules by each cell type. These tools and visual representations can represent a useful starting point for researchers looking to apply single-cell technology to their investigation of the maternal-fetal interface. Figure created using BioRender.