BEYOND THE TUBULE: E2F6 KNOCKOUT MICE REVEAL RESILIENCE IN FERTILITY DESPITE A DECREASE IN SPERM PARAMETERS

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Introduction

- Male factor contributes to ~50% of cases of infertility[1]
- Little is known about gene disorders affecting sperm development
- Transcriptional repressor E2F6 inhibits meiotic gene expression in somatic cells -> theorized to play a role in spermatogenesis[2]
- Published histology displays *E2f6* KO mice with fewer mature spermatocytes within seminiferous tubules[3]
- **Hypothesis: KO mice exhibit diminished sperm characteristics and lower fertile capacity compared with WT controls**

Context

Spermatogenesis

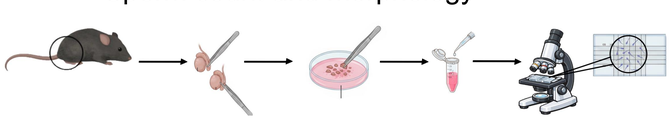
Methods

Part I: Controlled Breeding Experiments

- 6 exp. couples paired with KO mates and 6 control couples paired with WT mates
- Cages checked daily for litters
- DOB and number of pups recorded
- Cages retired at 150 days and mice 6-8 mo-old

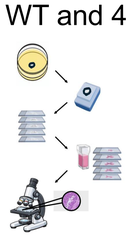
Part II: WT vs KO Reproductive Characteristics

- 9 WT and 4 KO male mice collected at 4-mo-old
  - Body and testis weight
  - Serum testosterone levels
  - Sperm count and morphology



Part III: WT vs KO Tubule Stages

- 4 WT and 4 KO male P10 mice testes collected



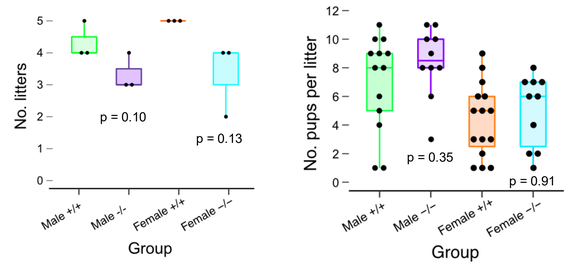
- Fixed (4% PFA/Bouins)
- Paraffin-embedded
- Sectioned onto slides
- Stained (HePAS)
- Imaged and staged

Despite lower testis weight and sperm counts in KO mice, fecundity, sperm morphology, and testosterone levels were unaffected by genotype.

Results Part I

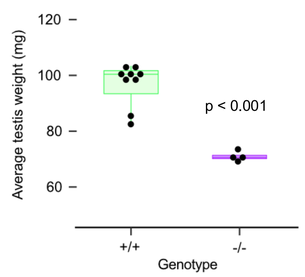
Number of litters Mean (sd)			
Sex	Control Pair (+/+)	Exp. Pair (-/-)	**p-value**
M	4.3 (0.6)	3.3 (0.6)	**p = 0.10**
F	5 (0)	3.3 (1.2)	**p = 0.13**

Total number of pups Mean (sd)			
Sex	Control Pair (+/+)	Exp. Pair (-/-)	**p-value**
M	30.3 (3.5)	28 (1.7)	**p = 0.35**
F	21.7 (2.5)	16.7 (11.4)	**p = 0.91**



All couples fertile with viable offspring.

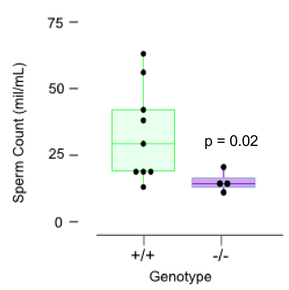
Results Part II

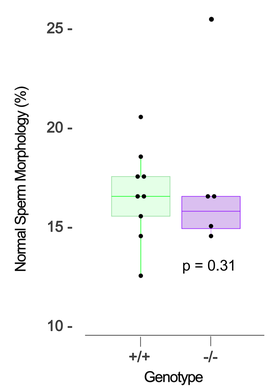


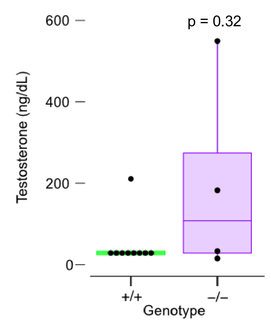
Testis weight (mg) Mean (sd)		
+/+	-/-	**p-value**
195.8 (14)	143.9 (3.7)	**p < 0.001**

*Adjusted for body weight, p < 0.001.

Average testis weight lower in KO mice.







Sperm Count x10^6 (mil/mL) Mean (sd)		
+/+	-/-	**p-value**
33.6 (17.9)	15.5 (4)	**p = 0.01**

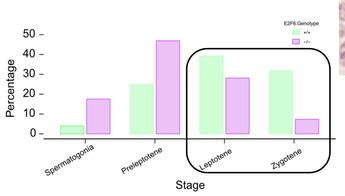
Normal Sperm Morphology (%) Mean (sd)		
+/+	-/-	**p-value**
17 (2)	16 (1)	**p = 0.31**

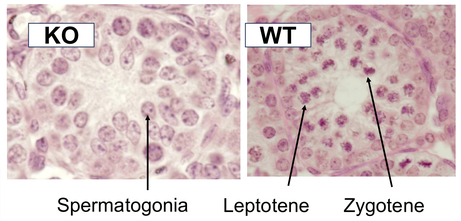
Serum Testosterone (ng/dL) Mean (sd)		
+/+	-/-	**p-value**
46.8 (61)	193.3 (247.6)	**p = 0.19**

Sperm count lower in KO mice compared with WT. Morphology and testosterone unaffected by genotype.

*Unpaired two-tailed t-tests were used.
**Sperm count and testosterone t-tests were done on log-transformed values.

Results Part III





KO testes exhibit less meiotic entry
$\chi 2(1) = 56.14$ p =< 0.05
(95% CI 3.0 – 6.69)

Conclusions

Part I

- Experimental breeding pairs were fertile and produced viable offspring, similar to controls. All pairs produced at least two litters with no differences observed in number or size of litters between groups.

Part II

- KO mice had significantly lower testis weight than WT mice, which remained significant after adjusting for body weight. This suggests altered testicular architecture.
- Despite a statistically lower sperm count in KO mice, sperm morphology and testosterone was unaffected.

Part III

- WT mice show more advanced stages of spermatogenesis indicating a delay or disruption in meiotic entry in the KO.

Limitations

- Sperm parameters from mice younger than 4 months were not assessed.
- Age-stratified studies are needed to determine if KO mice experience disrupted reproductive function throughout life.

Future Directions

- Immunostaining with stage specific antibodies to determine spermatogenesis disruption timing.
- Analyze additional stages to distinguish a delay in differentiation versus a disruption of meiotic entry.

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References