

Routine protocol to accompany transport semen:

- Name and address of the collection center - EU-number
- Name and identification (registration no.) of the stallion
- Date and time of collection
- Identification of the insemination dose (name on the “straw”)
- Volume pr. insemination dose (max. 40 cc)
- Concentration of sperm (x 10⁶ pr. cc)
- Motility a. at dilution
- b. at insemination
- Total number of motile sperm pr. insemination dose (min. 600 x 10⁶ pr. cc)

Copy to be returned to the stallion center

Routine protocol to accompany frozen semen:

- Name and address of the collection center - EU-number
- Name and identification (registration no.) of the stallion
- Date and time of collection
- Identification of the straw, EU-rules
- Volume pr. straw
- Concentration of sperm (x 10⁶ pr cc)
- Total number of motile sperm pr. straw
- Total number of motile sperm pr. insemination dose
- Motility: - at preparation
 - after thawing
- Thawing protocol to follow frozen semen
- Location of frozen semen within the container

Copy to be returned to the stallion center

Rules for use of insemination within the auspices of the WBFSH

Proposals for maximum quality assurance of equine semen for A.I. purposes

From a biological and medical as well as economical point of view it is important that irrespective of methods of dosing, storing and preservation, only semen of high quality should be available. Semen quality requires maximum hygienic standards of the semen donors and during the period of semen collection, processing, storage and insemination. The highest fertility capacity of the semen must be guaranteed. These principles are outlined in the actual legislative of the European Community (see Table 1). It seems wise to establish an international CODE of STANDARDS for EQUINE A.I. PRACTICE that meets the criteria necessary for a certified or accredited horse artificial insemination center. Therefore the veterinary committee specialising in reproduction has made the following rules regarding AI centers and insemination standards for semen from the members of the WBFSH. The quality of the semen should meet this minimum standard for export between the WBFSH countries.

An accredited or certified A.I.-centre must warrant to fulfil such standards and sign accordingly. An accredited centre will get an official certificate (logo, official formulars etc.) and agree with controls performed by independent A.I. experts. National committees are responsible for the application of the code within participant member countries. A.I.-centers from each country may join the code common for their countries.

This control system is supposed to minimize hygienic risks and give the best guarantee of optimal semen quality for the benefit and protection of the clients and reassurance of A.I. centres.

Table 1 : Actual legislation of Equine A.I.

- EU level: - council directive 92/65 EU
13.07.1992, nr. L268/54-72
- Commission decision 95/176/EU
06.04.1995 nr. L117/23-29
Annex I-V

A. Insemination Centres:

A.I.-centres follow a given natural structure. They are physically and functionally divided into the three subunits of semen collection, semen processing (laboratory) and insemination.

1. Subunit: Semen collection 95/176 EU Annex I cap. Nr. I

Site: sufficient floor space (min. 6 x 6 m) and height (min. 4 m) allowing safe semen collection at the dummy and/or teaser mare. Floor covering must be slip-proof and wall covering water resistant, enabling easy cleaning and disinfecting of floors and walls.

Caution: teaser mare has to fulfil the criteria concerning approved health status of semen donor stallions.

Separate units for cleaning (wet) and storing (dry) collection utensils and instruments must be available.

Equipment: Dummy, stock for teaser mare, AVs, hot water device, hot foam cleaner or other approved system of disinfection, heater.

Materials: AV inner liners, disposable gloves (rubber, plastic), thermometer, sperm compatible gel

2. Subunit : Semen processing 95/176 EU Annex IV cap. III

Site: Adjacent but separate from the semen collection room and with sufficient floor space; floor and wall covering water resistant and easily cleaned and disinfected. A laboratory subunit for semen examination, extending, packaging and storage must be available.

Caution: ensure sufficient ventilation when working with liquid nitrogen. (Follow national safety requirements.)

Equipment: microscope with heatable slide carrier, waterbath, photometer, centrifuge (stirring bench), utensils for dosaging and packaging, refrigerator, hot sterilization incubator, (water deionisator and distilled water available).

Storing facilities for miscellaneous laboratory materials, e.g. slides, pipettes, ampulles, tubes, chemicals etc.

Adjustment of semen diluting and dosage with sufficient floor space.

Proved systems for identification of semen (mechanical printer is not required for fresh or chilled semen).

3. Subunit: Insemination: 95/176 EU Annex I, cap. I nr. II 1&2

Site: Physically strictly separated from subunits 1 and 2 !!
Floor surface slip-proof, surface of walls and floor easily cleaned and disinfected
At least one set of stocks for recipient mares

Equipment: (vaginoscope), utensils and instruments for A.I.
Heat sterilization

Seminettes (paper/cotton), disposable gloves (rubber or plastic), syringes, semen compatible gel.

Teasing facilities

(caution: teaser stallion must not come into physical contact with semen donor stallions with approved health status !!)

Cleaning and disinfection of sites and utensils

Preferably disposable utensils should be used. Alternatively rubber material must be autoclaved or boiled; glass utensils (beakers, pipettes etc.) must be heat sterilized.

A separate room is required for cleaning and disinfection (95/176 EU, Annex I cap. I nr. I) !!

It is a general obligation that all materials or utensils in contact with semen and/or genital mucosa of the donor stallion or recipient mare must be sterile or disposable and discarded after use.

B. Semen donor stallions

95/176 EG Annex II A

- Principles:
1. Only reproductively healthy stallions are to be licensed to act as a semen donors for A.I.
 2. Simoultaneous natural covering and collection for A.I. must not take place.
 3. An annual preseasonal and random seasonal health check of donor stallions by a veterinary surgeon must take place.

Note: To fulfil the different legislations of A.I. (EU, national, federal) strict cooperation between official veterinary authorities and the local veterinarian under contract is absolutely necessary.

In most countries to which the code applies a special official license is needed.

The actual health state (general, genital, microbial) of the stallions and the condition of their housing must be under continuous control and surveillance from the local veterinarian contracted.

A stallion concomitently used for natural covering will lose his center health status. Before re-entering the center and be able to act as semen donor again the stallion has to repeat all the examinations necessary to achieve this status.

See schedule for semen sampling due to EU 62/65 Annex II A7

C. Veterinarian under contract

95/176 EU Annex I cap. I nr. I

The technical part of an A.I.-centre is to be subjected to the direction of an experienced veterinarian (employed or under contract). To meet with the assigned duty continuous presence is required in order to survey:

- health status of donor stallions
- hygienic requirements
- collection, quality control, processing and storage of semen
- detection of below average fertility results
- declaration of deficiencies to the head of the A.I.-centre

D. Seminal minimum standards

95/176 EU Annex IV cap. III

Semen collection should be performed continuously and regularly; no more than one collection per day is recommended since this collection frequency adjust the number of vital and fertile spermatozoa to the daily sperm production and the output forms a solid calculation base for planning, dosaging and portioning. The National Breeders Association will decide specific national requirements for the selection of stallions (for instance semen of 2×10^9 TNB).

Minimum standard requirements for semen for A.I.:

Fresh semen: Minimum 300 millions progressively motile spermcells

Chilled semen:

diluted/fresh

sperm dose : - minimum of 300 million progressively motile spermatazoa at time of portioning
- insemination within 12 hours of collection
- storage conditions maintained
- progressively motile spermatazoa at time of insemination no less than 35%

diluted/transported:

sperm dose: minimum of 600 million progressively motile spermatozoa at time of portioning
- maximum volume 40 cc (dilution 1:2)
- insemination 24-36 hours after collection
- storage conditions maintained
- progressively motile spermatozoa at time of insemination no less than 35%
All semen leaving the insemination center has to be treated as diluted/transport semen.

Frozen semen:

volume dose: dependent on manufacturing process

sperm dose: minimum of 35% progressively motile spermatozoa post-thawing

minimum of 250 millionss progressively motile spermatozoa per A.I. dose post-thawing
- A breeding dose for insemination of one mare has to contain a minimum of 3 insemination doses.

- free to sell a single insemination dose