ASPRA Asociación Peruana de Reproducción Animal

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SPERMOVA

Spermova 2021; 11(2): 96-102

Artículo de revisión – Review article DOI. 10.18548/aspe/0009.13

MAXIMIZING IN VITRO EMBRYO PRODUCTION IN CATTLE

Maximizando la producción de embriones in vitro en bovinos

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Recibido: 29/11/2021

Aceptado: 13/12/2021

Publicado: 31/12/2021

ABSTRACT

In vitro embryo production (IVEP) is used to develop high-quality genetics associated with intergenerational genetic gain. It is characterized by acquisition (in vivo or post-mortem) and maturation (MIV) of oocytes from donors, followed by fertilization (FIV) of matured oocytes and culture (IVC) of embryos, which are then sent to transferred or cryopreserved. Even with extensive knowledge on IVEP, some biochemical and hormonal regulations that involve embryonic development are still unknown, leading to a low overall efficiency of the biotechnological process. Although in vitro developed embryos have a lower quality than that produced in vivo, in terms of resistance to challenging events, IVEP presents itself as a potential biotechnology. In cattle breeding, reproductive biotechnologies are key to increase and improve the genetic improvement of the herd, associated with productive and reproductive efficiency. In this article, the steps and strategies of IVEP and its contribution to reproduction in the cattle sector are discussed.

Keywords: In vitro fertilization; bovine; reproductive efficiency; reproductive biotechnology.

RESUMEN

La producción in vitro de embriones (IVEP) se utiliza para desarrollar genética de alta calidad asociada con la industria genética. Se caracteriza por la adquisición (in vivo o post-mortem) y maduración (MIV) de ovocitos de donantes, seguida de fecundación (FIV) de ovocitos maduros y cultivo (IVC) de embriones, que luego se envían para transferir o criopreservar. Incluso con un amplio conocimiento sobre la IVEP, aún se desconocen algunas regulaciones bioquímicas y hormonales que involucran el desarrollo embrionario, lo que conduce a una baja eficiencia general del proceso biotecnológico. Aunque los embriones desarrollados in vitro tienen una calidad inferior a los producidos in vivo, en términos de resistencia a eventos desafiantes, IVEP se presenta como una biotecnología potencial. En la ganadería, las biotecnologías reproductivas son clave para incrementar y mejorar el mejoramiento genético del rebaño, asociado a la eficiencia productiva y reproductiva. En este artículo se discuten los pasos y estrategias de IVEP y su contribución a la reproducción en el sector ganadero.

Palabras clave: Fecundación in vitro; bovino; eficiencia reproductiva; biotecnología reproductiva.

INTRODUCTION

In recent decades, there has been a great technification of livestock farms to achieve greater productive efficiency. In this context, in vitro embryo production (IVEP) have played a great role and contributed to the genetic improvement and increased reproductive efficiency of bovine herds (Crowe, Lonergan, e Butler 2021). This importance is demonstrated in the 2017 International Society for Embryo Technology (IETS) records, with the total number of IVEP exceeding those derived in vivo (992,289 vs. 406,287, respectively) (Viana et al. 2018).

However, the efficiency of this technique is 30 –40% of embryos produced at the end of the process. Although the IVEP technique is well established, some adjustments, from in vivo collection (Ovum Pick up-OPU) and in vitro (post-mortem) to cryopreservation processes, to increase the production rate of bovine embryos are to be investigated, considering the influence of category and animal aptitude (heifers, cows, dairy farm, or beef) (Crowe, Lonergan, e Butler 2021).

In this review, we present the basic steps of in vitro embryo production and some of the new challenges to improve the efficiency of IVEP use in the cattle industry. We believe that a combination of full understanding of the process and future vision of the current challenges are the key tools to maximize the IVEP efficiency.

In vitro embryo production

In case of post-mortem cumulus-oocyte complex (COC) recovery, it is interesting to highlight the importance of a trained professional in providing the proper conditions during the transport of ovaries from the slaughterhouse to the laboratory. The transport conditions are variable, but normally the ovaries are deposited in thermos bottles with sterile physiological saline at a temperature of 22 –35 °C (Barberino et al. 2019). However, these variations do not appear to affect oocyte viability if a 4-hour period is not exceeded (Barberino et al. 2019; Crowe, Lonergan, e Butler 2021).

In the laboratory, COC aspiration should be performed with 30 × 8 mm or 40 × 12 mm needles, coupled with syringes or vacuum pump adjusted to 10 mL follicular fluid per minute (Morotti et al. 2014; Costa et al. 2020). The follicular fluid obtained, containing the COCs, is kept in a water bath at 35 °C for cell decantation. The obtained pellet is transferred to a Petri dish, and with the aid of a stereomicroscope, the COC's are recovered and stored in a maintenance medium. In this type of aspiration, only COC's surrounded by a minimum of 3 layers of cumulus cells and homogeneous cytoplasm are selected (grades 1 and 2; (Seneda et al. 2001; Crowe; Lonergan, and Butler 2021).

In contrast, COC's collected from live donors are selected less rigorously, based on morphological classification, as the number of recovered COCs is smaller compared to post-mortem aspiration. Basically, the follicular aspiration technique (OPU) is performed with a guide that supports the ultrasound convex probe (7.5 MHz) and a stainless-steel device with a needle, which picks-up the antral follicles (>2 mm in diameter) visualized on the ultrasound monitor. The needle is connected to a vacuum system and the aspirated follicular fluid is sent into a tube. The follicular fluid is filtered and deposited in Petri dish for search and recovery of COC's under stereomicroscope (Seneda et al. 2001; Seneda et al. 2003; Morotti et al. 2014).

The aspirated follicles are normally at different stages of development and the selected COCs have not yet undergone nuclear and cytoplasmic maturation, an essential process for fertilization to occur, so the in vitro maturation (IVM) step is considered extremely important for the success of embryo production (Pontes et al. 2011; Morotti et al. 2014) Generally, the IVM medium is supplemented with LH, FSH, amino acids, inorganic salts, FBS (fetal bovine serum), and vitamins. The supplementation of culture medium in IVEP is widely researched and is constantly evolving in order to define the ideal conditions for early embryonic development (Rosa et al. 2018; Pioltine et al. 2021).

Once selected, the COC's are deposited in drops, with a specific maturation medium (IVM), covered with mineral oil. The maturation stage lasts for approximately 24 hours in an oven with a controlled temperature of 38.5 °C in a humidified atmosphere and with 5% CO2, 5% O2, and 90% N2 (Cavalieri et al. 2018). The ideal atmosphere for embryonic cultivation has provoked discussions over the years. Regarding O2 tension, most labs use a concentration of 20% and 5%, or even a combination of both at different stages of embryonic development (IVM and in vitro culture (IVC), respectively) (Hirao et al. 2012; Bessi et al. 2021).

Other labs and researchers have already tested the use low O2 concentrations ranging from 1.5– 7%, and observed damage to cell metabolism, impairing the embryo development (Whitty et al. 2021). However, high concentrations of O2 can result in an increase in the reactive oxygen species (ROS). The ROS production is a physiological process, several cell enzymes and metabolic pathways, like oxidative phosphorylation in mitochondria, generates ROS, but its increase exacerbates cellular aging (Yong et al. 2020).

Factors that increase ROS levels, such as high oxygen tension and excess glucose in embryonic culture, impairs the functioning of mitochondria, endoplasmic reticulum, protein transport, and calcium homeostasis within the cell, which can lead to DNA fragmentation and apoptosis, and consequently embryo death (Chu et al. 2013; Landau et al. 2013; Yoon et al. 2014; Chiaratti et al. 2020; Whitty et al. 2021).To minimize cell damage caused by oxidative stress, some potent antioxidants such as tauroursodeoxycholic acid (TUDCA), phenylbutyric acid (PBA), salubrinal, and others are added to culture medium (Zhang et al. 2012; Pioltine et al. 2021). In addition, the co-cultivation of embryos with somatic cells, reduction of embryo exposure to incandescent light and/or adjustments of O2 tension, are also some strategies described to avoid oxidative stress (Bessi et al. 2021).

The next step, after in vitro maturation (IVM), is in vitro fertilization (IVF). The day of fertilization is defined as Day 0, and aims to promote an ideal environment for both COC's and sperm cells, and thus favoring the formation of the zygote. The COC's are transferred to drops of specific fertilization medium, submerged in mineral oil. The semen is selected and prepared before being co-cultured with the oocytes. Atmosphere and temperature conditions remain the same. The duration of the IFV step is approximately 20 hours (Crowe, Lonergan, e Butler 2021).

The effect of the bull on IVF has been suggested as a cause of variation in the final production of blastocysts, including differences between semen batches from the same bull (Siqueira et al. 2018). In this sense, some studies have been carried out to identify characteristics in sperm cells that indicate better performance under in vitro production conditions (Bucher et al. 2019; Kasimanickam 2021).

The last and the longest step in in vitro embryo production is embryo culture. A variety of methodologies, culture media, and atmospheric conditions have been tested over time in order to understand and provide an environment capable of increasing the rate of embryos produced (Costa et al. 2020).

Several parameters such as morphokinetics, lipid content, organelle rearrangement, and, more recently, microvesicles mediated intercellular communication are some of the factors that are commonly studied for the production of in vitro embryos (Chiaratti et al. 2020; de Ávila et al. 2020; de Andrade Melo-Sterza e Poehland 2021; Suzuki et al. 2021). Even so, it has not yet been possible to mimic the ideal physiological conditions of in vivo embryonic development, which is why a great increase in the final production rates of blastocysts is so difficult, even with enormous advances in basic and applied research.

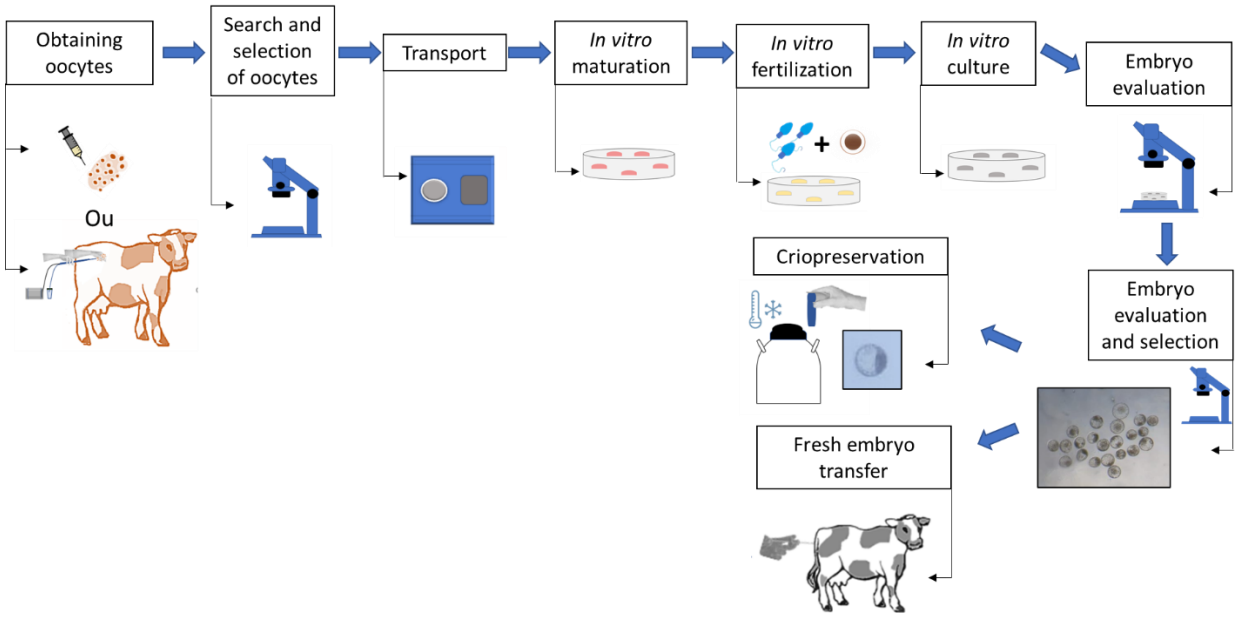

Figure 1. Steps of in vitro embryo production.

Genomic analysis: from animal selection to oocyte and embryo evaluation

Currently, genomic analysis is driving the development of several IVF laboratories worldwide (Sirard 2018). Based on genomic analysis, animal selection based on real genetic merits doubles the genetic progress of traits of economic interest (Wiggans et al. 2017; Yang et al. 2021).

With genomic analysis performed right after birth, the genetic value of the bull is determined early, and after reaching puberty and being able to reproduce, its semen can be used for IVF, shortening the time span between generations. The demand for producing embryos from young heifers and calves has also increased (Sirard 2018). The collection of oocytes from donors before puberty is possible with relatively high success (Landry et al. 2016; Baldassarre 2021). Additionally, genomic selection is helpful for choosing better embryo recipients according to the genes involved in the gestation maintenance, as well as for choosing females that lack markers related to neonatal mortality (Yang et al. 2021).

Genomic evaluations became official in 2009, and since then, more than 1 million animal genotypes have been evaluated (Council on Dairy Cattle Breeding 2016). Due to the popularity of genotyping chips, microsatellites have been replaced by SNPs, and the availaility to chips at a lower cost has made whole herd genotyping common in the US (Wiggans et al. 2017).

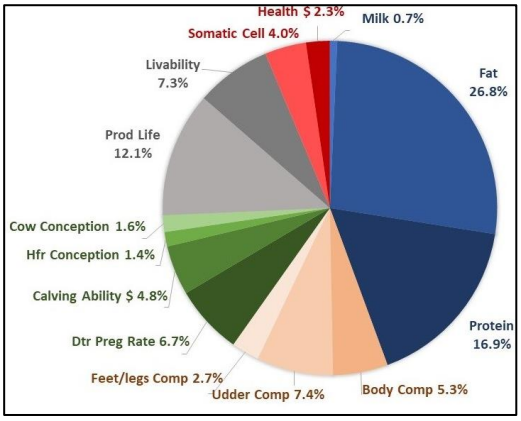

Figure 2. Composition and weighting of the 14 traits in Net Merit. Available on https://hoards.com/article-23717-net-merit-$-index-updated-to-include-health-traits.html accessed on November 15, 2021.

Additionally, cattle can be selected for any combination of traits, but total genetic progress will be the fastest if traits that add the greatest economic value are selected. In this context, the lifetime net merit (NM$) index, elaborated by the U.S. Department of Agriculture (USDA), ranks animals based on their combined genetic merit for economically important traits (Wiggans et al. 2017). The NM$ index includes economically important traits related to health, yield, longevity, and calving ease (Wiggans et al. 2017). The weighting and composition of the 14 traits that make up the net merit are shown in Fig. 5.

Cryopreservation

Cryopreservation is an efficient alternative for storing cells for a long period, in addition to facilitating the commercialization and dissemination of animal genetics with high zootechnical value (Sanches et al. 2016). Cryopreservation aims to keep the cells in a quiescent state, prolonging their viability and allowing their timely use, based on two main factors: cooling/heating rate and the choice of cryoprotectants (Marsico et al. 2019; Arshad et al. 2021).

Despite the use of cryoprotectants, the freezing process causes damage to cells. Additionally, in vitro produced embryos present differences at morphological, metabolic, and chromosomal levels from those produced in vivo, which influence the efficiency of cryopreservation (Sudano et al. 2011). Cryopreservation and rewarming processes are more critical for in vitro embryo than for those produced in vivo. Some studies indicate that low cryotolerance is mainly correlated with the accumulation of lipids in the cytoplasm of these embryos (de Andrade Melo-Sterza e Poehland 2021).

Embryo cryopreservation techniques differ according to the type, concentration, time of exposure to cryoprotectant, freezing speed, physical support, thawing speed, and others (Arshad et al. 2021). Among the cryopreservation methods, vitrification and direct transfer (DT) stand out (Sanches et al. 2017; Arshad et al. 2021).

Vitrification, known as the ultra-fast method, is one of the most used methods as it allows cryopreservation in a short period of time, in addition to having a low cost as it does not require the use of equipments (Yong et al. 2020). This technique uses cryoprotective solutions with a high degree of viscosity associated with high freezing speed; therefore, the medium passes directly to the vitreous state, avoiding the harmful effects of osmotic shock and ice crystal formation inside embryonic cells (Sanches et al. 2017). In contrast, high concentrations of cryoprotectants lead to high toxicity in the cells (Yong et al. 2020).

Due to the use of cryoprotectors in large concentrations, the reverse thermodynamic process of heating and devitrifying embryos requires the presence of a professional to manipulate and assess the quality of the embryo before transfer, which is a limiting factor of the technique (Sanches Et Al., 2017; Vajta; Kuwayama, 2006; Yong et al., 2020). For in vitro embryo cryopreservation, the most satisfactory results have been obtained by the vitrification technique; however, slow freezing is also a good option (Sanches et al. 2017; Arshad et al. 2021). The DT (direct transfer) technique has been used since the 1990s to simplify the rehydration step after thawing of embryos. Due to the low concentration of cryoprotectants, and consequently reduced toxicity to embryos (Voelkel e Hu 1992), the technique has been shown to be suitable for IVEP as it presents satisfactory pregnancy rates (Sanches et al. 2017). The slow freezing technique requires appropriate equipments to promote a gradual freezing curve. Its advantage is the practical and quick thawing, without having to manipulate the embryo, similar to the semen thawing (Arshad et al. 2021)

Advances in IVEP to increase reproductive efficiency

Within the animal food sector, cattle industry leads in the successful application of advanced technologies (Wiggans et al. 2017). The large sequence of genetic selection programs in the past aimed only at increasing milk and beef production, caused considerable losses in the reproductive efficiency of the animals. This created a demand for alternatives and technologies that could promote greater success in reproductive management of cattle (Baldassarre et al. 2018; Makanjuola et al. 2021). The precision of genomic analysis has helped in the evolution and development of new methodologies for harvesting gametes and producing embryos before the animals reach puberty, i.e.,at two months of age, in addition to promoting studies that indicate oocyte quality and embryonic viability (Landry et al. 2016; e Hasler 2017; Wiggans et al. 2017; Sirard 2018; Baldassarre 2021). Improved methods of IVEP and sexed semen have a considerable contribution in the selection and implantation of genetically superior animals, with the potential to maximize the sector's efficiency, as long as they are associated with other essential management techniques for the expression of animal potential (Hansen 2014; Sanches, Zangirolamo, and Seneda 2019; Yang et al. 2021).

The new perception of genomic analysis increased the interest among cattle producers in using prepubertal females for embryo production, in order to accelerate the genetic advancement (Baldassarre et al. 2018). This has been made possible by advances in genetic markers associated with improvements in equipment for retrieval of oocytes by OPU (e Hasler 2017; Baldassarre 2021), supplementation by more accurate culture medium, adjustments in embryo production stages, and improvement in cryopreservation and transfer of blastocysts (Baldassarre e Bordignon 2018; Yong et al. 2020).The medium used in the IVEP steps try to physiologically mimic the organism, having a determining power over the efficient production, quality of blastocysts, and embryonic cryotolerance (O'Shea et al. 2012; Morotti et al. 2014; Makanjuola et al. 2021).

In addition, embryos produced with sexed semen contributes considerably to intensification of selection of females or males in the herd and reducing the time between generations (Murphy et al. 2016; Ettema et al. 2017). The cost of sexed semen is usually higher in comparison to conventional semen. In addition, it has lower sperm viability, decreasing its efficiency when used for artificial insemination (AI). In the IVEP, sexed semen showed satisfactory blastocyst production rates and improved cost-benefits (Carvalho et al. 2010; Matoba et al. 2014; Pellegrino et al. 2016; Hall et al. 2017; Kasimanickam 2021).

The techniques IVF and ET (embryo transfer) have a particular contribution to the dairy herd. In the tropical countries, like Brazil, heat stress that is a great challenge. Since embryos are more resistant to high temperatures than gametes (Pontes et al. 2011), TE minimizes pregnancy losses, allowing for better

pregnancy rates in summer seasons (Stewart et al. 2011; Ferreira 2013; Marinho et al. 2015; Nanas et al. 2021).
There are several techniques that can and are being implemented in the cattle sector to ensure an increase in the reproductive and productive efficiency of the herd. However, the response of animals to these techniques is only possible when associated with greater compliance with vital requirements including well-being, nutrition, health care, and appropriate animal handling. Therefore, the use of reproductive biotechnology should be based on many factors for greater productive and economic efficiency, and sustainability of the cattle sector.

CONCLUSION

Despite the significant challenges of the cattle industry, the development of reproductive biotechnologies associated with the establishment of genomic analysis has been used as a potential tool to increase meat and dairy products to meet the world's demands.

Nowadays, in vitro embryo production has high applicability, contributing to its usage on a large scale. Thus, IVEP is no longer limited to elite animals or animals that do not respond to superovulation. The IVEP actively contributes to the production, improvement, and profitability of dairy and beef production.
The genomic selection of young animals, associated with sexed semen and frozen IVP-blastocysts and following direct transfer protocols, is driving a new era of IVF. However, since many of these processes are sensitive to operators or even the environment, the challenge of making IVF fully business-grade still remains.

Further studies and innovations are required to develop a personalized product, deliver a quality embryo, and ensure a high pregnancy rate.

Conflict of Interest

Authors declare no conflict of interest.

Author Contribution

C.B.C. and T.K.S. developed the first text. Both M.A.A. and D.N.Y. contributed to the final version of the manuscript. M.M.S. prepared the final review and edition.

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