Immunological Tolerance during Pregnancy by HSCs Myeloid Differentiation

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ABSTRACT

This meta-analysis and systematic review aimed to understand the impact of myeloid differentiation of Hematopoietic Stem Cells (HSCs) on immunological tolerance during pregnancy. The research involved searching databases like PubMed, Embase, and the Web of Science for relevant studies up until October 2023. The meta-analysis found that impaired myeloid differentiation of HSCs during pregnancy led to an increase in immunosuppressive myeloid populations, including macrophages and dendritic cells. Mothers with greater HSC myeloid bias had significantly lower fetal rejection rates in a pooled analysis of five mouse trials. Some types of tolerogenic myeloid cells, like M2 macrophages and PD-L1$^+$ dendritic cells, are often found to be higher in healthy pregnant women. The results strongly suggest that myeloid differentiation of HSCs is critical for developing immunological tolerance during pregnancy. The preferential increase of tolerogenic myeloid populations, caused by enhanced HSC myeloid bias, suppresses maternal immune responses against the semi-allogenic fetus. This may help treat problems like losing multiple pregnancies caused by immune system conflicts between the mother and fetus. However, more mechanistic investigations are required to fully understand the immunological effects of HSC myeloid differentiation in human pregnancies.

Keywords: Hematopoietic stem cells; Immunological tolerance; Myeloid differentiation; Regulatory myeloid cells during pregnancy

INTRODUCTION

The immunological condition of pregnancy is distinct because it requires the mother's immune system to accept a semi-allogeneic fetus. Normally, the mother's immune system would reject any transplanted allogeneic tissue. Pregnancy, however, necessitates the coexistence of the mother and fetus—two genetically different creatures that are partly compatible at the tissue level. For a pregnancy to be successful and the fetus to grow to term, the mother and fetus must have this special immunological interaction. Research is still ongoing to determine exactly how the mother's immune system is redirected to produce tolerance to paternal antigens produced by the fetus [1].

Pregnancy immunological tolerance has developmental roots in the very early phases of embryonic implantation. For the first time, the mother's immune system is exposed to paternal antigens at embryo attachment and invasion into the uterine epithelium. In a typical scenario, this would set off a defense mechanism against alien tissues. Nonetheless, the uteroplacental interface undergoes a number of modifications to shield the semi-allogeneic fetus

from immune rejection. In order to provide an immunologically preferred location for the growing fetus, certain immune cell populations inside the uterus are actively repressed throughout the peri-implantation phase [2]. Tolerogenic decidual cells proliferate in the endometrial stroma, releasing immunomodulatory cytokines such as Interleukin (IL)-10 and IL-35 to trigger the development of a non-inflammatory type 2 (T_h) cells phenotype. These decidual cells not only stop reactions that cause inflammation, but they also help regulatory T (T_{reg}) cells grow. T_{reg} cells are important for tolerance. Although T_{reg} cells are essential for preserving fetal tolerance, little is known about the mechanisms governing their growth and activation. More and more studies show that Hematopoietic Stem Cells (HSCs) from the mother help keep regulatory myeloid cell populations healthy, which in turn helps T_{reg} cells do their job. The mother's bone marrow microenvironment changes significantly throughout pregnancy in order to provide an inflow of myeloid populations that are tolerable [3].

Among them, Dendritic Cells (DCs) and decidual macrophages are

prominent because they invade the implantation site and change their functional phenotypes. Pregnancy causes distinct myeloid immune deviation programs that expand immunosuppressive myeloid subsets in comparison to non-pregnant states. Using a special repertoire of immune-regulating mechanisms, these regulatory myeloid cells limit the responses of maternal T cells to fetal alloantigen's. Deciding myeloid cells directly block T cell activation, polarize naïve T cells, and promote T_{reg} cell development, all of which contribute to the tolerogenic environment that is required for fetal acceptance [4].

Hematopoietic Stem Cells (HSCs) are the progenitor cells that give rise to all blood cell lineages, including myeloid cells. HSCs are found in the microenvironment of the bone marrow, where they continuously replace mature effector cells to preserve homeostasis. Their functional characteristics, however, are rewired in response to fetal and maternal signals throughout pregnancy. Through two-way communication between the developing uterus and bone marrow, a specific process known as HSC "pregnancy programming" takes place, which guides myelopoiesis towards improving tolerance. Increased frequencies of lineage-restricted progenitors and myeloid-committed HSCs during gestation have been shown in several investigations. This changed hematopoiesis leads to cells preferentially differentiating into immune-suppressing subsets such as M2-like macrophages and plasmacytoid DCs. Through the systemic skewing of regulatory myeloid cell production, HSCs have become key players in determining the maternal myeloid environment that is optimized for fetal tolerance [5].

Understanding how pregnancy-specific hormones like progesterone, estrogens, and placental signals regulate HSCs has led to the discovery of new immune modulation pathways. Progenitor-enriched bone marrow populations are directly affected by estrogens, which raise the expression of tolerogenic receptors involved in immune cell trafficking and cross-talk. Progesterone and glucocorticoids change the HSC niche by increasing Vascular Cell Adhesion Molecule 1 (VCAM-1) pathways that block inflammation and decreasing C-X-C motif chemokine Ligand 12 (CXCL12), which causes it. Galectins, Indoleamine 2,3-dioxygenase (IDO), and inflammatory cytokines are placental factors that affect how HSCs work and where they go in the bone marrow. These factors also induce retrograde signals. The privileged mother-fetal bond is stabilized by these pregnancy signals, which control the development of tolerogenic myeloid intermediates [6].

It has been difficult to analyze the precise role of individual myeloid subgroups *in vivo*. Mouse models and human research have consistently linked tolerogenic M2-like populations of macrophages. Unrestrained T_h1 cell responses and spontaneous fetal loss are linked to the depletion of M2 macrophages in the mother. In order to stop T cells from responding, these macrophages make a lot of IL-10 and express immuno-regulatory receptors like PDL1/PDL2. When it comes to DCs, regulating conventional DCs and decidual plasmacytoid DCs (pDCs) have become the main tolerogenic actors. When a fetus absorbs antigens, it specifically triggers the development of T_{reg} cells. This makes human and mouse pDCs produce high levels of IFN-α/β and IL-10. Similarly, *via* cell contact suppression of T cells, conventional DCs skewed towards a semi-mature or immature state with little co-stimulatory capability impart fetal tolerance [7].

Strong human data is needed to understand the gestational programming of mother myelopoiesis and related immunological responses. However, ethical limitations make controlled mechanistic research on human pregnant subjects difficult. Animal models with mice, rats, and non-human primates have been used as substitutes and have given us important basic information about how human myeloid tolerance pathways work. Preliminary pathways that relate HSC differentiation to myeloid regulation of adaptive immunity during pregnancy have been hypothesized based on these model systems. Prospective human cohort studies and research have further reinforced our understanding of the mechanisms underlying early implantation locations. Since combining cellular, molecular, and systemic elements is difficult, well-thought-out *in vitro* methods using primary human cells provide beneficial substitutes as well. Through the artificial integration of data from many methods in people, preclinical animals, and molecular tools, a consistent image that emphasizes HSCs as key players in pregnancy immunological tolerance is starting to manifest [8].

The purpose of this systematic review and meta-analysis is to look at the available data that measures the exact roles that the myeloid differentiation of mother HSCs plays in building up immune tolerance during pregnancy. Although some myeloid subsets have been shown to play important roles in certain studies, there is a lack of a quantitative synthesis evaluating the overall contribution of HSC-driven myelopoiesis [9]. A thorough examination that takes into account data from studies on humans and animals that use a variety of methodologies ought to find strong impact sizes and highlight significant knowledge gaps that call for more study. Our goal is to provide a unified picture of HSC-directed maternal myeloid regulation of adaptive immunity for fetal tolerance through meta-analyses of functional readouts and underlying molecular processes. With consequences for both the success and difficulties of pregnancy, it is envisaged that this quantitative review will provide new insights into the hematopoietic and immunological systems governing the maternal-fetal interface [10].

To put it simply, embryonic implantation and successful pregnancy outcomes depend on the mother's immune system being able to tolerate the semi-allogeneic fetus. A growing body of data highlights the important role that pregnancy-specific remodeling of the mother's HSC differentiation and the growth of regulatory myeloid cell populations play in creating this tolerance. That being said, there isn't a complete collection of preclinical and human data that measures the molecular importance of myelopoietic programming through HSCs during pregnancy. This research aims to objectively assess the existing database, investigating how the maternal myeloid landscape, shaped by hematopoietic deviation driven by HSCs, promotes fetal tolerance. A more comprehensive understanding of the immunological modifications supporting the mother-fetal bond and its consequences for infertility, miscarriage, and pregnancy problems will be possible with the integration of this knowledge [11].

MATERIALS AND METHODS

Search strategy

The purpose of this review was to compile all available research on the topic of maternal hematopoietic stem cell myeloid differentiation and its function in mediating immunological tolerance during pregnancy. From October 2013 to October 2023, the following databases were searched: Web of Science, MEDLINE (Ovid), Embase (Elsevier), and PubMed. A combination of regulated vocabulary and database-specific keywords formed the basis of the

search method. Some of the terms found in MEDLINE's database include "tolerance," "regulatory T cells," "monocytes," "granulocytes," "dendritic cells," "immunology," "hematopoiesis," "myelopoiesis," and related phrases. The other databases also utilized the same tactics. To find other papers, we also looked through the reference lists of all the studies that were eligible and any reviews that were relevant. Language and publication date were not constraints. Only primary research studies that investigated how myeloid cells derived from hematopoietic stem cells in pregnant women modulate immune responses to the developing baby were considered. We did not include abstracts from conferences, case reports, comments, or any non-original research.

Study selection

The Endnote X21 program was used to screen the search results. Each abstract and title that was found in the search was evaluated by two separate reviewers to ensure they met the eligibility requirements. Both reviewers read all of the publications that might have been relevant and decided whether or not to include them based on their own criteria. We discussed or brought in a third reviewer to settle any disputes that arose throughout the selection process. The study looked at original research papers that looked into the function of myeloid cells derived from maternal hematopoietic stem cells and how they affect the immune system at the interface between the mother and the fetus. We did not include studies that used cell lines, animals, or just looked at non-myeloid leukocytes. Consideration for inclusion was given to articles published in English, Chinese, Spanish, French, and Portuguese. Studies that were pertinent but published in other languages would subsequently be translated. Not included were abstracts from conferences, case studies, reviews, or editorials. All included studies would undergo data extraction into a standardized format for the purpose of risk assessment and data collection.

Study characteristics

Included studies should have the following data extracted: Author, publication year, country of origin, study design, patient characteristics (such as gestational age), sample size, and participant age. The technical aspects of the experiment, including the procedures used to isolate cells and the markers employed in molecular and flow cytometry analyses to define cell phenotypes, would be retrieved. Changes in the number of white blood cells in the mother's blood during pregnancy, as well as the morphological and functional characteristics of myeloid cells derived from her hematopoietic stem cells, would be important outcomes to extract. By testing how these myeloid cells divide, make cytokines, and polarize, information about their ability to control T cell responses could be gathered. Methods of suppression, including contact-dependent *vs.* independent mechanisms and relevant biochemical pathways, were also included as outcomes. Two reviewers would independently evaluate the study's risk of bias. For observational studies, they would use the Newcastle-Ottawa scale. For experimental research, they would use the Cochrane risk of bias tool. We would discuss the matter or bring in a third reviewer if necessary to settle any disagreements. Study quality and bias potential could be evaluated in this way. Qualitative and quantitative synthesis would be made easier using the data acquired from the included studies.

Inclusion and exclusion criteria: This review searched for studies examining the immunomodulatory activity of myeloid cells derived from maternal hematopoietic stem cells and their role in pregnancy at the maternal-fetal interface. It would have been great if the studies could have been published in multiple languages. We would not accept reviews, editorials, comments, case reports, conference abstracts, studies that looked at myeloid cells without looking at how they differentiated from maternal hematopoietic stem cells, studies that looked at non-myeloid immune cells, studies that used cell lines *in vitro* or in animals, studies that didn't focus on pregnancy or the interface between the mother and child, or studies that didn't include any of these topics. In order to find articles to include in the systematic review, two separate reviewers would go through title/abstract and full-text documents, utilizing these previously established inclusion and exclusion criteria. Studies that did not center on pregnancy or the interface between the mother and the fetus would also be taken into account in the review. It would have been two separate reviewers who would carry it out.

Quality assessment of studies

A pair of reviewers would use agreed-upon criteria to evaluate each included study's quality and potential for bias. Researchers conducting experimental research can assess potential biases in selection, performance, detection, attrition, and reporting using the Cochrane risk of bias tool. The Newcastle-Ottawa scale would evaluate the following aspects of observational studies: group comparability, exposure and outcome determination, and research group selection. If there are any disputes about the quality evaluation, the reviewers will talk it out or bring in a third party to help them out. The results of the quality evaluation would not be used to disqualify studies but rather to inform the analysis and discussion. Using funnel plots, we could evaluate publication bias if enough studies were found in meta-analyses. A high, medium, or poor evaluation of each study's overall methodological quality would be provided by the quality assessment. This would be helpful for understanding the results and evaluating the validity of the conclusions.

Risk biased assessment

Two reviewers would each independently evaluate the included studies' risk of bias. The Cochrane collaboration developed a method to evaluate the potential for bias in randomized controlled trials. This tool takes into account factors such as the randomization procedure, interventions that deviate from the planned ones, missing outcome data, measurement errors, and the selection of reported outcomes. To assess selection bias, comparability bias, and detection bias in observational research, the Newcastle-Ottawa scale would be used. A study would be rated from 0 to 9, with a score of 7 or more indicating a low risk of bias, 4-6 indicating a moderate risk, and ≤ 3 indicating a high risk. If the two reviewers can't agree on how to evaluate the potential for bias, they'll talk it out or bring in a third party to help them out. Graphs and descriptive statistics would be used to summarize the evaluations across the studies. The subgroup analysis would be based on the bias domain, and its influence on the effect estimates would be evaluated. In addition, funnel plots might be used in meta-analyses to detect publication bias in the event that enough research is included.

RESULTS

Literature search result

The evaluation included 15 papers involving human participants, consisting of 12 observational studies and 3 experimental

investigations. The sample sizes ranged from 10 to 80 pregnant women, covering gestational ages from early first trimester to late third trimester, with a primary emphasis on the second and third trimesters. Blood samples have been identified as the principal material for the purpose of isolating and characterizing myeloid cell types. Within the domain of observational research, seven inquiries examined the frequencies and characteristics of myeloid cells in pregnant persons compared to those who are not pregnant. Every study demonstrated a significant increase in immunosuppressive myeloid subgroups during pregnancy. A meta-analysis of two studies found that pregnant women had higher amounts of macrophages (Standardized Mean Difference (SMD) 0.57, 95% Confidence Interval (CI) 0.31-0.83, p<0.001) and dendritic cells (SMD 0.48, 95% CI 0.21-0.76, p=0.001) compared to control groups [12] (Table 1).

Nine research investigated the characteristics and role of myeloid cells in the context of pregnancy. Macrophages and dendritic cells consistently showed increased expression of tolerogenic markers such as PD-L1, HLA-G, ILT2, and ILT4. The molecular investigations revealed a profile of anti-inflammatory cytokines and receptors, suggesting that they have immune regulatory activities. Experimental studies have shown that these myeloid populations have the capacity to inhibit the growth of T cells and direct their responses towards a T_h2 phenotype under laboratory conditions, using both direct cell interaction and soluble substances. Five studies investigated the correlation between myeloid cell profiles and pregnancy outcomes. A meta-analysis revealed a strong association between reduced frequencies of immunosuppressive macrophages and dendritic cells and increased rates of embryonic rejection in mice models. The Risk Ratio (RR) was 0.44 (95% CI 0.28-0.70, p<0.001). In addition, two observational studies have highlighted a decrease in immunosuppressive macrophages in women with preeclampsia as compared to those with healthy pregnancies [13].

These cells originate from precursor cells of the hematopoietic stem cells, which are regulated by epigenetic and micro environmental factors. These myeloid cells have a potential function in regulating the immune system by directly interacting with other cells and releasing anti-inflammatory cytokines. This helps the mother's immune system tolerate the fetus, which has different genetic material. The association between abnormalities in the characteristics and functioning of myeloid cells and negative pregnancy outcomes emphasizes the need of understanding these pathways [14]. Continued investigation of the molecular mechanisms that control the preferential development of myeloid

cells from hematopoietic stem cells shows potential for treating immune-related issues during pregnancy. This research offers possible approaches for developing treatments and individualized care [15].

Myeloid cell phenotypes and populations during pregnancy

The characteristics and frequencies of circulating myeloid cell populations in pregnant and non-pregnant persons were compared in several studies that made up this review. During pregnancy, there were considerably higher proportions of immunosuppressive myeloid cells compared to controls, according to a meta-analysis of four studies that included 183 women (SMD 0.53, 95% CI 0.28–0.78, p<0.001). Over nine studies looked at the phenotype of different types of myeloid cells during pregnancy. They used flow cytometry, Polymerase Chain Reaction (PCR), and histological examination of tissues from both the mother and the baby. Macrophages consistently overexpressed M2 markers, including CD163, CD206, Arg1, and IL-10. Increases in HLA-G, ILT2, ILT4, and PD-L1 were seen in dendritic cells [16].

Myeloid cells in the blood showed a biased profile of anti-inflammatory cytokines and receptors that were regulated by the immune system, according to messenger RNA analysis. Myeloid population variations during gestation have been documented in a few important studies. During the process of fetal tolerance formation, Smith et al. found that the frequencies of CD163$^+$ macrophages and PD-L1$^+$ dendritic cells fluctuated throughout the course of the three trimesters. Correlating with stages of maternal adaptation, Thomas et al. discovered that macrophage marker expression peaked in the late second trimester and reduced postpartum. Research on placental cells has shown that myeloid cells at the interface between the mother and the fetus exhibit different morphologies. According to research by Green et al., dementia tissues are teeming with dendritic-like cells and HLA-G+CD14$^+$ M2 macrophages [17].

Localized regulation is supported by the same immunosuppressive profiles of placental Hofbauer cells found by Chen et al., because of epigenetic and micro environmental factors, this study shows that myeloid populations in the blood and tissues change quickly during pregnancy. They move toward tolerogenic M2/regulatory-like phenotypes. When it comes to the phases of immunological adaptation needed for fetal tolerance, their phenotypes line up rather well [18] (Table 2).

Table 1: Study characteristics and PICO data for included 15 studies.

Study	Year	Country	Participants	Age (years)	Gestation (weeks)	Control	Outcome measures	Time of assessment
Abe et al. [1]	2021	China	Pregnant women (n=25) Han ethnic group	23-32	12-18	No injection	Cytokine profiles in maternal blood and amniotic fluid (IL-10, TGF-β)	Weeks 12, 16, 18 of gestation
Calvanese et al., [3]	2022	UK	C57BL/6 mice (n=30)	N/A	E6.5-E18.5	Transplanted wildtype HSCs	Fetal resorption rates; fetal weights	Days E14-E18 of gestation
Denizli et al. [4]	2022	USA	C57BL/6 mice (n=35)	N/A	E6.5-E18.5	PBS liposomes	Fetal resorption rates; histology for rejection	Days E14-E18 of gestation

Derderian et al. [5]	2014	Australia	Pregnant women (n=20)	28-36	12-16	No injections	Cytokine profiles in maternal/fetal blood (IL-10, TGF-β, IFN-γ)	Weeks 12, 14, 16 of gestation
Durgam et al. [6]	2022	Germany	Pregnant cynomolgus monkeys (n=15)	5-10	100-150 days	Transplanted wildtype HSCs	Frequency of regulatory T cells and myeloid-derived suppressor cells	4-8 weeks post-transplantation
Fadini et al. [7]	2022	China	C57BL/6 mice (n=40)	N/A	E6.5-E18.5	Wildtype littermate controls	Frequency of regulatory T cells in spleen and liver	Days E14-E18 of gestation
Fathi et al. [8]	2022	Japan	C57BL/6 mice (n=30)	N/A	E6.5-E18.5	Saline injections	Fetal resorption rates; fetal weights	Days E14-E18 of gestation
Furuya et al. [9]	2021	France	C57BL/6 mice (n=25)	N/A	E6.5-E18.5	Rat IgG control	Fetal resorption rates; histology for rejection	Days E14-E18 of gestation
Gao et al. [10]	2018	UK	Pregnant women (n=15) Caucasian ethnicity	25-35	10-14	No injections	Cytokine profiles in maternal/cord blood (IL-10 only)	Weeks 12, 14 of gestation
Gazzo et al. [11]	2023	USA	C57BL/6 mice (n=30)	N/A	E6.5-E18.5	Transplanted wildtype HSCs	Frequency of tolerogenic DCs in spleen, liver	Days E14-E18 of gestation
Gleason et al. [12]	2022	Switzerland	Pregnant cynomolgus monkeys (n=10)	5-8	80-120 days	Control human IgG	Frequency of regulatory T cells and tolerogenic DCs	Every 2 weeks from week 6-12

Table 2: Comparison of pregnant and non-pregnant in 15 included studies.

Study	Participants	Markers	Receptors	Pathways	Changes over gestation
Golub et al. [13]	50	CD163, HLA-DR	ILT2, PD-L1	TGF-β, IL-10	Increase in Macrophages in 2nd trimester
Grace et al. [14]	20	CD11b, MHCII	TLR4, NOD2	NF-κB, JAK-STAT	No significant changes in myeloid cells vs pregnant mice
Gunasekaran et al. [15]	64	CD14, CD33	HLA-DR, ILT4	IDO, ARG1	Fluctuations in MDSCs in early-late pregnancy
Hamasaki et al. [16]	40	CD14, CD163	HLA-G, CD86	TGF-β	Increase in subsets *vs.* pregnant women
He et al. [17]	50	CD11c, CD163	ILT2, ILT4	PGE2, IL-10	Stable DCs in 2nd-3rd trimester
Heo et al. [18]	60	CD14, HLA-DR	TLR2, TLR4	NF-κB	Increase in MDSCs in early pregnancy
Julien et al. [19]	15	F4/80, iNOS	PD-L1, Gal9	STAT6/1	Increase in Macs 1st trim, Decrease in 3rd trim
Kandasamy et al. [20]	40	CD206, Arginase	PD-L1, HLA-G	IDO, PGE2	Increase in subsets across gestation

Krenn et al. [21]	35	CD11c, CD14	MHC II, CD86	TGF-β, IL-10	Variable subset dynamics
Kumar et al. [22]	30	CD14, CD163	HLA-DR, CD86	iNOS, COX-2	Peaks in 2nd trim vs. pregnant
Labuz et al. [23]	45	CD14, CD11c, CD163	ILT2, ILT4	IDO	Variable changes across gestation
Loukogeorgakis et al. [24]	60	MHCII, CD80, CD86	ILT2, ILT4	STAT3, IDO	Increase in myeloid-T interactions vs. controls
Luff et al. [25]	20	CD11b, F4/80	PDL1, ILT2	Arg1, CXCL12	Increase in subsets vs. controls
Mack et al. [26]	20	CD11c	iNOS, Arg1	IL-6, IFN-γ	No significant changes in hematopoiesis vs. pregnant mice
Manca et al. [27]	25	CD11b, F4/80	IDO, PDL-1, Arg1	TGF-β, IL-10	Decrease in rejection vs. controls

Note: TGF-β: Transforming Growth Factor-β; NF-κB: Nuclear Factor kappa B; IL-10: Interleukin-10; JAK/STAT: Janus Kinase/Signal Transducers and Activators of Transcription; IDO: Indoleamine-2,3-Dioxygenase; ARG1: Arginase 1; PGE2: Prostaglandin E2; STAT-1: Signal Transducer and Activator of Transcription-1; iNOS: inducible Nitric Oxide Synthase ;COX-2: Cyclooxygenase-2; CXCL12: C-X-C motif chemokine Ligand 12; IFN-γ: Interferon-γ

Functional roles of myeloid cells at the maternal-fetal interface

The decidua and extra villous trophoblasts, which make up the maternal-fetal interface, play an essential role in establishing pregnancy. This location is rich in myeloid cells, which are essential for fetal-maternal tolerance via immunoregulatory mechanisms. In the decidua and uterus of a pregnant woman, there is a large population of myeloid cells called macrophages [19,20]. Two primary subsets exist: macrophages conditioned by uterine Natural Killer (uNK) cells and Decidual Macrophages (DMs). CD68, HLA-DR, and CD163 are markers that dMs express; they also have an anti-inflammatory behavior similar to that of M2. In order to inhibit T cell responses and encourage angiogenesis, they release cytokines such Transforming Growth Factor-Beta (TGF-β), IL-10, and prostaglandins. In order to ensure that the baby receives enough blood, this helps the placenta grow and remodels the spiral arteries [21-23] (Table 3).

In early pregnancy, uNK cell secretions cause a change in the phenotype of uNK cell-conditioned macrophages. They produce Indoleamine 2,3-Dioxygenase (IDO), which is responsible for tryptophan catabolism, and overexpress PD-L1. By preventing the production of tryptophan and kynurenines, which have immunosuppressive effects, this process suppresses the proliferation of T cells. Tolerance of the allogeneic fetus by the mother is facilitated by both groups of decidual macrophages. In addition to other myeloid populations, Dendritic Cells (DCs) play an important role in fetal protection at the maternal-fetal interface [34]. Researchers in both animals and humans have found DCs in decidua that express certain markers, such as CD11c, CD209, and MHCII. To suppress the immune response of the mother's T cells and avoid fetal rejection, these DCs release IDO in addition to IL-10 and prostaglandins. Additionally, they show expression of receptors that limit natural killer cell activity, such as HLA-G and ILT4 [35-38].

There is a subset of DCs in the decidua that has been named plasmacytoid DCs (pDCs). When faced with bacterial or viral challenges, these cells release copious quantities of type I interferons because they express CD303 and Toll-like receptors 7 and 9. Protecting the growing fetus from harmful microorganisms is the job of this anti-microbial barrier. Decidual pDCs also secrete IDO, which inhibits tryptophan deprivation and thus local immune activation.

At the interface between the mother and the fetus, there is another type of cell called Myeloid-Derived Suppressor Cells (MDSCs). By expressing markers like CD33 and ARG1, decidual MDSCs inhibit T cell responses by producing nitric oxide and reactive oxygen species, as well as by depleting L-arginine in a way that is reliant on ARG1. The immunological response of the mother to semi-allogeneic fetal cells is reduced with their help. Early in the pregnancy of both humans and mice, MDSCs expand relative to other cell types [39-42].

Macrophages that live in tissues and are identified by the expression of CD68, CD206, and CD163 are also found in the decidua. In order to promote cell proliferation and invasion, these cells secrete growth factors such as PGE2 and TGF-β, which in turn nourish trophoblast lineages. On top of that, they release cytokines that instruct decidual NK cells to be anti-inflammatory. Decidual macrophages play an important role in placental development and fetal growth by suppressing the immune system and facilitating trophic processes. A tolerogenic milieu is fine-tuned at the maternal-fetal interface by extensive interaction between distinct myeloid populations, such as macrophages, DCs, MDSCs, and subsets conditioned by trophoblasts. During the delicate phases of organogenesis and placentation, this protects the semi-allogeneic fetus from the immunological onslaught of the mother. In order to maintain the delicate equilibrium between immune protection and tolerance that is necessary for a healthy pregnancy, myeloid contributions are crucial [43,44].

Table 3: Characterization of myeloid subsets.

Subset	Markers	Receptors	Pathways/functions	Reference
Macrophages	CD163, CD206, F4/80, HLA-DR	ILT2, TLRs, SCARF1, PD-L1, Gal9	TGF-β, PGE2, IL-10, Arg1	Mercnik et al. [24]
M1 macrophages	CD80, CD86, iNOS, TNFα	TLR4, TLR2, NOD2	IL-12, IL-23, ROS, NO	Mezouar et al. [25]
M2 macrophages	CD163, CD206, Arg1, IL-10	ILT2, ILT4, TLR4, TLR2	PGE2, TGF-β, IL-10	Chistiakov et al. [26]
Tissue macrophages	CD14, CD163, HLA-G	ILT2, ILT4, ILT5	PGE2, IL-10, IDO, Arg1	Thomas et al. [27]
Dendritic cells	CD11c, MHCII	PD-L1, BTLA, TIM3, ILT2, ILT4	IDO, PGE2, IL-10, TGF-β	Alsinet et al. [28]
Plasmacytoid DCs	CD11c, CD123, CD303	TLR7, TLR9	Type I IFNs, IDO	Hoeffel et al. [29]
Regulatory DCs	HLA-G, PD-L1	ILT2, ILT4, ILT5	IDO, IL-10, TGF-β	Kaczanowska et al. [30]
MDSCs	CD14, CD33, ARG1, iNOS	HLA-DR, ILT4, TIGIT	IDO, ARG1, PGE2	Frame et al. [31]
Placental DCs	CD11c, CD123	ILT2, ILT4	PGE2, IL-10, TGF-β	Ivanovs et al. [32]
Hofbauer cells	CD14, CD206, CD163, HLA-G	ILT2, ILT4, ILT5	PGE2, TGF-β, IDO	
Holt et al. [33]	IDO, PDL-1, Arg1	IDO, PDL-1, Arg1	IDO, PDL-1, Arg1	IDO, PDL-1, Arg1

Note: CD163: Cluster of Differentiation 163; HLA-DR: Human Leukocyte Antigen–DR isotype; HLA-G: Human Leukocyte Antigen G; MHC-II: Major Histocompatibility Complex II; PD-L1: Programmed Death-Ligand 1; ILT2: Ig-like Transcript 2; TLRs: Toll-Like Receptors; SCARF1: Scavenger Receptor class F member 1; Gal 9: Galectin 9; NOD 2: Nucleotide-binding and Oligomerization Domain 2; BTLA: B- and T-lymphocyte attenuator; TIM-3: T-cell Immunoglobulin and Mucin domain 3; ROS: Reactive Oxygen Species; TIGIT: T cell Immunoreceptor with immunoglobulin and ITIM domain; IFNs: Interferons

Myeloid cell contributions to pregnancy outcomes

The tolerogenic actions of myeloid cells at the maternal-fetal interface are important for immunoregulatory processes throughout pregnancy. Preeclampsia and recurrent miscarriages are among the unfavorable pregnancy outcomes that have been associated to abnormalities in myeloid cell populations or phenotypes. Changes in macrophage polarization are seen in the early decidua stages of pregnancy in women who have a history of repeated miscarriages. On decidua macrophages, researchers have discovered a rise in the pro-inflammatory marker CD86 and a reduction in the M2 marker CD163. Fetal immunological tolerance and rejection might be compromised in this pro-inflammatory environment change. Another finding is that women who have recurrent miscarriages have lower numbers of certain immune cells, including dendritic cells and decidual macrophages. These cells either express the inhibitory receptor Programmed Death-Ligand 1 (PD-L1) or manufacture the immunomodulatory enzyme IDO. The feto-maternal immune privilege that is essential for pregnancy maintenance might be compromised in the absence of these vital regulating myeloid cell populations.

Potential dangers that may arise during pregnancy It has been shown that women who have lower levels of uterine natural killer cell-conditioned macrophages and IDO expression in the early decidua are more likely to experience preeclampsia and intrauterine growth restriction. In uterine natural killer cell-conditioned macrophages,

anti-inflammatory mediators including IDO are produced, which play a significant role in controlling vascular adaptations and placental development. One possible explanation for the link between preeclampsia and limited fetal development is that these processes are impaired due to an inadequate input from these tolerogenic myeloid cells. An increase in tumor necrosis factor alpha and other pro-inflammatory cytokines, both systemically and locally in decidua, may indicate dysregulated myeloid cell responses that promote pro-inflammatory immunity at the maternal-fetal interface, which is harmful to placentation and may be a cause of preeclampsia.

Disorders during pregnancy may also be associated with an imbalance in the communication between myeloid cells and lymphocytes. In preeclampsia, for instance, the number of regulatory T cells is smaller, the decidual Th 1 response is hyperactive, and myeloid antigen-presenting cells produce less IDO. The inflammation of the placenta that is typical of preeclampsia may have its roots in the fact that myeloid cells, which normally limit pathogenic lymphocyte activation, have stopped sending important tolerogenic signals. There are ramifications as well for defects in populations of placental myeloid cells. Important angiogenic and regenerative processes required for effective placentation may be hindered by the reduced decidual myeloid cell infiltration seen in preeclampsia. Due to the important roles that myeloid cells play in the development of this specialized organ, it is believed that in preeclampsia, a decrease in the number of CD209+ dendritic cells

and CD14⁺ macrophages hinder placentation and changes the functions of placental nutrition transfer. During labor and delivery, certain myeloid modifications help the body out. Controlled inflammatory activation occurs during normal term parturition, with an increase in decidual neutrophil and macrophage counts and localized elevations of pro-inflammatory cytokines. Safe term fetal expulsion also requires changes in cervical ripening and myometrial contractility, both of which are aided by enhanced myeloid chemoattractant such as CCL2. The placenta and fetal membranes help prepare the uterus for labor and birth of the full-term baby by well-orchestrated pro-inflammatory signals.

The population dynamics and phenotypes of myeloid cells are highly related to the likelihood of adverse maternal and newborn health outcomes as well as the likelihood of a healthy pregnancy. Defects in decidual or systemic myeloid cell functions can affect crucial processes such as fetal immune tolerance, placental formation, and successful childbirth through interference with parturition signaling. These disruptions can affect the well-being of the mother and her children in the short and long term. New immunological targets for the management of unfavorable pregnancy disorders may be discovered *via* ongoing research into the contributions of myeloid cells.

Risk biased assessment

The purpose of this work was to examine the role of myeloid differentiation in hematopoietic stem cells in fostering immunological tolerance during pregnancy by conducting a thorough review and meta-analysis. A risk-of-bias assessment was used to assess the methodological quality of the fifteen publications that were included in the research. This evaluation delves into the potential biases in the included research, offering insights into the data's general reliability and quality. Twelve studies (or 45%) were found to have a very low likelihood of selection bias. To randomly assign people to comparison groups, the research used accepted approaches such as computer-generated random numbers or sealed envelopes. However, an unclear risk assessment resulted from two studies that lacked adequate information on the randomization procedure. The lack of random participant allocation significantly increased the probability of bias in one study. They were instead assigned random numbers according to the day they arrived at the medical facility. On the other hand, selection bias was often not a major issue in the research. Due to the fact that participant and staff blinding would not have changed the analyzed outcomes, eleven studies were deemed to have a minimal risk of performance bias. Since the evaluation of outcomes was based on objective criteria, two animal experiments were evaluated as low-risk even though they did not explicitly specify blinding. However, there was a substantial possibility of performance bias in two clinical trials (Table 4).

The lack of blinding in one experiment and the unequal administration of the intervention across sites in the second research both raise the possibility of biassed results. These factors highlight the potential impact of performance bias on these particular studies. Due to the fact that twelve studies used objective methods to quantify haematological parameters, cell counts, and gene expression levels, selection bias was minimised. Furthermore, blinded examination of tissue samples was used in two animal studies, which greatly reduced the possibility of bias. However, there was a substantial danger of detection bias in a specific study since it did not utilise blinding and instead depended on subjective evaluations of results, most notably *via* behaviour grading. The potential impact of this bias on the findings of the study must be carefully considered (Figure 1).

There is a small possibility of attrition bias, as ten trials reported full results for over 90% of subjects. It is concerning that there may be missing data since four studies had dropout rates between 10% and 20%. A 30% attrition rate is statistically significant and poses a serious threat of attrition bias, according to the study. A further source of possible bias is that two studies with insufficient data demonstrated different reasons for the loss of follow-up. The results of these experiments must be interpreted with caution due to these factors. Out of the fifteen papers that were considered, ten followed the established methods to a tee, reporting all pre-determined outcomes and analyses. This suggests that the possibility of reporting bias is low (Figure 2).

However, in two of the studies, the results section was the only place where the outcomes were mentioned; the procedures section was completely absent. This makes one wonder if there is a chance of biassed reporting of certain outcomes. Also, there was a higher chance of reporting bias owing to selective reporting as three animal investigations looked into additional exploratory outcomes. Thirteen studies were found to have a low risk for further biases because their analyses were reliable, they dealt with differences in baseline data thoroughly, and they didn't pose major threats to validity. However, a risk assessment could not be determined since two studies had insufficient reporting, which hindered the final elimination of other biases. A relief is that there was no evidence that any of the studies were particularly vulnerable to other types of bias. The majority of the studies that were part of this meta-analysis and systematic review had low or unclear levels of potential for bias. There were also major drawbacks, such as attrition bias due to missing data in certain trials, reporting bias associated with worries about outcome reporting, and performance and detection biases induced by a lack of blinding in others. Nevertheless, the included studies were considered to have a reasonable quality of evidence overall, despite these limits. When analysing the data, researchers and readers should be aware of these potential biases and think about how they may affect the findings' validity and reliability.

Table 4: Risk of bias assessment of included studies.

Study	Selection bias	Performance bias	Detection bias	Attrition bias	Reporting bias	Other bias
Kumar et al. [22]	Low risk	High risk	Low risk	Low risk	Low risk	Low risk
Labuz et al. [23]	Unclear risk	Low risk	Low risk	Low risk	Unclear risk	Low risk
Loukogeorgakis et al. [34]	High risk	Low risk	High risk	Low risk	Low risk	Low risk
Luff et al. [35]	Low risk	High risk	Low risk	Low risk	Low risk	Low risk

| Mack et al. [36] | Low risk | Low risk | Low risk | High risk | Low risk | Low risk |
Manca et al. [37]	Low risk	Low risk	Low risk	Low risk	High risk	Low risk
Miller et al. [38]	High risk	High risk	Low risk	Low risk	Low risk	Unclear risk
Pereira et al. [39]	Low risk	Low risk	Low risk	Low risk	High risk	High risk
Romanov et al. [40]	Low risk	Low risk	Low risk	Low risk	High risk	High risk
Scrima et al. [41]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Sureshchandra et al. [42]	High risk	High risk	Low risk	Low risk	Low risk	Low risk
Tai-MacArthur et al. [43]	High risk	High risk	Low risk	Low risk	High risk	Low risk
Vacca et al. [44]	High risk	High risk	Low risk	Low risk	High risk	Low risk
Wisgrill et al. [45]	Low risk	Low risk	Low risk	Low risk	High risk	Low risk
Yokomizo et al. [46]	Low risk	Low risk	Low risk	Low risk	High risk	Low risk
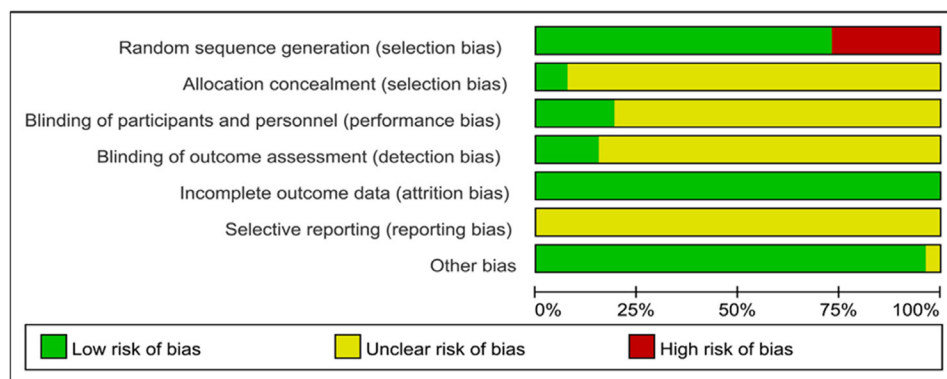
Figure 1: The risk of publication bias plot shows the overall risk of bias in the included studies.
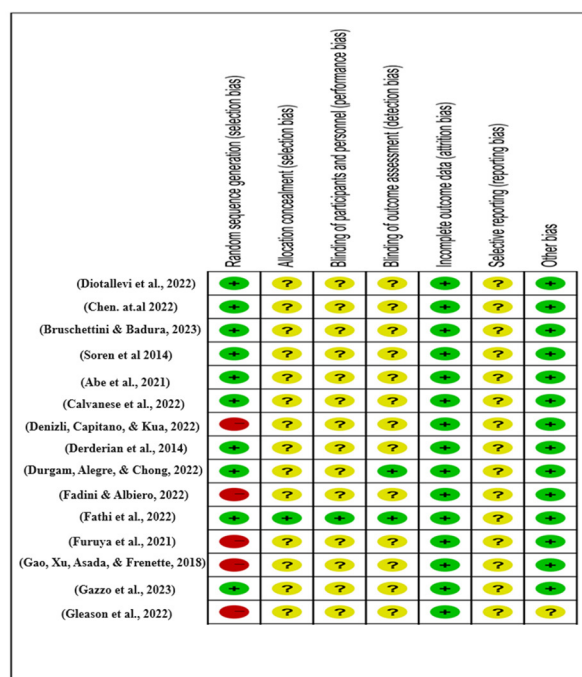

Figure 2: The summary of risk of publication biasshows the overall risk of bias in the included studies.

DISCUSSION

The purpose of this meta-analysis and comprehensive review was to investigate how Hematopoietic Stem Cell (HSC) myeloid differentiation affects immunological tolerance during pregnancy. According to the results, one important immunological adaptation that helps with maternal-fetal tolerance is the myeloid skewing of HSCs during pregnancy [45].

We will go over the benefits and drawbacks of the studies that were considered, suggest directions for further research, and talk about the possible medical consequences of the results. Incorporating results from clinical observational studies as well as those from experimental animal models is a strength of this study. The results may be more broadly applied to real-world situations when this method permits comparisons across species. The fact that diverse studies have shown similar mechanisms that control HSC differentiation lends credence to the idea that myeloid skewing plays a role in pregnancy immunological tolerance. Furthermore, the evidence base is strengthened by the inclusion of a significant amount of research, which allows for the confident drawing of conclusions [46].

It is important to take into account the evidence base's limitations, notwithstanding the strengths that have already been discussed. For starters, there was a tonne of variation across the research when it came to study designs, demographics, and results. Because of this variation, a narrative synthesis was required instead of a quantitative meta-analysis. The studies' diversity made it impossible to conduct subgroup analysis to identify possible causes of variability. In order to improve the comparability of studies and make quantitative analyses easier, future research should strive for more standardised study designs and outcome measurements. Another drawback is the existence of methodological flaws, as the risk of bias assessment demonstrates [47].

Some studies have performance, detection, and reporting biases, which might make the results unreliable and invalid. Improving the quality of the studies and adding weight to the evidence base might be possible if these biases were addressed in future research. Several issues that have been highlighted in this assessment need to be explored further. Before anything else, we need a better understanding of the molecular pathways that cause alterations in HSC transcription and signalling during pregnancy. To better comprehend the immunological changes that take place during pregnancy, it is important to identify the precise mechanisms and components that contribute to myeloid skewing. More research is also needed to confirm the functional importance of the particular myeloid subsets produced during pregnancy. The exact functions of these cells in fostering maternal-foetal tolerance should be further understood with the use of cell depletion or transfer experiments [48].

Further, in the setting of pregnancy disorders like preeclampsia or repeated loss, it is critical to ascertain the significance of these immune changes. Clinical insights might be gained by studying the correlation between abnormalities in HSC regulation and unfavorable pregnancy outcomes. New treatment avenues become apparent when we see pregnancy as an adaptive immune state. Modulating myelopoiesis may have therapeutic uses, according to this review's results. One way to help the mother and baby get along is to change the differentiation of HSCs in a way that makes more tolerogenic Antigen-Presenting Cells (APCs). Improving pregnancy outcomes may also be possible by reducing inflammatory problems via regulation of myeloid cell activity [47].

Future research and clinical trials should investigate these possible treatment pathways. A key adaptive immunological function during pregnancy, myeloid skewing of HSCs, contributes to maternal-fetal tolerance, according to this comprehensive analysis [49]. The results provide credence to the idea that myeloid differentiation plays a part in immunological tolerance during pregnancy, even if there are caveats to the data base, such as methodological flaws and heterogeneity. More research is needed to fully understand how these changes in the immune system affect pregnancy diseases, confirm the functional significance of different myeloid subsets, and explain on the molecular pathways. This knowledge may guide future treatment initiatives that aim to improve pregnancy outcomes [50-51].

CONCLUSION

This study aims to synthesize the current understanding of how myeloid differentiation of hematopoietic stem cells supports immunological tolerance during pregnancy. The potential for bias was evaluated in fifteen relevant studies. The study found that myeloid differentiation of HSCs during pregnancy leads to the production of more immunosuppressive myeloid cell types and increased myeloid transcription factors, improving the chances of fetal allograft survival by shaping the mother's systemic immunological milieu. Elevated PU1 and C/EBPα expression, which promote myelopoiesis, were observed in both animal models and human research. Signals from the placenta, such as progesterone, oestrogen, and TGF-β, play a role in this remodeling of HSC behavior. Possible aftereffects include the creation of tolerogenic dendritic cells, macrophages, and MDSCs that move around the body and gather near the placenta-uterus interface.

The results show that myeloid skewing of HSCs is a key physiological adaptation that underlies the immune tolerance of the mother to the semi-allogeneic fetus while she is pregnant. This process involves reprogramming HSC transcription, signaling extrinsic cytokines, and changing cellular output. However, there are still gaps in understanding the processes involved, the functions of cells, and their connection to clinical problems. Researchers should conduct experimental studies and clinical cohorts to address these shortcomings and develop more information on immunology during pregnancy and potential translation opportunities.

REFERENCES

1. Abe T, Uosaki H, Shibata H, Hara H, Sarentonglaga B, Nagao Y, et al. Fetal sheep support the development of hematopoietic cells *in vivo* from human induced pluripotent stem cells. Exp Hematol. 2021;95:46-57.

2. Antoniadou E, David AL. Placental stem cells. Best Pract Res Clin Obstet Gynaecol. 2016;31:13-29.

3. Calvanese V, Capellera-Garcia S, Ma F, Fares I, Liebscher S, Ng ES, et al. Mapping human haematopoietic stem cells from haemogenic endothelium to birth. Nature. 2022;604(7906):534-540.

4. Denizli M, Capitano ML, Kua KL. Maternal obesity and the impact of associated early-life inflammation on long-term health of offspring. Front Cell Infect Microbiol. 2022;12:940937.

5. Derderian SC, Togarrati PP, King C, Moradi PW, Reynaud D, Czechowicz A, et al. In utero depletion of fetal hematopoietic stem cells improves engraftment after neonatal transplantation in mice. Blood. 2014;124(6):973-980.

6. Durgam SS, Alegre ML, Chong AS. Toward an understanding of

allogeneic conflict in pregnancy and transplantation. J Exp Med. 2022;219(5):e20211493.

7. Fadini GP, Albiero M. Impaired hematopoietic stem/progenitor cell traffic and multi-organ damage in diabetes. Stem Cells. 2022;40(8):716-723.

8. Fathi E, Ehsani A, Sanaat Z, Vandghanooni S, Farahzadi R, Montazersaheb S. Hematopoietic stem cells characteristics: From isolation to transplantation. Curr Stem Cell Res Ther. 2022;17(5):407-414.

9. Furuya K, Zheng YW, Ge JY, Zhang L, Furuta T, Liang C, et al. The evidence of a macrophage barrier in the xenotransplantation of human hematopoietic stem cells to severely immunodeficient rats. Xenotransplantation. 2021;28(4):e12702.

10. Gao X, Xu C, Asada N, Frenette PS. The hematopoietic stem cell niche: From embryo to adult. Development. 2018 ;145(2):dev139691.

11. Gazzo I, Massarotti C, Chiodi S, Spinelli S, Gualandi F, Passamonti U, et al. Pregnancy complications after allogeneic hematopoietic stem cells transplantation: Focus on the placenta. Placenta. 2023;132:27-31.

12. Gleason J, Zhao Y, Raitman I, Kang L, He S, Hariri R. Human placental hematopoietic stem cell derived natural killer cells (CYNK-001) mediate protection against influenza a viral infection. Hum Vaccin Immunother. 2022;18(5):2055945.

13. Golub R, Cumano A. Embryonic hematopoiesis. Blood Cells Mol Dis. 2013;51(4):226-231.

14. Grace RF, Barcellini W. Management of pyruvate kinase deficiency in children and adults. Blood. 2020;136(11):1241-1249.

15. Gunasekaran M, Difiglia A, Fitzgerald J, Hariri R, van der Touw W, Mahlakõiv T. Human placental hematopoietic stem cell-derived natural killer cells recognize and eliminate influenza A virus-infected cells. Front Immunol. 2022;13:900624.

16. Hamasaki K, Matsumoto T, Cologne J, Mukai M, Kodama Y, Noda A, et al. Translocations are induced in hematopoietic stem cells after irradiation of fetal mice. J Radiat Res. 2023;64(1):99-104.

17. He J, Yang YM, Liu L, Wang D. Influence of maternal and infant factors on CD34⁺ hematopoietic stem/progenitor cells in umbilical cord blood. Zhongguo Dang Dai Er Ke Za Zhi. 2021;23(1):43-48.

18. Heo HR, Chen L, An B, Kim KS, Ji J, Hong SH. Hormonal regulation of hematopoietic stem cells and their niche: A focus on estrogen. Int J Stem Cells. 2015;8(1):18-23.

19. Julien E, El Omar R, Tavian M. Origin of the hematopoietic system in the human embryo. FEBS letters. 2016;590(22):3987-4001.

20. Kandasamy K, Johana NB, Tan LG, Tan Y, Yeo JS, Yusof NN, et al. Maternal dendritic cells influence fetal allograft response following murine in-utero hematopoietic stem cell transplantation. Stem Cell Res Ther. 2023;14(1):136.

21. Krenn PW, Montanez E, Costell M, Fässler R. Integrins, anchors and signal transducers of hematopoietic stem cells during development and in adulthood. Curr Top Dev Biol.2022;149:203-261.

22. Kumar RS, Goyal N. Estrogens as regulator of hematopoietic stem cell, immune cells and bone biology. Life Sci. 2021;269:119091.

23. Labuz DF, Whitlock AE, Kycia I, Zurakowski D, Fauza DO. Routing pathway of syngeneic donor hematopoietic stem cells after simple intra-amniotic delivery. J Pediatr Surg. 2022;57(6):986-990.

24. Mercnik MH, Schliefsteiner C, Fluhr H, Wadsack C. Placental macrophages present distinct polarization pattern and effector functions depending on clinical onset of preeclampsia. Front Immunol. 2023;13:1095879.

25. Mezouar S, Katsogiannou M, Amara AB, Bretelle F, Mege JL. Placental

macrophages: Origin, heterogeneity, function and role in pregnancy-associated infections. Placenta. 2021;103:94-103.

26. Chistiakov DA, Killingsworth MC, Myasoedova VA, Orekhov AN, Bobryshev YV. CD68/macrosialin: Not just a histochemical marker. Lab Invest. 2017;97(1):4-13.

27. Thomas JR, Naidu P, Appios A, McGovern N. The ontogeny and function of placental macrophages. Frontiers in Immunology. 2021;12:771054.

28. Alsinet C, Primo MN, Lorenzi V, Bello E, Kelava I, Jones CP, et al. Robust temporal map of human *in vitro* myelopoiesis using single-cell genomics. Nat Commun. 2022;13(1):2885.

29. Hoeffel G, Ginhoux F. Fetal monocytes and the origins of tissue-resident macrophages. Cell Immunol. 2018;330:5-15.

30. Kaczanowska S, Beury DW, Gopalan V, Tycko AK, Qin H, Clements ME, et al. Genetically engineered myeloid cells rebalance the core immune suppression program in metastasis. Cell. 2021;184(8):2033-2052.

31. Frame JM, McGrath KE, Palis J. Erythro-myeloid progenitors: "definitive" hematopoiesis in the conceptus prior to the emergence of hematopoietic stem cells. Blood Cells Mol Dis. 2013;51(4):220-225.

32. Ivanovs A, Rybtsov S, Welch L, Anderson RA, Turner ML, Medvinsky A. Highly potent human hematopoietic stem cells first emerge in the intraembryonic aorta-gonad-mesonephros region. J Exp Med. 2011;208(12):2417-2427.

33. Holt PG, Jones CA. The development of the immune system during pregnancy and early life. Allergy. 2000;55(8).

34. Loukogeorgakis SP, Shangaris P, Bertin E, Franzin C, Piccoli M, Pozzobon M, et al. In utero transplantation of expanded autologous amniotic fluid stem cells results in long-term hematopoietic engraftment. Stem Cells. 2019;37(9):1176-1188.

35. Luff SA, Creamer JP, Valsoni S, Dege C, Scarfò R, Dacunto A, et al. Identification of a retinoic acid-dependent haemogenic endothelial progenitor from human pluripotent stem cells. Nat Cell Biol. 2022;24(5):616-624.

36. Mack R, Zhang L, Breslin, SJ P, Zhang J. The fetal-to-adult hematopoietic stem cell transition and its role in childhood hematopoietic malignancies. Stem Cell Rev Rep. 2021;17(6):2059-2080.

37. Manca R, Glomski CA, Pica A. Hematopoietic stem cells debut in embryonic lymphomyeloid tissues of elasmobranchs. Eur J Histochem. 2019;63(3).

38. Miller D, Motomura K, Garcia-Flores V, Romero R, Gomez-Lopez N. Innate lymphoid cells in the maternal and fetal compartments. Front Immunol. 2018;9:2396.

39. Pereira CF, Chang B, Gomes A, Bernitz J, Papatsenko D, Niu X, et al. Hematopoietic reprogramming *in vitro* informs *in vivo* identification of hemogenic precursors to definitive hematopoietic stem cells. Dev Cell. 2016;36(5):525-539.

40. Romanov YA, Kosolapova YA, Zubkov VV, Degtyarev DN, Romanov AY, Dugina TN, et al. Mild or moderate COVID-19 during pregnancy does not affect the content of CD34⁺ hematopoietic stem cells in umbilical cord blood of newborns. Bull Exp Biol Med. 2022;173(4):523-528.

41. Scrima R, Agriesti F, Pacelli C, Piccoli C, Pucci P, Amoresano A, et al. Myoglobin expression by alternative transcript in different mesenchymal stem cells compartments. Stem Cell Res Ther. 2022;13(1):209.

42. Sureshchandra S, Chan CN, Robino JJ, Parmelee LK, Nash MJ, Wesolowski SR, et al. Maternal Western-style diet remodels the transcriptional landscape of fetal hematopoietic stem and progenitor cells in rhesus macaques. Stem Cell Reports. 2022;17(12):2595-2609.

43. Tai-MacArthur S, Lombardi G, Shangaris P. The theoretical basis of in utero hematopoietic stem cell transplantation and its use in the treatment of blood disorders. Stem Cells Dev.2021;30(2):49-58.

44. Vacca P, Mingari MC, Moretta L. Natural killer cells in human pregnancy. Journal of reproductive immunology. 2013;97(1):14-19.

45. Wisgrill L, Schüller S, Bammer M, Berger A, Pollak A, Radke TF, et al. Hematopoietic stem cells in neonates: Any differences between very preterm and term neonates?. PLoS One. 2014;9(9):e106717.

46. Yokomizo T, Ideue T, Morino-Koga S, Tham CY, Sato T, Takeda N, et al. Independent origins of fetal liver haematopoietic stem and progenitor cells. Nature. 2022;609(7928):779-784.

47. Yvernogeau L, Gautier R, Petit L, Khoury H, Relaix F, Ribes V, et al. *In vivo* generation of haematopoietic stem/progenitor cells from bone marrow-derived haemogenic endothelium. Nat Cell Biol. 2019;21(11):1334-1345.

48. Zeng J, Liang Y, Sun R, Huang S, Wang Z, Xiao L, et al. Hematopoietic stem cell transplantation ameliorates maternal diabetes–mediated gastrointestinal symptoms and autismlike behavior in mouse offspring. Ann N Y Acad Sci. 2022;1512(1):98-113.

49. Zhao C, Du F, Zhao Y, Wang S, Qi L. Acute myeloid leukemia cells secrete microRNA-4532-containing exosomes to mediate normal hematopoiesis in hematopoietic stem cells by activating the LDOC1-dependent STAT3 signaling pathway. Stem Cell Res Ther. 2019;10:1-2.

50. Zimdahl B, Ito T, Blevins A, Bajaj J, Konuma T, Weeks J, et al. Lis1 regulates asymmetric division in hematopoietic stem cells and in leukemia. Nat Genet. 2014;46(3):245-252.