

## **Guide to Artificial Insemination in the KFPS registered Friesian Horse**

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### **What is Artificial Insemination (AI)?**

Artificial insemination (AI) involves the collection of semen from a stallion into an artificial vagina (AV). The semen is then examined for sperm numbers and motility, diluted with extenders and is then introduced into the mare by a veterinary surgeon or licensed technician by means of an AI catheter at the appropriate time in her cycle.

Stallions used for AI semen collection are either trained to mount a dummy and ejaculate into an AV or a teaser mare may be used for the stallion to mount whilst ejaculating into an AV. AVs are in effect a bit a thermos flask with a rubber or latex lining. Water is introduced between the case and the lining in order to have the temperature of the AV between 45 – 50C at the start of the collection and an additional disposable plastic lining can be used to minimise the likelihood of infection transfer. The semen must be protected from sudden increases or decreases in ambient temperature following collection and all equipment must be scrupulously cleaned.

### **What are the advantages of AI and who can carry out AI?**

The use of AI has many advantages,

- It allows the use of top class approved studbook stallions from the Netherlands throughout the world.
- The stallion is able to “cover” a greater number of mares including many on the same day
- There is not the risk of injury to mare or stallion associated with natural service
- Theoretically, the risk of transmission of venereal and notifiable diseases should be lessened, provided of course that people observe the EU / DEFRA regulations on semen collection / shipment/ importation.

However, you do need to have a veterinary surgeon who is experienced in stud work, including ultrasound scanning and AI, including the use of frozen semen. You may use a DEFRA licensed AI technician to inseminate the mare, however, a veterinary surgeon still needs to carry out the trans-rectal scanning of the ovaries, uterus and foetus. The use of AI can also be very costly if your mare does not take first or second time around, and you are very much at the mercy of the couriers to ensure that the shipment of semen arrives on time. Indeed, many people have had everything in place only to be let down by the courier at the last moment!

### **What types of AI are there?**

Most of the studbook stallions are available by Artificial Insemination (AI) only, and to this end the stallions are licensed for various types of AI. Before the start of the breeding season, Approved stallions used by AI must have a semen sample assessed to determine its TNM (TNM is a value indicating the number of sperm cells exhibiting a normal motility as expressed in millions,). Bacteriological testing of the semen must also take place as well as having the stallion tested for

CEM. A stallion tested positive for CEM cannot be used for stud service until testing proves him to be free of CEM again.

During the breeding season, at least two visits will be made without previous notification by the Inspection and Monitoring Division of the Department of Animal Health Services to monitor semen quality and breeding administration.

- On Location AI (Fresh AI)

This involves collecting the semen from a stallion and then having the mare inseminated at that location as well. The semen may not be transported to another station or to where the mare is normally kept. This stallion may not be used for natural cover or teasing purposes. An Insemination dose will have a TNM (value indicating the number of sperm cells exhibiting normal motility as expressed in millions) of at least 300. This number is theoretically sufficient to result in a pregnancy. The semen will often be mixed with an extender fluid, containing protective, energy-rich substances to enhance the survival of the sperm. The whole ejaculate may be used for one insemination, or if sufficient quality, can be split into several samples. It is imperative that fresh semen is kept at 37C and used within 1-2 hours of collection.

- SWS AI – semen collection station (Chilled AI)

Semen from a stallion at a semen collection station can be used to inseminate mares at the insemination facility located at the semen collection station, to inseminate mares at the location of the mare owner, or to inseminate mares at another insemination facility. Due to the transport of semen, additional quality demands are placed on this semen. The semen must remain sufficiently viable for at least 48 and may last up to 72 hours in some cases, have a TNM of 600 before being shipped so that it will still have the required TNM of 300 at the time of insemination.

- EU station. (Chilled and Frozen AI)

At a European station, the stallions are quarantined so that their semen can be exported abroad outside the EU. These stallions may not come into direct contact with other horses, and they must be free of all kinds of pathogenic organisms, e.g. CEM, EVA, and infectious anaemia. They are monitored by the Inspection Authority for Livestock and Meat (the RVV...Dutch equivalent of DEFRA animal health office). If the semen is for export, the RVV pays a call to provide the deep freeze chest or the package for chilled semen with a leaden seal. Accompanying the transport are a health certificate for the stallion and information about the semen being shipped.

## **Frozen semen**

Frozen semen is available from some stallions. For semen to be frozen the spermatozoa must first be mixed with an appropriate extender, they are then slowly cooled from body temperature and exposed to cryoprotective agents such as glycerol or sugar, which will protect the sperm against freezing. They must then be packaged into appropriate single dose containers (usually called straws), and the slow initial freezing process is changed when the temperature reached 5C to a rapid freeze

to below -100C using liquid nitrogen, so that the straws can be stored at -196C. On thawing the straws must be rapidly warmed to 37C and used for AI shortly after thawing.

Whilst Frozen semen offers the advantage of export over greater distances and storage for a much longer time, which can be useful if the stallion dies suddenly but has a large contribution to pay genetically or if the mare is not regular in her seasons. The process of freezing and thawing semen is detrimental so there are more damaged and dead sperm cells. For this reason, there should still be at least 300 million TNM after thawing that can then be sufficient for inducing pregnancy.

To use frozen semen, it is essential that your vet or AI technician has constant temperature water-baths and be familiar with the use of frozen AI as the semen must be defrosted in a specific way. It is quoted that the success rate with frozen semen for pregnancy per cycle is 10% lower on average than using fresh semen, however in practice this can be as much as 40% lower. Present theory is that insemination should take place in the six hours prior to ovulation, or indeed right at ovulation with frozen semen, although new technology and findings are coming to the forefront including deep intra-uterine insemination where the semen is introduced deep into the womb close to the fallopian tube which the ova will be shed down.

A KFPS protocol for the use of frozen semen is given in Appendix 1.

### **Fresh & Chilled versus Frozen semen**

Higher pregnancy rates are achieved by the use of fresh / chilled semen; however, it should be used within 24 hours preferably and at the outside 48 hours of collection. Best pregnancy rates have been found when used 1-3 days before ovulation and ideally 12 hours before ovulation. New technologies such as deep intra-uterine insemination can increase the likelihood of a successful pregnancy, when carried out by a veterinary surgeon fully trained in this technique.

Although Many studs and vets will advise the use of hormone injections to regulate the mare's seasons and bring her on heat and bring about an ovulation at a given time to facilitate using imported AI, if your mare comes into season like clockwork, better to work around this and not give hormone injections, as they can in occasional circumstances lead to cystic ovaries, and retained corpora luteum.

As the number of EU licensed approved stallions is at this time limited, you will need to decide whether to send your mare to the Netherlands (she would need to stay to at least the start of her second trimester to prevent embryo loss during transit) in order to have a greater choice of stallion or to keep your mare at home and select from those which are EU licensed. It should be noted that livery bills in the Netherlands are nowhere near as high as the UK, and many studs offer packages where they will train your mare and take her to a grading plus inseminate her with an approved stallion's semen. It is also easier to re-inseminate should she not take first time around. However, it should be noted that the standards of care may not be as high as in the UK.

### Which Stallion Stations are EU Licensed?

You will need to check with the stallion stations the availability of semen for export. Semen from popular stallions may need to be reserved a year in advance. The KFPS are encouraging the other studs to become EU licensed so we hope that there will be a bigger choice of stallions in the near future. They may also have stocks of frozen semen for export from stallions which were previously standing at their stud.

Station	Stallions	Contact
Henswoude	Lute 304, Wobke 403	00 31 566 631364
Maijer	Brandus 345, Jorn 430, Loadewyk 431, Maeije 440	00 31 572 352648
Het Swarte Paert	Jasper 366, Anders 451	00 31 651 923992
Gebr. Van Mannen	Onne 376, Ielke 382, Beart 411,	00 31 318 513371
Toonen	Tsjerk 328 Pref, Tsjalke 397, Harmen 424, Sake 449	00 31 412 479242
Widjewormer	Fabe 348, Sape 381, Bente 412, Dries 421, Gjalt 426, Olgert 445, Sjerp 446	00 31 299 439443

### What is EU certification and why is it important?

The majority of Approved Friesian Studbook Stallions are only available by Artificial Insemination. Legally, you are only supposed to import semen from EU certified stallion stations, of which only a few Friesian studs in the Netherlands are. Obviously, this severely restricts the number of stallions as an option to use, and KFPS and its daughter organisations such as FHAGBI are keen that those studs which are non-EU licensed become so in the near future.

Up until the past couple of years, non-EU studs have sometimes also been willing to export the semen and due to legislation not being strictly adhered to, mares in the UK and Ireland have been in the past inseminated with semen from non-EU licensed studs. However, as a result of an EIA outbreak in Ireland the other year which was linked to imported semen used for AI, DEFRA and the other EU member states' Animal Health departments are cracking down on the use of semen from non-EU licensed studs and rightly so; nobody cares about equidae species wants the equestrian industry to be decimated by a notifiable disease.

If you import semen from a non-EU licensed stud and / or do not have the correct animal health certificates with the semen, then you are risking prosecution and being sued if an outbreak of a notifiable disease occurred. If the semen does not have an EU health certificate, then the attending veterinary surgeon or DEFRA licensed AI technician can have their license to practice revoked (i.e. be struck off) or be prosecuted / sued if they were to AI your mare with it and a notifiable disease outbreak occur. – **PLEASE DO NOT EXPECT YOUR CHOSEN VET / STUD / AI TECHNICIAN TO RISK THEIR CAREERS JUST TO INSEMINATE YOUR MARE!**

## **What are the regulations for importing semen for fellow EU states?**

There is a huge amount of information on the DEFRA website about the regulations:

Visit: <http://www.defra.gov.uk/animalh/int-trde/iins/genetic/genetic-a11.htm> and a list of Licensed AI Technicians may also be found on the DEFRA website.

However, a basic guide to the regulations is given in Appendix II

A guide to notifiable diseases including African Horse Sickness, CEM, EVA and EIA can be found on the DEFRA site: [http://www.defra.gov.uk/animalh/diseases/vetsurveillance/az\\_index.htm](http://www.defra.gov.uk/animalh/diseases/vetsurveillance/az_index.htm)

Additional advice can be obtained from:

Animal Health Import Team  
Government Offices,  
Beeches Road,  
Chelmsford,  
Essex  
CM1 2RU

Tel: 01245 358383

Email: [AHITchelmsford@animalhealth.gsi.gov.uk](mailto:AHITchelmsford@animalhealth.gsi.gov.uk)

References:

The Rules and Regulations of the Royal Association “Het Friesch Paarden-Stamboek” 2001

PHRYSO International (1<sup>st</sup> edition March 2009)

Horse Breeding – Peter Rosedale OBE PhD, FRCVS, David & Charles (The Equestrian Library) revised edition 2003, chapter 15 pp369-375.

Determining the specimen animal health certificate for trade in semen of the equine species. EU Commission Decision (95/307/EC)

Laying down animal health requirements governing trade in and imports into the Community of animals, semen, ova and embryos, not subject to animal health requirements laid down in specific Community rules referred to in Annex A (i) to directive 90/425/EEC. EU Council Directive (92/65/EC)

International Trade: Importer Information Notes- Information about the intra-community trade and importation of equine semen, ova and embryos into Great Britain (IIN A/11) DEFRA Website.

## Appendix I

### **KFPS Recommendations / Protocol for Handling Frozen Semen for Veterinary Surgeons / DEFRA Licensed AI technicians © KFPS 2009**

**It is Important to only import frozen semen from and EU licensed stallion station, as it is only EU stations which can send out frozen semen. In order to safeguard the quality of this semen, the Dutch Authorities apply strict supervision.**

Every EU station will send a certificate with a shipment of frozen semen, on this certificate is listed:

- The quality of the semen
- The number of doses to be used for one insemination
- A detailed description of how to handle the semen.

Thawing the frozen semen will need to take place according to a strict protocol. A general protocol is given below, but on the certificate, the specific protocol for the frozen semen purchased will be printed. By strictly adhering to the directions on the certificate then you will have the most chance of a pregnancy with the frozen semen used.

### **THAWING PROTOCOL FOR STALLION SEMEN FROZEN IN 0.5ML STRAWS.**

#### **Materials:**

Thermos flask, or other vessel with a wide neck filled with water at 37 -38 C. Or a water bath with a measuring cylinder filled with water (all at 37 - 38C).

Thermometer

Stop-watch

Scissors

Long Forceps

Protective clothing (gloves, visor etc.)

A Liquid Nitrogen container within which insemination doses are packaged individually within plastic tubes, that are themselves grouped into goblets and held in canisters.

- 1) for large numbers of straws (>8): 15ml tubes, 20ml syringes and conventional AI pipettes
- 2) for small numbers of straws: AI pipettes with stylets that remove the straw from the pipette after emptying.

#### **THAWING PROCEEDURE**

- 1) Bring the liquid nitrogen canister as close as possible to the water bath or the thermos flask.
- 2) If possible check the semen ID.
- 3) Take one straw out of the plastic tube and transfer it very quickly into the cylinder or thermos flask (with thermometer), upright with the sealed end upwards.
- 4) Check ID if this was not previously possible.
- 5) Transfer the straws one by one into the cylinder or thermos flask (not all at the same time!)
- 6) Start the stopwatch after the last straw.

7) Take the straws out after 30-40 seconds

8) Dry them well

9) cut off the sealed end and:

- for large numbers of straws; hold the straws upside down in a pre-warmed (37C), 15ml tube, cut off the other end of the straw – the straw will empty itself. Empty all of the straws in the same way. Before insemination, examine a drop of the semen using a microscope with a heated stage. Assuming adequate semen quality, use immediately.
- For small numbers of straws: examine a drop of semen from one straw for quality (in case of doubts of quality, examine drops from other straws). Keep the straws warm / protected – introduce into mare via a suitable AI pipette one by one, as soon as possible after thawing.

WARNING: DO NOT HOLD A STRAW OR EMPTY (NO NITROGEN) CANNISTER FOR MORE THAN 8 SECONDS ABOVE THE LIQUID NITROGEN.

***NB Check the certificate for the quality of the semen at the time of freezing, the number of doses to be used for one insemination, and a detailed description of how to handle this specific semen.***

**APPENDIX II: Regulations for the importation of equine semen from fellow EU states.**

For more detail visit: <http://www.defra.gov.uk/animalh/int-trde/iins/genetic/genetic-a11.htm>

The importation of equine semen, ova or embryos from other EU member states must comply with the provisions of The Animal and Animal Products (Import and Export) Regulations 2006.

#### **Dealer requirements:**

In order to import semen, you must first ensure that the stud which the semen is coming from is EU licensed. However, they are also required by the RVV (dutch equivalent of DEFRA Animal Health office) to be registered as an official trader before any trade begins. **If the semen is being delivered directly to you for your mares to be inseminated at home, then you must also register with your local animal health office as a trader / dealer. The stud from which the semen was sent are required to keep a record of all deliveries, consignments and their destinations, and you as a recipient should also keep a record of semen deliveries and their source.**

If your mare is being taken to your veterinary surgeons for AI or to a large stud with DEFRA AI technicians, then the vet or the stud if receiving the semen directly, will also need to register as a trader with their local animal health office.

**You will also need to check with the Customs Office about any HM Customs regulations in place.**

#### **Collection & Health Certificates**

Collection centres from which semen is collected from, must be approved and must be supervised and checked throughout the year by competent authorities.

To comply with EU regulations, the Stud, must on the date the semen was dispatched as fresh or chilled semen / or until the 30 days mandatory storage period for frozen semen elapsed:

- be situated in the territory of an EU Member State that was free of African Horse Sickness
- fulfilled the conditions of Article 4 or council directive 90/426/EEC (relates to identification / passports/ movement and transport).
- contained only equidae which were free of clinical signs of Equine Viral Arteritis (EVA) and contagious equine metritis (CEM).
- All equidae have been admitted onto the centre under the provisions of article 4 and 5 of Directive 90/426/EEC (relates to Identification/ passports/ movement and transport).

#### The donor stallion

- must not have shown clinical signs of infectious or contagious disease on the day that the semen was collected.
- During at least 30 days prior to the collection must not have been used for natural service.
- During the last 30 days prior to collection of the semen have been kept on holdings where no equidae showed signs of Equine Viral Arteritis (EVA).
- During the last 60 days prior to collection of the semen have been kept on holdings where no equidae showed signs of Contagious Equine Metritis (CEM).
- Have not been in contact with equidae suffering from an infectious or contagious disease during the 15 days immediately preceding the collection of semen.

The stallion must have undergone the following health tests carried out in a laboratory recognised by the competent authorities (In the Netherlands the RVV).

- A "Coggins" test for Equine Infectious Anaemia (EIA) with negative result
- Either a serum neutralization test for Equine Viral Arteritis (EVA) with a negative result at a serum dilution of 1 in 4 or a virus isolation test for EVA carried out with a negative result for on an aliquot of the entire semen.
- A test for CEM carried out on two occasions with an interval of 7 days by isolation of *Taylorella equigenitalis* from the pre-ejaculatory fluid or a semen sample and from genital swabs taken at least from the penile sheath, urethra and from the urethral fossa with a negative result in each case.
- The stallion must have been subject to one of the following test programmes:
  - If donor stallion was continually resident on the collection centre for at least 30 days prior to the semen collection period and during the semen collection period, and during the collection period no equidae at the collection centre came into direct contact with equidae of a lower health status than the donor stallions then the afore mentioned tests must have been carried out at least 14 days after the commencement of the above residence period and at least at the beginning of the breeding season.
  - If the donor stallion was not continuously resident on the collection centre, or if other equidae on the collection centre came into direct contact with equidae of a

lower health status than the donor stallions then the afore mentioned tests must have been carried out within the 14 days period before the first semen collection and at least at the beginning of the breeding season.

In addition:

- The Coggins test for EIA must have been carried out on a sample of blood taken not more than 120 days before the semen was collected.
- The last test for EVA must have been carried out not more than 30 days before the semen was collected or the non-shedder state of a EVA seropositive stallion must be confirmed by a virus isolation test carried out not more than 1 year before the semen was collected.

- In the case of frozen semen, tests for CEM, EIA and EVA must have been carried out during the 30 days mandatory storage period of frozen semen and not less than 14 days after the collection of the semen.

### **Shipment of the consignment**

All consignments of semen must be accompanied by an original, valid health certificate, issued in the country of origin. There must be sufficient information within the health certificates to ensure that the original collection centre / holding can be traced if required.

A link to a specimen copy of the required certificate can be found on the DEFRA website.

<http://www.defra.gov.uk/animalh/int-trde/iins/genetic/genetic-a11.htm>

(Health certificates / section 9)

The competent authority in the EU member state from which the shipment of semen originates (in the Netherlands, the RVV) must inform the destination Member State (the DEFRA Animal health office) of the intended shipment on the day of certification. Its arrival date should be informed by entering the certification details into TRACES by the RVV (TRACES is an electronic communication system that member states use to inform each other about consignments of animals and animal products moving between member states to and from non-EU members). This allows shipments of animals and animal products to be traced in the case of a notifiable disease outbreak.

If the consignment of semen is intended for several destinations, it must be grouped accordingly and have a separate health certificate for each group with a separate destination. I.E. - **You cannot arrange to have a bulk order of semen shipped to you, and then take half of it to your friend down the road for his mare, unless the semen for his mare has its own health certificate with his address as the destination.**

**Veterinary checks & arrival of semen into the UK.**

Veterinary checks are not mandatory, but may be randomly carried out by your local animal health office. Veterinary checks will usually involve document identity or physical checks. Samples may be taken as required. Ensure you or the stud / technician/ vet carrying out the AI keeps a record of all semen imported and a copy of all health certificates.

**IF YOU IMPORT SEMEN FROM A NON - EU LICENSED STALLION STATION THEN YOU ARE RISKING AN OUTBREAK OF CEM, EVA or EIA (or even worse AHS!) AND YOU MAY BE PROSECUTED.**