

REPRODUCTIVE PHYSIOLOGY & ARTIFICIAL INSEMINATION

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Visitors to this web site should note that Dr Lofstedt is happy to consult with veterinary colleagues and veterinary students but does not have the resources to be able to help animal owners with specific queries or problems. Please respect this limitation. This page was last edited on: May 22, 2001

REPRODUCTIVE PHYSIOLOGY OF BITCHES AND A.I. WITH FRESH SEMEN

(A general resource for breeders, veterinarians and veterinary students)

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ARTIFICIAL INSEMINATION WITH FRESH SEMEN

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BITCHES

General characteristics of the cycle

There is a single follicle wave in a bitch's cycle (as in mares, women, pigs, hamsters etc.) but the wave is protracted when compared with other animals and follicles only mature every three to eight months.

BITCHES are not really inactive between estrous periods. Their follicles are just small and produce small amounts of steroids. In fact, if a bitch is ovariectomized during the months between estrous periods, pituitary

gonadotropin (LH and FSH) concentrations rise dramatically, illustrating the recent effect of steroid feedback and providing proof that the ovaries were not inactive at all. In these bitches urinary sphincter tone also diminishes significantly within days after the removal of the ovaries.

Although the interestrous period varies between three and eight months and is quite variable between cycles, some breeds are known to have consistently shorter interestrous periods than others, e.g., German Shepards 26, versus Collies 36 weeks. Also, as bitches grow older, their interestrous periods tend to become longer. In fact, interestrous intervals of nine to 12 months seem to be normal for some old bitches. Unfortunately most of these reports are anecdotal and the specifics of this trend are not known.

Seasonal effects

In one Beagle breeding colony it was noticed that there were twice as many estrous periods in the spring and summer than during the second half on the year. This appears to be reflected by the incidence of reproduction cases in practice as well, i.e., more cases relating to breeding seem to occur in the spring and summer than later in the year. Such findings are not surprising because wolves & foxes are naturally springtime breeders. Interestingly, wild canidae (and perhaps domestic bitches too) are probably not responding to increasing photoperiod, but to the delayed effect of decreased photoperiod of the previous fall because they become reproductively active even before day length starts to increase in late December.

Note: Basenjis's (a barkless' breed native to central Africa) are different to all other canids, breeding most often during the period of shortening day length, i.e., the fall.

Predicting the onset of estrus

If one wishes to know if estrus is approaching and the signs of estrus (vulvar swelling, vaginal cytology etc.) are not yet obvious, it may be valuable to measure serum estradiol concentrations. According to one study (two others show some disagreement) estradiol concentrations remain almost undetectable until about 30 days before ovulation. Cut-off value have not been generated but current data suggest that estradiol 17 beta concentrations over 10 pg/ml indicate that ovulation will occur within 30 days.

Predicting the time of ovulation

Besides being the best method of achieving optimum fertility, the time of ovulation must often be predicted accurately to test-breed apparently "infertile bitches" under ideal conditions. (Interestingly this usually reveals that they are normal!). It is also used for AI with cooled-shipped or frozen-thawed semen. In the former case, air freight costs can be cut to a minimum. In the case of frozen semen where surgical insemination is most often done, only a single insemination is performed and therefore, it must occur at the best possible time for conception. Another advantage of knowing the time of ovulation is that the time of whelping can also be accurately predicted; it consistently occurs 65 days 1 day after the LH surge. This is much more accurate than monitoring the behaviour of the bitch in late gestation looking for a pre-partum decrease in body temperature. The probable time of whelping is valuable information for owners but is also useful to veterinarians to determine the time for pregnancy diagnosis (as early as 18 days after the LH surge) or elective C-sections (the last three days of gestation).

Detection of the LH surge is the diagnostic cornerstone of breeding in dogs.

It is now quite easy to detect the LH surge in routine veterinary practice because of the advent of rapid and easy patient-side progesterone and LH assays. In contrast, radioimmunoassay and ELISA tests for progesterone are widely available but radioimmunoassays for LH are specialized and not performed routinely.

Ovulation occurs during the first four days after the LH surge but canine oocytes ovulate in an earlier state of meiosis than other domestic animals. Therefore they have to complete the first, as well as the second reduction division of meiosis before fertilization can occur.

A brief review of meiosis follows for those who feels they need to know the inner workings of the process:

During meiosis I, oocyte DNA doubles to a 4n state and crossover of the chromatids occurs (to affect genetic diversity between parent and offspring) then the doubleDNA chromatids separate in two cells, completing the first stage of meiosis. In that process, an immature oocyte and the first polar body are formed. Both are haploid in genetic information but still contain twice the amount of DNA of a gamete. In most species and probably in dogs as well, when a sperm penetrates one of these newly ovulated, immature oocytes, it stimulates the onset of meiosis II, causing the chromatids to split again, forming haploid oocytes with half the amount of DNA and half the genetic code of its adult diploid form. The same process occurs irregularly in the polar bodies and up to three polar bodies may form in the first few days after ovulation.

With all these processes occurring, it takes several days for a canine oocyte to become fertilizable, at least four days after the LH surge. However, within two days after they have matured to a fertilizable state, the oocytes begin to degenerate. In contrast to sperm, they have a very short life. Therefore the fertile period in bitches is actually quiet short, only three to four days during estrus. It is fortunate that sperm can last for long periods in the uterine tubes (Fallopian tubes) because they are usually still ready and able to fertilize when at last, the oocytes have matured. In fact, single fertile matings up to seven days before the LH peak are common, indicating that sperm can remain fertile in the female tract for up to 10 to 12 days after breeding.

Detecting the LH surge

Vaginal mucosal shrinkage, vaginal cytology, vulva compressibility, vaginal electrical resistance, vaginal mucus "ferning" patterns and often be predicted accurately to testbreed apparently "infertile bitches" under ideal conditions. (Interestingly this usually reveals that they are normal!). It is also used for AI with cooledshipped or frozenthawed semen. In the former case, air freight costs can be cut to a minimum. In the case of frozen semen where surgical insemination is most often done, only a single insemination is performed and therefore, it must occur at the best possible time for conception. Another advantage of knowing the time of ovulation is that the time of whelping can also be accurately predicted; it consistently occurs 65 days "1 day after the LH surge. This is much more accurate than monitoring the behaviour of the bitch in late gestation looking for a prepartum decrease in body temperature. The probable time of whelping is valuable information for owners but is also useful to veterinarians to determine the time for pregnancy diagnosis (as early as 18 days after the LH surge) or elective Csections (the last three days of gestation).

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By themselves however, vaginal cytology, vulvar compressibility, ferning and vaginal mucosal shrinkage are too loosely correlated with the LH surge to be reliable indicators of the surge.

When it is essential that the time of the LH surge be accurately detected (surgical AI, shipped semen etc.) we use the following approach:

Vaginal cytology is only be relied upon during the first few days of proestrus and when there is obvious evidence of cornification (50% or more) daily blood samples are taken. To start, we suggest that progesterone should only be measured in every second serum sample. These can be run in your own practice using any of the commonly used progesterone ELISA assays. One that is made specifically for dogs is "Status Pro" [Synbiotics corporationSan Diego, Ca](#). Most of these assays change color when the concentration of serum progesterone exceeds 2ng/ml. This is fortuitous because serum progesterone concentrations usually exceed 2ng/ml on the same day as the LH surge! Some care must be taken in the interpretation of these assays but

they can be very useful.

In our clinic we tend to run radioimmunoassays for progesterone because they are easy to interpret and it is also possible to tell when progesterone probably began to rise above baseline concentrations. By contrast, when simple ELISA assays are used, it is impossible to determine how high the serum progesterone concentration is once the negative-positive color change has occurred, i.e., the initial rise in progesterone could have occurred on the day of sampling or the day before. If an actual number is available for serum progesterone concentration, the initial rise in progesterone is easier to determine. For example, if the serum progesterone concentration is 7.8ng/ml., we know that the rise probably began the day before (progesterone concentrations rise rapidly). In the case of a sample with a progesterone concentration of 3.1ng/ml, the progesterone concentrations are probably rising above baseline concentrations on that very day. This is the day of the LH surge!

When an ELISA assay such as "Status Pro" is being used for progesterone assay, testing should occur every day.

Ideally, the serum LH concentration should also be measured so the day of the LH surge is verified beyond doubt. Therefore, when the approximate first rise of progesterone is detected, the serum samples on that day and the day before should be tested using the "Status LH" test. The Status LH test is widely available in the US and Canada.

Occasionally, the LH surge cannot be detected because it is less than 24 hours in length and sampling intervals may straddle the surge. In such cases, one has to rely entirely on serum progesterone assays.

Even if the LH surge itself is not detected, detecting progesterone alone is far more accurate than performing vaginal cytology alone. With vaginal cytology, the first peak in cytology may occur three days before or after the LH surge, an unacceptable level of accuracy for the management of infertility, transported semen and certainly for the use of frozenthawed semen. By comparison, serum progesterone concentrations can indicate the day of the LH surge to within a standard deviation of about one day.

Once the LH surge detected (either directly or indirectly) transported cooled semen can be shipped to the bitch owner. It is inseminated on days four and six after the surge. Therefore, two shipments are required for optimal fertility. Non surgical insemination of frozenthawed semen should be done on the same days but in the case of surgical insemination, surgery is performed on day five after the LH surge.

The interval between the LH surge and the fertile period is very valuable in practice because it gives one plenty of time to obtain the results of hormone assays and make arrangements for shipping, anesthesia for surgical insemination etc. The corollary of this is that owners become nervous because their bitches are bred so long after they normally would have been bred, i.e., by sexual receptivity alone. Some client education is usually in order.

Levels of fertility to expect

Data generated in more than 200 bitches indicated that about 95% of normal bitches should become pregnant if they are bred at the optimal time. Even in the so called "problem" bitches in that study, pregnancy rates exceeded 75%! In another large group of bitches, it was shown that the pregnancy rate increased by 45% if breeding occurred more than once. The number of puppies per litter increased as well, presumably due to the fertilization of a few latematuring oocytes.

Other methods of detecting ovulation that you may have wondered about

Laparoscopy:

Although developing follicles can be seen on the surface of the ovary about 10 days before ovulation using

laparoscopy, the ovarian bursae normally cover the ovaries in bitches so ovarian structures can only be seen in surgically prepared bitches. Therefore, laparoscopy has no value as a routine clinical procedure to determine the time to breed a bitch.

Ultrasound:

The ovaries are situated caudal and ventral to the caudal poles of the kidneys therefore, the kidneys are primary landmarks to locate when one examines the ovaries. They are close to the abdominal wall in the standing bitch and caudolateral to the kidneys in recumbent bitches.

The ovaries are generally anechoic until about five days before the LH peak and at that time, with good equipment, follicles can be recognized as discreet anechogenic structures. Follicles reach about 0.5 cm in diameter around the day of the LH surge and within four days after the LH peak (with echogenicity varying greatly from one animal to another) they change gradually from anechoic spheres to a mixed population of echogenic structures as they ovulate.

Corpora lutea can be seen for the first time as soon as one day after the LH surge but by day five after the LH surge, they are present in all bitches.

Ovulation is seldom seen as a distinct event in bitches because canine follicles do not luteinize abruptly as is they do in farm animals. Cystic corpora lutea are also common, giving the impression that some follicles have not yet ovulated. In many cases, the only sign of ovulation is gradual thickening of follicle walls due to luteinization.

Interestingly, the number of follicles that are visible on ultrasound does not correlate well with the number of oocytes that actually ovulate. This is because 20 to 30% of canine follicles are "polyovular oocytes." This means that they contain more than one oocyte; the highest number recorded in one follicle being 17. Therefore, in theory at least, a whole litter of pups could be born from the product of a single ovulation!

Because of these challenges, we do not use ultrasonography to monitor bitches for breeding. However, if you do wish to examine the ovaries, for example in cases of cystic ovarian disease, a "standoff" should be used so that the ovarian stroma is in the focal zone for single zone transducers (otherwise the optimal focal zone is too deep for optimal resolution of ovarian structures). Some transducers have builtin standoffs.

Although a 7.5 MHz transducer can be used to observe canine ovaries, a 9.5MHz transducer is optimal. However, even with a 7.5 MHz transducer, the uterus can be imaged fairly easily, especially during proestrus and estrus when a "starburst" pattern similar to that in mares is seen.

Ultrasonography is also very useful to demonstrate pregnancy, pyometra and cystic ovarian disease.

ARTIFICIAL INSEMINATION WITH FRESH SEMEN

Semen Collection

Ideally, several days of sexual rest should be allowed prior to semen collection and evaluation.

Canine semen is most easily collected using masturbation with a gloved or bare hand, although some operators find the process distasteful and prefer to use an artificial vagina (AV). However, most artificial vaginas are made of latex and most latex is spermicidal. In addition, these artificial vaginas cannot be gas sterilized because the residues of gas sterilization are also toxic to spermatozoa. If an AV is used, it should be cleaned by washing and chemical disinfection then rinsed several times with distilled water and air dried.

The artificial vagina and attached plastic tube are warmed to body temperature and lubricated with a small amount of sterile aqueous lubricant.

It is much easier to obtain an ejaculate when an estrual bitch is present; therefore owners should be asked to bring in teasers when appointment are made. Alternatively, a non estrous bitch of the same breed or size may be used. A commercially available pheromone (methyl paraben "Eau d'estrus, [Synbiotics corporation](#) phone 18584513771, fax at 18584515719) may be used to stimulate the male but we have no experience in its use. It is sometimes very difficult to collect semen if no bitch is available at all can it can be done.

Optimally, the male and female are brought together on leashes in a quiet room with nonslip flooring. As the dog sniffs at the bitch's vulva or mounts her, the collector quickly moves the prepuce back, behind the bulbus glandis and directs the tip of the penis into the AV, held in the left hand. Once the artificial vagina is slipped onto the penis, the right hand is used to hold the artificial vagina onto the penis while exerting firm pressure around the back of the bulbus glandis. Once this occurs, the dog will usually show pelvic thrusting and normal ejaculation. An AV is certainly not essential to collect semen from dogs. Excellent ejaculates can be obtained by hand collection alone.

If a gloved or bare hand is used instead of an AV, the dog is masturbated rapidly for a few seconds until he gains a full erection. In the process, the prepuce is slipped behind the bulbus glandis. Masturbation ceases and the hand held behind the bulbus glandis using very firm pressure, until ejaculation is complete. The other hand is used to hold a plastic bag over the end of the penis.

Almost any warm receptacle can be used to collect the semen but most commonly sterile "Whirlpak" bags are used. Syringe casings and other hard objects should be avoided as the penis is very easily traumatised during collection and substantial bleeding may occur into the ejaculate. This does not seem to decrease fertility in dogs (Cf horses) but it interferes with semen evaluation and of course, alarm owners.

Semen can be collected when the bulbus glandis expands within the prepuce but some dogs object to this. Therefore, it is usually best to be sure that the bulbus glandis is out of the prepuce before it expands.

The reader can see therefore than the term "masturbation" is somewhat misleading. Most of the contact time consists of pressure exertion behind the bulbus glandis; a process identical to that used with an artificial vagina!

Ejaculation occurs intermittently over a variable period, perhaps five to 15 minutes, usually just long enough to deprive the collector (squatting on the floor) of all blood flow and feeling to the legs.

If pressure is maintained firmly around the bulbus glandis, pulsations can be palpated in the urethra. The anus will also be observed to contract in a rhythmic fashion. The dog may stop ejaculating for several minutes then pulsations will resume.

Initially, a few drops (one to 3 ml) of clear to slightly cloudy pre sperm fraction are ejaculated, followed by a whitish spermrich fraction (0.1 to 6.0 ml) but most often these fractions are mixed and only a homogeneous light greyopalescent ejaculate is obtained. The collector should try keep one hand around the collection vessel to keep it near body temperature. This is easiest when a plastic bag is used as a collection vessel.

Soon after the dog begins to ejaculate, he will often lift his hind limb as though attempting to step into the rump position that occurs during natural breeding. If this is observed, the collector should allow the dog to step over his/her arm so that the penis then extends out caudally from the dog. Soon the clear, third fraction of the ejaculate (mostly prostatic fluid) is ejaculated increasing the volume to as much as 60 ml. If the semen is being collected for artificial insemination as well as evaluation, enough prostatic fraction is collected to bring the total volume to three to 10 ml so large numbers of sperm are not lost in the insemination process and the insemination volume is comfortable to work with. Frequently, only a few ml of semen are collected but total sperm numbers, not semen volume, is what is important in A.I.

After collection is complete, the male is observed until his erection subsides. Paraphimosis may occur following collection, so the dog must never be kennelled or sent home until the penis is completely inside the prepuce. To prevent paraphimosis, one should lubricate the preputial opening liberally after semen collection.

Semen evaluation

Semen should be kept at a 35 to 37°C until progressive motility has been determined, after which it can be allowed to cool to room temperature. Semen volume is not important, but it is necessary to record the volume of the sperm-containing portion so that the total number of sperm per ejaculate can be calculated. Sperm cell motility, morphology, and concentration are determined in the conventional manner.

Normal prostatic fluid makes a good diluent if one is required for motility estimates. It can also be pooled and frozen for future use as a diluent for per vagina insemination of frozenthawed semen (a relatively new approach to the use of frozen semen).

A normal canine ejaculate:

Colour: Opalescent to milky white with a clear prostatic supernatant or homogeneous greyish white.

Volume: Pre sperm fraction: 0.1 to 3 ml, Spermrich fraction: 0.1 to 6 ml Prostatic fraction: one to 50 ml Total volume: one to 60 ml

Progressively Motile Sperm: 60 to 90%

Number of Sperm per Ejaculate: 200 to 3000 X 10⁶ (the population of the United States)

Morphologically Normal Sperm: 70 to 90%

Bacteria: Many; usually more than 10,000/ml. However, only the presence of many white blood cells is an indication for bacterial culture of the semen.

The presence of epithelial cells, red blood cells, inflammatory cells, and germinal epithelial cells are noted under low magnification. All cells other than sperm (COTS) are easy to see if a smear is stained with Wright's Giemsa or DiffQuick but difficult to differentiate using common sperm morphology stains.

Semen from males that have not ejaculated recently may contain more epithelial cells and debris than semen from a male that is used frequently but if large amounts of debris or dead sperm are present, a second sample should be collected 24 hours later.

Artificial insemination

Artificial insemination is performed when behavioural problems prevent mating (especially female dominance) when mounting is difficult or unlikely due to orthopedic problems or inexperience, or when transported or frozen semen is used.

Semen is collected from the dog using masturbation or an artificial vagina and only the sperm-rich fraction and a small amount of prostatic fluid is used (total volume three to 10 ml).

Semen collection should not be more frequent than once every two days. Daily ejaculation results in very low concentrations of ejaculated sperm after five to seven days.

Artificial insemination of the bitch is most easily performed by depositing the semen in the cranial vagina with a Cassou sheath shortened to about 25 cm. These sheaths are normally used to cover the rigid A.I. Cassou rods used for inseminating cattle. They are available from any veterinary supplier or A.I. cooperative. They are soft and flexible and therefore, far superior to the rigid plastic cattle inseminating pipettes sometimes used. In addition, they fit directly on a syringe and do not require adapters like the rigid pipettes. The Osiris apparatus from France is recommended by some operators but its superiority to other methods has not been demonstrated.

In all cases, the vulva is washed, rinsed and dried. The pipette is then inserted into the vagina, first dorsally for several centimetres, then cranially until the cervix is reached. If resistance is felt, the pipette is withdrawn one

or two centimetres, then reinserted at a slightly different angle. The vagina of the bitch is long and an insemination pipette may have to be inserted to a depth of more than 20 cm in large bitches. The hindquarters of the bitch are then elevated so that the spinal column is at an angle of 45 to 60 degrees and held there for as long as possible, up to 10 minutes.

One excellent study demonstrated that this was advantageous for sperm transport. Some inseminators also stroke the dorsal wall of the vagina with a gloved finger ('æfeathering') or massage the clitoris for about one minute when the hind quarters are elevated. This may promote semen transport within the uterus but it has not been objectively studied. Along the same line of thought, after insemination, the bitch should not be allowed to squat or jump for another 10 to 15 minutes.

It is usual to inseminate the total volume of undiluted ejaculate and prudent to use the ejaculate as soon as possible after collection. Insemination doses should contain at least 200 million motile sperm because fertility decreases with ejaculates containing less than 50 million live cells. Single estrus pregnancy rates up to 90% can be achieved with A.I. using fresh semen in fertile dogs. Optimal extension rates for dogs have not been studied but experience with horses suggests that it should be extended at a rate of between 1:1 to 1: 6 (semen: extender) for transport or if it is to be used more than an hour or two after collection. In most cases, a dilution rate of one part semen to two parts extender works well. It is also packed and transported in cooling containers like those used for equine semen transport. The "Equitainer" (Hamilton Thorne; sales@hamiltonthorne.com) cools the semen at 0.3oC/minute maintaining better motility than other cooling rates. Perhaps more important, this container has the best insulation on the market, a consideration when semen is to be transported through various temperature extremes.

Various semen extenders can be used for canine A.I. with satisfactory results. However a simple and effective extender can be made for shipped-cooled semen by heating skim milk to about 95 o C for ten minutes in a double boiler. It is then cooled to 37 o C for use. The heating step denatures a spermicidal albumin component in milk. It can be frozen in 50 ml aliquots for several weeks but its exact shelflife is unknown. A specific canine extender called "Fresh express" is available from the [Synbiotics corporation](#) (phone 18584513771 or fax at 18584515719). Commercial extenders used for equine semen transport can also be used; for example Kenney's or "EZ mix-in" extenders (arssales@dupreeinc.com or mntubcan@execulink.com) but no data exist to show that any of these extenders are superior to the others.

The extender is warmed to exactly the same temperature as the semen and slowly added to the semen until the final dilution rate is obtained. The semen is then packaged according to the instructions that come with the shipping system. Often this involves just placing syringes loaded with semen into a styrofoam shipper. The method used for the Equitainer is more involved.

Some operators recommend warming the chilled semen to room temperature just before insemination but this recommendation is not universal. It is a good idea to evaluate the motility of the semen when it is inseminated just so its status at the time of receipt is known. Occasionally all the sperm are dead! Motility should be evaluated in a drop of semen warmed to 37oC.

It is recommended that a longevity trial be conducted with the semen of any male animal (any species) before it is shipped over long distances in the chilled state.

Pregnancy rates of 50 to 60% higher have been reported with the use of chilled semen.