Evaluation of the tom: collection procedures, evaluation of sperm, and subsequent use
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Abstract
When managing catteries, it is important to understand appropriate behavior and management of the tom cat in order to diagnose and manage fertility problems. Semen collection and subsequent use in the domestic cat has its own unique challenges, but is important for analysis of fertility and the implementation of advanced reproductive techniques. Improved techniques for the collection and use of feline spermatozoa is vital as it allows the preservation of valuable genetics in pure breed catteries, and preservation of rare and endangered feline species. The domestic cat also serves as an important model for several human diseases and preservation and maintenance of these genetics is necessary. The intent of this review is to offer a detailed protocol for the successful collection, evaluation, and use of feline sperm.

Keywords: Feline, cat, tom, sperm collection, electroejaculation

Introduction
Interest in the domestic cat examination, semen collection, evaluation, and use has increased in the past ten years. Because the domestic cat can serve as a model for reproduction of exotic and endangered feline species, and as an animal model for human disease, research interest in assisted reproduction has increased. Additionally, preserving the valuable genetics in pure breed catteries has also increased the demand for quality reproductive management. Semen collection and evaluation is vital to diagnose and monitor progress for any reproductive problem. However, the process presents its own set of challenges unique to the cat due to the small volume and comparatively low sperm number produced in each ejaculate as well as the decreased overall sperm quality seen in many exotic species or research catteries. The demand for semen collection, evaluation, and subsequent use is growing as a way to preserve important or valuable genetic materials.

History
A complete reproductive and general management history is extremely important when evaluating the tom for infertility, as many of the issues may be management practices. Questions should include (but not limited to) type of housing, mating practices, time with female, number of matings observed, previous litters sired, litter size, and any previous sperm analysis performed. Also important to document is if the female shows the typical after reaction following mating. If this does not occur, the tom may not be achieving vaginal penetration of the queen and therefore not inducing ovulation or ejaculating within the vaginal vault.

Examination
In addition to a complete history, a general physical examination should be performed, possibly with a complete blood count and serum chemistry to evaluate the overall health of the animal. A reproductive examination should consist of palpation of the testicles, assessing for size, texture, and symmetry followed by ultrasound to assess any irregularities within the testicular parenchyma. The penis should also be evaluated for any discoloration, discharge, and the presence of spines. Exteriorization of the penis may be difficult in the unsedated animal, so this portion of the examination might be best accomplished just prior to sperm collection when the tom is sedated or anesthetized.

Semen collection methods
The artificial vagina (AV) for the domestic cat is most commonly constructed using an Eppendorf tube and a rubber pipette bulb. The male is allowed to mount a queen, and the AV is held in place to facilitate and collect the ejaculate. If the male has been adequately trained to the AV, or a queen in estrus is not available, the male may be allowed to mount a gloved arm. The advantages of using an AV are that
Semen collection can be performed readily in the unanesthetized tom and allows a complete ejaculate to be obtained. Disadvantages are the requirement for training and a frequent necessity of a teaser queen. Often, weeks to months of conditioning and training are required before a tom is consistently producing ejaculates, and training may not be successful in all toms. Valiente et al worked with toms in their cattery three times weekly for 20 minutes and reported a mean of 3.9 months (1.5-5.5 months) before the first ejaculate was produced. With patience, collection using an AV is an excellent method for situations where a single male or group of toms are collected on a regular basis, such as in a cattery or research colony, but is impractical for a single evaluation in a clinical setting using an untrained tom.

Electroejaculation has been the most common method of obtaining an ejaculate from a tom that is not trained to an AV. The procedure requires general anesthesia. This author’s preferred anesthesia protocol includes dexmedetomidine (30-40 µg/kg) and ketamine (3-5 mg/kg) in the muscle, followed by intubation and supplemental oxygen. Inhalant anesthesia (isoflurane) can be added if necessary, but the short procedure time generally does not require it. On occasion, electroejaculation using inhalation anesthetics may result in urination and contamination of semen samples. Zambelli et al compared the use of medetomidine alone to ketamine alone on the quality of ejaculates collected by electroejaculation. These researchers found that the use of an α₂ agonist (medetomidine; Domitor, Pfizer, Florham Park, NJ) produced higher numbers of spermatozoa in the ejaculate than using ketamine alone, and did not increase the incidence of retroejaculation. However, these researchers did not evaluate the use of these medications in combination. To prevent any perception of discomfort during the procedure, it is recommended that an anesthetic, such as ketamine, be added to balance the sedative and analgesic effects of dexmedetomidine. Each tom should be monitored appropriately while under general anesthesia to minimize anesthetic complications.

Procedure for electroejaculation

- Electroejaculation is performed using a rectal probe one cm in diameter and 12-13 cm long. Appropriate electroejaculators are available commercially (P-T Electronics, Boring, OR).
- Lubricate the rectal probe with non-spermicidal lubricant and insert the probe gently into the rectum approximately 5-7 cm. The electrodes should be oriented ventrally.
- If feces in the rectum prevent the placement of the probe, a lubricated gloved finger may be used to evacuate the rectum but is not always necessary.
- Manually extend the penis and clean with gauze moistened with saline (no alcohol or soap). Dry the penis with clean or sterile dry gauze.
- Place a sterile vial (Eppendorf) over the penis. Alternatively, one could use a sterile 5 ml sample collection vial but the small volume of ejaculate may be more difficult to manage in a larger vial.
- Turn on the ejaculator. Make sure the rheostat dial is set to zero prior to activating the power switch.
- Rotate the rheostat to provide a series of electrical stimuli by turning the dial to the desired voltage for 2-3 seconds, then abruptly back to zero for 2-3 seconds. The stimuli should be administered in the following order:
  - Set one: 10 times with 2 volts, 10 times with 3 volts, 10 times with 4 volts, rest 3-5 min.
  - Set two: 10 times with 3 volts, 10 times with 4 volts, 10 times with 5 volts, rest 3-5 min
  - Set 3: 10 times with 4 volts, 10 times with 5 volts, 10 times with 5 volts (or 6 if needed, depending on previous response).
- The sample obtained between each electroejaculation set should be evaluated for the presence of sperm. This is usually readily evident, as an ejaculate containing spermatozoa will be cloudy. A new, sterile tube should be used between each set after collection to prevent contamination or loss of the sample.
The tom’s response to the stimuli should be monitored and the probe location be adjusted accordingly. During the stimulation, both hind limbs typically would extend symmetrically. If they are not extending, or if one extends more than the other, confirm that the probe is in contact with the rectal wall, and that the electrodes are on ventral midline.

Because electroejaculation requires specialized equipment, collection using urethral catheterization has become very popular as a means to procure a sample for semen analysis. Following heavy sedation with medetomidine (130-140 µg/kg, IM), collection of semen was successful by urethral catheterization. A comparable dose of dexmedetomadine (Dexdomitor, Zoetis) would be 65-70 µg/kg IM. Once heavy sedation is achieved, the penis is exposed and cleaned with saline. A tomcat urinary catheter (open ended) is inserted into the urethra approximately 8-9 cm and removed. Care must be taken not to enter the bladder with the catheter. Once removed, the catheter can be flushed with appropriate extender and the sample evaluated. Although the total sperm collected was lower than what was obtained by electroejaculation, Zambelli et al were able to collect adequate numbers of spermatozoa for insemination or cryopreservation. This provides an excellent alternative method for semen collection in a practice setting.

As an alternative to an ejaculate, sperm collection by epididymal flushing after castration or postmortem followed by cryopreservation has been widely described. The research performed in the domestic cat has provided a model for the technique in endangered feline species in attempts to preserve valuable genetics. A recent report showed that sperm motility and membrane integrity was decreased when the cat was euthanized with pentobarbital prior to epididymis collection when compared to induction of general anesthesia. Therefore, if an animal is undergoing epididymal sperm collection followed by euthanasia, it is advised to anesthetize the cat for castration, and then administer the euthanasia agent after the testicles are removed. If cryopreservation is not feasible in the clinical setting, epididymal sperm recovery can be performed, processed, and cooled to 5°C, and shipped overnight to a facility for freezing. Cooling for 24 hours did not affect the post-thaw motility.

Spermatozoa for morphologic evaluation may be collected by aspiration or lavage of the queen’s vaginal vault following mating. Collection by this method may be useful to rule out azoospermia, but there may be a higher rate of morphological defects in this sample because normal sperm should be moving out of the vaginal vault and into the uterus very quickly following breeding. Because retroejaculation is common in the tom, cystocentesis and analysis of the urine after ejaculation may yield enough sperm cells for a limited analysis, but both sperm motility and morphology are likely to be compromised.

**Semen evaluation**

Following collection, the volume of the ejaculate is recorded, and the sample is extended at least 1:1 immediately (slow, drop-wise addition with mixing) with suitable media. This author prefers Ham’s F-10 with 25 mM Hepes, 1mM pyruvate and glutamine, penicillin/streptomycin/ neomycin, and 5% fetal bovine serum. An alternative that is commercially available is TEST yolk buffer comprising of tes, tris lactose and 20% egg yolk, however, analysis of the motion characteristics might be difficult due to the interface egg yolk droplets if the extender is not filtered prior to use. Motility (total and progressive) should be recorded by estimation under low power microscopy on a warmed microscope slide, or using a computer assisted analysis (CASA) calibrated for feline spermatozoa. Concentration is obtained using a hemacytometer at a 1:100 dilution. The NucleoCounter® (ChemoMetric A/S, Allerød, Denmark) has also been used clinically to determine feline sperm concentration.

Evaluation of sperm morphology is an integral part of the semen evaluation. Teratospermia in the domestic cat has been defined as less than 40% morphological normal sperm. There is a very high degree of teratospermia in many of the exotic feline species studied, complicating genetic preservation. Teratospermia is also observed in small populations of cats where inbreeding has occurred. Research has shown that a single generation of inbreeding (offspring bred to parent) produced male offspring with less than 15% morphologically normal sperm compared to 55% morphologically normal sperm in control animals, indicating that loss of genetic diversity leads to increased teratospermia in as little as one
The degree of teratospermia and the nature of the defects present affect the post-thaw survival of cryopreserved sperm, and the freezing method may need to be adjusted to compensate for these defects. For example, rapid cooling of sperm from teratospermic cats results in a large number of damaged acrosomes compared to normal controls. This damage is decreased when using a slower cooling rate. In other species, the number of morphologically normal sperm and motility of the sperm are closely correlated. In the cat, many teratospermic ejaculates demonstrate adequate motility (greater than 70%), in spite of low number of morphologically normal sperm. Other measured parameters of the ejaculate may include pH, osmolality, membrane integrity, sperm chromatin structure, bacterial culture, or seminal plasma chemistry.

The ejaculate characteristics expected for each collection method can be found in the Table. During natural matings, copulation occurs multiple times within a short period of time. This is necessary for the induction of ovulation. A suggested breeding dose using vaginal artificial insemination is $8 \times 10^6$ spermatozoa (motility greater than 80%) and achieved a pregnancy rate of 77.8%. Vaginal insemination with $20 \times 10^6$ and $40 \times 10^6$ obtained a conception rate of 6.6% and 33.3%, respectively, in that same report. If collecting via AV, two collections, 10-15 minutes apart should produce sufficient numbers.

**Use of semen**

Once the ejaculate is collected and the volume is measured, this author extends it immediately 1:1 to 1:3 with Ham’s F-10 with 25 mM Hepes, 1mM pyruvate and glutamine, penicillin/streptomycin/neomycin, and 5% fetal bovine serum. This extends the volume of the sample to make it easier to work with. Sperm collected following each ejaculation set is extended, and then all samples are combined at the end to make up the total ejaculate. In this author’s experience, the total volume of the ejaculate (without extender) obtained using electroejaculation ranges from 50-130µl (average 94.1µl). Once the motility and morphology are evaluated, the ejaculate can be centrifuged (300 x g for 8 minutes) if a smaller volume is needed for intrauterine insemination, or vaginally inseminated into an estral queen that has received ovulating induction agents. If cooling and transporting is necessary, the ejaculate can be extended with refrigeration medium component (TEST-Refrigeration Medium; Irvine Scientific, Santa Ana, CA) and cooled to 5°C for holding overnight or shipping. In this author’s experience, extending the ejaculate to a concentration of 30 million sperm per ml in TEST Refrigeration Medium extender produced acceptable motility characteristics after 24 hours at 5°C.

**Conclusion**

The amount of scientific information involving the domestic cat has greatly increased making the diagnosis, treatment, and management of fertility issues a reality in the tom. With improved semen collection techniques, complete evaluation of the spermatozoa is possible, as is the subsequent insemination of the sperm into a female to achieve pregnancies.

**References**


<table>
<thead>
<tr>
<th>Sperm Parameter</th>
<th>Artificial Vagina</th>
<th>Electroejaculation</th>
<th>Catheterization</th>
<th>Epididymal Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (µl)</td>
<td>102.6 +/- 14*</td>
<td>67.1 +/- 25.9*</td>
<td>10.5 +/- 5.3*</td>
<td>variable</td>
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<td></td>
<td>42.8 +/- 5.6§</td>
<td>94.1 +/- 40*</td>
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<tr>
<td>Concentration (10⁶/mL)</td>
<td>542.9 +/- 557.9*</td>
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<td>1868.4 +/- 999.8</td>
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<tr>
<td>Total Sperm Number (10⁶)</td>
<td>83+/-.0.4 †</td>
<td>33.6 +/- 34.5*</td>
<td>21.0 +/- 18.1*</td>
<td>variable</td>
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<td>Total Motility (%)</td>
<td>90.9 +/- 1†</td>
<td>78.1 +/- 10.3*</td>
<td>50.4 +/- 20.3†</td>
<td>71.5 +/- 9†</td>
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<td>92+/-.1.1 a</td>
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<td>+/- 9.6</td>
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<td>Progressive motility (%)</td>
<td>88.6 +/- 0.9‡</td>
<td>90†</td>
<td>30.5 +/- 18.3†</td>
<td>50 +/- 12.4†</td>
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<td>75+/-.5§</td>
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<td>Morphologically Normal Sperm (%)</td>
<td>90‡</td>
<td>25+/-.10†</td>
<td>41.5 +/- 18.5†</td>
<td>58.1 +/- 16.6†</td>
</tr>
</tbody>
</table>

Table: Sperm characteristics for various collection methods in the tomcat.
* Zambelli et al. (2008)
† Filliers et al (2010)
‡ Valiente et al (2014)
§ Oba et al (2011)
¶ Johnson, unpublished