The effects of pair bonding and monogamy on epididymal sperm characteristics in prairie voles (*Microtus ochrogaster*)

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Background

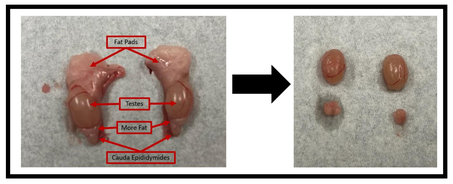
Sperm characteristics between and within species are both highly conserved as well as highly variable among mammals. Sperm competition is known to have substantial effects on sperm and gonadal characteristics as well as sperm production. A high level of sperm competition is associated with larger testes and greater sperm production (Horst and Maree 2014). Monogamy is relatively uncommon in mammals but is practiced among a few species, including humans, along with the formation of familial social bonds (Lukas and Clutton-Brock 2013). In contrast with sperm competition, little research has been done on the effects of monogamy or a lack of sperm competition on sperm and gonadal characteristics. The research that has been done indicates a relationship between generally monogamous species and a change in these characteristics compared to species with routine sperm competition. For example, this relationship is observed in pair-bonding humans when compared with other promiscuous primate species (Horst and Maree 2014).

Prairie Voles are a social, monogamous species - a rare feature in rodents - which have gained traction in the fields of neurobiology, neuroendocrinology, and psychology as a potential model species for studying human social behavior and associated biology, due to similarities in brain chemistry and social tendencies as well as the similarities between rodent and human physiology (Aragona and Wang 2004). Reproductive work in prairie voles is somewhat limited, with more emphasis placed on behavior than physiology. The female prairie vole estrus cycle is unique in that the females do not begin estrus cycling until exposed to a non-relative male (Carter et al. 1980). It is unknown if/how female exposure and pair bonding affect male sperm characteristics and/or production.

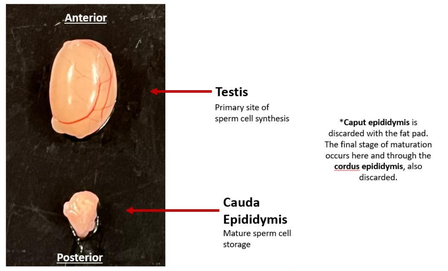
Here we utilize male prairie voles as a study model for if/how pair bonding and monogamy affect sperm production and characteristics. Prior to comparing sperm characteristics between mated and unmated males, we establish an effective methodology for epididymal sperm extraction and analysis utilizing established techniques in other rodents (Duselis and Vrana 2007), modern theriogenology concepts, personal experience, and trial and error.

Methods

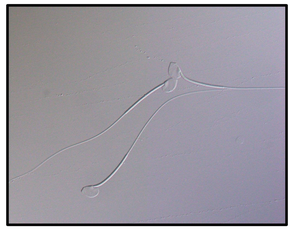
All prairie voles are lab raised and kept in temperature, humidity, and light controlled environments at the OSU Animal Resources MRSF facility. The males are harvested at a minimum of 95 days of age, up to the end of their lifespans (12-18 months) to allow for the consideration of the effect aging may have on sperm. The voles are euthanized via isoflurane, secondarily verified via decapitation.

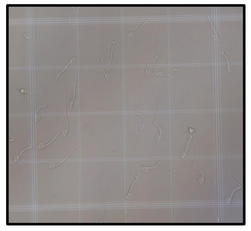


The gonads are removed, the fat is trimmed, and each testis is separated from its cauda epididymis. Each testis and epididymis is weighed, and the epididymides are placed in 0.5mL of PBS at 37 degrees Celsius. 18-gauge needles are used to destroy the epididymal tissue while keeping the tissue connected in 1 or 2 pieces. This step is crucial for the prevention of excessive tissue cells in the sperm suspension. At this point, the sperm is examined on a bright-field Leica microscope to ensure it is living and motile. 0.5mL of 10% buffered formalin is added to immobilize and preserve the sperm sample for future analysis.



The sperm samples are analyzed in the small animal theriogenology lab at the OSU Veterinary College via DIC (differential interference contrast) microscopy, which is the preferred method for sperm imaging, and a NucleoCounter. While hemocytometer slides are often used for cell counting, a NucleoCounter is designed for sperm cells, highly accurate, and helps decrease human error/bias and substantially increases time efficiency. However, it is designed for ejaculated/catheterized samples. In order to test its accuracy on our samples, a hemocytometer was used, then compared to the NucleoCounter results. The sperm counts of each method are consistently within 10-15% of each other, confirming the accuracy of the NucleoCounter for future use.





Results

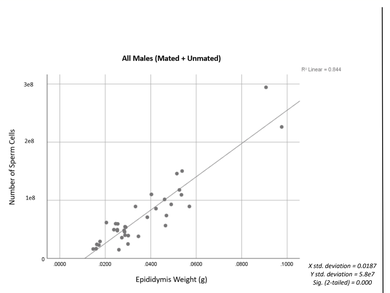


Figure 1. Relationship between epididymis weight and number of sperm cells

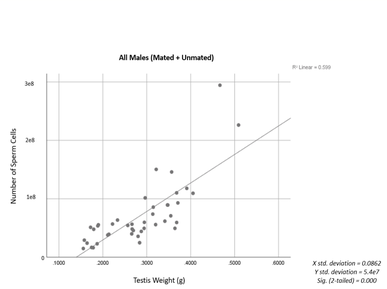


Figure 2. Relationship between testis weight and number of sperm cells

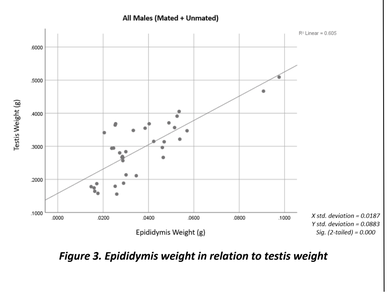


Figure 3. Epididymis weight in relation to testis weight

Looking Ahead to Unmated vs Mated Males:

	Average Total Sperm Count	Average Testis Weight	Average Epididymis Weight
Unmated Males	1.04e8	.25g	28mg
Mated Males	2.25e8	.36g	70mg

Figure 3: Comparison of unmated male and mated male gonadal weights and sperm counts

* The mated male sample size is not yet large enough for reliable statistical analysis, but preliminary results indicate a high likelihood of significance

Conclusions

Our results thus far indicate that **the methodology developed for this research is effective**. By utilizing established techniques in other rodents and modern theriogenology concepts, we were able to confirm the following:

- **Direct relationships between:**
  1. testes weight and sperm cell count
  2. epididymis weight and sperm cell count
  3. testes weight to epididymis weight
- **A NucleoCounter is an accurate method to quantify sperm cells** harvested by this methodology, as confirmed by cross-referencing with hemocytometer counts

Currently, our data indicates a **significant difference in the gonadal weights and total sperm counts between unmated and mated males**, yet to be confirmed by a larger sample size and statistical analysis.

Future Directions

- **Continue gathering sperm data on mated males**
  - Target sample size of 15-20
  - Currently at 7 males analyzed, 9 harvested
- **Perform sperm morphologies on all unmated and mated samples**
- **Determine cause of difference between unmated and mated males**
  - Copulatory-induced or neuroendocrine in nature?
  - Utilize dividers in final sample size that allow for olfactory cues and pair bonding but not copulation

References & Acknowledgements

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