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Preparation for Breeding

Bitch

Regardless of the type of semen being used, all bitches over 4 years of age should be screened for underlying health conditions by running a complete blood count; a serum chemistry panel (including SDMA if available at your lab); a urinalysis, including specific gravity, protein assessment on a reagent strip and sediment analysis; and either urine protein:creatinine ratio or urine microalbuminemia. Urine may be obtained by free catch if the bitch is not in heat; but if the bitch is in heat at the time the bloodwork is done, the urine sample should be taken by cystocentesis (by needle through the abdominal wall) so that the sample is not contaminated by blood and proteins from the reproductive tract. An alternative to the needle method would be using an urethral catheter, but there will be some blood contamination with this technique and this must be considered when interpreting the results. It is preferred to do this bloodwork just prior to the expected onset of the heat cycle. If the labwork is done too far in advance (more than 1 month), there may be some changes in organ function that may develop and will therefore be missed on the screening bloodwork.

All bitches should be screened for brucellosis regardless of their prior breeding status or the type of insemination or breeding being performed. Exposure to this organism occurs by aerosol contact about 60% of the time when the animal puts its nose into urine or secretions on the ground that contain the organism. The other 40% of the time it is spread venereally. Because of the 2 methods of infection, even bitches that have never been bred or will be bred by AI, have the same risk of prior exposure as do bitches that have been bred before. Screening testing is performed using either a rapid slide agglutination (RSAT), tube agglutination (TAT) or immune fluorescence assay (IFA). Any positives should be confirmed using an agar gel immunodiffusion test (AGID) or polymerase chain reaction test (PCR). Brucellosis is a disease caused by a bacteria called either *Brucella canis* or *abortus*. Brucellosis bacteria are infective to all animals, including humans, thus there is a risk of human infection if the bitch starts shedding the organism into the environment which is why screening is recommended. This bacteria prefers to infect the reproductive tract and remains clinically imperceptible until the bitch becomes pregnant or develops a uterine infection (i.e. pyometra). In a pregnant bitch, the bacteria will cause abortion and shedding of the bacteria into the environment; while if the bitch has a uterine infection, when the discharge is eventually expelled the environment will be contaminated.

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Depending on the type of semen that will be used for the breeding, there will be some differences in how we approach the management of the breeding. We normally use a combination of behavioral changes, vaginal cytology, speculum examination of the vaginal mucosa, progesterone concentrations and in some situations LH (luteinizing hormone) concentrations to monitor the progression of the bitch's cycle. The more modalities used the more accurately we can time the breeding and determine the onset and end of the fertile period to maximize breeding days and use of the male. When semen quality is good and longevity of the semen is expected to be longer (fresh or good quality fresh-chilled semen), ovulation timing doesn't need to be as rigorous; but when semen quality is poor or longevity is expected to be short (frozen semen or poor quality fresh-chilled semen) then timing needs to be much more intense.

Breeding is expensive and often clients want to try to save money by not utilizing all the tools we have to manage a breeding. Skipping procedures like vaginal cytology put us at a distinct disadvantage in breeding management. First of all, vaginal cytology and speculum examination (usually performed simultaneously) tell us whether the cycle is progressing normally or not. These 2 tests tell us about how much estrogen the follicles are producing and thus how mature they are. This helps us determine when to run the next bloodwork. If we know the bitch is still early in her cycle based on cytology and speculum exam we don't need to run progesterone concentrations as frequently. If we don't know how the bitch is progressing in the cycle, then we need to run a progesterone every other day to make sure we don't miss determining when ovulation occurs. It is much more expensive to run a progesterone every other day, then to do a vaginal cytology and speculum exam every 3-4+ days. Once it appears that follicles are producing maximal estrogen concentrations, we will see >50% cornification (also called anucleated superficial cells) visible on the cytology and crenulation or wrinkling and whitening of the vaginal mucosa, and then more frequent blood analysis of hormones will be needed.

Even after we know the bitch is at maximal estrogen concentration by the cytology, continuing to evaluate the cytology gives us excellent information about uterine health. For example, if bacterial populations don't decrease appropriately or if white blood cells continue to be seen on cytology, concern about uterine, cervical or vaginal infection should be elevated and appropriate diagnostic steps taken. Additionally, continued evaluation of cytology and speculum exam will tell us far in advance of progesterone, that something has gone wrong with the cycle in terms of follicle development and ovulation. That is, if a bitch is going to have a split heat or an anovulatory cycle, the cytology will alert us to this far in advance of progesterone concentrations dropping. This means that we don't continue to run progesterone for many days after the cycle abnormality occurs before recognizing the problem – and this can save a client a lot of money.

There are handouts on ovulation timing and types of artificial insemination techniques provided separately that owners are encouraged to read for information on these topics.

Fresh semen – side by side AI or natural breedings

Normally we start ovulation timing on day 8-9 of the cycle unless the bitch has a history of ovulating early in her heat cycle or if the owner is not sure when the bitch came into heat, in which case we may start earlier. When breeding naturally, it is important to remember that more is not always better in terms of number of matings. Each time a bitch is bred and sperm enter the uterus, an inflammatory reaction occurs because sperm are foreign proteins in the bitch's uterus. The uterine immune system responds by clearing any sperm, associated bacteria and cellular debris from the reproductive tract. The more sperm that are inseminated, the greater the immune response and inflammation. This inflammation may not be able to be cleared by some bitch's uterine immune systems before the embryos enter the uterus. If embryos enter a hostile environment, they may not survive.

Furthermore, breeding more than once every 48 hours for 2-3 breedings may deplete some male's sperm stores, especially in small – medium size dogs or in dogs with fertility issues. Thus, waiting to breed before the bitch until she reaches her fertile period and then only breeding 2-3 times is ideal for both the bitch and the dog.

For fresh semen, we will run progesterone every 2-3+ days until ovulation is confirmed (see handout on ovulation timing for details). If doing side by side AI, we try to breed twice – once toward the beginning of the fertile period and once in the middle to the end of fertile period. This replaces older sperm that may have lower fertilizing power with fresh ones.

Fresh-chilled semen

Ovulation timing begins a little earlier with fresh-chilled semen (usually day 7-8) so that we have a chance to contact the stud dog owner and collecting vet and get everyone on the same page in terms of a breeding plan. If the dog being used hasn't had a semen evaluation recently, we normally recommend asking the stud dog owner to have him collected and evaluated as soon as the bitch comes into heat to allow enough time to replenish sperm stores prior to breeding and to give us enough time to get a backup plan in place should there be a fertility issue with the desired male.

The bitch owner should bring the name of the stud dog owner and their contact information (phones [home and cell] and email addresses), the stud dog's call name, and the name of the clinic doing the collection along with their contact information (veterinarian or technician at the clinic to speak to, phone, fax and email address). We like to be the point of contact for all parties so we are sure that everyone is getting the same information and understands the plan. It is very easy for misunderstandings to occur if the bitch owner is trying to communicate with the dog owner and then the dog owner is communicating with the collecting vet's clinic.

Normally we recommend 2 breedings with fresh-chilled semen. This more closely approximates what we do with fresh semen or natural breeding and will get us the highest pregnancy rates and litter size. We breed at the start and again at the middle to the end of

the fertile period, typically 2 days apart, but sometimes a Sunday or holiday will interfere with shipping and we need to adjust, breeding either 2 days in a row, or only 1 time in a cycle. If owners only want to ship once to reduce costs or because of stud dog availability, then we tend to breed toward the middle to the end of the fertile period. We do not recommend splitting a collection in half to breed once on arrival and then again 1-2 days later. This practice is not recommended because sperm will survive longer in the bitch's tract than in the refrigerator. There is no benefit to splitting a dose of semen while there is a detriment to the longevity of the semen that is saved back for the second insemination.

Ovulation timing for fresh-chilled semen usually requires sampling every 2-3 days until we get up into a range where ovulation is likely to occur and then daily sampling until ovulation is confirmed. We normally ship the first dose the day after ovulation and the second dose 2 days later.

Frozen semen

Frozen semen can be used on maiden bitches or on bitches that have previously whelped. There is no requisite need for a bitch to be bred naturally or to have had a prior litter to use frozen semen except to prove that she can get pregnant. If the frozen semen being used is in limited supply, sometimes the stud dog owner will not want to waste it on an unproven bitch. Pregnancy rates with frozen semen are generally lower than with fresh or fresh-chilled semen for a few reasons: 1) the number of sperm used is MUCH lower (100-200 million for frozen semen, compared to 300 million – 2 billion+ with fresh or fresh-chilled semen depending on breed and semen quality); 2) the longevity of frozen semen is very short, usually 8-12 hours, making sperm-egg interaction time very limited; 3) sperm can be damaged during the freezing process which may decrease the ability of the sperm that reach the eggs or to be able to complete fertilization when they reach them; 4) the facility that performs the freeze may or may not perform an accurate pre-freeze evaluation or post-thaw evaluation, making the presumed quality of the semen very different from the actual quality which can result in sperm numbers being too low for acceptable fertility; 5) if frozen semen is not handled or shipped properly, partial or complete thawing may occur prior to use and this could effect post thaw motility when the semen is thawed for the breeding.

Trying to get some information about the frozen semen prior to use can be helpful in adjusting breeding management and assessing the expected success rate. Getting a copy of the pre-freeze semen evaluation and a copy of the post-thaw assessment is very helpful. In some cases, there will be an 'expected' post-thaw, rather than an actual post-thaw assessment and this may mean that when the semen was frozen, a post-thaw evaluation was not performed and the freezing facility is just assuming an average sperm loss after each freeze. This may or may not be accurate for every dog frozen. Furthermore, the accuracy of the pre-freezing evaluation is only as good as the evaluation that was performed. So, if inaccurate counting or assessment of morphology was performed, then the breeding dose that was calculated may be inadequate. The latter situation is a fairly common one.

Finding out if the semen has been used previously and if so, how was it used (vaginal, transcervical or surgical), the number of doses used or breedings performed; if it resulted in pregnancy or not; and how many pups were delivered will be helpful in determining the number of doses that should be requested for a breeding. In cases where the semen has been used unsuccessfully or resulted in small litter size, increasing the number of doses used may be necessary for a more positive result.

In some cases a small aliquot of semen may be evaluated before the breeding. For example, if the semen was frozen in pellets, a single pellet can be thawed and both motility and morphology can be assessed to confirm that the 'expected' post thaw is accurate. Total sperm numbers in the breeding dose cannot be assessed in this way because individual pellet size will vary and depending on the number and size of the pellets in the breeding dose received, sperm numbers cannot be determined until the entire dose is thawed. For semen frozen in straws, pre-breeding assessment is more difficult unless extras straws are available and one can be wasted for a pre-breeding evaluation. Alternatively, an experienced facility may be able to cut a small piece of a straw off for evaluation. This can damage the remaining straw and should only be entertained by an experienced facility.

Most semen frozen in Europe has been frozen in larger breeding units than that frozen in the US prior to the year 2000. Since the year 2000, many US facilities have increased the number of sperm included in a breeding unit to become more like the Europeans. Asking for and expecting a minimally accepted breeding dose is critical to the success of frozen semen. For toy and small breeds, 100 million motile sperm; for medium breeds 125 million motile sperm; for large breeds 150 million motile sperm; and for giant breeds 150-200 million motile sperm are recommended.

In the majority of cases a single breeding is performed. In situations where motility or morphology is low, or the fertility of the semen is questionable (used previously unsuccessfully), either 2 breedings may be performed, multiple doses of semen may be used for a single breeding, or multiple doses may be used for multiple breedings. The type and number of breedings should be discussed with the veterinarian performing the breeding prior to ordering and shipping the semen.

In cases where the inseminating facility has appropriate storage for the semen, the frozen semen may be shipped in advance of the breeding. In facilities where there is no storage available, semen should not be shipped until the bitch has been in heat for several days. If no storage is available, the shipping facility should be notified of this fact and a dry shipper with long holding times (14-28 days) should be used. The receiving facility should find out the required weight of the dry shipper holding an acceptable charge, and they should weigh the tank daily. If the tank reaches the critical weight that indicates an inadequate charge, the tank's liquid nitrogen supply should be replenished. Any veterinarian using frozen semen needs to have a contingency plan for obtaining nitrogen quickly (within 24 hours) if needed.

Ovulation timing for frozen semen breedings requires dedication on the part of the bitch owner and veterinarian. Daily blood draws are necessary (for LH testing – see Ovulation Timing handout for detail) once the bitch reaches peak estrogen production by the follicles. Missing the LH surge means that you are only estimating when the best time to breed occurs and this may not be accurate enough for good fertility. Frozen semen breedings are done late in the fertile period, usually on days 3-4 post ovulation (days 5-6 post-LH).

Dog

As a stud dog owner you have a number of responsibilities. If you are charging a stud fee for your dog's semen then the buyer of the semen or breeding has some rights. If the bitch owner asks for a semen evaluation prior to breeding it is only fair to provide a recent semen evaluation (within the last 30 days) or to have one performed. This should be a complete semen evaluation which includes motility assessment (total and progressive), concentration, total sperm in the ejaculate, and a stained morphology slide (preferably with eosin-nigrosin stain or by phase contrast microscopy using formal buffered saline). If the semen has already been frozen then the bitch owner should be provided a copy of both the pre- and post-thaw semen analysis.

You will need to work out with the bitch owner the type of semen to be provided (fresh via natural breeding or side-by-side AI; fresh-chilled; or frozen) and the number of breedings to be performed. Since the bitch owner pays for almost everything, if they want to do 2 breeding (fresh or chilled) it seems reasonable to comply with their wishes. Semen or breedings should be collected or performed, respectively, at the request of the bitch owner and their veterinarian. If they ask for assistance with breeding management, an opinion may be offered, but if they don't ask, opinions should not be forced upon them and semen should be shipped as requested.

The male should be tested for brucellosis either when the bitch comes into heat or within the 2 months prior to when the breeding is to occur. All males should be tested regardless of their breeding histories. Exposure to this organism occurs by aerosol contact about 60% of the time when the animal puts its nose into urine or secretions on the ground that contain the organism. The other 40% of the time it is spread venereally. Because of the 2 methods of infection, even dogs that have never been bred or will be bred by AI, have the same risk of prior exposure as do dogs that have been bred naturally before. Screening testing includes the rapid slide agglutination (RSAT), tube agglutination (TAT) or immune fluorescence assay (IFA). Any positives should be confirmed using an agar gel immunodiffusion test (AGID) or polymerase chain reaction test (PCR). Brucellosis is a disease caused by a bacteria called either *Brucella canis* or *abortus*. Brucellosis bacteria are infective to all animals, including humans, thus there is a risk of human infection if the male is shedding the organism into the environment which can occur at any time, particularly early in the infection. This bacteria prefers to infect the reproductive tract and prefers the testicles and epididymides of the male where it will cause inflammation and swelling. Another common symptom is scrotal dermatitis. The organism can be shed in the urine or via semen or preputial drippings.

If frozen semen is provided, it is incumbent upon the dog owner to provide what is considered an acceptable breeding dose (see the section above for detail on what is considered an acceptable breeding unit). As long as this is provided in at least 1 breeding dose, it should be acceptable to the bitch owner. However, if the male's semen has been used multiple times without success, providing additional doses should be considered.

Conclusion:

Being prepared for whatever type of breeding is planned is critical to a smooth and successful breeding process. Having a back-up plan, and a back-up plan for your back-up plan is ideal. There are many things that can happen to make the need for a back-up plan necessary – just to name a few common scenarios: 1) the semen quality is unexpectedly low at the time of collection or he cannot be collected at all; 2) the male unavailable for collection due to health or travel; 3) FedEx or UPS loses or fails to deliver the shipment on time; or 4) the frozen semen is non-viable at the time of thawing. Having a back-up plan or two provides another avenue to make a breeding successful, thus not wasting all the time, energy and expense involved in ovulation timing.

Working with an experienced veterinarian, especially with frozen semen, is also very important for success. Taking the time to locate and establish a relationship with an experienced reproductive veterinarian prior to the onset of the bitch's cycle is ideal. Locating a reproductive specialist can be expedited on the American College of Theriogenologist's member search page (www.theriogenology.org) or a general practitioner with an interest in reproduction can be found on the Society for Theriogenology "Find a Reproductive Vet" search page (www.therio.org).

Every breeding is very important and takes a lot of effort on both the dog/bitch owners' parts and the veterinarian's. Everyone's goal is to achieve a healthy pregnancy with normal litter size, so all parties should work together to achieve this end point.