

## An Overview of Artificial Insemination

### An Overview of Artificial Insemination in Dogs with Frozen Semen

It is over 40 years since dog breeders started to seriously consider the possibility of artificial insemination in dogs using frozen canine semen.

In America, in particular, travelling for days across the country with a bitch in season to mate a chosen stud dog was the norm for many breeders. Gradually enthusiasts embraced the work of a small group of scientists, often themselves dog breeders and show people, who started to develop techniques for chilling and freezing canine semen. This would avoid the need to move dogs around a vast country, yet still use their genetics. Improved timing of insemination that went alongside this process also led to more litters and numbers of pups being born. So things progressed, and the number of canine reproductive centres you can find in the US on a “google search” is one indicator of the success of this trend.

#### So where are we in the UK?

Well the answer is still quite a long way behind the Americans and some top European specialists like Catharina Linde Forsberg in Sweden. But there are some really eminent Vets in the UK field – Angelika von Heimandahl in Cambridge, Hector Heathcote in Surrey and Prof Gary England in Nottingham, for example. That is kind of where my enthusiasm for this area of science began and I strive to be a footstep behind these experts!

The basic sequence of Artificial Insemination in the dog is the use of freshly collected, chilled for frozen semen from your chosen stud to inseminate a bitch at her optimum time for breeding. Then sit back and wait 61 days from this AI for a litter of pups, that exceed all your breeding expectations. AI can also mean successful breeding with reduced chance of spread of disease.

Reality is a little harder than this and I will try to explain how in the UK we are quickly moving to successful AI litters, and this is helped even further with committed breeders who check their bitches in particular for optimum breeding time.

So to the stud dog.....

Storing frozen semen from a winning field or show dog is increasingly requested for reasons such as

- future use within that line,
- future use should the dog be sold abroad,
- future use should the stud have to be neutered for any health reasons.

- future use should the dog die

Many readers will be familiar with the collection process. At my Unit, for example, we liaise with the stud dog owner for a convenient appointment to visit, making sure the stud is in good health. Rarely do I collect from the dog without the help of a teaser "in season" bitch. Many a time I am fortunate to call upon the assistance of a good friend who owns a show terrier kennel. Often a terrier in standing heat will more than oblige to interest the male as we take a semen sample from him.

I was fortunate to train in dog breeding in the states and there the dogs we used were so trained that a teaser wasn't needed that often. Americans are always one stage in front!

The semen collection process is in 3 stages, with stage 2 being sperm rich and the part mainly needed, before stage 3, which is prostatic fluid is produced. I analyse various parameters include sperm motility, density and abnormalities to get a report and decide if the semen is fit for chilling or freezing.

Some notes here - older stud males can experience complications like prostatic disease where the sample has reduced counts, and often a bloody content. These are hard challenges to treat and get a freezable sample - so act whilst your stud is in his prime if you are going to freeze. Some males wont ejaculate fully without an in-season bitch and only produce prostatic fluid, which gives a false impression about their fertility.

Using the Clone method for semen freezing, the semen is frozen into 0.5 ml straws. The freezing process takes several hours and involves adding 2 sets of freezing buffers in timed sequence that give the sperm adequate nutrition and protection against its final freeze to -196 Celsius, at which temperature it can stay preserved for decades. Its rather like giving the sperm their tea first, before putting on their jackets for the cold.

Different specialists, and indeed Countries suggest different numbers of sperm for a normal breeding Unit. Europeans tend to favour 150 million sperm per breeding, whilst others, particularly Vets in the States, have great success with 50 - 70 million sperm. A lot depends upon how well the sperm thaw and what defects are present.

A test thaw is important to check the semen batch has survived the freezing process and returns to good motility and progression. This test thaw will give an indication as to how many straws are needed to breed a bitch with AI.

Semen can also be processed for chilled AI. Here it is collected in the usual way, but is preserved into a higher final volume for insemination. The buffer used here is different with no requirement for an antifreeze type of protectant in its composition.

Using chilled semen, the insemination is often into cranial vagina and 5- 8 ml is the common volume for AI. Chilled semen has better viability than frozen and placement is not so critical. (Those bitches being inseminated with frozen semen require TCI, or transcervical insemination, with smaller volumes, for optimal chance of pregnancy).

## So what pregnancy rates can you expect?

Well many factors influence the chances of pregnancy success:

Firstly – mating v chilled semen AI v frozen semen AI

This survey is of data from C Linde Forsberg and is a study of her work with 2210 canine inseminations.

Figures have been rounded to nearest whole number.

Semen type used How many dogs whelped out of 100%

Basic AI TCI AI

Fresh 48 % 62%

Fresh and Natural mating 83 % 89%

Chilled 45% 65%

Frozen 37% 56%

Basic AI = insemination with large volume into cranial vagina

TCI = transcervical insemination, placing approx. 2ml semen directly into uterus.

If you take a look at the figures, natural mating (after a fresh AI here in the table) produces pregnancies in 80 – 90% of matings. It is vastly more successful than even AIs with freshly collected, unprocessed semen.

Chilled semen shows a lower success rate, but this success goes to 65% with placement of the semen directly into the uterus.

Frozen semen, though, inevitably carries the hardest chance for success. Again, it significantly improves if insemination is via a transcervical technique.

Even these figures are a few years old and the success rates for AI are significantly better again, based upon a number of improvements – particularly improvements in semen handling techniques, operator skill and equipment to facilitate successful AI – cameras, monitors, shunt techniques, improved AI catheters, for example. The other key area that has improved figures is due diligence from the bitch owners – it is important to have progesterone testing and even vaginal swabs to make sure your bitch is ready.

We know that in a natural mating, or mating with fresh semen, the semen can last 7 days or so. Bitches may ovulate over 2 – 3 days. This range of timing means if you don't hit all the eggs being released when you mate your bitch, semen is still viable for several days to “mop up” the later eggs and lead to a positive pregnancy.

Chilled semen, such as you may send to a bitch owner based in Europe, will last for approximately 4 days. Again, there is some cross over here for sperm to meet eggs, but success becomes improved if semen is placed through cervix into uterus.

When frozen semen is used we are dealing with a much tighter window for success – frozen semen will only last 12 hours when thawed. Timing for AI is therefore critical and we also need to minimize any barriers to success by placing the semen into the uterus, most commonly through a TCI technique. Some operators in recent studies report a greater than 75% success with frozen semen TCI methods these days.

The other bonus of a TCI technique is larger litter sizes – for example, Dr Linde Forsberg found on average 2.9 pups with frozen semen AI into the vagina, whilst it was an average of 5.2 pups when frozen semen AI was through the cervix into the uterus in one study.

### **And what of the bitch?**

Well the bitch is at the centre of all the attention, and owners seeking AI are naturally keener than the stud dog owner to have a successful outcome. One of the biggest hurdles to success is adequate mapping of the bitch's cycle.

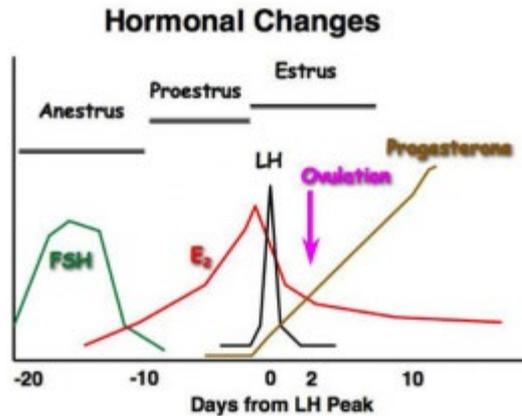
Progesterone tests are really important and the bitch owner must be prepared to spend on 2 or possibly more tests to pin point her ovulation. Each test may cost £40 – £50 but is well worth it.

Another point to remember is that all progesterone tests are not the same...

As Vets we tend to offer a laboratory progesterone test, where the sample result may take 24 hours to come back, but it is an actual number and extremely reliable. In some cases, the set up allows a same day result. Brilliant!

The “in house” tests that many of us Vets also offer do have their place, but are open to a lot of variation if not carried out by the same person each, time, at the same temperature and so on. Plus, they work on a “yes or no” result, so do not allow us any chance at predicting what may happen tomorrow.

The reason we need to progesterone test is that it is the only hormonal test commonly available to look for ovulation in the bitch. Testing for an LH (luteinizing hormone) surge is good but not easily available, except in research labs. But the bitch is unique in producing the hormone progesterone as her eggs get ready BEFORE she ovulates. So, the aim is to try to pick up the magic 15 – 20 nmol/l level of progesterone, which is the level noted at ovulation.



The Chart is courtesy of the University of Wisconsin and shows the LH peak and rising progesterone. The bitch's ovulation point is marked in pink.

In dogs the eggs need to mature a bit further before they are fertile. So, after predicting when ovulation occurs, we must wait 48 – 60 hours before inseminating the bitch – so all eggs are there and ready to fertilise. (progesterone may be 30 – 60 nmol/l at that point). If you remember, frozen semen only lasts around 12 hours on thawing. Hence our strict timing for AI.

Luckily, once we pick up a rise in progesterone from a baseline, the level tends to double every day – a so called exponential pattern of increase. This means that we can estimate when our target progesterone level has been reached in the bitch's season and it means we can save spending upon too many blood tests.

Soon to come to the UK market is a hand-held progesterone meter, which is being developed in New Zealand. I hope this will make progesterone tests much cheaper and therefore more frequently used.

### **And so to mating...**

Well if you are going to the trouble of AI with frozen semen, to preserve some long genetic line, or use an international dog, you need your progesterone tested bitch and to use a TCI, transcervical insemination technique.

In early days, bitches were AI'd using a "Norwegian catheter" – and Dr George Govette, in the States is absolutely brilliant at successful inseminations, passing this rigid catheter through the bitch's cervix, with no need for cameras and endoscopes.

Most ordinary Vets find this a really hard technique to master and cannot justify using it at the expense of pregnancies whilst they get experience.

Hence the advent of the endoscope and camera and TCI, where the bitch's cervix can be viewed and a fine catheter can be watched as it passes through the bitch's cervix. The bitch stays standing and since she thinks she is ready to mate, she very rarely even needs sedation. A non-pregnant uterus will only hold 2 – 3 ml of fluid. – less than ½ a teaspoon. Hence AI with frozen semen is with a small volume.

One further refinement has been the clever development of a TCI Shunt method, from a company in the States, where a special inflatable catheter is also used to guide the endoscope in the bitch's reproductive tract. It can seal the vagina and allow some air to temporarily inflate the tract, so Vets can work far more clearly, and is much more akin to the natural mating process.



Demonstration of the TCI shunt for canine AI

This photo shows the Shunt system on the endoscope from Minitube, USA. This system acts more like natural mating in the bitch.

One word about Kennel Club rules with mating by Artificial Insemination – their rules state that the stud dog must be out of the country or deceased when the semen is used. Exceptionally he may be immobile or a certain breed like an Irish Wolfhound. The Kennel Club will also only allow registration of litters bred by a surgical AI technique in very extreme circumstances.

Surgical AI has not been mentioned here since it involves the bitch being anaesthetised and operated upon. This is considered too large a “welfare debt” to the bitch to be justified.

Artificial Insemination is increasingly being used in the UK and readers may consider it has a place in their breeding programs in the future, allowing never before considered access to studs around the World

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