

Benefits of Frozen Semen

Frozen canine semen has gained in acceptance and demand since the American Kennel Club first recognized a litter conceived from frozen semen in 1981. The advantages to a breeding program using frozen semen are numerous. The most obvious benefit is long-term storage of a superior stud dog's genetic material. Researchers estimate that frozen semen could potentially remain viable for 10,000 years. Thus, frozen semen can bridge not only long distance breedings, international breedings, but also bridge the span of time. The preservation of select gene pools can help improve individual breeds and help eliminate problems that could develop within a breed.

Semen Longevity Factors

Breedings can be performed using three types of canine semen: fresh, fresh-chilled, and frozen. Fresh canine semen has the longest viable period and has been reported to live in the bitch's reproductive tract from 5 to 7 days.

Fresh chilled semen is a great alternative when the male is in a different location than the female and one does not wish to ship the female or travel great distances. The semen is collected at one location, mixed with a semen extender, and cooled down to 40°F. This process conserves the sperm's energy enough that it can be shipped overnight and artificially inseminated into the female. After warming back up to body temperature, fresh chilled sperm lives about 24 to 72 hours (we have seen longer).

Frozen semen uses liquid nitrogen to take the semen to the extreme temperature of -322°F. The semen is collected and analyzed for concentration and total numbers, morphology (normal structure), and motility. At CSBC the semen is preserved in precise units called "pellets". The pellets are then carefully labeled and stored in liquid nitrogen containers. After thawing, the sperm has a much-shortened lifespan of only 12-24 hours, although possibly longer.

As you can see, Mother Nature gives us a large margin for error on our timing for natural breedings because of the fresh semen's long lifespan. However, when dealing with frozen semen, it is critical to know exactly when ovulation has occurred and deliver the frozen semen to the "eggs in

waiting." For more information on ovulation timing, please read the section ["Progesterone Timing"](#) under *Topics of Interest*.

Factors Affecting Semen Quality

Many factors can affect the viability of frozen semen. While an older male can still be frozen, a better collection can be expected from a young, mature dog (two to six years old). Therefore, it is better to collect and store a dog at a young age based on potential, rather than wait until a male is in great demand but unfortunately much older. The overall health of a dog is extremely important as well. A sick, or stressed dog will not provide a quality collection. The quality of the collection can also be affected by the dog's libido (sex drive). Most dogs will perform better if a "teaser" dog (dog in active heat cycle) is present at the time of collection. Dogs that are accustomed to natural breedings may take several attempts to be collected. In most cases, with a teaser bitch and some patience the outcome is successful. Studies have shown, a dog's sperm count can decrease significantly after two ejaculations in a one-week period. A period of rest 10 to 14 days is suggested before semen freezing to restore the count to maximum potential. Also note, a dog that has not been used for breeding purposes for an extended time (several months) will benefit from having a collection performed 2 weeks prior to collecting for freezing. All dogs being collected for freezing must be checked for the bacterial infection Brucellosis. Brucellosis is highly contagious, causes infertility, abortion in females, and is zoonotic (people can catch too). Any male or female dog in an active breeding program should be tested yearly for brucellosis. It is wise to test in advance to collections or breedings, as false positives are possible and require retesting a month later.

Frozen Semen Insemination

Several breeding options exist for using frozen semen. Some individuals have reported success with thawing semen and inseminating intravaginally as with fresh chilled semen. This is usually performed on several successive days. While this method is less invasive, it also requires a greater amount of frozen semen. Most freezing centers would agree that a surgical insemination of the semen directly into the uterus provides a greater chance of pregnancy and increased litter size. This can be accomplished with a very small abdominal incision and injecting the semen directly into



the two horns of a female's uterus. A newer method called transcervical insemination (TCI) involves using a camera on the end of a rigid scope. A catheter, which is guided through the cervix, also deposits the semen directly into the uterus. Most bitches in heat will tolerate this procedure without sedation. However, certain breeds can prove more challenging to inseminate by this method due to differences in visualizing the angle of the cervix. Regardless of the type of insemination, the most critical variable is the timing of the procedure to match the fertile period of the eggs. Accurate ovulation timing is accomplished by a series of blood tests to identify the most fertile period. Please refer to ["Progesterone Timing"](#) for more details.

Progesterone Timing

Most female dogs cycle twice a year with an average interval being 5 to 11 months between cycles. Breed variations do exist, with the extreme case being the African dog breeds such as the Basenji, that only cycle once a year. Variation also exists between the lengths of the "heat cycle", with the range being as short as 3 days all the way up to 3 weeks in length! The average time however from the onset of heat (proestrus) to the actual mating period (estrus) is 9 to 11 days. To make matters more confusing, some dogs have "silent heats" meaning little or no obvious signs of heat such as swelling or bleeding are ever seen. Another phenomena called "split heats" exist where dogs go into a heat cycle but stop short without ovulating then go into fertile heat period weeks later. A female's fertile period can be different from heat cycle to heat cycle. Meaning that just because she was ready to be bred on the 11th day the last heat doesn't necessarily mean she will be "ready" on the 11th day this heat.

The variations presented above can present notable challenges in finding the correct breeding days. The type of breeding (type of semen) to be performed also must be considered when choosing the best days to breed. Fresh semen and natural breedings allow more room for error because the semen typically lives for 5 to 7 days. However, with fresh chilled and [frozen semen](#) the timing of the breeding is much more critical- because the sperm has a much shorter life span. Our goal is to find the maximum overlap between the female's eggs (which live 2 to 3 days) and the male's

sperm (lives less than 24 hours with frozen semen to 7 days with fresh semen). This is where progesterone testing (a blood test) and determining an exact ovulation date becomes paramount to our success. "In-house" progesterone tests only give a range based on interpreting the fading of dots in a test kit. While these are helpful, for maximum accuracy we use a quantitative test- meaning it measures the exact progesterone level and gives us a number.

Basically, progesterone is a hormone produced by the ovaries that rises as the heat cycle progresses. Early in the heat cycle the progesterone values will usually read less than 1.0 ng/ml. The first significant, sustained rise in progesterone usually coincides with the "LH Surge". The LH stands for luteinizing hormone and is released by the pituitary gland in the brain. This is important because ovulation occurs about 48 hours after the LH surge. The progesterone value at the time of the LH surge is usually about 2-3 ng/ml. The progesterone will rise to about 5-8 ng/ml at the time of ovulation. Canine eggs are not ready to be fertilized at the time of ovulation and take about 2 days to mature. Once mature, the eggs remain fertile for 2 to 3 days and then begin to deteriorate. At the time of insemination, the progesterone can be in the teens and into the 20's (ng/ml). Progesterone stays elevated for about 2 months whether the bitch is pregnant or not. For accurate timings a baseline progesterone is helpful between day 3 and day 5 of the heat cycle or when vaginal cytology shows about 50% cornified cells. After interpreting the baseline number, we will decide when to resume testing and then test every 48 hours to best pinpoint ovulation. Some females will "hover" in the 2 to 3 range for longer the two days because of stress or just their own ovulatory pattern. It is important to see the progesterone level peak "5" ng/ml to feel confident that ovulation has occurred.

Fresh chilled breedings are usually performed 48 hours after ovulation and frozen breedings about 72 hours after ovulation.

LH tests are helpful, but need to be run daily and can get very expensive. The "old way" of doing vaginal cytology is based on rises in estrogen not progesterone. The vaginal cytology is still helpful but not accurate enough for frozen or fresh chilled semen.

Due dates can be determined by counting forward 65 days from the LH surge (LH surge is day 0) or 63 days from ovulation. This is accurate +/-

one day.

A cesarean section can be safely performed if the progesterone is less than 2.5 ng/ml- Assuming full term.

Progesterone levels should be maintained above 2 ng/ml to support pregnancy. If a bitch is confirmed pregnant by ultrasound but cannot maintain a pregnancy, a progesterone test should be performed. Literature states that progesterone should be supplemented if it falls below 5 ng/ml. Supplementation can be performed either by a progesterone in oil injection at 2mg/kg every 72 hours, or an oral supplement (Regumate) at 0.088mg/kg (0.2cc/10 pounds daily). The oil can be measured in blood tests but the oral form cannot. Stop all supplementation 3 days prior to due date. Note that progesterone levels can drop as the result of other problems (fetal death etc) and it is often difficult to realize if the progesterone drop was the primary problem or secondary to something else. Any supplementation of progesterone should be discussed with your veterinarian and monitored closely.

(Reference: Purswell BJ. Management of apparent luteal insufficiency in a bitch. J Am Vet Med Assoc 1991: 199:902-903.)

Summary

- LH surge coincides with first rise in progesterone -usually around "2" ng/ml
- Ovulation occurs about 2 days after LH surge- usually around "5-8" ng/ml
- Eggs take about 2 days to mature
- Eggs remain fertile about 2 to 3 days
- Fresh semen lives 5 to 7 days
- Fresh Chilled Semen lives 24 to 48 hours- occasionally longer
- Frozen Semen lives 12-24 hours post-thaw
- The fertile period begins two days after ovulation and lasts about 2 to 3 days