Studies of SUMO Protein and Spermatogenesis using Transgenic Mouse Models

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Introduction

Infertility affects approximately 8% to 12% of couples; 50% of male cases are idiopathic, highlighting the need to study spermatogenesis. This biological process involves the formation of sperm cells, starting with the mitotic division of spermatogonia, followed by meiotic division of spermatocytes, and concluding with the maturation of spermatids into fully developed sperm (Fig.1). **Understanding these stages is crucial for comprehending male fertility and its regulatory mechanisms**.

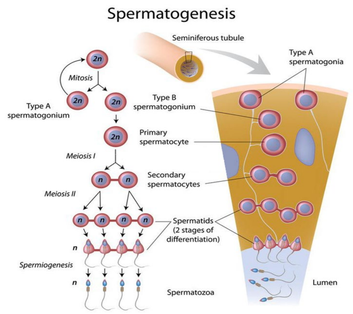

Figure 1: Spermatogenesis process

Protein functions during spermatognesis are regulated by posttranslational modifications (PTMs), including SUMOylation, which adds small ubiquitin-like modifiers (SUMO) to proteins (Fig. 2). SUMO proteins are highly expressed in testicular cells, modifying key proteins involved in spermatogenesis, but their role in male fertility remains largely unexplored.

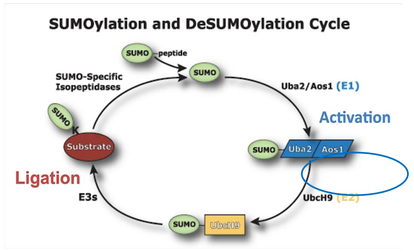

Figure 2: SUMOylation and DeSUMOylation Cycle

We have previously shown that inhibition of SUMOylation *ex vivo* arrested the completion of meiosis in purified mouse spermatocytes. Inhibition of SUMOylation also affected the secretion of the primary mouse Sertoli cells and activated their apoptotic response. However, the question of whether SUMOylation is required for spermatogenesis *in vivo* is not currently understood.

Specific Aim:
To confirm the role of SUMOylation during spermatogenesis in mice *in vivo*.

Methods

In-Vivo SUMOylation Inhibition:

- UBA2 is the enzyme that initiates SUMOylation. To study this process, we inactivated UBA2 in mouse cells using the Cre-LoxP method.
- LoxP sites were placed around exon 3 of the UBA2 gene (Fig.3) The two Cre models used were Stra8-Cre, which inactivates UBA2 in meiotic germ cells, and AMH-Cre, which inactivates it in Sertoli cells. These models permit investigating SUMOylation in a cell-specific manner. We produced two sets of male mice, each set with one of the changes.

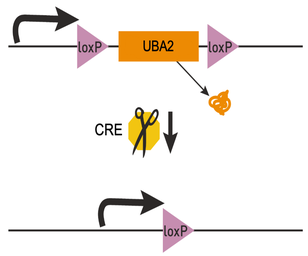

Figure 3: Cre-LoxP Mechanism

Results

- **Fertility:** The altered male mice were housed with wild-type females for several weeks and showed a complete male infertility.
- **Morphological and quantitative analysis of Seminiferous Tubules** Testicular weight was reduced by 50% in Stra8-Cre mice and 75% in AMH-Cre mice (Fig.4) without changes in body weight (Fig.5).There were no sperm observed in the epididymis.

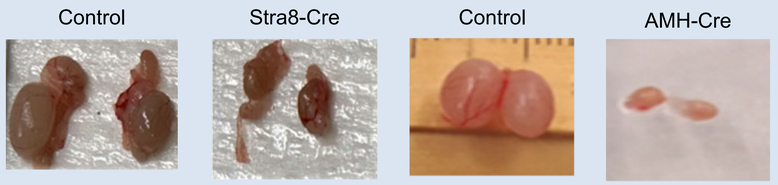

Figure 4: Testes size of Control and Experiment Mice

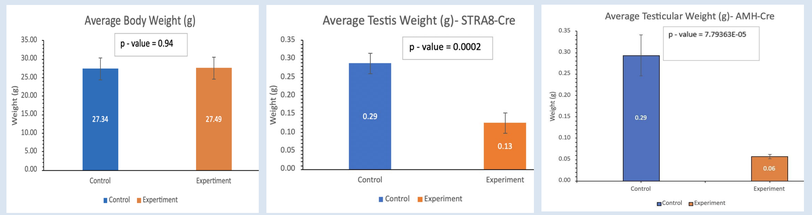

Figure 5: Body weight and Testes weight of Control and Experiment Mice

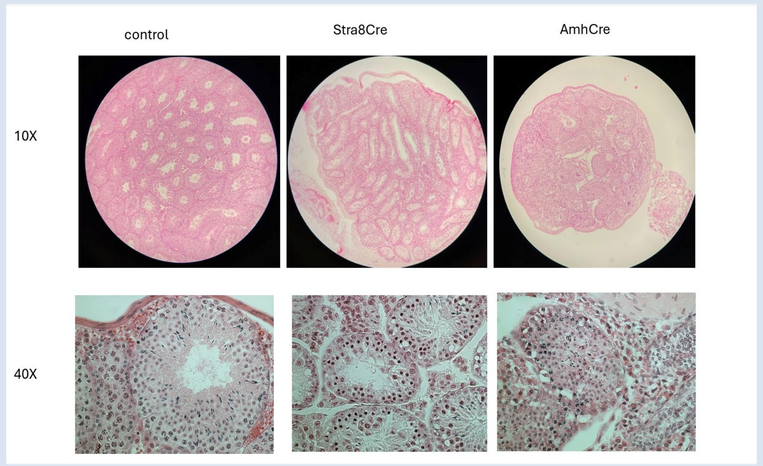

Figure 6: Hematoxylin & Eosin Staining of Testicular tissue of Control and Experimental Mice

Both models showed decreased tubular diameter.(Fig. 6) Stra8- Cre model has shown spermatogenesis mostly arrested at spermatocyte stage, while AMH-Cre model has shown a gradual testicular degeneration and atrophy (Fig. 6)

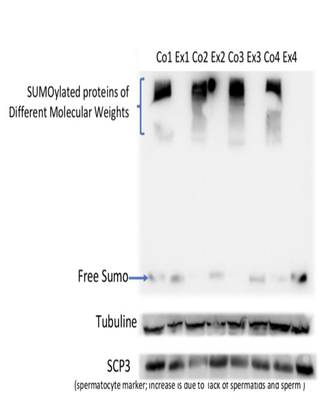

Figure 7: Western blot analysis

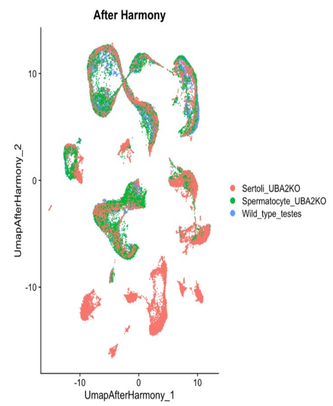

Figure 8: Single-nucleus RNAseq experiment Umap After Harmony

As shown in Fig. 7, SUMOylation was inhibited in the germ cell fraction of Stra8-Cre mice, confirming successful gene inactivation in this population. The results of single cell RNA seq revealed that in both models (Fig. 8), important genes involved in spermatid maturation and differentiation were significantly downregulated while genes involved in cytokine signaling and stress response increased.

Conclusions

- SUMOylation is essential for meiosis and the supportive functions of Sertoli cells during spermatogenesis in mice. It regulates key genes necessary for the successful progression of spermatogenesis.
- In mouse models, inactivation of SUMOylation resulted in complete infertility, arrest of spermatogenesis, and testicular atrophy.
- To further investigate the affected molecular pathways, transcriptomic profiling of testicular cells was performed using single-cell sequencing techniques.

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