



Health and safety of IVF embryos: challenges for the international ET industry

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Abstract

The current world population is increasing at a fast rate. In order to feed this larger population, food production must increase by 70 percent. Recent reports show a record global production of 58.9 Carcass Equivalent Weight million metric tonnes of beef expected for 2014. It becomes clear that the worldwide agricultural community will have to integrate new technologies to assure the sustainability of global livestock and meat demands. Agriculture has benefited tremendously from the innovation of reproductive technologies such as semen artificial insemination and cryopreservation, embryo transfer and cryopreservation, and *in vitro* fertilization. Only recently have some developed countries accepted the import and export of frozen IVF embryos and more countries are currently evaluating this. Before 2003, *in vitro* embryos represented not more than 20% of all embryos produced. After 2003, this jumped to 30 to 39% of all embryos produced, and is increasing. It is clear that South America, and more specifically Brazil, is driving this increase. However, most people in this field would agree that the trend is true for many regions active in this field. International movement of gametes or embryos must be performed in biosecure manners to make certain that pathogenic organisms are controlled and that transmission of infection to recipient animals and progeny is avoided. The embryo transfer industry has adopted appropriate procedures to manage the biosecurity risks and hence mitigate risks of pathogen transmission through international trade of bovine embryos. Techniques for biosecure production of *in vivo* bovine embryos have been well established. However, as *in vitro* embryos are relatively new to this business, there are not many papers on the subject of pathogen-interaction with this type of embryo. Certain studies demonstrate that the decontamination of *in vitro* embryos using recommended procedures is effective for specific pathogens while others have shown that this is not as evident in other conditions. All agree that more research is needed regarding washing protocols for *in vitro* embryos. It is imperative that the scientific community continues its research to validate current embryo sanitary washing procedures and recommend any modifications that would be necessary for IVF embryos. As embryos are becoming an important component of international trade of bovine genetics, such research must not only continue but augment if key

parties want to assure they meet the worldwide rising need of meat and dairy products.

Keywords: biosecurity, embryo trade, *in vitro* fertilization.

Introduction

According to a recent press release from the United Nations Department of Economic and Social Affairs, the current world population is at 7.2 billion and projected to increase by almost one billion people within the next twelve years, reaching 8.1 billion in 2025 and 9.6 billion in 2050 (United Nations, 2013). Most of the population growth will occur in developing regions, which are projected to increase from 5.9 billion in 2013 to 8.2 billion in 2050. During the same period, the population of developed regions will remain largely unchanged at around 1.3 billion people. Growth is expected to be most rapid in the 49 least developed countries, which are projected to double in size from around 900 million inhabitants in 2013 to 1.8 billion in 2050. Needless to say, feeding the planet is an international concern.

According to a report from the Food and Agricultural Organization (FAO), nearly all of the population increase will occur in developing countries (FAO, 2009). Urbanization will continue at an accelerated pace, and about 70 percent of the world's population will be urban (compared to 49 percent today). Income levels will be many multiples of what they are now. In order to feed this larger, more urban and richer population, food production (net of food used for biofuels) must increase by 70 percent. Annual cereal production will need to rise to about 3 billion metric tonnes from 2.1 billion today and annual meat production will need to rise by over 200 million metric tonnes to reach 470 metric million tonnes.

International trade

What are current livestock and meat productions worldwide? For this review, the author will focus mainly on beef and dairy data. The USDA Foreign and Agricultural Service (2014b) published a Livestock and Poultry World Market/Trade Report in April 2014 and the results are shown in Fig. 1. The USDA forecasts a record global production of 58.9 Carcass Equivalent Weight (CWE) million metric tonnes of beef for 2014. In Fig. 1, only Brazil and India

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Received: May 21, 2014
Accepted: June 26, 2014



have higher production of beef and veal when compared to national consumption. In fact, a more favorable demand outlook for a wide range of countries such as China, Venezuela, Angola, Chile, Saudi Arabia and the EU will stimulate greater shipments by Brazil and India. For the United States and Australia, significant changes in demand and tight supplies will impact trade patterns, but not necessarily volumes. Russia on the other hand under produces livestock and meat products when compared to its national consumption. The livestock and meat production in the EU has dropped between 2010 and 2013. This could be explained by the elevated milk price and approaching abolition of the milk quotas in 2015 which will encourage farmers to retain animals to expand their dairy herds. On the other hand, India's production has increased in the same period mainly due to increased emphasis on efficiency gains in the dairy sector such as the utilization of more nutritious feed. Strong global demand and depreciation of the Real has bolstered Brazil's livestock slaughter industry and thus facilitate competitive export pricing and improve packer margins. As well, the outlook has improved for Argentina as its production has risen in 2013 and was driven by higher slaughter. Figure 2a and 2b highlights the import and export activities of selected countries between 2010 and 2013. Figure 2a demonstrates how countries such as Russia, Japan, South Korea, EU, Canada and USA continue to be big importers of livestock and meat. Additionally, countries such as China, Hong Kong Venezuela and Chile have increased

significantly their import activities creating greater demand for meat and livestock. Figure 2b highlights how countries such as Paraguay, Uruguay, New Zealand, USA, and especially Australia, Brazil and India, have increased very significantly their export activities during the same period.

Needless to say, with the growing global population especially in developing countries, combined with the clear evidence of increased demand in certain developed countries, the worldwide agricultural community will have to integrate new technologies to assure the sustainability of global livestock and meat demands. Agriculture has benefited tremendously from the innovation of reproductive technologies in the last 60 to 70 years. The first of these was the introduction of Artificial Insemination (AI) and semen cryopreservation in the 1940s and 1950s. This was the first true innovation to permit the exportation of animal genetics worldwide in a safe and biosecure way. Then embryo transfer (ET) and embryo cryopreservation were introduced in the 1960s and 1970s and thus permitted the trade of not only genetics from the sires through semen. Then in the 1980s and 1990s, the development of *in vitro* fertilization (IVF) and associated biotechnologies permitted to accelerate the production of key genetic crosses. Only recently have countries such as Canada, USA, China and the EU accepted the import and export of frozen IVF embryos and this niche continues to expand as more countries, such as Brazil, are currently evaluating the import and export of frozen IVF embryos.

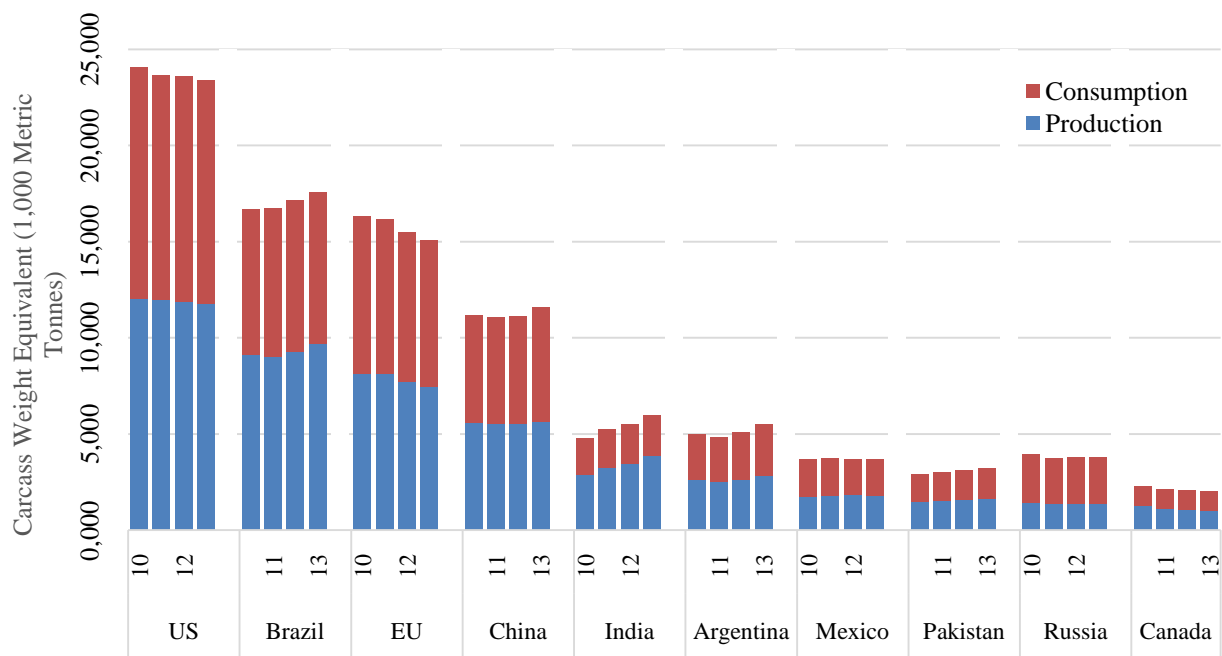


Figure 1. Beef and veal production and consumption for selected countries.

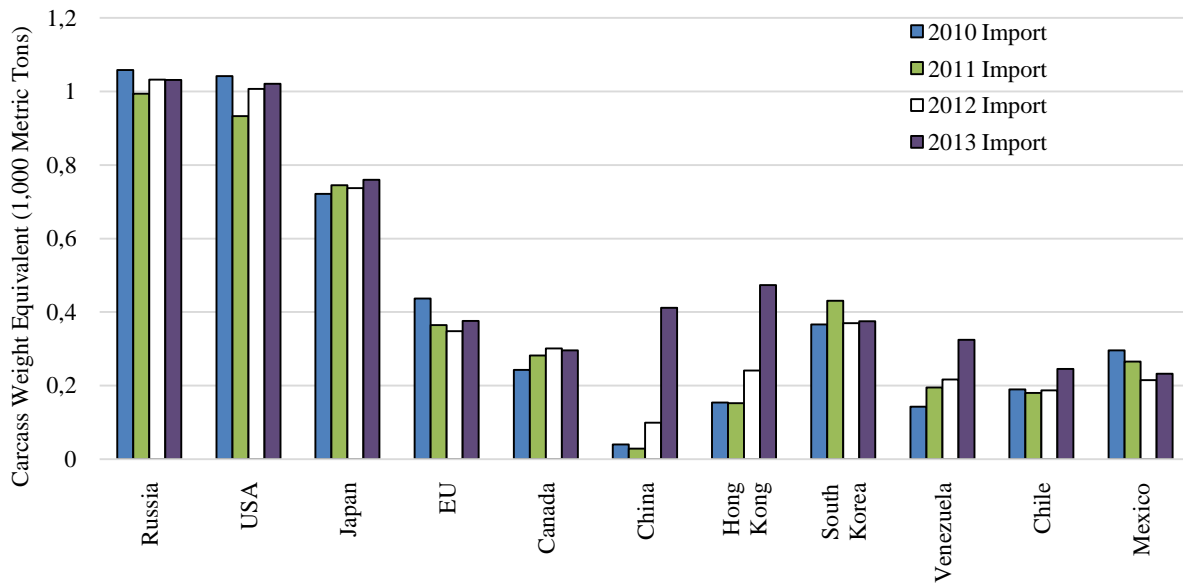


Figure 2a. Livestock and meat import activities for selected countries.

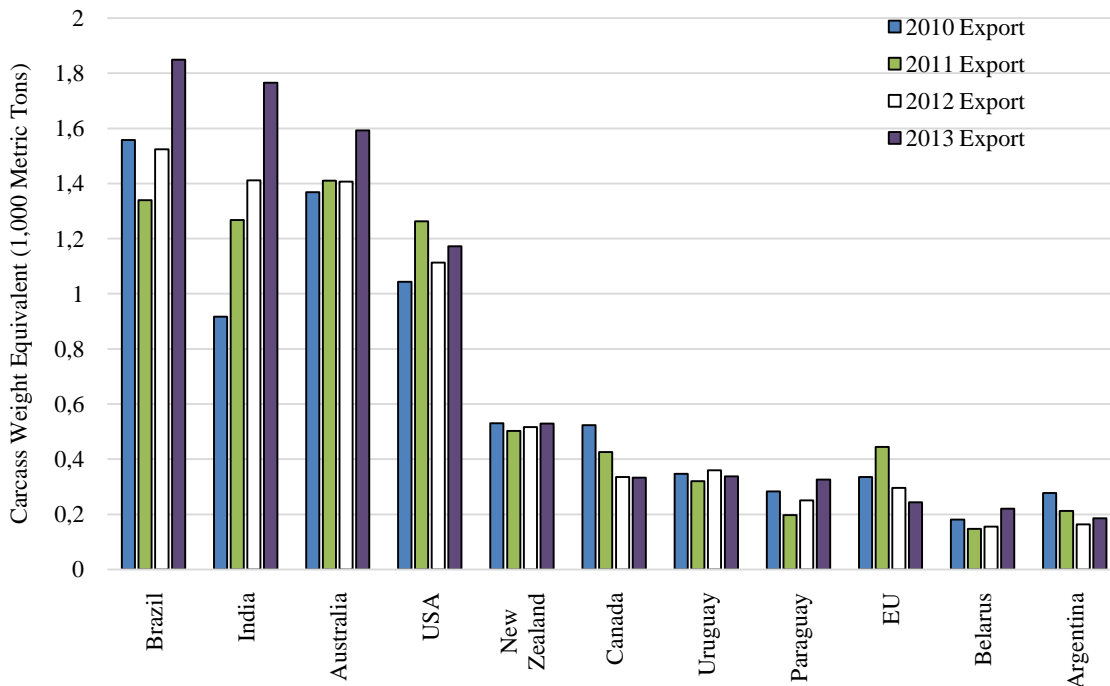


Figure 2b. Livestock and meat export activities for selected countries.

***In vivo and in vitro* embryo statistics**

The International Embryo Transfer Society’s (IETS) Data Retrieval Committee is a group of individuals from around the world that collect and present worldwide data on activities related to embryo transfer technologies in domestic farm animals. The current chair of this committee is Dr. George Perry from

Australia and the past chair was Dr. Brad Stroud from the USA. This committee published the 2012 statistics of embryo collection and transfer in domestic farm animals report and data relevant to this review are included in Fig. 3a, 3b and 3c (IETS Newsletter, 2013). Figure 3a and 3b detail the number of *in vivo* and *in vitro* embryos produced between the years 1997 and 2012 for different regions worldwide. For *in vivo*



embryo production, North America remains the region with the most production followed by Asia, Europe and South America as the other active regions (Fig. 3a). As for *in vitro* embryo production, South America clearly is the region with the most growth followed by North America and Asia (Fig. 3b). It is important to note that the data retrieved by this committee relies on the voluntary involvement of the representatives of each region. Thus, the decrease for Asia in 2012 probably reflects a lack of access to *in vitro* embryo production data rather than a reduction of these activities as seen on Fig. 3b. Figure 3c highlights the worldwide *in vivo* vs.

in vitro embryo production for the same time periods. Before 2003, *in vitro* embryos represented not more than 20% of all embryos produced, and increasing! It is clear that South America, and more specifically Brazil, is driving this increase. However, most people in this field would agree that the trend is true for many regions active in this field. In fact, North America will probably be the next region to go through a significant increase in *in vitro* embryo production in the next three years, most probably followed by Europe and Asia in 3 to 5 years.

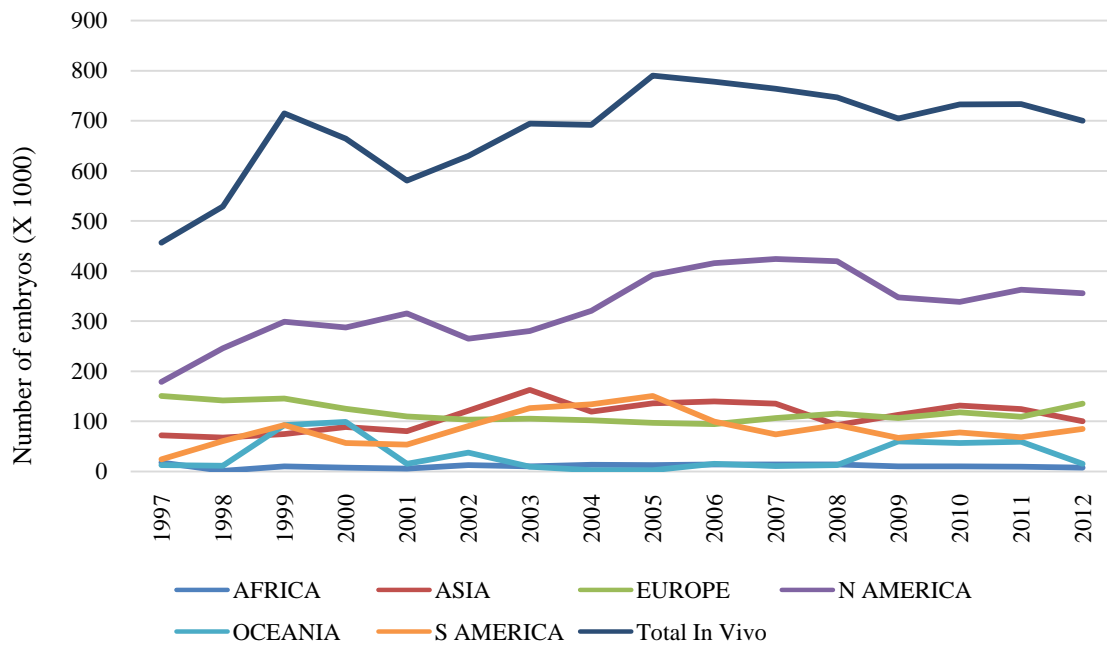


Figure 3a. *In vivo* produced transferable embryos for different regions for the period between 1997 to 2012.

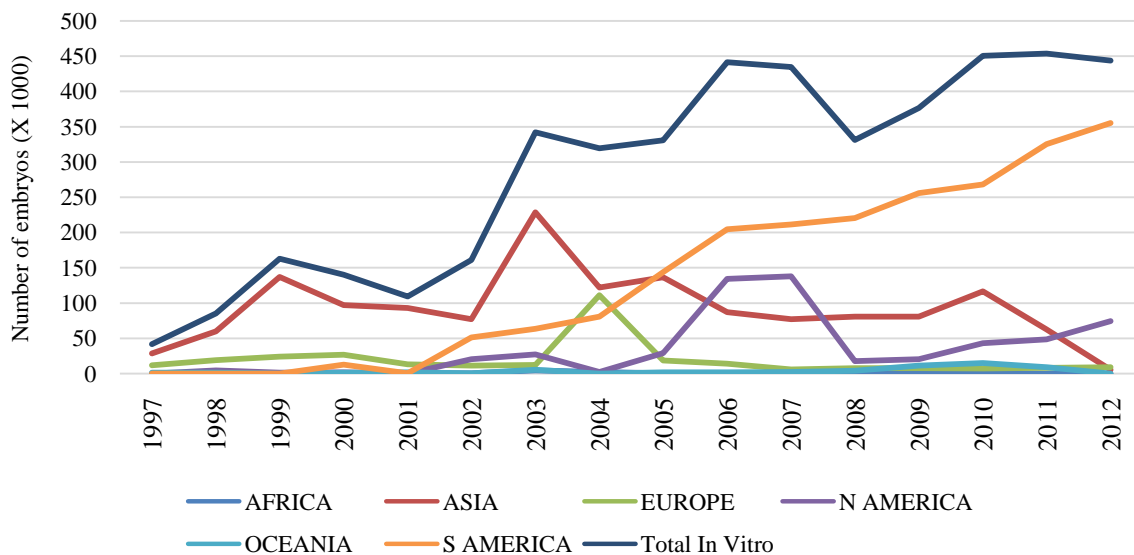


Figure 3b. *In vitro* produced transferable embryos for different regions for the period between 1997 to 2012.



Figure 4 depicts the Canadian and US export markets between 2010 and 2013 of bovine embryos, bovine semen or cattle according to USDA (USDA Foreign and Agricultural Service, 2014a) and AgCanada statistics (Statistics Canada, 2014). In Canada, the export of bovine genetics are accomplished mostly through semen exports with a 25% increase between 2010 and 2013. Live cattle is the second most active export of bovine genetics, followed by a lower activity through embryos. In the USA, although semen represents an important way of exporting bovine genetics with a

30% increase between 2010 and 2013, live cattle export remains the most important export activity for this country. Interestingly, a significant 30% drop in live cattle export occurred between 2012 and 2013. Although Fig. 4 depicts also an increase in transactions for embryo export in 2012 and a drop in 2013, IETS embryo data depict an increase in over 50% in *in vitro* embryo produced between 2011 and 2012 in North America (Fig. 3b). This could be an indication of how embryos are becoming an important source of export of bovine genetics for different countries worldwide.

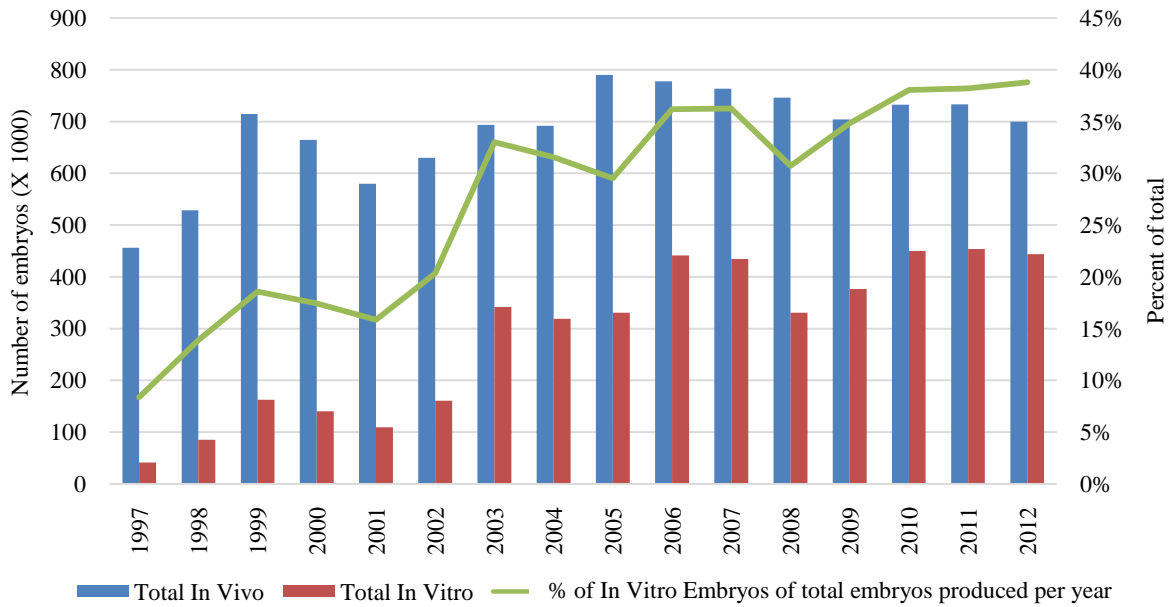


Figure 3c. In vivo vs in vitro produced transferable embryos for different regions for the period between 1997 to 2012.

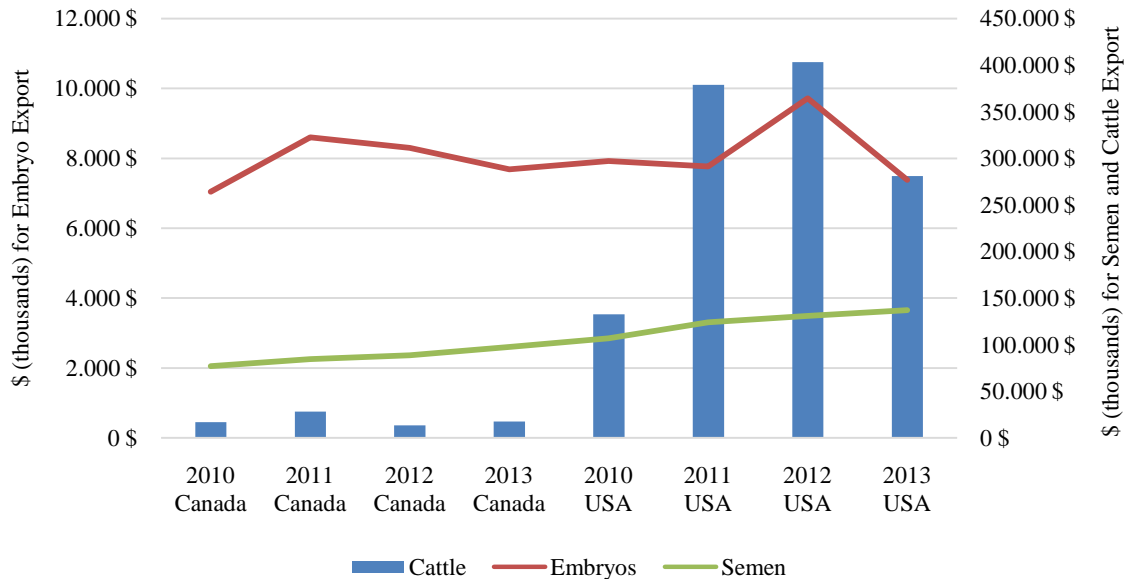


Figure 4. Canadian and US export markets of bovine embryos, bovine semen or cattle.



International movement of embryos

Of course, international movement of gametes or embryos must be performed in biosecure manners to make certain that pathogenic organisms are controlled and that transmission of infection to recipient animals and progeny is avoided. The international organization that is responsible for improving and monitoring animal health worldwide is the World Organisation for Animal Health, or more commonly known as its French acronym, Office International des Epizooties - OIE. It is recognised as a reference organisation by the World Trade Organization and maintains permanent relations with 35 other international and regional organisations. OIE's Terrestrial Animal Health Code addresses specifically the collection and processing of *in vivo* and *in vitro* produced intact embryos/oocytes from livestock and horses (World Organization for Animal Health, 2013; Chapters 4.7 and 4.8).

This review does not intend to review the different pathogenic agents that can interact with oocytes and embryos as there are very good reviews in the scientific literature that cover these subjects (Bielanski, 2006; Van Soom *et al.*, 2008, 2010a; Ponsart and Pozzi, 2013). The more common pathogens remain Bovine Brucellosis, Blue Tongue Virus, Foot and Mouth Disease, Bovine Tuberculosis, Contagious Bovine Pleuropneumonia, Enzootic Bovine Leucosis, Infectious Bovine Rhinotracheitis/Infectious Pustular Vulvovaginitis and Lumpy Skin Disease. Pathogens can be found in embryos due to contamination of the oocytes, the semen or adhesion to the zona pellucida of embryos. Semen collection and storage centers are strictly regulated by national agencies and are under veterinary and official supervision to produce semen for international export. Additionally, the general requirements for semen production for these centers are also covered by the OIE Terrestrial code (World Organization for Animal Health, 2013; chapters 4.5 and 4.6). All *in vivo* and *in vitro* embryos produced for export must obtain certificates by the semen production centers that testify that the semen used was produced under biosecure conditions and this free of any pathogens. Thus, the semen component is easily controlled with regards to embryo health status.

IETS - Health and Scientific Advisory Committee

The Health and Scientific Advisory Committee (HASAC) of the IETS is an advisory Committee composed of technical specialists in reproductive biotechnologies, animal health and food safety. It responds to the IETS members' questions and requests for advice related to those matters both at a national and international level. It submits recommendations based on current scientific knowledge to the IETS Board of Governors to further provide guidance and advice to international Agencies, such as those of the United

Nations or of the OIE. The current Chairwoman of HASAC is Dr. Claire Ponsart-Posiere from France and the immediate past-chair woman was Dr. Pascale Chavatte-Palmer of France. Information can be found at the website http://www.iets.org/comm_hasac.asp.

HASAC's mission statement is

- to review regularly and extensively all the literature relevant to biosecurity issues, in order to evaluate the potential risks at stake, based on science, for safe international trade of embryos and human consumption of animal or animal products derived from embryo transfer and related technologies.
- to develop and provide guidelines such as codes of practice, recommendations and other information pertinent to the safe movement of embryos and safe introduction into the food chain of animal or animal products derived from reproductive biotechnology without unduly restricting technological advance and commerce,
- to communicate to the IETS members all its achievements and upon acceptance of the IETS Board, to all the relevant International Agencies.

Dr. Michel Thibier, past HASAC chairman, has published an excellent review of critical points of *in vivo* and *in vitro* embryo production procedures and how practitioners can operate in high degrees of safety for international trade of embryos (Thibier, 2010). As stated in this review, through an active participation of the IETS HASAC, acting as an ad hoc committee to the OIE, it was possible to produce guidelines for general procedures for bovine embryo transfer, identifying minimum standards for hygienic handling of embryos, a continued update of results on embryo-pathogen interactions, and make recommendations for standardization methods of labeling of containers of frozen embryos. In fact, IETS has published these recommendations in the 4th edition IETS Manual available in English, French, and Spanish. This resulted in the ET industry adopting appropriate procedures to manage the biosecurity risks and hence mitigate risks of pathogen transmission through international trade of bovine embryos.

In vivo embryos vs. *in vitro* embryos

One thing is clear in the scientific literature on the subject, all authors agree that regarding the techniques used for production of pathogen-free genetic material, we cannot extrapolate from one species to another, from one pathogen to another, even from one embryo production technique to another (*in vivo* embryos to *in vitro* embryos). Techniques for biosecure production of *in vivo* bovine embryos have been well established and described in the papers referenced in this mini-review. For the purpose of this discussion, it is important to note a few aspects of *in vitro* produced embryos.



As *in vitro* embryos are relatively new to the ET business, there are not many papers on the subject of pathogen-interaction with this type of embryo. Dr Van Soom and collaborators (Van Soom *et al.*, 2010b) published a paper describing in details the importance of the embryo zona pellucida (ZP) as an efficient barrier to viral infection. She states that the ZP is a dynamic structure that can vary according to species, origin, embryonic stage and other factors. It is also demonstrated that the ZP of *in vitro* produced embryos is more porous than *in vivo* embryos. Dr Van Soom speculates that these pores may entrap small viruses and thus increase the risks of pathogen transmission. The most recent HASAC report to the IETS has clearly stated that IVF embryos do not react in the same way to decontamination procedures and that it is impossible to extrapolate data gained from *in vivo* embryos. Although certain studies demonstrate that the decontamination of *in vitro* embryos using IETS recommended procedures is effective for specific pathogens (Bielanski *et al.*, 2013), others have shown that this is not as evident in other conditions (Penido *et al.*, 2014). As stated above, all agree that more research is needed regarding washing protocols for *in vitro* embryos.

The HASAC 2014 report made the following recommendation to the international export of *in vitro* embryos:

- If the oocyte donors meet the requirements for importing live cattle into that country and if the semen used for *in vitro* fertilization also meets the country's import requirements, then it is safe to use the IETS sanitary procedures associated with *in vitro* production of bovine embryos (IETS Manual; Stringfellow and Givens, 2010).

Thus, it is safe to conclude that in such conditions, there is no reason for all countries to not accept frozen IVF embryos processed according to IETS's sanitary procedures. This should permit most countries to import and export more genetics from pathogen-free donors.

- HASAC strongly urges government agencies and private institutions related to ET and international trade of animal genetics to finance important research on embryo-pathogen interaction, especially with *in vitro* produced embryos. The drop of scientific literature on this subject is very alarming.

Therefore it is imperative that all individuals and organizations related to the beef and dairy industries to communicate to their representative government and private partners the importance to finance research and development on embryo-pathogen interactions, specifically when related to new evolving biotechnologies such as IVF.

Conclusion

This mini-review was written simply to give readers a quick update of the challenges faced by

different groups worldwide with the international trade of bovine *in vitro* produced embryos. It is clear that many countries are under pressure to open the import and export markets for IVF embryos but scientists have expressed relevant concerns regarding the risks with such trade. For over 40 years, groups such as IETS and the OIE have overseen the risk management of the exchange of *in vivo* bovine embryos between different countries by relying on the expert advice of renowned scientists in this field. Understanding that there is insufficient scientific data regarding the efficiency of current sanitary protocols for all IVF embryos produced, IETS's HASAC have proposed that ET groups use current IETS sanitary embryo washing procedures when using oocytes and semen from animals that meet the requirements for importing live cattle into that country. Thus, if the importing country would accept the animals into their territory, there is very little sanitary risks to propagate pathogens when the accredited IVF laboratories produce the embryos using IETS procedures. As for donor animals that may not meet these criteria, it is imperative that the scientific community continues its research to validate current embryo sanitary washing procedures and recommend any modifications that would be necessary for IVF embryos. This of course applies as well to any new and emerging diseases or pathogens that can be transmitted by *in vivo* or *in vitro* bovine embryos. Overall, as embryos are becoming an important component of international trade of bovine genetics, such research must not only continue but augment if key parties want to assure they meet the worldwide rising need of meat and dairy products.

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