



## AN OWNER'S GUIDE TO ARTIFICIAL INSEMINATION

### Introduction

Artificial insemination (AI) is the technique used to transfer appropriately processed semen collected from a stallion into the uterus of the mare at the correct time in her oestrus cycle in order to obtain a single pregnancy.

The semen can either be fresh, chilled or frozen. Many of the techniques used are common to all three types of semen. Fresh semen is usually collected, extended and stored in an airtight, light free container for up to 8 hours at room temperature. Semen that is to be used longer than 8 hours after but within 48 hours of collection should be chilled to 4°C and stored for shipping in a special container. Semen that is required to last longer than 48 hours is frozen in liquid nitrogen at a temperature of -196°C.

### Advantages of AI

1. AI allows mare owners to use stallions from all over the world without having to go to the cost of sending their mare to the stallion. Semen from deceased stallions can also be used. These factors thus increase the available gene pool.
2. AI allows the safe mating of mares or stallions with injuries. It prevents injury to valuable stallions by mares of poor temperament.
3. The intensive veterinary management of AI mares can in some circumstances improve the chances of obtaining a pregnancy by employing minimum contamination practices.
4. The addition of extenders and antibiotics to the semen can improve the fertility of some stallions by improving the longevity of the sperm.
5. AI reduces the risk of transfer of venereal infection.
6. AI allows more efficient usage of busy stallions by the division of single ejaculates into multiple doses
7. The main advantage is that the mare and foal at foot, if there is one, can be kept at home under the owner's supervision.
8. Frozen semen can allow a stallion to compete internationally without having to worry about stud duties and temperament changes whilst covering.
9. Significant savings are often made on transporting the mare long distances to the stallion and then having to leave it at the stud with the resultant livery fees

### Disadvantages of AI

1. The veterinary input is relatively intensive and as such the veterinary costs will often be higher than for natural service.
2. There is still the risk of some venereal infections such as equine viral arteritis being transmitted from the stallion to the mare.
3. Logistically things can be difficult if clear lines of communication are not open, for example the stallion may be away at competition or the semen may be required at a weekend when it cannot be shipped. The use of frozen semen can negate these problems as it can be stored near the mare prior to its requirement. The use of agents to sort out the nomination agreement can help.
4. The semen of some stallions will not withstand chilling or freezing and thus these processes reduce fertility.



### **Expected Fertility with the AI Different Techniques**

The success of any AI program is dependent on factors such as the quality of the semen used and the fertility status of the mare. Also important is the routine used by the inseminating vet. The expected fertility with chilled and fresh semen AI should be the same if not a bit better than that for natural service, that is to say that approximately 60% of mares will go in foal on the first cycle. The expected fertility with frozen semen is between 30 and 60%; this is very dependent on the previous factors mentioned and the particular stallion.

Prior to embarking on an AI program it is important that the mare is examined to assess whether there are factors that may reduce fertility, such as poor vulval conformation. A plan can then be formed as to the measures that need to be implemented to maximise the chances of conception. The stallion should have his semen examined at every collection to assess the quality of the ejaculate, however bear in mind that some stallions' semen does not freeze or chill well.

### **Balancing the Advantages and the Disadvantages of AI**

Prior to embarking on an AI program with a mare it is important to become aware of all the facts and to balance out the pros and cons. One of the most important factors to consider is the cost. The keep and transport of the mare costs must be balanced against the veterinary costs of AI. Many of the veterinary costs will be incurred anyway. Some veterinary practices will have a fixed price scheme for AI programs. These can vary considerably, they may require the mare to go to a stud that the vet attends regularly, they may not include pre-breeding swabs or pregnancy scans.

### **Semen Collection and Processing**

Semen is collected from the stallion using an artificial vagina. In order for this to happen the stallion must mount either a mare in season or a phantom mare. Some trained stallions will allow collection from the ground without having to mount a mare. The use of a phantom mare reduces the risk of injury to the stallion and negates the necessity to have an in season mare available all the time for collection. Collection from the ground can be used for stallions with problems such as a bad back.

There are various designs of artificial vagina (A.V.). The basic principal is that they have a water-jacket filled with warm water to bring the internal temperature of the A.V. to between 45 and 48°C. Inside the water-jacket is a disposable liner, which is replaced before each collection to prevent the risk of infection. At the end to the liner is usually a filter to remove the gel fraction of the ejaculate. The semen is then collected in a warmed container at the end of the A.V.

It is essential that the semen is collected properly as there are many factors that will damage the sperm cells and thus render the dose infertile. Temperature fluctuations must be prevented, all collection vessels must be dried well to avoid water contamination and the exposure to air and light should be kept to a minimum. Ideally the stallion stud should have a dedicated lab for handling the semen.

Once the semen has been collected the volume of the ejaculate is estimated, it is examined for contamination with either blood or urine and it is examined under a microscope to assess the motility, concentration and to look for signs of problems with the sperm cells.

Following evaluation the ejaculate is mixed with a semen extender at a ratio dependent on the concentration of the ejaculate. The semen extender usually contains an energy source for the sperm cells and antibiotics to reduce the chance of propagating infection. The extender also dilutes the sperm cells thus providing a buffer against temperature fluctuations and for the harmful metabolites produced by



the sperm. Also included in the semen extender for freezing is a cryoprotectant to prevent crystallisation of the liquid in the sperm cells and thus damage to the sperm cell membranes.

The aim is to dilute the sperm for chilling to between 25 and 50 million progressively motile sperm per millilitre. An insemination dose may then be between 10 and 20 millilitres resulting in a total dose of 500 million progressively motile morphologically normal sperm. With frozen semen the aim is to achieve a concentration of 120 million progressively motile morphologically normal sperm per millilitre, an insemination dose is approximately 600 million sperm i.e. between 5 and 8 half millilitre straws.

### **Semen Transport and Storage**

Once the semen has been collected and processed appropriately for either chilling or freezing it is then packaged. For chilling it is either placed in plastic bags, tubes or special syringes without black rubber plungers that would damage the sperm cells. The semen must then be placed in a container for storage and shipping. The "Equitainer" is a special designed transport box that will chill the semen at a controlled rate and maintain the semen at a temperature of around 4°C for at least 48 hours.

Recently disposable polystyrene containers have been used very successfully. The semen is placed in preloaded syringes within the container along with frozen cool blocks. The box can then be posted inexpensively, overnight to the mare owner. The semen will last a good 24 hours in this type of container.

For freezing the semen is typically loaded into 0.5ml straws. Recently there have been techniques developed to allow larger volumes of semen to be frozen. Once packaged the straws are then submersed in liquid nitrogen at -196°C. The semen can then be stored indefinitely and transported in the liquid nitrogen.

### **Mare Preparation**

The success of an AI program is dependent on placing semen in the uterus at the correct stage in the mare's cycle. The mare must be in season, with plenty of oedema in her uterus on an ultrasound scan and a relaxed open cervix. The size of the follicles on the ovary are then monitored to ascertain the optimum time for insemination. This is done using rectal palpation and ultrasound examination of the uterus and ovaries, and examination of the cervix.

Some mares will cycle all year round, but the vast majority will cycle from March to October naturally. The breeding season can be altered using artificial light to extend the day length. Some mares show well in season some don't, some mares will not show in season when teased with a foal at foot. As such a veterinary examination prior to embarking on an A.I. program will help to ascertain at what stage in her cycle she is. I usually carry this out on a Friday, unless the mare shows in season, if she is not in season at the time of the examination then a prostaglandin injection can be given to bring the mare in season. An examination the following Monday is performed to check she is in season and to see what size follicles are present on the ovaries.

At this stage a swab and smear are usually taken from the endometrial lining of the uterus to check for infection.

The sizes of the follicles present on the ovaries are then recorded. These sizes will dictate when the mare next needs examining. The follicles grow at between 3 and 5 mm per day. The aim is to get to a follicle measuring 3.5cm in two planes perpendicular to each other on the ultrasound scan. At this stage the mare's cycle can be controlled, an injection of luteinising hormone now will stimulate ovulation in the vast majority of mares within 24 to 48 hours. As such we can then relatively easily predict the best time for insemination.



### **Timing Chilled and Fresh Semen Insemination**

The aim is inseminate the mare prior to ovulation. Good quality semen will last at least 48 hours in the mare's oviduct after insemination, however we usually aim to inseminate the mare within 24 hours prior to ovulation. A typical plan would be as follows:

1. Friday – Record any uterine cysts, examine for signs of infection or uterine disease, examine for signs of being in season. If not in season administer prostaglandin hormone to induce oestrus.
2. Monday – Check for signs of oestrus, record follicle sizes and collect a swab and smear from the uterus if appropriate. Plan next examination depending on follicle sizes.
3. Take action as indicated by laboratory results.
4. Once a follicle 3.5cm in diameter is present, and injection of luteinising hormone can be given. At this stage the semen is ordered to arrive the following day.
5. Inseminate mare.
6. Within 6 to 24 hours after insemination check mare for signs of inflammation in the uterus and treat with intrauterine antibiotics or saline lavage if indicated. Also check that the mare has ovulated. It is also important at this stage to record the number of ovulations as this can give an indication of the chance of multiple pregnancies being present at the first pregnancy diagnosis scan.

### **Timing of Frozen Semen Insemination**

After freezing and thawing the semen will last approximately 12 hours. After ovulation the mare's egg is viable for conception for up to 6 hours. This results in a window 12 hours before to 6 hours after ovulation in which insemination with frozen semen should take place. The hardest thing to do is to predict exactly when ovulation will take place. As such the conventional approach with frozen semen is to examine the mare every day, when in season until a follicle 3.5cm in diameter is reached. Then monitor every 4 to 6 hours after injection with luteinising hormone until she ovulates and then inseminate her knowing that you are within 6 hours of her ovulating.

Due to the huge amount of time and effort required for this technique the cost can be quite high. Recently a fixed time routine has been used. The mare is examined daily when in season until a 3.5cm follicle is reached, a luteinising hormone injection is then given. The mare is then inseminated 32 hours after this injection. The basis for this technique is that 85% of mares will ovulate in the correct window around 32 hours, after injection with luteinising hormone if they have a 3.5cm follicle.

### **Insemination Technique**

Prior to inseminating the mare it is essential that the semen used has paperwork detailing which stallion it is from, how it has been processed and how it should be used. Usually the stallion owners will require markings to be taken from the mare to confirm which mare has been inseminated. Also included in the stallion paperwork should be a certificate confirming the stallion to be free of the venereal disease equine viral arteritis. This is especially important in stallions from abroad, without it the semen cannot be used. It should be remembered that even freezing semen does not kill the virus.

With chilled semen the vulva is cleaned and tail bandaged. The semen is then introduced into the uterus aseptically using a long pipette. There is no need to warm the semen prior to insemination. Many studs send 2 doses one for today and one for tomorrow, there is no need for this as the semen will last better in the mare than in the container and only a single insemination dose will be required.



With frozen semen, the mare is usually resident at a veterinary practice or well-equipped stud. The mare's vulva is cleaned and the tail bandaged. The semen is removed from the liquid nitrogen and thawed in a water bath for the appropriate length of time and at the correct temperature, as instructed by the stud that froze the semen, this is usually 30 seconds at 37°C. A deep intrauterine insemination technique is employed where the semen is delivered to the opening of the oviduct on the side that ovulation took place, either using a special pipette or an endoscope. This technique can then be used to reduce the dose of semen required and can enhance success rates as the semen dose does not need to get from the cervix to the oviduct by itself, it therefore cuts down wastage.

### **Post Insemination Examination**

This examination is as important as the work carried out prior to insemination. Stallion sperm cells can cause an inflammatory reaction in the mare's uterus, post-inseminating endometritis. Some mares are susceptible to this problem and it will result in failure to conceive. Endometritis will result in the production of fluid within the lumen of the uterus visible on an ultrasound scan. If this is detected then it will indicate that intrauterine lavage and antibiotics may be required, in conjunction with oxytocin injections to make the uterus contract and empty the fluid.

It is also important to confirm ovulation has occurred and that further inseminations are not required. It is also important to check the number of ovulations as this will help in ruling out multiple pregnancies at later examinations.

The time that the post inseminating examination is carried out is dependent on the mare. A non-susceptible mare will need to be examined 24 hours after insemination. A problem mare may need to be examined and treated 6 hours after insemination and on several further occasions.

### **Pregnancy Diagnosis**

Transrectal ultrasound examination at between 14 and 16 days after ovulation is the best method of determining pregnancy. This is the most appropriate time as multiple pregnancies can be detected and relatively easily dealt with. After 17 days the pregnancies attach to the lining of the uterus, invariably twins end up lying next to each other and as such they become difficult to manage as they cannot be separated. If twins are detected the smaller or most irregular of the pregnancies is pinched out.

A further scan at between 25 and 30 days is important to check that there is still only one healthy developing pregnancy, with a heart beat visible on the scan. Some owners like to have their mare scanned again at 42 days.

If the mare is not pregnant on the first scan then the insemination program can be repeated, as she should be back in season imminently. Subsequent attempts may include more veterinary intervention, particularly after insemination to deal with post inseminating endometritis. Accurate recording of the program as it happens will indicate what improvements can be made on further cycles.