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(RESEARCH ARTICLE)

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PRENATAL DEVELOPMENT OF THE HUMAN LIVER THROUGH THE LENS OF SINGLE-CELL TECHNOLOGIES

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ABSTRACT

Background: Prenatal liver development integrates hepatobiliary differentiation, vascular and stromal assembly, and fetal hematopoiesis.

Methods: This structured narrative review evaluated peer-reviewed human embryonic and fetal-liver studies identified in PubMed/MEDLINE and PubMed Central through July 2026. Claims were weighted by donor-level replication, spatial or protein corroboration, and functional validation.

Results: Single-cell and spatial atlases consistently resolve epithelial, endothelial, mesenchymal, stromal, hematopoietic, and immune compartments and place selected states within ductal-plate, vascular, and hematopoietic niches. The review examines hepatobiliary hybrid progenitors, ID3-positive hepatoblast candidates, stellate-endothelial transitional states, and function-associated fetal HSPC populations. Agreement is strongest for major compartments and broad maturation patterns, but weaker for rare states, lineage direction, and inferred cell-cell signaling. Ambient RNA, dissociation bias, donor composition, and integration strategy can materially alter marker and pathway estimates.

Conclusion: These atlases provide a robust framework for prenatal liver biology, but most trajectories and communication networks remain inferential. Progress requires donor-level replication, standardized developmental-age metadata, orthogonal spatial or protein confirmation, causal perturbation, and public release of reusable data.

Keywords: Human Fetal Liver; Single-Cell RNA Sequencing; Spatial Transcriptomics; Developmental Trajectories; Cellular Atlas.

1 INTRODUCTION

Prenatal liver development couples' hepatobiliary differentiation with vascular, stromal, and hematopoietic assembly. These processes depend on tissue architecture, extracellular matrix, blood flow, and communication among epithelial, endothelial, mesenchymal, and blood-cell populations [1].

Histology and bulk profiling established major structures and averaged molecular programs but cannot resolve rare or transient states. Single-cell and spatial methods now distinguish cell states and localize them within developmental niches, supporting the Human Developmental Cell Atlas [2].

Human fetal-liver atlases consistently identify epithelial, endothelial, stromal, erythroid, myeloid, lymphoid, and progenitor compartments [3–8]. However, clusters are not lineages, pseudotime is not lineage tracing, and ligand-receptor co-expression does not prove signaling.

This review provides a claim-specific synthesis based on donor-level reproducibility, spatial or protein corroboration, and functional validation. It focuses on normal human prenatal tissue; adult, animal, disease, and organoid studies are used only as contextual or mechanistic evidence. Its aims are to identify reproducible cell states, assess orthogonal support for trajectories, niches, and markers, and explain cross-study disagreement.

Accordingly, this review addresses three questions: which prenatal human liver cell states are reproduced across datasets; which inferred trajectories, niches, and markers receive orthogonal or functional support; and which technical or sampling differences explain residual disagreement. The scope is normal human prenatal development. Disease, adult, animal, and organoid evidence is used only to test interpretation or translational relevance and is not treated as equivalent to direct evidence from prenatal human tissue.

2 MATERIALS AND METHODS

PubMed/MEDLINE and PubMed Central were searched through July 2026 using combinations of fetal/foetal, embryonic, or prenatal liver; human; and single-cell, single-nucleus, spatial transcriptomic, scATAC-seq, or multi-omic terms. Supplementary searches covered hepatoblasts, cholangiocytes, hematopoiesis, endothelium, stellate cells, trajectories, cell-cell communication, organoids, and named methods or datasets. Bibliographic metadata, sample characteristics, and accession information were checked against Crossref, publisher pages, GEO, ArrayExpress/BioStudies, and Human Cell Atlas records.

Table 1: Key single-cell and spatial studies of prenatal human liver development

Study	Human material and window	Technology	Principal contribution
Popescu et al., 2019 [3]	Human fetal liver, 7–17 pcw	scRNA-seq	Atlas of hematopoietic and immune states; no lineage tracing
Segal et al., 2019 [4]	Human fetal and adult liver	scRNA-seq; spatial and functional assays	Ductal-plate hybrid progenitors with protein, organoid, and xenograft support
Wang et al., 2020 [5]	Developing human liver; mouse comparison	scRNA-seq	Cross-species ID3-positive hepatoblast candidate
Hou et al., 2021 [6]	Human fetal liver, 8 and 17 pcw	scRNA-seq; spatial transcriptomics	Localized hepatocyte and erythroid programs; two-stage sampling
Wesley et al., 2022 [7]	Human fetal liver, 5–17 pcw	scRNA-seq; trajectory analysis	Hepatic and non-parenchymal programs across 17 fetal livers
Bailey et al., 2026 [8]	Nine FFPE prenatal liver samples	Single-cell spatial imaging	Mapped ductal-plate and multicellular niches with RNA validation
Ranzoni et al., 2021 [9]	Fetal liver and marrow HSPCs, 17–22 pcw	scRNA-seq; scATAC-seq	Linked HSPC RNA states to chromatin accessibility
Vanuytsel et al., 2022 [10]	Human fetal-liver hematopoietic cells	CITE-seq; flow cytometry; xenografts	Identified RNA-protein phenotypes enriched for engraftment

Abbreviations: CITE-seq, cellular indexing of transcriptomes and epitopes by sequencing; FFPE, formalin-fixed paraffin-embedded; HSPC, hematopoietic stem and progenitor cell; pcw, post-conception weeks; scATAC-seq, single-cell assay for transposase-accessible chromatin sequencing; scRNA-seq, single-cell RNA sequencing.

Priority was given to peer-reviewed primary studies from 2020 onward that analyzed human embryonic or fetal liver using single-cell, single-nucleus, spatial, epigenomic, or multimodal approaches. Earlier studies were retained when they established a foundational atlas or method. Adult-liver, cancer, animal, and organoid-only studies were excluded

as direct evidence and used only for method comparison or explicitly labeled mechanistic context; reference lists of eligible reports and relevant reviews were also screened.

Claims were graded qualitatively at the level of each major biological statement. Confidence increased with replication across donors or independent datasets and with spatial, protein, histological, perturbational, organoid, transplantation, or engraftment support. Single-dataset states, cross-sectional trajectories, co-expression-based communication networks, and markers vulnerable to ambient RNA or reference-dependent annotation were treated more cautiously.

This was a structured narrative review rather than a registered systematic review; no meta-analysis, PRISMA flow diagram, or numerical risk-of-bias score was used. Study selection and evidence appraisal were designed for transparent interpretation rather than exhaustive effect estimation. Table 1 summarizes the principal studies.

3 RESULTS AND DISCUSSION

3.1 Developmental framework of the prenatal human liver

3.1.1 Epithelial specification and hepatobiliary differentiation

Hepatic specification from foregut endoderm is followed by liver-bud expansion and gradual divergence of proliferative hepatoblast, hepatocyte-biased, and biliary-associated states [1, 5–7]. Developmental ages should remain in their original convention because post-conception and gestational weeks are not interchangeable.

Fetal hepatocytes progressively acquire synthetic and metabolic programs, whereas cholangiocyte differentiation localizes to the ductal plate [4, 7, 8]. Hybrid states suggest developmental competence but require donor replication, tissue localization, protein confirmation, or functional testing before lineage potential is inferred.

3.1.2 Vascularization and the hematopoietic niche

The fetal liver is a major site of hematopoietic stem and progenitor-cell expansion before hematopoiesis shifts toward bone marrow. Single-cell studies place this phase between embryonic HSC generation and marrow colonization [3, 11–13].

Endothelial, sinusoidal, perivascular, mesenchymal, and stellate-like cells develop alongside hematopoietic populations. Portal structures and ductal plates organize interacting epithelial, vascular, stromal, and blood-cell neighborhoods that dissociation alone cannot reconstruct [3, 6–8].

3.2 High-resolution technologies

Table 2 summarizes the complementary information and main limitations of the principal technologies.

Table 2: High-resolution technologies used to study prenatal human liver development

Technology	Information	Main strength	Main limitation
scRNA-seq	Whole-cell RNA	Scalable state discovery	Dissociation bias, dropout, ambient RNA, doublets; no spatial context
snRNA-seq	Nuclear RNA	Frozen or fragile tissue	Nuclear bias; weaker cytoplasmic and immune signals
Spatial transcriptomics	RNA with coordinates	Anatomical localization	Cell mixing, segmentation, and reference dependence
scATAC-seq and multi-omics	Chromatin with RNA/protein	Regulatory and surface context	Sparsity, cost, batch effects, and integration assumptions
Imaging-based profiling	Targeted RNA/protein in situ	Single-cell neighborhoods	Predefined panels, segmentation, and field-of-view limits

Abbreviations: scATAC-seq, single-cell assay for transposase-accessible chromatin sequencing; scRNA-seq, single-cell RNA sequencing; snRNA-seq, single-nucleus RNA sequencing.

3.2.1 Single-cell and single-nucleus transcriptomics

scRNA-seq enables broad cell-state discovery but is affected by dissociation bias, ambient RNA, doublets, dropout, and donor or library-depth differences. Recovered cell proportions need not reflect tissue proportions, and annotation

should use developmental rather than solely adult markers [14]. Ambient-RNA correction can materially change differential-expression and pathway results [15].

snRNA-seq supports frozen or difficult-to-dissociate tissue but captures nuclear-biased RNA and may resolve cytoplasmic or immune programs less well. Direct prenatal liver applications remain limited, so method-specific conclusions require caution. Adult paired analyses inform method comparison, not fetal biology [16].

3.2.2 Spatial and imaging-based methods

Spatial transcriptomics links expression to tissue coordinates but may mix cells or depend on reference-based deconvolution. In fetal liver, integration with scRNA-seq localized hepatocyte and erythroid programs at 8 and 17 pcw [6]. Imaging-based profiling, including spatial molecular imaging, provides single-cell or subcellular localization but uses predefined panels and requires careful segmentation [17, 18]. Bailey et al. combined spatial imaging of fixed prenatal liver with single-cell references and orthogonal RNA validation, enabling direct localization of epithelial, endothelial, stromal, and hematopoietic states [8]. Spot-level signals and imaging-defined single cells should not be treated as equivalent analytical units.

3.2.3 Epigenomics, multi-omics, and computational inference

scATAC-seq and multimodal assays connect RNA states with chromatin accessibility or surface phenotype. Fetal-liver studies have linked HSPC expression states to regulatory accessibility and engraftment-associated phenotypes [9, 10]. Broader multi-omic frameworks provide methodological context [19].

Trajectory and RNA-velocity methods infer ordering from cross-sectional data and depend on sampling, preprocessing, root selection, and kinetic assumptions [20, 21]. Ligand-receptor tools likewise generate hypotheses; database and scoring choices can substantially change predicted interactions [22, 23].

3.3 Cellular atlas of the developing human liver

3.3.1 Epithelial populations

Hepatoblasts are identified by combined fetal hepatic, epithelial, and proliferative programs rather than a single gene. An ID3-positive state was proposed in a human-mouse comparison [5], while fetal-liver atlases inferred hepatoblast-to-hepatocyte and hepatoblast-to-biliary paths [7]; both remain computational candidates.

Segal et al. localized hepatobiliary hybrid progenitors to the ductal plate and supported them with protein, organoid, and xenograft assays [4]. This exceeds clustering alone but is not equivalent to in situ lineage tracing. Fetal hepatocytes retain proliferative and immature metabolic programs, so isolated adult-associated genes do not establish maturity.

3.3.2 Endothelial, mesenchymal, and stellate populations

Endothelial and stromal atlases reveal heterogeneity linked to vascular and portal anatomy [5–8]. A proposed stellate-endothelial progenitor state lacks definitive lineage tracing [7]. Comparisons should prioritize expression programs and spatial evidence because dissociation and inconsistent labels affect collagen-rich, pericyte-like, fibroblast-like, portal, and stellate populations.

3.3.3 Hematopoietic and immune populations

Fetal-liver hematopoiesis includes stem and multipotent progenitors, erythroid and myeloid differentiation, mast cells, innate lymphoid and natural-killer precursors, and B- and T-lineage states [3]. Cross-organ analyses caution that many immune states are not liver-exclusive [24, 25], while chromatin and multi-omic studies extend transcriptomic annotation [9, 26].

3.3.4 Rare and transitional states

Rare-state claims require cross-donor and preferably cross-method support. Hepatobiliary hybrid progenitors have the strongest combined spatial and functional evidence [4]. ID3-positive hepatoblast and stellate-endothelial states remain candidates [5, 7]. GPI-80- and CD201/EPCR-associated HSPC phenotypes enrich for engraftment competence [10], and MYCT1 perturbation provides direct functional support for a fetal-HSPC regulatory program [27]. No single surface marker should be treated as an absolute measure of functional competence.

3.4 Temporal trajectories and spatial organization

Cross-sectional atlases reveal age-associated changes but not longitudinal lineage histories. Hematopoietic composition, hepatic and biliary paths, and regulatory accessibility therefore represent candidate temporal sequences that should be tested for donor dependence and algorithmic stability [3, 7, 9].

Spatial data connect dissociated states to ductal plates, sinusoidal endothelium, stroma, and hematopoietic niches. Multicellular neighborhoods and chemokine-associated features have been mapped in fixed prenatal liver [8], but spatial proximity does not prove receptor activation. Mouse HSC/MPP expansion units remain contextual hypotheses [28].

3.4.1 Evidence-weighted cross-study synthesis

Across human studies, agreement is strongest for major cell classes and broad age-associated shifts, and weaker for rare-state boundaries, exact trajectories, and signaling networks [3–10]. Differences in developmental windows, cell enrichment, dissociation, sequencing, integration, and annotation explain much apparent discordance; absence from one atlas does not establish biological absence. This is especially important for rare populations that may be lost during dissociation or merged during integration.

Evidence is strongest when donor-level transcriptomic replication is followed by spatial or protein confirmation and functional testing. Hepatobiliary hybrid progenitors and engraftment-associated HSPC phenotypes meet more of these criteria [4, 10], whereas ID3-positive hepatoblast and stellate-endothelial states remain provisional [5, 7]. MYCT1 perturbation adds causal support [27].

Concordance is claim-specific. Hematopoietic studies broadly agree on stem/progenitor, erythroid, myeloid, and lymphoid organization, while chromatin, surface-protein, and transplantation assays add independent evidence [3, 9, 10, 26, 27]. Epithelial atlases consistently recover hepatoblast, hepatocyte-biased, and biliary programs but differ in intermediate-state nomenclature [4–8]. Spatial studies support ductal-plate and multicellular niches [6, 8] without making spot-level and single-cell neighborhoods equivalent. Consensus at compartment level should not be extrapolated automatically to subclusters, trajectories, or signaling.

3.5 Representative developmental markers and regulatory programs

Table 3: Major cell populations, developmental states, and representative markers in the prenatal human liver

Cell population or state	Representative markers	Developmental interpretation	Refs.
Hepatoblasts	AFP, LGR5, MKI67	Proliferative fetal epithelial progenitors	[7]
Fetal hepatocytes	ALB, AFP, TTR, APOA1	Fetal synthetic programs; incomplete adult maturation	[4–7]
Cholangiocyte and ductal-plate populations	EPCAM, KRT19, SOX9, CD24	Biliary specification and ductal-plate organization	[4, 7, 8]
Endothelial and sinusoidal populations	PECAM1, KDR, EMCN, VWF	Vascular organization and niche support	[5–8]
Mesenchymal, stellate, and perivascular cells	COL1A1, COL3A1, RGS5, PDGFRB	Matrix production and perivascular support	[5–8]
Hematopoietic stem and progenitor populations	CD34, SPINK2, HLF, GATA2	Expansion and lineage priming; markers alone do not establish function	[3, 9, 10, 26, 27]
Erythroid populations	GYPA, KLF1, HBB, HBA1	Age-associated fetal erythropoiesis	[3, 26]
Myeloid and macrophage populations	CSF1R, LYZ, CD68, FCER1G	Tissue- and age-associated immune states	[3, 25]

Notes: Marker panels are representative and non-exclusive. AFP, LGR5, and MKI67 mark hepatoblast-associated states [7]; fetal hybrid progenitors combine hepatic (ALB, APOE, TF, HNF4A) and biliary (KRT19, SOX9, CD24) programs [4]. Expression alone does not establish lineage potential or clinical validity.

Markers should be interpreted as panels within developmental and anatomical context. Identity markers support annotation, state markers vary with maturation, and function-associated markers require experimental validation.

Combinations of transcripts, tissue localization, protein confirmation, and functional assays are stronger than isolated genes. Table 3 summarizes representative examples.

The combined hepatic and biliary program of hepatobiliary hybrid progenitors illustrates why marker panels must be interpreted in anatomical and experimental context [4]. Developmental transcripts such as AFP and DLK1 indicate developmental state rather than disease specificity [4]. In hematopoietic studies, joint RNA-protein profiling, perturbation, and transplantation provide a stronger validation ladder, although enrichment remains assay- and model-dependent [10, 27].

3.6 Data integration and translational implications

3.6.1 Integration and reproducibility

Multimodal integration links RNA states with protein, chromatin, or spatial information but may either remove genuine developmental differences or retain technical effects. Credible populations should remain detectable within donors and across reasonable analytical choices [29–33]. Benchmarking must balance batch correction against conservation of genuine developmental variation.

3.6.2 Organoids, regenerative medicine, and congenital disease

Fetal references help benchmark stem-cell-derived hepatocytes and organoids; multilineage communication also supports liver-bud development [34]. Recent models co-developed hepatobiliary, endothelial, mesenchymal, and hematopoietic lineages, while cross-protocol atlases showed incomplete and protocol-dependent recapitulation of native fetal compartments [35, 36]. Organoids remain limited by perfusion, immune complexity, stromal architecture, and developmental timing [37]. Native fetal tissue therefore remains the reference standard for developmental fidelity.

Normal prenatal atlases also provide stage-matched references for congenital disease. A fetal-blood multi-omics study in Down syndrome illustrates how affected cells can be compared with normal developmental states [38]. Similar approaches may inform congenital cholangiopathies, metabolic disorders, vascular abnormalities, or disrupted hematopoietic support, but clinical utility requires independent cohorts, reproducible assays, and evidence beyond transcript clustering. The principal claim-specific evidence gaps and corresponding validation priorities are summarized in Table 4.

Table 4: Claim-specific evidence gaps and validation priorities

Priority claim	Current support	Main uncertainty	Most informative next test
Hepatobiliary hybrid progenitors	RNA, spatial, protein, organoid, and xenograft evidence [4]	Persistence and in situ lineage potential	Independent spatial validation and lineage-resolving perturbation
ID3-positive hepatoblast state	Cross-species transcriptomic candidate [5]	Human reproducibility and function	Human-only replication, protein localization, and perturbation
Stellate-endothelial progenitor state	Trajectory candidate with selected organoid support [7]	Directionality and stable in vivo existence	Spatial localization plus clonal or perturbational testing
Engraftment-associated fetal HSPCs	RNA-protein, flow, xenograft, and MYCT1 evidence [10, 27]	Model dependence and clinical transferability	Independent donors and standardized transplantation
Predicted niche signaling	Spatial proximity and ligand-receptor compatibility [6, 8, 22, 23, 28]	Activation, directionality, and causality	Spatial perturbation with pathway readouts

Note: This author-generated synthesis distinguishes replicated observations from unresolved lineage or signaling claims and identifies the experiments most likely to reduce claim-specific uncertainty. Key supporting studies are cited directly in the table.

3.7 Current limitations and future directions

Human prenatal tissue is scarce, developmental windows and donor numbers are limited, and age may be confounded with tissue handling, platform, or center. Demographic representation is also limited. Dissociation, nuclear sampling, spot mixing, predefined imaging panels, doublets, and stress responses can distort recovered populations.

This narrative review did not use duplicate screening, PRISMA reporting, or a quantitative risk-of-bias instrument, and its biomedical and English-language focus may have missed non-indexed studies. Heterogeneity in age reporting, tissue source, enrichment, assay design, and validation precluded meta-analysis. These constraints may contribute to apparent non-replication.

Future atlases should treat the donor as the biological replicate, preserve exact developmental-age metadata, test annotation and integration sensitivity, confirm rare states spatially or at protein level, and use perturbation for causal claims. Standardized reporting of tissue source, handling, and developmental age would improve cross-study comparison. Dense developmental sampling, cross-cohort replication, and release of raw data, matrices, coordinates, annotations, and code are priorities.

4 CONCLUSION

Single-cell, spatial, and multimodal studies reproducibly define the major compartments and broad developmental shifts of the prenatal human liver. Rare-state boundaries, trajectory direction, and niche causality remain less secure. Confidence is highest when donor-level replication is combined with anatomical, protein, and functional validation. The priority is not additional clustering alone, but denser sampling and causal validation. Until then, developmental markers are best used for research and organoid benchmarking rather than as clinical biomarkers.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Disclosure of conflict of interest

The author declares no conflict of interest.

Statement of ethical approval

Ethical approval and informed consent were not applicable because this review analyzed previously published studies and did not collect new data from human participants or animals.

Data availability statement

No new datasets were generated or analyzed. All evidence discussed in this review is available in the cited publications and, where applicable, in their associated public repositories.

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