90

Investigating Regulation of Spermiogenesis Using a Novel Method of Spermatid Isolation for Comparative Omics Analysis

Tracy Clement, Ph.D.

T3: TEXAS A&M TRIADS FOR TRANSFORMATION
A President's Excellence Fund Initiative

Abstract

Background and significance: Two significant needs in reproductive health remain identifying causes of male infertility and increasing male contraceptive options. Disruptions in spermiogenesis impact germline contributions to offspring and male fertility and thus understanding the mechanisms involved is critical. This phase of sperm development includes significant chromatin remodeling during a highly coordinated cellular morphogenesis including ordered histone replacements for chromatin compaction, ultimately producing a sperm with chromatin 6 times more compact than in a metaphase cell. As spermiogenesis progresses and chromatin is compacted, transcription is repressed and the developing spermatid must increasingly rely on post-transcriptional regulation for continued development. However, the mechanisms controlling chromatin dynamics and post-transcriptional regulation in the spermatid are only beginning to come to light. Understanding the mechanisms important for spermiogenesis has been hindered by current testicular cell density gradient cell isolation techniques, which fraction the developing spermatids into only two semi-purified populations. Previous characterization of factors critical for spermiogenesis have relied on identification from these semi-pure populations and the availability of specific labels, such as antibodies and oligo probes, to identify specific key steps of development in histological and cytological samples. While single-cell technologies have recently allowed refined inquiry into spermatid transcriptomes, methods for refined separation of pooled spermatid populations is still required for key investigations including translational and post-translational regulation. Two major hinderances for assessment of spermatogenesis in a developmentally refined step-wise manor are the co-occurance of all developmental stages in the testis, and the adherence of the germ cells to the seminiferous epithelium by cell-cell junctional complexes. In this study, techniques routinely used to isolate spermatids, are modified and combined in a novel way to obtain populations of cells in specific refined steps of development. These techniques will now be utilized for isolating spermatid populations in refined steps of development for comparisons of transcriptomes, ribonomes and proteomes, to investigate translational regulation, and histone modifications in spermiogenesis and male fertility.

Introduction

Spermatogenesis in mice starts with spermatogonia (A-B), which enter meiosis (Pl, L, Z, P), to form haploid spermatids (1-16) which are released from the seminiferous epithelium at spermiation as sperm. In mice the develop of a spermatogonia into sperm takes about 35 days. All developmental steps of spermatogenesis are present in the adult testis simultaneously.

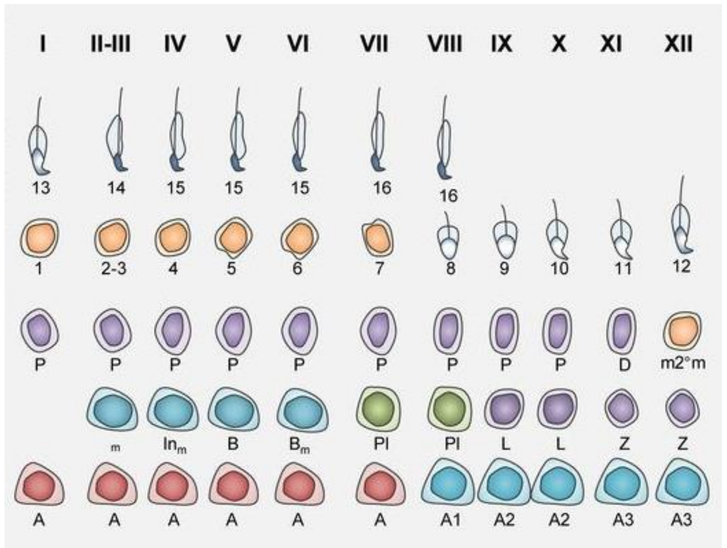
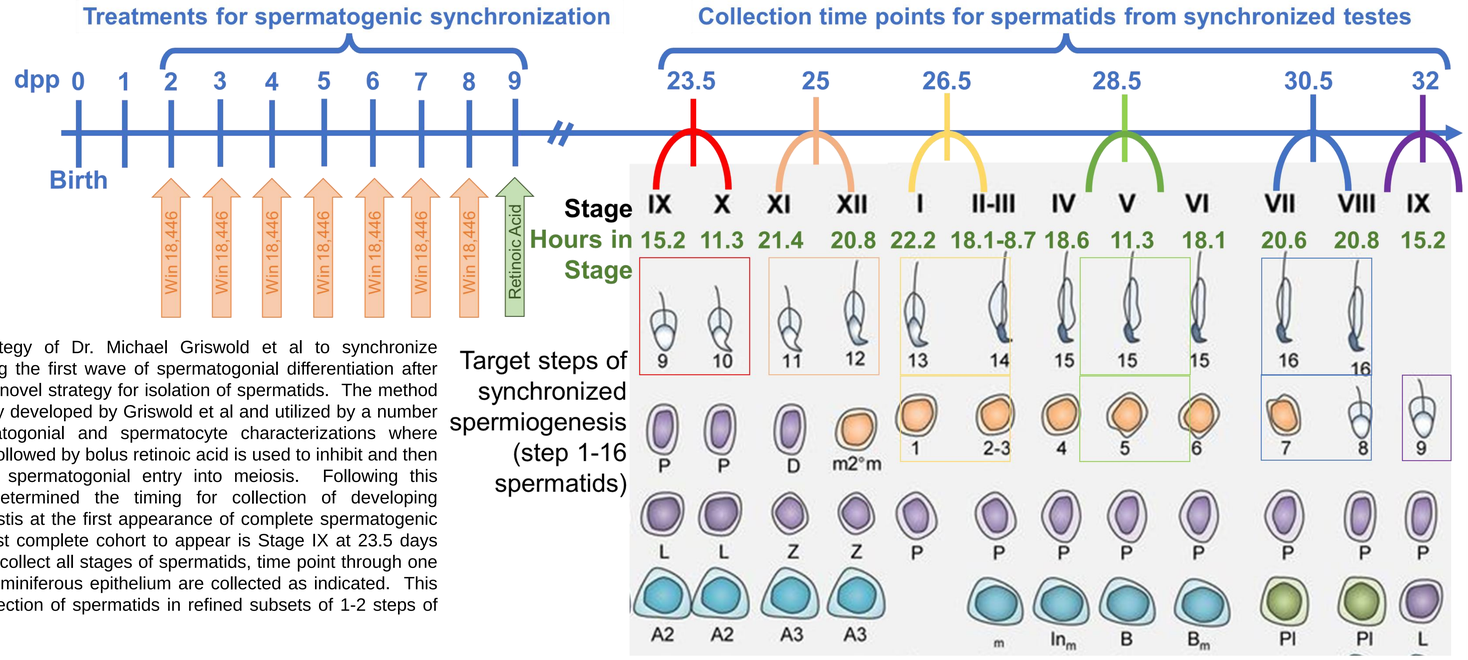


Figure from Griswold, 2016; "Spermatogenesis: The Commitment to Meiosis"

Traditional methods of separation of germ cells from the testis involve enzymatic digestion with trypsin and collagenase, followed by mechanical disruption using the sheer force of pipetting. The resulting mixed cell populations are enriched for germ cells, while excluding cells of the testis interstitial compartment. The mixed germ cells can then be further separated by density or gravity sedimentation, or by FACs sorting. Using these techniques, the 16 steps of spermatids can be crudely separated into two populations (round spermatidsand elongating/condensed spermatids) with about 90% purity.

Novel Collection Strategy from Spermatogenic Synchronized Testes



Starting with the strategy of Dr. Michael Griswold et al to synchronize spermatogenesis during the first wave of spermatogonial differentiation after birth, we determined a novel strategy for isolation of spermatids. The method begins with the strategy developed by Griswold et al and utilized by a number of groups for spermatogonial and spermatocyte characterizations where retinoic acid inhibition followed by bolus retinoic acid is used to inhibit and then simultaneously induce spermatogonial entry into meiosis. Following this synchronization we determined the timing for collection of developing spermatids from the testis at the first appearance of complete spermatogenic stage cohorts. The first complete cohort to appear is Stage IX at 23.5 days post RA treatment. To collect all stages of spermatids, time point through one 8.6 day cycle of the seminiferous epithelium are collected as indicated. This strategy allows for collection of spermatids in refined subsets of 1-2 steps of development.

Target steps of synchronized spermiogenesis (step 1-16 spermatids)

Germ Cell Isolation Optimization

The developing spermatids are anchored in the seminiferous epithelium by a number of junctional complexes including adherens junctions, tigh using retinoic acid inhibition t junctions,

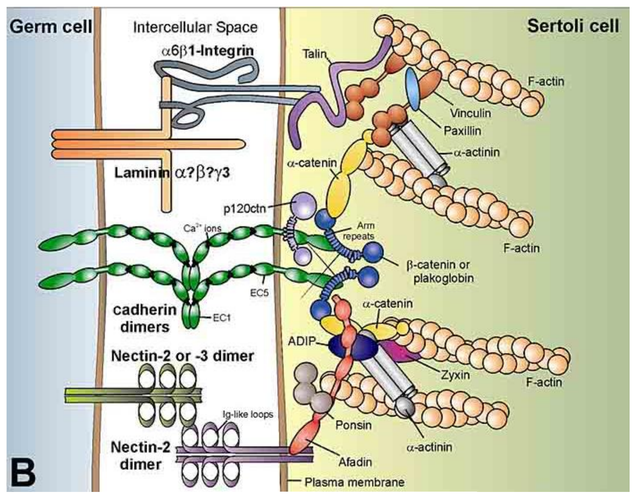
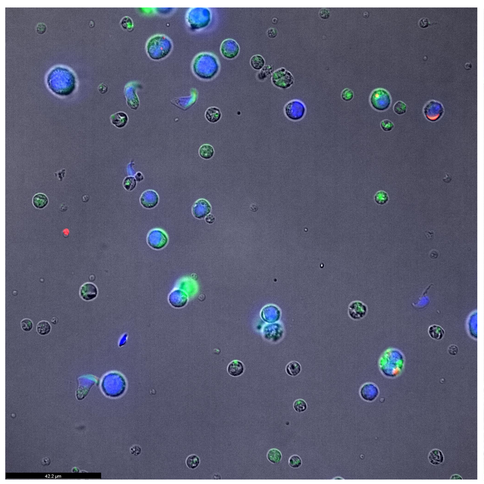
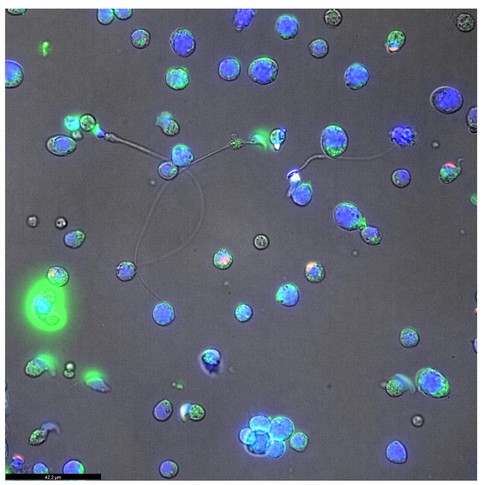


Figure from Goossens and Van Roy, 2005

To optimize germ cell isolation with minimal damage of germ cells or loss of cytoplasmic contents, the use of EDTA, DTT, and acrylamide in conjunction with collagenase, accutase, papain, and or trypsin treatments was explored. In addition to the enzymatic digestions. The chelating agent EDTA was used to disrupt Ca++ sensitive cadherins, adherens and tight junctions. The reducing agent DTT was used to disrupt covalent disulfide linkages between junctional complex proteins, and acrylamide was used to disrupt desmosomes as it has previously been shown to disrupt integrin containing junctional complexes.



Mixed germ cells from standard isolation procedure



MGC from optimized procedure on asynchronized testes

Optimized cell isolations contain more spermatids with more retained cytoplasm, suggesting that the integrity of the spermatid cell membranes and cytoplasmic contents are better preserved during isolation. The improved isolations are now being combined with the modified testis synchronization strategy. This will now allow for unique aspects of the highly ordered process of spermiogenesis to be investigated across the developmental continuum of the developing spermatid such as to compare spermatid ribonomes and proteomes over the course of spermiogenesis. Development of the mammalian spermatid depends heavily on post-transcriptional regulation and these studies will serve as the foundation for extended research efforts and collaborations. Isolating refined populations of spermatids will also allow inquiry into histone modifications affecting chromatin dynamics, regulation of RNA storage and translation, and post translational regulation in the developing spermatid that is much needed toward understanding male infertility and identifying potential contraceptive targets.