

ARTIFICIAL INSEMINATION WITH CANINE SEMEN

Dr. Fiona Hollinshead BVSc (Hons) PhD Diplomate ACT
Registered Specialist - Small Animal Reproduction
Glenbred
C/- Matamata Veterinary Services
26 Tainui Street
Matamata 3400



The most important aspect when discussing artificial insemination (AI) is to realise that there are many factors that play a role in whether or not your bitch becomes pregnant after AI. These factors include; type of semen (fresh vs frozen), semen quality and quantity, age and fertility of both the stud dog and bitch, site of semen deposition (intrauterine vs vaginal) and time of insemination.... just to name a few! As you can see, it is very important that when comparing "conception" or more correctly, "pregnancy rates" that all of these factors should be considered. Usually a combination of these factors are acting together in any one case.

1: Types of Semen: Fresh, Chilled and Frozen

Insemination can be performed using three types of canine semen: fresh, fresh-chilled and frozen.

i) **Fresh** ejaculated canine semen has the longest viability. Semen from young, fertile stud dogs can survive for up to 5 to 7 days in the bitch's reproductive tract!

ii) **Fresh-Chilled** semen involves the dilution of ejaculated semen in special canine extenders which contain egg yolk and buffers that protect the sperm during the cooling process. The extended semen is then slowly cooled to 4°C and can remain viable at 4°C for up to 3-4 days. Semen from some stud dogs has been reported to maintain viability and fertility after storage at 4°C for 10 days! This ability to preserve semen at fridge temperatures facilitates the shipment of canine semen either within Australia or internationally to New Zealand. It is a great alternative to shipment of either the stud dog to the bitch or vice versa when each are located at a distance from each other, which can be very costly and impractical. Once the semen is collected from the stud dog it is evaluated and then extended to an appropriate concentration and volume in special "canine semen chilling extender". The extended semen is then placed in a special transport box that cools the semen to 4°C while it is in transit to the bitch's location. Once the semen reaches 4°C it is then held at this temperature for up to 24-36 hrs depending on the transport system used and the outside temperature. The objective of chilling semen is to conserve the sperm's energy and hence prolong its longevity and viability. After warming back up to body temperature, fresh

chilled semen lives about 24 to 72 hours in the bitch although with some stud dogs the sperm last a lot longer.



Fig 1: Examples of transport systems used for shipment of chilled canine semen.

iii) Frozen semen is stored in liquid nitrogen at the extreme temperature of -196°C . Semen is preserved in a frozen state in either "straws" or "pellets". After enduring the freeze-thawing process, the sperm has a much reduced lifespan of only 12-24 hours compared to fresh or chilled semen. This is because the freeze-thawing process causes some degree of sperm damage.



Fig2: Examples of liquid nitrogen tanks in which frozen semen is stored indefinitely

Mother nature gives us a large margin for error in our timing for natural breedings because of fresh semen's long lifespan. However, when dealing with frozen semen, it is critical to know exactly when ovulation has occurred so that we can make sure the frozen semen is deposited into the uterus when the oocytes or "eggs" are ready to be fertilised.

2. Timing of Insemination: Monitoring your bitch's heat/oestrous cycle

Timing of insemination is very important especially when you are using frozen-thawed semen as it has a significantly reduced longevity compared to fresh or chilled semen.

Monitoring your bitch's heat or oestrous cycle in order to determine the optimal time to inseminate involves carrying out one or ideally a combination of the following procedures:

1. Blood samples to measure the hormone *progesterone* .
2. Vaginal smears for *cytological evaluation* to help not only determine when to inseminate but to make sure no infectious or inflammatory process is going on that needs to be addressed.
3. *Vaginoscopy* to assess the vaginal lining, which can be correlated to the stage of the oestrous cycle and is also a very helpful tool to use in maiden bitches to make sure there are no anatomical abnormalities before breeding.

The number of samples that need to be taken to determine when to inseminate varies between bitches. At Glenbred we recommend to start testing approximately 5 days after the onset of heat and then every 2nd or 3rd day until we determine when the bitch has released her ovulatory hormone called "luteinising hormone" or "LH". The day this occurs is called the "LH Surge" = " Day 0 of oestrus or the fertile period". The day or days on which we recommend artificial insemination to be carried out depends on the type of semen that is to be used. For example, insemination with frozen-thawed semen is carried out on "Day 5" (post LH 0) if only one insemination is to be carried out or on Days 4 and 6 or 5 and 6 (post LH 0) if the TCI technique is used and two inseminations can be performed. For more information see "*Tips to help make insemination with frozen-thawed semen successful*".

3. Site of Semen Deposition: Artificial Insemination Techniques

When using frozen-thawed semen or low numbers of potentially compromised fresh or chilled semen, deposition directly into the UTERUS is carried out to maximize the chance of pregnancy. Two intrauterine insemination techniques include:

a. Transcervical Insemination (TCI)

This is a non-surgical, non-invasive insemination technique whereby a catheter is passed through the cervix into the uterus using a specialised rigid endoscope. The semen is then flushed through the cervix and deposited directly into the uterus. This procedure can be visualised by both the veterinarian performing the procedure and the owner of the bitch because the endoscope has a camera fitted onto it which projects an image onto a television mounted on the wall (see Fig 3). This *internationally* recognised technique was developed by Dr Marion Wilson in New Zealand. It has been used for breeding dogs very successfully for over 20 years. It has the advantages of being anaesthetic and sedation free, non-surgical and relatively stress free in most bitches. It also has the distinct advantage in comparison to surgical AI in that more than one insemination can be carried out during the bitch's heat.

However, there are a few bitches, often for behavioural reasons where it may not be a feasible option.

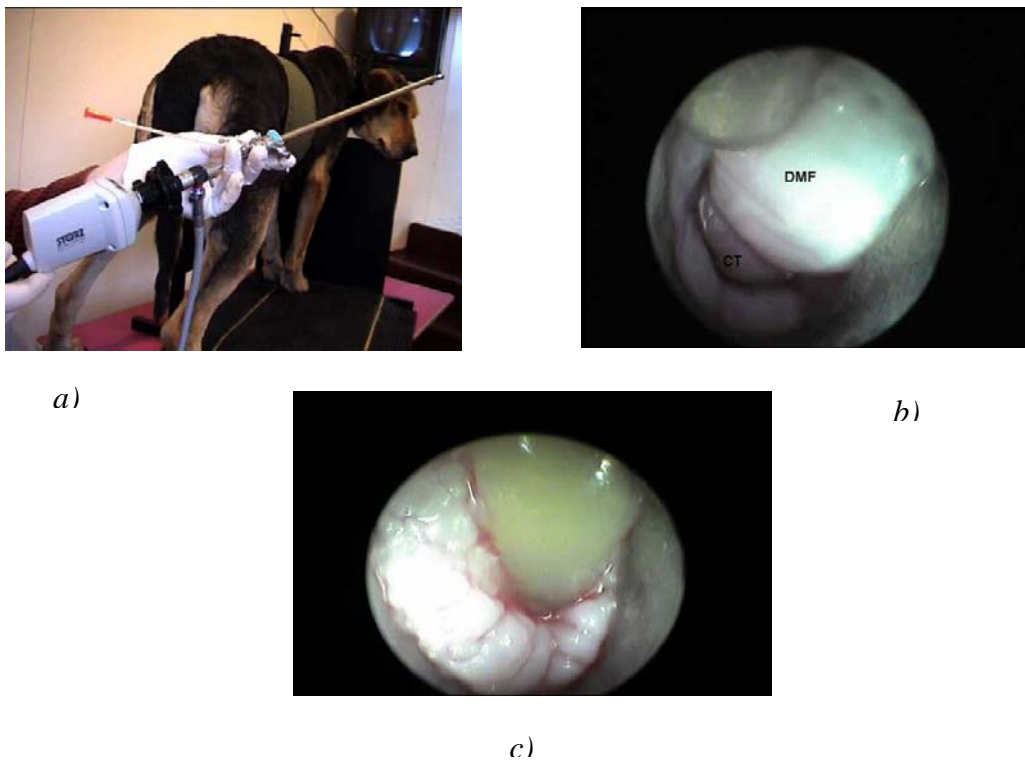


Fig 3: These pictures illustrate:

- The minimal restraint required for TCI and the rigid endoscope used to visualize the reproductive tract;
- Endoscopic view of the end of the vagina with the cervix (CT) appearing just below the large vaginal fold (DMF) and;
- Catheterization of the cervix and flushing of semen into the uterus.

* Courtesy of Dr Marion Wilson, 2003

There have been some recent developments with the canine TCI endoscope. A new scope which is longer and thinner is now available (the technical term is "renourethroscope"). This not only facilitates TCI in large breed and maiden bitches but its narrow and slender tip is able to be easily passed through the cervix and into the uterus allowing visualisation and sampling of any abnormalities that are detected. This is a fantastic diagnostic tool for investigation of female infertility cases.

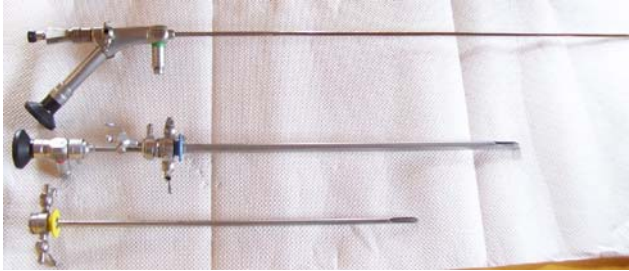


Fig 4: Photograph of the new longer and thinner TCI renourethroscope alongside the original rigid TCI urethroscope.

b. Surgical Intra-uterine Insemination

This technique involves a general anaesthetic and surgery and the associated risks. A small incision is made into the abdomen and the uterus identified and exteriorized. The semen is then injected directly into the uterine horns. This technique has the advantage of not requiring any specialized equipment but due to the invasive nature of this technique it can only be carried out once during the bitch's heat which makes timing of insemination very important.

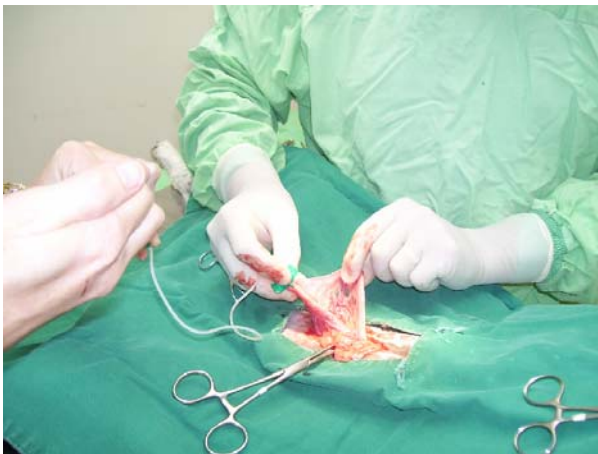


Fig 5: This picture shows the exteriorisation of a bitch's uterus through a surgical incision in the abdomen. Thawed semen is then injected through a catheter into the uterine horn.

c. Vaginal Insemination

When using fresh or chilled semen with adequate sperm numbers and sperm quality, deposition of semen into the very end of the vagina can result in pregnancy and litter sizes equivalent to natural matings.

There are many different types of catheters that can be used for vaginal AI. At GlenBred we insert a special artificial insemination catheter to the very end of the vagina. At this point a bulb at the end of the catheter is inflated to mimic the swelling of the dog's bulbus glandis during mating (see Fig 4). This not only prevents back flow of semen but stretches the vaginal wall resulting in oxytocin release and stimulation of vaginal and uterine smooth muscle contractions which facilitates transport of the sperm into the uterus. After the bulb is inflated, fresh semen is slowly injected down the catheter into the far end of the vagina. This is then followed by warm canine semen extender which functions to help flush the semen into the uterus in the same way prostatic fluid does during natural mating.

This is a technique that can be used in all bitches, is non-invasive, requires less skill and equipment than the intra-uterine AI techniques and therefore is also economical. It is often used in maiden bitches and studs that fail to "tie" or mate, which can be due to a number of reasons.

Vaginal AI is not routinely used for AI with frozen semen. Semen is frozen in significantly lower numbers (i.e. 100 million motile sperm per "AI dose") than what a fresh or chilled insemination dose contains. Furthermore, the viability and longevity of frozen semen is significantly less than that of fresh or fresh-chilled semen. Therefore deposition of low numbers of fragile frozen-thawed sperm into the hostile vaginal environment results in low numbers migrating through the cervix into the uterus and reaching the fallopian tubes (oviduct), which is where fertilisation takes place. This obviously results in a lower pregnancy rate and litter size.



Fig 6: One type of catheter that can be used for vaginal AI. It is called the "MAVIC" catheter (Minitube) and has a special "balloon" at the end that helps prevent semen backflow

4. Importance of Semen Handling and Assessment

No matter what type of semen (fresh, chilled or frozen) or the method of artificial insemination that is used to deposit the semen, careful and skillful handling of the semen is essential. Furthermore, assessment of a sample of semen by a qualified and knowledgeable person prior to insemination also plays a critical role in the maximisation of pregnancy rates.



Fig 7a: A high powered microscope connected to a monitor screen so that semen samples can be analysed by a number of people at the same time.



Fig 7b: Dr Fiona Hollinshead examining a semen sample with the microscope and monitor set up as shown in figure 5a.

In conclusion, AI is a very useful reproductive management tool. It allows the transport of semen both nationally and internationally. However, management of both the stud dog and bitch is critical to maximise both pregnancy rate and litter size after AI.