

# **PROGRESSIVE INCORPORATION OF CENEXIN IS RELATED TO SPERM MATURATION DURING EPIDIDYMAL TRANSIT IN THE DOMESTIC CAT**

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Smithsonian



# Assisted reproductive techniques

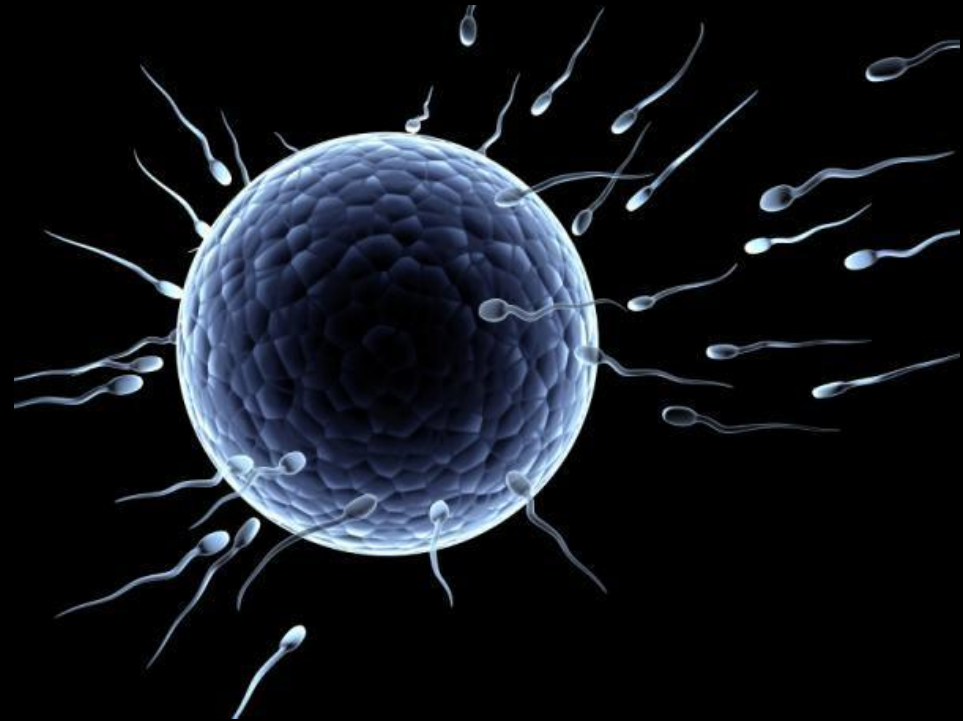
- Use of assisted reproductive techniques has become crucial across zoological institutions
- Research aim: improve understanding of natural sperm maturation to improve in vitro culture methods



# Sperm Flagellum

## □ Flagellum Function

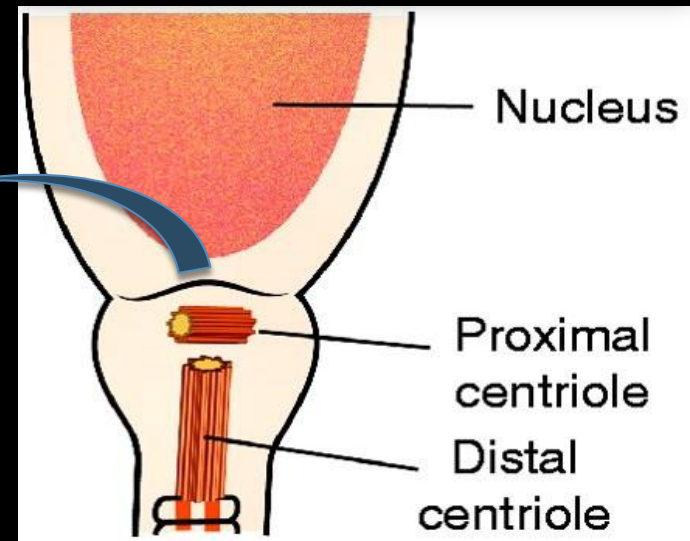
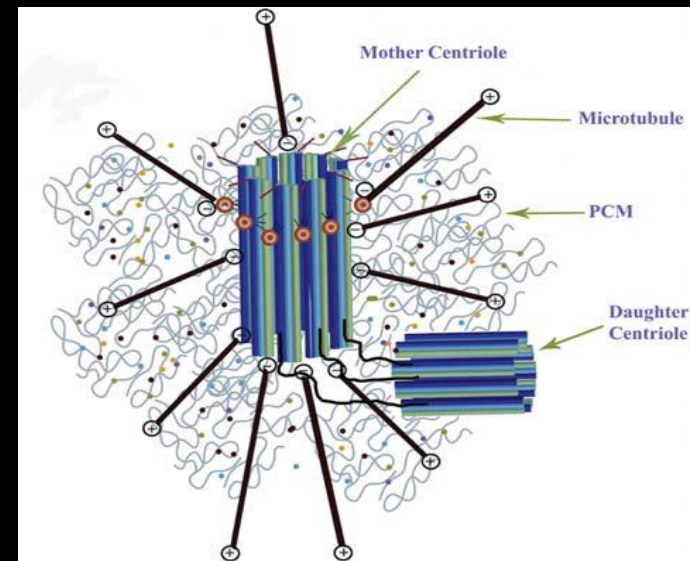
- Propels sperm through female's tract for oocyte fertilization
- Sperm motility is dependent on a properly matured flagellum



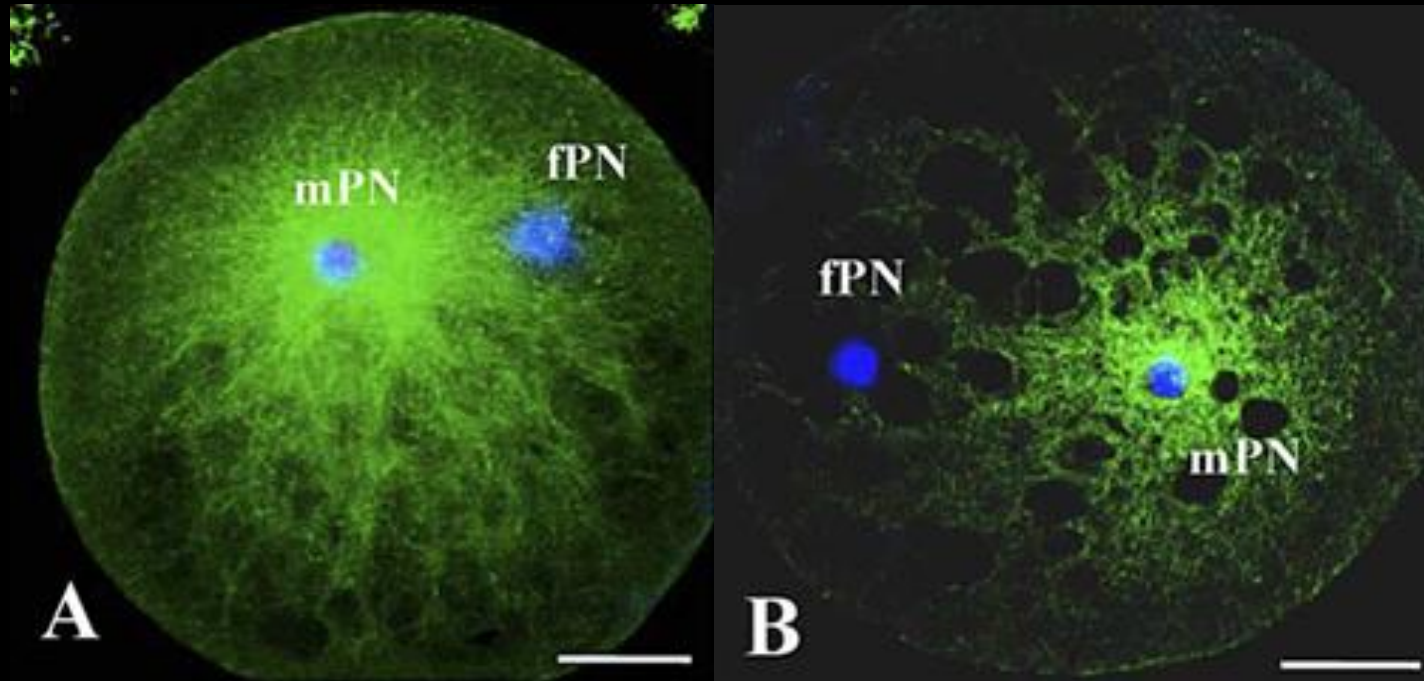
# Sperm Centrosome

## □ Centrosome Function

- ▣ Production of a large sperm aster is required for male/female pronuclear fusion after oocyte penetration
- ▣ Size and function of aster is dependent upon maturation of sperm centrosome



# Sperm Aster



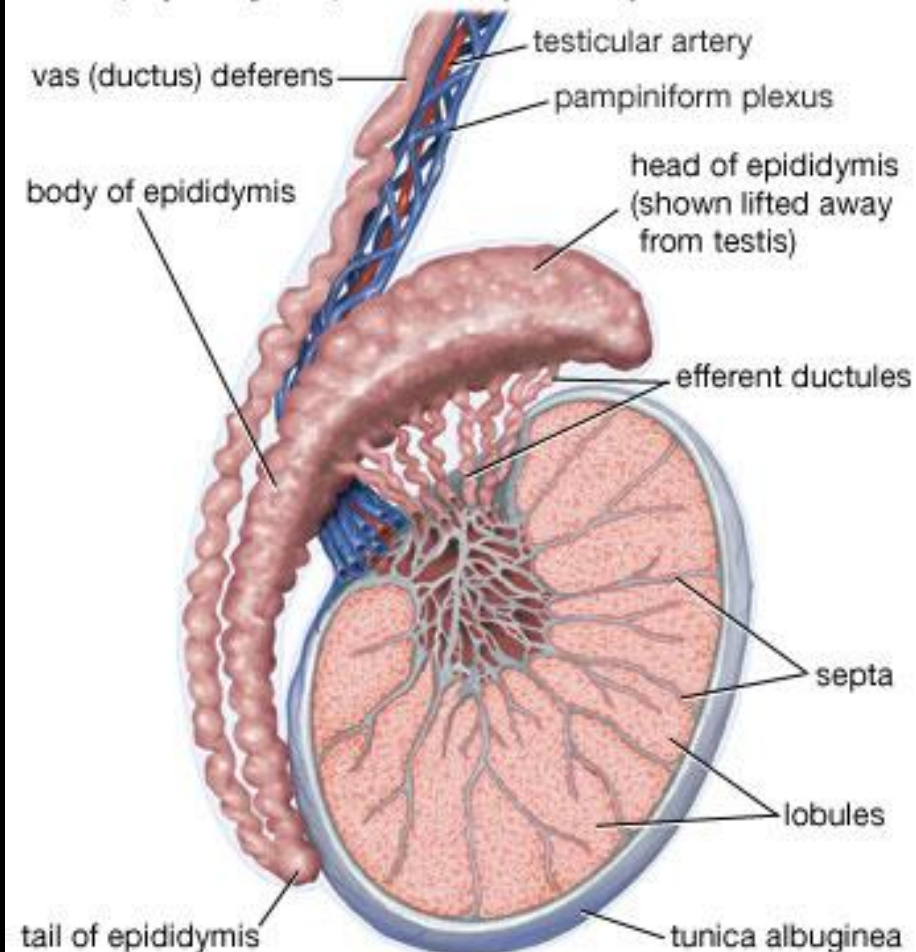
A. Large sperm aster produced with mature, ejaculated spermatozoa

B. Short sperm aster produced with immature, testicular spermatozoa

# Epididymis

- Hypothesis: Epididymal factors are produced and integrated by the sperm flagellum and centrosome sequentially during transit.

**Testis, epididymis, and vas (ductus) deferens**



# Epididymis

- Current issue:
  - ▣ Most key epididymal factors have not been identified
  - ▣ This renders it difficult to understand functional sperm abnormalities and conduct artificial maturation
- Objective: better understand flagellum and centrosome maturation and identify key epididymal proteins that may be used to improve artificial culture techniques.

# Cenexin- Outer Dense Fiber 2

- Previously identified as a centrosome-associated protein in somatic cells
- Localizes exclusively to mature centriole
- Surrounds sperm flagellum to protect against sheer forces and maintain flexibility

*Does cenexin aid in sperm flagellum and centrosome maturation?*



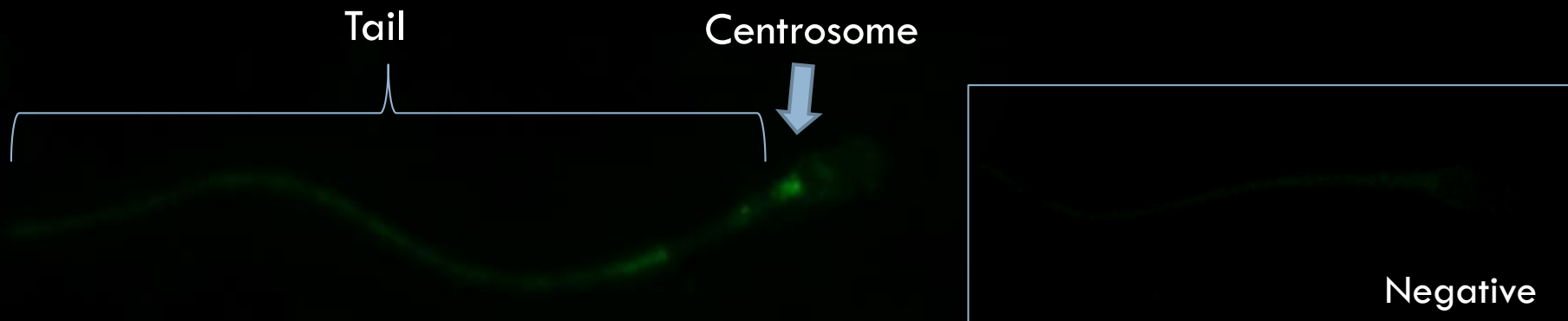
# Materials & Methods

- Model species: domestic cat
- Tracts were dissected and sperm samples collected from each segment (testis, caput, corpus, cauda, and vas deferens) by slicing with scalpel blade in PBS medium at 37°C



# Materials & Methods

- After fixation in 4% paraformaldehyde, sperm were immunostained with anti-cenexin antibodies labeled with a fluorescent probe
- Images were recorded using Spot Basic 5.1 software (Diagnostics Instruments)
- Immunofluorescence intensity analyses were carried out using ImageJ 1.47 software



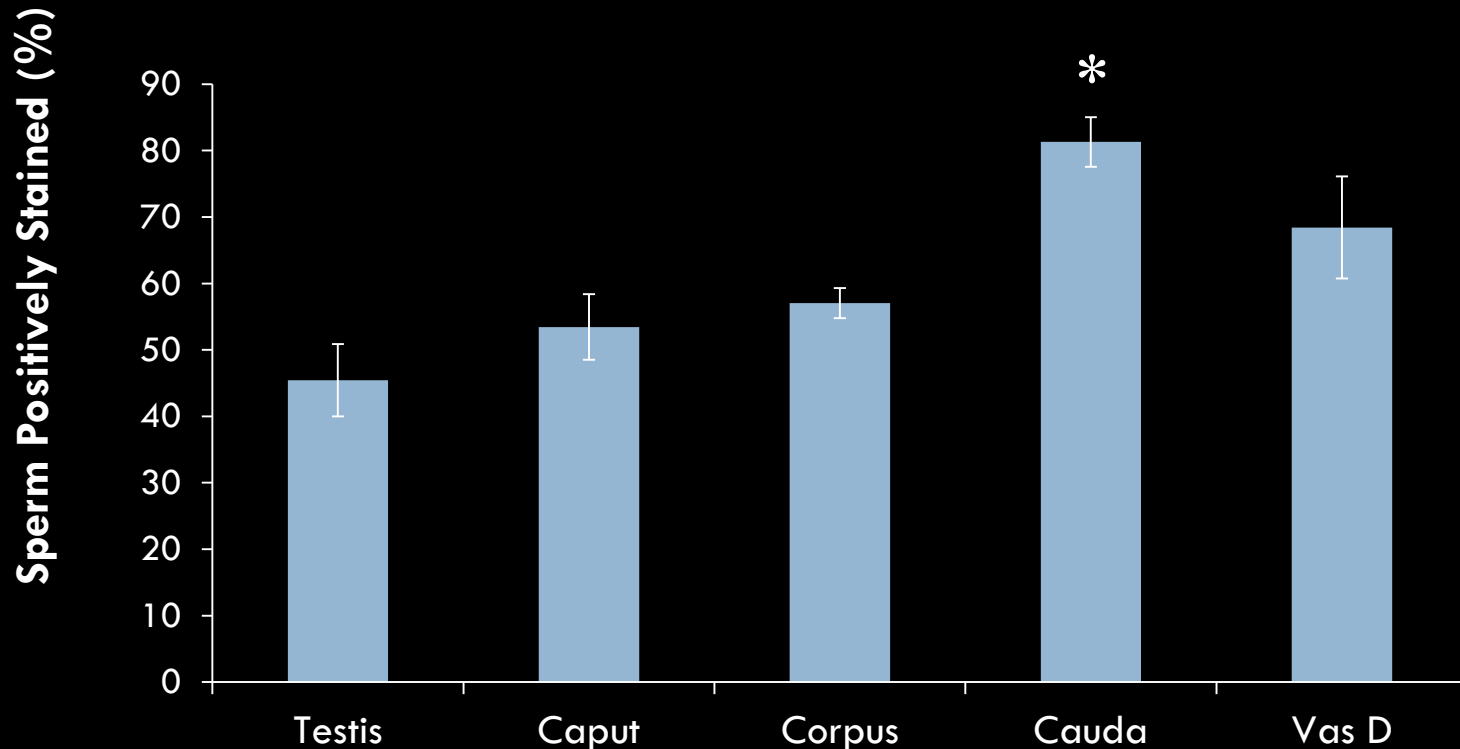
# Materials & Methods

- Statistical analyses conducted using SAS 9.3 software following a mixed model procedure
- Repeated measures was performed accounting for individual male variation
- Following these results, differences were further compared using protected F tests for orthogonal contrasts



# Results- Centrosome

## Sperm Positively Stained for Cenexin at Centrosome (Mean $\pm$ SEM)

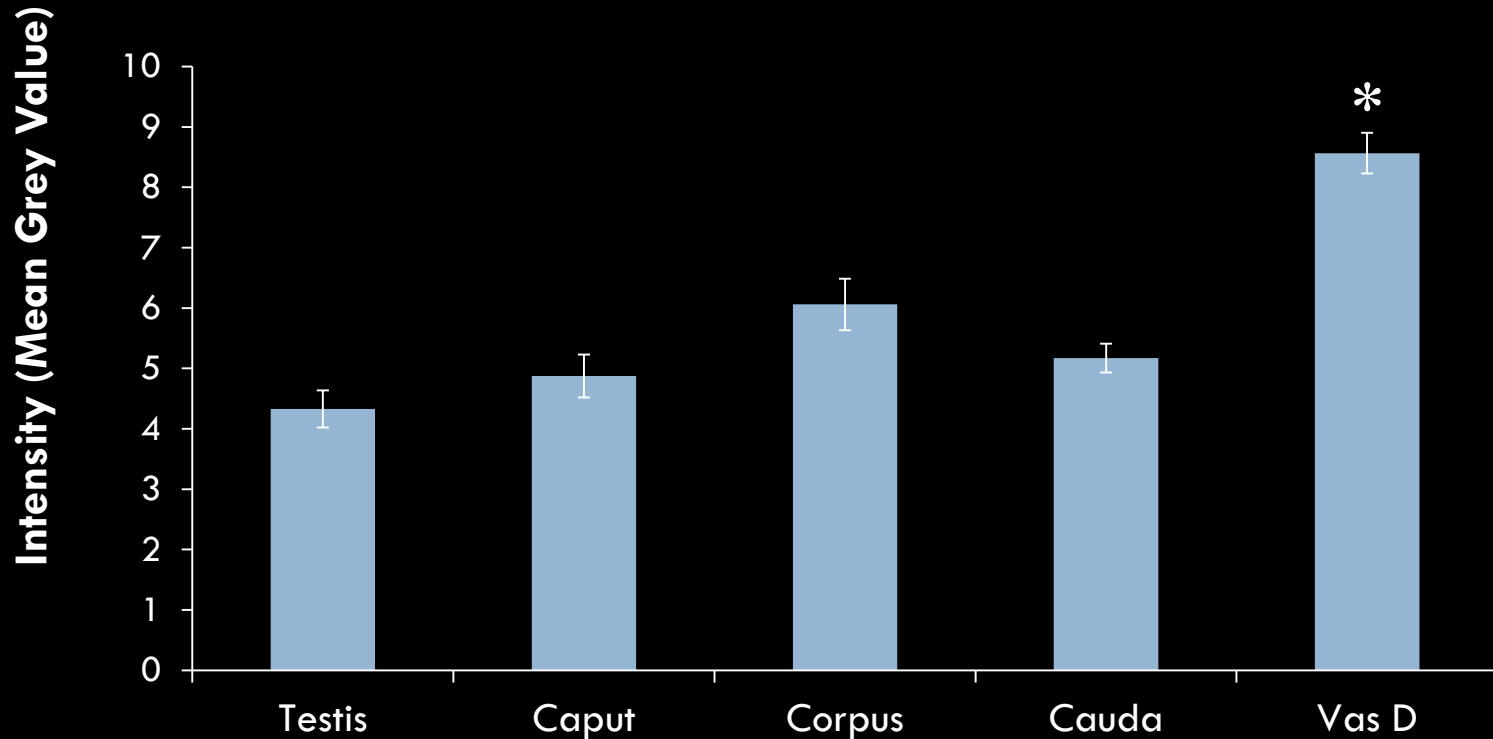


n=8 testes

\* $T_{28}=4.65$ ,  $p<0.0001$

# Results- Centrosome

## Intensity of Cenexin Immunofluorescence at Centrosome (Median $\pm$ SEM)

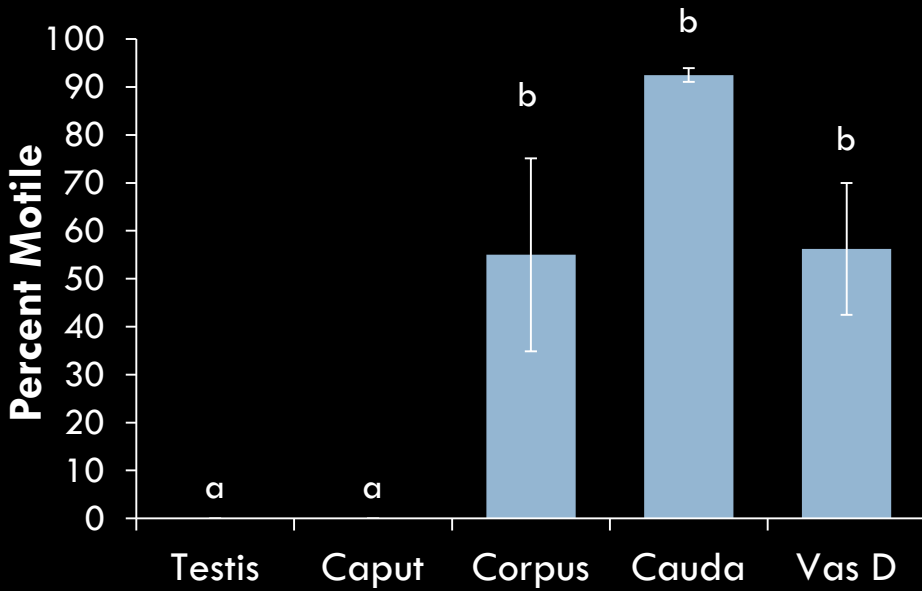


n=4 testes

\* $T_{12}=3.29$ ,  $p<0.0065$

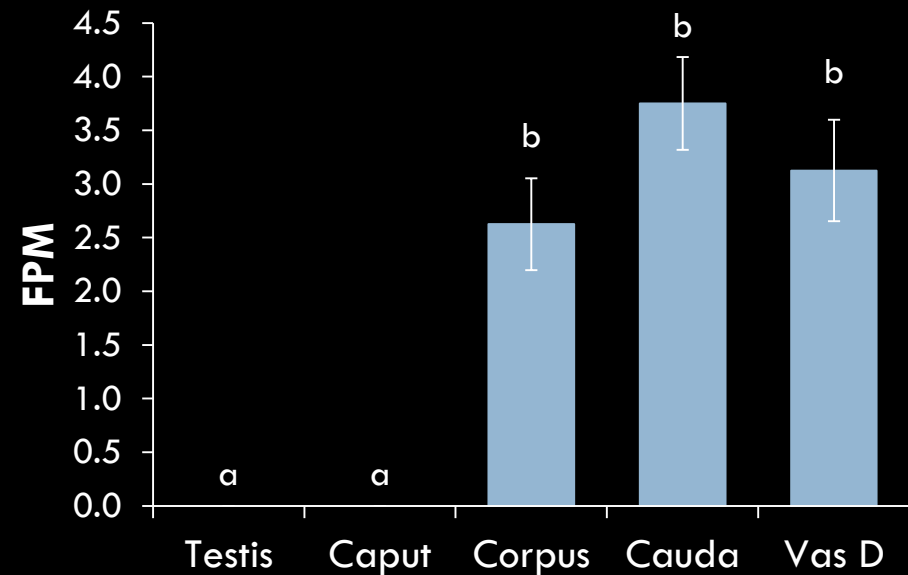
# Results- Tail

**Sperm Motility**  
(Mean  $\pm$  SEM)



$F_{4,15}=13.53, p<0.0001$

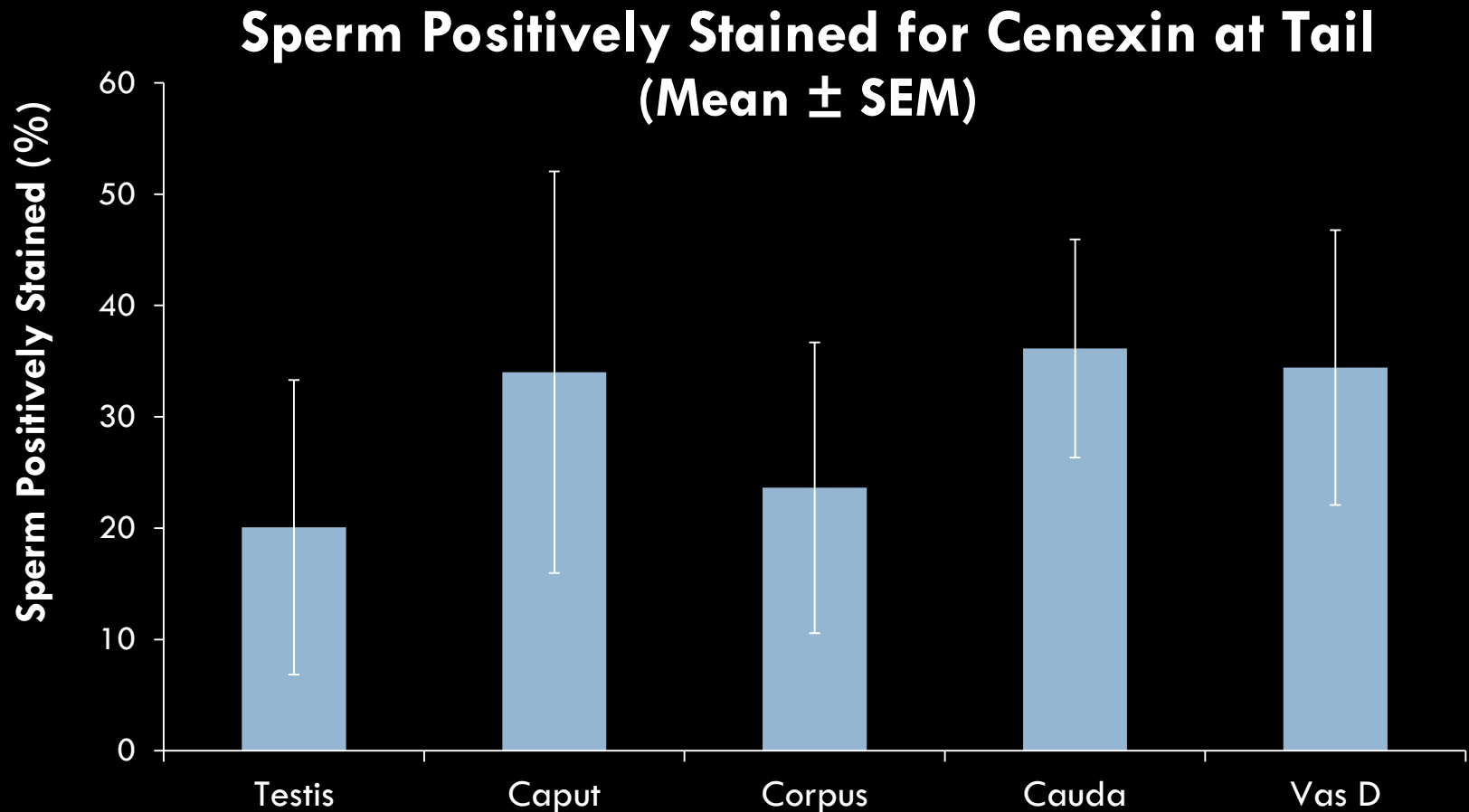
**Sperm Forward Progressive Movement**  
(Mean  $\pm$  SEM)



$F_{4,15}=26.67, p<0.0001$

n=4 testes, Protected Tukey's test following repeated measures

# Results- Tail

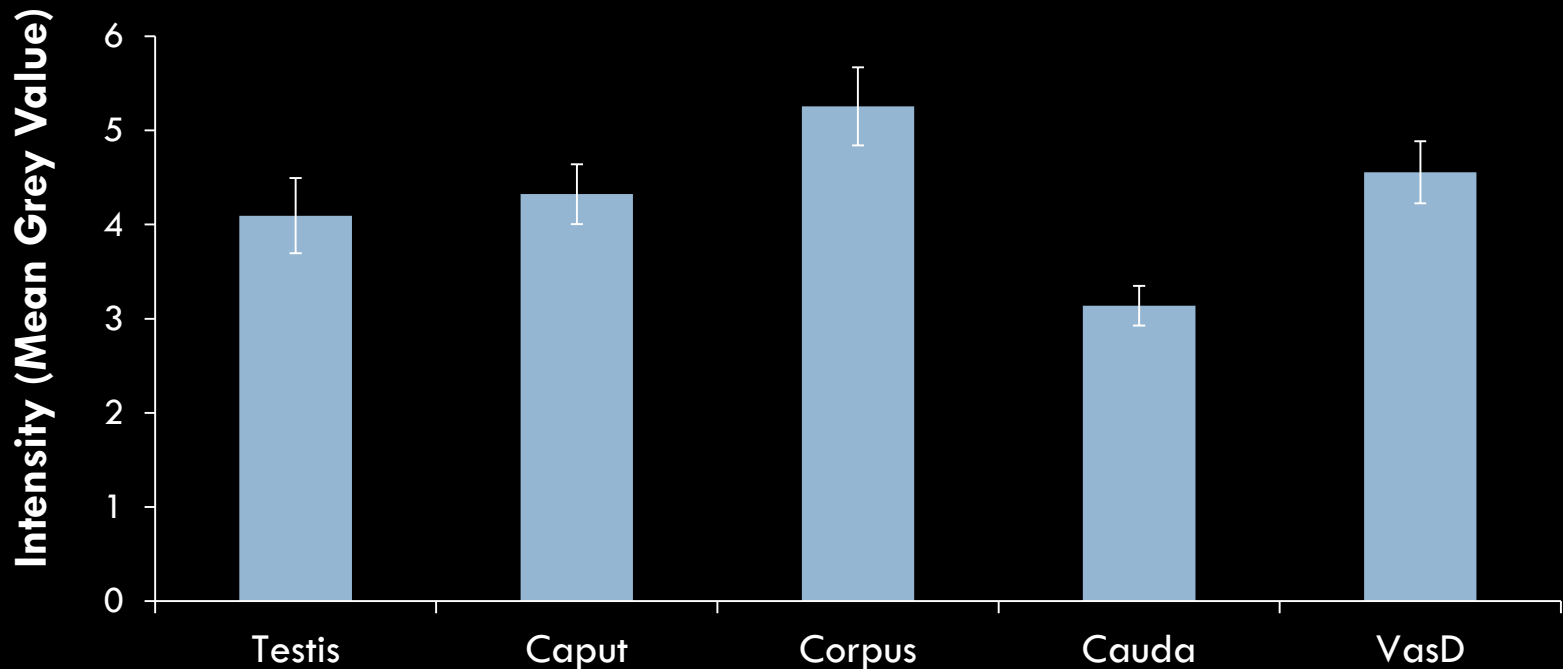


n=4 testes

$F_{4,15}=0.39, p<0.815$

# Results- Tail

## Intensity of Cenexin Immunofluorescence at Tail (Mean $\pm$ SEM)



n=4 testes

$F_{4,15}=0.56, p<0.698$



# Discussion- Centrosome

- Both the number of sperm stained positive for cenexin and the intensity in which they were labeled increased along the reproductive tract
- This indicates that cenexin may play a role in sperm centrosome maturation
- Therefore, supplementing cenexin in culture media could potentially improve artificial maturation of sperm cells

# Discussion- Tail

- Sperm motility and FPM significantly increased along the tract
- Despite this, the proportion of sperm tails labeled with cenexin as well as the fluorescent intensity did not significantly change ( $p > 0.05$ ) across segments
- These results indicate that cenexin is not associated with sperm motility

# Discussion

- Questions left to be answered:
  - What is the characterization of cenexin secretion along the epididymal tract?
  - Does presence of cenexin affect centrosome and subsequent function of sperm aster following oocyte penetration?
  - Which other proteins could be responsible for flagellum maturation?

# Acknowledgments



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# References

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