Experiences with exporting embryos: comments related to health certification*

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Summary: Experiences with the export of deep frozen embryos are discussed with reference to the logistical and management problems encountered when having donor cows comply with health certificate requirements. International experience is that the greatest demand is for embryos from dairy cows and that they must be competitively priced compared to live animals.

The differences between a donor cow and a bull in an AI centre, the need to maintain an acceptable calving interval, the trend towards fewer specialised embryo centres in favour of “on farm collections”, the age and previous management of donor cows, the efficiency of embryo production and the semen to be used for embryo collections are discussed.

The conclusion is drawn that, if the use of frozen embryos is to make a contribution to the international movement of superior genetic material, then the logistics of embryo health certification should not be excessively restrictive nor the costs prohibitive.

KEY-WORDS: Cow - Disease control - Embryo - Legislation - Semen - Trade in animals - Transplantation - USA - Veterinary Services.

INTRODUCTION

Since 1979 I have been involved with the export of fresh and deep frozen embryos from the United Kingdom and the USA. This involvement has on occasions included discussing the health certificate with veterinarians of the importing Government. In almost every case I have been responsible for both the collection and deep freezing of the embryos as well as the thawing and transfer of them in the importing country.

Embryos have been exported to the following countries:
— From the United Kingdom to: South Africa, Portugal, Northern Ireland, Kenya, Zimbabwe, Australia.
— From the USA to: Hungary, South Korea, Indonesia, the Philippines, Israel, Egypt, Venezuela.
— From the Gambia to Kenya (N’Dama project for ILRAD).

When many of these exports took place, information on the lack of disease transmission by embryos as currently available was not published or not accepted as being definitive.

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It is hoped that the comments in this paper will assist those people involved in drafting health certificate regulations in understanding some of the management and cost problems confronting the exporters of embryos.

**REASONS FOR EMBRYO IMPORTATIONS**

The indications most often quoted for the importation of deep frozen embryos are:

— To obtain relatively small numbers (100 or less) of embryos from high quality donor cows sired by very good progeny tested bulls. This is the programme being implemented in Europe and England for the importation of Holstein embryos from Canada and the USA. Generally these embryos are expensive but represent genetic material that would otherwise not be available for purchase.

— To obtain relatively large numbers (300 or more) of very good quality embryos to ultimately provide a base herd of new genetic material. This process is often coupled with the above, the superior embryos being used to supply bulls for artificial insemination and cows as potential donor cows.

— To obtain large numbers (1,000 or more) of embryos over a period of two or three years to rapidly introduce new genetic material into a population of cows in which AI would take too long to have a significant effect. This process is being used in Israel and Asia to introduce new breeds to these regions.

**COST AND EFFECTIVENESS OF THE TECHNIQUE**

The technique of exporting deep frozen embryos has four main cost components. These are:

1. The cost of health testing, including quarantine costs.
2. The collection and deep freezing of the embryos.
3. The thawing and transfer of the embryos in the importing country.
4. The cost of purchasing the genetic material from the farmer who owns it.

The cost of health testing is variable and depends on the complexity of the health certificate and the diseases being tested for. The cost can range from negligible to prohibitive.

The second two cost components are easily assessed as they are the costs of the technical services provided with additional charges for air fares, etc.

The cost of the genetic material is, however, variable and depends on the quality of the donor and sire used as well as current "fashion" in the beef or dairy world.

For international projects with deep frozen embryos a realistic overall pregnancy rate is 40%. This means that, on average, it takes 2.5 frozen embryos to produce a pregnancy or that each donor collection will produce 1.5 to 2.0 pregnancies in the importing country.

Generally clients importing embryos are very cost conscious as the price of pregnancies must compare with the sale of live animals with a discount for the sex ratio and rearing time.
It is important that each cost component of the export process is controlled and that unnecessary charges are not made. These comments apply to the cost of health certification as well as every other aspect. For the export of embryos from the UK to China, the cost of meeting the health certificate has been an important factor in our inability to agree on a price with the client.

It is often difficult to get the client to appreciate that somebody must pay for the health tests including those on the animals that fail the tests. If, in addition to the actual tests, a fee for attendance during collection and freezing by an official veterinarian of the exporting country is imposed, then it is possible that the cost of the health certification will be the most expensive component of the pregnancy.

If the export of deep frozen embryos is to become a significant method by which genetic material is moved around the world, then it is desirable that the cost of administering the health tests does not become prohibitive.

OBSERVATIONS ON DONOR COWS

The nature of a donor cow

Because of existing expertise and familiarity with the export of deep frozen semen, some health certificates have been modelled on existing semen certificates. This concept can raise significant logistical, financial and practical problems for embryo exports that do not exist with semen exports. This is an important consideration if significant progress is to be made with the quality of genetic material available for export.

In simple terms, an embryo donor cow is not a bull in an AI station. For this reason it is difficult to apply the same management practices to a donor cow as one would to a bull resident at an AI station for the collection of semen. The important differences can be summarised as follows.

General treatment

A donor cow is invariably a proven animal. This means that she is at least 6 years of age or older. To be proven she must have survived in the general herd, been vaccinated with the herd, perhaps been taken to agricultural shows, been in a large group of animals since birth and have produced several calves. It is unlikely that a donor cow would have been recognised as being suitable for embryo collection until she had proven herself.

In contrast, the bull for AI is recognised as such from his pedigree from before birth, is invariably placed in isolation from a very early age, can be vaccinated or not vaccinated because of his isolation and generally has little or no direct contact with other animals.

Embryo production

On average a cow will produce 5 to 6 good embryos per collection. When embryos are collected for export, and only those embryos suitable for deep freezing are frozen, it is my experience that the average number of embryos frozen is 4. This includes both Bos indicus and Bos taurus breeds. Generally the number of times a
donor can be collected in a year is 6 giving an average number of embryos available for export per full time donor cow per year of 24.

This contrasts with the large number of straws of semen that can be collected from a bull in one year.

**The lactating dairy cow**

Most of the international demand for embryos is from dairy cows. In all cases there are production and sometimes type requirements placed on the donor cows. Normal farming practice is for a cow to be put back in calf as soon as possible after calving to maintain an acceptable calving interval. This is as true of an embryo donor cow for export as it is of any other cow. The practical point is that the cow must have embryos collected within 100 days of calving and then be put back in calf again immediately. This timing usually coincides with the peak of lactation and farmers are reluctant to allow their cows to leave their care and attention to go to an embryo centre during this time. Generally farmers will not allow their good cows to leave their farm for an embryo centre. For this reason most dairy cow embryo transfers are undertaken in “on the farm” operations. It is unusual for dairy cows to be full time in an embryo collection programme. The usual pattern is for them to be collected on one or two occasions and then put back in calf. It follows that very few commercial embryo transfer centres exist and those that do are not designed for the collection of embryos for export.

What is important is that the lack of embryo centres reflects the current status of the embryo transfer industry after 12 or 13 years of experimenting with various options. It appears that this is the option that works best within the constraints of working with cows. Any health regulations should consider this aspect of management.

This contrasts with the management of bulls where AI centres are an accepted and easy way of isolating the animals and collecting and processing the semen for either domestic or export sales.

**Pregnancy status of the cow**

If a dairy cow is to maintain an acceptable calving interval then for 9 months (280 odd days) out of every 380 to 400 days she must be pregnant. The collection of embryos is naturally precluded during the pregnancy. Also embryos cannot be collected until at least 60 days post calving (with 80 to 100 days being the more usual) and on average it takes a minimum of 30 days to get a cow in calf. Therefore to collect embryos from a dairy cow, and at the same time preserve her calving interval, presents management problems. If the embryo collection technical requirements plus the clients' specifications are added to this formula a complicated picture emerges in order to collect a few embryos for export.

This contrasts with the collection of semen from a bull whereby semen can be collected all year if necessary and in many cases all the semen required can be collected and the bull removed from the centre until such time as he is needed again.

**The beef cow**

For beef cows the comments above do not apply to the same extent, as the beef farmer is able to have foster or recipient mothers rear his calves while the donor
cow is in full time embryo production. It follows, therefore, that it is easier to have non-lactating beef cows in an embryo centre than it is to have dairy cows.

**Source of embryos for export**

To meet the client’s specifications it is often necessary to collect embryos from many different donor cows located on many different farms. Often a client will specify how many different donors and sires must be used to make up the consignment. Animals meeting these specifications will be at different stages of lactation and gestation and it is likely, therefore, that the embryo collections will take place over an extended period of time as donors become available. For this reason collections are undertaken on the farm where the donor is resident as bringing one or two donors at a time to a centre is expensive and impractical.

It is my experience that, following movement from one farm to another, a donor cow requires a settling-in period to ensure that her oestrous cycles are normal again. All this takes time and it is naive to think that a donor can arrive at a centre, be collected quickly and sent home again with no upset in milk production or disturbance to the embryo programme. It is a brave person who “fiddles” in such a fashion with a high producing dairy cow. There are exceptions but they are not the rule.

**Probability of collecting embryos**

In my experience 25% of all donor cows will at any one time fail to produce a good embryo. There is no way of predicting in advance whether or not a donor is going to produce embryos at any one collection. This does not mean that an individual cow will not produce embryos at the next attempt as the reasons for failure are complex and tend to relate to any one collection attempt. Some cows will always be very poor embryo donors and some will be very good.

For these reasons any tests required of donor cows before they are collected will add significantly to the cost of embryo production as the tests will be undertaken on those animals that fail to produce embryos as well as those that do. When it is considered that, for export, a cow will on average produce 4 embryos per collection, the cost of the health testing per embryo can be very high. This, of course, contrasts with the health tests on a bull when the costs are averaged over hundreds of straws of semen.

**SEmen TO BE USED FOR COLLECTIONS**

It was in a health certificate for the export of embryos to East Africa in 1980 that I first encountered the requirement that the semen used for the production of embryos must itself qualify for export to the country importing the embryos.

This principle is not always required by countries importing embryos and can cause severe logistical problems if included in a certificate without consideration of the consequences. For example, at that time it cost us £50.00 (US $70.00) per embryo exported to Africa for the health certification of the semen. This cost was just the fee from the AI Centre for producing the certificates for each batch of semen used and did not include the health tests as semen meeting the requirements existed.
These fees are understandable when certifying large quantities of semen for export but in an embryo programme with each donor being inseminated with a different sire and using only 3 to 6 straws of semen from each bull the costs become prohibitive. In this case the cost of this semen certification made up one quarter of the cost of the pregnancy.

The concept of semen meeting all the export requirements to the country importing embryos creates problems for the following reasons:

1. Some countries do not have semen health certificates but wish to import embryos.
2. Some countries have complex semen health certificates that relate to the dangers of importing semen. If a bull has not been tested to all these requirements, then special testing will need to take place for the sake of 6 to 10 (or even less) straws of semen. This could necessitate the bull returning to the AI centre, if no longer resident, for testing and collection, the costs of which would be prohibitive.
3. In the case of semen imported from a third country what happens? For example, in the UK we wish to use Holstein semen imported from Canada on our export donor cows. This semen is highly tested for import to the UK, but these tests might not be identical to those required by the third country.
4. Is this requirement really necessary as what is the likelihood of semen collected under AI conditions infecting the embryo? It is the embryo that is being exported which contains the remnants of one spermatozoa 7 days after fertilisation.

For the above reasons I have favoured the following wording in relation to the semen used for the collection of embryos for export: "That the semen used on the donor cows was collected and stored at a recognised AI centre and meets the health standards for general distribution and use."

When it is considered that individual batches of semen must be certified and that perhaps only 3 to 6 straws of semen might be required, then serious consideration must be given to whether or not the semen used in embryo collections does constitute a disease transmission risk.

**GENERAL COMMENTS**

**Approved centres**

With the decline in the number of commercial domestic embryo transfer centres in favour of on-farm work, what will now be defined as a centre and who will approve it? China, New Zealand and the USSR require embryos to be collected in centres and impose the requirement that no co-mingling of cows not similarly tested should occur, which precludes using an existing domestic operation.

Some farms have built their own facilities for their own use in which embryos could be collected for export. Will these become approved centres for export? On these farms it is difficult to isolate individual cows in the milking herd from all others as cattle are herd animals and farms are designed for cattle to be housed and milked together.

**Tests requiring no rise in titre between two blood samples at a 30 to 40-day interval**

We have recently exported about 1,000 embryos on a certificate with this requirement for bluetongue. The donors were beef cattle and were in a continuous
embryo collection programme for the project. It was our experience that the test 
was adequate for a single collection situation but when the same donors were being 
repeatedly collected at 45 to 60-day intervals the repeat testing became a logistical 
nightmare to organise as well as being relatively meaningless. Often the second 
sample for one collection was the first sample for the next collection. In this parti­
cular project small rises in titre occurred that the laboratory advised were sampling 
variations and did not indicate a recent infection. These results, however, constitut­
ed a rise in titre within the terms of the certificate.

It is important, therefore, to be specific as to what rise in titre constitutes indi­
cation of infection or recent infection for a particular disease and also to make the 
testing pertinent for both “one off” and “continuous” embryo collections.

Stockpiling of embryos for export

Many of the problems raised could be overcome if embryos could be stockpiled 
for export. Todate the industry has not been able to achieve this for the following 
reasons:

— The cost of health testing is prohibitive for the small number of embryos that 
can be collected and stored.
— It is impossible to know what health certificate to test the donors for. The 
same comment applies as to what bull to use for the collections.
— The clients’ specifications for the donor and sire need to be anticipated in 
advance and this is very difficult to do.

With domestic embryo transfers usually all embryos are transferred fresh to 
recipients. Surplus embryos are usually deep frozen but they generally do not qua­
lify for export as no health tests are undertaken for domestic embryo collections.

Authority to sign certificates

In my experience it is possible that, in an embryo export operation, three types of 
veterinarian may be involved in the production of the health certificate. These are:

— The full-time government employee, if required to supervise the collections 
and freezing of the embryos or to undertake the health tests.
— The veterinarian in practice who undertakes the health tests on the cattle.
— The veterinarian who collects and freezes the embryos (usually different to 
the above).

It is important that health certificates are designed so that a veterinarian is 
asked to sign for only those items that are his responsibility or are under his con­
trol. In one export shipment the government veterinarian was asked to sign that the 
embryos were placed in identified glass ampoules and deep frozen. As all this work 
was undertaken by the veterinarian who collected the embryos and the government 
veterinarian had no embryo experience, he felt that he could not sign this certificate 
as there was no way he had actual knowledge of what was done. This was overcome 
by the veterinarian who collected the embryos supplying a support certificate to the 
government veterinarian.

This same principle applies to asking veterinarians to certify the status of biolo­
gicals used in the embryo collection and freezing procedures as often they will have 
no actual knowledge of the manufacturing process.
CONCLUSION

The collection of bovine embryos for export presents problems in health certification that relate to the normal management of cows as well as the technical requirements of an embryo transfer programme. The potential clients for deep frozen embryos are very cost conscious. It is therefore important to ensure that the health tests required for the export of embryos are not so expensive as to be prohibitive. For progress to be made in the international movement of genetic material in the form of deep frozen embryos these problems need to be addressed while at the same time safeguarding the animal health status of the importing country.

EXPÉRIENCES D’EXPORTATION D’EMBRYONS ET COMMENTAIRES CONCERNANT LA CERTIFICATION SANITAIRE. — G.D. Mahon.

Résumé : L’auteur présente son expérience en matière d’exportation d’embryons congelés en insistant sur les problèmes de logistique et de conduite d’élevage à résoudre pour que les vaches donneuses satisfont aux exigences de la certification sanitaire. Au niveau du commerce international, la plus grande demande porte sur les embryons de vaches laitières et leur prix doit rester concurrentiel avec celui des animaux vivants.

Plusieurs aspects sont à prendre en considération : les différences de contexte d’intervention entre une vache donneuse et un taureau d’insémination artificielle, la nécessité de conserver un intervalle acceptable entre vêlages, la tendance à restreindre le nombre de centres de transfert spécialisés au profit de « collectes sur l’exploitation », l’âge et les conditions d’élevage antérieures des vaches donneuses, le rendement de la collecte d’embryons et le choix de la semence à utiliser pour leur production.

En conclusion, si l’emploi d’embryons congelés doit contribuer à la circulation internationale d’un matériel génétique de qualité supérieure, la logistique de la certification des embryons ne doit pas être trop contraignante ni les coûts trop prohibitifs.

MOTS-CLÉS : Commerce d’animaux - Embryon - Etats-Unis - Prophylaxie sanitaire - Réglementation - Semence - Services vétérinaires - Transplantation - Vache.

EXPERIENCIAS DE EXPORTACIÓN DE EMBRIONES Y COMENTARIOS SOBRE LA CERTIFICACIÓN SANITARIA. — G.D. Mahon.

Resumen : Expone el autor su experiencia en materia de exportación de embriones congelados, haciendo hincapié en los problemas de logística y de manejo de ganado que se han de resolver para que las vacas donantes cumplan con los requerimientos de la certificación sanitaria. A nivel del comercio internacional, la demanda mayor se refiere a los embriones de vacas lecheras y su costo debe ser competitivo con el de los animales vivos.
Se han de tomar en consideración varios aspectos: las diferencias de contexto de intervención entre una vaca donante y un toro de inseminación artificial, la necesidad de conservar un intervalo aceptable entre pariciones, la tendencia a reducir el número de centros especializados de transferencia a favor de «recolecciones en la granja», la edad y anteriores condiciones de cría de las vacas donantes, el rendimiento de la recolección de embriones y la elección del semen para la producción de éstos.

En conclusión, puesto que el uso de embriones congelados debe contribuir a la circulación internacional de un material genético de calidad superior, la logística de la certificación de embriones no deberá ser demasiado apremiante ni los costos demasiado inasibles.

PALABRAS CLAVE: Comercio de animales - Control sanitario - Embrión - Estados Unidos - Reglamentación - Semen - Servicios veterinarios - Transplante - Vaca.