Technique Report

Canine semen processing for chilled shipment

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Abstract

Advanced reproductive techniques for dogs have become commonplace in general practice during last decade. Most procedures performed in these practices are based on what is known for semen processing and shipping in the equine industry. This presentation reviews current literature of what is known on canine semen processing and attempts to standardize techniques to maximize their successful use in clinical settings.

Keywords: Canine, chilled semen, processing, shipping, veterinarian role

Importance of semen shipment as an advanced reproductive technique

Over the last decade, advanced reproductive techniques (ART) have blossomed in multiple species, especially in equine and canine patients. The shipping of gametes throughout the world aids in diversifying genetic pools and overcoming geographical or time-related challenges. Specifically for dogs, semen processing after collection has become commonplace in both general and reproductive practices. However, techniques that are used and the process by which semen is handled and shipped is not standardized. Due to the uniqueness of the canine reproductive cycle and the distinct disadvantage of resistance to in vitro manipulation, fertility related to semen processing or shipping are difficult to predict.[1] Many practitioners are using techniques from equine semen processing and shipment and applying those concepts to canine semen. Current research areas of canine ART are numerous and geared toward minimizing the knowledge gap between canine and equine semen processing and handling in order to assure better fertility outcomes.

Importance of semen evaluation at collection

Semen evaluation is still the primary step of any semen processing. Using semen from animals that have not had semen evaluation can lead to missed pregnancies due to poor concentration, motility, viability, or morphologic defects. Semen that is frozen without a full evaluation may lead to future genetic losses due to unproductive frozen-thawed semen used for breeding. Over the past several years, there have been presentations and publications related to canine semen evaluation and also in the context of infertile or subfertile male dogs.[2-4] Referring to those resources will be helpful to practitioners conducting canine andrology work and details will not be discussed.

A dog that is collected for the purpose of a current or future breeding, shipping, or frozen semen should have a full semen evaluation. A single evaluation parameter is not accurate in predicting the performance of a stud dog.[5] In the event that semen from the dog is shipped or frozen, the semen should be evaluated in the same manner after shipping or thawing to document the performance of that individual collection. These evaluation(s) should include the following:

- Full physical examination, paying attention to possible systemic, congenital or heritable diseases that can be observed, heard, or palpated.
  - Examples: heart murmurs, ocular abnormalities, pain or reduced range of motion in joints during the examination, or endocrinopathies.
- Evaluation of libido and gross appearance of the collection that may lead to diagnosis of disease specific to penis, urethra, or prostate.
  - Examples: hemospermia, penile lesions, persistent frenulum
- A full spermiogram to include concentration, motility, and morphology. Ancillary tests such as viability (plasma

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membrane integrity), hypo-osmotic swelling test (HOST), or computer-assisted sperm analysis may also be used to further describe sperm functionality.

- These will require additional training or equipment to benefit from their advanced evaluation capabilities. Examples include a phase contrast microscope, flow cytometer-based machine, or additional reagents needed to perform additional tests.
- These tests will identify extremely poor or excellent semen, although there are no assurance of fertilizing capability based on a single evaluation.[6]
- The HOST test is positively correlated with normal progressive motility and normal morphology in fresh semen.[7]
- In stallions, the phase contrast technique used for morphologic evaluations resulted in better agreement between evaluators when compared to other morphologic evaluation methods.[8]

Comparison (canine and equine) and application of semen processing studies

Canine andrology work is currently in its infancy compared to what is known for other species. Specifically, the equine and porcine industries use chilled semen commonly in routine breeding management, so dog breeders have some 'catching up' to do. There are several experiments that have been performed over the past 15–20 years that shed some light on what is known and that can be implemented in practices to use chilled semen as a routine technique in routine breeding management.

Currently, what is used as a standard for semen evaluation, centrifugation, processing, and dilution for shipping is based on what is known from stallions and a handful of studies in dogs. Due to the size disparity in both the animal and testicular volume between stallions and dogs, andrology research about processing and shipment is difficult in dogs due to their smaller size and semen volume. Most studies that have evaluated canine semen for centrifugation, processing, chilling, or freezing have used pooled samples. This is an astute way to maneuver around small volumes from each individual dog collected; however, has the weakness of making it impossible to measure the effect of the experimental parameter on an individual dog.[6] This must be kept in mind when discussing papers moving forward.

Stallions produce large volumes of ejaculate and sperm (20–160 ml and 0.08–0.35 x 10^9/ml) in a collection.[9] This lends itself to splitting a single ejaculate and using that ejaculate for multiple breeding doses. There are also many advances related to reducing the number of nonmotile or abnormal sperm during processing, so only superior sperm are contained within the shipment, such as cushions, filters, or sperm washing, to name a few.[9] Currently these technologies can be used in canine collections for the same reason, but they have several disadvantages:

- They drastically reduce the number of sperm within the sample that can reduce the sample to be below a normal breeding dose.
- The process may be cost prohibitive to a typical client as the price of the apparatus may be as much as the appointment to collect and process the semen without advanced processing.

Currently, equine semen is processed in the following manner:[9]

- Collected, extended at a 1:1 volume:volume ratio and centrifuged at a speed of 400–900 g for 9–15 minutes.
- Removal of seminal fluid to 5–20% of the original volume and diluted with extender to 25–50 x 10^6/ml.[10,11]
- Packaged in unique containers to slow cooling at a rate of 1.8–6°C/hour down to 4–5°C for holding. Semen retains its fertilization capacity for 24–48 hours.
- Newer research currently evaluating the practicality of higher temperature storage of stallions (17°C) for 7 days appears promising.[9]

Many decades of research have gone into optimizing the conditions in which stallions are collected, the semen processed, and then chilled for shipping or short-term storage. These studies are ongoing and constantly changing. In contrast, canine studies evaluating different parameters of semen collection and processing have been limited. Centrifugation speeds and amounts of seminal plasma have been represented.[12–14] More recently, studies compared individual performance of canine semen, rather than pooled samples. These results will possibly shed light on the future of canine semen shipping. Centrifugation speeds have varied for canine semen processing; however, for pooled samples and fertile individuals 400–900 g for 5–10 minutes is adequate for sperm recovery rate, minimized sperm damage, and viability at 24 hours after cooling with standard procedures.[15] An ongoing study is determining the optimal concentration that canine semen requires for cooled shipping. Higher concentration(s) (compared to equine semen) is necessary during cooling. Dilution and cooling at 100–200 x 10^6/ml rather than 25–50 x 10^6/ml had significant effects on the 24 hour evaluations.[16] The current recommendations for canine semen processing in practice based on these studies and others are as follows:

- Collection with immediate evaluation and processing of the collection if the total volume is > 2 ml. This will reduce the seminal plasma volume that has been, similar to stallions, detrimental at high concentrations in chilled or frozen semen.[13]
- Centrifugation speeds of 400–900 g for 5–10 minutes are acceptable for processing.
- Dilution at a final concentration of 200 x 10^6/ml, or dilution at a 1:3 volume:volume, for cooling and shipping.
- For quality assurance and liability purposes, retaining a small aliquot of the processed and extended semen sample at the facility for assessment at 24 hours. This could help assess if handling had a factor in poor arrival quality. Insemination with any cooled semen at 24 hours after collection and no longer.

Further studies are needed to optimize the extender types or additives, practical semen filtration procedures, and optimization of in vivo fertility parameters.

Owner-collected semen may affect fertility outcomes

Those breeders that are bypassing semen collection, evaluation, and processing for shipment at a veterinary clinic may be placing their dogs at risk for missed pregnancies or reduced litter sizes without warning. Failure to detect changes in their stud dog's systemic status that may affect semen quality and/or concentration, poor performance with certain types of

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extenders or lack of formal processing prior to shipping may negatively affect a planned breeding or kennel's breeding plan. Discussing the importance of the following with clients is key to achieving success with stud dog management:

- All canine collections should contain the first and second or second fractions only.[12] This allows for reduced volumes and easier dilutions for shipment as well as reduction of possible detrimental components within the other fractions. Remember that higher volumes do not mean that more sperm have been collected.
- Evaluation of collected semen allows the veterinarian and owner to determine the best breeding practices for the stud dog and/or if adjustments are necessary.[2,4] For example, an aging stud dog may not be able to adequately provide semen for 2 female dogs in a single collection due to declining concentrations as testicular degeneration progresses. Communicating this to prospective female dog owners is key to transparency when managing a stud dog, but if routine spermiograms are not performed, an owner may be unaware of declining numbers or quality.
- Processing should occur to reduce the amount of seminal plasma that may be detrimental to sperm during shipping and to achieve the appropriate shipping concentration. The ideal concentration of seminal plasma should be ~ 10–20% of initial volume. Centrifugation is the only way to achieve this reduction in seminal plasma and overall volume. Volume to volume dilutions is known to be inadequate, since canine sperm needs more concentrated shipping conditions, so collection and addition of extender without processing and evaluation should be avoided.

Role of veterinarians in assisting clients

The main mission of reproductive veterinarians is to help clients achieve their goals for their breeding programs while educating them on the current, best practices necessary to accomplish those goals. To achieve the results that a breeder wants from their stud dog(s) program, it is necessary to ensure that proper collection and evaluation techniques are used every time. This allows for real time adjustments to occur and to prevent any unwanted or surprising outcomes. Test shipments for any stud dog that is offering chilled semen as a method of breeding should be performed. Collection, evaluation, and processing of the semen occurs in a standard format, then the collection is divided between different extenders, at an appropriate dilution, packaged for shipment and evaluated at 24–72 hours. If adequate performance is not achieved with standard processing technique, altering centrifugation speed, final concentration, or seminal plasma concentration can be adjusted until either the animal is deemed inappropriate for chilled semen shipment or the adjustment(s) result in acceptable quality after the test shipment is concluded. This allows for optimization of semen processing parameters (centrifugation speeds and time, appropriate concentration and preferred semen extender for each individual) and performance metrics when the shipment arrives at its intended destination. It also allows the breeder some evidence that due diligence occurred as steps were performed to ensure the best quality product was provided to the female dog owner.

Extension and outreach to local clubs is essential. Education and current literature dissemination is extremely helpful to breeders, and aids in preventing the dissemination of 'breeder lore.' These types of events reach a large cohort of breeders and ensure that they are receiving correct information that is science based. They may also boost client numbers for clinics, so it is a 'win' for both veterinarian and breeder.

It is important to support research that is ongoing for canine reproductive studies. Referrals to programs conducting research are essential for the growth of the knowledge base, which will translate into real time positive change in the field of canine reproduction. Practitioners should start conversations with universities on things that are wanted or needed and discuss if a study is necessary or feasible. Many private practices can perform or assist in studies without having to refer the animal to a different facility. Also request continuing education in the form of wet labs or didactic instruction to learn new techniques or refresh on things that are not often performed or that are additions to services that a practice offers.

Conclusion

Canine semen processing standards are rapidly changing as scientific data highlight best practices. As animals are continued to be bred globally, with exchange of genetic material over vast distances and generations, it is important to continue to research and adjust to current trends in semen processing. Discussing these trends often with breeders and their clubs will assure that the veterinary and breeding communities are maximizing their breeding potential over large distances.

Conflict of interest

Authors report no conflict of interest.

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