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Effect of liquid storage and cryopreservation on the morphometry of spermatozoa in the West Indian manatee (*Trichechus manatus*)

While the conservation status of the West Indian manatee (*Trichechus manatus*) has been downgraded from 'endangered' to 'threatened', the continuation of the population still remains at risk. In addition to both natural and anthropogenic threats that result in high numbers of injury and mortality on an annual basis, the West Indian manatee faces potential challenges to reproduction related to the moderate level of inbreeding occurring within the population. In a first effort to mitigate the effects of inbreeding within this population, a pilot study was conducted to determine the best methods for liquid storage and cryopreservation of West Indian manatee semen. As part of this, the current study aims to understand the morphometric changes associated with liquid storage and cryopreservation on the spermatozoa of Florida manatees.

**Specific Aim 1:** Compare the influence of different semen extenders on the morphometric parameters of West Indian manatee spermatozoa.

**Specific Aim 2:** Determine the effects of cryopreservation on the morphometric parameters of West Indian manatee spermatozoa.

**Specific Aim 3:** Compare the influence of different parameters, such as storage temperature, storage time, and presence/absence of seminal plasma on the morphometric parameters of West Indian manatee spermatozoa.

Whole semen was collected from a wild-born, adult West Indian manatee under managed care at the Puerto Rico Manatee Conservation Center in Bayamón, PR as part of a doctoral research project. Semen samples were diluted in one of four liquid extenders under a variety of parameters including storage temperature (room temperature vs. 4°C), storage time (initial, 6hrs, 12hrs, 24hrs), and presence or absence of seminal plasma. In addition to liquid storage, two samples were successfully cryopreserved. For each of the liquid storage and cryopreservation samples, sperm smears were made and stained with SpermBlue® for morphometric analysis. The student tasked with this research project will undergo training on operating the AAH program's Sperm Class Analyzer (SCA) computer-aided sperm analysis (CASA) system to complete morphometric analyses for each of these representative samples. The student will ultimately be responsible for: (1) morphometric analysis of liquid storage and cryopreservation samples using the CASA system and (2) completion of representative portion of larger scientific manuscript for publication. Student will work independently while under the guidance of supervisor Dr. Iske Larkin and postdoctoral research associate, Dr. Jonathan Cowart, for the completion of this project.