THE OHIO STATE UNIVERSITY

University of Michigan

Sue Hammoud, Ph.D.

Assistant Professor of Human Genetics

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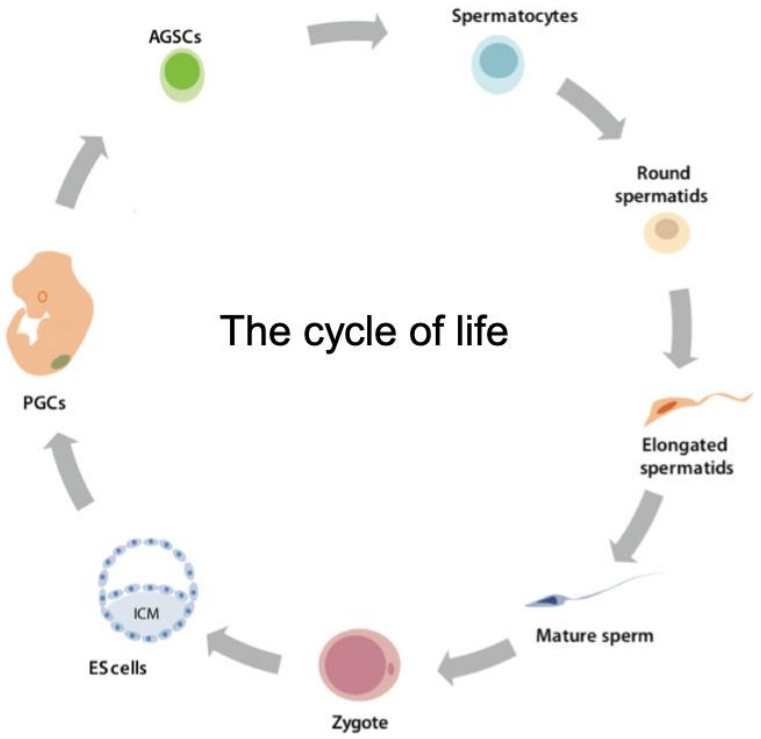
Hammoud Lab



Yunhao Wang Ph.D.

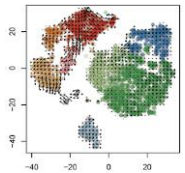
October 16, 2020

Department of Biomedical Informatics, Ohio State University

Research @ Hammoud Lab

➢ Molecular mechanisms and pathways required for proper germ cell development (single-cell + lineage tracing techniques)

- Germ cell comparative biology
- Dissecting germ cell-soma communication
- Making somatic cells of the testis



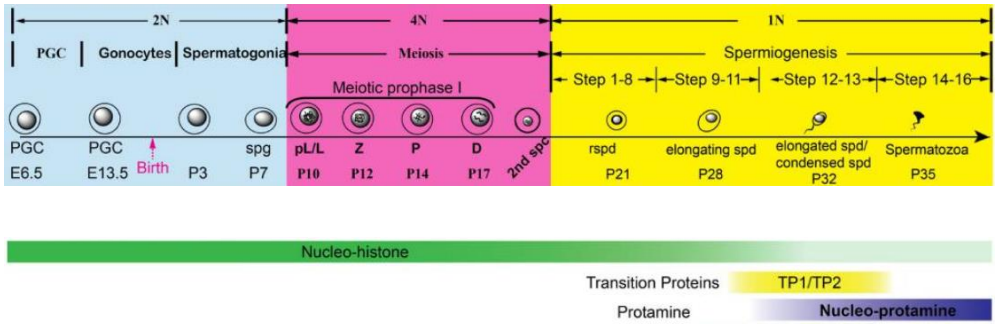
➢ Contribution of the male germline chromatin to development and disease (genetic, genomic and biochemical techniques)

- Protamine (small arginine-rich, sperm-specific nuclear protein): structure *vs* function
- Epigenetic inheritance:

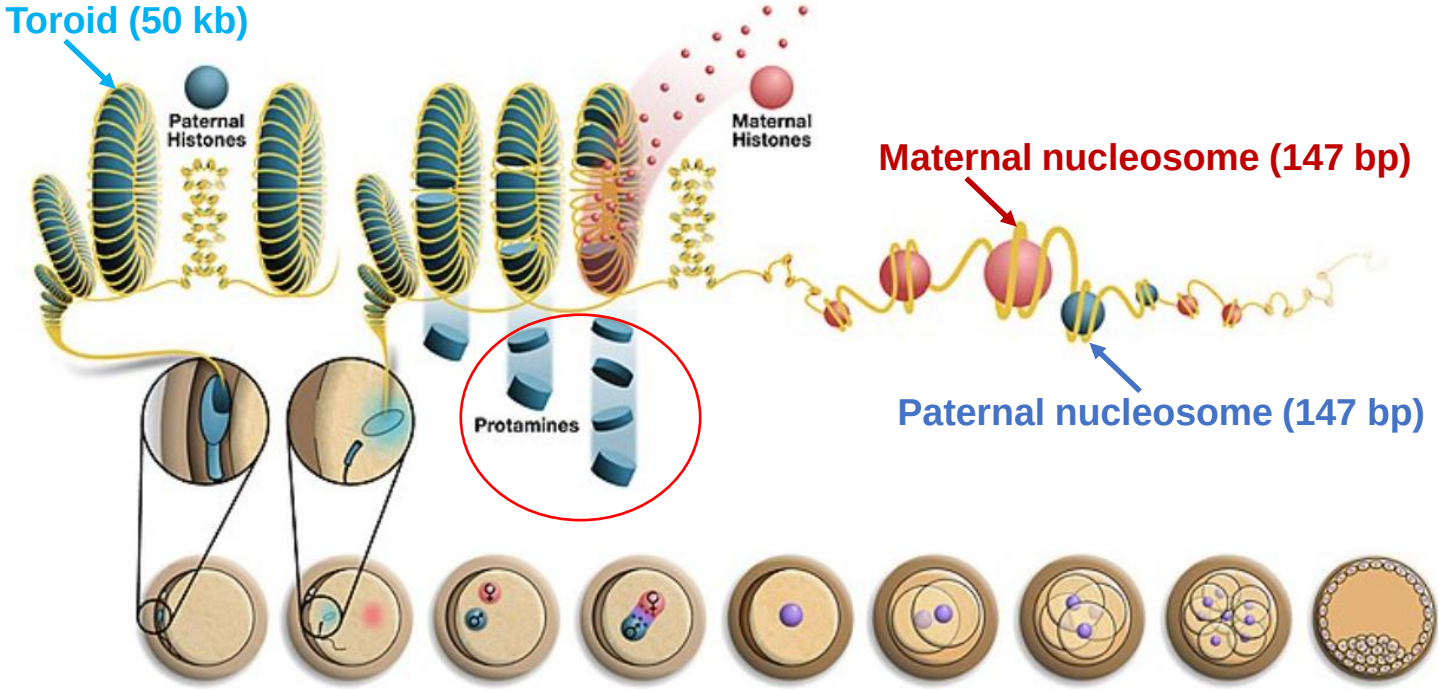
1) “histone-to-protamine transition”

Spermatogenesis

Histone-to-protamine



2) “protamine to histone exchange” after fertilization



Bao, J. and M. T. Bedford, *Reproduction* (2016); https://en.wikipedia.org/wiki/Protamine

Single-cell RNA sequencing of human, macaque, and mouse testes uncovers conserved and divergent features of mammalian spermatogenesis

Adrienne Niederriter Shami, Xianing Zheng, Sarah K. Munyoki, Qianyi Ma, Gabriel L. Manske, Christopher D. Green, Meena Sukhwani, Kyle E. Orwig, Jun Z. Li, Saher Sue Hammoud

doi: https://doi.org/10.1101/2020.03.17.994509

Posted March 18, 2020.

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Supplementary Material

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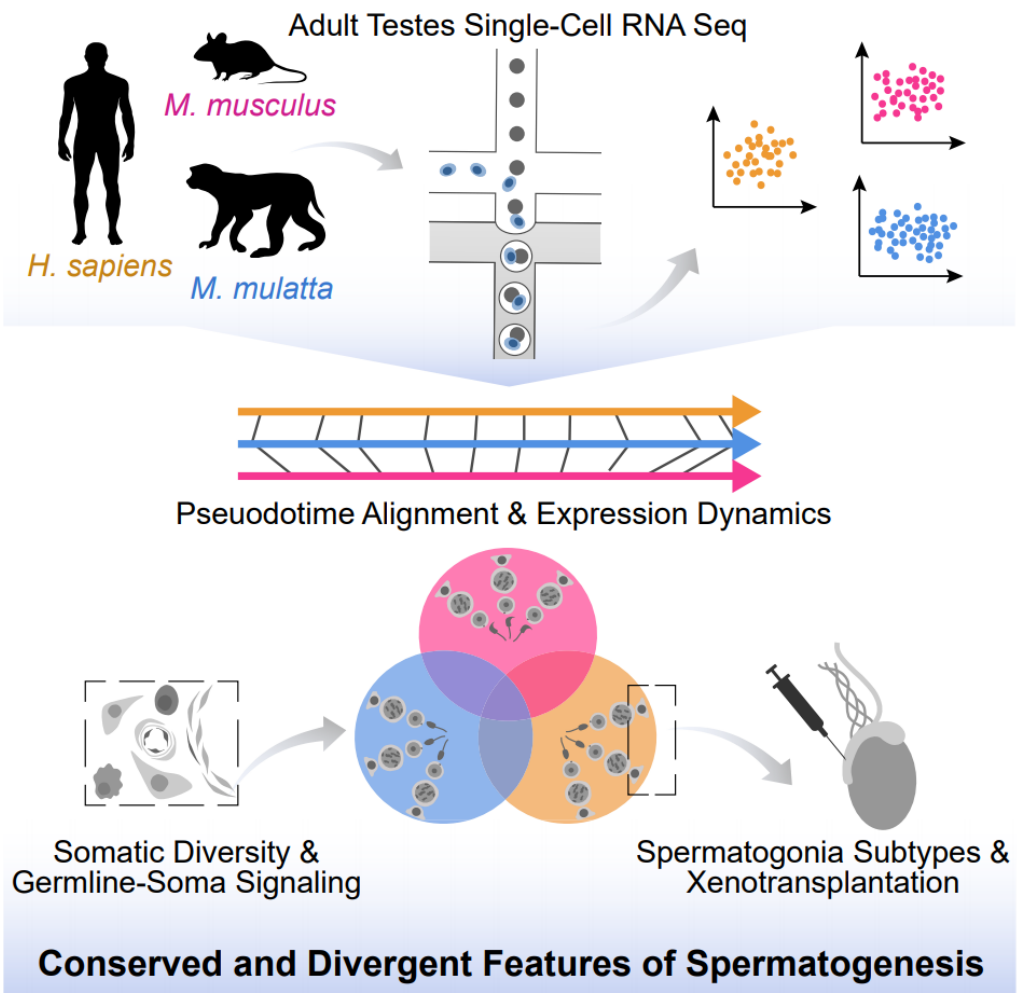

$Tcf21^{+}$ mesenchymal cells contribute to testis somatic cell development, homeostasis, and regeneration

Yu-chi Shen, Hailey Larose, Adrienne Niederriter Shami, Lindsay Moritz, Gabriel L. Manske, Qianyi Ma, Xianing Zheng, Meena Sukhwani, Michael Czerwinski, Caleb Sultan, Jourdan Clements, Haolin Chen, Jason R. Spence, Kyle E. Orwig, Michelle Tallquist, Jun Z. Li, Saher Sue Hammoud

doi: https://doi.org/10.1101/2020.05.02.074518

Posted May 03, 2020.

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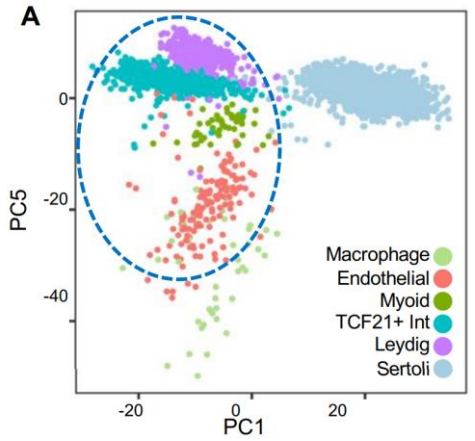
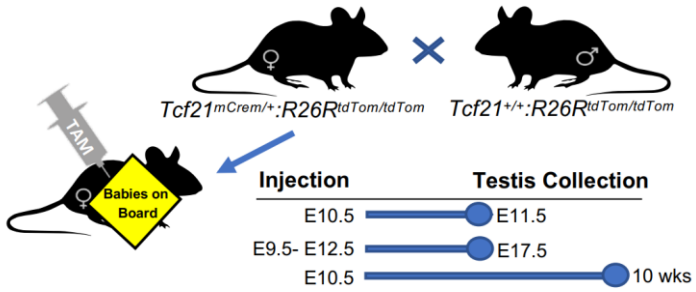
Supplementary Material

XML

Tcf21+ cells



Genetic lineage tracing



- **What is the origin**
  - **Mesenchymal** by DEG + GO analyses
- **What is the property/function**
  - **Multipotent somatic stem cell (to Leydig and myoid)**
  - **Somatic lineages in the male gonad**
  - **Multiple fetal and adult ovarian somatic cell types**
  - **Regenerate somatic cell types in the adult mouse testis**
  - **Somatic turnover in the testis during aging**
  - **Resembles resident fibroblast populations in other tissues**

Towards a Framework for the Characterization of Cellular & Spatial Relationships in Development and Disease

Gabriel Manske[1], Wenxin Xie[2], Sue Hammoud[1,2]

1 Cellular and Molecular Biology Graduate Program 2 Department of Human Genetics, University of Michigan, Ann Arbor, MI

MICHIGAN MEDICINE

Abstract

Mammalian spermatogenesis occurs in a structurally complex organ, where the germline stem cells undergo a highly ordered series of mitotic divisions, meiotic divisions, and morphological changes to give rise to sperm. The differentiation process is elaborately choreographed and requires intricate interactions and ongoing communication between the germ cells and the supporting somatic cell structures. Our recent single-cell RNA analysis has afforded us a systematic survey of the cellular diversity and developmental states in the testis. However, single cell sequencing does not provide positional information or retain cell to cell relationships. Nor does the data reveal the spatio-temporal dynamics of germline-soma interaction and communication. As a result, we cannot observe and measure critical molecular signals received by germ cells from neighboring somatic cells at each developmental stage or transition, or how this information is processed within germ cells. To overcome these limitations, we have adopted a spatial transcriptomic platform to understand the interplay among intrinsic signaling programs, single cell transcriptomic composition and dynamics, and cell-cell communication at key time points during a male's reproductive lifespan. These experiments will allow us to reconcile our previously identified molecular subtypes and predicted germ cell classifications with their spatial location and provide the inter-cellular context to their development within the testes. To demonstrate feasibility we applied our framework to analyze the expression patterns of a handful of molecular markers previously discovered in our single cell atlas.

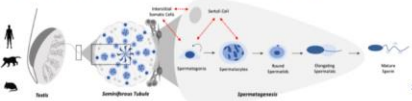


Figure 1. Schematic summary of the spermatogenesis developmental trajectory and cellular heterogeneity within the testis. Successful navigation through the germ cell developmental program requires ongoing juxtacrine, paracrine, and endocrine signaling between germ cells, supporting somatic cells, and the pituitary. Within the testis there are multiple somatic cell types that produce growth factors that influence neighboring cells, either by direct contact or by indirect, ligand-mediated signaling. These include: Sertoli cells (secreting products such as Gdnf, Fgf, Egf), macrophages (Csf1), endothelial cells (Vegf), steroidogenic Leydig cells (testosterone, Igf1, Csf1), and peritubular myoid cells. Together this unique heterogeneity and interaction network makes the testis one of the most complex tissues of the human body.

Background

Sperm are unique, highly specialized terminally differentiated cells that are produced by the complex male reproductive system. They carry the genetic information from father to offspring and are a continuous link between the past, present and future of a species. Although essential for the survival of our species – and all species – we do not clearly understand the process for the generation of the billions of sperm produced in a male's lifetime. Decades of scientific effort have begun to chip away the layers of complexity in this process- uncovering pieces of the puzzle, yet many pieces are missing, and the full picture remains hidden.

To date, our molecular understanding of the process has relied on genetic transcript profiling. Prior work has examined either complex tissues in bulk, or specific cell populations that have defined cell surface markers. Research has missed the specific, time-dependent interplay of RNA gene expression and intercellular communication. To fill the gaps in knowledge in the spermatogenesis program, we and others have applied high-throughput single-cell RNA sequence profiling. Single-cell RNA analysis affords us a systematic survey of the cellular diversity and developmental states in active testis. Our work specifically in mice has led to a new single-cell molecular 'atlas' containing: (1) a catalog of seven distinct somatic cell types (including two newly-discovered cell types), (2) the identification of multiple spermatogonial stem cell states, and (3) a continuous time-course trajectory of germ cell differentiation leading to mature sperm. The atlas includes several cellular subtypes within the major cell types, and markers to specify these cell types for labeling and enrichment (Figure 2). Although a significant advance in observing the unique cell characters in the testis, single cell RNA-sequencing requires whole tissue dissociation and thus the positioning information and cell to cell relationships are not retained.

To complement our single cell RNA-seq datasets with spatial information we plan to apply intron sequential fluorescent in situ hybridization (seqFISH) (Figure 2). The traditional FISH method, uses short fluorescently conjugated synthetic oligonucleotide to detect and quantify a single mRNA molecule within a cell with high specificity and accuracy. In order to exponentially scale the FISH technology to analyze the full transcriptome we will utilize a technique pioneered by the Cai lab where they combined FISH with combinatorial barcoding and serial hybridization (a.k.a Intron SeqFISH, see Figure 2). This will allow us to image and quantify the expression level of up to 10,000+ genes in single cells. By leveraging our previous single cell, we plan to design probes for 10,000 genes highly variable in the soma and germline, allowing us to link scRNA-seq molecular clusters with spatial and histological molecular subtypes.

Methods & Results

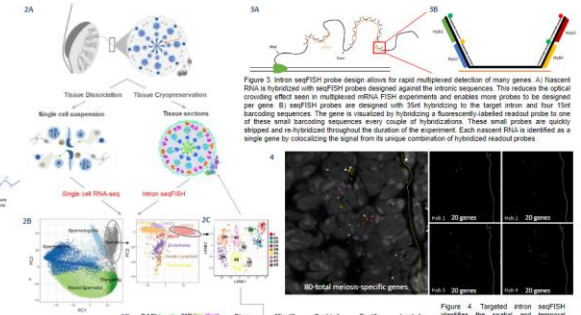


Figure 2. Single cell RNA-seq can inform the discovery of distinct somatic sub-types which support different stages of spermatogenesis. A) Testes from either mouse, monkey, or human have been collected and either dissociated or cryopreserved. The dissociated testes were used for single cell RNA-sequencing and the cryopreserved testes were used for highly multiplexed intron seqFISH. B&C) Data from these two techniques can be utilized to identify and compare major cell types and individual cell subtypes. D) seqFISH identifies the spatial and temporal relationship of these individual cell subtypes across the stages of spermatogenesis.

Figure 3. Intron seqFISH probe design allows for rapid multiplexed detection of many genes. A) Nascent RNA is hybridized with seqFISH probes designed against the intronic sequences. This reduces the optical crowding effect seen in multiplexed mRNA FISH experiments and enables more probes to be designed per gene. B) seqFISH probes are designed with 35nt hybridizing to the target intron and four 15nt barcoding sequences. The gene is visualized by hybridizing a fluorescently-labelled readout probe to one of these small barcoding sequences every couple of hybridizations. These small probes are quickly stripped and re-hybridized throughout the duration of the experiment. Each nascent RNA is identified as a single gene by colocalizing the signal from its unique combination of hybridized readout probes.

Figure 4. Targeted intron seqFISH identifies the spatial and temporal expression patterns of 80 genes dynamically expressed in meiotic cells. Left) The seqFISH signal from 80 dynamically expressed meiotic genes. Right) Signal from multiple different hybridizations is combined, colocalized, and de-barcoded reveal gene identity.

CONCLUSIONS

- Intron seqFISH will provide a high-resolution view of major testis cell types at the molecular level.
- Known and newly discovered markers provide a resource for spatiotemporal characterization by intron seqFISH.
- Small scale (80-gene) intron seqFISH identifies spatial distribution and dynamics of 80 meiotic genes.

FUTURE DIRECTIONS

- Scale seqFISH from 80 genes to 10,000+ genes.
- Analyze expression across gonadal development.
- Combine intron and exon seqFISH to compare the current vs nascent transcriptome.
- Perturb molecular signaling pathways and reevaluate spatial effects.

FUNDING

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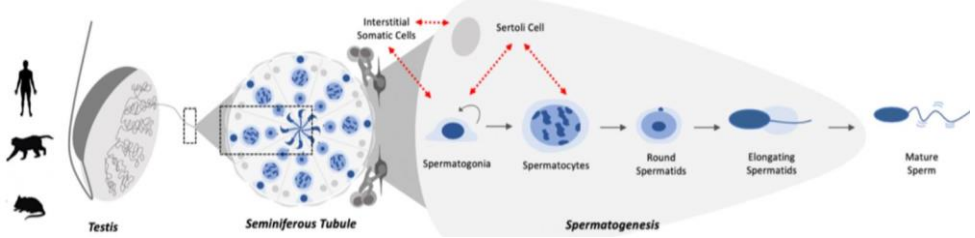
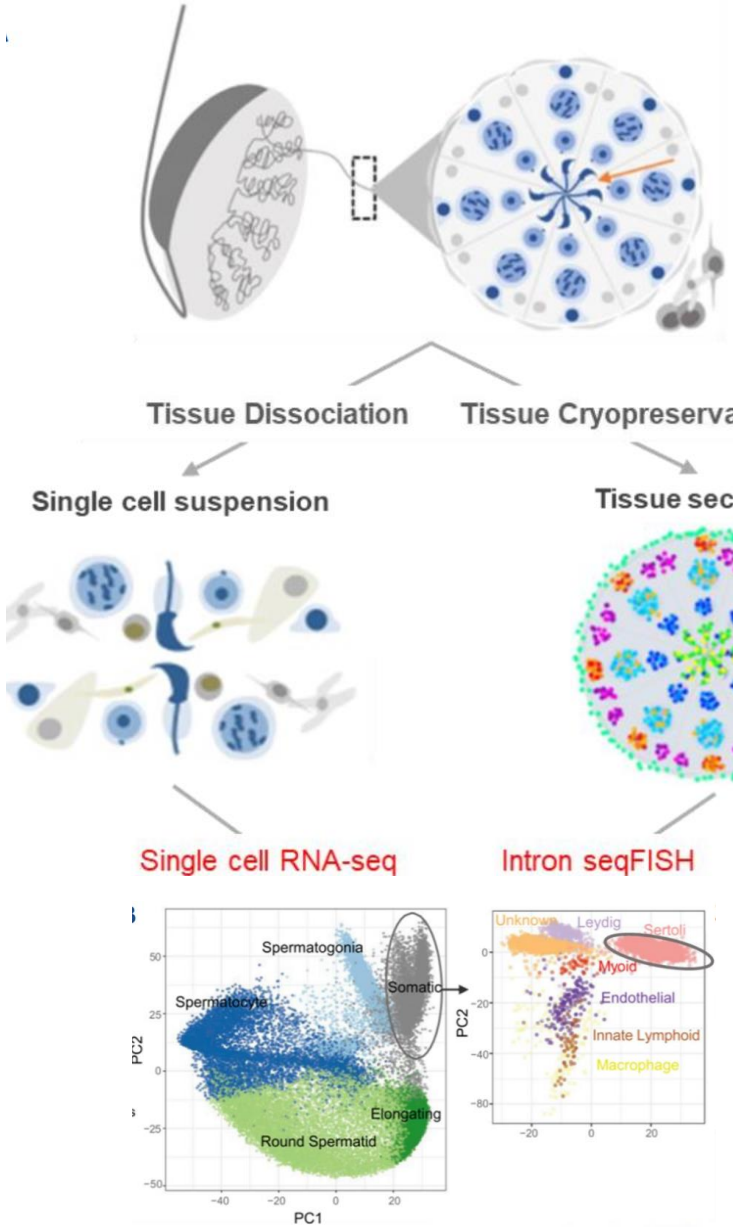
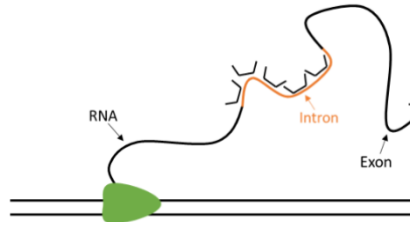
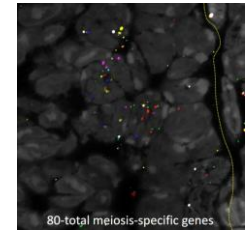


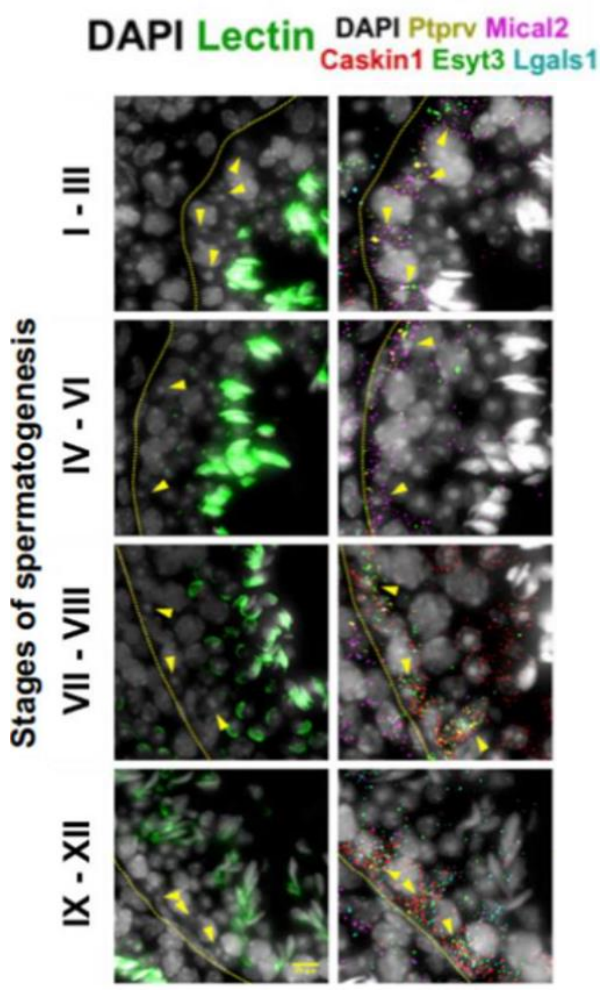
QUESTION: the spatial communication between germ cell and somatic cell during germ cell development

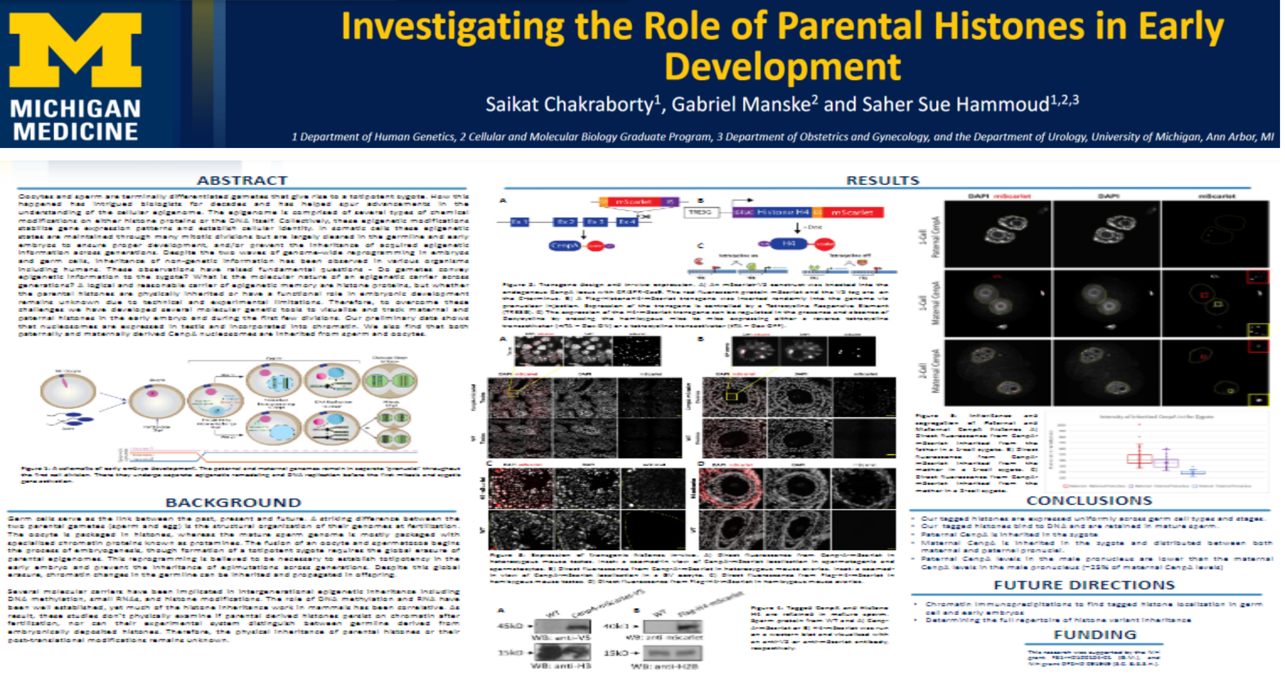


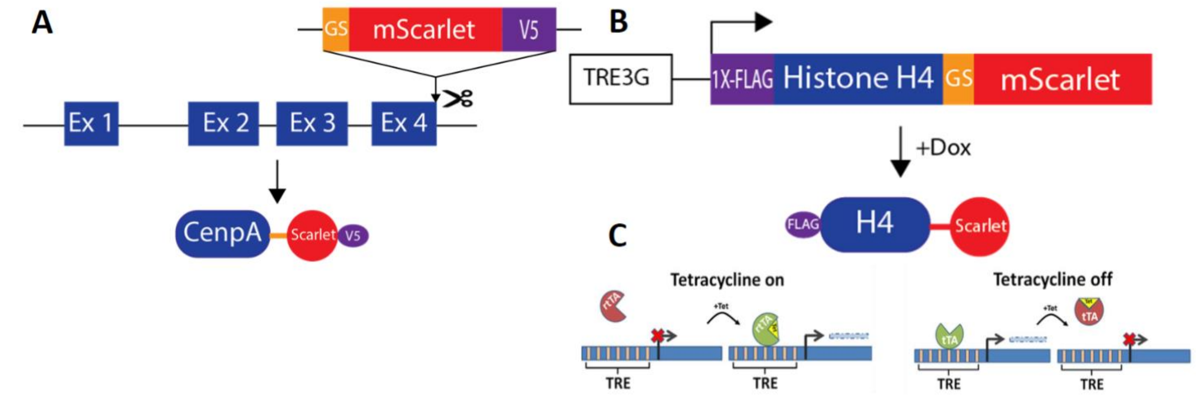
Intron sequential fluorescent in situ hybridization

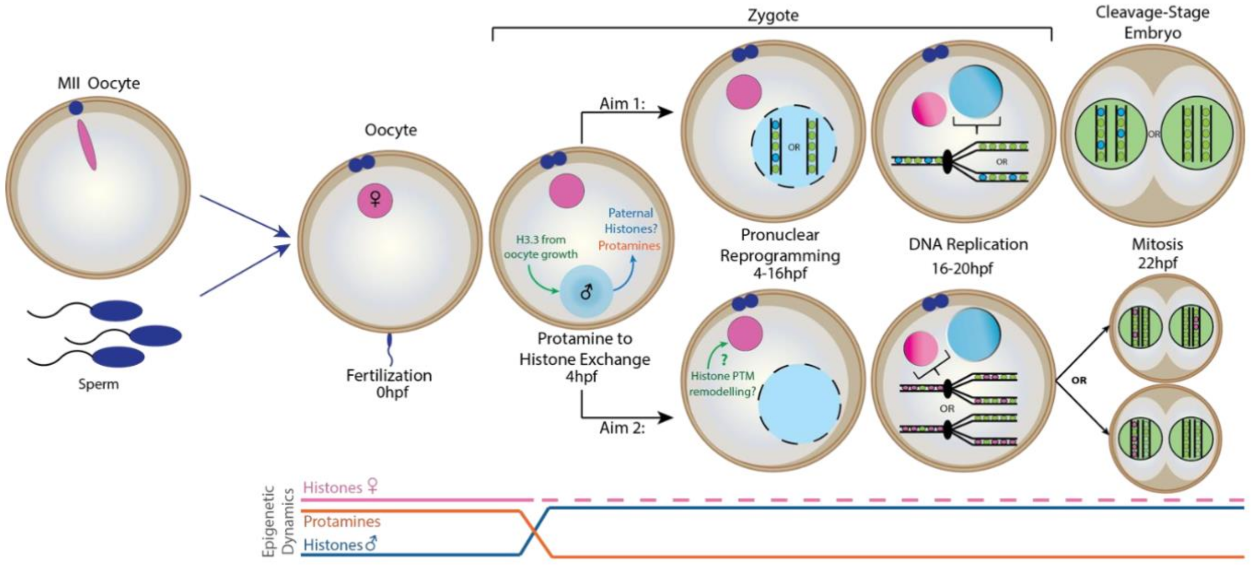


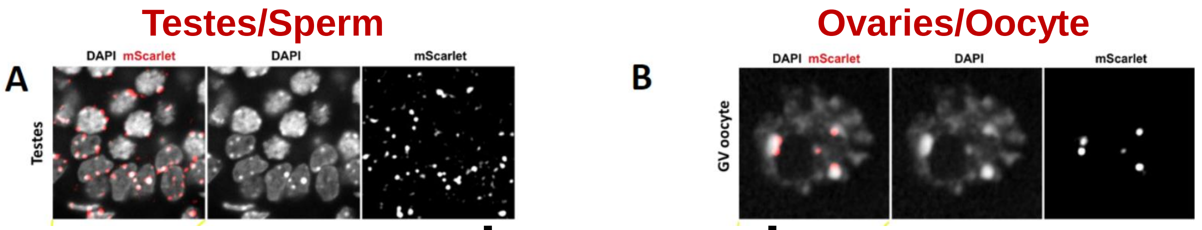




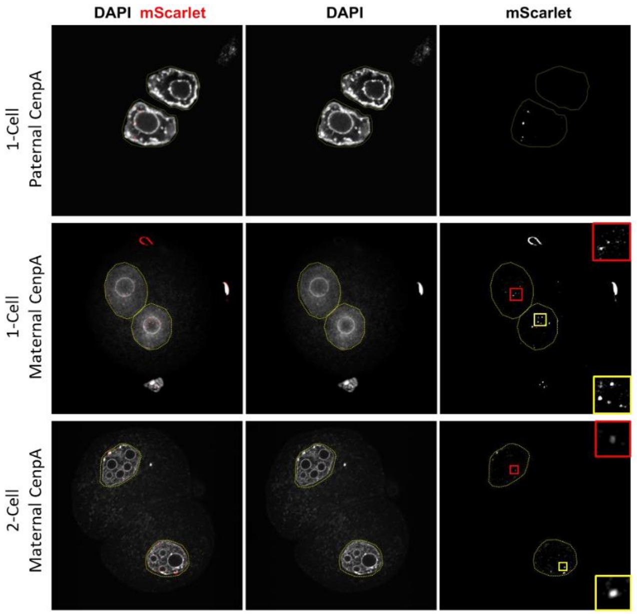








Fertilization



1-cell (Paternal)

1-cell (Maternal)

2-cell (Maternal)

QUESTION: during early development

1) "Protamine to histone exchange" ; 2) Histone PTM remodeling

Thanks