

Maximizing success using fresh chilled semen

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The practice of using fresh chilled semen has been done for many years to allow breeding of dogs from different geographical areas. This helps avoid the inconvenience and often stress to the animal of shipping a bitch or stud, or allows a breeding when availability of a stud is difficult. As the popularity of this modality has increased over the years, understanding how to maximize success with this technique is an important consideration. Several factors should be discussed.

As with any breeding, both the bitch and the stud need to be considered. Criteria for the bitch include appropriate health examination, brucellosis testing, and ovulation timing. These will help ensure pregnancy in any insemination attempt. Ovulation timing is paramount, as longevity of viability of fresh chilled semen is potentially lower than that of naturally inseminated semen. One may have easier success without specific ovulation timing with natural breedings or “side by side” breedings when multiple inseminations are convenient over the fertile period. Even in these breedings, however, appropriate timing increases not only the level of success of pregnancy but also helps to predict gestational length and determine whelping dates. In fresh chilled breedings, quantitative progesterone testing, and when convenient, luteinizing hormone testing are critical to optimize insemination at an appropriate time post-ovulation. Interpretation of these tests as to when ovulation has occurred is a matter of understanding when luteinizing hormone has peaked, stimulating the ovulatory event, and based on the luteinizing hormone surge, and or a concomitant rise in progesterone levels. It has often been simplified by many that ovulation occurs at 5 nanograms of progesterone, or insemination should occur at 15-18 nanograms for example. These statements are not accurate and actual values can vary from bitch to bitch and cycle to cycle of the same bitch. It is important to note that ovulation does not occur at a specific value, nor should insemination be done at a particular value of progesterone. Rather, it is the surge in luteinizing hormone, and rise in progesterone, and “the slope of the curve” of that rise which helps determine when ovulation has likely occurred. This requires a bit of experience, and should be done by a veterinarian familiar with ovulation timing. Luteinizing hormone testing requires daily serum samples to be drawn and held, whereas progesterone levels can be drawn less frequently such as every other day. For this reason, many veterinarians, based on the convenience of the breeder to present the bitch for daily blood drawings, may opt to do only progesterone timing unless convenient to draw daily luteinizing hormone levels.

Once it is determined when the ovulatory event has occurred, coordinated communication with the collecting individual is important. For determining when to ship semen, one needs to know that the canine egg is unique in that it requires maturation, and meiotic division causing a polar body shed before it is viable for conception. This takes twenty four to forty eight hours post-ovulation, and for the purpose of fresh chilled insemination, shipment should be arranged for the first sample to be sent to arrive at least twenty four and preferably forty eight hours post-ovulation. Therefore, appropriate progesterone testing, and timely results help the individual collecting semen to be informed in time to collect and ship the sample. Constant communication between parties during ovulation timing is paramount to achieve this.

In terms of ensuring viable semen is available when the bitch has been appropriately timed for ovulation, similar criteria exist for the male. Health examination, brucellosis testing, semen evaluation, and his current semen’s viability as a fresh chilled sample are critical. Importantly, not all males that can produce a litter with a natural breeding, or “side by side” breeding will necessarily do well with a fresh chilled shipment. Semen performance in a fresh chilled extender can be variable, and affected by several factors. Ideally, either knowledge of viability in a chilled extender based on recent shipment experience, or a fresh chill test done when the decision has been made to use this male and the bitch is soon expected to be in estrus, or has just started her cycle, ensure viable semen at the time of insemination. Therefore a current semen evaluation and or chill test are important. Semen production is a dynamic changing event, and simply because a male has produced a litter in the last several months or year, does not guarantee

current viability of his semen. Many factors can contribute to semen viability. Ambient heat during summer months, if the male is kept in outdoor kennels can affect sperm viability. Age of the male, disease, and genetic factors can also affect his viability. The sperm cycle from spermatogenesis to sperm maturity is about two months in the male dog. If traumatic events such as heat damage or infection occur, it can take two to three months to see a full cycle of sperm development and improvement of the ejaculate. A current sperm evaluation which includes progressive motility, morphology, and viability in the fresh chilled extender to be used, are important to maximize success. This will require the use of a microscope, and experience in evaluating morphological abnormalities, grading progressive motility, and evaluating concentration of a semen sample. A semen count requiring specific equipment such as micropipetters, and hemocytometers, or machines is desirable when inseminating with fresh chilled semen, but not required for this particular procedure if the analyzing individual is experienced in estimating concentration levels.

Chill testing to determine viability for shipment is the next step after determining initial viability. Many veterinarians practicing reproductive medicine will have a variety of extenders available, and can do a chill test in each of them to determine in which extender the male's semen best performs. Both initial evaluation and chill testing should be done as close to the potential shipment as possible to ensure no changes or insult to the semen may have occurred affecting its structure or viability in an extender prior to shipment. With inexperienced males, the initial semen evaluation and chill test also gives the collector a chance to make sure the male is amenable to collection of a semen sample. It is not uncommon if the male has not been previously collected, for the bitch owner to call for a collection, and receive a call back that the male was not able to be collected due to inexperience, stress with a particular facility or collector, or other factors. This can be devastating to the bitch owner unless a standby male is available. There are several techniques to allow a male dog to be collected, from use of teaser bitches, to the use of and injection of the prostaglandin F₂-alpha if a teaser bitch is not available. Some male dogs are not amenable to collection though, and this should be determined ahead of time.

Once the individual collecting the semen has been notified of the need for shipment, several factors are to be considered. Ideally, the inseminating individual has notified the collector of what type of insemination is expected, allowing for proper extension in the appropriate volume of extender. For surgical insemination, or transcervical insemination a smaller amount of total volume of sample may be desired than for vaginal insemination. This volume will depend on the preference of the individual inseminating, and should be discussed prior to collection. Preparation of the semen rich fraction of the ejaculate may include centrifugation to remove undesired volumes of seminal or prostatic fluid, and allow for proper final volume as discussed earlier. Please note that most canine reproductive tracts (uterine body and horns) will hold no more than up to two to three milliliters, and any larger volume will simply flush back out into the vaginal tract taking sperm cells with it. Too large a volume shipped to the inseminating individual may require centrifugation, and re-extension to a smaller volume because of this fact.

Proper labeling of the sample tube, and inclusion in the shipment of a record of semen morphology, and progressive motility will help the individual inseminating to determine how well the sample survived the shipping process, and allow for determination of viability, and which type of insemination to perform. While determination of which type of insemination will be done depends on personal preference and is often subjective, samples with lower overall or progressive motility, low percentage of morphologically normal sperm, or low total concentration often will be inseminated by transcervical or surgical insemination to avoid losing viable numbers of sperm more distally in the reproductive tract such as the vaginal vault. It is advisable on shipment that the collecting individual note the tracking number of the shipment and inform the inseminating individual so they may track the shipment on the receiving end. Several entities exist for shipment of semen, but in the domestic US, Federal Express is the most commonly used company. Alternatively, direct "counter to counter" shipment can be used to ship semen "same day". This requires the collector to be identified as a "known shipper" however, which will require a facility inspection and registry with the shipping agent. The author has had success with Delta Dash in the continental US for this purpose. The process of becoming

a “known shipper” must be done well previously to an expected collection and shipment and can be a cumbersome process. The success of becoming a “known shipper” can also depend on the airline being used. It can be difficult to achieve this status. If possible, “counter to counter” semen shipment provides for sending semen for example on weekends when Federal Express cannot deliver. It also allows the semen to be inseminated in a timely fashion on the same day it was collected, when ovulation timing does not ensure enough time for a twenty four hour shipment to be effective. Pick up at the local airport, however, is required and should be planned as a part of the process. What method of shipment to be used will also be affected by the environmental conditions currently present geographically. If extreme heat or cold are expected, “counter to counter” shipment in a well-insulated shipping container may be preferable over a delivery with an overnight shipper where samples may not be kept in a controlled environment during shipment. If the collecting individual does not have “known shipper” status, then packaging, and type of container are critical. Semen should be in a well-insulated container, and never be placed directly in contact with a frozen “cooling block” as it may freeze the sample and kill the sperm. The sample should always be kept separated from the cooling block by newspaper, bubble wrap, or some other insulating material. Cold ambient temperature has also been known to freeze a chilled sample. An Equitainer[®], a shipping vessel commonly used by equine veterinarians, can be used to protect the sample. There are several shipping containers available from various manufacturers, and personal preference, based on experience can help determine which to utilize.

How many shipments should be sent with the use of fresh chilled semen often arises as a common query. Of course, the more samples shipped, the more expensive the venture, but generally multiple samples covering the fertile period offer the best opportunity for success. For the purpose of vaginal insemination, which is the most common method used, two samples are often sent to inseminate the bitch on day one and three, or more commonly day two and four after suspected ovulation. At diestrus, which is usually expected at day five to six after ovulation, the cervix closes, and vaginal delivery of sperm to the uterus and horns can be compromised. Diestrus may be determined to some extent by reading a vaginal smear. The infiltration of segmented neutrophils into the vaginal lumen is a hallmark of diestrus and can be visualized from a vaginal swab evaluation under a microscope. For this reason, it is usually not recommended to inseminate after day four post-ovulation. This can be circumvented by surgical insemination, effectively bypassing the closed cervix, and allowing for conception sometimes up to eight or nine days post-ovulation. While this has been documented, it is not advisable to make such a late attempt at insemination if possible. If it is not possible to collect two samples for insemination at day one and three, or two and four post-ovulation, and only one shipment is to be made, it is best to do this single insemination at day two or three post-ovulation. Which type of insemination to perform is again personal preference of the inseminating individual, and may be decided based on the overall viability of the sample as determined by overall count, progressive motility percentage, and morphology.

A common practice in the related field of equine medicine is sometimes to “split” a single collection into two samples to be sent separately a couple of days apart. This is not advisable with canine semen. The differences lie in the fact that equine samples contain tremendously higher numbers of sperm than canine samples. By splitting a canine sample, you are simply decreasing the number of sperm introduced by half for each insemination, and with lower overall numbers than equine sperm you are decreasing your success rate for conception. It is better to inseminate a full collection and collect a second sample for insemination a couple of days later, effectively doubling total sperm inseminated, and providing a more nurturing environment in the bitch’s reproductive tract, than in a refrigerated test tube. The chilled sample in a test tube sample will still be two days older and in a less hospitable environment than in the bitch’s reproductive tract which is designed to maintain the sperm cells appropriately. If only one shipment is expected, then all of the sample should be inseminated at the same time as mentioned above. Extenders do exist currently that allow for chilled samples to be held for longer periods. These are advised to only be used when availability of the male for collection when the bitch is ready is simply not achievable. They are not to be used routinely with fresh chilled insemination when the male will be available to be collected when needed. The best environment for semen is still either the male’s

epididymis, or the bitch's reproductive tract. Long term extension in a test tube should be avoided unless necessary as described above.

Once received, the semen sample should be kept at a constant cool temperature until it is inseminated. Refrigeration is advisable and if the container is still at appropriate temperature and the cooling blocks are still cold, it may be kept in the shipment container. Alternatively the sample may be kept in the refrigerator (not the freezer) until the bitch is available for insemination. Insemination should, however, be done as soon as possible upon shipment arrival, unless there is specific reason as mentioned earlier such as use of a longer effective extender in case the male will not be available when the bitch is ready, and semen has been shipped a bit early. On insemination, directions if provided with the shipment, for warming or activation should be followed as they usually have been developed and tested for that particular extender. Insemination can be achieved as mentioned by vaginal, transcervical, or surgical insemination as determined by the individual inseminating. This again will be decided based on several factors such as viability and concentration of the semen, how many inseminations are to be performed, and personal experience and preference. It therefore is very important for the sample to again at insemination be evaluated for viability. This is no less critical at this point, than it was on initial collection. Semen progressive motility, morphology, and concentration should be analyzed. If less than desirable, several interventions can be achieved, such as different insemination technique, different extender for the next sample, or an increased number of shipments during the fertile period. Sometimes re-suspending the sample in fresh extender has helped to boost the sample's motility.

While fresh chilled semen has been collected and shipped in a variety of ways by a variety of individuals, with or without thorough evaluation, ovulation timing, or careful packaging, maximizing the effectiveness of this technique can be achieved by consideration of the techniques and factors listed in this text. Due to the costs of collecting, packaging, and shipping fresh chilled semen, and often the desire to achieve a pregnancy on a particular cycle, it is advisable to be as thorough and careful in the process as possible. When performed in this manner, fresh chilled insemination is a very effective means of achieving pregnancy in the canine.