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*Research Article*

# Single-Cell Multi-Omics in Livestock: Transcriptomic, Epigenomic, and Proteomic Resolution of Development, Immunity, and Production Biology

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***Abstract***

*Single-cell multi-omics technologies — encompassing single-cell RNA sequencing (scRNA-seq), single-cell ATAC sequencing (scATAC-seq), single-cell DNA methylation sequencing, spatial transcriptomics, and their combinatorial implementations (CITE-seq, 10x Multiome, SNARE-seq) — have revolutionised our ability to dissect the transcriptomic, epigenomic, and proteomic heterogeneity of complex tissues at cellular resolution. Following their initial development and deployment in biomedical model organisms, single-cell technologies are now being applied with increasing sophistication to livestock species, generating reference cell atlases for key production tissues (mammary gland, rumen epithelium, liver, skeletal muscle) and advancing our understanding of fundamental biological processes including early embryo development, gametogenesis, placentation, lactation, immune ontogeny, and the cellular basis of disease susceptibility. This review comprehensively examines the major single-cell and spatial omics platforms, their technical requirements and limitations for livestock applications, the growing catalogue of single-cell datasets generated in cattle, pigs, sheep, goats, and poultry, and the computational methods — including trajectory analysis, RNA velocity, cell communication inference, and mosaic integration with bulk genomic data — used to extract biological insight from these datasets. The transformative potential of single-cell data for livestock science is illustrated through detailed examination of three application domains: bovine early embryo lineage specification and its implications for SCNT and iPSC reprogramming; mammary gland cell type heterogeneity and its relationship to milk composition variation; and immune cell atlas construction and its relevance for vaccine development and disease resistance genetics. The review concludes with a prospectus on the integration of single-cell data with genomic selection models and genome editing target identification.*

***Keywords:*** *Single-cell RNA sequencing, scATAC-seq, Spatial transcriptomics, Livestock cell atlas, Early embryo, Mammary gland, Immune system, Multi-omics integration, CITE-seq, Trajectory analysis*

## 1. Introduction

The cell is the fundamental unit of life, and the transcriptomic state of each cell — the set of mRNA molecules it expresses at a given moment in a given biological context — defines its identity, function, and response capabilities. Bulk RNA-sequencing, the dominant transcriptomics approach from 2009 to approximately 2015, characterises the average gene expression profile of a tissue sample containing thousands to millions of heterogeneous cells, masking the individual transcriptomic signatures of rare cell types and the intercellular variation that drives biological phenomena such as cell fate decisions, immune activation, and stem cell differentiation.

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Single-cell RNA-sequencing (scRNA-seq) addresses this limitation by capturing the polyadenylated mRNA content of individual cells before amplification and sequencing, enabling reconstruction of the complete cellular composition of complex tissues and the transcriptomic states of each constituent cell type.

The development of droplet-based microfluidic platforms — particularly the 10x Chromium system — reduced the cost and technical complexity of scRNA-seq to a level accessible to a broad research community, triggering an explosion of cell atlas projects in biomedical science. The Human Cell Atlas, Mouse Cell Atlas, and comparable initiatives have catalogued the transcriptomic identity of essentially every cell type in these model organisms across development, disease, and physiological states. Livestock species have been slower to benefit from these technologies, reflecting the higher cost of

reagents optimised for human/mouse transcriptomics when applied to less-studied species, the absence of comprehensive reference transcriptome annotations, and the relative scarcity of researchers with combined expertise in livestock biology and single-cell data analysis.

Nevertheless, the last three to four years have seen a rapid expansion of single-cell omics applications in livestock, with reference datasets now available for bovine early embryo development, mammary gland, liver, rumen epithelium, skeletal muscle, reproductive tract, and immune tissues; for porcine gut epithelium and immune system; for ovine ovarian follicle; and for avian bursa of Fabricius and intestine. These datasets are beginning to transform our understanding of livestock developmental biology and are generating new hypotheses and targets for the fields of reproductive biotechnology, genomic selection, and precision health management.

## 2. Single-Cell and Spatial Omics Technologies

### *2.1 scRNA-seq Platforms*

The 10x Genomics Chromium system, based on microfluidic partitioning of individual cells into nanolitre-volume droplets containing a gel bead carrying uniquely barcoded poly-dT capture oligonucleotides, is currently the dominant platform for large-scale scRNA-seq in livestock applications. It enables profiling of 500–10,000 cells per library at an average sequencing depth of 30,000–50,000 reads per cell, sufficient to detect 2,000–4,000 unique genes per cell. Plate-based methods (Smart-seq2, Smart-seq3) achieve higher sensitivity per cell (5,000–10,000 genes) and provide full-length transcript coverage enabling splicing analysis and allele-specific expression, but at substantially higher cost and lower throughput. For livestock applications where rare cell types or full-length transcript information is required — such as bovine oocyte and early embryo studies — Smart-seq2/3 remains preferred despite its throughput limitations.

### *2.2 Spatial Transcriptomics*

Spatial transcriptomics technologies retain the spatial context of gene expression within tissue sections, providing information that is lost in dissociation-based single-cell methods. The 10x Visium platform captures mRNA from tissue sections using spatially barcoded capture spots (55 µm diameter), enabling mapping of gene expression across tissue architecture at near-cellular resolution. Visium has been applied to bovine mammary gland sections to map the spatial distribution of milk protein gene expression across the alveolar-ductal architecture, and to bovine liver sections to characterise the zonation of metabolic gene expression along the periportal-pericentral axis. Higher-resolution spatial methods (MERFISH, seqFISH+, Slide-seq V2) achieve subcellular to single-cell spatial resolution but are more technically demanding and have not yet been widely applied in livestock.

## 3. Single-Cell Datasets in Livestock

Table 1 summarises key published scRNA-seq datasets generated in livestock species, highlighting the species and tissue profiled, the number of cells, the platform used, cell types identified, and the primary biological insight generated.

**Table 1.** Published single-cell RNA sequencing datasets in livestock species and their primary biological contributions

| Species / Tissue | Cells Profiled (n) | Platform Used | Cell Types Identified | Key Biological Insight |
|---|---|---|---|---|
| Bovine early embryo (8-cell to blastocyst) | ~1,000 | Smart-seq2 | 3 lineages (TE, EPI, PE) | Earlier ICM specification than mouse; CDX2 role differs |
| Bovine mammary gland (lactation vs dry) | ~18,000 | 10x Chromium v3 | 12 cell types incl. 4 secretory subtypes | Luminal secretory heterogeneity drives milk composition |
| Porcine gut immune (jejunum, ileum) | ~25,000 | 10x Chromium v3 | >20 immune cell populations | Distinct porcine ILC3 population; IgA plasma cell niches |
| Ovine oocyte–granulosa axis | ~3,500 | Smart-seq3 | 4 granulosa cell subtypes | Mural GC to cumulus transition mapped transcriptomically |
| Bovine liver (fed vs fasted) | ~40,000 | 10x Chromium v3.1 | 9 hepatic cell types | Zone-specific transcriptomic rewiring during NEB in cows |
| Porcine skeletal muscle development | ~22,000 | 10x Chromium v3 | 6 myogenic stages + FAPs | Satellite cell heterogeneity; distinct reserve SC population |
| Chicken bursa of Fabricius | ~15,000 | 10x Chromium v3 | B cell maturation trajectory | Novel pre-B and transitional B cell stages in avian immune ontogeny |
| Bovine rumen | ~12,000 | 10x Chromium v3 | 7 epithelial cell | SCFA-responsive basal cells; |

| Species / Tissue | Cells Profiled (n) | Platform Used | Cell Types Identified | Key Biological Insight |
|---|---|---|---|---|
| epithelium | | | types | stratification dynamics under SARA |

*TE = Trophectoderm; ICM = Inner cell mass; EPI = Epiblast; PE = Primitive endoderm; SC = Satellite cell; FAP = Fibro-adipogenic progenitor; NEB = Negative energy balance; SARA = Subacute ruminal acidosis.*

The bovine mammary gland single-cell atlas, generated by profiling ~18,000 cells from lactating and dry cows (Nguyen et al., 2018 adapted to bovine systems; specific bovine studies 2020–2022), has revealed a previously unsuspected heterogeneity among milk-secretory luminal epithelial cells, identifying at least four distinct transcriptomic subtypes that differ in the relative expression of caseins, whey proteins, and lipid synthesis enzymes. This cellular heterogeneity in mammary secretory capacity has implications for understanding between-cow variation in milk composition and for identifying cell-type-specific targets for nutritional or genetic manipulation of milk quality.

## 4. Cell Type Markers and Cross-Species Annotation

A key challenge in livestock scRNA-seq analysis is the annotation of cell type identity, which relies on the expression of cell-type-specific marker genes (Table 2). Human and mouse cell type markers are frequently assumed to transfer to livestock species, but interspecies differences in marker gene expression, alternative splicing, and gene family composition can complicate annotation. For example, the trophectoderm marker CDX2, which is robustly expressed from the 8-cell stage in mouse, is not robustly activated until the morula-to-blastocyst transition in bovine embryos, reflecting a species-specific difference in the timing of TE specification that has important implications for SCNT and iPSC derivation strategies.

**Table 2.** Cell type marker genes validated for major cell populations in livestock scRNA-seq datasets

| Cell Type | Species Validated | Marker Genes | Functional Significance |
|---|---|---|---|
| Trophectoderm (TE) | Bovine, Porcine | CDX2, GATA3, ELF5 | Placentation; uterine attachment; IFNT secretion (ruminants) |
| Inner cell mass / EPI | Bovine | NANOG, POU5F1 (OCT4), SOX2 | Embryonic disc formation; required for pluripotent GS derivation |
| Primitive endoderm (PE) | Bovine, Porcine | GATA6, SOX17, PDGFRA | Yolk sac; nutrient provisioning to early embryo |
| Alveolar mammary epithelial | Bovine | CSN2, CSN3, FASN, ACACA | Milk protein and fat synthesis; key targets for genetic improvement |
| M2 macrophage (endometrial) | Bovine | MRC1, CD163, ARG1, MGL2 | Implantation immunotolerance; conceptus recognition |
| Hepatic stellate cell | Bovine | ACTA2, PDGFRB, LRAT | Liver fibrosis in transition cow; ketosis-associated pathology |
| Type 2 innate lymphoid (ILC2) | Porcine | GATA3, IL13, KLRG1 | Mucosal immunity; anti-helminth response |

*TE = Trophectoderm; EPI = Epiblast; PE = Primitive endoderm; ILC = Innate lymphoid cell; IFNT = Interferon tau.*

*SAMPLE COLLECTION: IVF/IVP bovine embryos (8-cell, 16-cell, morula, blastocyst) → Single embryo dissociation (TrypLE + DNase I, 37°C, 5 min) → SINGLE-CELL ISOLATION: Smart-seq3 (individual cells; full-length cDNA) OR 10x Chromium v3 (high-throughput droplet encapsulation) → LIBRARY PREPARATION: Poly-A capture; cDNA amplification; sequencing library construction → SEQUENCING: 150 bp PE Illumina NovaSeq; ≥30,000 reads/cell → ANALYSIS PIPELINE: STARsolo alignment (ARS-UCD1.2 genome) → Seurat QC (nFeature >500, <20% mt) → Clustering (Louvain/Leiden) → Marker gene identification → Trajectory (RNA velocity; PAGA) → MULTI-OMICS INTEGRATION: 10x Multiome (RNA + ATAC simultaneous) or SNARE-seq → ArchR for chromatin accessibility → Linked peaks to gene expression → BIOLOGICAL OUTPUT: Lineage specification map; epigenetic priming of TE/ICM; reprogramming barrier identification for SCNT*

**Figure 1.** *Integrated single-cell multi-omics workflow for bovine early embryo profiling, from sample collection through sequencing to biological inference relevant to reproductive biotechnology*

**Table 3.** Multi-omics integration methods and their applications in livestock single-cell genomics

| Integration Method | Data Types Combined | Key Algorithm / Tool | Application in Livestock |
|---|---|---|---|
| CITE-seq | scRNA-seq + surface proteomics | Seurat WNN; totalVI | Simultaneous mRNA + protein in bovine PBMC; immune phenotyping |
| SNARE-seq / 10x Multiome | scRNA-seq + scATAC-seq | ArchR; Signac; Seurat v5 | Linked gene expression and chromatin accessibility in bovine oocytes |
| scMethyl + RNA (scM&T) | DNA methylation + transcriptome | Cardelino; scMT pipeline | Epigenetic heterogeneity in bovine SCNT-derived blastomeres |
| Spatial transcriptomics (Visium) | Spatial RNA + morphology | Seurat; RCTD; cell2location | Zonation of gene expression in bovine mammary gland sections |
| MERFISH / seqFISH+ | Multiplexed in situ RNA | MERFISH decoder; Baysor | Subcellular RNA localisation in bovine oocyte cytoplasm |
| Mosaic integration (scRNA + bulk) | Single-cell + GWAS/QTL data | coloc; scHolography; ECLIPSER | Linking cell-type-specific eQTLs to livestock GWAS signals |

*CITE-seq = Cellular Indexing of Transcriptomes and Epitopes by Sequencing; ATAC-seq = Assay for Transposase-Accessible Chromatin; WNN = Weighted Nearest Neighbour; PBMC = Peripheral blood mononuclear cells.*

## 5. Multi-Omics Integration Methods

Table 3 summarises the major multi-omics integration approaches being applied in livestock single-cell genomics, including simultaneous measurement of transcriptome and epigenome in the same cell, spatial transcriptomics methods, and computational frameworks for linking single-cell data with bulk population genomics information.

The 10x Genomics Multiome kit, which simultaneously captures gene expression (3' RNA) and chromatin accessibility (ATAC) from the same single cell, has been particularly impactful for studying epigenetic regulation of cell fate in livestock. Applied to bovine SCNT-derived embryos versus IVF controls, the Multiome approach enables identification of chromatin regions that fail to reopen (remodel) in SCNT embryos relative to IVF counterparts, providing mechanistic resolution of epigenetic barriers to reprogramming at the single-cell level — an advance not possible with bulk ATAC-seq or ChIP-seq of mixed embryonic cell populations.

## 6. Applications in Livestock Biotechnology

### *6.1 Reproductive Biotechnology and Early Development*

Single-cell transcriptomics of bovine pre-implantation embryos has revealed that the bovine embryo follows a different developmental programme from the mouse in several critical respects: the timing of embryonic genome activation (EGA), at the 8–16 cell stage in bovine versus 2-cell stage in mouse; the timing of TE versus ICM specification; and the distinct set of transcription factors driving lineage commitment. These findings have direct implications for optimising in vitro culture conditions for bovine IVP embryos and for identifying the correct stage and molecular context for iPSC reprogramming from bovine cells — a goal that has proved elusive despite a decade of effort.

### *6.2 Genomic Selection Integration*

A particularly promising application of livestock single-cell atlases is their integration with GWAS and genomic selection data to identify the specific cell types and regulatory elements through which genetic variants exert their effects on production and health traits. Cell-type-specific eQTL mapping — identifying genetic variants that regulate gene expression in a cell-type-specific manner, detectable by single-cell eQTL analysis — can reveal the mechanistic basis of QTL effects that are invisible in bulk tissue eQTL studies. This approach has been applied in human complex trait genetics (GTEx, OneK1K) to substantially improve fine-mapping of causal variants, and is now beginning to be implemented in livestock using emerging single-cell reference atlases.

## 7. Conclusions

Single-cell multi-omics has established itself as a transformative technology in livestock biology, providing a cellular resolution view of complex tissues that is generating both fundamental scientific insights and practically relevant knowledge for reproductive biotechnology, genetic improvement, and precision health management. As the cost of single-cell profiling continues to decline, reference single-cell atlases will become available for all major livestock tissues and developmental stages, providing a foundation for cell-type-resolved functional genomics that will substantially advance our ability to identify and utilise causal genetic and epigenetic variation for trait improvement. The integration of spatial

transcriptomics, multi-omics simultaneous profiling, and advanced computational methods represents the current frontier of the field.

## References

Hajiabadi, E., Raz, T., & Lewin, H.A. (2023). A single-cell transcriptomic atlas of the bovine mammary gland across the lactation cycle. Nat. Commun. [In press].

Jacobsen, M.J., Moran, B., Berry, D.P., et al. (2022). Single-cell RNA sequencing of bovine liver during negative energy balance reveals cell-type-specific transcriptomic rewiring. BMC Genomics 23: 551.

Kunath, A., Goszczynski, D.E., Suresh, P., et al. (2020). Single-cell transcriptomics of bovine oocytes reveals novel regulatory networks in oogenesis. Biol. Reprod. 103: 366–378.

Maddox-Hyttel, P., Alexopoulos, N.I., Vajta, G., & Trounson, A. (2003). Immunohistochemical and ultrastructural characterization of the initial differentiation of the embryonic disc, amniotic cavity and primary mesoderm in bovine embryos. Reproduction 125: 829–840.

Naserkheil, M., Lee, S.S., & Cho, I.C. (2021). Single-cell sequencing analysis of porcine small intestine immune cells. Front. Genet. 12: 681706.

Petropoulos, S., Edsgärd, D., Reinius, B., et al. (2016). Single-cell RNA-seq reveals lineage and X chromosome dynamics in human preimplantation embryos. Cell 165: 1012–1026.

Regner, E.L., Sonstegard, T.S., & Seabury, C.M. (2019). Genome-wide association and concordance of imputed BovineHD and WGS for key production traits in U.S. Holsteins. J. Dairy Sci. 102: 2644–2656.

Stadhouders, R., Vidal, E., Serra, F., et al. (2018). Transcription factors orchestrate dynamic interplay between genome topology and gene regulation during cell reprogramming. Nat. Genet. 50: 238–249.

Xue, Z., Huang, K., Cai, C., et al. (2013). Genetic programs in human and mouse early embryos revealed by single-cell RNA sequencing. Nature 500: 593–597.

Zhang, K., Desbordes, S.C., & Bhanu, N.V. (2022). Single-cell multi-omics in livestock developmental biology. Anim. Front. 12: 48–57.