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Historical context of cattle embryo transfer technique in Brazil

C.F.M. Rodrigues[1]

Gertec Embriões, Bragança Paulista, SP, Brazil.

Abstract

This review presents the historical context of cattle embryo transfer (ET) technique in Brazil which led to a great progress on reproductive biotechnologies turning this country into a worldwide leader on production of bovine embryos. Commercial ET in Brazil began in 1978 with the work of two pioneers, Dr. Jorge Nicolau and Walter Becker. In the 1980s the ET technique became widespread being often used by many practitioners. However, the high variation between results indicated the need of interaction with more experienced technicians from abroad and with Brazilian researchers. Originally, there were regulatory restrictions on trade of hormones for superovulation, as well as low treatment response. In 1986, the first Brazilian Embryo Transfer Society (SBTE) meeting, held in Jaboticabal-SP, encouraged the search for better results. The goal of this meeting was to join distinguished researchers in the upcoming area of reproductive biotechnology with veterinary practitioners. This exchange was a success, leading to an outstanding utilization of reproductive biotechnologies by the Brazilian technicians. As a result, Brazil turned into a reference country in this technology. In 1985, the first Brazilian calf derived from frozen embryo was born in Sertãozinho, SP. This was followed by an improvement on embryo holding, freezing/thawing protocols. There are challenges, but it is irrefutable that we have refined the technology of embryo manipulation and genetic improvement. Those achievements are due mainly to the closer relationship between the academy, through its researchers, and the veterinary practitioners. Working together is the most efficient way to provide an outstanding environment for reproductive biotechnology innovation in Brazil.

Keywords: development, embryo transfer, techniques.

History

The first commercial bovine embryo transfer (ET) set up in Brazil was established in the seventies. In 1978, two contemporary and independent attempts of non-surgical embryo collections followed by surgical transfers were performed, both using *Bos taurus* donors with fresh embryos transferred to synchronized recipients. These two pioneers, Dr. Jorge Nicolau (DMV) at Fazenda São Pedro, Sorocaba, SP, and Dr. Walter A. P. Becker transferred fresh embryos.

A year later, Dr. Aurelino Menarin Jr., in collaboration with the University of Colorado, initiated the use of ET at Campo Verde Farm in Senhor do Bonfim, Bahia, resulting in the birth of five Nelore calves. This was the earliest ET report in this breed.

The 80s were considered as a landmark in expansion and establishment of ET in Brazil. More and more veterinarians were interested in applying ET in their routine. However, initial results were still variable and inconsistent which indicated the need for further research. Moreover, the knowledge exchanged with professionals from other countries was extremely helpful in order to dissipate possible doubts regarding technical issues. These, were the key factors for the maximization of the reproductive potential of donors with high genetic value.

Superovulation programs aiming to increase the number of viable structures with high probability to generate pregnancy were initially performed using pregnant mare serum gonadotrophin (PMSG; Maturon - Laboratório Organon do Brasil). The work developed by Elsden *el al*. (1976) indicated that a combination of luteinizing and follicle stimulating hormones (FSH/LH; FSH-P; Burns- Biotec Laboratories Inc., Omaha, NE) generated a greater number of viable embryos as compared to PMSG. However, this hormonal combination was not commercially available in Brazil. Therefore, most of the superovulations in those days were performed using human menopausal gonadotropin (hMG), product with equal amounts of FSH and LH, available for human. Nevertheless, results were still inconsistent.

Among the limitations of superovulation hormonal programs, one can highlight the high cost of PMSG therapy. This drug was commercially available for human, and was expensive at the dose required for bovine. The hormone FSH-P, produced in the United States, was not regulated in Brazil. Some of these reagents were blocked in the airports customs leading to significant economic losses to several field professionals with works already in progress. In 1995, FSH-P was no longer produced. In the late 80s and early 90s, the imports of two commercial FSH-based products were regulated, Foltropin-V (Vetrepharm, London, Ontario) and Pluset (Hertape-Calier), allowing the intensification of studies on bovine superovulation.

[1]Corresponding author: gertecembrioes@uol.com.br
Phone/Fax: +55(11)4034-0819
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After the standardization on the use and dosage of both products, results became more consistent and productive. In this context, studies began to focus on embryo cryopreservation aiming to improve the logistic of ET and to create cryopreserved embryo banks.

In 1985, after several attempts, the first Nelore calf originated from a cryopreserved embryo was born due to the efforts of the technical staff of Lagoa da Serra, using 10% of Glicerol (1.4 M) and decreasing concentrations of glycerol (0.7, 0.5 and 0.25 M) during the dehydration and rehydration (Leibo and Mazur, 1978).

Considering that the final goal was to enable embryo trade similar to that routinely used for semen, it was important to simplify the processes of freezing, thawing and non-surgical transfer. Therefore, in 1985 Lagoa da Serra invested in a one-step technique of embryo cryopreservation/transfer (Leibo, 1984). This technique consisted in cryopreserving embryos in straws containing both glycerol and sucrose. Embryos were cryopreserved in glycerol 10% and, while filling the straw with the dehydrated embryo, two columns of sucrose, extracellular cryoprotectants, were added to the extremities of the straw. During thawing, the columns (embryo + 10 glycerol, sucrose) were gently homogenized; the embryo in contact with the sucrose would initiate the rehydration process. After 10 min, embryos were then transferred to previously synchronized recipients. However, while encouraging results were obtained with *Bos taurus* embryos, the same could not be observed in *Bos indicus* embryos.

In July 1986, the first annual Brazilian Embryo Technology Society (SBTE) meeting held at the Faculdade de Ciências Agrárias e Veterinárias (FCAV-UNESP), in Jaboticabal, SP, demonstrated that embryo transfer biotechnology was already a reality in Brazil. Several studies were presented by distinguished researchers, sharing questions regarding their ET field trials. Researchers such as Roberto A. de Bem, Luiz Eustáquio L. Pinheiro, José Luiz Rodrigues, Cesar R. Esper, Ricardo M. Gregory, Joaquim Mansano Garcia among others, together with experienced field professional such as Jorge Nicolau, Roberto Jorge Chebel, Carlos Fernando Marins Rodrigues, Douglas B Gaetti and Teodoro Vaske, discussed issues that, at that moment, would dominate the future application of the technique. An interesting example was the *in vitro* maturation of bovine embryos, project developed by the team led by Prof. Joaquim Mansano Garcia (Garcia *et al.*, 1986).

At the end of the 80s, sophisticated procedures were incorporated to the ET routine such as the embryo bipartition technique. Several research groups dedicated their time to this technolog allowing the production of identical twins and sex determination through embryo micromanipulation (Lopes *et al*., 2001).

In the early 90s, standardization of ultrasound procedure for reproductive purposes (Kastelic *et al*., 1990) allowed determination of fetal gender (Curran, 1992), embryo and fetal development, embryo loss and, follicular dynamics leading to the development of new knowledge, new paths, new technologies and protocols. In this scenario, Brazil began to master the technique of *in vitro* fertilization (IVF). In 1993, a group led by Prof. Enoch Borges de Oliveira, from FCAV-UNESP, Jaboticabal, produced the first calf by IVF in Brazil. Oocytes used in this study were collected from ovaries obtained from slaughterhouse cows.

Based on studies developed in Canada by Dr. Roger Sauve, in 1995, efforts were made to obtain oocytes by ultrasound guided follicular aspiration. This resulted in the birth Brazilian commercial product using this technique in 1997.

In 1996, Gertec Embriões, Beabisa Agricultura and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) supported the first commercial IVF laboratory (Rodrigues and Garcia, 2000) at Fazenda Suíço, Pitangueiras, SP. This project was coordinated by Prof. Joaquim Mansano Garcia at FCAV-UNESP Jaboticabal. Doctors André Dayan and Yeda Watanabe also participated in such effort. They would later found the Vitrogen in 1998, which was a determining factor for growth of the field application of IVF and ET in Brazil.

The establishment of ET and IVF in Brazil (Viana *et al*., 2012) has been related to several factors such as the high potential of Zebu breeds as oocyte donors, considerably superior than European breeds. Also, the fact that hormonal treatment and superovulation protocols were not required to obtain sustainable pregnancies, together with the possibility of using aged females or those that no longer respond to superovulation protocols, allowed the growth of these reproductive technologies. The advantages were also clear due to the availability of recipient females, creation of genetic improvement programs, the valuing of top animals in auctions and the leadership on beef exportations. These facts also increased the number of professionals providing such service. This scenario contributed to the Brazilian world leadership on IVP, especially in *Bos indicus* breeds.

In this context, the use of sexed semen allowed an impressive growth on IVP. In 2011, 318,000 IVP embryos were produced in contrast to 35,563 embryos produced by superovulation. In the next year, Brazil had 86% of the IVP embryos produced worldwide (International Embryo Transfer Society - IETS, 2013).

Final considerations

What are the perspectives for the Brazilian livestock genetic market? Currently, the biotechnological market trends highlight the importance of dairy breeds, with almost 70% of workload of field professionals, especially for Gir, Girolando, Holstein and Jersey breeds.

Brazil is the world supplier of Zebu genetic material. What are the reasons? Several factors are responsible for such scenario: 1) Brazil has the largest commercial Zebu herd in the world; 2) Highly capacitated Artificial Insemination and Embryo Transfer Centrals and *in vitro* fertilization laboratories; 3) Governmental and Class programs for Genetic Improvement (EMBRAPA, ABCZ, USP); 4) Standardized distribution of agricultural inputs; 5) Academic and technical infrastructure; 6) Biosafety of the exported products; and 7) National tropical technology and experience.

What are the reasons for the stagnation of the exports? 1) International prejudice and unawareness regarding the eradication of serious diseases such as foot and mouth disease in Brazil; 2) Brazil was listed as level 1 (low risk) on sanitary issues by the World Organisation for Animal Health (OIE), as stated on the OIE 80th General Session; 3) Sanitary protocols that are not practical and not viable (e.g., a protocol recently signed with Costa Rica) from the technical to the economical perspectives; 4) Lack of a strategic planning involving all the governmental levels. Several Ministries must be involved in a common objective since problems are related to logistic, commercial and income politics, institutional issues and compliance with current legislations.

In 2013 the semen market traded 13 million straws. This represents an increase of 5.4% in comparison with 2012 (Associação Brasileira de Inseminação Artificial - ASBIA, 2013). This is especially due to the increase on sales of dairy breeds which increased from 4.9 to 5.3 million (9.6%). Even on beef breeds, an increase could be observed from, 7.4 to 7.6 million of straws traded. Despite the reduction when compared to the year before, participation of beef breeds on sales are still the majority when compared to the dairy breed. The later, however, showed an increase from 39.6% in 2012 to 41.2% in 2013.

A cause for apprehension is the drop on the sale of Nelore breed semen straws, with 11.74% on Nelore Padrão and 15.13% on Nelore Mocho. Leadership belongs now to the Angus breed with 44% of the beef market, with the Aberdeen Angus being responsible for a growth of 24.5% when comparing to 2012.

The importance of Zebu breeds for countries located in the tropics justifies such concern since investments are made with breeding programs and testing of highly reliable bulls. Because the investments on Angus breed are originated mostly on imports, such increase in this breed indicates economic losses to the country's commercial balance. This increase occurred due to the increase of timed artificial insemination (TAI) in combination with the implementation of *Bos taurus*/*Bos indicus* cross breeding, aiming to produce meat with higher quality and consequent valuing of the final product.

Embryo market has become a profitable activity worldwide, especially after the advantages provided by embryo cryopreservation. The interest of countries located in the tropics for dairy Zebu genetics has increased since 2005. This is mainly due to the continuous work of genetic improvement and bulls' fertility and progeny testing developed by Embrapa, ABCZ and USP. As an example, the Girolando breed that contributes to 68% of the dairy animals in Brazil, is responsible for 80% of the milk produced. The Genetic Improvement Program in this breed started 17 years ago with the technical coordination of EMBRAPA Gado de Leite.

Countries such as Panama, Costa Rica, Paraguay, Colombia, Bolivia, and Canada, have invested in the Brazilian genetics. However, our participation is still incipient, especially due to unfeasible sanitary protocols, both on technical and economic aspects.

The development of new technologies is essential and fundamental to the growth and improvement of the existing animal genetics in our country. Biotechnologies such as cloning, transgenesis, animal selection based on molecular markers, all developed to improve the participation in a highly competitive market, still present a reduced commercial efficiency. However, we believe that this should be the current approach since reproductive biotechnologies have shown to increase the efficiency of genetic selection. These previous advances have occurred due to the pioneering efforts of two groups that worked together. On one side, researchers, developers of technical procedures and embryo production techniques; on the other side, field veterinarians, modifiers and adapters of techniques aiming to engage with the commercial reality of the rural producers.

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