Prevention of Disease Transmission by the Use of Semen in the Porcine AI Industry

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Abstract

For more than 40 years the artificial insemination (AI) in pigs has been used on piglet producing farms. It has become predominant over natural service only in the last 20 years. Boar ejaculates can be extended on average 20 times or more. One boar can produce more than 2000 doses of semen annually. AI studs with more than 300 boars are therefore required to fulfill all regulations to prevent the spreading of diseases. Regulations of the Office International des Epizooties (OIE), a directive of the European Union (EU), are enforced to establish a common standard in semen production all over Europe. In addition, certain disease prevention programmes exist, e.g. against the Porcine Reproductive and Respiratory Syndrome (PRRS) or Parvo virosis.

The methods of preventing the spread of diseases by AI centres are documented and valued. Both the animal hygiene and the personal hygiene of people working with the boars are required.

To a large extent the success of a prevention programme is due to the regional situation of the diseases. Only when semen producing organisations value disease prevention methods highly in their work can infection of boars and semen doses be prevented. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Insemination; pigs; transmission of diseases; prevention methods

1. Introduction

More than 40 years ago the artificial insemination in pigs reached a level of practicality but it has developed into the most important biotechnology in the porcine industry only in the last 20 years. As in other species, e.g. cattle, at least 5 advantages of using AI can be mentioned:

- Improvement of product quality by implementing breeding and testing programmes
- Better on-farm-organisation of the breeding process in comparison to natural service especially under group-housing and -weaning conditions
- Better hygiene of semen doses compared to natural service or self-collected semen
- Economical reasons, since AI boars have a better inheritance of the relevant parameter daily gain, percentage of valuable carcass and meat/fat-relation or food conversion
- Better fertility, since each ejaculate is tested before use.
Similar to the cattle industry the awareness of the AI-biotechnology arose in the pork area when disease problems spread in Europe and the USA. Classical swine fever, Aujeszky’s disease, Brucelosis or PRRS are some of the most important threats in this regard. Breeding institutions did not show much favour towards AI, since the breeding farmers lived very much in fear of losing markets. The breeding of boars by private farmers has lost market share in the last years much faster than in the cattle industry, and this is not necessarily due to AI as the most important reason.

Piglet producing farmers were the first to use AI for economical reasons and have been the most important clients for pig AI-organisations ever since. On specialized farms, with sometimes thousands of inseminations per year, special epidemical risks arise which can be controlled best by using AI.

At present, although facing a long time of low piglet or pork sales prices, the percentage of AI compared to natural service still rises, exceeding 50% in most of the countries world-wide and in many of them more than 70%.

The basis for using AI from these huge farms down to the pig-smallholder is the confidence of the farmer in buying a disease-free semen dose, which is free of transmissible viruses or bacteria that could cause economical harm to the herd. To ensure this, semen-producing organisations have to guarantee certain quality criteria, which are mainly but by far not all, related directly to the live animal as will be shown. Only organisations that follow the best possible way of production, controlled by neutral authorities if possible, can be considered as serious market partners for the farmers.

### 2. Risk analysis

Before the special methods of disease prevention or transmission are discussed, it is important to show the potential risk of contaminated semen in the boar.

The physiological potential of semen production in pigs is shown in Tables 1 and 2. These show the importance of keeping the semen strictly free of all relevant transmissible diseases. Average sized semen

<table>
<thead>
<tr>
<th>Table 1</th>
<th>The boar ejaculate</th>
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<tr>
<td>Volume:</td>
<td>250 ml</td>
</tr>
<tr>
<td>Density:</td>
<td>0.2 Mio./mmCHR.4.26;</td>
</tr>
<tr>
<td>Colour:</td>
<td>white or grey</td>
</tr>
<tr>
<td>pH-value:</td>
<td>6.8-7.4</td>
</tr>
<tr>
<td>Motility:</td>
<td>70% or more</td>
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<td>Anomalies:</td>
<td>20% plus 25% plasmic droplets</td>
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<th>Table 2</th>
<th>Calculation of semen doses per boar and year</th>
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<tr>
<td>One boar has a potential of producing 50 billion sperm per ejaculate.</td>
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<tr>
<td>From one ejaculate about 20 doses of semen can be produced.</td>
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<tr>
<td>Two ejaculates can be produced per week.</td>
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<tr>
<td>20 doses × 2 ejaculates/week</td>
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<tr>
<td>× 52 weeks = 2080 doses/year.</td>
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<tr>
<td>With 2080 doses of semen about 1000 sows can be inseminated.</td>
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production units collect about 100 ejaculates or more per day five times a week. In case of infection this will cause an immediate spreading of the disease into several hundred herds.

### 3. Diseases (the animal factor)

There are a lot of possible risks in animal production. By far, not all of them must be considered to be dangererous for pigs. In addition, only a small number of pig diseases can be spread by semen.

To clearly understand the pig situation, one should follow the systemization of the International Animal Health code of the OIE, which gives the fundamentals for all animal diseases. The most relevant parts under our aspects are as follows (see also Annex A: OIE Animal Health Code, 1998 edition):

In parts 2 and 3 of the Code the relevant pig or multispecies diseases worldwide are identified. In part 4 health control and hygiene principles are outlined out especially for pigs in appendix 4.2.2.1. Here we have a very good definition of what we are aiming at in our disease transmission efforts in AI organisations:
The purpose of official sanitary control of semen production is to maintain the health of animals on an AI centre at a standard which permits the international distribution of semen free of specific pathogenic organisms which can be carried in semen and cause infection in recipient female swine.

Artificial insemination centres should be officially approved and under the direct supervision and sanitary control of an official veterinarian. The Veterinary administration is also responsible for routine checks of health and welfare at least every 6 months. Only swine associated with semen production should be permitted to enter and should be adequately isolated from farm livestock on adjacent land or buildings. Entry of visitors must be strictly controlled. Personnel should be technically competent and observe high standards of personal hygiene to preclude the introduction of pathogenic organisms. Protective clothing and footwear for use only on the centre should be provided.

The entering boars must fulfill the specific requirements of the Veterinary administration. No boar with genetic defects can be used for export. Pre-entry tests within 30 days of entry into isolation are required; the isolation period being as long as 30 days before re-testing. The standards of testing are given in the OIE-code. After successful completion and in clinical health, boars can enter the programme.

Testing programme: The tests cover a minimal range of diseases of which the routine tests have to be applied at regular intervals to confirm the continued freedom from disease of the stud. Claims of country freedom from some viral and bacterial infections of swine may be given consideration provided that serological survey data and epidemiological investigation back such claims. AI centres may be required by the Veterinary administration to include other diseases in their prophylactic programme, either through vaccination or by requiring negative results to serological tests.

Importing countries may require assurance of freedom from other diseases based on negative serology or other tests. Bilateral agreements are to be applied in this case. Where a disease is covered by a Chapter of the OIE-code, the testing requirements of the respective disease of this Chapter should be followed.

4. Prevention of disease transmission in the European Union

In a certain way, these regulations and recommendations of the OIE can be applied to any region of the world. But, as they are covering all possible risks, to make it more practicable for regions with specific epidemiological problems, directives can be laid out. For example, it is practiced in the EU-region since the introduction of the so-called COUNCIL DIRECTIVE of 26 June 1990 laying down the animal health requirements applicable to intra-Community trade and imports of semen of domestic animals of the porcine species (90/429/EEC).

Following an EU-directive on bovine semen and one on bovine embryos, the pig semen directive brought clarity and a high degree of comparability into a large number of the pig-semen producing centres all over the EU-member countries (and to a number of centres in neighbouring or trade-keeping countries).

At first, only trading organisations were meant to qualify as EU-recognized sites. But it turned out that the EU-recognition is such a quality label, that nowadays more or less all seriously producing organisations aim at gaining this qualification. As this EU-regulation is the standard for the biggest piglet-producing area in the world, the regulations will be discussed in more detail as follows. For a better understanding the whole EU-directive is added as an Annex.

For the EU the reason to introduce such a directive was the existence of a certain risk of the spread of transmissible diseases in the trade of semen. In addition, the Community policy of harmonizing national animal health provisions governing intra-Community trade in animals and animal products required the creation of such a system for the porcine semen trade.

In principle, the Member States where the semen is collected have the obligation to ensure that such semen has been collected and processed at approved and supervised collection centres. Semen must be obtained from animals whose health status is such as to ensure that the risk of spread of animal disease is eliminated. In addition, semen must be collected, processed, stored and transported in accordance with
rules that preserve its health status and must, finally, be accompanied by an animal health certificate.

One problem is the difference in the policies pursued within the Community with regard to vaccination against certain diseases. This justifies maintenance of derogations for a limited time, authorizing the requirement by the Member State of additional protection against those diseases.

For importing semen from third countries a list of these countries was drawn up taking into account animal health criteria. But this is not enough; import is authorized only from semen collection centres of such countries which reach certain standards and which are officially supervised. So-called on-the-spot checks are assured in these countries.

In order to prevent the transmission of certain contagious diseases import controls can be carried out when a consignment of semen arrives in the EU. Also, a Member State is permitted to take emergency measures in the event of an outbreak of a contagious disease in another Member State or in a third country. The dangers associated with such diseases and the protective measures that necessitate have to be assessed in the same way throughout the EU. Exceeding this directive, an emergency Community procedure under which the necessary measures are taken was instituted within the Standing Veterinary Committee (SVC). The SVC is the place of cooperation between the Commission and the respective Member States.

Clarity is one of the most important prerequisites in understanding the precautions of the directive (Table 3).

The principles laid down by the Commission are in almost every detail enforced in the Articles and Annexes of the EU-directive. On the way through the directive the important points in connection with disease transmission are marked and mentioned (see Annex B: EU-directive 90/429/EEC at the end of this article).

Finally, one point must never be forgotten. Several times in the last years it turned out that efforts to amend EU-directives were very difficult. Prerequisites for this procedure are clarity in decision finding, consultancy of scientists of international reputation, the balancing of economical and scientific positions or interests and the existence of new methods of disease prevention. As far as possible these principles have been followed by the EU-Commission.

### 5. National regulations

In addition to these mentioned regulations in many countries disease prevention programmes were introduced to improve the sanitary conditions in the respective country.

Many countries e.g. try to be recognized as Aujeszky disease-free and work on a non-vaccination programme. Larger countries, like Germany, start with a regionalization programme to be followed in the next step hopefully by total eradication all over the country. For an AI-organisation situated in a vaccinating region but working in a larger area this can cause big problems in the transition period as some regions may ask for semen from non-vaccinated boars (as proposed by the new EU-directive Annex enforced from October 1999).

Another example of an effort to get rid of a disease is the national anti-PRRS campaign in Denmark, which had to be postponed for several reasons shortly afterwards.

In this respect it should not be forgotten that

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<thead>
<tr>
<th>Table 3</th>
<th>EU-directive 90/429/EEC</th>
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<tbody>
<tr>
<td>Chapter I</td>
<td>General provisions</td>
</tr>
<tr>
<td>Chapter II</td>
<td>Intra-community trade</td>
</tr>
<tr>
<td>Chapter III</td>
<td>Import from 3rd countries</td>
</tr>
<tr>
<td></td>
<td>- list of countries</td>
</tr>
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<td>- list of semen collection centres</td>
</tr>
<tr>
<td>Chapter IV</td>
<td>Precautionary and control measures</td>
</tr>
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<td>Chapter V</td>
<td>Final provisions</td>
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<td>- advance in technology</td>
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<tr>
<td>Annex A</td>
<td>Approval of semen collection centres</td>
</tr>
<tr>
<td>Chapter I</td>
<td>Admission of animals to approved centres</td>
</tr>
<tr>
<td>Chapter II</td>
<td>Compulsory routine tests on approved centres</td>
</tr>
<tr>
<td>Annex C</td>
<td>Conditions which semen must satisfy for intra-community trade</td>
</tr>
<tr>
<td>Annex D</td>
<td>Semen shipment certificate</td>
</tr>
</tbody>
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fighting a certain disease is always dependent not to the least extent on the economical possibilities of the country or the animal insurances, which are meant to pay an eradication campaign.

6. Other relevant problems

6.1. Porcine reproductive and respiratory syndrome (PRRS)

Although not mentioned earlier in the contagious diseases catalogue, PRRS has caused possibly the biggest harm to the piglet producer at least in many parts of Europe, if not elsewhere, in the last decade.

PRRS was first observed in the United States in 1986 and in Europe in 1990. The syndrome was initially called Mystery Pig Disease since no known pathogen could be implicated.

The etiology was confirmed in the Central Veterinary Institute in the Netherlands in 1991. Many strains of the PRRS-virus have been found since then. Diagnosis can be difficult in many cases. The knowledge about transmission with semen is limited, although some strains of PRRS-virus persisted in semen up to 92 days post-infection. Fresh semen from such an infected boar caused infection; extended semen did not.

In areas with a prevalence of infection estimated at 60–80% (and sometimes more), it is difficult to find the right way of handling the PRRS problem for an AI organisation because it is not possible to stay totally free of seropositive boars unless the loss of breeding material is accepted. On the other hand, to ensure the prevention of the disease, the length of quarantine has to be extended up to 3 months for such boars.

If there is any chance of infection, only seronegative boars from seronegative herds are acceptable to be introduced to a centre.

6.2. Others

Almost every country has diseases that are included in the quarantine screening process to obtain knowledge about the situation and, of course, to protect the high-value AI-boars in the producing sites, although an infection might not cause a transmission with semen.

An AI-organisation has to deliver semen every day. Many farmers totally rely on this semen supply. Precautions have to be taken to ensure semen production. An outbreak of any disease can interrupt this chain. Therefore, non-transmissible diseases have to possibly be eradicated or at least controlled by vaccination programmes.

In addition, precautions should be made to ensure a stand-by-system in case of outbreak of a disease in an AI-centre (worst case) or in an area where an AI-centre is situated that leads to an interruption of production. Neighbouring AI-units can help in such a case for a short while. Good examples for this are the help of neighbouring countries for the AI-industry in the Netherlands on the occasion of the last big swine-fever outbreak or a stand-by-contract system in Germany where almost all AI-organisations participate and which had to be used several times since its introduction.

7. Special emphasis on the human factor

In a discussion about transmission of diseases with semen one should never forget that the human being is in many cases the decisive factor in both prevention and spreading of the above mentioned diseases. Continuous information and education of farmers and personell on-farm or in AI-studs is required to ensure the acceptance and continuous application of regulations which are not always convenient for the said persons. The understanding that the job is heavily dependent on reliable labour, intensive sanitary control not only on the spot but also at home, and personal hygiene like requiring showering every time before entering the barn is required has to be kept alive every day.

The best disease control programme either internationally or in an area of one country or even in one AI-organisation will not work unless the people understand it perfectly well and are in favour of cooperation.

Part 2
List A Diseases
Foot and mouth disease, Vesicular stomatitis, Swine vesicular disease, African swine fever, Classical swine fever

Part 3
List B Diseases
Aujeszky’s disease, Leptospirosis, Atrophic rhinitis of swine, Porcine brucellosis, Trichinellosis, Enterovirus encephalomyelitis, Transmissible gastroenteritis, Salmonella enteritidis and Salmonella typhimurium

Part 4
Section 4.2. Health control and Hygiene-General
Appendix 4.2.2.1. Porcine semen

A. Aims of control
The purpose of official sanitary control of semen production is to maintain the health of animals on an artificial insemination (AI) centre at a standard which permits the international distribution of semen free of specific pathogenic organisms which can be carried in semen and cause infection in recipient female swine. The disease position in one country generally differs from that in another, thus prophylactic programmes vary widely in the range of organisms for which donor boars are tested before admission to an AI centre, while in isolation, and periodically after full admission into the stud.

B. General conditions
Official sanitary control has to be practised on the lines of the following requirements as a prerequisite of an AI centre being eligible for the export of semen and the designation of ‘accredited’.

Artificial insemination centre
1. The centre should be officially approved by the Veterinary Administration.
   a. The centre should be under the direct supervision and sanitary control of an Official Veterinarian.
   b. The centre should be under the overall supervision of the Veterinary Administration, which is responsible for routine visits to check the health and welfare of animals, and the procedures and prescribed records at the centre at least every 6 months.
   c. Only swine associated with semen production should be permitted to enter the centre. Other species of livestock may exceptionally be resident on the centre provided they are kept physically apart from the swine.
   d. Swine on the centre should be adequately isolated from farm livestock on adjacent land or buildings for instance by natural or artificial means.
   e. The entry of visitors should be strictly controlled and personnel at a centre should be technically competent and observe high standards of personal hygiene to preclude the introduction of pathogenic organisms. Protective clothing and footwear for use only on the centre should be provided.
   f. Individual semen containers and storage rooms should be capable of being disinfected.

2. Boars
   a. Boars should only enter an AI centre if they fulfil the requirements laid down by the Veterinary Administration.
   b. The semen from boars with genetic defects or associated with genetic defects in near relatives may not be eligible for export.
   c. Boars must be clinically healthy and physiologically normal and must pass pre-entry tests within the 30 days prior to entry into isolation at an AI centre. The prescribed diseases and tests are listed in paragraph B.3. Boars may only enter the stud on the successful completion of these tests and must be clinically healthy.

3. Testing programme for boars on AI centres
   a. Definitions: Prescribed tests cover a minimal range of diseases from which all boars on an AI centre must be free. Routine tests are tests applied at regular intervals to confirm the continued freedom from disease of the stud.
b. Prescribed tests:
   i. Bovine tuberculosis: Boars to give negative results to intradermal tuberculin tests with mammalian tuberculin in accordance with the OIE-Manual.
   ii. Brucellosis (B. abortus, B. suis). Boars to give negative results in accordance with the Manual.

   c. Routine tests
   i. Swine vesicular disease. Boars to give negative results to a serum-neutralisation test in accordance with the OIE-Manual. Routine tests to be applied at least every 12 months.
   ii. African swine fever. Boars to give negative results to enzyme-linked immunosorbent assay and indirect immunofluorescent tests in accordance with the OIE-Manual. Routine tests to be applied at least every 6 months.
   iii. Enterovirus encephalomyelitis (ex Teschen disease). Boars to meet certification standards of the OIE-Code. Routine tests to be applied at least every 12 months.
   iv. Vesicular stomatitis. Boars to give negative results to a complement fixation test in accordance with the OIE-Manual. Routine tests to be applied at least every 12 months. Claims of country freedom from some viral and bacterial infections of swine may be given consideration providing such claims are backed by serological survey data and epidemiological investigation.

C. Optional tests and requirements

   AI centres may be required by the Veterinary Administration to include in their veterinary prophylactic programmes a number of other diseases, either through vaccination or by requiring negative results to serological tests.

   Additionally, some importing countries may require assurances of freedom from a disease (for example: classical swine fever, Aujeszky’s disease) based on negative serology or other biological tests. The range of infections to be covered is extensive and beyond the capacity of AI centres to support totally. Thus, optional tests remain to be applied and interpreted by bilateral agreement when the importation of semen is being considered.

   Where a disease is covered by a Chapter in this Code, the testing requirements of the Chapter should be followed.

   D. Diluents

   Whenever milk, egg yolk or any other animal protein is used in preparing the semen diluent, the product must be free of pathogens or sterilised; milk heat-treated at 92°C for 3–5 minutes, eggs from SPF flocks when available. The inclusion of penicillin, streptomycin, polymixin etc. is permitted provided this is declared in the international animal health certificate.

   E. Semen

   Deep frozen semen for export should be stored separately in fresh liquid nitrogen in sterilised flasks for at least 28 days. The examination of ejaculates and the dilution and freezing of semen must be carried out in a laboratory maintaining the hygienic standards set by the Veterinary Administration. The pre-sperm fraction should not be included in material to be stored. Only semen of a health standard equivalent to that produced in an AI centre should be handled. The semen straws or pellets shall be code marked in line with national standards. The containers must be sealed before export and accompanied by an international animal health certificate listing the contents.

F. Donor boar

   Records of the progeny of a donor boar should be maintained as far as possible to determine that he is not associated with any genetic defect. The records of the boar should indicate his fertility. The semen must be obtained from a boar with a normal libido.

   Model Certificate No. 3

   Animal health certificate for semen of porcine species

   (Information concerning the donor animal. Information concerning the semen, Origin of the semen, Destination of the semen, sanitary information)
Annex B: EU-Directive 90/429/EEC from 1990:

CHAPTER I General provisions

Article 1
This Directive lays down the animal health conditions applicable to intra-community trade in and imports from third countries of semen of domestic animals of the porcine species.

Article 2
For the purposes of this Directive, the definitions contained in Article 2 of Directives 64/432/EEC, 72/462/EEC, 80/407/EEC (9) and 90/425/EEC (10) shall apply as necessary.

Moreover, ‘semen’ means the ejaculate of a domestic animal of the porcine species, in the unaltered state or prepared or diluted.

CHAPTER II Intra-Community trade

Article 3
Each Member State shall ensure that only semen, meeting the following general conditions, is intended for trade:

a. it must have been collected and processed, for the purpose of artificial insemination, in a collection centre approved from the point of view of animal health for the purposes of intra-Community trade in accordance with Article 5 (1);

b. it must have been collected from domestic animals of the porcine species whose health status complies with Annex B;

c. it must have been collected, processed, stored and transported in accordance with Annexes A and C.

Article 4

1. Until 31 December 1992, Member States in which all collection centres contain only animals which have not been vaccinated against Aujeszky’s disease giving a negative reaction to the serum neutralization test, or to the ELISA test for Aujeszky’s disease, in accordance with the provisions of this Directive:

a. may refuse admission to their territory semen from collection centres which do not have that status,

b. may not prohibit the admission of semen from boars which have been vaccinated in the collection centre with the GI deleted vaccine, provided that:

c. such vaccination has only been carried out on boars that were serum-negative with regard to the virus of Aujeszky’s disease,

d. serological examinations carried out at the earliest three weeks after vaccination of such boars do not reveal the presence of antibodies induced by the disease virus.

e. In this event a sample of semen from each daily collection intended for trade may be subjected to a virus isolation test in an approved laboratory in the Member State of destination. The provisions of this paragraph shall not come into effect until such time as the Commission, acting in accordance with Article 18, not later than 1 July 1991, has laid down the protocols for the tests to be used for these examinations following the opinion of the Scientific Veterinary Committee, in particular in connection with the frequency of the tests to be carried out in the centre, the virus isolation tests and the effectiveness and safety of the GI deleted vaccine.

2. In accordance with the procedure referred to in Article 18, it may be decided to extend the provisions of paragraph 1 to part of the territory of a Member State if all the collection centres in that part of the territory contain only animals giving a negative reaction to the serum neutralization test or the ELISA test for Aujeszky’s disease.

3. The Council shall, before 31 December 1992, review this Article on the basis of a report from the Commission, accompanied by any proposals.

Article 5

1. The Member State on whose territory the semen collection centre is situated shall ensure that the approval provided for in Article 3 (a) is granted only if it meets the conditions of Annex A and satisfies the other provisions of this Directive. The
Member State shall also ensure that the official veterinarian supervises the observance of those provisions. The official veterinarian shall propose that approval be withdrawn when one or more of the provisions is no longer observed.

2. All approved semen collection centres shall be registered, each centre being given a veterinary registration number. Each Member State shall send a list of semen collection centres and their veterinary registration numbers to the other Member States and to the Commission and shall notify them of any withdrawal of approval.

3. The general rules for applying this Article shall be adopted in accordance with the procedure laid down in Article 18.

Article 6

1. Member States shall ensure that each consignment of semen is accompanied by an animal health certificate drawn up in accordance with the specimen in Annex D by an official veterinarian of the Member State of collection. This certificate must: (a) be drawn up in at least one of the official languages of the Member State of collection and one of those of the Member State of destination; (b) accompany the consignment to its destination in its original form; (c) be drawn up on a single sheet of paper; (d) be made out to a single consignee.

2. The Member State of destination may, in addition to measures provided for in Article 8 of Directive 90/425/EEC, take the necessary measures, including storage in quarantine, provided this does not affect the viability of the semen, in order to obtain definite proof in cases where semen is suspected of being infected or contaminated by pathogenic organisms.

CHAPTER III: Imports from third countries

Article 7

1. A Member State may authorize importation of semen only from those third countries which appear on a list drawn up in accordance with the procedure laid down in Article 19. That list may be supplemented or amended in accordance with the procedure laid down in Article 18.

2. In deciding whether a third country may appear on the list referred to in paragraph 1, particular account shall be taken of:
   a. the state of health of the livestock, other domestic animals and wildlife in that country, with particular reference to exotic animal diseases, and of the environmental health situation in that country, which might endanger animal health in the Member States;
   b. the regularity and rapidity of the information supplied by that country concerning the existence of contagious animal diseases in its territory transmissible by semen, in particular those diseases mentioned in lists A and B of the International Office of Epizootic Diseases;
   c. that country’s rules on animal disease prevention and control;
   d. the structure of the veterinary services in that country and their powers;
   e. the organization and implementation of measures to prevent and control contagious animal diseases; and the guarantees which that country can give with regard to compliance with this Directive.

3. The list referred to in paragraph 1 and all amendments thereto shall be published in the Official Journal of the European Communities.

Article 8

1. Under the procedure laid down in Article 19, a list shall be drawn up of semen collection centres from which Member States may authorize the importation of semen originating in third countries. The list may be amended or supplemented according to the same procedure.

2. In deciding whether a semen collection centre in a third country may appear on the list referred to in paragraph 1, particular account shall be taken of the veterinary services and the supervision to which semen collection centres are subject.

3. A semen collection centre may appear on the list provided for in paragraph 1 only if: (a) it is situated in one of the countries on the list referred to in Article 7 (1); (b) it fulfils the requirements of Chapters I and II of Annex A; (c) it has been officially approved for exports to the Community by the veterinary services of the third country.
concerned; (d) it is placed under the supervision of a centre veterinarian of the third country concerned; and (e) it is subject to inspection by an official veterinarian of the third country concerned at least trace a year.

Article 9
1. Semen must come from animals which, immediately prior to collection of their semen, have remained for at least three months in the territory of a third country on the list referred to in Article 7 (1).
2. Without prejudice to Article 7 (1) and paragraph 1 of this Article, Member States shall not authorize the importation of semen from a third country on the list unless the semen complies with the animal health requirements adopted under the procedure laid down in Article 18, for imports of semen from that country. In adopting the requirements referred to in the first subparagraph, consideration shall be given to:
   a. the health situation in the area surrounding the semen collection centre, with particular reference to the diseases appearing on list A of the International Office of Epizootic Diseases;
   b. the state of health of the herd in the semen collection centre and testing requirements;
   c. the state of health of the donor animal and testing requirements;
   d. testing requirements in relation to semen.
3. The reference basis for fixing animal health conditions shall be the standards laid down in Chapter II and the corresponding Annexes. It may be decided, in accordance with the procedure laid down in Article 18, on a case-by-case basis, to waive these conditions where the third country concerned provides similar animal health guarantees, that are at least equivalent. Article 4 shall apply.

Article 10
1. Member States shall authorize the importation of semen only on submission of an animal health certificate drawn up and signed by an official veterinarian of the third country of collection. This certificate must:
   a. be drawn up in at least one of the official languages of the Member State of destination and one of those of the Member State where the import control provided for in Article 11 is carried out;
   b. accompany the semen to its destination in its original form;
   c. be drawn up on a single sheet of paper;
   d. be made out to a single consignee.
2. The animal health certificate must correspond to a specimen drawn up under the procedure laid down in Article 19.

Article 11
1. Member States shall ensure that each consignment of semen entering the customs territory of the Community is subject to control before being released for free circulation or placed under a customs procedure and shall prohibit the introduction of the semen into the Community if the import control made on arrival reveals that:
   • the semen does not come from the territory of a third country on the list drawn up in accordance with Article 7 (1),
   • the semen does not come from a semen collection centre on the list provided for in Article 8 (1),
   • the semen comes from the territory of a third country from which imports are prohibited in accordance with Article 15 (2),
   • the animal health certificate which accompanies the semen is not in conformity with the conditions laid down in Article 10 and fixed pursuant thereto. This paragraph shall not apply to consignments of semen which arrive in the customs territory of the Community and are placed under a customs transit procedure for consignment to a destination situated outside the said territory. However, it shall be applicable where customs transit is waived during transport through the territory of the Community.
   • The Member State of destination may take the necessary measures, including storage in quarantine provided that this does not affect the viability of the semen, in order to obtain definite proof in cases where semen is suspected of being infected or contaminated by pathogenic organisms.
   • If the admission of semen has been prohibited
on any of the grounds set out in paragraphs 1 and 2 and the exporting third country does not authorize the return of the semen within 30 days in the case of deep-frozen semen, or immediately in the case of fresh semen, the competent veterinary authority of the Member State of destination may order it to be destroyed.

Article 12
Each consignment of semen authorized for admission into the Community by a Member State on the basis of the control referred to in Article 11 (1) must, when sent to the territory of another Member State, be accompanied by the original certificate or an authenticated copy thereof, suitably endorsed, in either case, by the competent authority which was responsible for the control carried out in accordance with Article 11.

Article 13
If it is decided to take destruction measures pursuant to Article 11 (3), any costs incurred shall be chargeable to the consignor, the consignee or their agent, without compensation by the State.

CHAPTER IV: Precautionary and control measures

Article 14
The rules set out in Directive 90/425/EEC shall apply in particular with regard to checks at origin, the organization and the monitoring of the checks to be carried out by the Member State of destination.

Article 15
1. The precautionary measures provided for in Article 10 of Directive 90/425/EEC shall apply to intra-Community trade.
2. Without prejudice to Articles 8, 9 and 10, if in a third country a contagious animal disease which can be carried by semen and is liable to endanger the health of the livestock in a Member State breaks out or spreads or if any other reason connected with animal health so justifies, the Member State of destination concerned shall prohibit the import of that semen, whether imported directly or indirectly through another Member State, either from the whole of the third country or from part of its territory. Measures taken by the Member States on the basis of the first subparagraph and the repeal of such measures must be communicated immediately to the other Member States and the Commission together with the reasons for such measures. Under the procedure laid down in Article 18, it may be decided that those measures must be amended, in particular in order to coordinate them with measures adopted by the other Member States, or that they must be repealed. If the situation envisaged in the first subparagraph arises and if it is necessary that other Member States also apply the measures taken under that subparagraph, amended where necessary in accordance with the third subparagraph, appropriate steps shall be taken under the procedure laid down in Article 18. Resumption of imports from the third country concerned shall be authorized under the procedure laid down in Article 18.

Article 16
1. Veterinary experts from the Commission may, in cooperation with the competent authorities of the Member States and third countries, make on-the-spot checks in so far as that is indispensable for ensuring uniform application of this Directive. The country of collection within whose territory a check is being carried out shall give all necessary assistance to the experts in carrying out their duties. The Commission shall inform the country of collection concerned of the results of the checks.

The country of collection concerned shall take any measures which may prove necessary to take account of the results of this check. If the country of collection does not take those measures, the Commission may, after the situation has been examined by the Standing Veterinary Committee, have recourse to the provisions of the third subparagraph of Article 6 (2) and of Article 5.
2. The general provisions for implementing this Article, especially as regards the frequency and method of carrying out the checks referred to in the first subparagraph of paragraph 1, shall be laid down under the procedure set out in Article 19.
Chapter v final provisions

Article 17

The Annexes to this Directive shall be amended in accordance with the procedure set out in Article 18 to adapt them to advances in technology.

Article 18

1. Where the procedure laid down in this Article is to be followed, matters shall without delay be referred by the chairman, either on his own initiative or at the request of a Member State, to the Standing Veterinary Committee (hereinafter referred to as 'the Committee') set up by Decision 68/361/EEC (11).

2. Within the Committee, the votes of the Member States shall be weighted as provided for in Article 148 (2) of the Treaty. The chairman shall not vote.

3. The representative of the Commission shall submit a draft of the measures to be adopted. The Committee shall deliver its opinion on the draft within a time limit which the chairman may lay down according to the urgency of the matter. Opinions shall be delivered by a majority of 54 votes.

4. The Commission shall adopt the measures and apply them immediately where they are in accordance with the Committee’s opinion. Where they are not in accordance with the Committee’s opinion, or in the absence of any opinion, the Commission shall forthwith submit to the Council a proposal relating to the measures to be taken. The Council shall adopt the measures by a qualified majority. If, on the expiry of 15 days from the date on which the matter was referred to it, the Council has not adopted any measures, the Commission shall adopt the proposed measures and apply them immediately, save where the Council has decided against the said measures by a simple majority.

Article 19

1. Where the procedure laid down in this Article is to be followed, matters shall without delay be referred to the Committee by the chairman, either on his own initiative or at the request of a Member State.

2. Within the Committee, the votes of the Member States shall be weighted as provided for in Article 148 (2) of the Treaty. The chairman shall not vote.

3. The representative of the Commission shall submit to the Committee a draft of the measures to be adopted. The Committee shall deliver its opinion on such measures within two days. The opinion shall be delivered by a majority of 54 votes.

4. The Commission shall adopt the measures and apply them immediately where they are in accordance with the Committee’s opinion. Where they are not in accordance with the Committee’s opinion, or in the absence of any opinion, the Commission shall forthwith submit to the Council a proposal relating to the measures to be taken. The Council shall adopt the measures by a qualified majority. If, on the expiry of 15 days from the date on which the matter was referred to it, the Council has not adopted any measures, the Commission shall adopt the proposed measures and apply them immediately, save where the Council has decided against the said measures by a simple majority.

Article 20

1. This Directive shall not be applicable to semen collected and processed in a Member State before 31 December 1991.

2. Until the date of entry into force of the decisions adopted pursuant to Article 8, 9 and 10, Member States shall not apply to imports of semen from third countries more favourable conditions than those resulting from application of Chapter II.

Article 21

Member States shall bring into force the laws, regulations and administrative provisions necessary to comply with this Directive by 31 December 1991 at the latest. They shall forthwith inform the Commission thereof.

Article 22

This Directive is addressed to the Member States.

Done at Luxembourg, 26 June 1990. For the Council
The President
M. O’KENNEDY
(In the following the annexes A–D as amended in 1999.)

Annex A

Chapter I

Conditions for the approval of semen collection centres

Semen collection centres must:
1. be placed under the permanent supervision of a centre veterinarian; have at least
   a. animal housing including facilities for the isolation of animals which have failed tests described in Annex B, Chapter II, or which show clinical signs of disease,
   b. semen collection facilities including a separate room for the cleaning and disinfection or sterilisation of equipment,
   c. a semen processing room, which need not necessarily be on the same site,
   d. a semen storage room which need not necessarily be on the same site;
   be so constructed or isolated that contact with livestock outside is prevented; be so constructed that the animal housing and semen collection, processing and storage facilities can be readily cleaned and disinfected; be so designed that the animal accommodation is physically separated from the semen processing room and both are separated from the semen storage room.

Chapter II

Conditions relating to the supervision of semen collection centres

The collection centres must:
1. be so supervised that they contain only animals of the species whose semen is to be collected;
2. be so supervised that a record, file or computer record is kept of all porcine animals at the centre, giving details of the breed, date of birth and identification of each of the animals, and also a record, file or computer record of all checks for diseases and all vaccinations carried out, giving also information from the disease/health file of each animal;
3. be regularly inspected by an official veterinarian, at least twice a year, at which time checks on the conditions of approval and supervision shall be carried out;
4. be so supervised that the entry of unauthorised persons is prevented. Furthermore, authorised visitors must be required to comply with the conditions laid down by the centre veterinarian;
5. employ technically competent staff suitably trained in disinfection procedures and hygiene techniques relevant to the control of the spread of disease;
6. be so supervised that:
   a. only semen collected at an approved centre is processed and stored in approved centres, without coming into contact with any other consignment of semen;
   b. collection, processing and storage of semen takes place only on the premises set aside for the purpose and under conditions of the strictest hygiene;
   c. all implements which come into contact with the semen or the donor animal during collection and processing are properly disinfected or sterilised prior to use;
   d. products of animal origin used in the processing of semen - including additives or a diluent - are obtained from sources which present no animal health risk or are so treated prior to use that such risk is prevented;
   e. storage flasks and transport flasks are properly disinfected or sterilised before the beginning of each filling operation;
   f. the cryogenic agent used has not been previously used for other products of animal origin;
   g. each collection of semen, whether or not it is separated into individual doses, is clearly marked in such a way that the date of collection of the semen and the breed and identification of the donor animal, as well as the name and the registration number of the centre, preceded by the name of the country of origin, where appropriate, in the form of a code, can be readily established; the characteristics and form of this marking will be established under the procedure laid down in Article 19.
Annex B

Chapter I

Conditions applying to the admission of animals to approved semen collection centres

1. All animals admitted to a semen collection centre must:
   a. have been subjected to a period of quarantine of at least 30 days in accommodation specifically approved for the purpose by the competent authority of the Member State, and where only animals having at least the same health status are present;
   b. prior to their entering the quarantine accommodation described in (a) have been chosen from herds or holdings;
      • which are free of brucellosis in accordance with the Article 3.5.2.1 of the International Animal Health Code,
      • in which no animal vaccinated against foot and mouth disease has been present in the preceding 12 months,
      • in which no clinical, serological or virological evidence of Aujeszky’s disease has been detected in the preceding 12 months,
      • which are not situated in a restricted area defined under the provisions of the Community legislation due to the emergence of a disease in domestic pigs. The animals may not previously have been kept in any herd of a lower status.
   c. Before the period of quarantine specified in a) and within the previous 30 days have been subjected to the following tests performed in accordance with standards laid down in relevant Directives, with negative results:
      • a complement fixation test or a buffered brucella antigen test in respect of brucellosis (from the 1st January 2001, the buffered brucella antigen test will be the only authorised test),
      • a serum neutralisation or an ELISA test using all the Aujeszky’s Disease viral antigens in the case of non-vaccinated pigs, or an ELISA test for Aujeszky’s Disease GI antigens in the case of pigs vaccinated with a GI deleted vaccine,
      • an ELISA test or a serum neutralisation test for the presence of classical swine fever.
   d. during the last 15 days of the period of quarantine of at least 30 days specified in (a), have been subjected to the following test with negative results:
      • in respect of brucellosis, a complement fixation test or a buffered brucella antigen test (from the 1 January 2001 the buffered brucella antigen test will be the only authorised test).
      • a serum neutralisation or an ELISA test using all the Aujeszky’s Disease viral antigens in the case of non-vaccinated pigs, or an ELISA test for Aujeszky’s Disease GI antigens in the case of pigs vaccinated with a GI deleted vaccine.

Without prejudice to the provisions applicable in cases where foot and mouth disease or other list A disease are diagnosed, if any of the above mentioned tests should prove positive, the animal must be removed forthwith from the quarantine accommodation. In the case of group quarantine, the competent authority must take all necessary measures to ensure that the remaining animals have a satisfactory health status before being admitted to the collection in accordance with this Annex.

However, with regard to brucellosis when animals are positive, the following protocol is implemented:
   i. the positive sera are subjected to a sero-agglutination test as well as the test mentioned at the first indent above which has not been carried out,
ii. an epidemiological survey is carried out on the holdings of origin of the reacting animals, iii. the positive animals, a second series of tests (buffered brucella antigen test, sero-agglutination, complement fixation) is carried out on samples collected more than seven days after the first collection.

The suspicion of brucellosis will be ruled out in the light of the results of the survey carried out on the holdings of origin and the comparison of the results of the two series of tests.

When the suspicion of brucellosis is ruled out, the animals negative to the first brucellosis test can be introduced into the center. Animals positive to one test may be accepted if they answer negatively to two series of tests (buffered brucella antigen test, sero-agglutination, complement fixation) carried out with an interval of at least seven days.

2. All tests must be carried out in a laboratory approved by the Member State.

3. Animals may only be admitted to the semen collection centre with the express permission of the centre veterinarian. All animal movements, both in and out, must be recorded.

4. No animal admitted to the semen collection centre may show any clinical sign of disease on the day of admission; all animals must, without prejudice to paragraph 5, have come directly from quarantine accommodation as referred to in paragraph 1.a) which, on the day of consignment, officially fulfils the following conditions:
   a. not situated in a restricted area defined under the provisions of the Community legislation due to the emergence of a disease in domestic pigs;
   b. no clinical, pathological or serological evidence of Aujeszky’s disease has been recorded for the past 12 months;

5. Provided that conditions laid down in paragraph 4 are satisfied and the routine tests referred to in Chapter II have been carried out during the previous 12 months, animals may be transferred from one approved semen collection centre to another of equal health status without quarantine or testing if transfer is direct. The animal in question must not come into direct or indirect contact with cloven-hoofed animals of a lower health status and the means of transport used must have been disinfected before use.

6. In case of trade between Member States, animals will be accompanied by an animal health certificate in conformity with the model 2 in annex F of Directive 64/432/EEC, the disinfection of the mean of transport being certified in section C point 4 as one of the following additional guarantee, corresponding to their status; animals come directly from a semen collection centre complying with the Directive 90/429/EEC animals come directly from a quarantine accommodation and comply with the semen collection centre admission conditions provided in annex B chapter 1 of Directive 90/429/EEC animals come directly from a holding where they were undergoing the pre-quarantine admission protocol and comply with the quarantine admission conditions provided in annex B chapter I points 1, 2, c) and 2 of Directive 90/429/EEC.

CHAPTER II

Compulsory routine tests for animals kept at an approved semen collection centre

1. All animals kept at an approved semen collection centre must be subjected to the following tests with negative results:
   a. a serum neutralisation or an ELISA test using all the Aujeszky’s Disease viral antigens in the case of non-vaccinated pigs, or an ELISA test for Aujeszky’s Disease GI antigens in the case of pigs vaccinated with a GI deleted vaccine,
   b. in respect of brucellosis, a complement fixation test or a buffered brucella antigen test (from the 1 January 2001 the buffered brucella antigen test will be the only authorised test),
   c. an ELISA test or a serum neutralisation test for the presence of antibodies of classical swine fever.

These tests shall be carried out either on all animals when leaving the centre, but not later than 12 months after admission where they have not left the centre before this time. The sampling may be carried out in the abattoir, or 25% of the animals in the centre are tested every three months.

In this case, the centre veterinarian shall ensure
that the samples taken are representative of the total population on the centre, in particular with respect to age group and boar accommodation. Furthermore, the centre veterinarian shall also ensure that all animals are tested at least once during their stay at the centre and at least every 12 months if their stay exceeds a year.

2. All tests must be carried out in a laboratory approved by the Member State.

3. If any of the above tests should prove positive, the animal must be isolated and the semen collected from it since the last negative test may not be the subject of intra-Community trade.

Semen collected from each animal at the centre since the date of that animal’s last negative test shall be held in separate storage and may not be the subject of intra-Community trade until the health status of the centre has been re-established.

Annex C

Conditions which semen collected at approved centres must satisfy for the purposes of intra-Community trade

1. Semen must be obtained from animals which:
   a. show no clinical signs of disease on the day the semen is collected;
   b. have not been vaccinated against foot and mouth disease;
   c. satisfy the requirements of Annex B, Chapter I;
   d. are not allowed to serve naturally;
   e. are kept in semen collection centres which must not be situated in a restricted area designated under the provisions of the Community legislation relating to contagious diseases in domestic pigs,
   f. have been kept in semen collection centres which, during the 30 day period immediately prior to collection, have been free from Aujeszky’s Disease.

2. An effective combination of antibiotics, in particular against leptospires and mycoplasmas, must be added to the semen after final dilution or to the diluent. In case of frozen semen, antibiotics must be added before the semen is frozen.

This combination must produce an effect at least equivalent to the following dilutions: not less than: 500 µg streptomycin per ml final dilution
500 IU penicillin per ml final dilution
150 µg lincomycin per ml final dilution
300 µg spectinomycin per ml final dilution.

Immediately after the addition of the antibiotics, the diluted semen must be kept at a temperature of at least 15°C for a period of not less than 45 minutes.

3. Semen for intra-Community trade must

   a. be stored as laid down in Chapters I and II of Annex A prior to dispatch;

   b. transported to the Member State of destination in flasks which have been cleaned and disinfected or sterilised before use and which have been sealed prior to dispatch from the approved storage facilities.

4. Member States may refuse admission of semen from collection centres where boars vaccinated against Aujeszky disease are admitted to their territory or to a region of their territory, when it has been recognised as free of Aujeszky’s disease in accordance with Article 10 of directive 64/432/EEC.

Annex D: Semen shipment certificate