Analysis Tutorial Overview

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1. Title

Compare single cell RNA transcriptomic profiles across different stages of spermatogenesis using Shiny app

1. Research question(s)

How can we identify and analyze gene markers in distinct stages of spermatogenesis? How can we more effectively visualize scRNA-seq data to interpret gene expression profiles throughout this process?

1. Objective(s)
2. Access and reorganize publicly available scRNA-seq dataset of testicular tissue to enable easy sorting of gene markers by assigned cell types or defined stages of spermatogenesis
3. Create an interactive Shiny application to display the reorganized scRNA-seq dataset and allow flexible exploration and manipulation of gene expression profiles
4. Provide public access for application download and customized analysis of spermatogenesis-related genes expression profiles
5. Approach

A selected single-cell RNA-seq dataset of mouse testis, obtained from a previously published study (Lukasse et al., 2018), is publicly available through the NCBI-GEO scRNA-seq database. This dataset, formatted as a Seurat object (Satija Lab, n.d.)—a widely used R data structure for processing scRNA-seq data—will be accessed and reorganized using distinct biomarkers to distinguish cell types. Selected gene markers, including *Stra8* (Ma et al., 2018), *Sycp3* (Ozaki et al., 2011), and *Cd46* (Mizuno et al., 2005), will be used to identify and classify cells into spermatogonia, spermatocytes, and spermatids, respectively. A Shiny app (Posit, n.d.) will then be developed to load the reorganized dataset and display gene expression profiles by cell type, enabling comparison across spermatogenesis stages and facilitating the identification of potentially novel gene markers. A previously developed Human Infertility Single-Cell Atlas (HISTA) will serve as a reference for the types of gene profiles and features that can be incorporated into our Shiny app (Mahyari et al., 2024).

1. Selected References

Lukassen S, Bosch E, Ekici AB, Winterpacht A. Single-cell RNA sequencing of adult mouse testes. *Sci Data.* 2018;5:180192. doi:10.1038/sdata.2018.192

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