In Vitro Embryo Production: Overview

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Abstract

Reproductive biotechnologies, also known as assisted reproductive technologies (ART), have undergone significant development over time, reaching a remarkable level of evolution in the 20th century. Currently, several biotechnologies stand out, among which Artificial Insemination (AI), widely spread globally, Embryo Production (EP) both in vivo and in vitro for embryo transfer, Intracytoplasmic Sperm Injection (ICSI), sexing, among other innovations. In Vitro Production (IVP) of embryos has emerged as the biotechnology that has experienced the greatest development and evolution in recent decades, covering various fields such as research in assisted human reproduction, obtaining transgenic animals for biomedicine, preservation of endangered species and conservation of animals of high genetic value. This research aims to analyze the relevance of IVP, as well as the fundamental elements that make up this complex process. IVP of embryos involves several stages, from oocyte procurement, oocyte selection and in vitro maturation, sperm preparation, in vitro fertilization, to the culture of the resulting zygotes to blastocysts. These blastocysts, once transferable to recipients, culminate in the birth of a new being. However, this process has both advantages and disadvantages, which motivates continuous innovation in the field.

Keywords: In vitro production, in vitro maturation, fertilization, in vitro, in vitro embryo culture.

1. Introduction

The existence of animals of high genetic value, which have completed their life cycle, genetic improvement, the preservation of the genetics of endangered species, as well as the emotional state of a couple that cannot have offspring due to multiple factors, also the use of transgenic pig embryos for biomedicine, have been of interest to the scientific community (Romar, et al. 2015), for the development of multiple research projects leading to the evolution of reproductive biotechnologies or assisted reproductive technologies (ARTs). There is a variety of

biotechnologies used in assisted reproduction, among which we can mention: AI, PE for embryo transfer, ICSI, sexing, among others. Currently, IVP is the biotechnology that is being studied and developed the most in several countries, where the costs of assisted reproduction are very high, and it is also booming for livestock farms, this process includes obtaining and MRV (In vitro maturation) of oocytes, capacitation in vitro of spermatozoa, the co-culture of both gametes or IVF (In vitro fertilization) and the CEIV (In vitro culture of resulting embryos) (Ho et al., 2023; Sato et al., 2023; Lee et al., 2023). Sexing, together with IVP, constitutes the third generation of reproductive biotechnologies (Campanholi et al., 2023; Rosa et al., 2023). This review describes historical background to the process of IVP of bovine and porcine embryos including, oocyte retrieval, oocyte selection, oocyte IVM, sperm preparation, IVF and culture of the resulting zygotes to blastocysts (Mendes et al., 2023). The objective of this review is to provide an overview of bovine and swine IVP; and is aimed at professionals who are immersed in the techniques used in embryo IVP.

DEVELOPMENT

The history of assisted reproduction dates back to 1878, where Schenck tried to fertilize in vitro rabbit and guinea pig eggs without achieving any success (Magata, 2023). IVF has its origins in the late nineteenth century, mainly in 1890 when the first IVF experiments were carried out, Heape successfully performed the transfer of rabbit embryos (Bajpai & Chaturvedi, 2023). Heape then retrieved rabbit embryos by washing oviducts, then transferred them to a Belgian rabbit that were successfully viable (Carrillo-González et al., 2023). In 1959, thanks to the experiments carried out by Chang on rabbits, the unequivocal results of IVF could be verified, because for some time there were many doubts about its results (Izady et al., 2022; Falchi et al., 2022). The studies carried out by Heape opened a door for scientists to show them a wider field in assisted reproduction making it possible to culture embryos in the laboratory, perfecting the study of early embryonic development (Mendes et al., 2023). The first attempts at artificial fertilization in mature bovine oocytes in vitro, with pre-incubated bull sperm reported by Sreenan in Ireland (Mehbub et al., 2022). Edwards in 1965 published the first works on maturation in vitro, of bovine oocytes (Duarte-Da-Fonseca Dias et al., 2022). Thanks to the efforts of Iritani and Niwa, in 1977 in Japan they reported the first success of IVF, from an artificially matured bovine oocyte (Rakha et al., 2022). In 1981, the birth of the first IVF calf is reported, from an embryo obtained in vitro (Roshan Krishna et al., 2022).

Techniques used for embryo IVP

IVP of embryos requires techniques such as: oocyte retrieval, oocyte selection, oocyte IVM, sperm preparation, IVF and culture of the resulting zygotes to blastocysts (Dellaqua et al., 2023).

Oocyte collection

The importance of oocyte collection for IVF lies in the fact that it allows the recovery and use of anovulatory follicles that, under physiological conditions, in vivo, would end up as atretic follicles, due to the feedback of endocrine regulation (Dellaqua et al., 2023; Dode et al., 2023). There are basic procedures for obtaining oocytes, among which we can mention: Aspiration of follicles, from females slaughtered in a slaughterhouse (Dode et al., 2023). Oocyte collection by means of the SLICING, from ovaries from slaughterhouse cows (Boruszewska et al., 2020),

ultrasound-guided transvaginal aspiration also called OPU (Ovum Pick-up), which allows us to collect oocytes in females over 6 months of age, also during the first 3 months of gestation and from 2 to 3 weeks postpartum, which is why it does not interfere with the productive and reproductive cycles of donor females (Carrillo-González et al., 2020). Oocytes can also be obtained laparoscopically, in females less than 6 months old (Grycmacher et al., 2019).

Oocyte selection

All oocytes obtained by any method are destined to degenerate either into mono-ovulatory species such as cows or multi-ovulatory species such as sows (Huang, Zhang, Mei, et al., 2023), for this reason it is important to estimate its quality. These oocytes obtained are immature and very necessary for IVP, and also present variations in the appearance of the cytoplasm, in the cluster and in its morphology (Besenfelder & Havlicek, 2023). In the selection of viable oocytes, three criteria are generally taken into account: the diameter of the oocyte, the appearance of the cytoplasm and the characteristics of the clusters surrounding the oocyte (Ammari et al., 2022). In addition, a morphological evaluation should be carried out using a magnifying glass between 40 and 60 magnifications (Stamperna et al., 2021). The most competent or viable immature oocytes are those that are surrounded by a cluster, made up of the cells of the crown radiated in a compact way, forming several layers of cells (Travnickova et al., 2021). These characteristics ensure higher percentages of maturation, fertilization and development up to blastocyst (Silva et al., 2021).

In vitro maturation

IVM has become a topic of great importance, due to its numerous clinical applications in the field of assisted reproduction (Wang et al., 2023), as well as in the development of research into the cloning and production of transgenic animals (de Aquino et al., 2023). In addition, it has become the decisive stage in the embryo production process in vitro, but in spite of this, a lot of literature can still be found from many laboratories in which they still use the procedure of Fukui and Ono (1989), which consists of using (Tissue culture medium) TCM-199, as a medium for the culture of immature oocytes (Ho et al., 2023). Naspinska et al (2023) mention about the supplementation of sodium pyruvate, antibiotic, luteinizing hormone, 17β estradiol and 10% fetal bovine serum (FBS) to TCM-199 achieving excellent results.

Likewise, Reshi et al (2023)It states that TCM-199 was designed to meet the needs of somatic cells during prolonged periods of culture, which makes it inappropriate for the needs of maturing oocytes. Currently, what is being used as a culture medium for bovine oocytes is mSOF (Synthetic Oviductal Fluid modified), the OF (Oviductal Fluid) obtained from the culture of oviductal cells, for maturation of bovine and porcine oocytes (Rabaglino et al., 2023). While for the IVM of pig oocytes the medium that is being used is the NCSU-37 (Miglio et al., 2023).

Sperm preparation

The sperm used for IVF can be obtained from cryopreserved straws, epididymal or in turn fresh ejaculate (Wysok et al., 2022), which must be separated from both the seminal plasma as well as defective spermatozoa, taking into account the normal morphology, adequate motility and excellent DNA integrity of the rest of the population (Alcaráz et al., 2022). For pre-training

sperm selection and preparation in vitro, there are different systems that can be used, depending on the species, among which we can mention: albumin washes, migration technique (Swim-up, Swim-down, Self-Migration, Migration-sedimentation), filtration technology (fiberglass filtration and filtration in glass columns). Sephadex), density gradients (Percoll).

Regardless of the sperm selection method used, sperm will be capacitated later since the selection systems mentioned above do not lead to sperm capacitation in the different species (Shi & Sirard, 2022). However, it has been shown in humans, rodents and pigs that spermatozoa washed through Percoll gradients have a higher level of fertilization unlike those obtained only by centrifugation washing (Shi & Sirard, 2022).

In vitro fertilization

Sperm capacitation in vitro, tends to simulate the phenomena that occur in the oviductal environment, in such a way that spermatozoa are incubated under certain conditions of humidity, temperature and CO_2 (Huang, Zhang, Li, et al., 2023), among the media that are generally used are: Tyrode, supplemented with energy sources (pyruvate, lactate and glucose) and serum albumin, depending on the species (Kępka et al., 2023). Fertilization in vitro, also known as insemination, is the procedure in which mature oocytes are cultured with selected sperm, leading to fertilization. Just as it happens in physiological conditions or in vivo, sperm reach their fertilizing capacity, in vitro Similar phenomena must occur, for which sperm capacitation must occur, the acrosome reaction must be triggered, penetration of the zona pellucida, formation of pronuclei and syngamy (Reshi et al., 2023).

In vitro embryo culture

The decade of the 80's was key for the development and evaluation of numerous culture media, in order to obtain adequate rates of transferable and freezable blastocysts on day 7 (Martin-Pelaez et al., 2023). In the first studies on swine ECs, it was possible to reach the stage of 4 cells from an embryonic cell obtained in vivo (de Lima et al., 2023), which coincides with the transmission of genetic control from the mother to the embryo (Tayefeh et al., 2023).

Subsequently, thanks to co-culture with oviduct epithelial cells and obtaining FO during CE, the 4-cell stage was overcome (Melo-Báez et al., 2023). Subsequent studies on obtained embryos in vivo and cultivated in vitro, showed more than 70% developing to the blastocyst stage using the following means (dos Santos et al., 2023): modified Whitten's medium (Beckmann and Day, 1993), NCSU23 medium, medium Iowa State University (Youngs et al., 1993) and medium BECM-3 (Jaworska et al., 2023). The first studies carried out in cattle EC in vitro, did not exceed the stage of 8 to 16 cells (developmental block), and did not imply the immediate death of the embryo. Subsequently, co-culture with somatic cells supplemented with serum was used to avoid this blockage. However, over time it was shown that this medium did not cover the needs of the embryo in its early development stage, in turn it incorporated pathogens during culture and was responsible for the presence of the syndrome of excess fetal volume and low resistance to cryopreservation (Velázquez, 2023). To perform the CE of pigs there are different alternatives both in the culture system using (mixtures of gases, mineral oil, number of embryos); or in the composition of the medium, culture dynamics (static or dynamic system, tranquility or simple or sequential stress).

The culture methods have been performed on plates of 20mm in diameter under different conditions of medium volume and embryo density, which physiologically are not ideal since they do not resemble the conditions in vivo, for this reason new methods have been developed, among which the following can be mentioned (Janini et al., 2023): microwells (Vajta. et al, 2000), PCR tubes (Polymerase Chain Reaction; Roh. et al, 2008), glass capillaries (Martin-Pelaez et al., 2023), microfluidics in microchannels (Pohjanvirta et al., 2023). The culture media should imitate as much as possible the oviductal environment in which the embryo is found in vivo, to achieve optimal embryonic development. Generally, the culture media must be made up of water, inorganic salts, energy compounds, proteins, amino acids, chelators, growth hormones or vitamins, antibiotics, etc., and must also be fully sterilized by membrane filtration with a pore diameter of 0.22 μm. (Hitit et al., 2023), in turn, the osmolarity required by the cells (245mOsm/kg) must be taken into account, as well as an optimal pH (7.2-7.6).

Macromolecules or substrates are also being used in culture media such as fetal bovine serum (FBS) and bovine serum albumin (BSA) (González-Rodríguez et al., 2022). The culture media that are being used in pigs for embryonic development, from zygote to blastocyst in vitro, are: Whitten's medium, NCSU23, BECM-3 and Bavister, and PZM 5 (Colombo et al., 2022). While in the simplest media most used for the culture of bovine embryos are: KSOM (Sahoo & Gupta, 2023) and the SOF (Hammoud & Jebur, 2022). Several authors point out that the use of a single culture medium during the entire culture period does not show excellent results, for this reason modifications are made (Ju et al., 2023). However, it is necessary to continue developing more research in order to improve the culture media, which meet all the needs of the embryo during its different stages of development.

2. Methodology

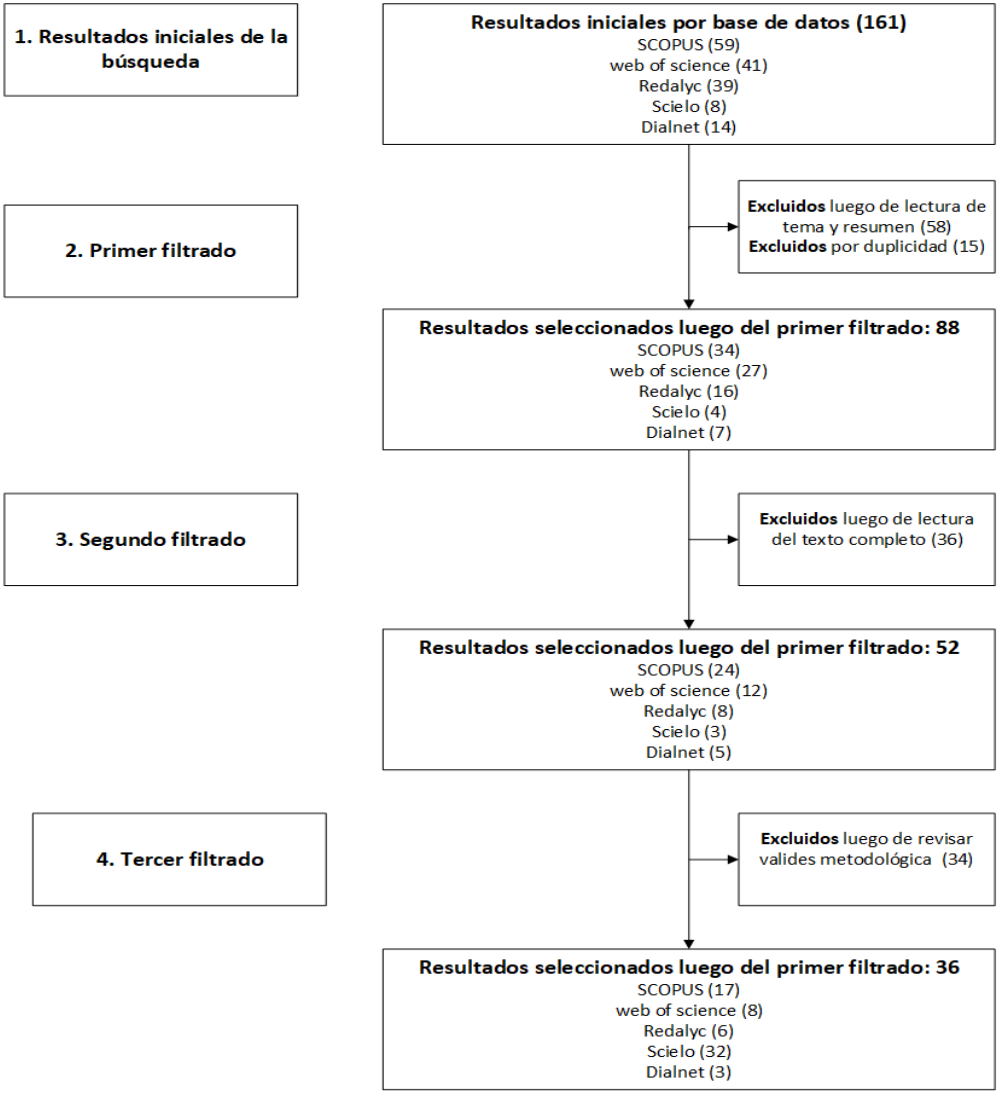
Within the research, a systematic review was carried out, following the recommendations of the (Higgins JPT, 2011), the recommendations of the PRISMA Report and the steps that have been proposed by various authors for the systematic reviews of scientific articles (Ferreira González et al., 2011; Perestelo-Pérez, 2013). In order to have a greater scope for scientific production on the topic of "In vitro production of embryos", a survey of information was carried out in Scopus, Web Of Science, Redalyc, Scielo as they are the search engines that contain the largest amount of information on the subject analyzed.

The search process was carried out through keywords such as "In vitro reproduction of embryos", "In vitro production of embryos", "In vitro fertilization (IVF)" and "In vitro fertilization (IVF)", another inclusion criterion was access to scientific production of the last five years, of the same information from scientific articles, leaving aside review articles, procedure books, conference proceedings, publications, book chapters to have a better delimitation of access to scientific production.

3. Results

By applying the criteria and recommendations established by the PRISMA 2020 declaration (Page et al., 2021), the following results were established as described in Figure 1:

Figure 1 Search results for scientific articles . In original language Spanish



A total of 36 articles that met the inclusion and exclusion criteria contributed to an understanding of IVP, from which the abstract, methods, results, and conclusions were chosen.

Board 1 Articles that complied with inclusion and exclusion

Article	Year	Summary	Methods	Results
Association between the morphokinetics of in-vitro-derived bovine embryos and the transcriptomic profile of the derived blastocysts	2022	The article analyzes the association between the morphokinetics (developmental patterns) of in vitro derived bovine embryos and the transcriptomic profile of the resulting blastocysts. It does not specifically focus on the process of in vitro reproduction of bovine embryos.	- Time-lapse system for continuous evaluation of embryonic development - Microarray analysis to study the transcriptomic profile of blastocysts	- The study found an association between the morphokinetics of bovine embryos and the transcriptomic profile of blastocysts. - Abnormally split embryos had a lower ability to develop into blastocysts.
Ovum Pick-Up and In Vitro Embryo Production in Bovine	2021	The article discusses in vitro embryo production in cattle, including the egg collection process (OPU) and the potential to integrate genomics and artificial intelligence into OPU-IVEP technology.	- Egg collection (OPU) - In vitro embryo production (IVEP)	- Egg collection (OPU) and in vitro embryo production (IVEP) have the potential to improve milk production globally. - The efficacy of OPU-IVEP can be improved by ovarian stimulation and media modulation.
In Vitro Generation of Bovine Embryos	2022	The article discusses two methods for generating bovine embryos: one that uses zygotes retrieved from donor cows through surgery or slaughter, and the other that uses in vitro generation of zygotes from oocytes collected from slaughterhouse ovaries.	- Pronuclear microinjection of bovine zygotes for the production of transgenic cattle. - In vitro generation of bovine embryos using oocytes collected from ovaries from slaughterhouses.	- In vivo generated bovine zygotes have a greater capacity for development. - Transvaginal ultrasound-guided oocyte retrieval can combine the advantages of different protocols.
Method for selecting bovine in-vitro transplantable embryos	2020	The article discusses a method for selecting in vitro transplantable bovine embryos, but does not provide information on the in vitro reproduction process of bovine embryos.	- Method for selecting in vitro transplantable bovine embryos - Selection of embryos at six and seven days of culture	- The method improves embryo quality in vitro and the pregnancy rate after transplantation. - It reduces the rate of presence of non-pregnant cattle and production costs.
Pre-Implantation Bovine Embryo Evaluation—From Optics to Omics and Beyond	2023	The paper discusses the use of in vitro production (IVP) of bovine embryos in the context of embryo transfer technologies. It mentions that approximately 80% of the bovine embryos	- Microscopic techniques (differential interference contrast, electron microscopy, fluorescent, time-lapse, and AI-based) - Non-microscopic techniques (genomics, transcriptomics, epigenomics, proteomics, metabolomics, and	- The article reviews several techniques for embryo evaluation in bovine reproduction. - The document highlights the need for accurate, non-invasive and field-friendly assessment methods.

		transferred in 2021 were produced in vitro.	nuclear magnetic resonance)	
The proteomic analysis of bovine embryos developed in vivo or in vitro reveals the contribution of the maternal environment to early embryo	2022	The article analyzes the proteomic analysis of bovine embryos developed in vivo or in vitro, comparing the levels of protein expression between the two. It provides insights into the molecular contribution of the maternal environment to the developmental ability of early embryos and suggests improvements for in vitro culture systems. However, specific details about the in vitro reproduction of bovine embryos are not mentioned in the paper.	- Nanoliquid chromatography combined with tagless quantitative mass spectrometry - Multivariate analysis of quantified proteins	- Proteomic analysis showed clear differences in protein expression between embryos in vivo and in vitro. - In vivo embryos showed increased degradation of mitochondrial proteins and upregulation of carbohydrate metabolic pathways.
Effects of single or serial embryo splitting on the development and morphokinetics of in vitro produced bovine embryos	2022	The article discusses the effects of individual or serial embryo division on the development and morphokinetics of bovine embryos produced in vitro. It does not provide information on the in vitro reproduction process of bovine embryos.	- Embryo division on day 2, day 3 and blastocyst stage - Single division and serial division of embryos in the excision phase	- Dividing the third day embryos into four parts produces the highest production of viable embryos. - Simple division is better than serial division in terms of embryonic viability.
Dissecting the molecular features of bovine-arrested eight-cell embryos using single-cell multi-omics sequencing	2023	The article discusses the molecular characteristics of eight-cell bovine embryos, including the causes of developmental failure in preimplantation embryos after in vitro fertilization. However, it does not focus specifically on the in vitro reproduction process of bovine embryos.	- Single-cell multi-omics sequencing - Profiling of copy number variations (CNV), transcriptome, DNA methylome, and chromatin status	- Blocking the development of eight-cell embryos may be due to multiple molecular layers, including CNVs, abnormal DNA methylation and chromatin accessibility, and insufficient expression of EGA genes. - Aneuploid embryos inferred by CNVs tended to lose their ability to develop.
Preimplantation Genetic Testing for Aneuploidy Improves Live Birth Rates with In Vitro Produced	2021	The article discusses the use of in vitro production (IVP) of bovine embryos as a method to enhance genetic gain in cattle. He	- Three preimplantation genetic testing approaches were used to detect aneuploidy (PGT-A). - SNP genotyping and calculation of estimated	- Aneuploid embryos are unlikely to establish a pregnancy and be born alive. - Preimplantation genetic testing for aneuploidy (PGT-

Bovine Embryos: A Blind Retrospective Study.		mentions that approximately one million IVP bovine embryos are transferred worldwide each year.	breeding values (GEBVs) were applied.	A) improves pregnancy and live birth rates.
Oocyte Selection for In Vitro Embryo Production in Bovine Species: Noninvasive Approaches for New Challenges of Oocyte Competence.	2021	The paper discusses the efficiency of in vitro embryo production in bovine species, but does not provide specific details on the in vitro reproduction process of bovine embryos.	- Identification of non-invasive markers associated with oocyte quality in bovine species. - Combination of oocyte and zygote selection using various techniques.	- Non-invasive markers associated with improved oocyte competence in cattle species. - Combination of oocyte and zygote selection methods for better evolutionary competence.
Genomic selection in beef cattle creates additional opportunities for embryo technologies to meet industry needs.	2022	The paper mentions that the number of embryos created with in vitro technologies has increased considerably since 2015 and now accounts for about 30% of all calves with embryo transfer. Therefore, the article does analyze the in vitro reproduction of bovine embryos.	- Genomic selection - Reproductive technologies (AI, embryo transfer, in vitro technologies)	- Genomic selection has improved the predictability of the expected progeny difference. - Genomics has allowed embryonic technologies to have a greater impact on cattle.
Recent progress in bovine in vitro-derived embryo cryotolerance: Impact of in vitro culture systems, advances in cryopreservation and future considerations.	2020	The paper discusses the cryopreservation of in vitro derived bovine embryos, but does not provide specific information on the in vitro reproduction process of bovine embryos.	- Programmable slow freezing - Vitrification	- In vitro derived bovine embryos are sensitive to cooling and cryopreservation. - No suitable protocol for cryopreservation has yet been developed.
Deciphering two rounds of cell lineage segregations during bovine preimplantation development	2021	The article discusses an optimized in vitro culture method for bovine embryos, which allows the successful reproduction of blastocyst embryos and their subsequent transfer to surrogate mothers.	- "Gel" culture method using agarose gel filled with nutrient-rich media - Immunofluorescence studies and comparison of RNA sequences for molecular analysis	- Blastocyst embryos derived from a "gel" culture successfully implanted in surrogate mothers. - The proportion of TE cells expressing the pluripotent ICM marker, OCT4, decreased over time.
Update on the vitrification of bovine oocytes and invitro-produced embryos.	2019	The paper discusses the vitrification of bovine oocytes and in vitro produced embryos (IVPs), but does not provide specific details on the in vitro	- Vitrification of oocytes and embryos produced in vitro (IVP) - Maturation of bovine oocytes in the presence of trans-10 and cis-12 conjugated linoleic acid (10T,12c-CLA)	- The article discusses the challenges and improvements in the vitrification of bovine oocytes and embryos produced in vitro. - The document highlights the need for better cryopreservation

		reproduction process of bovine embryos.		procedures to improve pregnancy rates.
Gene expression analysis and in vitro production procedures for bovine preimplantation embryos: Past highlights, present concepts and future prospects.	2019	The paper discusses improvements in in vitro production (IVP) of bovine embryos, which has been widely applied under field conditions. He mentions that more than half a million IVP embryos are generated each year, which demonstrates the potential of this technology.	- In vitro production (IVP) of bovine embryos - Transcriptomic profiling for gene expression analysis	- In vitro production of bovine embryos has improved significantly. - Bovine embryos are used as a model system to study early embryogenesis in mammals.
In Vitro Culture Alters Cell Lineage Composition and Cellular Metabolism of Bovine Blastocyst	2023	The article discusses the effects of in vitro culture on bovine blastocysts, including differences in cell lineage composition and cell metabolism. However, it does not specifically address the process of in vitro production of bovine embryos.	- Single-cell transcriptomic analysis using scRNA-seq - Gene pool enrichment (GSEA) analysis to examine developmental potential	- In vitro culture delays the engagement of cell fate with the inner cell mass (ICM). - Nutrient-reduced culture medium improves blastocyst development compared to conventional culture medium.
Cathepsin-L Secreted by High-Quality Bovine Embryos Exerts an Embryotrophic Effect In Vitro	2023	The article analyzes the in vitro production of bovine embryos and explores the role of L-cathepsin as an embryotrophic factor to improve embryo development and quality.	- Proteomic screening of conditioned culture media by bovine embryos - Liquid chromatography and tandem mass spectrometry (MS) analysis	- Cathepsin-L is secreted by embryos of excellent and good quality. - L-cathepsin supplementation improves blastocyst development and quality in bovine embryos.
Influence of fetal calf serum on the production of bovine embryos in vitro	2023	The article discusses the effect of fetal calf serum (FCS) on the production of bovine embryos in vitro. He found that the addition of FCS to embryo culture media improved the efficiency and quality of bovine embryo production. The optimal time to add FCS was immediately after fertilization.	- Two experiments were conducted to evaluate the effect of fetal calf serum (FCS) on the production of bovine embryos in vitro. - The experiments consisted of dividing mature bovine oocytes in different embryo culture media.	- The addition of FCS to embryo culture media improved the production of bovine embryos. - The addition of FCS at 0 h of fertilization resulted in higher embryo quality.
Comparison of in vitro Maturation Media on Cattle Oocytes after in vitro Embryo Production	2022	The article analyzes the in vitro production of bovine embryos using different maturation media and their effects	- Follicular fluid recovery using aspiration and cutting techniques - Oocytes were sorted and matured into four different media groups	- TCM 199, BO-IVM®, and VitroMat Protect® media had higher oocyte polar body extrusion rates compared to EGF medium. - Vitrofert® medium had a higher total

		on embryonic development.		fertilization rate compared to other media.
An updated protocol for in vitro bovine embryo production	2022	The paper provides a protocol for the maturation, fertilization and in vitro culture of bovine embryos up to the blastocyst stage, with high rates of excision and blastocyst.	- Maturation and fertilization of bovine oocytes - Culture of presumed zygotes to the blastocyst stage	- Excision rate greater than 70% - Blastocyst rate greater than 20%
Effects of serum addition to culture medium on efficiency of producing in vitro embryos in cattle	2022	The article analyzes the efficiency of in vitro embryo production (IVP) in cattle using the BO-IVC medium. It is concluded that the medium is highly effective in obtaining IVP embryos in cattle.	- In vitro embryo production (IVP) and transfer to recipient animals - Fetal bovine serum (FBS) supplementation in culture medium	- The addition of fetal bovine serum (FBS) to the culture medium increased the viability of blastocysts. - BO-IVC medium is very effective for obtaining embryos produced in vitro in cattle.
Vitrification of Bovine Oocytes and Embryos	2023	The document does not provide information on the in vitro production of bovine embryos. The article focuses on improving the outcomes of bovine oocyte vitrification and on analyzing the long-term effects of vitrification on gene expression in bovine blastocysts.	- Extended culture after heating, addition of EGTA to vitrification solutions, and resveratrol supplementation. - Analysis of global gene expression by RNA sequencing of elongated embryos.	- The study aimed to improve the results of vitrification of bovine oocytes. - The long-term effects of vitrification were analysed using global gene expression.
Effects of fetal bovine serum on trophectoderm and primitive endoderm cell allocation of in vitro-produced bovine embryos	2022	The article discusses the effects of fetal bovine serum on cell differentiation in the early development of bovine embryos, but does not provide information on the in vitro production process of bovine embryos.	- Bovine embryos were produced in vitro and randomly distributed into three experimental groups. - Experimental groups included FBS supplementation, medium volume renewal, and no supplementation.	- The blastocyst rate was higher in the KSOM-FBS group compared to the KSOM-zero group. - FBS supplementation altered cell allocation during the early stages of bovine embryonic development.
Effect of culture conditions on gene expression in manipulated bovine embryos	2022	The article analyzes the effects of different culture systems on the developmental competence of bovine embryos produced in vitro.	- Experiment 1: Comparison of embryos cultured in synthetic oviductal fluid (SofAA) or in optimized potassium simplex medium (KSoMaa) supplemented with amino acids. - Experiment 2: Comparison of embryos cultured in sofaA or ksomAA with or without	- Different culture systems and protein sources affected gene expression in embryos. - OCT-4 and GLUT-1 were upregulated in blastocysts cultured under certain conditions.

			the addition of calf serum (CS).	
The Effect of Co-Culture Systems From Non-Reproductive Origins on the Development of Superovulated and in Vitro Fertilized Bovine Embryos.	2022	The article analyzes the in vitro development of bovine embryos obtained from superovulation and in vitro fertilization (IVF) using different co-culture systems. It does not specifically focus on the production of bovine embryos in vitro.	- Two unique monolayer co-culture systems were used: monolayers of bovine fetal spleen cells (BFS) and monolayers of chicken embryo fibroblasts (CEFs). - The developmental capacities of the IVF-cleaved bovine embryos were compared between four treatments: cluster cell monolayers (treatment A), BFS (treatment B), CEF (treatment C) and a control in tissue culture medium (TCM-199) alone (treatment D).	- The BFS and CEF co-culture systems had no detrimental effects on the development of murine embryos. - The CEF treatment was as effective as the cluster cell monolayer treatment in terms of the development rate of the embryos fertilized by in vitro fertilization of cows until the hatched blastocyst stage.
Improvement of bovine in vitro embryo production by fetal calf serum and cysteamine supplementation and investigation of freezability	2021	The article investigates the effects of cysteamine and fetal calf serum on the production and freezing capacity of bovine embryos in vitro.	- Collection and selection of oocytes by the cutting method - Maturation of oocytes in TCM-199 medium buffered with HEPES, with or without cysteamine	- The highest cleavage rate was observed in the CR1aa+Cys group. - The highest blastocyst rate was observed in the SOF+FCS+Cys group.
Transcriptional Profiling of Porcine Blastocysts Produced In Vitro in a Chemically Defined Culture Medium.	2021	The paper provided deals with the transcriptional profile of porcine blastocysts produced in vitro, not bovine embryos. The document does not provide information on the in vitro production of bovine embryos.	- Transcriptome analysis of cultured porcine blastocysts in defined and undefined media. - Microarray analysis to compare gene expression under different culture conditions.	- There are no differentially expressed genes between the PF4 and BSA groups. - 2780 and 2577 degrees were detected when comparing PF4 or BSA with group IVV.
In Vitro Bovine Embryo Production: The Role of FCS and Cysteamine on Cleavage Rate	2021	The article discusses the effects of cysteamine and fetal calf serum (FCS) on cleavage rates in the in vitro production of bovine embryos.	- Oocytes obtained from ovaries sacrificed using the cutting method - Embryos cultured in incubators containing CO2, N2 and moisture	- The cleavage rates of bovine embryos were not significantly affected by the addition of cysteamine and fetal calf serum (FCS) in different culture media. - The highest cleavage rate was achieved in group B2 (CR1 CF-) with 69.9%, followed by group A2 (SOF CF-) with 67.8%.
Application of platelet-rich plasma in the in	2020	The article discusses the use of platelet-rich plasma (PRP) in the in vitro production of	- Platelet-rich plasma (PRP) used as a substitute for fetal bovine serum (FBS) - PRP is added to	- Excision was higher in the group with 5% PRP. - The percentage of blastocysts was

vitro production of bovine embryos.		bovine embryos. It is suggested that the addition of PRP to the embryonic culture medium at a concentration of 5% may increase the quantity and quality of bovine embryos produced in vitro.	bovine embryo maturation and culture media	higher in the group with 5% PRP.
Culture Medium and Sex Drive Epigenetic Reprogramming in Preimplantation Bovine Embryos.	2021	The article discusses the impact of in vitro culture on DNA methylation in bovine embryos, but does not specifically mention the process of in vitro production of bovine embryos.	- Whole genome DNA methylation profiles - Hierarchical cluster analysis	- In vitro culture affects DNA methylation in bovine blastocysts. - Differences in methylation by sex were observed at the blastocyst stage.
Developmental and molecular response of bovine embryos to reduced nutrients in vitro	2021	The paper discusses the development and molecular response of bovine embryos to in vitro nutrient reduction, but does not focus specifically on the in vitro production of bovine embryos.	- Blastocyst formation, hatching, and cell allocation were assessed on day 7. - Western blot analysis and RT-qPCR were used for molecular analysis.	- Blastocyst formation and hatching were inhibited with a nutrient concentration of 6.25%. - Reduced nutrient conditions led to increased AMPK activity and altered the abundance of certain transcripts.

4. Discussion

The literature review provides a comprehensive overview of in vitro reproduction of bovine embryos, covering various facets ranging from morphokinetics and transcriptomic profiling to transplantable embryo selection and improvements in cryopreservation. These studies collectively highlight the complexity of this field and suggest key strategies to improve the efficiency and quality of in vitro production of bovine embryos.

The association found between the morphokinetics of bovine embryos and the transcriptomic profile of blastocysts highlights the critical interrelationship between morphological development and gene expression. These findings open the door to more accurate approaches to assessing the viability and developmental potential of embryos, which may be instrumental in increasing success rates in in vitro reproduction (de la Fuente et al., 2023).

The comparison of methods for the in vitro generation of bovine embryos reveals the importance of oocyte retrieval guided by transvaginal ultrasound. This technique, combined with other protocols, offers a promising prospect for improving process efficiency. The study also highlights the need for further research into new strategies that can overcome current limitations and increase the rate of embryonic development (Contreras-Benicio et al., 2022).

The comprehensive review of embryo assessment techniques, ranging from microscopic methods to omics approaches, highlights the diversity of tools available to analyse embryo

viability. The need for precise and non-invasive methods stands out as a critical aspect for the advancement of in vitro reproduction, especially considering the high rates of embryos generated in vitro being transferred globally (Sahoo & Gupta, 2023).

Proteomic analysis of embryos developed in vivo and in vitro reveals significant differences in protein expression. These findings suggest the crucial contribution of the maternal environment to protein expression and highlight the importance of improving in vitro culture systems to more accurately reflect the uterine environment (Zacchini & Heber, 2021).

Analysis of cryopreservation of in vitro derived bovine embryos reveals that although progress has been made, a suitable protocol has not yet been developed (Rabel et al., 2023). This area remains a critical challenge, and the need for better cryopreservation procedures is highlighted as a priority to improve pregnancy rates and embryo viability after cryopreservation (Marsico et al., 2023).

The study addressing the impact of genomic selection highlights the considerable increase in the in vitro production of bovine embryos and their significant contribution to the livestock industry. Genomics not only improves the predictability of the expected progeny difference, but also amplifies the impact of embryonic technologies on cattle (Baños, 2023).

5. Conclusion

IVP is a biotechnology that is constantly innovating and developing, and is also standardized around the world, achieving significant advances in research, human assisted reproduction, biomedicine, preservation of endangered species, conservation of animals with high genetic value, among others. The culture media used for both IVM and CE do not fully satisfy all the needs of the embryo, so there are certain disadvantages when it comes to obtaining a fully viable embryo.

The association between embryo morphokinetics and blastocyst transcriptomic profile highlights the importance of adequate morphological development for embryonic success. The identification of abnormal patterns in cell divisions can serve as an indicator of the development capacity of embryos.

Different approaches to embryo generation, whether through in vivo zygote retrieval or the use of slaughterhouse oocytes, offer valuable insights. The choice of method should be carefully considered, taking into account the developmental capacity and the specific advantages of each approach.

The integration of genomics and artificial intelligence into in vitro egg collection and embryo production (OPU-IVEP) technology shows great potential to improve the efficiency and quality of bovine embryo production. Strategies such as ovarian stimulation and media modulation can further optimize these processes.

The development of methods for selecting in vitro transplantable embryos has a significant impact on embryo quality and post-transplant pregnancy rate. This not only improves reproductive efficiency, but also reduces costs and the presence of non-pregnant cattle.

The quality of bovine embryos produced in vitro is influenced by nutrition and culture media. Nutrient depletion can negatively affect blastocyst formation and hatching, highlighting the importance of optimal conditions for embryonic development.

A diversity of strategies and approaches is evidenced in the studies reviewed, from the application of co-cultures with different cell types to the addition of specific supplements. Optimization of growing media is a constant concern, with an emphasis on finding effective combinations of nutrients, substrates, and growth factors.

The addition of supplements such as cysteamine, fetal calf serum (FCS), and platelet-rich plasma (PRP) emerges as a promising strategy to improve the rate of development and quality of embryos produced in vitro. The importance of epigenetic considerations in the in vitro production of embryos is highlighted, with studies examining DNA methylation and its impact on embryonic development.

In vitro cryopreservation of bovine embryos remains a challenge, with the need to develop effective protocols that preserve embryo viability and quality during the process. Studies on in vitro culture reveal significant effects on cell lineage composition, metabolism and gene expression, highlighting the importance of culture conditions in embryonic development.

The lack of standardized protocols for the in vitro production of bovine embryos is evident, and the continuous search for improvements and optimizations reflects the complexity and challenges associated with this technique. The use of emerging technologies, such as transcriptional analysis and the application of PRP, opens up new avenues to improve the in vitro production of bovine embryos. In vitro production of bovine embryos has direct applications in assisted reproduction in cattle, with implications for genetic improvement and reproductive efficiency in the livestock industry.

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