

## TIMING THE FERTILE PERIOD OF THE BITCH: BRIEF REVIEW

Canine Reproduction Seminar  
Dr. Scarlett Gotwals

### Stages of the Estrous Cycle

- Proestrus:* Vulvar swelling and bloody vaginal discharge marks the beginning of proestrus. The first day of bleeding is called the first day of heat. The duration of proestrus can vary from as little as 1 day to 21 days. Proestrus is a time of rising estrogen levels preparing the reproductive tract for breeding.
- Estrus:* Is the period of receptivity. Behaviorally it begins the first day the bitch stands. Hormonally it begins on the day of the LH surge and is marked by rising progesterone levels and decreasing estrogen levels.
- Diestrus:* Normally begins 7 to 9 days post the actual LH surge. Progesterone levels continue to rise and the vaginal cytology cornification abruptly declines. The diestrus period lasts until the bitch whelps or the serum progesterone returns to a basal level.
- Anestrus:* Is the period of time between the end of diestrus and the next proestrus period.

### Important Hormones of Estrous

*Estrogen:* Estrogen is the first significant hormone of the estrous cycle. Rising estrogen levels result in the clinical signs of estrous. Estrogen causes vaginal and vulva swelling and bloody discharge. Under the influence of estrogen, the vaginal lining thickens resulting in the changes seen on exfoliative vaginal cytology. Estrogen rises during early proestrus, reaches a peak level and then declines. Changes in estrogen level cause the bitch to show behavioral changes. Even a spayed bitch administered estrogen will develop vulvar swelling, become fully cornified on vaginal cytology, stand, flag, and accept a male. However, estrogen blood levels are quite variable from bitch to bitch and do not correlate accurately with ovulation.

*LH (Luteinizing Hormone):* LH is the biological trigger leading to the events resulting in ovulation. The LH surge is the central timing event of the bitch's cycle. After a variable period of elevated estrogen the bitch will be stimulated to have a surge in LH. Ovulation occurs 2 days post the LH surge. In the bitch the ova are not mature and capable of being fertilized until an additional 2 days have passed. The mature ova live another 1 to 3 days. Counting the LH surge as day 0, the bitch's peak fertile period is days 4, 5 and 6 post the LH surge. Determining the actual day of the LH surge requires daily blood testing. The LH surge causes the follicular cells to convert from secreting estrogen to secreting progesterone. The time of the LH surge can be estimated by monitoring progesterone serum levels.

*Progesterone:* Progesterone is needed to maintain pregnancy. In the dog progesterone levels are baseline prior to the LH surge (typically < 1.0 ng/ml). Corresponding to the beginning of the LH surge serum progesterone levels will increase (initial rise) to a typical range of 1.5 to 2.0 ng/ml. After the LH surge, progesterone will continue to rise, it usually rises to a level > 5 ng/ml by day 2 to day 4 post the LH surge and continue to rise to >10-15ng/ml by the end of the peak fertile period. The initial rise in progesterone correlates closely to the LH surge and can be used reliably to plan breedings during the bitch's peak fertile period.

### **Gestation Length**

Bitches whelp 65 +/- a day from the LH surge, regardless of the day bred. Bitches that whelp at no less than 59 days were bred very late and those that whelp over 65 days were bred early. When bred ideally a bitch should whelp 60-61 days from the first of two breedings. If the day of the LH surge is not known a due date can be estimated from the first day of diestrus. Bitches whelp 57 +/- a day from D 1 (the first day of diestrus).

### **Advanced Ovulation Timing Tools:**

#### **Vaginoscopy**

The vaginal mucosa becomes edematous as a result of high estrogen levels in proestrus. On vaginoscopy the vaginal wall has a billowing pillow appearance. Close to the LH surge the decreasing serum estrogen level and rising progesterone level causes a decrease in vaginal edema. The loss off edema in the previously swollen vaginal folds results in a wrinkled or crenulated appearance of the surface. Maximal crenulation or angulation of the folds occurs a few days later corresponding to the optimum fertile period. As diestrus approaches, the vaginal mucosa takes on a blotchy white and pink appearance. Vaginoscopy is a very useful tool in estimating the LH surge.

#### **Blood Progesterone Levels**

The blood progesterone level is the number one way to accurately determine the optimum fertile period in the bitch. Progesterone is also the best tool for quickly staging where a bitch is in her estrous cycle. The standard is quantitative progesterone levels (numerical values reported by an outside lab). There are many veterinary as well as human labs that offer timely, cost effective quantitative progesterone analysis.

For areas of the country without a quick lab turn around, or affordable outside testing, there are several semiquantitative progesterone kits commercially available. These kits include Stat-Pro (ICG/Synbiotics), Target (Biometallics), PreMate (Camelot Farms) and others. The in-office kits are useful for providing a quick estimate of progesterone levels. In-office kits can and do work well for many uncomplicated breedings. For fresh chilled breedings, frozen semen breedings, bitches with previous infertility, or breedings to dogs with decreased semen quality, quantitative progesterones provide a greater degree of accuracy.

Progesterone levels are baseline prior to the LH surge; increase to 1.5 to 2.0 around the time of the LH surge and increase to >5 ng/ml by the peak fertile and often are >10 to 15 ng/ml by the end of the fertile period. The time of the LH surge can be closely estimated from the initial rise in progesterone levels. Accuracy is related to the testing interval.

## **LH (Luteinizing Hormone)**

To accurately find the day of the LH surge blood must be drawn daily once the bitch is at least 60 percent cornified on vaginal cytology. Intensive blood testing is expensive and time consuming. For frozen semen breedings and some chilled semen breedings it can greatly increase the chance of success. Quantitative LH testing is not widely or readily available. ICG/Synbiotics has the only commercially available semi-quantitative in-office LH test. The test generally performs well.

## **The Need for Advanced Ovulation Timing (OVT)**

Whenever breeding **must** work it is advisable to use the tools of ovulation timing. Proper ovulation timing reduces the variables of breeding and provides the best chance for conception and maximum litter size. Proper ovulation timing puts the bitch owner in the driver's seat.

### *Natural Breedings:*

Many dog breeders are very busy and have to plan in advance for a breeding. They need the breeding to work when planned. They do not have time for missed breedings. OVT maximizes conception rate and provides a more accurate due date.

### *Subfertile bitches or bitches who previously missed*

The number one cause of a missed breeding is improper timing. Whenever a bitch does not conceive, OVT should be required for the next breeding.

### *Bitches with abnormal cycles or who have split heats*

Without OVT it is very difficult to know when a bitch is definitely ovulating.

### *Bitches requiring planned C-sections*

The best way to accurately predict a due date is with accurate ovulation timing. The majority of bitches whelp 65 +/- one day from the day of the LH surge regardless of when bred. If the OVT is accurate the C-section date can be narrowed to a 2 or 3 day window the day the breeding is actually done. This is a big help in assuring mature puppies at surgery.

### *Stud dogs that have low-fertility*

Many stud dogs are able to maintain a high conception rate with OVT despite having subnormal semen quality or low counts. For these dogs OVT tailors breeding days to their particular problem.

### *Busy Stud Dogs*

When access is limited, OVT may make the difference between success and failure.

### *Fresh Chilled and Frozen Semen Breedings*

Fresh chilled **or** frozen/thawed semen lives for a much shorter time than fresh semen. It is imperative to use advanced OVT for these breedings.

## **Ovulation Timing Protocol**

### *When to start testing:*

Call the veterinarian who will be doing the timing when the bitch comes in to season to plan the breeding. If you need a Brucellosis test take the bitch in within the first 3 days of heat. There are a few general steps to follow; if a bitch has an ultra short season (5 to 7 days of bleeding) start timing immediately, if she normally has 9 to 11 day seasons start timing by day 5, if she normally has 14 day seasons start timing by 8 days, if her seasons last 3 weeks start timing by day 10. For fresh chilled or frozen breedings you always want to be sure to start ovulation timing early enough. When in doubt, start ovulation timing early. If the stud dog is close by and you do not have to allow for travel time you have more flexibility in when to start ovulation timing.

### *Testing Frequency:*

The higher the stakes the more frequently you need to test. If the breeding is local and there is unlimited access to the stud dog you may need to only check a progesterone level every 3 to 4 days. For breedings requiring the bitch to travel testing is generally done on alternate days. Frozen semen breedings usually require daily blood testing.

### *Value of even one progesterone:*

A single progesterone value will give one of three results: low, mid range, or a high progesterone level. If the level is very low then you know you have at least four days or longer before a breeding will be needed. If the level is mid range (2.0 to 4.0 ng/ml) you need to start breeding within two days; if the level is high (>5.0 ng/ml) you should start breeding immediately; if the level is very high (> 15.0 ng/ml) you need to evaluate if the bitch is still in season and if she is, breed ASAP. Breedings beginning when the bitch already has a very high progesterone may be too late and you will need to start sooner for subsequent breedings.

## Ovulation Timing FAQ's

### *What is ovulation timing?*

Ovulation timing is the process of using blood hormonal values in the bitch to predict when her optimum fertile period is. The bitch ovulates two days after her LH surge (Luteinizing Hormone-the biological trigger resulting in ovulation). Following ovulation the eggs require an additional two days to complete maturation and to be fertile. Therefore the optimum fertile period in the bitch is days 4, 5 and 6 post the LH surge (day 0). Since LH blood levels are often only present for a short time, 16 to 24 hours, we use a second hormone, progesterone, to estimate when the LH surge occurred. Blood progesterone levels are generally less than 1.0 ng/ml prior to the LH surge and rise to 1.5 to 2.0 ng/ml, around the time of the LH surge. Post the LH surge progesterone levels continue to rise and are usually >5.0 ng/ml by the beginning of the fertile period and are often >10-15ng/ml by the end of the fertile period. Blood progesterone levels are the single best indicator of the fertile period in the bitch.

### *How are blood progesterone levels monitored?*

The gold standard for measuring progesterone levels is quantitative assessment by an outside lab. In areas of the country where access to timely progesterone results are limited there are several in office semi-quantitative kits available. The in-office kits are less expensive than lab values but are more subject to errors in interpretation. I personally prefer actually knowing what the value is. I am fortunate to have access to a lab that runs progesterones daily. Samples collected in the morning have same day results usually by 6 to 7 PM.

### *When should I use ovulation timing?*

Ovulation timing (OVT) should be utilized any time a breeding is important. OVT provides control over a breeding and documents breedings occurring during the bitch's optimum fertile period. OVT is essential any time where there is a reduction in the quality of semen used (e.g. fresh chilled semen breedings, frozen semen breedings, older stud dog, heavily used stud dog, etc.). Another advantage with OVT is knowing more accurately when the bitch is expected to whelp. The bitch whelps 65 +/- a day from her LH surge regardless of the days bred.

### *How many visits are required to time a bitch?*

Generally the first progesterone level is checked during the first 5 to 7 days of heat. Subsequent progesterone levels are checked every 2 to 4 days until the fertile period is determined. If a bitch has a very short season (<9 days) she needs to be checked at the first sign of blood. You can expect 1 to 4 visits for routine OVT. The expense typically is in the \$75 to \$255 range for ovulation timing. Artificial inseminations or other procedures incur an additional charge.

### *How do I set up timing appointments?*

If you are a new client please call before your bitch comes into season so we can establish a plan. If it will be your bitch's first breeding, or she has had reproductive problems in the past, a consultation visit should be set up 4 to 6 weeks before you expect your bitch to come into season.

## **Successful Breeding Tools: Selecting a Healthy and Fertile Stud Dog** *By Scarlett Gotwals, DVM*

Successful breeding is about reducing and controlling as many variables as possible. A very important variable to control is selecting a fertile stud dog. To get useful answers, you need to ask the right questions:

1. How old is the dog?  
*Peak fertility is usually 18 months to 6 years old; many dogs experience a drop in fertility over 7 years old. Older stud dogs with viable semen can be used but they require a higher level of reproductive management.*
2. How old are his most recent pups? Are any bitches pregnant now? What was the litter size?  
*Currently pregnant bitches, normal litter size, and young pups on the ground suggest the dog is currently fertile.*
3. If not used in past 6 months to a year, or if the dog is of questionable fertility, what are his semen analysis results?  
*Typical sperm counts can be estimated at -1 0 million sperm per pound of body weight. Normal semen is > 70% motile and has < 20% morphologic defects.*
4. Most recent negative brucellosis?  
*Dogs should be tested at least annually and repeated every four to six months for heavily used dogs. All bitches bred to the stud should have been tested prior to breeding.*
5. Is the dog currently in strenuous competition/training?  
*Some dogs will experience a temporary decline in semen quality while being heavily trained, competing or when exposed to heat stress. The semen quality may take 4 to 6 months to recover.*
6. How often is the dog used? Are there other bitches being bred at the same time as your bitch? How does the owner handle other requests when your bitch is ready to breed?  
*If the dog is over-used he may not have a sufficient sperm count when it comes time to breed your bitch.*
7. By what method does the dog breed? Natural or AI? If AI who does it?  
*Many stud dog owners perform their own AI's successfully. You want to know what their success has been. Having an inexperienced person do the AI can potentially cause a breeding to be unsuccessful.*
8. Has the dog had any recent health problems? High fevers?  
*Sperm cell production is a dynamic process. Any health problem can have an affect on semen quality. A high fever (>104) can knock out the sperm count for 4 to 6 months.*
9. Does the dog have all the necessary genetic clearances?  
*Seeing is believing. The stud owner should readily give you copies of all genetic clearances. Know what you need to ask for.*

**Fresh Chilled Semen Breedings**  
***Canine reproduction Seminar***  
**By Scarlette Z. Gotwals, D.V.M.**

Fresh chilled semen breedings are a great way to accomplish breedings over a distance without shipping the bitch. The highest success is achieved with proper preparation, selection of adequate candidates and finding experienced veterinarians for the collections and inseminations.

**Selection of Candidates**

Ideal candidates for chilled semen breeding are dogs and bitches free of any underlying health condition and in their reproductive prime (2 to 4 years old). Bitches that have any history of reproductive problems are best bred naturally to a highly fertile dog and do not generally make good candidates for chilled semen breeding. Likewise the semen quality needed for successful chilling is much higher than what is needed for a successful natural breeding. There are many dogs with marginal semen quality that have successful stud careers. These dogs do not chill well and will not produce offspring with this breeding method. The trick is to select high quality candidates thereby maximizing your chance for success.

**Selection of Stud Dogs in Advance**

Before a breeder offers their dog at stud for fresh chilled semen breeding they have several questions they need to answer:

*Does their dog have suitable semen?*

Fresh chilled semen breedings require average or above quality semen. The dog should have > 70% motile semen with at least medium speed and average or better quality of forward progression. A dog with >80% motile semen is generally a better candidate. The stud's semen count should be well into the normal accepted range for the breed. There should be less than 20% morphologic defects and few additional cells. The dog also needs to be free of prostatitis. A general rule of thumb to estimate a normal sperm count is 10 million sperm per pound of body weight. For example:

Breed	- Weight	Typical Sperm Counts/ Good Sperm Count	Typical Range
Bull Terrier	60-70 lb.	>600 million	400 to 1,500 million
Labrador Retriever	70-80 lb.	>700 million	400 to 1,500 million
Mastiff	150 lb.	> 1 billion	800 to 2,500 million
English Cocker	30-35 lb.	>300 million	300 to 600 million

A Chill Check performed in advance is a good way to predict semen quality on arrival. Chill Checks should be performed as part of a routine semen analysis on any dog where the breeder plans to make the dog available by this breeding method. To perform a chill check the semen is processed exactly as it would be for an actual chilled breeding except the extended semen is placed in the refrigerator instead of shipping. Small aliquots are removed every 12 hours for three days, and evaluated, after sufficient warming, for percent motility, speed and quality of forward progression. Generally a good candidate for a chilled semen breeding should start out >70% motility and have only a 10 - 15% drop in motility in the first 24 hours and have a sperm count in the normal to high range for the breed.

*Is the dog easy to collect? Will a suitable teaser bitch be available when needed?*

The success of a chilled breeding is very dependent on getting the best collection possible. Although many experienced studs will provide a semen sample without a teaser bitch, the count and quality of the ejaculate is maximized when a suitable teaser bitch is available. Clearly it is the stud owners responsibility to find a teaser!! **An estrous teaser bitch can make or break a fresh chilled semen collection!!** Performing a test collection in advance provides the opportunity to address these issues.

*Example of Semen Collection with and without an estrus teaser bitch:  
(68 LB Bull Terrier)*

	Sperm Count	Motility and Quality
Without teaser	254 million	70%, average
With teaser (next day)	864 million	80%, good

Which collection would you want shipped to you?!

*Will the stud owner be able to make the dog available when needed for the breeding? Will the collecting vet be flexible? Who will take the semen to FedEx or to the airport?*

When the semen is needed will affect the best time of day for the collection. Generally collections should be performed as close to the shipping time as possible. The goal is to have the shortest interval from collection to insemination as possible. For FedEx shipments this will be late afternoon; for counter to counter airline shipments this may be early morning. The stud owner and collecting vet need to be very flexible. If you know you have limitations on availability discuss this well in advance so contingency plans can be made.

*Has the dog had sufficient sexual rest to provide a suitable collection? Will the stud owner commit not to use the dog on another bitch, at the same time collection is needed for shipping?*

It takes 60 days to form a new sperm cell and another two weeks for epididymal transport. It is true that dogs are continually releasing new sperm but dogs do experience a significant decrease in total sperm cells with successive collections. Fresh Chilled semen breeding success can be optimized by assuring there will be sufficient sperm numbers. A high sperm count can offset a reduction in quality on arrival at the destination. Depending on the dog a few days rest may be all that is needed, but a week or longer is better. Also do not do a test collection within 2 to 3 days of when the dog will be needed; why waste the best collection for testing at that point?

### **Ovulation Timing: Selection of Insemination Days**

Ovulation timing is a discussion of its own. The best success with chilled semen breedings is achieved when bitches are bred during their optimum fertile period 3 to 6 days post LH surge. Chilled semen does not live as long as fresh semen and is not as forgiving of error in the selection of breed days. Considering the expense, as well as the emotional drain, it is important to be as accurate as possible in selecting breeding days.

It is important to utilize the best tools available to you in ovulation timing. In my opinion quantitative progesterones are the most accurate, but there are also several in-office progesterone kits (Synbiotics/ICG, Biometallics, Camelot Farms etc.) that are helpful for areas in the country that do not have timely, inexpensive, serum progesterone values available. There is also an in-office LH serum test available (Synbiotics/ICG) that will identify the day of the LH surge with daily testing. LH testing may be overkill for some fresh chilled semen breedings, however it increases the accuracy and may be added assurance for veterinarians new at the fresh chilled process or in cases where less than ideal candidates are being bred.

### **Semen Collection and Processing**

*Collections should be scheduled to allow for the shortest time interval possible between collection and insemination.* For FedEx shipping, schedule the collection as late as possible, allowing time for unforeseen problems and time to easily make shipping. It is often better for the stud owner to hand deliver the semen to the FedEx center. Check last shipping times well in advance and confirm that the destination does have AM delivery available. Also allow for the possibility that the dog may have to be collected twice to get a sufficient sample.

Whenever possible only the sperm rich fraction should be used. The best way to isolate the sperm rich fraction is to have an assistant fractionate at the time of collection. In cases where clear separation does not occur, it is important to remove excess prostatic fluid. Including excess prostatic fluid can significantly decrease semen quality on arrival at the destination. A general rule of thumb is to centrifuge the ejaculate when there is > 1 cc of sperm rich fraction for small breeds, >1.5 - 2.0 cc sperm rich for medium breeds, >2 cc sperm rich for large breeds and >2.5 cc sperm rich for giant breeds. The semen can safely be centrifuged at slow speed, the supernatant removed, and the pellet re-suspended in the extender.

Semen should be diluted according to the buffer directions. Take into consideration the size of the bitch and the insemination method. Generally 2 to 3 cc is adequate for small breeds, 4 to 6

for larger breeds and up to 10 cc for giant breeds. If a surgical insemination is planned the volume may need to be adjusted. When reconstituting a sperm pellet you do not need to calculate a ratio, just dilute up to a suitable volume for the breed.

Evaluate the semen to be certain it is sufficient to get the job done. If there is any question concerning whether the dog released completely, perform a second collection. If full release was achieved the ejaculate will be fairly clear. If he did not release fully, a second collection will have a significant number of sperm. When in doubt collect a second time!! Usually centrifuging will be necessary. If significantly more sperm cells are obtained and are of the same quality the two fractions can be combined. If the collections are different in quality clearly label the tubes and ship both tubes to the inseminating vet.

If the semen quality is obviously questionable call the inseminating vet or bitch owner and get their authorization prior to shipping. Nothing is more frustrating than to receive poor quality semen that doesn't have any chance of working.

### **Semen Packaging (ICG Fresh Express or similar kits)**

When packaging the extended semen be sure the tube seals completely and does not leak. Placing the tube inside of a plastic bag will allow the semen to be recovered should the tube leak. It is important not to place the inner box containing the semen in direct contact with the frozen Kool-it bricks. Place newspaper around the inner box on one side of the container and the frozen bricks on the other side. If this is not done the semen could freeze, within the first few hours, and be DOA (dead on arrival)!! Remember there is only a few degrees temperature difference between frozen and a safe chilling temperature.

When the environmental temperatures are below freezing, there are a few things that can be done to reduce the risk of the semen freezing and being DOA. You can use only one frozen Kool-it brick in the kit to reduce the influence of internal freezing. If using FedEx or other courier service it is safest to deliver and pick up the kit directly from the nearest FedEx center. The delivery trucks do not have temperature controlled storage compartments. At the centers, the packages are kept indoors which reduces the risk of freezing. In extremely cold climates you can also use refrigerated instead of frozen bricks. Refrigerated bricks will serve as a heat sink to absorb cold while still functioning to keep the semen chilled.

When there is sufficient extended semen I will often keep a small aliquot behind, about 1/2 cc. The sample is placed in the refrigerator until the next day. In the morning it can be warmed and checked for viability. If there is a discrepancy between the quality you have versus the quality at the destination, it can provide information as to where the problem may have occurred.

### **Semen Shipping**

In selecting a shipping method the goal is to have the shortest interval possible from collection to insemination. Dogs with high quality chilled semen often work fine with FedEx overnight shipping. FedEx generally provides delivery within 18 to 24 hours of collection. Dogs with marginal chilled semen work best if the time from collection to insemination can be minimized. Counter to counter airline is best in these cases. Airline shipping provides the shortest interval from collection to delivery often allowing insemination within 6 to 8 hours of collection. Check in advance whether the airline servicing your area will accept chilled semen. Some airlines will not ship chilled semen. I have had the best luck with Delta Dash and US Air.

### **Insemination Method**

The fresh chilled semen method is designed to work with vaginal inseminations. With proper selection of candidates the semen quality on arrival should be more than adequate for vaginal inseminations. The success rate for vaginal inseminations should be 80% when you receive good quality semen that has a high count and is >70% motile. The success is strongly related to the quality of semen you receive.

Surgical inseminations will increase the pregnancy rate. You have to decide at what point the gain in success rate is worth subjecting the bitch to surgery. A surgical insemination when the chilled semen quality is excellent (>80%) barely increases your chances. A surgical insemination when the chilled semen quality is good (60-70%) slightly increases your chances.

A surgical insemination when the chilled semen quality is marginal (40 to 50%) may make the difference between success and failure. A surgical insemination when the chilled semen quality is poor (<20%) may not make any difference at all.

Transcervical Emanations (TCI) are a viable alternative to surgical intrauterine inseminations. An endoscope is used to visualize the cervical Os and a catheter is placed visually through the cervix into the uterus. TCI achieves direct intrauterine insemination without surgery. Currently there are only a handful of veterinarians offering this service. TCI is the method of the future and will become more widespread. Over time this should replace surgical insemination in the majority of cases.