Trends in Immunology

CellPress

Spotlight

# Towards decoding the space-time continuum of pregnancy

Kristin Thiele[1,2,*] and Petra Clara Arck[1,2,*]

**Pregnancy is likely nature's most elaborate model of dynamic adaptations occurring over a distinct period of time at specific sites of the maternal body. Spatially-resolved transcriptomic analyses now enable the comprehensive decoding of these dynamic adaptations during the early onset stages of mammalian pregnancies and throughout fetal development.**

Successful embryo implantation at the onset of pregnancy requires essential adaptations of the uterine lining, the endometrium. These adaptations, referred to as decidualization, evolve from the cellular and molecular reprogramming of endometrial stromal cells (ESCs). Simultaneously, immune cells residing in the decidualized endometrium begin to adopt an altered, immune tolerance-prone phenotype. This local immune tolerance is expanded by the targeted recruitment of tolerogenic cells to the decidua [1,2]. Decidualization of ESCs also encompasses the secretory transformation of uterine glands and vascular remodeling of maternal arteries. Here, placentation, which is a key requirement for the vertical transfer of nutrients, oxygen, cells and other factors to the fetus, can progress. Hence, akin to Einstein's definition of the space-time continuum, the uterus (space) and the early gestational period (time) are inextricably merged into a transient single continuum that spans multiple dimensions: decidualized ESCs, tissue-resident and emigrated immune cells, the vascular system, and the implanting embryo (Figure 1).

Despite its importance, decidualization of ESCs has not yet been systematically investigated. This gap in knowledge can be explained by the unavailability of tools allowing to decode such intricate spatial adaptations over a period of weeks or months (dependent on the species). However, spatial technologies have now emerged to enable high-resolution transcriptomic profiling at the single-cell level. In this context, Yang *et al*. recently used single-cell Stereo-sequencing (scStereo-seq) technology combined with single-cell RNA sequencing (scRNA-seq) to create a spatiotemporal atlas of implantation sites during distinct periods of early and mid-pregnancy decidualization in mice [3]. Hereby, functional hubs at implantation sites could be visualized at the single-cell level and tracked over time. The insights arising from these unbiased analyses confirm pathways underlying successful embryo implantation, for example, leukocyte-driven decidual angiogenesis or the suppressive function of galectin-9, which were previously identified using hypothesis-driven, traditional molecular or cellular approaches [4,5]. Additionally, the study by Yang *et al.* amends our understanding of how successful embryo implantation is achieved and places endometrial and decidual adaptations to the implating embryo into spatiotemporal trajectories. In this regard, the authors could categorize subclusters of decidual stromal cells (DSCs) according to distinct molecular features and along transdifferentiation processes. The immune functions of DSCs were decoded and categorized into three functional subclusters, termed iDSC0, iDSC1, and iDSC2. iDSC0 and iDSC1 promoted the recruitment of immune cells in synergy with endometrial stromal fibroblasts, as well as angiogenic sprouting and vascular maturation, while iDSC2 activated apoptotic signaling. The functional role of these subclusters could be confirmed in an abortion-prone mouse model [6], where dysfunctional and spatially disordered iDSCs were identified, along with an enhanced recruitment of immune cells and the disruption of vascular maturation. The availability of this spatiotemporal decidualization atlas in mice now advances our understanding of the complexity around embryo implantation in mouse pregnancies and highlights what needs to be addressed next in research objectives, such as the confirmation of the observed mRNA expression changes at the protein level [7].

Interestingly, Chen *et al*. developed a similar approach as Yang *et al*. by using spatial enhanced resolution omics-sequencing (Stereo-seq) combining DNA nanoball-patterned arrays and *in situ* RNA capture to exemplify embryogenesis and organogenesis in fetal mice at mid to late gestation [8]. Here, cell type-specific transcriptional visualization in whole embryo sections generated a mouse organogenesis spatiotemporal transcriptomic atlas. Such an approach may revolutionize the investigation of mammalian genetic diseases that are often complex and affect multiple organ systems. Hence, screening the developmental origin of tissue- and area-specific transcription factors across different stages of embryogenesis might contribute to unearth the pathogenesis of diseases. One example is the functional connection between *Wnt5a* and *Msx1* gene expression, especially in mesenchymal cells that differ in maxilla and limb, which might help to explain the skeletal phenotypic features of Robinow syndrome by different disease mechanisms) [9].

Embryo implantation and decidualization are pivotal milestones during early pregnancy. These are followed by the maintenance of immune tolerance toward the embryo throughout gestation, which ensures adequate embryogenesis, organogenesis, and fetal development. Lastly, mounting a surge of inflammation is the last milestone to be completed during pregnancy, as it is required to induce labor. While these milestones have been

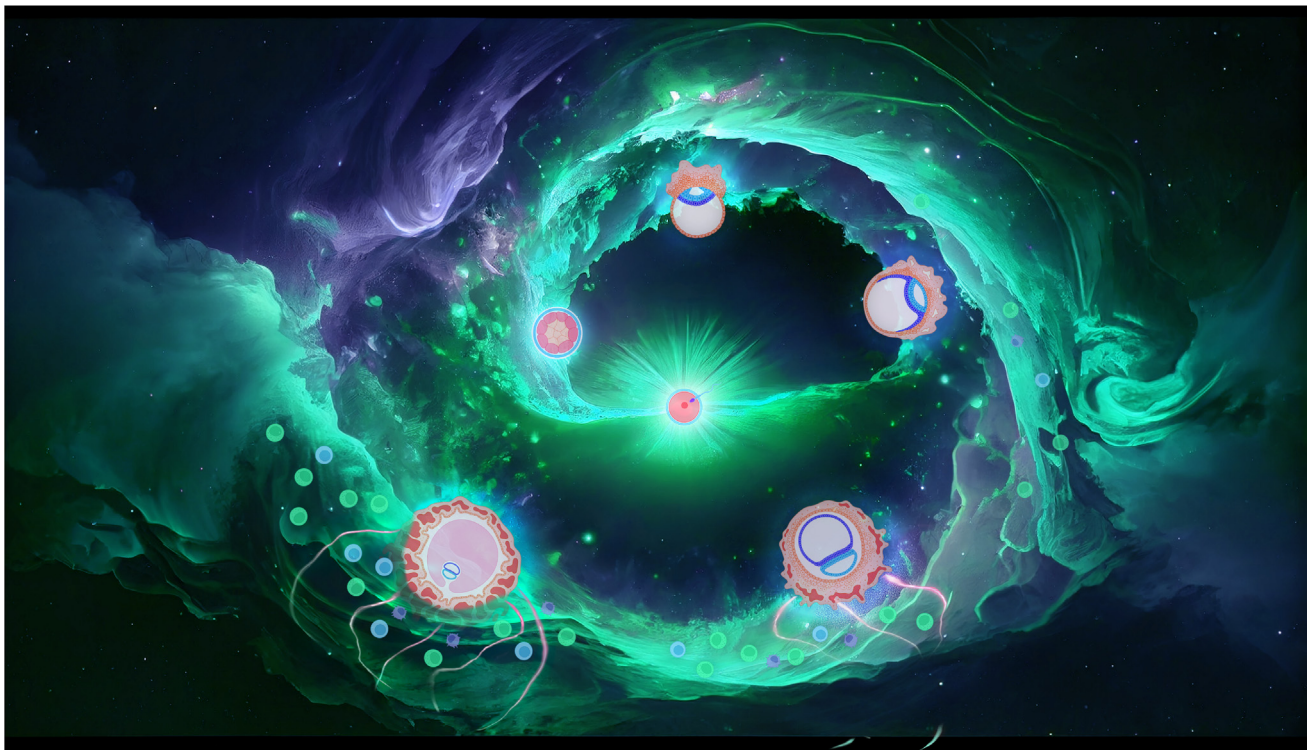


Figure 1. Space-time continuum of mammalian pregnancy. The uterus (space) and the early gestational period (time) are inextricably merged into a transient single continuum that spans multiple dimensions: decidualized endometrial stromal cells (ESCs), tissue-resident and emigrated immune cells, including uterine NK cells, $CD4^+$ regulatory T cells (including a memory subset), tolerogenic dendritic cells (sketched by the colours green, blue and purple), the growing vascular system, and the implanting and developing embryo (the latter created with BioRender.com).

described in a number of mammalian species, considerable species differences need to be considered. For example, in contrast to mice, humans regularly shed the endometrium during menstruation. Also, placental cells show a greater invasiveness into the human decidua. Humans also have a higher risk for infertility and pregnancy complications, such as spontaneous abortion or preterm birth. Thus, genes underlying such milestones (i.e., as seen by the decidualization analyses by Yang *et al*. in mice [3]) may differ between eutherian mammals. This is especially relevant for species with highly divergent gestational lengths, such as mice with rather short pregnancies, opposed to humans, which have a relatively long gestational period. Marinić *et al*. addressed this aspect by using evolutionary methods to identify changes in endometrial gene expression during pregnancy in eutherian mammals. Here, the heart- and neural crest derivatives-expressed protein 2 (HAND2) expression in ESCs in the eutherian stem lineage was documented to critically regulate gestation length [10]. Of note, the spatiotemporal atlas on implantation in mice described by Yang and colleagues also identified iDSCs coexpressing HAND2 [3]. These $HAND2^+$ iDCSs were detectable among the vascular and immune cell-assembling hubs of early to mid-gestational implantation sites. To understand the translational relevance of HAND2 and other endothelial markers identified during decidualization in mice, endometrial organoids models may be helpful. Organoids derived from human endometrium from women with normal fertility or impaired endometrial receptivity have been established. Until now, this model was mostly used to understand endometrial receptivity upon modulation using steroid hormones [11]. Here, analyses also included single-cell and spatial transcriptional profiling of the endometrial organoids, allowing the spatial localization of ESCs during the menstrual cycle of women to be decoded.

Taken together, tools such as scStereo-seq technology combined with scRNA-seq are now available for decoding the space-time continuum of pregnancy by comprehensively dissecting the dynamic adaptations of ESCs during the early onset stages of mammalian pregnancies, as recently shown in mice [3]. This spatiotemporal atlas of implantation sites now offers an important source of information, which will aid to improve our understanding of normal and impaired uterine receptivity in humans, as well as the risk for adverse

pregnancy outcomes and transmission of pathogens from mother to fetus. In the future, technical prerequisites will have to be advanced (e.g., enlarging Stereo-seq chips,) to control for species-specific differences.

## Acknowledgments

This work was supported by grants provided by the Deutsche Forschungsgemeinschaft (German Research Foundation: Clinical Research Unit KFO296: AR232/25-2 to P.C.A. and Research Unit FOR5068: AR232/29-2 to P.C.A. and TH 2126/1-1 to K.T.) and the Authority for Science, Research and Equality, Hanseatic City of Hamburg, Germany (LFF-FV73) to P.C.A. Graphical support was provided by Doreen Martens.

## Declaration of interests

The authors declare no conflicts of interest.

[1]Division of Experimental Feto-Maternal Medicine, Department of Obstetrics and Fetal Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[2]Hamburg Center for Translational Immunology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

*Correspondence:
k.thiele@uke.de (K. Thiele) and p.arck@uke.de (P.C. Arck).

https://doi.org/10.1016/j.it.2023.09.011



## References

1. Arck, P.C. and Hecher, K. (2013) Fetomaternal immune cross-talk and its consequences for maternal and offspring's health. *Nat. Med.* 99, 548–556
2. Yüzen, D. *et al.* (2022) Tissue-resident immunity in the female and male reproductive tract. *Semin. Immunopathol.* 44, 785–799
3. Yang, M. *et al.* Spatiotemporal insight into early pregnancy governed by immune-featured stromal cells. *Cell* 186, 4271–4288.e24
4. Lima, P.D. *et al.* (2014) Leukocyte driven-decidual angiogenesis in early pregnancy. *Cell. Mol. Immunol.* 11, 522–537
5. Heusschen, R. *et al.* (2013) Profiling Lgals9 splice variant expression at the fetal-maternal interface: implications in normal and pathological human pregnancy. *Biol. Reprod.* 88, 22
6. Blois, S.M. *et al.* (2007) A pivotal role for galectin-1 in fetomaternal tolerance. *Nat. Med.* 13, 1450–1457
7. Koussounadis, A. *et al.* (2015) Relationship between differentially expressed mRNA and mRNA-protein correlations in a xenograft model system. *Sci. Rep.* 5, 10775
8. Chen, A. *et al.* (2022) Spatiotemporal transcriptomic atlas of mouse organogenesis using DNA nanoball-patterned arrays. *Cell* 185, 1777–1792
9. Roifman, M. *et al.* (1993-2023) Autosomal dominant Robinow syndrome, 2015. In *GeneReviews* (Adam, M.P. *et al.*, eds), University of Washington, Seattle (WA)
10. Marinić, M. *et al.* (2021) Evolutionary transcriptomics implicates HAND2 in the origins of implantation and regulation of gestation length. *eLife* 10, e61257
11. Fitzgerald, H.C. *et al.* (2019) Self-renewing endometrial epithelial organoids of the human uterus. *Proc. Natl. Acad. Sci. U. S. A.* 116, 23132