Successful breeding management includes appreciation of the reproductive peculiarities of the canine species. The estrus cycle of the bitch is classically divided into four stages: proestrus, estrus, metestrus/diestrus and anestrus. Proestrus begins on the day a vaginal hemorrhage first can be seen and lasts on average 9 days (3-27 days). The bitch is inviting the male, but is not ready to mate. In estrus, by definition, the bitch allows mating, usually for a period of 9 days (1-21 days). In metestrus/diestrus the bitch rejects the male again. The progesterone stimulated uterine epithelium desquamates as the progesterone concentration subsides over 2 to 3 months. The endometrial repair process is completed after 4 1/2 to 5 months (corresponding to the human menstruation period). Anestrus lasts for from 1 to 9 months, depending on if the bitch has 1, 2 or 3 cycles per year. A too short interval between estrus periods may cause fertility problems. This interval is usually prolonged by around 2 months after a pregnant cycle.

With the great individual variation in length of estrus it is not possible to determine the fertile days of the bitch’s cycle based on the days from onset of proestrus. Some bitches may ovulate as early as day 3 to 4, and others as late as day 26 or 27 from the beginning of proestrus. The only consistent relationship is the time from the LH peak until the onset of ovulation, ovulation in most bitches beginning 24 to 72 hours after the LH peak. Ova may be released over 24 to 96 hours. Unlike most other mammals the dog ovulates primary oocytes that are at the germinal vesicle (GV) stage and meiotic resumption occurs after about 48 hours spent in the oviduct. The oviductal transit takes 5-10 days. Fertilization occurs during this passage, in the distal part of the oviduct, when the oocytes reach the MII stage, 48-60 hours post ovulation. Mature canine ova may remain fertilizable for 2 to 4.5 days. Vaginal exfoliative cytology is used to identify the stage of the estrus cycle of the bitch. The thickness of the vaginal mucous membrane increases from 2-3 to 20-30 cell layers due to the rising estradiol levels during proestrus, with a lag-time of 3-6 days. The cells change during proestrus from small parabasals, with a high nucleus to cytoplasm ratio, over the larger intermediary cells, which still have a large nucleus, to the fully cornified superficial cells, which usually are irregular in shape and sometimes have folded borders and contain either a small pycnotic nucleus or are anuclear. Maximal cornification can be seen from late proestrus or early estrus and remains during the period of the abrupt fall in estradiol and rise in progesterone preceding ovulation, and throughout estrus. In metestrus there is a quick shift from merely superficial cells to intermediary cells and parabasals. Characteristic for metestrus is the appearance of a large number of polymorphonuclear leucocytes. The changes of the vaginal cells are caused by estradiol and not by progesterone. Vaginal cytology can, therefore, not be used to determine whether, or when, the bitch ovulates, and is thus not an exact enough method for timing of the bitch for mating or artificial insemination. The technique is, however, useful in that a smear will show whether the bitch is still in proestrus or already in metestrus. Measurements of peripheral plasma LH levels may be the most exact method to predict ovulation in the bitch. LH assays are available, but because the LH peak only lasts for 1-2 days in the bitch, blood samples would have to be taken daily or every second day during proestrus, which makes the method impractical. Measurement of the peripheral plasma progesterone concentration is the method that best combines the practical and economical aspects with the requirement of exactness. The level of progesterone is basal (< 0.1 ng/mL) until the end of proestrus, when the follicles change from producing estradiol to producing progesterone shortly before the LH-peak. When the LH peaks the progesterone level usually is between 2 and 3 ng/mL. Ovulation occurs 1 to 2 days later at a progesterone level of between 4 and 8 ng/mL. Progesterone then rapidly rises to a maximum of around 50 ng/mL in about a week’s time, then to slowly decrease during the ensuing 2 to 3 months. Canine ova are released as primary oocytes and need 2 to 5 days to mature. Canine spermatozoa have been reported to survive in the uterus of the female for at least 4 to 6 days. Theoretically, thus,
the bitch could conceive after one mating from about 1 or 2 days before until about 7 or 8 days after the LH peak, a period referred to as “the fertile period” (Fig). The best time for mating or AI would be 2 to 5 days after ovulation, during the period of optimal fertility. The progesterone level then is usually between 10 and 20 ng/mL.

It should, however, be remembered that plasma levels of progesterone fluctuate considerably during the day, with up to 20-40%, but not in a regular diurnal fashion. Thus, even though the values obtained by a validated assay may be very accurate, they should be interpreted with this daily variation in mind. In conclusion: In a bitch that has a short 10-day heat the best time for mating or AI is on days 8-9, in a 14-day heat on days 11-13, in a 21-day heat on days 18-20, in a 24-day heat on days 21-23 and in a 28-day heat on days 25-27.

Dogs ejaculate in 3 fractions, with mainly prostatic fluid in fractions 1 and 3, and with the spermatozoa in the 2nd fraction. Depending on the size/breed of the dog the ejaculate contains between 100x10⁶ and 5,000x10⁶ spermatozoa. Of the several hundred million spermatozoa that are deposited at mating maybe only a thousand will finally reach the oviducts. The percentage of abnormal spermatozoa should not exceed 20-40% and motility should be at least 70%. The relative significance of different types of sperm defects in the dog has been little studied. It seems to be generally agreed, however, that dog spermatozoa with proximal droplets are immature and lack fertilizing capacity, while those with distal cytoplasmic droplets function normally. It has been suggested that a higher number of spermatozoa to some extent may compensate for a higher percentage of abnormal spermatozoa. Semen quality may vary between breeds and was e.g. found to be poor in Irish Wolfhounds. It is generally considered that mature, healthy stud dogs can accomplish a mating every second day without a decrease in ejaculate volume or number of spermatozoa.

Effects of age: Bitches are usually bred when they are between 2 and 7 years of age. The largest litters are produced at between 3 and 4 years of age (Gavrilovic et al., 2008). Male dogs also are commonly used for breeding from about 2 years of age, but unlike bitches they may keep breeding into old age. Middle aged and older dogs commonly suffer from prostatic problems, which lowers their fertility.

Effects of season: A significant variation in fertility between months and/or seasons of the year have been shown both in bitches and male dogs, with lowest whelping rates and poorest semen quality in summer.

In conclusion: Best results are obtained by breeding from healthy dogs and bitches at their most fertile age, practising proper timing of the mating and mating the bitches twice with a day’s interval and, if possible, by avoiding breeding during the low-fertile season of the year.

References:
Tips and tricks for artificial insemination in the bitch

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The keys to obtaining good results by canine artificial insemination are proper timing of the insemination, the use of an adequate number of spermatozoa of good quality, good semen handling and preparation methods, and to apply an intrauterine insemination technique. Whelping rates by intrauterine AI in the dog are significantly better than those obtained by vaginal AI not only for frozen-thawed semen (+51%) but also for chilled (+44%) as well as for fresh semen (+30%). Litter size using intra-uterine AI of frozen-thawed semen is also significantly larger than by vaginal AI. Litter size has been estimated to be 25-30% smaller in bitches receiving frozen semen compared to fresh and chilled (6,8,10,11,12).

AI techniques in the dog.

Methods for AI in bitches include vaginal deposition of the semen, transcervical intrauterine deposition (TCI) using the Norwegian/Scandinavian catheter or with the aid of a rigid endoscope, and intrauterine (IU) insemination by laparoscopy or full abdominal surgery. (see 5,7).

Figure 1. Three sizes of the Norwegian/Scandinavian AI catheter for non-surgical TCI in dogs, and two sizes of rigid plastic single-use catheters for vaginal AI.

Figure 2. Transcervical intrauterine insemination can also be accomplished with the aid of a rigid fiberoptic endoscope, with or without a deflector and camera and monitor, and a canine 6-8 Fr gauge urinary catheter.

Intrauterine AI using TCI

To learn the techniques for non-surgical TCI using the Norwegian/Scandinavian catheter (Fig 1.) or a rigid endoscope (Fig. 2) have a long learning curve, but once learned they are quick procedures, performed in the unsedated, standing bitch, and usually being accomplished within minutes (7,8,9,20).
Intrauterine AI using surgery

Surgery to effect intrauterine insemination is still widely used. The method is, however, considered by many to be unethical and unacceptably stressful for the bitch. The risks for infection, etc. associated with surgery in general and the limited number of surgical AI’s that can be performed in a given bitch are two obvious disadvantages. The method is illegal in several European countries.

Intrauterine insemination using laparoscopy

Abdominal laparoscopy should offer a somewhat more acceptable alternative to full surgery for AI in the dog, but this method has not met with acceptance from practitioners, most likely because they are more used to the surgical technique.

A recent survey on the web-lists for small animal reproductionists reveals that surgical AI probably currently still is the most commonly used technique for AI in the dog, especially when frozen-thawed semen is used, or when the number of spermatozoa is low or semen of inferior quality is used. More and more veterinarians are, however, learning one or the other of the TCI techniques. It appears that the use of a video endoscope especially attracts the younger colleagues, and is also appreciated by breeders who can watch on the screen as the semen is injected into the uterus. Those who learn to use the Norwegian/Scandinavian catheter especially appreciate its simplicity.

Timing of the insemination

Timing of the AI is crucial, especially when frozen semen is used, which has a short survival after thawing. The most commonly used method to determine the optimal days for breeding or AI is to measure the peripheral plasma concentration of progesterone. Especially in the USA LH-tests are also used. The bitch should be inseminated 2-5 days after ovulation, when the progesterone concentration is between 10-20 ng/mL (30-60 nmol/L). However, progesterone values should be interpreted bearing in mind that plasma levels of progesterone fluctuate during the day by up to 30-40%, in a not diurnal fashion (13).

Semen dose per AI

In Europe the recommended number of normal spermatozoa per breeding unit is 150 to 200 x 10^6 (1,5), and to do 2 AIs per oestrus cycle, whereas in for instance the USA commonly 100 x 10^6 progressively motile spermatozoa (>50%) and a single AI is considered adequate. Pregnancies have, however, been achieved with as few as 20 x 10^6 fresh spermatozoa deposited surgically at the tip of the uterine horn and with two doses of 50 x 10^6 frozen-thawed spermatozoa deposited into the uterus through the cervix with the aid of an endoscope (20). Vaginal deposition of fresh as well as frozen-thawed semen appears to require approximately 10 times as many spermatozoa to obtain the same whelping rate as by intrauterine deposition (12,18). Because the uterine lumen in the bitch is small, the final volume of extended semen should not exceed 1-3 ml in intrauterine AIs and 3-5 ml in vaginal AIs, depending on the size of the bitch. This is clearly an area where more research needs to be done, aiming at determining the minimum number of normal spermatozoa for optimal results, in different breeds and sizes of dogs.

Results by AI in the dog

Pregnancy rate in the dog after well controlled natural matings has been shown to be as high as between 85% and 90%. Results from AI generally are still not as good, although when semen of good quality is inseminated into the uterus at the right time in healthy bitches up to 80-87.5% whelping rate has been reported by TCI also for frozen-thawed semen (8,16,17). Whelping rates by intrauterine AI compared to vaginal AI is significantly higher both for fresh semen and for frozen-thawed semen (6). Obviously, results will also depend on many other factors such as breed, age, fertility of the dog and bitch, season of the year etc (2).
How many times should the bitch be inseminated?

Repeating the AI after 24-48 hrs results in a significantly higher pregnancy rate and litter size (3,6,10,11). If only one insemination can be made, for instance when performing surgical AI, emphasis should be placed on making sure that the bitch is inseminated at the optimum time, i.e. 2-5 days after ovulation.

References:

Further reading
Things to know before using preserved semen; chilled or frozen; and regulations for international shipment.

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Significantly higher whelping rates are obtained when fresh (+30%), chilled (+44%) and frozen-thawed (+51%) semen is deposited IU compared to in the vagina. (Linde Forsberg 2011). Litter size using intra-uterine AI of frozen-thawed semen is also significantly larger than by vaginal AI (by 80%). It has been estimated that 10 times as many spermatozoa are required to obtain similar results by vaginal AI as by intrauterine AI in the dog (Linde Forsberg 2011).

Canine semen international shipment

It is becoming increasingly more common to freeze and store dog semen for future use at home, or for use in another country. At the time of semen collection it is not always known whether the frozen semen later will be exported. Therefore, it is a good practice to use a protocol that takes this possibility into consideration. National legislation as well as Kennel Club regulations pertaining to the use of frozen stored dog semen may change at any time. Before undertaking to freeze and store dog semen the dog owner should, therefore, always be advised to contact the Ministry and the Kennel Club to get accurate information about the latest rules and regulations, so that the necessary health certificates and blood tests can be made in accordance with the requirements. Within the European Union is now for the most part a free exchange of dog semen. One exception is Italy.

Ministry of agriculture requirements

When it comes to the national legislations pertaining to the use or importation of dog semen different countries basically fall into one of the following five categories:

Category 1. No restrictions.
Category 2. A veterinary health certificate including the ID-tattoo or microchip marking of the dog.
Category 3. An import permit is required.
Category 4. An import permit and a veterinary health certificate for the semen donor issued at the time of semen collection, or at a stipulated time interval before, and/or after the semen collection are required.
Category 5. An import permit and a health certificate and a serological test for leptospirosis (usually L. canicola and L. ichterhaemorrhagica) and/or for brucella canis are required. Note that the blood samples in some cases should be taken prior to (usually within 15-45 days before), in other cases at the time of, and sometimes after (usually 20-30 days, or 3-6 weeks after) the semen collection.

Kennel Club requirements:

♦ Most Kennel Clubs request that the semen donor should be registered by an officially recognized Kennel Club.
♦ The Kennel Clubs usually request that the semen donor is permanently identified by an ID-tattoo or a microchip, and that the veterinarian in charge of the semen collection certifies that the dog’s identity has been checked.
♦ Most Kennel Clubs request that the semen donor has a normal testicular status.
♦ Some Kennel Clubs request prior application for permission to use imported semen.
The American Kennel Club requests that a DNA sample is submitted. It is taken with the aid of a special cheek-swab supplied by the AKC.

Some Kennel Clubs have an eradication scheme for various hereditary diseases (for instance Hip Dysplasia, Progressive Retinal Atrophy, and Progressive Nephropathy) for some breeds, and request that the semen donor has been tested free from any such disease.

Other requirements may be that the dogs have shown that they can mate normally or have proved that they are fertile.

General recommendations for minimum documentation on semen doses to be stored in the semen bank.

In order to comply with the majority of the various national rules and regulations, and as a courtesy to the colleague who will perform the AI, it is recommended that each dose of dog semen is accompanied by the following documents, and that the following procedures are adhered to, also when this is not formally required:

- A veterinary health certificate, including a statement that the dog has a normal testicular status, and that the identity of the dog was properly confirmed.
- A semen quality assessment form, and in the case of frozen semen also thawing instructions, together with a recommendation of how many straws, or vials in the case of pelletted semen, should be used for each AI.
- The official, Kennel Club registered, name of the dog should always be used for all official documents, including the blood test report, never the pet name.

How to mark the semen straws or vials

To comply with the requirements in most countries the semen-containing straws or vials should always be marked with the following information:

- the breed (which may be abbreviated),
- the dog’s registered name (which may be abbreviated),
- the dog’s KC registration number or microchip number (for Australia the microchip number is necessary),
- the date of semen collection (necessary when blood tests and veterinary certificates are required),
- where the semen was collected/processed.

Example:

<table>
<thead>
<tr>
<th>Breed</th>
<th>Dog's Name</th>
<th>Registration Number</th>
<th>Date of Collection</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afghan hd</td>
<td>B.S. Sandstorm</td>
<td>SE-76689-12</td>
<td>1-5-2014</td>
<td>CaniRep</td>
</tr>
</tbody>
</table>

The exact identification markings of the straws/vials should also appear in the certificates.

Cooperation between the semen banks and the inseminating veterinarians.

Semen banks must provide the practitioners with adequate information about the quality of the semen they send and, in the case of frozen semen, information about how the semen should be thawed, because methods vary between freezing agencies.
References:

Diagnostic approach and treatment of canine genital infection and its infertility aspect

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**Brucella canis.** Abortion caused by *B. canis* usually occurs between 45 and 55 days of gestation and is accompanied by a highly contagious brown to greenish-gray vaginal discharge that lasts for 1 to 6 weeks. Infected bitches may not exhibit other clinical signs. *B. canis* may also cause conception failure, fetal death, and absorption in early stages of gestation or the birth of weak, infected pups. The infection is chronic and mostly asymptomatic in adult animals. Infected animals have intermittent bacteremia and shed bacteria through body fluids. **Diagnosis** is most commonly based on serologic testing using RSAT and ME-RSAT, bearing in mind that it can take several months after infection before antibody titers rise and that antibiotic treatment may create false-negative test results. Additionally, surface antigens of *B. canis* cross-react strongly with antibodies to several other nonpathogenic bacterial species. Blood cultures and PCR on blood and semen samples are the most accurate methods presently (Keid et al., 2007). **Treatment:** There is no effective treatment. Long-term broad spectrum antibiotics will suppress antibody formation temporarily. Euthanasia. **Strategy in infected kennel:** Test all dogs. All positive animals are euthanized. Testing of all dogs continues every month until 3 months have lapsed with no more positives, thereafter at 3 months’ intervals for one year. All new dogs introduced to the kennel should be tested in advance.

**Canine Herpesvirus (CHV1).** The canine herpes virus is species specific. It is presumed to be enzootic in dogs all over the world and the seroprevalence is as high as 88% in some European countries, the incidence not being higher in breeding kennels than among non-breeding dogs.

CHV1 usually spreads as a contact infection via the upper respiratory tract, but also at mating, or via saliva, urine, and vaginal discharge. It can also spread transplacentally to the fetuses. Typical for all the herpesviruses is that they create a latent infection. Activation of the infection may be induced by stress. Symptoms in adult dogs are usually mild: conjunctivitis, cough, genital infection, and possibly abortions. Experimental infection in early stages of pregnancy causes fetal death and mummification; in midpregnancy it may result in abortion, and in later stages in premature birth. More common in naturally occurring cases is that the pups are born apparently healthy but become ill during the first 1 or 2 weeks and succumb within days. Often the whole litter is affected; however, some of the pups may survive but develop defective vision, deafness, neuromuscular disorders, or kidney malfunction at 7 or 8 months of age. Normally immunity is incurred, and bitches that have lost a litter due to herpesvirus infection subsequently give birth to normal litters. Young puppies (<2-3 weeks old) that are infected i.u. or at birth usually die within 1-3 days after showing the first symptoms. Surviving puppies may develop CNS-symptoms, ataxia, blindness and deafness due to a meningoencephalitis. **Diagnosis:** Autopsy will show typical multifocal haemorrhages and necroses of inner organs. **Serology** from adults. **Virus culturing**. A first time infection will result in a rise in antibody production over 2-3 weeks. CHV1 can no longer replicate when the a.b. titre is high, which then prevents further replication, and thus also the spread of disease. If a male dog with a low a.b. titer mates a positive bitch with an activated CHV1 infection he will be boosterized already within 2-3 days, and vice versa. Thus, if a bitch is infected at the time of mating she will either have a protective a.b. titer after 2-3 weeks if she has not been infected with CHV1 before, or if boostered already after 2-3 days. That will give her plenty of time to produce a.b. enough in colostrum to protect her puppies from infection. Problems really only appear when a CHV1-negative bitch is infected during the last 3 weeks of pregnancy, or when a litter is infected before 3 weeks of age. **Treatment:** Most important is prevention. Possibly the use of Eurican CHV1 vaccine for pregnant bitches, and hyperimmune serum 1-2 mL for the puppies. **Strategy in a kennel:**
Isolate pregnant and lactating bitches. Either vaccinate all dogs, or test all dogs. If all are seronegative either try keeping the kennel free from CHV1, or vaccinate those that are not positive or have a low titer. If, as is usually the case, some dogs are positive and some negative this is a risk situation, thus vaccinate as above. If all are seropositive there is no need for vaccination, but all new dogs being introduced to the kennel should be tested and those not positive should be vaccinated.

**Transmissible Venereal Tumor (TVT).** TVT or Sticker Tumor consist of cells of mesenchymal origin. It has just recently been shown to have existed for 11,000 years. TVT can be found anywhere in the body. The tumors are very fragile and spread by transplantation of cells at contact with other tissues. They can also occur in and spread via semen. **Diagnosis:** The characteristic appearance, it bleeds easily, a cell impression smear, or a biopsy. May spread leishmania. **Treatment:** Cytostatics, (e.g. vinkristin, cyclophosphamide, doxorubicin), radiation therapy or surgical removal.

**Ehrlichia canis** A severe, tick borne disease present in various body organs. It may possibly be spread in monocytes and lymphocytes in and around the reproductive system (e.g. the prostate). May lead to orchitis and testicular degeneration.

**Canine leishmaniasis (CanL).** A severe disease spread by the sand fly. It is a zoonosis. It has been detected in the testis of a dog affected by orchitis. If shedding occurs into the semen it may be venerally transmitted. It has been isolated in TVT cells and associated macrophages in a bitch and might be an alternative route of venereal spread of CanL in dogs. Australia now has a requirement for testing the dog for CanL for importation of dog semen.

**Commensal/opportunistic bacteria.** There is bacterial growth on all normal mucous membranes. The vaginal and preputial bacterial microbiota in the dog is similar. The most commonly isolated bacteria in these regions in healthy dogs and bitches are: *Pasteurella multocida* (100%/98%), beta-haemolytic streptococci (73%/90%) and *Escherichia coli* (53%/85%). The microbiota varies between breeds of dogs. It also varies with stage of the estrous cycle, with more growth in proestrus and estrus. In the post-partum period growth of enterococci and *Staphylococcus aureus* is found more commonly. The commensal bacteria are opportunistic pathogens, and may cause problems in susceptible or immune-suppressed animals. To be meaningful vaginal or preputial bacteriological sampling should only be done when the bitch or male dog has clinical symptoms indicating that it suffers from an infection.

**References:**


