Electroejaculation in wild felines

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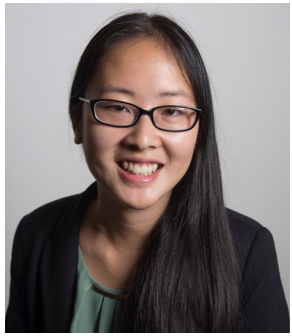

Writer's Comment: Animal reproduction, especially semen collection, has been a favorite topic of mine ever since I was first exposed to it in my introductory animal science classes. When charged with the task of a literature review in Dr. Rodger's UWP 104E class, I chose electroejaculation because it is a method that most people haven't heard about. I hope my paper will shed light on the electroejaculation process, and how it can be used to help with conservation of wild felines.

Instructor's Comment: Early in the winter quarter, Courtney approached me after class one day and said she wanted to run her idea for a paper topic by me. Knowing that the assignment—a technical description written for a professional audience—included a visual poster that would be presented to our class, she was concerned that the topic might be inappropriate. As I've taught UWP 104E many times, I was certain that there was no topic that could surprise me; my students have written about everything from bioethics to biogas. So needless to say, I was doubly surprised when Courtney stated that she wanted to write a technical description about electroejaculation. This is a process that is a vital part of selection for breeding in the dairy industry—a cornerstone application of animal science research here at UC Davis. Courtney took on the topic with professional attention to detail and design, and created an effective and impressive poster. She continued to work on the topic for the other assignments in class, including in the essay included here. Courtney's work reflects the best of our science students, and I am pleased that we can share this piece

of professional writing.

– Katie Rodger, University Writing Program

Introduction

Electroejaculation has long been used in the dairy industry to obtain semen from bulls. The process involves inserting a probe into the animal's rectum and applying an electrical current in order to stimulate muscle contractions and induce erection and ejaculation (Dziuk et al. 1954; Marden 1954). In the dairy industry, obtaining semen is important for use in artificial insemination, as it eliminates the need to transport animals to each other, enables dairies to not own a bull, and allows access to the best genetics from anywhere in the world.

Current research is looking to apply electroejaculation to the conservation of wild species, specifically small felines. Applications of electroejaculation include fertility assessment (Kheirkhah et al. 2017) and, more importantly, in vitro fertilization (IVF). IVF is important in wildlife conservation for many of the same reasons as in the dairy industry. By shipping genetic materials such as semen or eggs, IVF eliminates the need to ship animals to each other (Wildt and Roth 1997). Animals who are socially incompatible can be bred using IVF (Tajima et al. 2016; Wildt and Roth 1997). Cryopreservation of genetic material allows long-term storage, and could be used to help populations recover genetic diversity in the event of disease or a natural disaster. Sexually transmitted disease risk is eliminated when using IVF. IVF also allows researchers to introduce wild genes into captive populations, or vice versa, without bringing new animals into captivity (Wildt and Roth 1997).

Despite the potential power of IVF, small feline conservation is challenging. Since populations of endangered cats are small, there is inbreeding and little genetic diversity. The former can be especially problematic because of inbreeding depression, while a lack of genetic diversity increases the risk that one disease can easily wipe

out an entire population (Frankham 2015; Swanson 2006). Inbred populations therefore have a higher risk of extinction (Frankham 2005). Further, small populations are more susceptible to genetic drift, or a loss of alleles in a population due to random chance (Swanson 2006).

Procedures

Through experimentation with domestic cats, a procedure was described for electrical stimulation when electroejaculating wild felines. Electrical stimulation starts at zero volts, then linearly increases from zero to the maximum amplitude in one second, maintains at the maximum amplitude for two seconds, drops instantly back to zero, and stays at zero for one second before beginning again. The maximum amplitude needs to be determined by the operator for the different species and individuals that are being collected (Oana et al. 2016).

In the domestic cat model, the probe is an 8mm plastic tube, with disposable copper wire used to conduct electricity. The wires are disposable for ease of cleaning when electroejaculating multiple animals. Empirically, this design has been found to minimize stress and pain felt by the animal, and also to deliver the electricity in the most effective way (Oana et al. 2016). In wild felines, probes must be tested for each species. Probes are often custom-made and some variables to consider are the number of electrodes, ring versus parallel electrodes, and the materials from which the probe and electrodes are made (Tajima et al. 2016). Probe placement inside the rectum is also important; if the probe is inserted too far into the rectum, the bladder may be stimulated, resulting in urine contamination of the semen sample (Tajima et al. 2016).

Various collection vessels have been described. One method is a urethral catheter inserted into the urethra and attached to a syringe which collects semen via capillary action (Oana et al. 2016). A less invasive collection vessel is a simple canonical tube, which is placed around the penis to collect the semen as it drips out during

electroejaculation. In order to prevent urine contamination using this method, a catheter should be used to collect the urine prior to electroejaculation (Tajima et al. 2016).

In an Amur leopard cat model, electroejaculation was considered successful in obtaining viable semen for IVF. Sperm motility was high, and abnormalities were low unless blood or urine contamination occurred. The sperm count was about 19 million per ejaculate, which is less than the 40-80 million typically collected from domestic cats (Tajima et al. 2016). It should be noted, however, that there is no baseline data for Amur cats. This reduced volume could possibly be attributed to Amur cats in general ejaculating fewer sperm than domestic cats (Tajima et al. 2016). Electroejaculation in a jaguar model tells a similar story: semen collected via electroejaculation was found to have high sperm motility and quality, and the sperm count was about 10 million (Gonzalez et al. 2017).

Seasonal differences were found in the Amur leopard cat model, depending on whether or not the electroejaculation occurred during breeding season, January-April. It was found that collections performed during May-August, after breeding season, had the lowest sperm count and sperm motility, as well as the highest rates of deformities. The testicular volume, calculated based on measurements of major axis, minor axis, and thickness, by the formula $4/3\ \pi \times$ (major axis)$/2 \times$ (minor axis)$/2 \times$ thickness$/2$, was also found to be significantly smaller after rather than during breeding season. Additionally, differences due to age were not found to be significant (Tajima et al. 2016).

An optional step in the electroejaculation process is cryopreservation. This step is not needed if the semen is to be used right away, but cryopreservation allows semen to be shipped or stored long-term (Wildt and Roth 1997). For cryopreservation, semen is first centrifuged to remove seminal fluids, and then a semen extender is added. The solution is equilibrated for one hour at 4 degrees Celsius, then stored in liquid nitrogen until ready for use. Thawing is accomplished using a water bath at 37 degrees Cel-

sius to mimic body temperature (Thuwanut et al. 2016). A problem with cryopreservation is that semen quality, as measured by sperm motility, is often poor after the freezing and thawing. In an Indochinese leopard model, it was found that semen quality was protected by the addition of adenosine 5'-triosphate (ATPe). It is thought that ATPe affects intracellular calcium and ATP levels, which give energy to sperm acrosomes that are responsible for motility (Thuwanut et al. 2016).

Alternatives

One alternative to electroejaculation is the artificial vagina (AV) method. In AV, the male animal mounts a teaser animal or a phantom, and his penis is deflected into an AV that provides physical stimulation and serves as a reservoir to collect the semen. Compared to electroejaculation, collections using AV typically have a lower volume of total semen but higher concentration of sperm (Kheirkhah et al. 2016; Tajima et al. 2016). AV is less expensive than electroejaculation, since electroejaculation requires specialized equipment and a trained operator (Kheirkhah et al. 2016). Additionally, AV is more natural, as electroejaculation requires anesthesia (Tajima et al. 2016) and, in some countries, illegal (Tajima et al. 2016; Zambelli et al. 2008). However, the main obstacle to AV is that the animal must be trained to service the AV, which is often not feasible with wild animals (Kheirkhah et al. 2016; Tajima et al. 2016).

Another alternative to electroejaculation is collection directly from the caudal epididymis. This method is not repeatable, since it requires harvesting the entire testicle. Therefore, this method can only be used in conjunction with a castration, or when an animal is dead. This method has the advantage of being able to preserve the genetics of a dead animal (Tajima et al. 2016). Caudal epididymal collection is performed by cutting the vas deferens and pampiniform plexus to remove the entire testicle, then cutting the caudal epididymis in order to drain the sperm (Toyonaga and Tsutsui

2012). A problem with this method is that the epididymis is a storage site for immature sperm, and the sperm do not fully mature until ejaculation. Collecting directly from the caudal epididymis leads to samples with immature sperm. However, researchers found that the maturation is due to contact with seminal fluids, so exposing the immature sperm to seminal fluids can remedy the problem. After this remedy, semen collected from the caudal epididymis is similar to semen from other collection methods (Toyonaga and Tsutsui 2012).

A newer alternative to electroejaculation is urethral catheterization (UT) alone. UT can be used in conjunction with electroejaculation (Oana et al. 2016), but in this method, the stimulation comes from drugs instead of electrical current. The male animal is injected with ketamine and medetomidine. Ketamine is an anesthetic, but medetomidine serves a dual purpose, acting both as an anesthetic and to displace sperm from the epididymis to the urethra. Following an ultrasound to determine correct placement, a urinary catheter is inserted into the urethra. The sperm are then collected by capillary action and a syringe attached to the catheter (Kheirkhah et al. 2016; de Schepper 2016). Compared to electroejaculation, the ejaculate volume obtained using this method is typically very small (10-100 uL), but the sperm concentration is higher (Zambelli et al. 2008; de Schepper 2016). The motility and abnormality rates are similar (Zambelli et al. 2008). UT is noninvasive, repeatable, and convenient to perform in the field (de Schepper 2016), so it can be a good alternative in situations where electroejaculation is not permitted by law or by ethics committees (Zambelli et al. 2008).

Conclusion

Electroejaculation is becoming an important tool in feline conservation. Electroejaculation and other semen collection techniques make IVF possible, which is critical for helping endangered species through captive breeding programs and restoring popula-

tion numbers and genetic diversity in wild populations. In the future, scientists are looking to further refine semen collection techniques or to discover new ones that can efficiently collect semen from endangered species.

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