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# APPLICATIONS OF EMBRYO TRANSFER BIOTECHNOLOGY IN DAIRY CATTLE

## Aplicaciones de la biotecnología de transferencia de embriones en ganado lechero

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## ABSTRACT

The dairy sector plays an important role in global socioeconomic scenarios. However, world milk production experienced a modest growth rate of only 0.5% during 2017, a smaller increase than any time in the last decade. Therefore, the more effective application of biotechniques related to bovine reproduction is an alternative to support the large-scale production of dairy cattle. Among the different reproductive biotechnologies, embryo transfer (ET) maximizes the reproduction of the herd through improved genetics and the ability to guarantee the birth of a greater number of females. In general, embryos are more resistant to thermal stress compared to gametes, representing an advantage over the application of biotechniques such as artificial insemination (AI) and fixed-time artificial insemination (FTAI). Thus, ET together with the benefits from the application of sexed semen and in vitro embryo production (IVEP) are some of the main strategies for the necessary modifications that aim to improve the dairy cattle sector. This review summarizes the main advantages of embryo transfer biotechnology for increasing reproductive efficiency and production in dairy cattle.

Keywords: Bos taurus taurus, bovine embryo transfer, reproductive biotechniques

### RESUMEN

El sector lácteo tiene un papel importante en el escenario socioeconómico global. Sin embargo, la producción mundial de leche experimentó una tasa de crecimiento moderada del 0,5% durante 2017, lo que representó un aumento menor en comparación con el logrado en la última década. En vista de esto, la aplicación más efectiva de biotecnologías relacionadas con la reproducción bovina es una alternativa para apoyar la producción de ganado lechero a gran escala. Entre las diferentes biotecnologías reproductivas, la transferencia de embriones (TE) maximiza la reproducción del rebaño, con un alto beneficio genético, al mismo tiempo que garantiza el nacimiento de un mayor número de hembras. En general, el embrión es más resistente al estrés térmico en comparación con los gametos, lo que representa una ventaja sobre la aplicación de biotécnicas como la inseminación artificial IA e inseminación artificial en tiempo fijo - IATF, por ejemplo. Por lo tanto, la TE junto con los beneficios generados por la aplicación de semen sexado y la producción in vitro de embriones (PIVE) son algunas de las estrategias clave para las modificaciones necesarias para la mejora en la producción lechera. Esta revisión resume las principales ventajas de la biotecnología de transferencia de embriones para aumentar los aspectos reproductivos y la producción en el ganado lechero.

Palabras clave: Bos taurus taurus, bovino, transferencia de embriones, biotécnicas reproductivas.

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#### INTRODUCCION

The dairy sector plays an important role in global socioeconomic scenarios. According to the Food and Agriculture Organization of the United Nations - FAO (2018), dairy cows produced three-quarters of the milk in sub-Saharan Africa, approximately 60% in Asia and practically all the milk produced in Latin America. However, the Organization for Economic Cooperation and Development - OECD and FAO (2018) showed that in 2017, world milk production achieved a growth rate of only 0.5%, a smaller increase than any time in the last decade. Therefore, it is necessary to search for practices and alternatives that improve dairy cattle production. Increasing the production efficiency of a herd is one of the great challenges for dairy producers. In the past, genetic selection programs have searched for characteristics that are essential for increasing milk production, with effective gains in milk quantity and quality, but reproductive efficiency has been disregarded. In recent years, some papers have been presented with strategies to further increase milk production and also increase reproductive performance, a key factor for efficient dairy farming.

Taking into account the importance of a sustainable, intensive and economically viable production system, achieving reproductive efficiency of the dairy herd determines the profitability from the number of offspring produced, the genetic progress and the shorter interval between lactations. This is a great challenge because there is a low heritability of production and reproduction characteristics. The crucial importance of precision reproductive care is therefore highlighted and is capable of providing maximum production efficiency in the smallest area possible, in addition to improving animal welfare.

Roche et al. (2018) showed that dairy herds were selected and managed over the years for high milk production yields, but reproductive efficiency has been disregarded. On the other hand, according to Viana (2012) and Baruselli (2016), improvements in the efficiency of biotechnology in recent years has gradually increased the use of techniques such as fixedtime artificial insemination (FTAI) and in vitro embryo production (IVEP). Another application that is gaining popularity in dairy cattle, as shown by Ettema et al. (2017), is the use of sexed semen, which allows for an increase in the proportion of females born, an increase in the intensity of selection and a decrease in the interval between generations. In this context, Phillips and Jahnke (2016) described embryo transfer (ET) technology in cattle, and it has attracted much attention and research over the past 50 years. This biotechnique maximizes the reproduction of the herd, increases the high genetic gain of the herd, and guarantees the birth of a greater number of females.

In general, embryos are more resistant to thermal stress when compared to gametes, and this represents an advantage over the application of biotechniques such as artificial insemination (AI) and FTAI. The use of ET together with the benefits generated by the application of IVEP and sexed semen are some of the main strategies for modifications to improve production in the dairy cattle industry. This review summarizes the main advantages of embryo transfer biotechnology to increase reproductive efficiency and production in dairy cattle.

#### The dairy cattle scenario

A few decades ago, the dairy sector consisted basically of an extensive system with the herds kept in the pasture. Metabolic requirements were lower, and critical factors included inadequate sanitation and many reproductive failures, resulting in a context of low productivity. The proposal established in the last few years has been to initiate a set of strategies to modify production; aiming at high production and reproduction efficiency and providing animal comfort and environmental sustainability. However, at first, the excessive intensification of the system subjected the animals to stressful conditions but now with a very high metabolic demand. This scenario caused a decrease in reproductive efficiency. In addition, it was again observed that only a balanced set of nutrition, environment, management, sanitation, and reproduction could provide adequate conditions for the milk production supply chain. These are the current goals for the dairy industry: find a model capable of providing high production efficiency while maintaining animal welfare; do so without damaging the environment; and utilize the most advanced reproductive techniques for improving gestations and breeding.

# Use of AI / FTAI versus in vivo production of embryo (ET) in dairy cattle

Artificial insemination is known as the simplest and lowest cost technique of reproductive biotechnology. However, European dairy breeds in conditions of high temperature and humidity exhibit failures in estrus cycling and demonstration, which compromises AI results. On the other hand, with the advent of FTAI protocols that synchronize follicular growth and ovulation, it is possible to achieve a rate of 100% of inseminated animals without the need to observe estrus (Colazo and Mapletoft, 2014), thus providing an increase in the service rate and avoiding the occurrence of human failures in the detection of estrus. However, gametes can undergo degeneration in the extreme temperatures of summer (Chebel et al., 2008), which makes the transfer of embryos produced in vivo (ET) a strategy to avoid the deleterious effects of this period and provide a higher productive index than with AI (Vasconcelos et al., 2011).

Finally, in Al / FTAI, the genetics of selected bulls are multiplied. In the production of embryos, the offspring of females with high quality genetic traits can also be generated, increasing the improvement to each generation in a qualitative and quantitative way (Marinho and Seneda, 2012). Thus, by offering higher pregnancy rates and economic advantages, beyond accelerating the genetic gain between generations, many dairy farms have partially replaced Al with embryo transfer (Marinho et al., 2012).

#### Expansion of in vitro embryo production (IVEP)

Despite the advantages described, the efficiency of bovine embryo production using superovulation protocols (in the transfer of embryos produced in vivo) is also negatively affected during the periods of high ambient temperature. Climatic variations in the period of activation of the ovarian follicular development and the time of the fertilization can affect the production of embryos, for example.

Until a few years ago, some obstacles impeded the use of large-scale IVEP in dairy cattle. One was the large number of calves born of unwanted sex, greatly increasing the cost of producing these animals. Furthermore, due to metabolic and morphological differences when compared to those produced in vivo, pregnancy rates are lower in the in vitro production of embryos. Likewise, cryopreservation and rewarming processes are more critical for IVP embryos (Abe et al., 2002). However, in the last 40 years the obstacles have been overcome, and IVEP in cattle has gone from a little-used procedure for the treatment of infertile animals to a means to accelerate the genetic gain for many breeds (Sirard, 2018). In the last decade, IVEP use in the world has expanded dramatically when compared to in vivo embryo production (Watanabe et al., 2017). In the study conducted by Perry (2017), they showed that in the year 2016, a total of 666,215 in vitro embryos were produced – and thus, for the first time, surpassed the volume of embryos produced in vivo.

The most advantageous aspect of embryos produced in vitro refers to the great success of the use of sexed semen in this biotechnology. In AI, the in vivo efficiency of sexed semen production has been unsatisfactory. In addition to the greater genetic gain of IVEP when compared to AI, the reduction of costs have contributed to making IVEP a more widely used option in dairy farms. Thus, progress in the use of this biotechnology has enabled the production and generation of millions of valuable products from animals of high genetic value (Sirard, 2018).

#### Sexed semen and cryopreservation in IVEP

In dairy cattle, the common male calf has little to no agricultural value when compared to the female. In this context, many studies have focused on strategies to favor the birth of offspring of the desired sex and consequently increase the efficiency in the production systems. The first studies involving the use of sexed semen dated from the 1980s when Johnson and his team reported on the efficiency of flow cytometry in separating X sperm from Y based on the amount of DNA present in each sperm cell (Seidel, 2007).

Currently, the use of sexed semen represents a commercial scale tool of high applicability. There are still challenges for increasing the use of sexed semen. For example, many sperm are discarded during the sexing process. Although this loss occurs and the cost of the treatment is higher in IVEP, the use of sexed semen provides better results than AI and ET in vivo, because spermatozoa are submitted to fewer challenges at the time of transport to the oocyte (Pellegrino et al., 2016; Hall et al., 2017).

Another technique used in the IVEP is cryopreservation. This process enables the indeterminate storage of embryos after their production, as well as the transport of the embryo for long distances. The cryopreservation of bovine embryos generated in vivo has very well-established and effective protocols; the slow freezing process allows for the storage and easy transport of embryos, thus expanding the dissemination of genetic material. However, IVP embryos are more susceptible to damage caused by rewarming when compared to those produced in vivo, as they present higher lipid accumulation in the cytoplasm (Abe et al., 2002). For this reason, vitrification, a faster and less expensive technique, has been created to obtain an acceptable pregnancy rate.

Alternatives have been successfully employed aiming at the physical or chemical delipidation of embryos and thus increasing embryonic cryotolerance and pregnancy rates on vitrification. Another practice developed was the direct transfer method (DT), which allows the transfer of the IVP embryo without the need for slow thawing, becoming one of the most promising techniques in the field of reproductive biotechnology (Sanches et al., 2016). In the study conducted by Sanches et al. (2016), it was demonstrated that IVP embryos with sexed semen could be transferred directly to recipient cows with similar rates compared to vitrified embryos, providing a more practical approach to IVEP and ET and making logistics easier for the dairy farms.

#### **Final comments**

In the dairy herd, embryo transfer biotechnology is an interesting alternative that can allow good pregnancy rates to be obtained year-round. There have been great advances of this biotechnique with a focus on accelerating the genetic gain between generations. The gains are possible through the development of advanced reproductive technologies. Thus, if the goal is to combine the transfer of embryos and sexed semen, the best strategy at the moment is the technique of in vitro production. Finally, the use of ET together with cryopreserved IVEP embryos or sexed females has a very specific potential for donor replacement and genetic improvement of the herd.

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