Androgen receptor mutation affect testes organization in an African cichlid *A. burtoni*

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Background

- The African cichlid fish, *Astatotilapia burtoni*, serves as a model species to study the role of androgens (1).
- Androgen receptors (AR) act as a ligand-dependent transcription factor which binds androgens that regulate gonad differentiation and development (2).
- *A. burtoni* have 2 paralogs of AR (ARα and ARβ) that are a result of a whole genome duplication in teleost fish (1).
- Spermatogenesis is the process by which haploid sperm form through meiosis. This process occurs in the seminiferous tubules (Figure 1).
- In zebrafish, *Danio rerio*, mutation of their single AR gene leads to small testes and disorganized seminiferous tubules (Figure 2); on a cellular level, compared to WT, spermatogenesis was delayed as indicated by more spermatogonia, fewer spermatocytes, and fewer spermatozoa (2, 3).
- In Alward et. al 2020, CRISPR/Cas9 gene editing was used to ultimately yield the homozygous mutants ($AR\alpha^{d50/d50}$;$AR\beta^{d5/d5}$) used in this study. As shown in figure 3, these mutants have extremely small testes compared to WT fish; this study aims to elucidate the reason for this effect (1).
- **How does the mutation of both ARα and ARβ affect the organization of the testes and the process of spermatogenesis?**

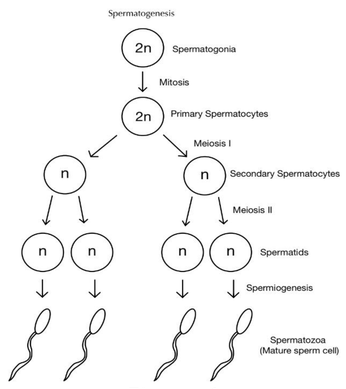


Figure 1

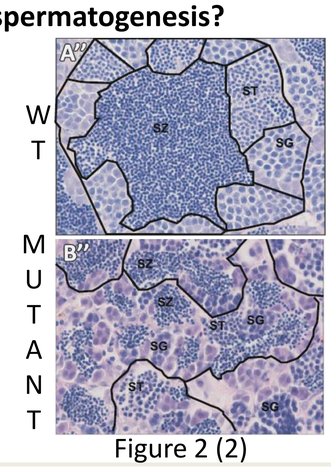


Figure 2 (2)

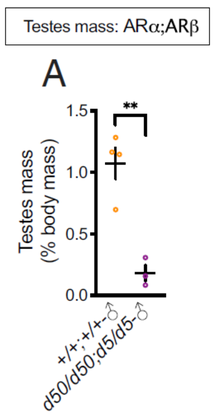
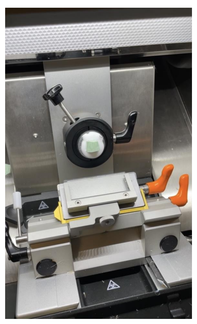
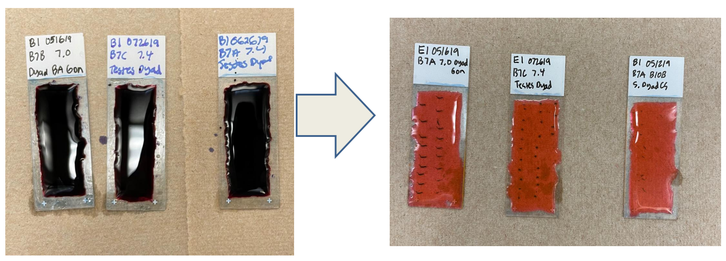


Figure 2 (3)

Methods



1) WT (n=3) and AR Mutant (n=3) testes were cryosectioned at a thickness of 10 microns.

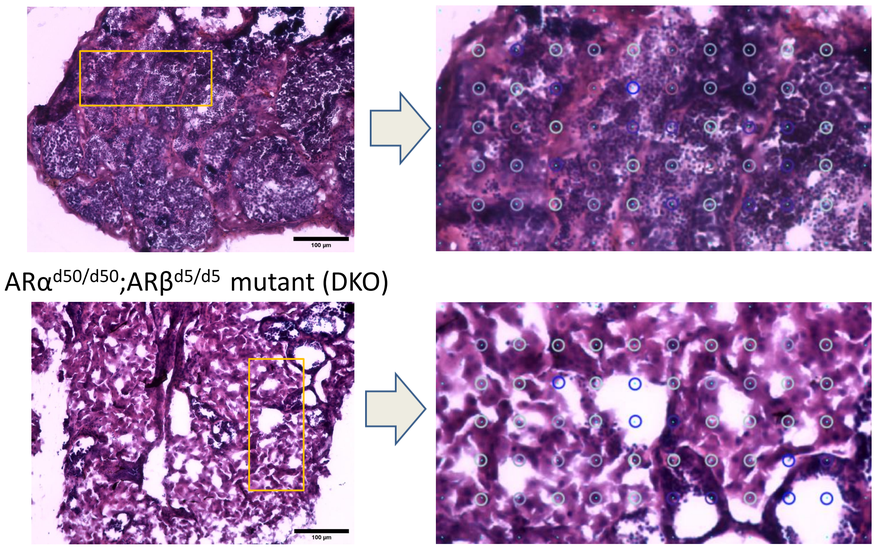


2) Sections were stained with Hematoxylin and Eosin (H&E) using standard histological technique protocols provided by Vector Laboratories in order to differentiate cell types within the testes.

3) Photomicrographs were taken of multiple sections on each slide to examine testicular cell composition.

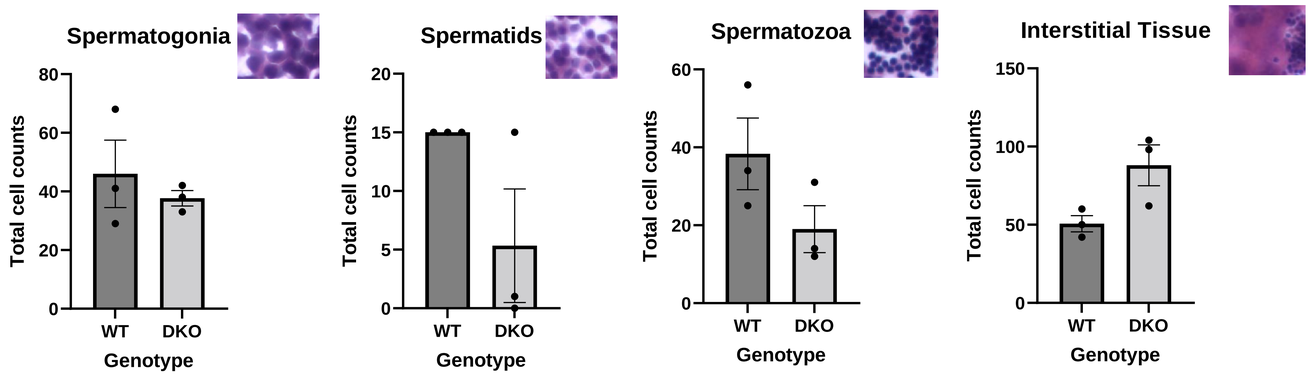
Cell Count Analysis/Results

Wild Type (WT)



$AR\alpha^{d50/d50}$;$AR\beta^{d5/d5}$ mutant (DKO)

A grid of randomly generated points was overlaid on each image in ImageJ. Starting at a random point on the tissue, the cell type underneath 50 points were counted on a random section as analyzed in previous work in A. burtoni (4). Using QuPath, images were analyzed for 3 cell types (Spermatogonia, Spermatids, Spermatozoa), and interstitial tissue. If some points landed on an open space, additional points were counted until there were 50 total.



Observations and Future Aims

- AR mutant testes sections appear to have numerous holes, possibly a product of the phenotype.
- In AR mutants the interstitial tissue surrounding the seminiferous tubules appears to lack structure relative to the WT fish.
- One AR mutant had cell counts comparable to WT, an anomaly to be investigated in future work.
- In the future, the area of the holes and its percent composition of the tissue will be quantified as a measure of analysis of organization of the testes.
- We also look to test the fertility of the sperm in $AR\alpha^{d50/d50}$;$AR\beta^{d5/d5}$ mutants *in vitro* as they have been shown to lack any mating behavior (1).

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