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Regulations and Recommendations for International Shipment of Chilled and Frozen Canine Semen (22-May-2001)

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Introduction

Interest in the international shipment of dog semen is steadily increasing as is the number of storage facilities for frozen canine semen, although at a somewhat slower pace. Dog semen can be shipped as extended and chilled, or frozen. Which of these is more convenient will depend on a number of factors, such as shipping distance, and whether the shipped semen is intended for artificial insemination (AI) of one or of several bitches, but also the rules and regulations of the country in question. In several countries different rules apply to different countries regarding importation/exportation of semen. The rules for importation of chilled semen may be different from those which apply to frozen semen. Sometimes the regulations in the importing country preclude the possibility of using chilled semen. The national Kennel Clubs may request prior application for permission to use imported semen, and they usually request proper identification of the semen donor by microchip or ID-tattoo, or a DNA test.

It should be kept in mind that both national legislation and Kennel Club regulations may change at any time. Before undertaking to export dog semen, the owner of the bitch or importer should, therefore, always be advised to contact the Ministry of Agriculture (or the corresponding authority) and the Kennel Club in the importing country to receive information about the latest set of rules and regulations. These contacts should be made well in advance of the planned semen export, so that all the necessary health certificates and blood tests can be made in accordance with the requirements. A list of addresses can be found in Appendix 1.

Ministry of Agriculture Requirements

When it comes to the national legislations pertaining to the importation of dog semen, countries basically fall into one of the four categories listed below. These categories are also used in the characterizations of requirements for individual countries provided in Appendix 1.

Category 1

No regulations, which may mean either that anything is allowed or that nothing is allowed in the way of semen import. Thus, the prevailing policy obviously needs to be checked. In general, however, the lack of regulations means that the importation of dog semen is not subject to any restrictions.

Category 2

A request for an import permit is needed, with no further requirements.

Category 3

There is a requirement for an import permit and a veterinary health certificate for the semen donor issued at the time of semen collection, and/or at a stipulated time interval before, and/or after semen collection.

Category 4

There is a requirement for an import permit and a health certificate and for a serological test for leptospirosis (usually *L. canicola* and *L. icterhaemorrhagica*) and/or for *Brucella canis*. Note that the blood samples in some cases should be taken prior to (usually within 15 - 45 days before), in other cases at the time of, and sometimes after (usually 20 - 30 days, or 3 - 6 weeks after) semen collection.

Additional requirements

Some additional requirements can include the following (see also categories marked with a "+" in Appendix 1):

- the semen donor must not have been outside the exporting country for a period varying from 2 to 12 months prior to semen collection
- the donor has not been used for natural breeding from the time of the blood test until semen collection
- the semen donor has, or has not, been vaccinated with different vaccines for a varying time prior to semen collection
- the inseminated bitch must not be bred by another dog during the same cycle and in case it should abort the Ministry must be immediately notified and the fetuses examined

- the original import permit must accompany the shipment
- the liquid nitrogen must be new or unused
- the liquid nitrogen container must be new or unused
- eggs used for semen extenders must be from a flock of hens free from Newcastle disease

Kennel Club Requirements

Some Kennel Clubs have no regulations which, again, may mean that everything is allowed, or that registration of litters by imported semen is not officially allowed. The prevailing policy should therefore be checked well in advance.

However, the lack of regulations generally means that there are no restrictions, or that there are no controls made of whether the bitch has been abroad to be mated, or the semen has been imported and the bitch artificially inseminated.

Most national Kennel Clubs request that the semen donor should be registered by an officially recognized Kennel Club.

They also usually request that the semen donor is permanently identified by an ID-tattoo or a microchip, and that the person performing the semen collection testifies that the dog's identity has been checked.

Most Kennel Clubs request that the semen donor has a normal testicular status.

Some Kennel Clubs request prior application for permission to use imported semen.

The American Kennel Club requests that a DNA sample is submitted. It is taken with the aid of a special cheek-swab kit supplied by the AKC (see Appendix 1).

Some Kennel Clubs have an eradication program against various hereditary diseases in some breeds (for instance hip dysplasia, progressive retinal atrophy and PNP), and request that the semen donor be tested and free from any such diseases.

Other requirements may include that dogs show that they can mate normally or have proved that they are fertile.

General Recommendations for Minimum Documentation to Accompany International Shipments of Dog Semen

In order to comply with the majority of the various national rules and regulations, and as a courtesy to the colleague who will perform the AI, it is recommended that each international shipment of dog semen is accompanied by the following documents, and that the following procedures are adhered to, even when one or more is not formally required:

- A general veterinary health certificate, including a statement that the dog has a normal testicular status, and that the identity of the dog was confirmed. An example of such a certificate is given in Appendix 2.
- A semen quality assessment form, and in the case of frozen semen also thawing instructions, together with a recommendation of how many straws, or vials in the case of pelleted semen, should be used for each AI. An example of such a certificate is given in Appendix 3.
- Always use the correct, registered name of the dog, not its pet name, for all official documents, including the blood test report.
- Always enclose a set of copies of the certificates, either attached on the outside of the shipment, or inside in case the airway bill with the original certificates is lost under way, or removed by mistake, for instance by customs in a transit country.
- Straws (or vials with pellets) should be packaged so that they are easy to move from the shipping dewar to the storage tank, i.e. they should be in goblets and canes, or in plastic sheaths, which are properly marked (for marking, see below). If semen is frozen in pellets, do not forget to enclose the plastic bags used for the thawing (Whirl-pak, Nasco, USA) as these are not readily available in all countries.
- Always apply a seal to the thermos flask, styrofoam box, or liquid nitrogen (LN₂) dewar, even if it is not an official requirement. This prevents unauthorized persons from opening the shipment and, thus, minimizes the risks of damage. Whenever possible do not put the seal on the outer case, but directly on the flask, box, or dewar. The LN₂ dewars usually are equipped with a separate ring for seals.

Identification of Semen Straws or Vials

To comply with the requirements from most countries, the semen-containing tubes, straws, or vials should always be identified with the following information:

- the breed (which may be abbreviated),
- the dog's registered name (which may be abbreviated),
- the dog's registration number,
- the date of semen collection (obligatory when blood tests and veterinary certificates are required), and
- where the semen was collected/processed.

Example:

	Afghan hd B.S. Sandstorm S-06689-99 18-5-2001 SLU
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The identification markings of the straws or vials should also appear on the certificates.

Chilled Extended Dog Semen

Most commonly canine semen is shipped extended and chilled. Chilled extended dog semen can retain its motility and membrane integrity for at least 4 days [1], and probably considerably longer, although it still remains to be determined for how long it can also retain its fertilizing capacity.

Extenders for Chilled Dog Semen

A number of chilled semen extenders are available and some are proprietary such as those used for the Cryogenetic Laboratories Of New England (CLONE) Chilled System and the Synbiotics Fresh Express. A commonly used non-proprietary TRIS-Egg Yolk extender for chilled dog semen is described in Appendix 4. You might ask your pharmacist to help you make the extender. Dog semen extenders should always contain some antibiotics to prevent the spread of infections.

Chilled Semen Preparation

The second, sperm-rich fraction of the ejaculate is collected and checked for total number of spermatozoa, sperm morphology and motility, and then centrifuged at 300 - 700 G for 5 - 6 min. The supernatant seminal plasma (which is detrimental to spermatozoa during storage) is discarded, 2 - 5 ml extender are added (at the same temperature as the semen) to the sperm pellet and this is well mixed. Using larger volumes for AI will only lead to loss of spermatozoa due to backflow of semen from the uterus or vagina. Semen is then chilled to around +5°C over 30 - 45 min, or put it in a styrofoam box with an ice-pack, to chill during transport. If a thermos flask is used for the shipment this should also be chilled. Check a re-warmed drop of semen for vitality after chilling, before putting the sample in the flask or box. Semen of originally good quality preserved in this fashion and stored chilled has been used successfully after 2 - 4 days, and can likely be used for considerably longer. Mark the semen vial with the dog's breed, registered name, registration number, and date and place of collection (see above). Whenever this is practically possible a numbered seal should be applied to the thermos flask or the styrofoam box. This is required by some countries, and it prevents unauthorized persons from opening the inner package containing the semen. The number on the seal should be stated in the veterinary certificate.

Shipment of Chilled Dog Semen

Use sterile plastic tubes for the semen, for instance Nunc tubes (Nalge Nunc International) which do not break during transport. The chilled semen is usually sent in an ordinary thermos flask or a styrofoam box with ice-packs. It is imperative that the temperature does not drop below 0°C to ensure that the semen does not freeze. Therefore, the tube containing the semen must be protected from direct contact with ice cubes or cold-packs, for instance by wrapping it in a piece of cotton wool. The thermos flask and the styrofoam box weigh little and usually need not be returned, which keeps the shipping costs low.



Figure 1. Thermos flask and plastic Nunc tube for chilled semen shipment. - To view this image in full size go to the IVIS website at www.ivis.org . -



Figure 2. Styrofoam box with cold-pack for chilled semen shipment.. - To view this image in full size go to the IVIS website at www.ivis.org . -

Results Using Chilled Extended Dog Semen

Whelping rates from 374 artificial inseminations using chilled extended semen were reported to be 45.1 % when the semen was deposited in the cranial vagina and 65.6 % by transcervical intrauterine semen deposition. The average litter size was 5.8±3.0 pups by vaginal AI and 6.4±3.2 by intrauterine AI (P<0.001) [2]. The results of this study emphasize the importance of intrauterine semen deposition not only for frozen-thawed semen [3], but also when using chilled extended and fresh dog semen.

Frozen Dog Semen

Frozen dog semen can be stored practically indefinitely. Dog semen can be frozen in 0.5 or 0.25 ml French straws or in pellets. The 0.5 ml straws are preferred by most semen freezing agencies and by practitioners performing the AIs.

Extenders for Freezing of Dog Semen

There are a number of facilities or agencies which freeze dog semen, using proprietary extenders, such as for instance Canine Cryobank, CLONE, International Canine Semen Bank (ICSB), and Synbiotics. Others use Triladyl (Minitüb, Tiefenbach, Germany) or the non-proprietary systems like the Norwegian Tris extender described by Andersen [4], or the Uppsala-Equex extender [1], and its modification the Uppsala-Equex 2 extender. The compositions of the two Uppsala-Equex extenders are described in Appendix 5.

Frozen Semen Preparation

Semen is collected and centrifuged as described above for chilled semen preparation. It can be further processed in many different ways, using various extenders and one or two-step dilutions (the first at room temperature and the second after chilling and just before freezing), equilibrating for 1 to 4 hours and freezing for instance, either in liquid nitrogen vapour on a rack 4 cm above the LN₂ surface in a styrofoam box [4-6] or in three steps directly into a liquid nitrogen tank [3,6,7]. Most agencies freeze dog semen at a final concentration of 50 - 100 million spermatozoa per 0.5 ml straw, and use 2 - 4 straws per AI. Some use a thaw medium, usually 0.5 - 1 ml for each straw or 1 - 2 ml per pellet vial. Semen is thawed in a waterbath either at 37°C for 15 - 60 sec or at 70°C for 8 sec. It is important to adhere to the thawing instructions provided by the semen freezing agency, as the thawing method is dependent on the freezing method. The freezing and thawing methods presently used in Uppsala are described in Appendix 6.

Shipment of Frozen Dog Semen

To ship frozen semen a liquid nitrogen container is required. The container must maintain the temperature at around -197°C. Today most semen freezing facilities use the so-called dry-shippers, which absorb the liquid nitrogen into a porous material in their walls. These will not spill and therefore need not to be shipped as dangerous goods, which is more expensive. They should, however, always be sent as fragile goods, because they are easily broken by rough handling. The tank is usually shipped in a plastic box for protection. They come in different shapes, and it appears that the ones that are mushroom-shaped offer the best protection, because their shape prevents them from being transported lying on the side, and from having something stored on top of them. Some semen processing facilities request that the dewar is insured against damaging during freight. The shipping code for the shipper's declaration for liquid nitrogen is: UN1977, which informs of its "Restricted Goods" status. The regulations pertaining specifically to the dry-shippers are found in the IATA Packing Instruction 202 Note, which reads: "Insulated packagings containing refrigerated liquid nitrogen fully absorbed in a porous material and intended for transport, at low temperature, of non-dangerous products are not subject to these Regulations..."



Figure 3. Different types of liquid nitrogen dewars and outer protective cases. - To view this image in full size go to the IVIS website at www.ivis.org . -

Check the Liquid Nitrogen Tank

Before shipment it should always be made a routine to check that the liquid nitrogen dewar is not broken. Therefore:

- Always fill the tank with liquid nitrogen at least 24 hours before the shipment is due. The dry-shippers have to be filled over a period of time, and left to saturate between fillings. When some LN₂ remains in the bottom of the tank it means that it is full.
- Weigh the tank both before and after filling to control that it contains the proper amount of LN₂. The most commonly used dry-shippers take from 1 to 4 kg of LN₂, and have a holding time of 10 - 20 days. Always check the manufacturer's information.
- Fill the tank and leave for 24 h. Check its weight again before shipping.
- Also when receiving a tank, it is a good routine to check its weight on arrival in case something has gone wrong with the shipment.

Liquid nitrogen containers are expensive and are usually on loan from the semen processing facility and should be returned as soon as possible. To avoid problems with the returning of the tank at Customs Offices, instructions for its return and a certificate should accompany it for the return freight. For instance, a statement such as the following can be attached:

"The LN₂ tank and shipping case are both the sole property of.....
and on loan to.....
and will be returned to.....
as duty free Return Goods."

When the dry-shippers are full and in their outer protective casing they usually weigh between 7 and 15 kg, and the freight costs can be quite high. On the other hand several doses of semen, or semen from several dogs can be sent in one shipment.

Results using Frozen-thawed Dog Semen

Whelping rates of 84% from 327 frozen-thawed semen AIs [3] and 71% from 312 frozen-thawed semen AIs [8] have been reported when the semen was deposited in the uterus using the Scandinavian catheter [9]. In a 10-year field study including 286 frozen-thawed semen AIs with semen of variable quality that had been processed by a large number of different agencies world-wide and AIs performed in bitches with varying fertility by many different veterinarians the whelping rate by vaginal AI was 35% compared to 52% by intrauterine AI [2].

How Much Semen Should Be Sent?

How much semen should be sent mainly depends on what has been agreed. It is still generally recommended to use at least 150 - 200 million motile, morphologically normal spermatozoa per AI, and to inseminate the bitch twice. If the ejaculate contains abnormal spermatozoa or the post-thaw motility is low the total number of spermatozoa per breeding unit should be increased accordingly in an attempt to compensate for the less-than-average semen quality. If, however, the semen is of unsatisfactory quality it should not be shipped unless the bitch owner or importer is informed about the situation and has given consent.

If the AI will be performed by surgery, which is still routine in some countries, fewer spermatozoa may be required per AI and usually only one AI is done, while vaginal AI may require up to 10 times more spermatozoa [3,10]. Chilled semen can be stored for at least 2 - 4 days, if originally of good quality. Therefore, two or more semen collections can be made and enough semen sent in one shipment for repeated AIs. Considering the high costs involved in the shipping of frozen semen it might be a good idea to send enough semen for a repeat breeding at the next oestrus in case the bitch doesn't get pregnant at the first attempt. If the importer has paid for the freezing, it may also be reasonable to send all the resulting semen doses, unless other agreements have been made.

Who Should Organise the Shipment?

Some canine semen facilities and agencies deal directly with freight companies. Freight charges, however, appear to be quite negotiable and it can be very time consuming to organize the shipments, as they almost always involve customs clearance and other procedures. For these reasons other processing facilities chose to leave it to the dog or bitch owner to organize shipments. For the "shipper's declarations" they will need to know that the code for liquid nitrogen is UN1977, and for the dry-shippers that the IATA Packing Instruction 202 Note clarifies that this kind of tank can be shipped as non-dangerous goods. There should also be an accompanying statement for customs that the tank is on loan and that it will be returned as an empty packaging, to avoid taxes or custom fees. Consider insuring the tank against damages during transportation.

Who May Perform A.I.?

Both national legislation and the Kennel Clubs may have regulations regarding the right to perform AI in dogs, and where semen may be stored. In several countries this is exclusively a veterinary matter, and in some countries the veterinarians are expected to have taken and passed a special course for AI in dogs. In other countries, the breeders themselves may inseminate their own dogs. When it comes to frozen semen, however, this is unlikely to happen, because of the special qualifications needed for the handling of the semen and the generally poor results obtained with vaginal AI.

Cooperation Between the Semen Processing Facilities and the Inseminating Veterinarians

Semen processing facilities or agencies must provide practitioners with adequate information about the quality of the semen they send and, in the case of frozen semen, information about how the semen should be thawed, because the method of thawing is dependent on how the freezing was done, and the methods vary among freezing facilities.

The responsibility of choosing the right time during the bitch's oestrous cycle to perform the AI, on the other hand, lies with the inseminating practitioner. It is strongly recommended to check the bitch's peripheral plasma level of progesterone when either chilled or frozen-thawed semen is to be used. The optimal time for AI is considered to be 4 to 7 days after the LH-surge, i.e. 2 - 5 days after the estimated time of ovulation. The level of progesterone is then usually between 30 and 75 nmol/L (10 and 25 ng/ml).

The AI-technique is also extremely important for obtaining good pregnancy rates when using dog semen. Significantly better results are obtained with intrauterine compared to vaginal deposition of fresh, as well as chilled extended or frozen-thawed canine semen [2]. Intrauterine AI in the dog can be done with the Scandinavian catheter (for more information see Intra-Uterine Insemination in the Dog Using the Scandinavian Trans-Cervical Catheter and a Comparison with other Methods by C. Linde-Forsberg) [9] or with the aid of a rigid endoscope and a dog urinary catheter [11]. Surgical AI and AI by laparoscopy are also used. Whether these latter methods are ethically acceptable is under some debate, and no field results have been reported.

An important part of the cooperation between the semen processing facilities and practitioners, and one that seldom is satisfactory, is the reporting back of the results obtained. Although time-consuming, this follow-up obviously is of great value to both parties, as well as critical for any objective evaluation of a semen processing system, an insemination facility, or of a specific procedure or modification thereof.

Appendixes

- Appendix 1 - A Guide to Regulations for International Shipment of Chilled and Frozen Semen
 - Appendix 2 - Veterinary health certificate for export of dog semen
 - Appendix 3 - Certificate of collection of dog semen
 - Appendix 4 - Tris-egg yolk extender for chilled dog semen
 - Appendix 5 - The Uppsala extenders for freezing of dog semen
 - Appendix 6 - The Uppsala freezing procedure
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Appendix 1 - To print this section separately go to the IVIS website at www.ivis.org

A Quick Guide to Regulations for International Shipment of Chilled and Frozen Canine Semen

In parallel with the growing interest in international shipment of canine semen there is a growing demand for a resource providing a quick reference to the varying rules and regulations that apply in different countries. This guide, although far from being complete, aims at summarizing much of this information and lists useful websites and addresses for the relevant national organizations. It is important to remember that the rules and regulations as they appear in this appendix have been substantially abbreviated, and that they may be changed at any time. Always check for the latest official versions. Please note that when it is stated that there are no rules or special requirements, this can imply either that everything is allowed, or that nothing is allowed.

Some Useful Addresses

The addresses and information for a large number of Kennel Clubs world-wide can be found on the website of the Fédération Cynologique Internationale, FCI. Website:www.fci.be

Australia (category 4+)

Australian Quarantine and Inspection Service (AQIS) Phone: +61 93115315; Fax: +61 94552325.

Australian National Kennel Council (ANKC) P.O. box 1005, St. Marys, New South Wales 2760, Australia. Phone: +61 2 98 34 40 40; Fax: +61 2 98 34 60 38; E-mail:dogsaust@ozemail.com.au Website:www.ankc.aust.com

Requirements: An import permit is required. The semen shipment must be accompanied by the import permit in original. Each permit is valid for 6 months, and for one consignment only. The liquid nitrogen container must be new/unused. A veterinary health certificate is required. A blood test for *Brucella canis* taken within 45 days before the semen collection and the dog not be naturally mated between sampling and the last semen collection. A blood test for leptospirosis taken within 21 days prior to semen collection, or if the dog is vaccinated against leptospira two blood tests should be taken with an interval of at least 14 days and the second being within 21 days prior to semen collection, with a titre of not more than 1:400 on each occasion and no increase in titre between the first and second tests.

Austria (category 4)

Federal Ministry for Social Security and Generations (Bundesministerium für Soziale Sicherheit und Generationen), Veterinärverwaltung, Radetskystrasse 2, A-1030 Vienna. Phone: +43.17.11.00; Fax: +43.17.10.41.51.

Website:www.bmsg.gv.at

Austrian Kennel Club (Österreichischer Kynologenverband, ÖKV), Johann-Teufel-gasse 8, a-1230 Vienna. Phone: +43 18.88.70.92; Fax: +43 18.88.26.21; E-mail:office@oekv.at; Website:www.oekv.at

Requirements: An import permit, a health certificate and a blood test for leptospirosis and *Brucella canis*. For frozen semen the health certificate and the Brucella test should be made at the time of semen collection and repeated after 14 days.

Belgium (category 1)

The Belgian Ministry of Agriculture (Ministère de l'Agriculture), Service de l'Inspection Vétérinaire, Avenue du Boulevard 21 (5ème étage), 1210 Bruxelles, Belgium. Website:www.cmlag.fgov.be/

The Belgian Kennel Club (Union Royale Cynologique Saint Hubert), 98, Avenue Albert Giraudlaan, B-1030 Bruxelles, Belgium. Phone: +32 22.45.48.40; Fax: +32 22.45.87.90

Requirements: None.

Canada (category 3+)

Canadian Food Inspection Agency (CFIA), P.O. Box 6088, 1081 Main Street, Moncton, N.B. E1C 8R2, Phone: +1 506 851 7652; Fax: +1 506 851 2689.

Canadian Kennel Club (CKC), 100-89 Skyway Avenue, Etobicoke, Ontario M9W 6R4, Canada.

Requirements: An import permit and a veterinary health certificate. The permit in original should accompany the semen

shipment to Canada. An export certificate should be issued by a Government veterinarian of the exporting country. The LN₂-tank must have a numbered tamper proof seal, and the number must be recorded on the export certificate. The dog must not have been vaccinated during the 30 days immediately preceding collection. The dog must not have been used for natural service for a minimum of 14 days prior to and until completion of semen collections. The semen sample(s) must be certified free from contaminating pathogenic microorganisms. All equipment used to collect, handle, wash, freeze and store the semen must have been new, or sterilized prior to use. Specified antibiotics must have been used in the extenders, and the semen have been stored in this for specified times, depending on which antibiotics were used, before freezing. The shipment of the semen through another country requires written authorization from CFIA.

Czech Republic (category 4+)

Czech Republic State Veterinarian (Státní Veterinární Správa České Republiky), Tesnov 17, 117 05 Praha 1, Czech Republic. Phone: +420 248.012.735; Fax: +420 218.129.74

The Czech Kennel Club (Ceskomoravská Myslivecká Unie), U Pergamenky 3, CZ-170 00 Praha 7, Czech Republic. Phone: +420 2.667.108.29; Fax: +420 2.667.128.27; E-mail:cmku@cmku.cz; Website:www.cmku.cz

Requirements: An import permit, a veterinary health certificate and a blood test for leptospirosis and *Brucella canis*, taken not earlier than 20 days and not later than 30 days after the collection of semen. Semen straws should be sealed at the time of collection and marked with details of the donor dog. The donor dog should be vaccinated against rabies.

Denmark (category 3+)

The Danish Veterinary and Food Administration, (Ministeriet for Fødevarer, Landbrug og Fiskeri), Morkhøj Bygade 19, DK 2860 Soborg, Denmark. Website:www.foedevaredirektoratet.dk/vnyt, E-mail:foedevaredirektoratet@fdir.dk, Phone: +45 33.95.60.00, Fax: +45 33.95.62.89.

The Danish Kennel Club (Dansk Kennel Klub), Parkvej 1, Jersie Strand, DK-2680 Solrod Strand, Denmark. Phone: +45 56.18.81.00; Fax: +45 53.18.81.91, Website:www.dansk_kennel_klub.dk

Requirements: The importer needs to apply for a registration as importer in writing to the "Veterinaerdirektoratet, 1. Afdeling, Rolighedsvej 25, 1958 Frederiksberg C, Denmark" at least 5 days before the import, stating the country of origin of the semen. The import permit **will** thereafter be sent out together with the relevant regulations and veterinary forms. A veterinary health certificate is required. For semen from rabies countries the dog must also be vaccinated against rabies at least 30 days and not more than 12 months before the collection of semen. The Kennel Club has no rules for litters by chilled imported semen. Frozen semen to be imported must have been collected by a veterinarian, who also issues a health certificate, and states that the dog has normal testicles. The dog must be ID-tattooed or micro chipped. The semen should be frozen in straws, which must be clearly marked. A special certificate is necessary for registration of the litter, and can be ordered from the KC.

Finland (category 1)

The Finnish Ministry of Agriculture and Forestry (Jord- och skogsbruksministeriet), P.O. Box 30, 00023 Valtioneuvosto, Street address: Kluuvikatu 4A, Helsinki, Finland. Phone: +358 9-1601;+358 9-1602432; Fax: +358 9-1603338; E-mail:outi.tyni@mmm.fi; Website:www.mmm.fi

The Finnish Kennel Club (Suomen Kennelliitto-Finska Kennelklubben r.y.) Kamreerintie 8, FI 02770 Espoo, Finland. Website:www.kennelliitto.fi, Phone: +358 9.887.300; Fax: +358 9.8873.0331.

Requirements: None.

France (categories 1 and 2)

The French Ministry of Agriculture (Direction Générale de l'Alimentation, Mission de Coordination Sanitaire Internationale), Rue de Vaugirard, 75732 Paris Cedex 15, France. Phone: +33 - 1.49.55.84.84; Fax: +33 - 1.49.55.83.14. Website:www.agriculture.gouv.fr

The French Kennel Club (Société Centrale Canine de France) 155, Avenue Jean Jaurès, F-93535 Aubervilliers Cedex, France. Phone: +33 1.49.37.54.00; Fax: +33 1.49.37.01.20. Website:www.scc.asso.fr

Requirements: There are no requirements from the ministry for dog semen from EU-countries. Semen from non-EU-countries needs an import permit from: Service des Titres du Commerce Extérieur 8, Rue de la Tour des Dames, 75436 Paris Cedex 09, France. Fax: +33 1.55.07.46.59. The French Kennel Club registers litters provided that the insemination is performed by a veterinarian who has followed a special course at one of the veterinary schools in Lyon, Maisons-Alfort or Nantes. Imported semen must be kept in an official dog semen bank before being sent to the inseminating veterinarian. Banks are located in the 3 veterinary schools mentioned above.

Germany (category 1)

The German Ministry of Agriculture (Bundesministerium für Verbraucherschutz, Ernährung und Landwirtschaft) Referat 327 Rochusstr. 1, D-53123 Bonn, Germany. Phone: +49 22.85.290, Fax: +49 22.85.29.44.01, E-mail:327@bml.bund.de. Referatsleiter: Dr. Valder (Tel +49 228 529 3618).

The German Kennel Club (Verband für das Deutsche Hundewesen, VDH), Westfalendamm 174, Postfach 10 41 54, D-44041 Dortmund, Germany. Phone: +49 231.565.000; Fax: +49 231.592.440. Website:www.vdh.de. The relevant Breed Club should also be contacted, as some of them have their own regulations.

Requirements: None.

Hungary (category 1)

The Hungarian Board of Agriculture (Földművelésügyi és Vidékfejlesztési Minisztérium) H-1860 Budapest 55.Pf.1, Hungary. Phone: +361 301.4000; Fax: +361 302.0408; E-mail: miniszter@f-m.x400gw.itb.hu

The Hungarian Kennel Club (Magyar Ebtenyésztők Országos Egyesülete, MEOE) H-1116 Budapest, Tétényi út 128/b-130. Phone: +361 208.2300

Requirements: None. The Kennel Club does not officially accept litters by AI, however, there is no control.

Italy (category 1)

The Italian Ministry of Agriculture, Phone: +39 06.599.438.23, E-mail: dav.u10@sanita.it;

Website: www.politicheagricole.it

The Italian Kennel Club (Ente Nazionale della Cinofilia Italiana, ENCI) Viale Corsica 20, 20137 Milano, Italy. Phone: +39 02.70.020.341; Fax: +39 02.70.202.321; E-mail: mond2000.enci@enci.it; Website: www.enci.it

Requirements: None specified/officially not allowed, but no control.

The Netherlands (category 1)

The Dutch Ministry of Agriculture, Nature Management and Fisheries The Dutch Kennel Club (Rad van Beheer op Kynologisch Gebeid in Nederland), Postbus 75901, 1070 AX, Amsterdam Z, The Netherlands. Phone: +31 20 664 4471; Fax: +31 20 671 0846; Website: www.minlnv.nl

The Dutch Kennel Club (Rad van Beheer), Website: www.kennelclub.nl

Requirements: None.

New Zealand (categories 3+ and 4+)

The Ministry of Agriculture and Forestry P.O. Box 2526, Wellington, New Zealand.

Website: www.maf.govt.nz/AnimalHHS/countries3-5html

The New Zealand Kennel Club, The New Zealand Kennel Club, Prosser Street, Eldsø, Private bag 50903, Porirua, New Zealand. Phone: +64 4 237 4498; Fax: +64 4 237 0721; Email: nzkc@nzkc.org.nz; Website: www.nzkc.org.nz

Requirements:

- From Australia: An import permit, a Zoo-sanitary certificate and a veterinary health certificate. Semen should have been collected in the presence of a veterinarian. The ampoules, straws or pellets containing the semen must be sealed following collection, identified with the details of the donor dog, securely packed in a new/unused container using coolant which has not been used for any other purpose, and been stored under veterinary supervision until the time of export.
- From The United Kingdom and The Republic of Ireland: An import permit. The original permit should accompany the consignment to New Zealand. A veterinary health certificate issued immediately before, and again between 3 - 6 weeks after the collection of the semen. A blood test for leptospirosis within 6 weeks before semen collection. A blood test for *Brucella canis* between 3 - 6 weeks after collection of the semen. The donor dog must have been resident in the country for at least 2 months prior to the semen collection. Identification of the dog and straws etc as above. The country of export must have been free from rabies for at least 12 months, and the donor dog must not have been vaccinated against rabies with a live vaccine during the 6 months preceding collection of the semen.
- From Hawaii, Norway and Sweden: An import permit. The original permit should accompany the consignment to New Zealand. A health certificate issued immediately before, and again between 3 - 6 weeks after collection of the semen. A blood test for *Brucella canis* taken during 14 days prior to semen collection. The donor dog must have been resident in the country for at least 2 months prior to the semen collection. The semen must be held frozen for at least 21 days after collection and a new veterinary certificate be made stating that the donor dog is still healthy. Identification of dog and straws etc as above. The country of export must have been free from rabies for at least 12 months, and the donor dog must not have been vaccinated against rabies with a live vaccine during the 6 months preceding collection of the semen.
- From Canada and The United States: As above with the following additions: The donor dog must not during the 6 months prior to semen collection have been on any property on which rabies has been diagnosed in the previous 12 months. The dog should be vaccinated against rabies with an inactivated cell culture origin vaccine, and the vaccination programme be completed not more than 12 months and not less than 30 days prior to semen collection. A blood test for *Brucella canis* within 3 - 6 weeks after semen collection. A blood test for leptospirosis within 3 - 6 weeks after semen collection, or two tests not less than 30 days apart with no rise in titre between tests and treatment with specified antibiotics during the 30 days prior to semen collection.

Norway (categories 1, 3+ and 4+)

The Norwegian Ministry of Animal Health (Statens Dyrehelsetilsyn) Postboks 8147 Dep., N-0033 Oslo, Norway. Phone: +47 22.24.19.40; Fax: +47 22.24.10.45; Website: www.dyrehelstilsynet.no

The Norwegian Kennel Club (Norsk Kennel Klub, NKK) Pb 163 Bryn, 0611 Oslo, Norway. Phone: +47 21 600 900. Website: www.nkk.no

Requirements: No restrictions apply to semen from Sweden. From EU/EFTA countries: An import permit. A veterinary

health certificate issued on a standard form accepted by the Ministry. The dog must be ID-tattooed or microchipped and this number appear on all certificates. The dog must be vaccinated against leptospirosis and rabies (unless from a rabies free country) within 365 days prior to semen collection. For dog semen from outside of EU/EFTA in addition: a blood test for *Brucella canis* within 21 days prior to semen collection, and the dog must not have mated naturally in the period between the blood sampling and the semen collection. Bitches inseminated with the imported semen must not mate with another dog in the same oestrus period. Should an abortion occur the Ministry must be immediately notified.

Spain (category 1)

The Spanish Ministry of Agriculture, Paseo Infanta Isabel 1, 280 14 Madrid, Spain. Phone: +34 91.347.5185; Fax: +34 91.347.5761; Website:www.mapya.es

The Spanish Kennel Club (Real Sociedad Central de Fomento de Razas Caninas en España), Lagasca 16, Bajo derecha, 28001 Madrid, Spain. Phone: +34 91 426 49 60; Fax: +34 91.435.11.13 / +34 91.435.28.95. Website:www.rsce.es

Requirements: Zoosanitary certificate. Health certificate. The Spanish Kennel Club is currently working on their rules.

Republic of South Africa (category 4)

National Department of Agriculture (Departement van Landbou), Directorate of Animal Health, Import-Export Control, Private Bag X138, Pretoria, 0001, Republic of South Africa. Phone: +27 012.319.75.14; Fax: +27 012.329.82.92.

Kennel Union of Southern Africa, P.O. Box 2659, Cape Town 8000, South Africa. Phone: +27 21.23.90.27; Fax: +27 21.23.58.76.

Requirements: An import permit. The original permit should accompany the semen shipment. The permit is valid for 6 months and for one consignment only. A veterinary health certificate. A blood test for *Brucella canis* taken at the time of semen collection.

Sweden (category 4)

Swedish Board of Agriculture (Statens Jordbruksverk), SE 551 82 Jönköping, Sweden. Phone: +46 36 15 50 00; Fax: +46 36 15 50 05; Website:www.sjv.se

Swedish Kennel Club, SE 163 85 Spånga, Sweden. Phone: +46 8.795.30.00; Fax: +46 8.795.30.40; Website:www.skk.se;

Requirements: An import permit, which is valid for 12 months. A veterinary health certificate issued not more than 15 days before shipment. Blood tests for leptospira canicola and ichterohaemorrhagica, and *Brucella canis*, to be marked with the identity of the dog. For fresh semen the blood sample must not be taken more than 15 days before semen collection and shipment; for frozen semen it should be taken not earlier than 20 days or later than 30 days after semen collection and freezing (i.e. during a 10-day-span). The Kennel Club requires that the identity of the dog is certified by the veterinarian collecting the semen. If the dog is not microchipped or ID-tattooed either a photograph of the dog should be signed by its owner and by the veterinarian collecting the semen, or a nose print is taken and signed as the photo. Photo or nose print should accompany the shipment of semen.

Switzerland (category 2)

Federal Veterinary Office (Bundesamt für Veterinärwesen), Schwarzenburgstrasse 161, CH-3003 Bern, Switzerland. Website:www.bvet.admin.ch. Phone: + 31-323.85.24/09; Fax: +31-323.85.22.

Swiss Kennel Club (Schweizerischen Kynologischen Gesellschaft), Postfach 8276, 3001 Bern, Switzerland. Phone: +31 306.62.62; Fax: +31 306.62.60, E-mail:skg@hundeweb.org, Website:www.dogweb.org

Requirements: Just a formal import permit.

United Kingdom/Great Britain (category 3+)

Ministry for Agriculture, Fisheries and Food (MAFF), 1A Page Street, London, SW1P 4PQ, United Kingdom.

Website:www.maff.gov.uk, E-mail:germplasmimports@ahvg.maff.gov.uk or, E-mail:germplasmexports@ahvg.maff.gov.uk, both Fax: +44 20.7904.6395.

The Kennel Club, Clarges street, London. Website:www.the-kennel-club-org.uk

Requirements: An import permit, which is valid for 8 months. From rabies free countries: Country disease clearance. A veterinary health certificate. The donor dog must have been resident in the country for the last 12 months and not been vaccinated against rabies with a live vaccine during the 6 months prior to semen collection. The semen must not be despatched until a new health check has been made after 14 days. From non rabies free countries: The dog must be microchipped. The semen must not be despatched from the exporting country until 6 months after the semen collection, and a new health certificate then be issued. The dog must not have been vaccinated against rabies with a live vaccine during the 6 months prior to semen collection. The dog must not be under any quarantine restrictions. The Kennel Club requests prior application for permission to perform AI.

United States of America (category 1)

United States Drug Administration (USDA) P.O. Box 3220, Minneapolis, MN 55403-1503, USA.

American Kennel Club (AKC), 5580 Centerview Drive, Raleigh, NC 27606-3390, USA. Phone: +1 919.233.9767 or +1 919.854.0124; Fax: +1 919.233.3627 or +1 919.854.0102; Website:www.akc.org

Requirements: As from October 2000 no import permit is required for dog semen. The AKC requests a prior application to permit AI by imported semen. They also request a DNA sample, which can be ordered via E-mail:dna@akc.org.

Appendix 2 - To print this section separately go to the IVIS website at www.ivis.org

VETERINARY HEALTH CERTIFICATE FOR EXPORT OF DOG SEMEN

From: To:

This is to certify that I on (day/month/year):

collected and processed/froze semen from the dog (breed/name/registration number):
.....
.....

owned by (name/address):
.....
.....

At the time of semen collection the dog was identified by:
.....

ID-tattoo no: Microchip no:

Photograph Nose-print

At the time of semen collection the dog was clinically examined and found to be free from any signs of disease, contagious or other. The dog is not suspected of carrying any disease, including Parvovirus-infection, which can be transmitted via semen. The dog has two testicles, normally developed, in the scrotum.

The shipment consists of either:

- a. ml of fresh, chilled semen
- b. (no.) of medium (or mini) straws of semen
- c. (no.) of vials with pelleted semen
- d. (no.) of vials containing thaw medium (when applicable)

The semen tube/straws/vials are marked (Breed / Name / Reg.no / Date / Place):
.....
.....

The shipment is closed with a seal wearing the number):

Date (day/month/year):

Signature of veterinarian:

Printed name:

Address:
.....

Phone: Fax:

Appendix 3 - To print this section separately go to the IVIS website at www.ivis.org

CERTIFICATE OF COLLECTION OF DOG SEMEN

Name of dog:..... KC Reg.No:.....

Breed:..... Date of birth:.....

ID-tattoo/microchip No:..... Nose print: Photograph:

VETERINARY CERTIFICATE

I hereby certify that the dog described above was presented for semen collection on..... (date)

From the collection resulted a) medium (0.5 ml) or mini (0.25 ml) straws or vials
or b) ml of fresh semen

The semen can be identified as follows [breed, name (may be abbreviated), KC reg. No., date and place of collection]:

.....
.....

Colour of straws/plug:.....

Type of extender/cryoprotective:
.....

Recommended thawing procedure:.....
.....
.....

Recommended number of straws or vials per artificial insemination:
.....

Semen quality (if frozen, state post-thaw quality): Excellent Good Acceptable Sub-standard

Total number of spermatozoa:..... Motility:.....%

Number of spermatozoa/straw or vial:..... Abnormal sperm.....%

At the time of semen collection the semen donor was clinically examined and was found free from signs of disease. The dog has two normal testicles fully descended in the scrotum.

Signature of veterinarian:.....

Printed name:.....

Address:.....

Phone:..... Fax:.....

DECLARATION BY STUD OWNER

I hereby certify that the dog mentioned above, from which semen has been collected, is the alleged dog, and that it rightfully belongs to me.

Signature of owner:.....

Printed name:.....

Address:.....

Phone:..... Fax:.....

Appendix 4 - To print this section separately go to the IVIS website at www.ivis.org

You might ask your pharmacist to help you make the extender. If it is prepared without egg yolk, the extender can be stored in smaller portions in an ordinary freezer, and 20% egg yolk can be added at the time of use. If it is freeze-stored containing egg yolk it should probably be used within 2 to 3 months.

Tris-egg Yolk Extender for Chilled Dog Semen	
Tris(hydroxymethyl)aminomethane	3.025 g
Citric acid	1.7 g
Fructose	1.25 g
Distilled water	to 100 ml
Egg yolk	20 ml
Benzylpenicillin	1 mg/ml
Dihydrostreptomycin sulphate	1 mg/ml

Appendix 5 - To print this section separately go to the IVIS website at www.ivis.org

The Uppsala Extenders for Freezing of Dog Semen

A. Uppsala Equex System.			
	Extender 1	Extender 2	Thaw Medium
Tris	2.4 g	ditto	ditto
Citric acid	1.4 g	ditto	ditto
Glucose	0.8 g	ditto	ditto
Streptomycin	0.1 g	ditto	ditto
Distilled water	to 77 ml	to 72 ml	to 100 ml
Bensyl penicillin	0.06 g (in 0.3 ml distilled water)	ditto	ditto
Glycerol	3 ml	7 ml	none
Equex *	none	1 ml	none
Egg yolk	20 ml	ditto	none
pH	6.53	6.48	6.60
Osmolarity	740 mOsm	1370 mOsm	253 mOsm

* (Rota A, Ström B, Linde-Forsberg C & Rodriguez-Martinez H. Effects of Equex STM Paste on viability of frozen-thawed dog spermatozoa during in vitro incubation at 38°C. *Theriogenology* 1997; 47:1093-1101). The Equex STM Paste described in the above recipe is produced by Nova Chemical Sales Inc., Scituate, MA, USA. Note that the formula of the Equex paste produced by Minitüb is different.

List of additional reading material on this topic:

- Rota A, Linde-Forsberg C, Vannozzi J, Romagnoli S & Rodriguez-Martinez H. Cryosurvival of dog spermatozoa at different glycerol concentrations and freezing/thawing rates. *Reprod Dom Anim* 1998; 33:355-361.
- Rota A, Iguer-Ouada M, Versteegen J & Linde-Forsberg C. Fertility after vaginal or uterine deposition of dog semen frozen in a TRIS extender with or without Equex STM Paste. *Theriogenology* 1999; 51:1045-1058.
- Peña A, Johannisson A & Linde-Forsberg C. Post-thaw evaluation of dog spermatozoa using a new triple fluorescent staining and flow cytometry. *Theriogenology* 1999; 52(6):965-980.
- Rota A, Peña A, Linde-Forsberg C & Rodriguez-Martinez H. In vitro capacitation of fresh, chilled and frozen-thawed dog spermatozoa as assessed by the chlortetracycline assay and changes in motility patterns. *Anim Reprod Sci* 1999; 57:199-215.
- Peña A and Linde-Forsberg C. Effects of Equex, one- or two-step dilution, and two freezing and thawing rates on post-thaw survival of dog spermatozoa. *Theriogenology* 2000; (54)6:859-875.
- Ström Holst B, Larsson B, Linde-Forsberg C & Rodriguez-Martinez H. Sperm binding capacity and zona pellucida ultrastructure of stored canine oocytes. *J Reprod Fertil* 2000; 119:77-83.
- Ström Holst B, Larsson B, Linde-Forsberg C & Rodriguez-Martinez H. Evaluating chilled and frozen-thawed dog spermatozoa using a zona pellucida binding assay. *J Reprod Fertil* 2000; 119:201-206.
- Peña A and Linde-Forsberg C. Effects of spermatozoal concentration and post-thaw dilution rate on survival after thawing of dog spermatozoa. *Theriogenology* 2000; 54(5):703-718.
- Peña A, Johannisson A & Linde-Forsberg C. Validation of flow cytometry for assessment of viability and acrosomal integrity of dog spermatozoa. *J Reprod Fertil Suppl* 2001; 57:371-376.
- Ström-Holst B, Larsson B, Rodriguez-Martinez H, Lagerstedt A-S & Linde-Forsberg C. Zona pellucida binding assay - a method for evaluation of canine spermatozoa. *J Reprod Fertil Suppl* 2001; 57:137-140.

B. Uppsala Equex - 2 System.			
	Extender 1	Extender 2	Thaw Medium
Tris	3.025 g	ditto	ditto
Citric acid	1.7 g	ditto	ditto
Fructose	1.25 g	ditto	ditto
Streptomycin	0.1 g	ditto	ditto
Distilled water	to 77 ml	to 72 ml	to 100 ml
Bensyl penicillin	0.06 g (in 0.3 ml distilled water)	ditto	ditto
Glycerol	3 ml	7 ml	none
Equex	none	1 ml	none
Egg yolk	20 ml	ditto	none
pH	6.72	6.74	6.76
Osmolarity	865 mOsm	1495 mOsm	324 mOsm

(Linde-Forsberg C., 2001. Unpublished)

Note - You might ask your pharmacist to help you make the extender. If it is prepared without egg yolk it can be stored in smaller portions in an ordinary freezer, and 20% egg yolk can be added at the time of use. If it is freeze-stored containing egg yolk it should probably be used within 2 to 3 months.

The pH will vary slightly between batches. The high osmolarities of the extenders containing glycerol etc. are outside the measuring range for ordinary osmometers, and have been measured after dilution, and are not exact.

Appendix 6 - To print this section separately go to the IVIS website at www.ivis.org

The Uppsala Freezing Procedure

The second or sperm-rich fraction of the ejaculate is collected. After assessing morphology and motility and counting the total number of spermatozoa, the ejaculate is centrifuged at 700 G for 6 min. The supernatant is removed (if it still contains spermatozoa it can be centrifuged again) and the pellet diluted at room temperature in Uppsala Equex-2 System's Extender 1 (or Uppsala Equex System Extender 1) to a concentration of 400×10^6 spermatozoa/ml and allowed to equilibrate for 60 - 75 min to +4°C. An equal volume of Uppsala Equex-2 System's Extender 2 (or Uppsala Equex Extender 2) is also cooled to +4°C and added after the equilibration period, immediately before filling 0.5-ml straws, resulting in a final concentration of 200×10^6 spermatozoa/ml. The straws are frozen in LN₂-vapor in an Apollo SX-18 or a TW-10 XT or similar LN₂-tank (Minnesota Valley Engineering, Inc., New Prague, MN, or Taylor-Wharton, Theodore, AL, USA) containing 15 - 18 ml of LN₂. Freezing is performed in three steps, with the goblets at the top of the canes and with the top of the canes 7, 13 and 20 cm (for 2, 2 and 1 min) below the opening of the tank, respectively, whereupon the canister is placed in the LN₂. Not more than 4 straws should be placed in each goblet, and not more than 4 goblets (i.e. a total of 16 straws) should be frozen in each batch.

The straws can also be frozen using a styrofoam box containing LN₂ with the straws lying horizontally on a rack placed 4 cm above the surface of the LN₂ for 10 min whereupon the straws are immersed into the LN₂.

The semen is usually frozen so that the final number of spermatozoa per straw is between 100 and 200×10^6 . Depending on the semen quality usually 2-3 straws are used for each AI. In smaller breeds producing fewer spermatozoa per ejaculate it may be desirable to freeze a less concentrated semen to obtain more straws.

Thawing of the Straws - The straws are best thawed in a water bath at +70°C for 8 sec. If this is impractical they can also be thawed at +37°C for 30 - 60 sec. Any water remaining on the outside of the straw is carefully wiped off before opening the straw. Each straw is emptied into 0.5 - 1 ml of the Uppsala Equex-2 Thaw medium (or Uppsala Equex Thaw medium) at +37°C and left at this temperature for approximately 5 minutes before assessing semen quality and performing the AI.

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