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Placental single cell transcriptomics: Opportunities for endocrine disrupting chemical toxicology

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Abstract

The placenta performs essential biologic functions for fetal development throughout pregnancy. Placental dysfunction is at the root of multiple adverse birth outcomes such as intrauterine growth restriction, preeclampsia, and preterm birth. Exposure to endocrine disrupting chemicals during pregnancy can cause placental dysfunction, and many prior human studies have examined molecular changes in bulk placental tissues. Placenta-specific cell types, including cytotrophoblasts, syncytiotrophoblasts, extravillous trophoblasts, and placental resident macrophage Hofbauer cells play unique roles in placental development, structure, and function. Toxicant-induced changes in relative abundance and/or impairment of these cell types likely contribute to placental pathogenesis. Although gene expression insights gained from bulk placental tissue RNA-sequencing data are useful, their interpretation is limited because bulk analysis can mask the effects of a chemical on individual populations of placental cells. Cutting-edge single cell RNA-sequencing technologies are enabling the investigation of placental cell-type specific responses to endocrine disrupting chemicals. Moreover, *in situ* bioinformatic cell deconvolution enables the estimation of cell type proportions in bulk placental tissue gene expression data. These emerging technologies have tremendous potential to provide novel mechanistic insights in

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a complex heterogeneous tissue with implications for toxicant contributions to adverse pregnancy outcomes.

Keywords

Placenta; villous tissue; endocrine disrupting chemicals; single cell analyses; cellular deconvolution

Introduction

The placenta is a transient organ that develops during pregnancy and plays a key role in not only the health of the fetus but also the mother (Gingrich et al., 2020; Padmanabhan et al., 2021). Defects in placental development are associated with poor pregnancy outcomes, intrauterine growth restriction, and programming of offspring health and disease. Gestational exposures to endocrine-disrupting chemicals (EDCs) can perturb placental functions contributing to adverse pregnancy outcomes and later onset diseases. Understanding the molecular pathways linking EDC exposures to health outcomes via placental dysfunction is a highly active area of promising and ongoing toxicologic and epidemiologic research. These studies typically use bulk placental samples, ignoring the complex cellular heterogeneity of the placental tissue. This review summarizes the current status of EDC exposures on bulk placental tissue gene expression, as measured by RNA-sequencing, highlighting the diverse cellular functions of different placental cell types and cutting-edge technologies available for single cell measures, enabling cell type resolution of placental gene expression. Lastly, we provide actionable recommendations to advance research on EDC exposure effects on placental tissue and development.

Impact of prenatal exposure to endocrine disrupting chemicals (EDC) on perinatal and childhood outcomes

EDCs are chemicals that interfere with hormone signaling through multiple mechanisms (Ho et al., 2022; Kabir et al., 2015), including disruption of synthesis, transport, or binding of endogenous hormones in the body (Kavlock et al., 1996), as well as direct activation of hormone receptors (Kiyama and Wada-Kiyama, 2015). Environmental contaminants that are classified as EDCs include pesticides (Brander et al., 2016), heavy metals (Jia et al., 2021), plasticizers (Rattan et al., 2017), and pharmaceuticals (Ho et al., 2022). Exposure to one or more EDCs during gestation is linked to pregnancy complications including preeclampsia (Cantonwine et al., 2016), gestational diabetes (Shaffer et al., 2019) and preterm birth (Welch et al., 2022). It is also associated with offspring outcomes including hypospadias, cryptorchidism (Grady and Sathyanarayana, 2012; Sathyanarayana et al., 2016; Wu et al., 2022), male (Bonde et al., 2016) and female (Rattan et al., 2017) reproductive disorders, behavioral problems (Philippat et al., 2017), autistic traits (Day et al., 2021), decreased IQ scores (Tanner et al., 2020), liver injury (Midya et al., 2022) and cardiometabolic dysfunction (Abrantes-Soares et al., 2022). Depending on the chemical or chemical mixture studied, EDC exposure is also associated with both increased (Pearce et al., 2021) and decreased (Hu et al., 2021) birth weight, highlighting the need to improve

our understanding of specific chemical impacts and the molecular mechanisms underpinning these associations. Epidemiological evidence for links between many specific chemical exposures and diseases remain mixed, however, and further research is required into specific mechanisms and thresholds of exposure that may contribute to disease outcomes.

In general, as described by the Developmental Origins of Health and Disease (DOHaD) hypothesis (Barker, 2007), exposures that disrupt growth and development in early life can contribute to lifelong health effects (Almond and Currie, 2011). The prenatal period of development represents a uniquely susceptible life stage for EDC exposure, as endocrine signals during this period are critical for fetal growth and healthy organ development (Gicquel and Le Bouc, 2006; Scott et al., 2009; Toivanen and Shen, 2017). For example, maternal exposure to EDCs is linked to birth weight in human studies and mechanistic toxicology studies, demonstrating EDC impacts body weight and energy metabolism. Pregnant mice exposed to EDCs like tributyltin have offspring with altered differentiation potential of multipotent cells, leaving them predisposed to differentiate into adipocytes (Kirchner et al., 2010). These cell-type differentiation impacts of EDCs can reveal mechanisms for their contribution to disease etiologies. For example, the EDCs that predispose multipotent cells to differentiate into adipocytes potentially contribute to obesity (Hao et al., 2012; Kirchner et al., 2010). In addition, EDCs like phthalates and bisphenol A stimulate cell proliferation in prostate (Corti et al., 2022) and breast (Williams and Darbre, 2019) cells, providing a plausible mechanism for contribution to cancer progression. Virtually all of the adverse health endpoints implicated by prenatal EDC exposures are at least partly mediated by the placenta, which may be unsurprising given the placenta's multifunctional role in fetal development. There is therefore a great need to understand the role the placenta plays in mediating EDC effects on maternal and fetal health during pregnancy.

Placental function, structure, development, and cell types

The placenta is a transient and multifaceted organ specially adapted to carry out multiple life-supporting and regulatory functions for the fetus throughout pregnancy. Perhaps the most fundamental functions of the placenta are to facilitate fetal oxygen, nutrient, and waste exchange at the maternal-fetal interface, which consists of a selective interhaemal barrier separating maternal and fetal circulations (Caruso et al., 2012). The placenta is also responsible for regulating many physiological processes of pregnancy through its roles in hormone synthesis, immune protection, and xenobiotic metabolism, all necessary for normal fetal development during pregnancy. Additionally, placental growth, development, and function are highly dependent on maternal and fetal hormone signaling and crosstalk (Murphy et al., 2006).

Originating from the earliest stages of pregnancy, the fetal components of the placenta, including the placental disk, umbilical cord, and amniotic and chorionic membranes, derive from the blastocyst, and therefore share genetic makeup with the fetus (Caruso et al., 2012). Initial placental development, known as placentation, begins shortly after fertilization as the blastocyst undergoes implantation into the endometrial layer of the uterine wall (Kim and Kim, 2017). The placental disk originates from two tissues: the stromal extraembryonic

mesoderm and the epithelial trophectoderm. Importantly, the extraembryonic mesoderm differentiates into fibroblasts, placental blood vessels and immune cells, including Hofbauer cells, the resident macrophages of the placenta (Burton and Fowden, 2015). Hofbauer cells are one of several notable placental cell types that confer unique structure and function to the organ. In addition to serving immune cell functions, Hofbauer cells promote critical angiogenesis throughout the placenta across the entire pregnancy (Loegl et al., 2016; Reyes and Golos, 2018; Seval et al., 2007). The maternal component of the placenta, the decidua, is derived from the endometrial lining of the uterus. Thus, together the placenta and associated membranes represent an example of parabiosis with cellular components derived from the fetal and maternal compartments.

As the placenta grows throughout pregnancy, finger-like projections called chorionic villous trees with extensive placental vasculature extend from the chorion of the placental disk into the interstitial space that houses the fetal-maternal interface (Figure 1). The trophectoderm, which makes up the wall of the blastocyst at the implantation face, differentiates into trophoblast. The trophoblast layer gives rise to cell types which constitute the epithelial barrier and covering of the villous trees (Burton and Fowden, 2015). Multiple cell types of trophoblast lineage perform essential placenta functions unique to the maternal-fetal interface and are often implicated in placental dysfunction. The innermost trophoblast layer covering villous trees consists of proliferative and undifferentiated cytotrophoblasts. These cells fuse to form and replenish the syncytiotrophoblast layer, a multinucleated, semi-continuous syncytium covering the surface of villi and some parts of the basal and fetal plates (Castellucci and Kaufmann, 2006; Midgley et al., 1963). The syncytium makes up the interhaemal membrane separating maternal and fetal circulations where gas and nutrient exchange takes place. Cytotrophoblasts also differentiate into extravillous trophoblasts that invade from anchoring chorionic villi that connect to the maternal component of the placenta, termed the decidua, which derives from the uterine endometrium. Extravillous trophoblasts, in conjunction with maternal immune cells, facilitate critical remodeling of the maternal spiral arteries necessary to optimize maternal blood flow to the placenta (Burton and Fowden, 2015; Maltepe and Fisher, 2015). The extravillous trophoblasts also form the outermost cell columns at the tips of chorionic villi that anchor the villi to the maternal basal plate and stabilize them in the intervillous space (Pijnenborg et al., 1981). Given the complexity of the placental tissue, to gain insight into placental function and/or dysfunction from EDC exposure, effects on placenta-specific cell types should be prioritized for interrogation.

The placental microenvironment is an important factor in determining its structure and cell type composition. For example, placental oxygen levels change throughout pregnancy with severe hypoxic conditions prevailing during the first trimester. The first trimester is a period when implantation and cytotrophoblast invasion occurs and uterine spiral artery remodeling begins. Oxygen tension influences the destiny and function of several of the placental cell types during early pregnancy (Zhao et al., 2021). High oxygen tension in the spiral artery promotes endovascular trophoblast invasion (Sato, 2020). The ability of the placenta to cope with environmental challenges highlights its plasticity to safeguard a healthy pregnancy. For instance, hypoxia-inducible factor (HIF) (Fryer and Simon, 2006) is induced to allow for adaptation to low oxygen tension. Maternal blood pressure is another

potential mediator of placental cell type composition. For example, elevated blood pressure has been associated with a reduced mesenchymal stromal cells-to-syncytiotrophoblast ratio (Broséus et al., 2022). Recent histopathologic findings indicate SARS-CoV-2 infection leads to significant placental hypoperfusion and inflammation (Di Girolamo et al., 2021). A systematic review found that 12% of pregnant women with SARS-CoV-2 infection had the virus present in syncytiotrophoblasts (Ashary et al., 2020). The consequence of this and other inflammatory disorders in modulating cell type composition is an avenue for future research.

Placenta as an endocrine organ and a target organ for EDC toxicity

One of the key roles of the placenta is its endocrine function, including the ability to synthesize and secrete hormones that are central for establishment and optimal maintenance of pregnancy, fetal development, and parturition (Costa, 2016). Early placental defects underlie several disorders of pregnancy such as miscarriage, fetal growth restriction, and pre-eclampsia (Dimitriadis et al., 2023). The placenta's secretome comprises of a plethora of hormones, steroids (progesterone, estrogen), proteins (human chorionic gonadotropin [hCG], human placental lactogen [HPL], placental growth hormone [HPGH], leptin, adiponectin, inhibin, activin, placental growth factor [PIGF]), and cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1). The roles placental hormones play during pregnancy are myriad and beyond the scope of this review. However, some of their most important functions include regulation of growth and differentiation of trophoblasts, support of fetal growth, protection of the fetus from infection, modulation of maternal adaptations, and preparation of the uterus and mother for parturition (Mesiano, 2009).

The syncytiotrophoblast layer is the predominant site of the production of progesterone, a key player in pregnancy maintenance. The functions of progesterone include placentation, immune tolerance, inhibition of myometrium contractility, and preparing the mammary gland for lactation. During early pregnancy, the corpus luteum is the site of production of progesterone, under the influence of placental hCG (Garner and Armstrong, 1977). hCG also plays a role in placental angiogenesis, trophoblast invasion, myometrial quiescence, and immunomodulation (Nwabuobi et al., 2017). Other placental hormones such as HPL regulate maternal lipid and carbohydrate metabolism (Costa, 2016), while HPGH takes on growth hormone functions (Caufriez et al., 1993), namely, metabolic regulation, induction of insulin resistance, as well as promotion of gluconeogenesis and nutrient availability for the growing fetus (Alsat et al., 1998). Estrogens are the other major class of steroids produced by the placenta and promote embryo implantation, angiogenesis, vasodilation, and syncytialization (Berkane et al., 2017). The endocrine function of the placenta extends to the support of maternal immunologic (Daniel et al., 1987), nutritional, metabolic (Parrettini et al., 2020; Stern et al., 2021), and cardiovascular (Melzer et al., 2010) adaptations. In addition to its endocrine function, the placenta also synthesizes several chemicals that influence inflammation, oxidative stress response, angiogenesis, and innate defense mechanisms. MicroRNAs (Jin et al., 2022) and extracellular vesicles (Jin and Menon, 2018) that regulate post-transcriptional gene expression and facilitate intracellular communication between mother and placenta have the potential to serve as biomarkers of disease, and are also part of the placental secretome.

The placenta is a plausible target organ for EDC toxicity because of multiple structural and functional characteristics. The placental structure consists of a large surface area and a thin interface separating maternal and fetal circulation, which provides an ideal environment for maternal transfer of compounds (Burton and Jauniaux, 2015). Because the placenta is highly perfused at rates up to 700 ml/min by the third trimester (Wang and Zhao, 2010), systemically available toxicants are readily delivered to the placenta. Additionally, multiple different types of EDCs can cross the placenta, as previously reviewed (Yang et al., 2019). Beyond its role in material transfer, the placenta plays a role in biotransformation and chemical metabolism. The placenta is capable of metabolizing toxicants, such as EDCs, into reactive metabolites because it expresses an abundance of metabolizing enzymes such as cytochrome P450s, glutathione-s-transferases, lipases and other enzymes, thereby increasing the risk of tissue-generated metabolites (Myllynen et al., 2005). Due to the unique characteristics of the placenta, EDCs have the potential to disrupt critical signaling and cause significant placental injury, as previously reviewed (Gingrich et al., 2020; Yang et al., 2019).

Use of bulk placental tissue in environmental exposure gene expression studies

Human epidemiology studies frequently sample biopsies of placental villous tissue for measurement of molecular biomarkers of exposure or adverse pregnancy outcomes. Gene expression is frequently selected as a molecular biomarker because it is highly responsive to chemical exposure and represents a potential mechanistic link between exposure and function. In particular, bulk placental tissue gene expression has been investigated with a multitude of environmental exposures using candidate gene quantitative PCR, targeted or genome-wide microarray, and genome-wide RNA-sequencing technologies in animal models and humans (Rosenfeld, 2021). Recently, there have been several human population studies evaluating mRNA, lncRNA, or microRNA expression with RNA-sequencing in bulk placental tissue with the majority focusing on prenatal exposure to metals (Table 1) (Lapehn and Paquette, 2022). Importantly, these prior RNA-sequencing and exposure studies did not estimate cell composition or include cell composition in their analytic framework, which represents a future opportunity. RNA-sequencing analyses of prenatal chemical exposures, including EDCs, are an understudied category of environmental exposure with only three papers evaluating prenatal chemical exposures (phthalates, polycyclic aromatic hydrocarbons, and organophosphate pesticides) with the placental transcriptome at birth in a human population (Li et al., 2023; Paquette et al., 2023, 2021). Therefore, there is still a substantial breadth of knowledge to be gained through new studies of the full placental transcriptome and its associations with environmental exposures.

Environmental toxicology studies, similar to epidemiology studies, have also used bulk placental tissue to evaluate gene expression following *in vitro* EDC treatment. Placental villous explant tissue obtained from human pregnancies is a common model because it allows for measurement of toxicological endpoints in intact human tissue and has the advantage of retaining some aspects of the placental microenvironment such as cell-to-cell interactions. Use of this model generally consists of excising out villous tissue from the *ex*

vivo placenta in small chunks, floating tissues in appropriate tissue culture media, exposing explants to toxicant(s) of interest, and flash-freezing tissues or isolating mRNA for future analysis. Multiple studies have used this model to evaluate toxicant-induced changes in gene expression for several compounds including the trichloroethylene metabolite S-(1,2-dichlorovinyl)-l-cysteine (DCVC) measured with RNA-sequencing (Elkin et al., 2021), candidate gene PCR studies of Bisphenol A (Sieppi et al., 2016; Zou et al., 2022) and the cholesterol-lowering drug Pravastatin (Brownfoot et al., 2015), among others. Animal models have also been used extensively to study effects of toxicant exposure on placenta tissue *in vivo*, though most animal placentas differ in structure and/or function relative to human placentas. In these models, pregnant animals are exposed to toxicants through various methods, animals are euthanized, and bulk placental tissues are dissected out and processed for further analysis. For example, animal models have been used to assess toxicant-induced changes in gene expression for different EDCs, including phthalates (Xu et al., 2021), bisphenols A and S (Mao et al., 2020), trichlorethylene (Elkin et al., 2021), PCBs (Laufer et al., 2022) and dexamethasone (Lee et al., 2017). Similar to epidemiological studies, prior toxicology studies did not take into account differences in placental cell composition. Therefore, it is unknown which cells within the bulk placental tissues analyzed contributed to gene expression changes reported in each study. Because many of these studies have their raw data stored in data repositories such as the National Institutes of Health's Gene Expression Omnibus, there is a {Updating}future opportunity to re-analyze these data using innovative computational methods that account for cell composition, as described in subsequent sections.

To date, bulk placental tissue makes up the vast majority of RNA-sequencing data (Table 1). Although gene expression insights gained from bulk placental tissue RNA-sequencing data are useful, their interpretation is somewhat limited because bulk analysis can mask the effects of a chemical on individual populations of placental cells which could be identified through RNA-sequencing of sorted cell types or single cells (Zhang et al., 2019). Because the placenta undergoes such rapid growth, development, and ultimately, aging over the course of a pregnancy, cell type composition of placental tissue can vary greatly depending on when sample collection occurs (Lim et al., 2017; Sitras et al., 2012; Suryawanshi et al., 2022). Another challenge of interpreting data from bulk placental tissue is the extreme heterogeneity of tissue resulting in vastly different gene expression patterns which can fluctuate solely based on the location within the tissue of sample collection (Coorens et al., 2021). Moreover, placental tissue presents a unique set of challenges for data interpretation because the organ is embedded into the maternal decidua to varying degrees throughout pregnancy, which results in the unavoidable co-mingling of placental cells and maternal cells when placental tissue is procured for experimental purposes (Heazlewood et al., 2014; Lamb et al., 2012; Sardesai et al., 2017). Due to the unique conditions of placental tissue *in vivo*, interpreting gene expression data from bulk placental tissue samples is particularly challenging. Use of the emerging single-cell RNA-sequencing technologies when evaluating placental tissue gene expression changes is a way to avoid some of the pitfalls of interpreting bulk placenta RNA-sequencing data.

Single-cell molecular analyses and the placenta

Single-cell RNA-sequencing is a relatively new technology that measures genome-wide gene expression in a series of individual cells, as opposed to sequencing all transcripts collected from bulk tissue homogenate. Single-cell RNA-sequencing improves on bulk RNA-sequencing by capturing cell specific and cell state specific gene expression, allowing for the detection of differences in cell composition or differential expression within cell types, which improve the potential biologic relevance of inferences of results. However, single cell technologies typically require increased cost and expertise and limit throughput and sequencing depth relative to bulk tissue RNA-sequencing (Hedlund and Deng, 2018). Single-cell RNA-sequencing data analysis typically begins by annotating individual cells using defining genes or similarity indices with established cell type-specific samples. Once cells are annotated to cell types, investigators can perform cell type specific differential expression analyses or even compare the cell type composition of their samples (Luecken and Theis, 2019). Furthermore, single-cell RNA-sequencing measures are not affected by cellular heterogeneity differences that confound bulk tissue analyses (Campbell et al., 2020). The increasing adoption of single-cell RNA-sequencing across tissues has led to the discovery of new cell types and subtypes with distinct gene expression profiles from previously discovered cell types. Most single-cell RNA-sequencing approaches require dissociation of the tissue to a single cell suspension. Consequently, cell composition and novel cell type results must be interpreted with caution as tissue dissociation bias, the differential resilience of cell types to the tissue dissociation process, may distort the appearance and abundance of cell types (Hedlund and Deng, 2018). This is especially true in the human placenta since large, multi-nucleated syncytiotrophoblasts may be less likely to remain intact through processing and are generally incompatible with microfluidic-based whole cell single-cell RNA-sequencing platforms. Single nucleus RNA-sequencing, which relies on only capturing more stable nuclei instead of whole cells, has emerged as an alternative to potentially address dissociation bias as well as capture large cells not amenable to single-cell preparation protocols (Kim et al., 2023). Though single-cell RNA-sequencing is more susceptible to dissociation bias, the technology provides key insight into cell states and distributions and overcomes critical limitations of bulk analyses.

We identified 15 studies that performed single-cell RNA-sequencing on human placental tissue (as of December 2022) (Table 2), including 9 studies involving placentas collected at the end of gestation (Campbell et al., 2023; Chen et al., 2022; Pavličev et al., 2017; Pique-Regi et al., 2019; Tsang et al., 2017; Wang et al., 2022; Yang et al., 2021; Zhang et al., 2021; Zhou et al., 2022) and 6 studies involving placentas collected earlier in pregnancy (Li et al., 2022; Liu et al., 2018; Ray et al., 2022; Sun et al., 2019; Suryawanshi et al., 2018; Vento-Tormo et al., 2018). All studies were conducted in humans, except one study which used mice (Tosevska et al., 2022). While the majority of early pregnancy placental samples were from aborted fetal tissue, Sun et al collected first trimester samples using chorionic villi sampling, which provides an opportunity to follow pregnancies to the end of gestation (Sun et al., 2019). Syncytiotrophoblasts are multinucleated cells which would be anticipated to cause inherent challenges for single-cell RNA-sequencing. Only one study attempted to directly address this issue by isolating syncytiotrophoblasts using laser

microdissection followed by conventional bulk RNA-sequencing (Pavličev et al., 2017). This challenge may explain why syncytiotrophoblasts are not well represented as a major portion of cell types in clustering-based analyses of these cell types, despite their prominent role in placental physiology and function. As an alternative, single-nucleus RNA-sequencing has been successfully employed to capture transcriptomes generated from an *in vitro* syncytiotrophoblast model (Khan et al., 2021). Another key consideration for all placental studies is the reporting of key clinical characteristics and covariates of interest, and only a few studies reported this type of data for important factors such as maternal BMI, maternal age, and/or race/ethnicity of participants (Chen et al., 2022; Pique-Regi et al., 2019; Yang et al., 2021). Four studies also collected and generated single-cell RNA-sequencing data of the maternal decidua (Pique-Regi et al., 2019; Sun et al., 2019; Suryawanshi et al., 2018; Vento-Tormo et al., 2018), which provided opportunities to explore interactions between the maternal-fetal interface using receptor-ligand networks. While earlier single-cell RNA-sequencing studies characterized typically developing placental and decidual cell types and their interactions, more recent single-cell RNA-sequencing studies have begun to apply this approach to pregnancy complications, including preeclampsia (Campbell et al., 2023; Tsang et al., 2017; Zhou et al., 2022), preterm birth (Pique-Regi et al., 2019) and gestational diabetes (Yang et al., 2021). One study applied single-cell RNA-sequencing to investigate functional changes to the placenta related to severe COVID-19 infection ultimately resulting in fetal demise (Chen et al., 2022). Meta-analysis or other integrative analyses in single-cell RNA-sequencing studies of the placenta are currently limited. However, one study performed an integrated analysis of newly collected uncomplicated placentas alongside 2 previously published studies (Pique-Regi et al., 2019; Tsang et al., 2017) to develop a deconvolution reference for bulk placental gene expression measures (Campbell et al., 2023). However, no human placenta studies to date have utilized single-cell RNA-sequencing data in the context of environmental exposures analysis.

Future opportunities and recommendations in single-cell placental toxicology

The generation of bulk transcriptomics data in well powered cohorts with rich phenotypic data provides an unprecedented opportunity to study environmental exposures, but the ability to gain mechanistic insight into toxicity is limited due to a lack of cell-specific information. Generating single-cell data in a cohort setting may not be feasible due to cost, timeline constraints, and logistics of sample collection, but cellular deconvolution tools can provide an opportunity to reveal the cellular proportions of bulk sequencing data (Figure 2). Deconvolution refers to the bioinformatic process of estimating the distribution of cell types that constitute bulk tissue. Campbell et al recently developed a human placental deconvolution tissue reference panel based on cell type-specific gene expression profiles generated from new and existing single-cell RNA-sequencing data (Campbell et al., 2023). This panel was developed for use with the bioinformatic deconvolution tool CibersortX (Newman et al., 2019) but may be applied in other deconvolution algorithms and includes gene expression signatures for 8 placental cell types, 11 other fetal cell types, and 8 co-mingled maternal peripheral immune cell types. The rapid development of publicly available, cell type-specific sequencing and microarray data and conveniently

packaged deconvolution algorithm software have made deconvolution increasingly available to the research community. Deconvolution references are now increasingly available across species such as mice (Marsh and Blelloch, 2020; Nelson et al., 2016), and other tissues and biological molecules, including brain, umbilical cord blood, and DNA methylation (Campbell et al., 2023). The use of deconvoluted cell composition estimates in placental research is evolving but has largely focused on modeling cell composition proportions as a primary outcome or as a covariate in genome-wide association studies of gene expression. For example, the Campbell placental RNA reference panel applied to a previously published case control microarray study of preeclampsia revealed that the proportion of extravillous trophoblasts was overrepresented among preeclampsia cases compared to controls, a cell type whose dysfunction has previously been implicated in preeclampsia. Further, a differential gene expression analysis of the same dataset suggested that upregulation of preeclampsia relevant genes *FLT1*, *ENG*, and *LEP* among preeclampsia cases was partially mediated by differences in cell composition (Campbell et al., 2023). Another tool developed using single cell RNA-sequencing data is “PlacentalCellEnrich”, which allows the user to characterize if a list of genes is enriched for genes with placental cell specific expression patterns (Jain and Tuteja, 2021). These approaches may be applied to bulk placenta tissue studies to test whether and how EDCs affect placental cell composition and gene expression.

Adopting widespread use of single-cell RNA-sequencing technologies to assess cell-specific differential gene expression in the placenta induced by toxicant exposures offers important advantages over current methods using bulk tissue or specific cell types. Similar approaches have been successfully deployed in lead (Pb) toxicology of the hippocampus brain region in mice (Bakulski et al., 2020), and has recently been applied in a mouse air pollution exposure study of the placenta (Tosevska et al., 2022). Evaluating gene expression changes at single cell resolution from tissues containing a full breadth of placental cell types will allow researchers to assess cell type-specific gene expression changes. This will lead to in-depth analysis of cell-by-cell transcriptional responses to a toxicant. Researchers will be able to pinpoint responses and/or impairment of specific cell types that likely play a role in placental pathogenesis. Single-cell approaches can even identify novel cell types and states. Moreover, changes in relative cell type abundance resulting from toxicant exposure can be determined using single-cell RNA-sequencing.

Single-cell approaches can be integrated with a chemical risk assessment framework for translational impact. One such framework is the Adverse Outcome Pathway (AOP) approach, used by researchers in regulatory and academic institutions. AOPs organize biological effects of toxicant exposure into a logical sequence of events of increasing scale of biological complexity from molecular, to cellular, and finally to organism or even population level effects (Ankley et al., 2010; Spinu et al., 2020; Vinken, 2013). Each step in an AOP is linked by Key Events that ultimately link early molecular events (e.g. toxicant binding to hormone receptor) to adverse health outcomes (e.g. fetal growth restriction). Single cell RNA-sequencing allows researchers to identify molecular (transcriptomic) responses at single cell resolution within the complex microenvironment of the placenta. Single-cell transcriptomics methods present a powerful method for identifying Key Events in specific placental cells, allowing researchers to build new AOPs that demonstrate how EDC exposure leads to adverse pregnancy outcomes. Thus, these methods have the potential

to significantly improve our ability to assess the risks posed by exposure to EDCs during pregnancy.

An additional future opportunity of single cell approaches is to model interactions between cells. Cell-cell interactions can be modeled through the use of receptor-ligand networks, which is a mathematical model detailing the relationship between different cells within a tissue, which is reflected in the ligands and their expressed receptors through both single cell and bulk RNA-sequencing data (Armingol et al., 2021). A network is a mathematical collection of objects composed of “nodes” connected by “edges”. Here, “nodes” represent cytokine ligands or receptors from specific cell types, and “edges” quantitatively encode the relationships between nodes. Since different placental cells release protein ligands, ligands bind to cell surface receptors, and receptors pass information to cells, these edges can be considered ‘directed’. Computational methods have been developed to analyze directed networks, estimating global properties of the network as indicators of the ‘immune state’. Receptor-ligand interactions have been curated in various databases, including cellphoneDB (Efremova et al., 2020), iCellNet (Noël et al., 2021), and the Human Connectome DB (Hou et al., 2020). These databases can act as a network scaffold for individual cell type networks, which can be refined through expression thresholding, expression product, expression correlation, or differential combination approaches (Armingol et al., 2021). This network architecture provides a framework to overlay tissue-specific expression levels and correlations to determine the immune cell cross talk in the decidua and placenta.

One exciting potential application of single-cell RNA-sequencing in placental toxicology is to use it to understand how EDCs disrupt the unique signaling pathways that occur at the maternal-fetal interface during pregnancy. Cellular communication within and between maternal and fetal cells is essential to the routine functioning of the maternal-fetal interface, and EDCs may disrupt these molecular signals. Placental cells and maternal cells undergo a carefully orchestrated ligand-receptor mediated crosstalk program with the specific goal of regulating some physiological processes unique to pregnancy. An example of maternal-fetal tissue coordination is the selective modulation of the maternal immune system in response to the presence of the semi-allogeneic fetus. Although complex and not fully understood, tissue and cell-specific immunomodulation is regulated by interactions between uterine natural killer cells and regulatory T cells on the maternal side and extravillous trophoblasts and syncytiotrophoblast on the placental side (Napso et al., 2018). Additionally, hormones synthesized and released by the placenta regulate maternal T cells (Morelli et al., 2015). Using single-cell RNA-sequencing to interrogate how EDCs may disrupt maternal-fetal signaling will lead to new mechanistic insights by potentially identifying new cells involved and revealing cell-specific signaling through gene expression.

Spatial transcriptomics is an emerging single-cell technology that does not require tissue dissociation and thus preserves the tissue structure and cellular geographic organization. The technology was highlighted by Nature Biotechnology as the 2021 method of the year (Marx, 2021) and is now available and scalable through several commercially available platforms. Spatial transcriptomics works by integrating existing sequencing based approaches with microscopy based techniques such as fluorescence in situ hybridization (FISH) (Tian et al., 2022). Spatial transcriptomics can provide resolution into the cell-type composition

of tissues, the rules and patterns of how individual cell types spatially interact, and also elucidate molecular interactions between tissue components (L. Tian et al., 2022). Given the significance of the maternal-fetal interface in fetal development and placental function, spatial transcriptomics approaches could provide novel insight into the interactions of cells and tissues at this critical interface. Recently, two studies were published detailing spatial transcriptomics applied to chorionic villous placental tissue (Liu et al., 2022) and trophoblast development in early pregnancy (Arutyunyan et al., 2023). Eventually, spatial transcriptomics approaches should be used to provide insight into how EDCs may interfere with processes at the maternal fetal-interface, representing a critical research gap.

Looking ahead, additional recently developed genomics technologies have the potential to yield novel mechanistic insight into how EDCs may disrupt cellular functions in the placenta. Single cell CUT&TAG is a novel experimental approach that can profile single cell DNA accessibility, histone modifications, and transcription factor occupancy at a single cell resolution (Bartosovic et al., 2021), representing a major technological improvement over chromatin immunoprecipitation and sequencing (CHIP-Seq). This may be highly relevant in the field of EDCs, based on their known mechanisms of toxicity involving disruption of nuclear hormone receptor transcription factors which can result in changes to the synthesis and signaling of downstream genes (Hall and Greco, 2019). Single-cell Assay for Transposase-Accessible Chromatin (ATAC)-sequencing is another emerging technology that can be used to understand the regulatory elements that drive cell-type gene expression (Fang et al., 2021). Endocrine disrupting chemicals including phthalates have been shown to disrupt histone acetylation and chromatin accessibility (Kuhl et al., 2007; Zhang et al., 2014), but to our knowledge this has not been profiled on a genome scale or single-cell level. Researchers may also consider other applications of single-cell methodologies as they are developed, such as single cell proteomics, or epigenomics. We anticipate that these emerging technologies may further elucidate the mechanisms of toxicity for EDCs by pinpointing how they disrupt maternal-fetal communication and transcriptomic regulation of gene expression.

Conclusions

Exposures during pregnancy to EDCs can result in adverse pregnancy outcomes and postnatal complications for children. Molecular and cellular changes to the placenta are likely central to these disorders. Given the placenta is constituted of multiple cell types of unique form and function, greater biologic and mechanistic insights can be gained by incorporating measures of cell composition or cell-specific measures (Figure 2). Specifically for large epidemiologic studies using archived bulk placental tissue measures, we recommend that investigators use single cell placental reference panels to estimate cell composition in their samples. With this approach, investigators can test for differences in estimated placental cell composition by exposure or outcome, and they can incorporate measures of cell composition in their tests of differential gene expression. For smaller epidemiologic studies or in vitro toxicologic studies with fresh placental tissue available, we recommend investigators consider a single nuclei approach to assess cell type specific differential gene expression. This allows for the identification of cell type specific adverse outcome pathways. As new technologies emerge and throughput increases, innovative opportunities for placental EDC toxicology will continue to evolve. This manuscript has

focused on placental villous tissues, however the placenta coordinates with additional tissues (fetal membrane, decidua). Many of the principles presented are applicable in these tissues, and fully capturing the biology will require considering tissue-tissue interactions. In addition, the concepts of this manuscript are applicable to toxicants beyond EDCs. The field of placental toxicology is in an exciting position to be able to build on prior successes in bulk tissue and in cell culture and now able to extend and consider cell specific responses and interactions in the tissue.

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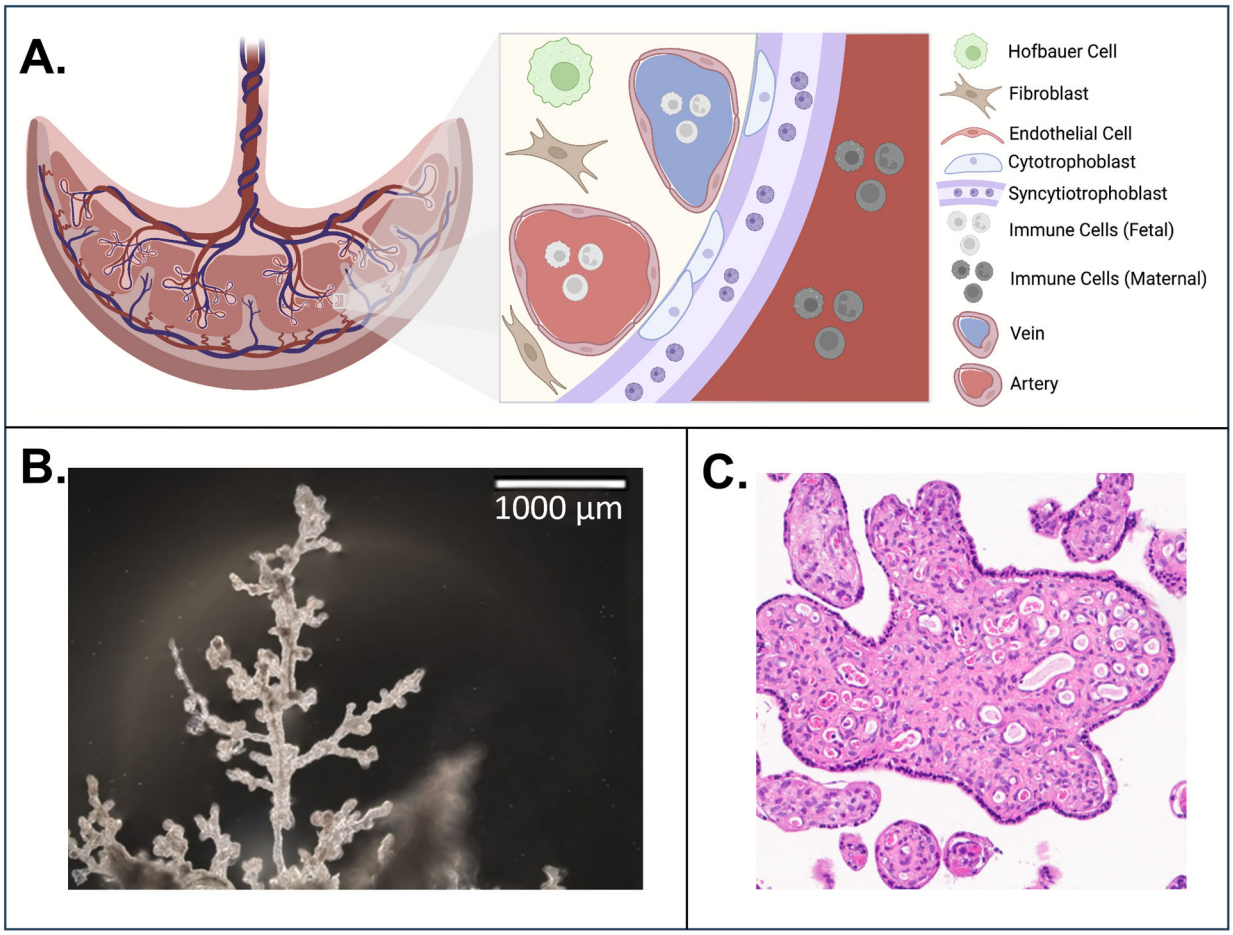

Figure 1. Human term placental tissue cellular heterogeneity.

A. Major cell types of the placenta include the resident macrophage Hofbauer cells (green), mesenchymal cells including fibroblasts (brown), and endothelial cells that line the fetal vasculature (pink). The outermost layer is the multinucleated semi-continuous syncytiotrophoblast (purple), which is formed by the syncytialization of cytotrophoblasts (light blue). The syncytiotrophoblast is the direct barrier between the placental villous tissue and the intervillous space perfused with maternal blood (dark red). Created with BioRender.com. **B.** Human term placenta villous tree excised from human placenta in Harris Lab. **C.** Cross-section of human term villous tree isolated by Harris Lab and visualized using hematoxylin and eosin stain.

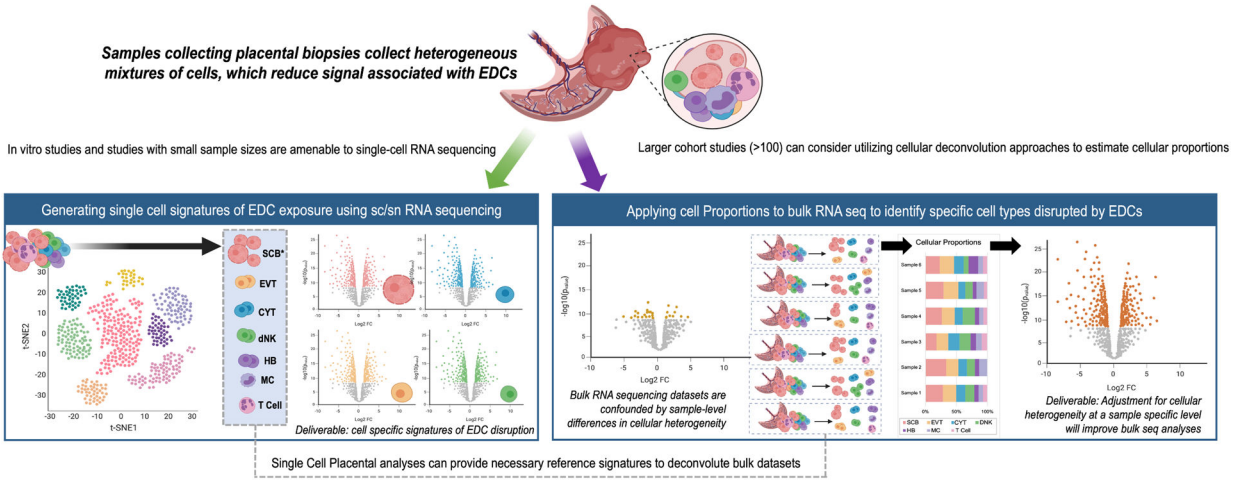

Figure 2. Recommendations for placental RNA-sequencing implementation based on sample size and research question.

Single-cell RNA-sequencing data is most amenable to in vitro studies and studies with small sample sizes, and can produce cell specific signatures of EDC disruption. Large cohort studies may find it more challenging to deploy single-cell sequencing, but can leverage single cell analyses to adjust for cellular heterogeneity and examine cell proportions within their data. *Syncytiotrophoblasts are multi-nucleated and may be challenging to capture using standardized single-cell approaches. EDC=Endocrine Disrupting Chemicals, SC=Single Cell, SN=Single Nuclear, SCB=Syncytiotrophoblast, EVT=Extra villous trophoblast, CYT=Cytotrophoblast, dNK=decidual natural killer cell, HB=hofbauer cell, MC=macrophage”

Table 1.

Summary of human population molecular epidemiology studies on environmental exposures and placental tissue RNA-sequencing at birth from 2017–2023.

Exposure	Timing of exposure	Exposure matrix	RNA-sequencing type	Cohort	Largest N*	Placental Sampling	Birth Outcomes Assessed	Main RNA-seq and Birth Outcome Findings	Reference	Data Availability
Selenium, Cadmium	2.8 months postpartum	Toenails	mRNA	RICHS	173	Fetal side near cord insertion	Fetal Growth	• Negative associations between Se & Cd with gene expression related to steroidogenesis and TNF genes. • Se associated with decreased odds of IUGR.	(Everson et al., 2017)	No
Cadmium	Birth	Placenta	mRNA	RICHS	200	Fetal side adjacent to cord insertion	Birth Weight	• 6 of 32 genes within 100kb of a Cd associated CpG with differential methylation exhibited expression level associations with CpG methylation levels. • 2 of these genes were associated with decreased birth weight and 1 with increased birth weight.	(Everson et al., 2018)	No
Arsenic	2nd trimester	Urine	mRNA	NHBCS	46	Fetal side at base of the umbilical cord	Birth Weight	• 606 genes associated with arsenic in males, but none in females. • There were 103 overlapping pathways that were associated with arsenic and birth weight in females.	(Winterbottom et al., 2019)	No

Exposure	Timing of exposure	Exposure matrix	RNA-sequencing type	Cohort	Largest N*	Placental Sampling	Birth Outcomes Assessed	Main RNA-seq and Birth Outcome Findings	Reference	Data Availability
Cadmium	Birth	Placenta	lncRNA	RICHS	199	Fetal Side <2cm from cord	Birth Weight	• 46 lncRNAs were associated with birth weight with 4 also being associated with placental Cd.	(Hussey et al., 2020)	dbGaP phs001586.v1.p1
PM2.5	12 weeks preconception through birth	Residential Address at Birth	mRNA	RICHS	471	Fetal Side <2cm from cord	Fetal Growth	• Birth weight associated with PM2.5 during the period of 12 weeks preconception through 13 weeks of pregnancy. • PM2.5 during this critical window was associated with 5 placental gene expression modules of which 2 were also associated with birth weight.	(Deyssenroth et al., 2021)	By request
Phthalates	2nd and 3rd Trimester	Maternal Urine	mRNA & lncRNA	CANDLE	760	Fetal Villous Tissue	-	• 2nd trimester urinary concentrations of 3 phthalate metabolites were associated with 18 genes. • 3rd trimester urinary concentrations of 2 phthalate metabolites were associated with 20 genes.	(Paquette et al., 2021)	No
Selenium	Birth	Placenta	miRNA	NHBCS, RICHS	393	Fetal Side	-	• 2 microRNAs were associated with placental selenium. • 30 predicted mRNA targets of these microRNAs were enriched in	(F.-Y. Tian et al., 2022)	dbGaP phs001586

Exposure	Timing of exposure	Exposure matrix	RNA-sequencing type	Cohort	Largest N*	Placental Sampling	Birth Outcomes Assessed	Main RNA-seq and Birth Outcome Findings	Reference	Data Availability
								Selenium related pathways.		
Cadmium	Birth	Placenta	miRNA	NHBCS, RICHS	396	2cm from cord insertion	NNNS Score	• Placental Cd associated with expression of 5 microRNAs in NHBCS. • Expression of some of these microRNAs were associated with NNNS score or one of its 13 characteristics.	(Tehrani et al., 2022)	doi.org/10.15139/S3/KHXJ2G
Polycyclic Aromatic Hydrocarbons	2nd Trimester	Maternal Urine	mRNA & lncRNA	CANDLE	629	Fetal Villous Tissue	-	• Urinary concentration of 6 OH-PAHs were associated with placental expression of 8 genes. • The positive association between TRIP13 expression and phenanthrene metabolites was validated in vitro.	(Paquette et al., 2023)	By request
Organophosphate Pesticides	1st, 2nd, and 3rd trimester	Maternal Urine	mRNA	SAWASDEE	254	Umbilical side, 2cm from cord insertion	-	• Urinary DEP was associated with expression of 2 out of 21 placental gene co-expression modules at early and/or late pregnancy time points.	(Li et al., 2023)	By request

*Sample size may have varied throughout the papers based on the specific analysis presented (exposure-gene expression relationship, or gene expression-outcome relationship). This table presents the single largest N used in one of these analyses.

Table 2.

Existing single cell RNA-sequencing studies in the human placenta

Reference	Population Information	Gestational Time Point	Number of Samples/Cells	Single cell approach and Sequencing Platform	Main Findings	Data Availability
(Pavličev et al., 2017)	Non-pathological pregnancies delivered via c-section in the absence of labor	Samples collected at the end of term gestation 39.14–39.28 weeks	2 Placentas/87 Cells	Used smart-seq2 approach followed by Illumina HiSeq2500	• Identified five clusters of cells from cytotrophoblasts, decidual cells, and extravillous trophoblasts complimented by separate RNA-sequencing data generated of the syncytiotrophoblast • Generated a ligand-receptor network that revealed cell-specific expression of G-PCRs, and that uterine decidual cells are a major cell-cell interaction hub. • Demonstrated that decidualization enhances fetal communication, and most ligands/ receptors upregulated during this process have corresponding ligands/receptors in the fetal placental tissue.	GSE87726
(Tsang et al., 2017)	Placentas were collected from 2 in patients not in active labor that were delivered via C-section at the Prince of Wales Hospital in Hong Kong Primary Outcome: Early onset preeclampsia (PE)	Samples collected at the end of preterm/term gestation Gestational Age (PE) 28.14 weeks-32.56 weeks Gestational Age (controls) 38–38.28 weeks	8 Placentas (4 normal/4 PE), 2 normal placentas were additionally sampled peripherally/ 20,518 cells	10x genomics library preparation followed by sequencing via Illumina miseq and nextseq	• Identified 12 clusters of placental cells, which were predominantly stromal, vascular and trophoblastic cells. • Demonstrated the differentiation of CVTs into extravillous trophoblasts and syncytiotrophoblasts using pseudotime analysis • Identified cell-free signatures of individual cellular components in maternal plasma RNA profiles • · Found changes in the extravillous trophoblast signature in patients with early preeclampsia compared to term controls, including genes involved in cell migration, cell death, and proliferation	N/A
(Liu et al., 2018)	Placentas gathered in early pregnancy from participants in 306th Hospital of PLA	First and Second Trimester 8 weeks and 24 weeks	8 Placentas /1471 cells	used smart-seq2 approach followed by Illumina Hiseq 4000	• Identified 14 discrete clusters of placental cells, including 3 new subtypes of cytotrophoblasts present in first trimester placentas • Identified 3 subtypes of extravillous trophoblasts present at 8 weeks of gestation and 2 extravillous trophoblast subtypes present at 24 weeks	GSE89497

Reference	Population Information	Gestational Time Point	Number of Samples/Cells	Single cell approach and Sequencing Platform	Main Findings	Data Availability
					• Identified 2 novel subtypes of villous mesenchymal stromal cells and macrophages	
(Suryawanshi et al., 2018)	Placentas gathered from elective termination samples. Only male samples used.	First Trimester 6–8 weeks	8 Placentas/ 14,341 cells Also collected 6 decidual samples/ 1542 cells	DropSeq or 10X processing followed by sequencing using Illumina NextSeq 500 platform	• Villous trophoblasts were the abundant trophoblast cell type, followed by syncytiotrophoblasts and extravillous trophoblasts. • Identified unique TFs specific to individual cell types, including members of the Krüppel-like family of TFs • Generated a ligand-receptor network using matched placental-decidual data	N/A
(Vento-Tormo et al., 2018)	Placental and decidual samples were collected from the Human Developmental Biology Resource from the UK tissue authority	First Trimester 6–14 weeks	5 placentas/ 70,000 cells	Single-cell libraries prepared using 10x genomics chromium system as well as plate-based smart-seq2 followed by sequencing using Illumina HiSeq 2000	• Identified regulators involved in extravillous trophoblast differentiation • Identified interacting receptors at decidual-placental interface using cellphoneDB tool • Identified 3 specific subclasses of decidual NK cells	E-MTAB-6701 ; E-MTAB-6678 ; E-MTAB-7304
(Sun et al., 2019)	Placental and decidual samples were collected from chorionic villi sampling of patients recruited into the Cedars-Sinai prenatal biorepository. All patients went on to deliver healthy term live births. Primary Outcome: Fetal Sex	First trimester samples (10–13 weeks)	10 Placentas (5 female/5 male) / 7,245 cells	Single cell libraries prepared using 10x genomics kit followed by NovaSeq 6000	• Identified 5 major placental cell types with unique cross-talk at the maternal fetal interface • TGFB1 and β-estradiol were significant upstream regulators of all cell types • Identified 17 genes upregulated and 27 genes downregulated in male placentas compared to females	N/A
(Pique-Regi et al., 2019)	Placental and decidual samples were collected from women from Detroit Medical Center Primary outcome: Preterm and term delivery	Samples collected at end of preterm/ term gestation 6 term samples (3 in labor, 3 not in labor-Age 38–40 weeks) 3 PTL (33–35 weeks) samples	9 samples/79,906 cells	Single-cell libraries prepared using 10x genomics chromium system followed by sequencing using Illumina HiSeq X Ten System	• Identified 2 new placental cell types including lymphatic endothelial decidual cells and non-proliferative interstitial cytotrophoblasts • Largest number of genes associated with labor status was in maternal macrophages • Largest number of DEGs related to prematurity was in extravillous trophoblasts and cytotrophoblasts • Demonstrated that single-cell signatures were modulated by advanced maternal age	phs001886

Reference	Population Information	Gestational Time Point	Number of Samples/Cells	Single cell approach and Sequencing Platform	Main Findings	Data Availability
(Yang et al., 2021)	Samples were collected from singleton full term placentas after cesarean section without any other pregnancy complications Outcome of interest: gestational diabetes mellitus	Samples collected at the end of term gestation (38.14–40.56 weeks)	4 Placentas (2 gestational diabetes mellitus cases/2 control)/ 27,220 cells	Single-cell libraries prepared using 10x genomics chromium system followed by sequencing using illumina novaseq 6000	• Identified 15 unique cellular clusters, including primarily villous cytotrophoblasts • No difference in the number of cell types/ clusters between gestational diabetes mellitus samples and controls • Identified 235 genes that were differentially expressed between gestational diabetes mellitus and control samples, including genes involved in estrogen signaling and antigen processing and presentation, and IL-17 signaling • Identified ligand-receptor interactions between trophoblast and immune subtypes	GSE173193
(Zhang et al., 2021)	Samples collected from singleton pregnancies delivered via cesarean section without other pregnancy complications at Weifang Traditional Chinese Hospital in Shandong, China Outcome of interest: PE	Samples collected at the end of preterm/term gestation (34.7–38.7 weeks)	6 Placentas (3 PE/3 controls)/ 11,518 cells	Single cell libraries prepared using Singleron GEXSCOPE system followed by sequencing via Illumina Hiseq X	• Identified 13 major clusters of cells • Identified 610 genes that were differentially expressed in syncytiotrophoblasts between preeclampsia and control samples, including genes involved in protein processing and preeclampsia • Captured differentiation of villous cytotrophoblasts into extravillous trophoblasts and syncytiotrophoblasts in control and preeclampsia placentas	Data available upon request
(Khan et al., 2021)	Human ESCs (H1; WA01) originated from WiCell Research Institute. Cells exposed to bone morphogenetic protein 4 (BMP4) in presence of inhibitors of ACTIVIN/ TGFB; A83–01 and FGF2; PD173074 (BAP) Primary Outcome; Different cells with trophoblast lineage. In low (5%) and normal (20%) oxygen conditions	Cell from early pregnancy	2 replicates for each treatment group/5000 nuclei	Single cell libraries prepared using 10x Genomics Chromium Next Gel Bead-in-Emulsion (GEMs) Single Cell 3′ Kit v3.1 followed by sequencing via Illumina NovaSeq S1 flow cell	• Identified two major groupings, one comprised of 5 and the second of 3 clusters. • The number of differentially expressed genes between 5% oxygen and 20% oxygen for clusters 1–8 ranged from 37 to 188 genes. • The BAP model for deriving trophoblast from embryonic stem cells, reveals a relatively complex picture of TB emergence, including the appearance of at least two kinds of syncytioTB nuclei plus multiple cytoTB populations.	GSE171768
(Zhou et al., 2022)	Placentas were collected from patients delivering via cesarean section at Changzhou Maternal and Child Health Care	Samples collected at the end of preterm/term gestation (gestational age	4 Placentas (2 preeclampsia cases/2 controls)/ 29,006 cells	Single-cell libraries prepared using 10x genomics system	• Identified changes in biological processes involving hormone secretion and immunity in the preeclampsia group	GSE173193

Reference	Population Information	Gestational Time Point	Number of Samples/Cells	Single cell approach and Sequencing Platform	Main Findings	Data Availability
	Hospital Primary outcome: PE	36.14–38.43 weeks)			• Identified 3 TFs that regulated genes whose expression in extravillous trophoblasts was associated with preeclampsia	
(Chen et al., 2022)	Samples were collected from a patient who experienced incomplete uterine rupture and fetal demise at 28.56 weeks and 2 preterm controls. Exposure: Severe COVID-19 infection in mid-pregnancy (20–24 weeks)	Samples collected at the end of preterm gestation (28.56–30 weeks)	3 Placentas (1 COVID infected vs. 2 Preterm)/ 17,481 cells	Single-cell libraries prepared using 10x genomics chromium system followed by sequencing using illumina HiSeq X	• Identified 20 subsets of cell types, including predominantly trophoblasts. • Identified changes in trophoblastic genes in COVID-19 patient, including cell invasion related genes, and increased expression of genes involved host-defenses at the maternal-fetal interface	Data available upon request
(Ray et al., 2022)	Samples were collected from Mount Sinai hospital in Toronto, CA	First Trimester Samples (6–8 weeks)	2 Placentas/cell number not reported	Single-cell libraries prepared using 10x genomics chromium system	• Identified 22 cell clusters • Examined Hippo signaling cofactors in single cell clusters, and found that VGLL1 was highly expressed across all single trophoblast cells. • Other Hippo genes were only expressed in differentiating cytotrophoblasts	GSE145036
(Tosevska et al., 2022)	Samples were collected from C57BL/6J mice following intranasal exposure to PM2.5	Placentas collected Late stage pregnancy (E19) following laparotomy and hysterectomy	N=6 samples (3 exposed, 3 controls)/40,739 cells	Single-cell libraries prepared using 10x genomics chromium system	• 25 distinct clusters, with variation amongst the three main compartments: decidual, junctional and labyrinthine • Air pollution exposure associated with increase in certain proliferating trophoblasts including NK cells, spongiotrophoblasts and decidual cells • scRNA-seq could successfully be used to deconvolute bulk data	GSE178233)
(Li et al., 2022)	Samples collected from elective terminations at Shanghai First Maternity and Infant Hospital	First Trimester Samples (6–16 weeks)	N=11 Placentas/ 52,179Cells	Single-cell libraries prepared using 10x genomics chromium system followed by Illumina NovaSeq 6000	• Identified 8 major cell types, including trophoblasts, macrophages, Hofbauer cells, erythroid cells, fibroblasts, and endothelial cells (3 subclusters) • one subset of Endothelial cells were primarily in first trimester placentas and decreased after 11 weeks of gestation • Identified a unique transcription factor network related to endothelial cell clusters • described mechanisms of differentiation of VCTs to EVTs	Data available upon request

Reference	Population Information	Gestational Time Point	Number of Samples/Cells	Single cell approach and Sequencing Platform	Main Findings	Data Availability
(Wang et al., 2022)	Samples collected at the end of gestation from Shenzhen Second People's Hospital	Samples collected at the end of gestation (28–40 weeks)	N=3 placentas/ 13747 cells	Single-cell libraries prepared using 10x genomics chromium system followed by sequencing on MGI-seq platform	• Examined subtypes localized to different regions of the placenta (fetal side, middle, maternal side) • Proposed that the PRDM6 may be involved in promoting enEVT differentiation through cell cycle arrest signals • demonstrated that stromal cells from the maternal side had higher proliferative ability	Data Availability CNSA: ID # CNP0000878
(Campbell et al., 2023)	Samples collected from singleton pregnancies delivered via cesarean section without complications at Von Voigtlander Women's Hospital in Ann Arbor, MI	Samples collected at the end of term gestation	N=2 placentas (1 male, 1 female), each with a technical replicate/9,244 cells Analyzed in conjunction with N=3 term, non-laboring placentas from Pique-Regi et al, 2021 and N=4 control placentas from Tsang et al, 2017/31,250 cells	Single-cell libraries prepared using 10x genomics chromium system followed by Illumina NovaSeq 6000	• An integrated analysis identified 19 fetal and 8 maternal cell types • Used to develop a deconvolution reference for bulk tissue data, and verified with in silico testing • Deconvolution analysis of microarray case-control study of preeclampsia identified preeclampsia composition changes and that cell composition mediated preeclampsia gene expression differences	GSE182381