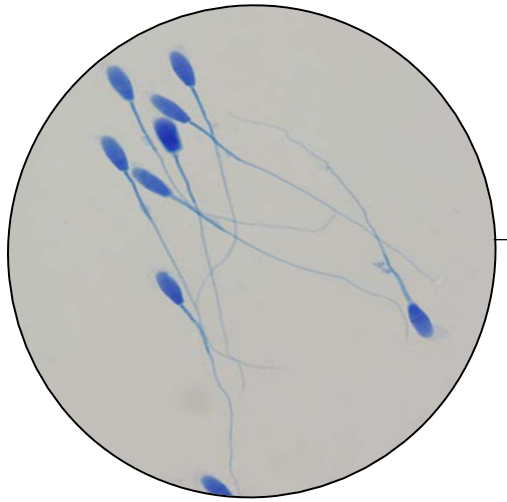
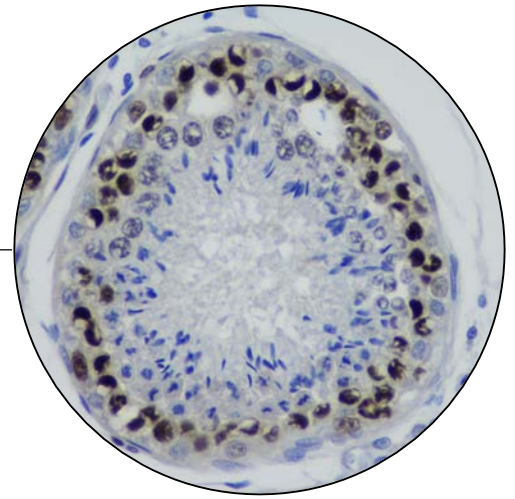


What is normal? Establishing baseline data for reproductive parameters in male Florida manatees

Jonathan R. Cowart, Danielle Arnold, Maria Quinones, Antonio Mignucci-Gionnani, Tamara Alejandro-Zayas, Paul S. Cooke, Iske Larkin



Can we see the problem?

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2. Miller, D. L., et al. (2001). "Ultrastructure of the spermatozoa from a Florida manatee (Trichechus manatus latirostris)." Anatomia, histologia, embryologia **30**(4): 253-256.

3. Reynolds, J. E., et al. (2004). "The likelihood of sperm competition in manatees - explaining an apparent paradox." Marine Mammal Science **20**(3): 464-476.

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5. Chavez, H. I. P. (2015). Reproductive anatomy and histology of the male Florida manatee (Trichechus manatus latirostris). Large Animal Clinical Sciences, University of Florida**:** 85.

Gaps in basic knowledge of male reproductive physiology

Deficiency of baseline data or normal reference points

PART I:

IMMUNOHISTOCHEMICAL ANALYSIS

Objective:

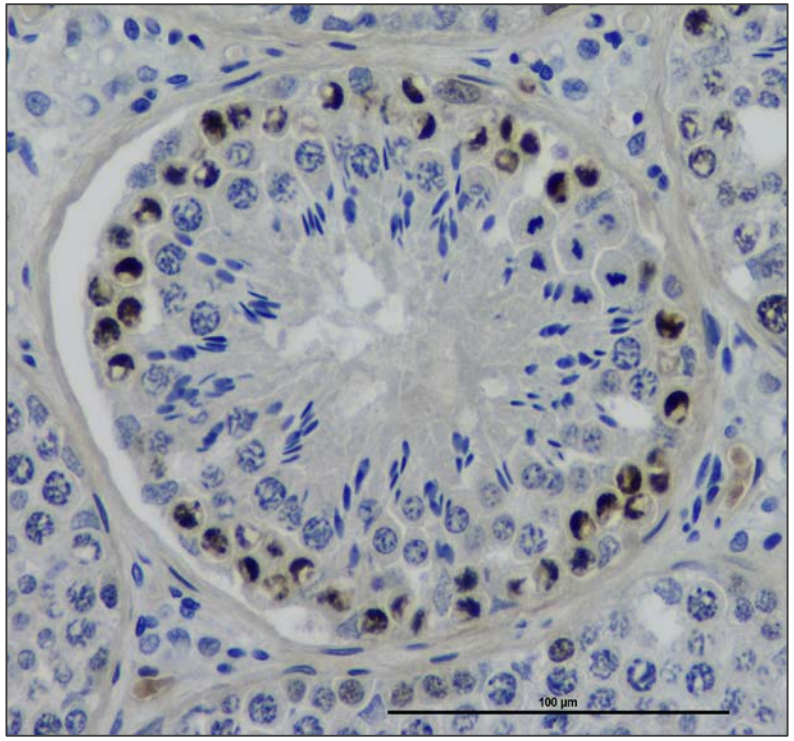
Assess the proliferative activity of spermatogonia within the seminiferous epithelium of male West Indian manatees

Sample Collection & Antibody Selection

- Gonadal tissues (24-48hrs post-mortem) collected between 2006-2017
- Samples separated by age class and season
- Anti-human rabbit monoclonal Proliferating Cell Nuclear Antigen (PCNA) aligned to Florida manatee PCNA immunogen

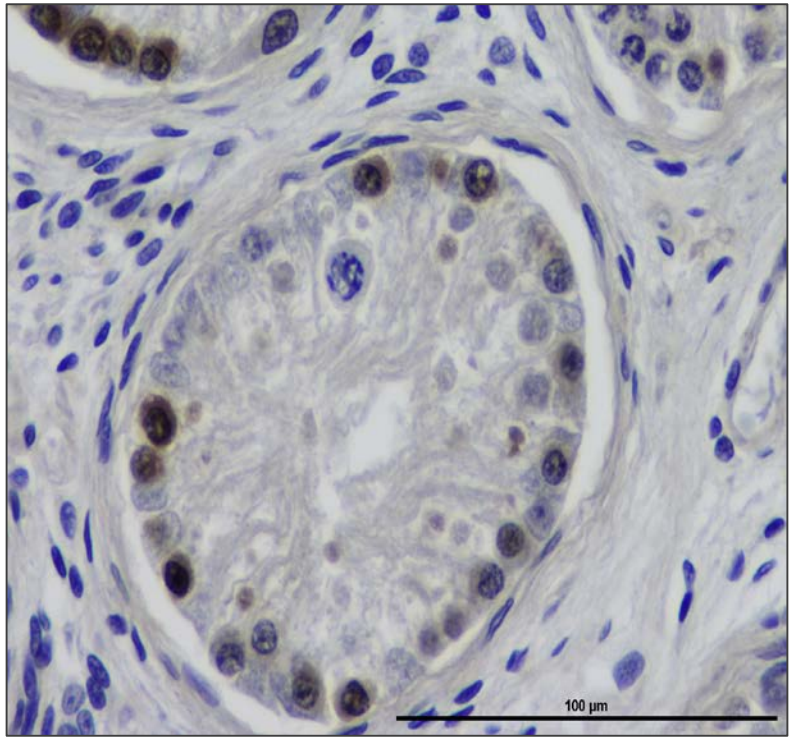
Sample size by age-class & season				
	Calf	Juvenile	Adult	Total
Non- Winter	17	9	10	59
Winter	8	6	9	

Age-class differences in expression



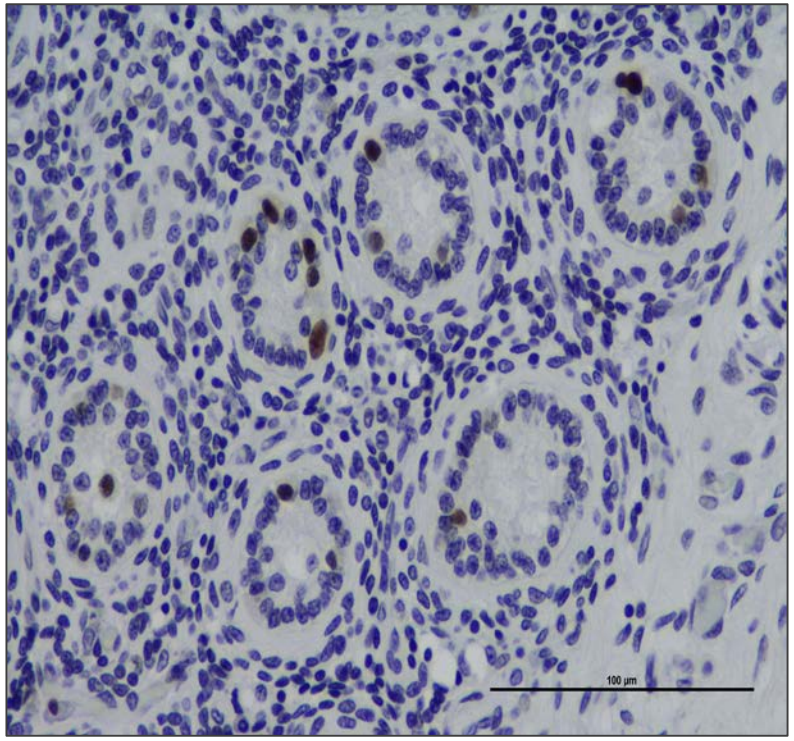
Adult

Adults exhibited **highest** levels of spermatogonia proliferation



Juvenile

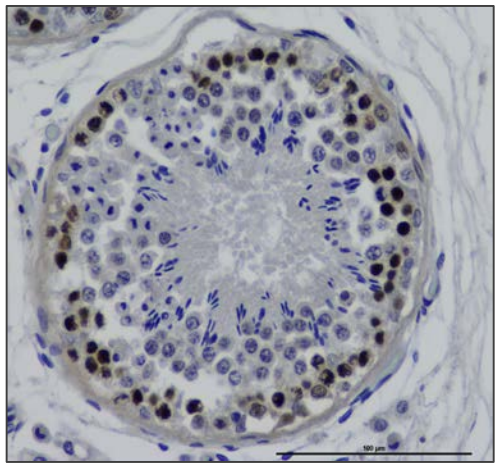
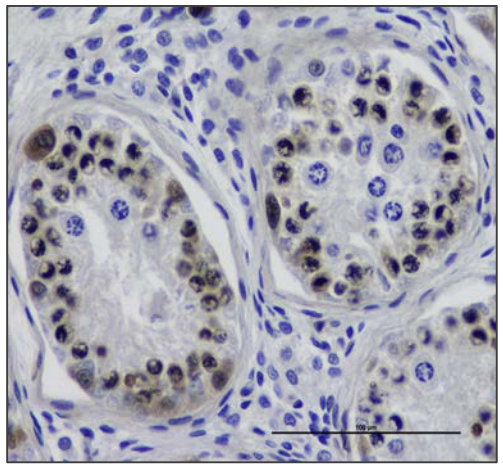
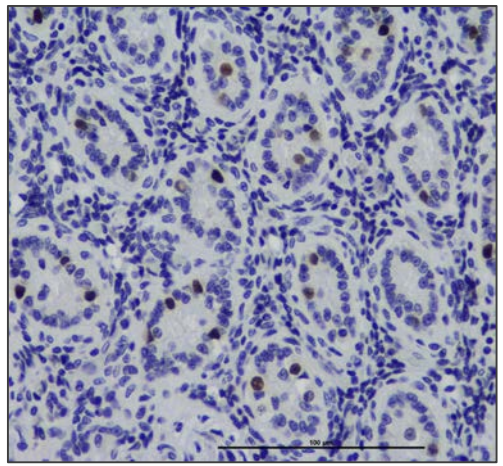
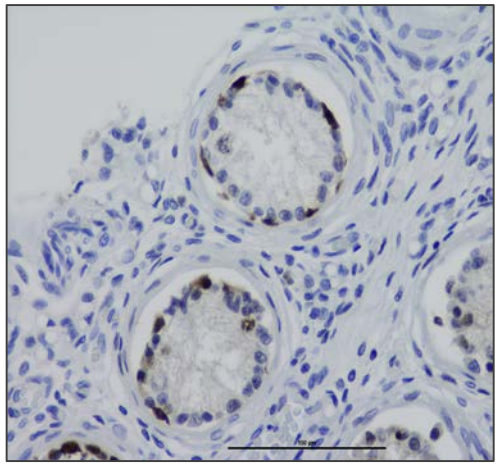
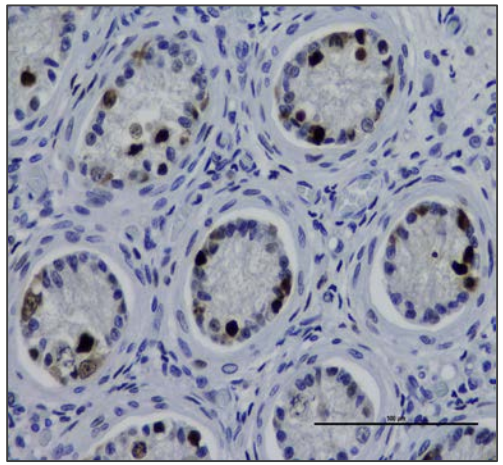
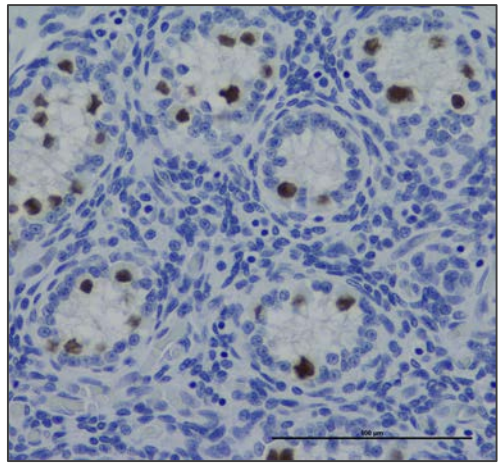
Juveniles exhibited **moderate** levels of spermatogonia proliferation



Calf

Calves exhibited **low to absent** spermatogonia proliferation

Seasonal differences in expression

	Adult	Juvenile	Calf
Non-winter			
Winter			

Part I: Conclusions

- PCNA immunohistochemical technique is viable method for assessing gonadal function in FL manatees
  - wide applicability for assessing proliferation in any tissue type
- Marked seasonal changes in spermatogenesis
  - active state of spermatogenesis during non-winter season
  - repression of spermatogenesis during winter season
    - continuation of spermatogonial proliferation

PART II:

SEMEN ANALYSIS

Objective:

Characterize spermiogram parameters of ejaculates collected from mature male West Indian manatee

- Semen Parameters:
  - **Volume**
  - **Color / appearance**
  - **pH**
  - Viscosity
  - **Concentration**
  - **Motility**
  - Agglutination
  - Mass movement
  - **Vitality**
  - Hyperactivation

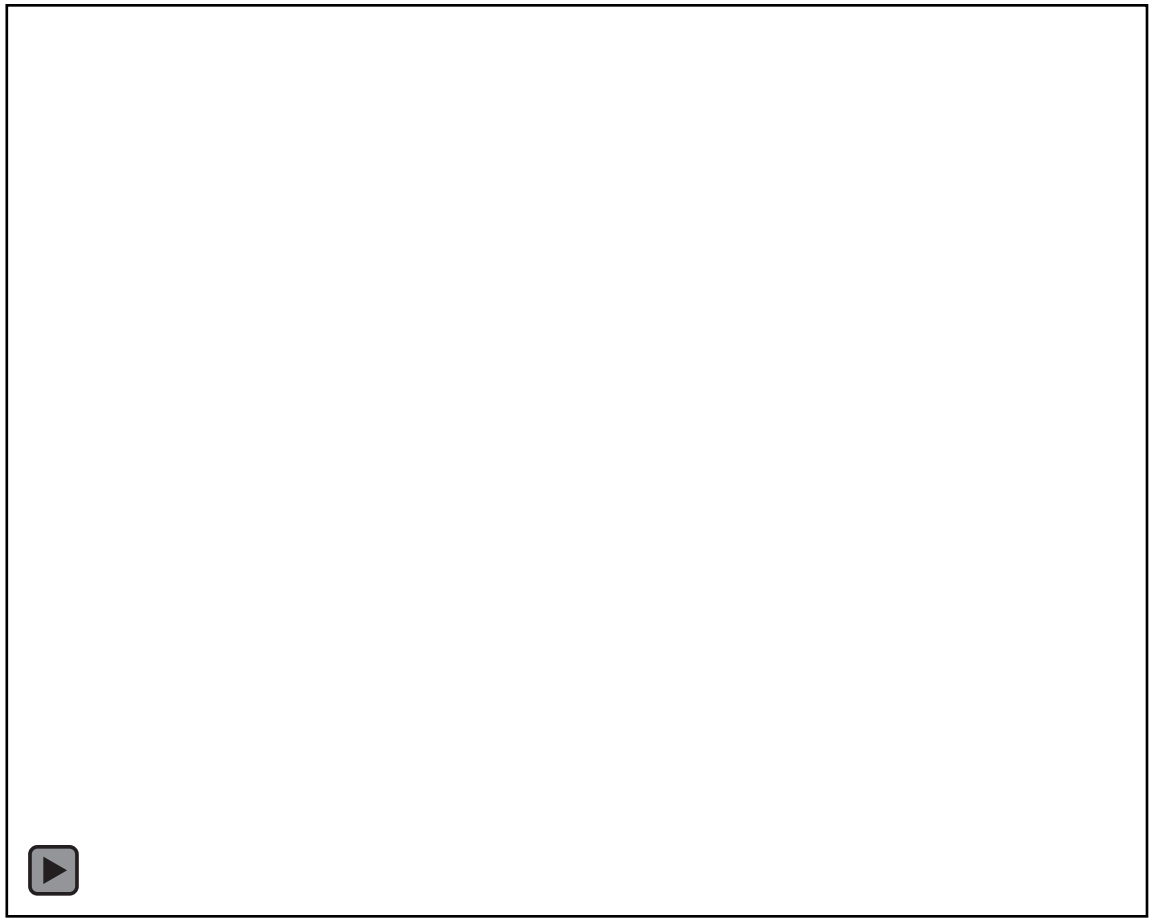
- Sperm Morphometry:
  - **Head length & width**
  - **Midpiece length**
  - **Total length**
  - Area
  - Perimeter
  - Acrosome coverage

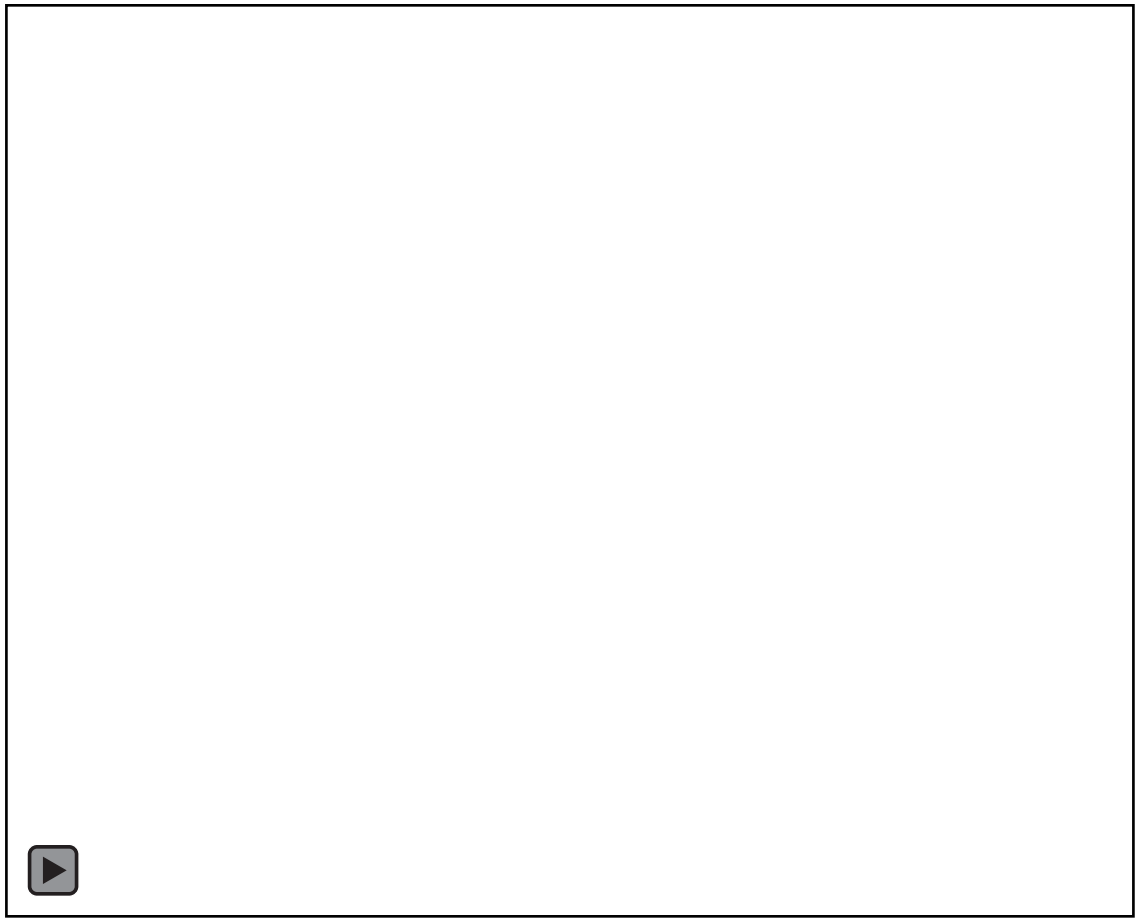
Photos taken under Federal Fish & Wildlife Permit #MA067116-2

Macro Analyses

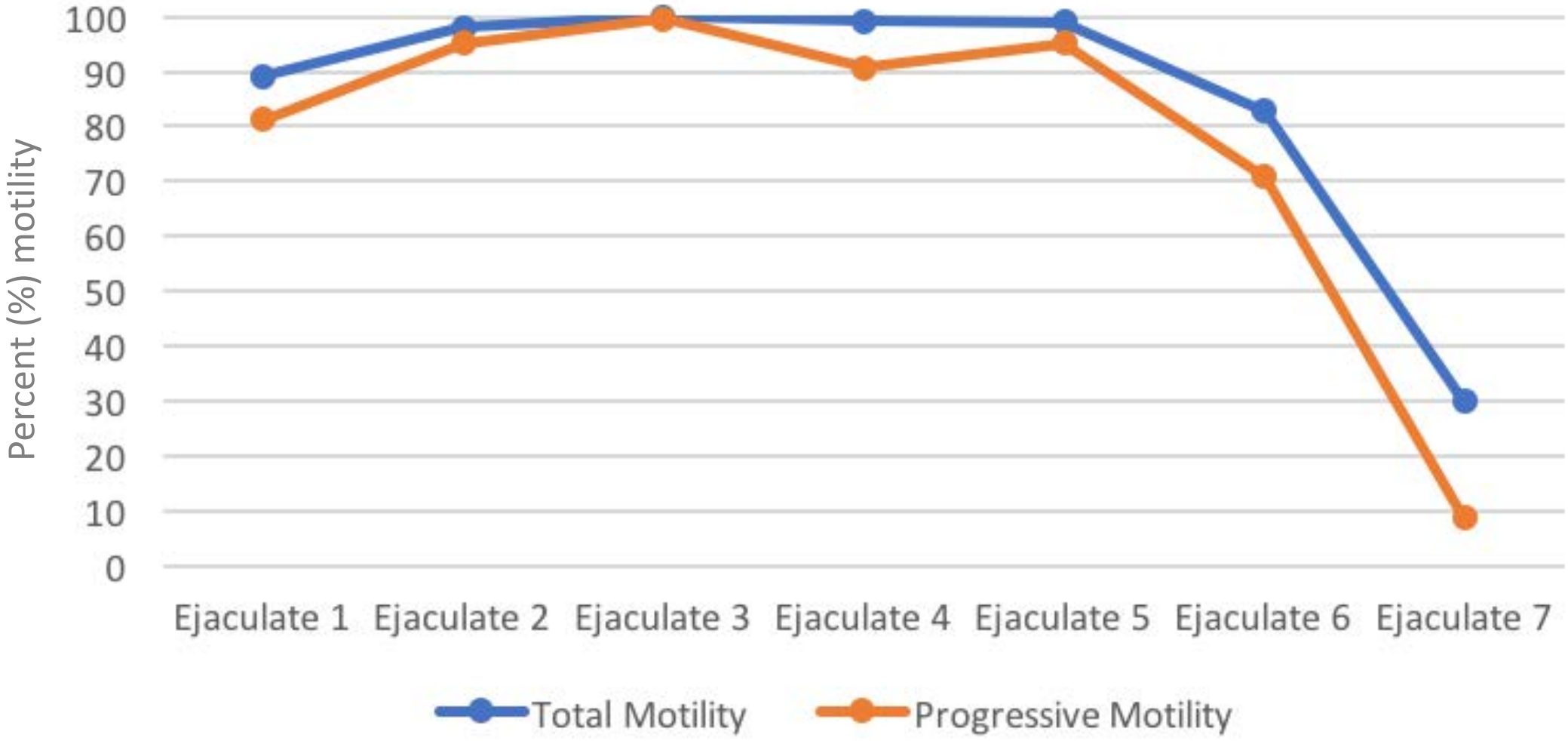
Sample	Volume (ml)	Color / Appearance	pH	Concentration	Comments
Ejaculate 1	45	Moderately translucent	8.5	97.47×10^6	
Ejaculate 2	44	Slightly milky	8.5	201.0×10^6	
Ejaculate 3	39	Moderately translucent	8.5-9.0	99.75×10^6	Potentially urine contaminated
Ejaculate 4	26	Slightly milky	8.0-8.5	71.85×10^6	Potentially urine contaminated
Ejaculate 5	44	Moderately translucent	8.5	70.0×10^6	
Ejaculate 6	30*	Moderately translucent w/ clumps of sperm	8.5	TBD	
Ejaculate 7	33	Moderately translucent	8.5	TBD	

Sperm Motility

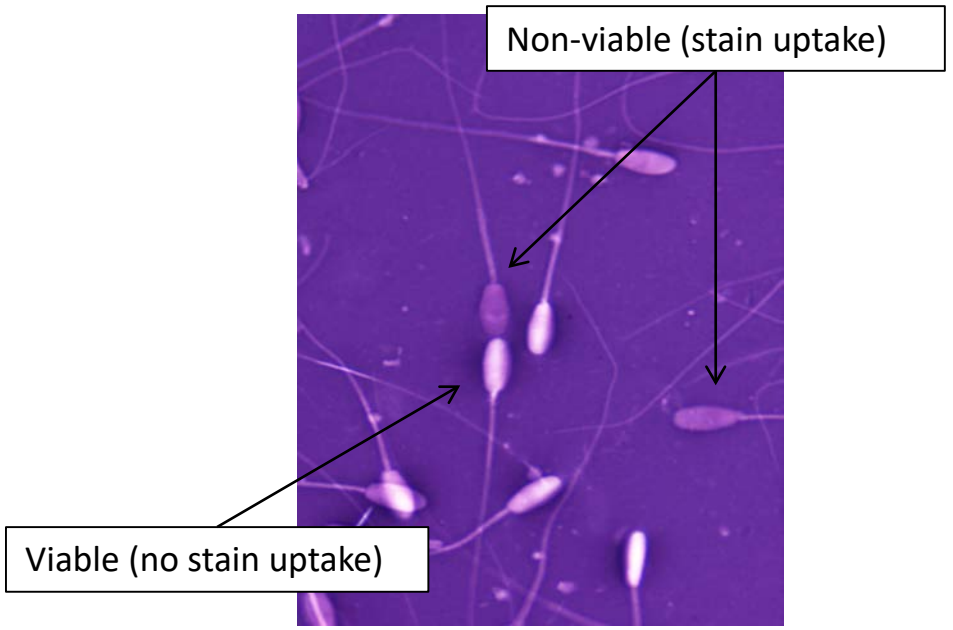


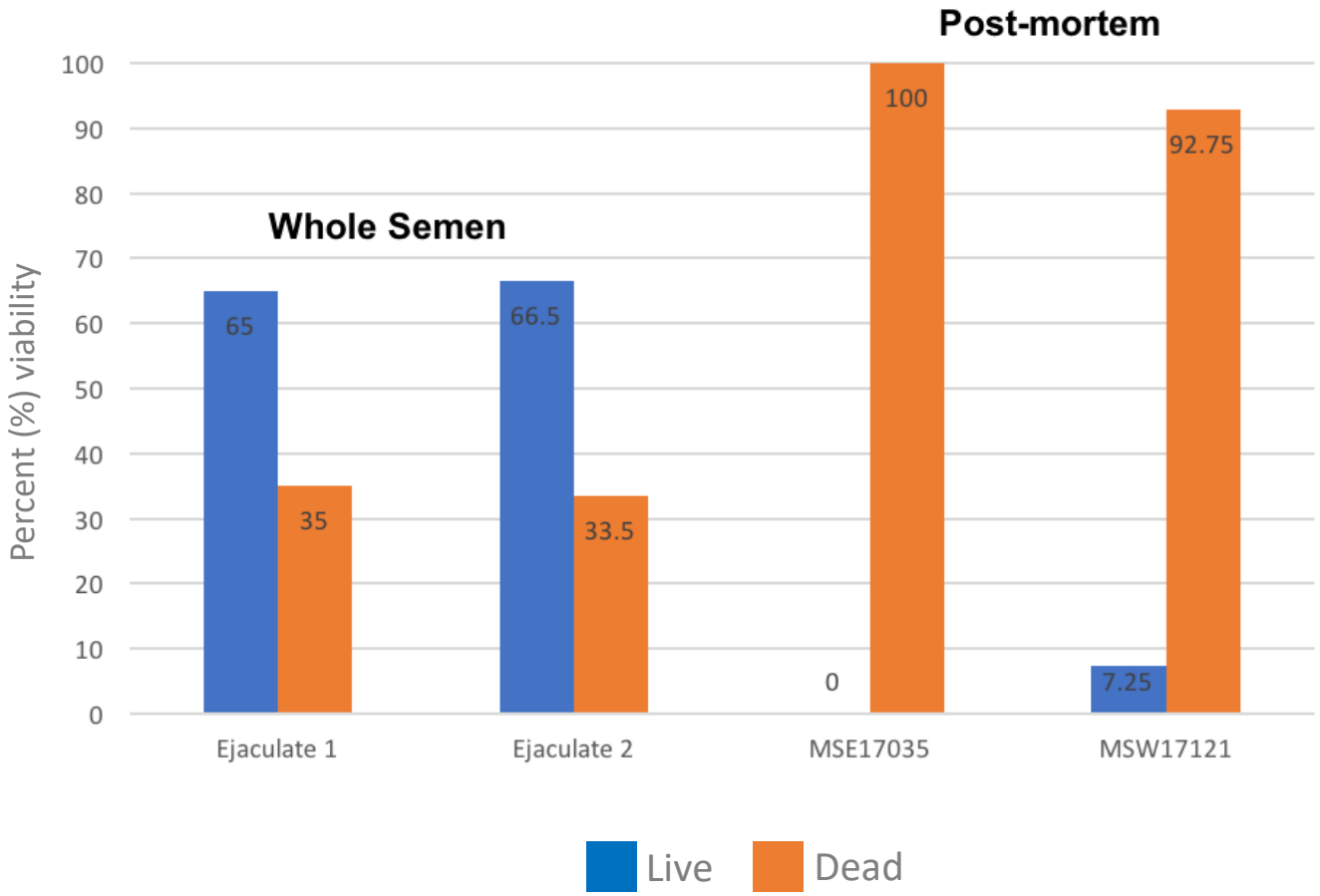


Sperm Motility

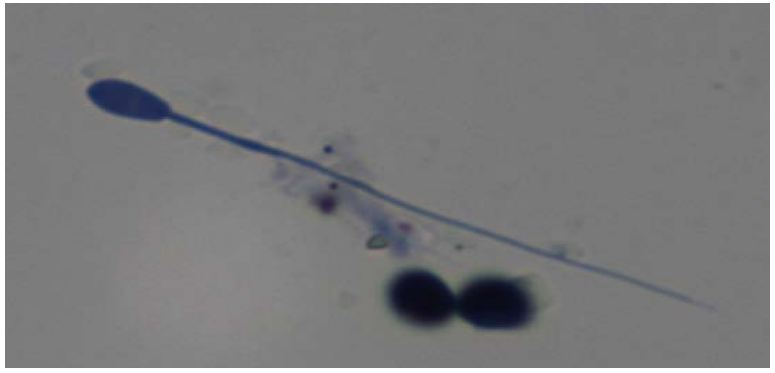


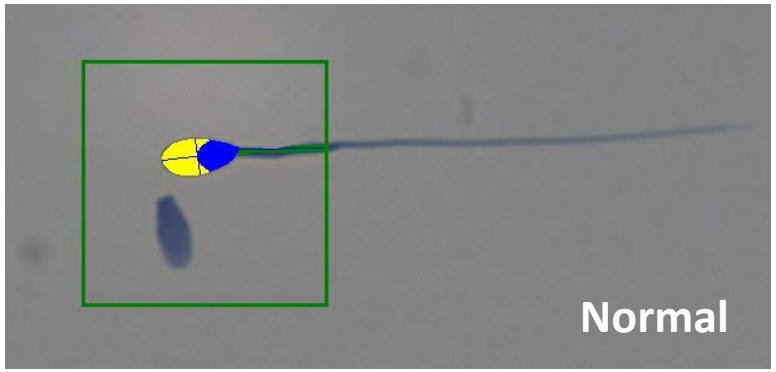
Sperm Viability
(plasma membrane integrity)

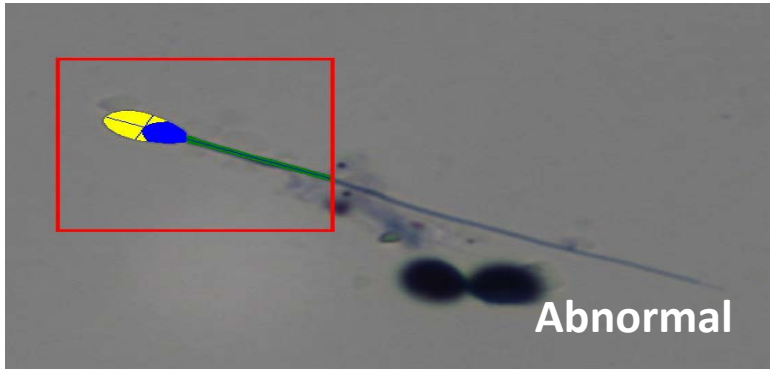


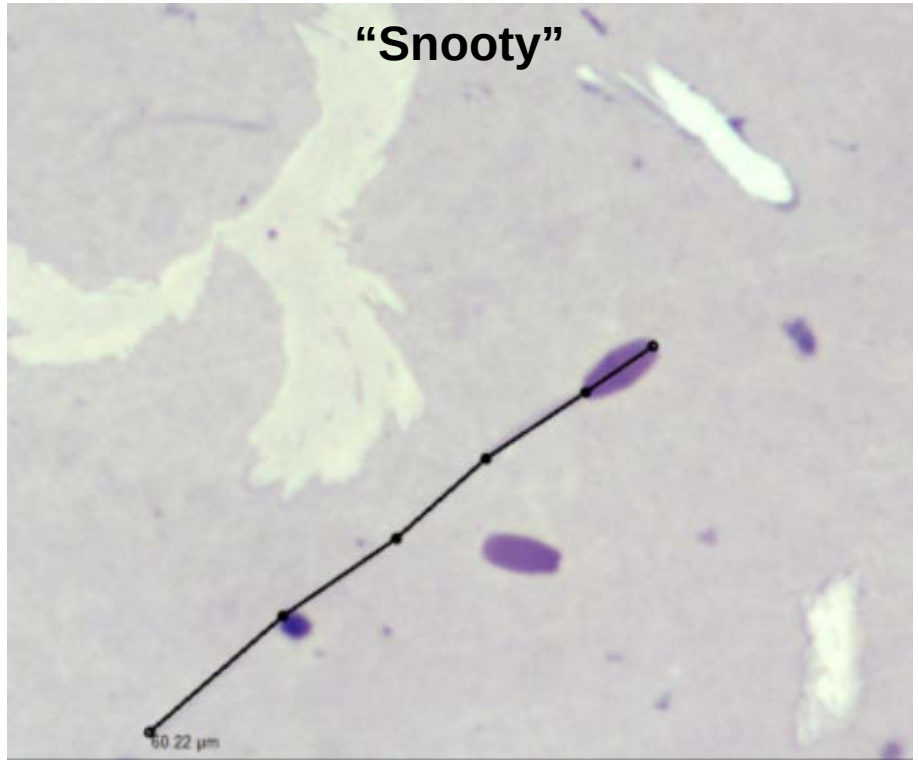


Sperm Morphometry









Sperm Morphometry

Collection Method	Analysis	Animals Analyzed	Mean Head Length (µ)	Mean Head Width (µ)	Mean Midpiece Length (µ)	Mean Total Length (µ)
Manual stimulation / Post-mortem	Computer – aided sperm analysis	3	7.42	3.45	10.47	59.21

Species	Collection Method	Analysis	Animals Analyzed	Mean Head Length (µ)	Mean Head Width (µ)	Mean Midpiece Length (µ)	Mean Total Length (µ)	Source
Florida manatee	Present in urine	SEM/TEM	1	4.9	2.9	5.5	30.1	Miller et al. (2001)
Amazonian manatee	Present in urine	Phase contrast	1	7.49	3.53	11.36	60.08	Amaral et al. (2010)
Bottlenose dolphin	Electro-ejaculation	SEM/TEM	1	4.5	2.0	4.0	65	Fleming et al. (1981)

Morphological Defects

Part II: Next steps…

- Further analysis of semen data
  - Hyperactivation
  - Viscosity
  - Motility kinematics

- Liquid storage & cryopreservation
  - Completed 4 full semen extender trials
  - Successfully cryopreserved 2 samples
  - Need to optimize

“… although our small sample sizes limit firm conclusions, this work nevertheless represents a useful contribution to the study of ~~mammalian~~ manatee ejaculate quality”

-modified from: Curren, Weldele, & Holekamp (2013) *Journal of Mammalogy*

Acknowledgements

Sample Collection & Analysis

Dr. Martine de Wit & entire staff of MMPL

Entire staff of PRMCC

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IHC Protocol Preparation & Optimization

Mrs. Pat Lewis

Dr. Elizabeth Whitley

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