World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(REVIEW ARTICLE)

Check for updates

Unveiling maturation biomarker dynamics: A comprehensive review on *In Vitro* embryo production in cattle

Shilpa Doultani [1,*], Prachi Sharma [2], SP Patil [2], LB George [1] and HN Highland [1]

[1] *Department of Zoology, Gujarat University, Navrangpura, Ahmedabad – 380 009, Gujarat, India.*
[2] *Department of Veterinary Gynaecology and Obstetrics,College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand - 388 001, Gujarat, India.*

World Journal of Advanced Research and Reviews, 2024, 21(01), 579–585

Publication history: Received on 21 November 2023; revised on 02 January 2024; accepted on 05 January 2024

Article DOI: https://doi.org/10.30574/wjarr.2024.21.1.2677

Abstract

In vitro embryo production (IVEP) is a revolutionary reproductive biotechnology in bovine species which enables rapid genetic gain and conservation of genetic resources. IVEP has numerous applications in field of animal breeding, conservation and research, making it a powerful tool for enhancing reproductive efficiency and genetic progress in livestock industry. The process involves manipulating gametes, including oocytes and sperm, in a laboratory setting. Success of IVEP largely relies on optimization of each step, including gamete quality, sperm preparation techniques, fertilization conditions and culture medium. Advancements in culture media formulations by additions of growth factors, antioxidants along with co-culture systems and advanced imaging techniques have improved efficiency and success rates. In this article, the authors have reviewed the importance as well as biomarkers associated with *in vitro* maturation for a more successful outcome of IVEP in bovines.

Keywords: *Biomarkers, In Vitro* Embryo Production; *In Vitro* Maturation; Oocytes

1. Introduction

In vitro embryo production (IVEP) mimics the natural process of fertilization and early embryonic development outside the maternal reproductive tract. It encompasses various steps, including oocyte collection, *in vitro* maturation (IVM), sperm preparation, *in vitro* fertilization (IVF), and *in vitro* embryo culture. This comprehensive review aims to provide an in-depth understanding of the principles, techniques, and applications of *in vitro* embryo production in bovine species [1, 2].

IVEP offers numerous advantages over traditional breeding techniques and has become a powerful tool for hastening genetic progress in bovines. IVEP has several key benefits. Firstly, it allows for the rapid production of a large number of embryos from genetically superior animals, thus accelerating the rate of genetic gain. Secondly, it enables the preservation and propagation of valuable and endangered bovine genetic resources. Furthermore, IVEP facilitates the use of elite sires with limited semen availability, as it enables the production of embryos from a single ejaculate. Additionally, it offers the opportunity for sex selection, allowing breeders to produce embryos of the desired sex, which is particularly useful in the dairy industry [3]. The success of IVEP relies on optimizing each step of the process. Oocyte quality is crucial for successful embryo production, and various factors, such as the source, maturation conditions, and culture media, influence oocyte competence. The choice of sperm preparation techniques and fertilization conditions greatly impacts fertilization rates and subsequent embryo development. Moreover, the culture environment, including culture media composition, culture conditions, significantly affects embryo quality and its developmental competence [4,5].

* Corresponding author: Shilpa Doultani

In recent years, several advancements have further improved the efficiency and success rates of IVEP. These include the use of novel culture media formulations, the supplementation of growth factors and antioxidants, the application of co-culture systems, and the utilization of advanced imaging and molecular techniques for embryo selection and assessment. These advancements have contributed to the optimization of embryo production, increased pregnancy rates, and improved the overall quality of the resulting embryos.

2. The Importance of *In Vitro* Maturation in Bovine Reproductive Biotechnology

In vitro maturation (IVM) is a critical component of bovine reproductive biotechnology that plays a pivotal role in the success of *in vitro* embryo production (IVEP). IVM involves the culture and maturation of immature oocytes harvested either from morbid ovaries or from live donor animals by ovum pick up (OPU) technique. This technique has gained significant importance in bovine breeding programs and research due to several key reasons [6,7].

2.1. Increased Availability of Oocytes

OPU allows for the collection of a larger number of oocytes compared to natural cycles or hormonal stimulation of donor animals. OPU enhances oocyte availability for IVM and is crucial for maximizing the number of embryos produced and increasing the efficiency of assisted reproductive technologies.

2.2 Genetic Progress

OPU-IVM offers the opportunity to utilize genetically superior animals that may not be available for repeated oocyte collections or have limited reproductive capacity. It enables the production of embryos from donors with exceptional genetic traits, such as high milk production, disease resistance, or superior growth rates. This technique facilitates the rapid dissemination of desirable genetic material and accelerates genetic progress within the bovine population [1].

2.3 Simplified Management

OPU-IVM simplifies the management of oocyte collection procedures by eliminating the need for hormone treatments in donor animals. This reduces the stress and potential health risks associated with hormonal manipulation. Moreover, it provides greater flexibility in scheduling oocyte collections, making it easier to synchronize multiple donors for embryo production.

2.4 Conservation of Genetic Resources

OPU-IVM plays a vital role in the conservation of endangered or valuable genetic resources in bovine species. It allows for collecting and preserving oocytes from genetically significant animals, including rare breeds, elite individuals, or those with specific genetic traits. These collected oocytes can be cryopreserved and stored for future use, ensuring the long-term preservation of valuable genetic diversity [1].

2.5 Optimization of Oocyte Quality

The IVM process can enhance the developmental competence and quality of oocytes. Through appropriate culture conditions, including media composition, growth factors, and environmental parameters, IVM can promote the maturation of high-quality oocytes. This, in turn, improves the likelihood of successful fertilization, embryo development, and subsequent pregnancy rates [8].

2.6. Research and Experimental Applications

IVM provides a valuable tool for conducting research and experimental studies in bovine reproduction. It enables researchers to investigate the cellular and molecular aspects of oocyte maturation, evaluate the effects of various culture conditions, and explore novel techniques for improving embryo production and quality.

In vitro maturation is a fundamental technique in bovine reproductive biotechnology, offering numerous advantages for genetic progress, reproductive management, and research purposes. By increasing the availability of oocytes, optimizing oocyte quality, and facilitating the conservation of genetic resources, IVM contributes to the advancement of assisted reproductive technologies and enhances our understanding of bovine reproductive physiology [9].

Earlier it was impossible to retrieve the matured oocytes *in vitro.* The phenomenon was well understood during the nineties. The first IVF was performed with immature rabbit oocytes. During the 1960s Edwards carried out work of IVM human COCs. IVM is the progenitor of the current IVEP treatment.

3. Methods of identification of maturation in COCs [10]

3.1. Morphological assessment

One of the most common methods is to visually examine the COCs under a microscope and assess their morphological characteristics. The stages of COC maturation can be identified based on the appearance and organization of the cumulus cells and the oocyte itself.

3.2. Nuclear morphology

The nuclear morphology of the oocyte can be used as an indicator of its maturation stage. Immature oocytes have a germinal vesicle (GV) nucleus. As they mature, the nucleus progresses through various stages, such as germinal vesicle breakdown (GVBD), metaphase I (MI), and metaphase II (MII) where it becomes fully mature [11].

3.3. Cytoplasmic changes

Maturing COCs undergo specific cytoplasmic changes that can be observed under a microscope. These changes include the redistribution of organelles and the appearance of cortical granules, which are indicative of maturation.

3.4. Cumulus expansion

As COCs mature, the cumulus cells that surround the oocyte undergo expansion due to the accumulation of hyaluronic acid. The degree of cumulus expansion can be used as an indicator of the maturation stage of the COCs.

3.5. Meiotic progression markers

Certain molecular markers or proteins associated with meiotic progression can be used to identify the maturation stage of COCs. For example, specific protein expression patterns or phosphorylation events can indicate the oocyte's meiotic stage.

3.6 *In vitro* maturation (IVM) assays

In certain assisted reproductive technologies (ART) procedures, COCs are collected and subjected to *in vitro* maturation. The maturation status of these COCs is monitored by assessing the above-mentioned morphological and molecular indicators.

3.5.1. Hormonal stimulation

In some cases, hormonal stimulation is used to induce the maturation of COCs in a controlled manner. The response of COCs to hormonal cues can be an indicator of their maturation stage.

3.5.2. Cumulus expansion-related markers

Biomarkers associated with cumulus cell expansion, such as hyaluronic acid synthases (HAS), hyaluronan synthase 2 (HAS2), and tumor necrosis factor alpha-induced protein 6 (TNFAIP6), can be used to assess the maturation status of COCs.

It's important to note that the assessment of COC maturation is a complex process and may require a combination of the above-mentioned methods to ensure accurate identification. Properly identifying the maturation stage of COCs is critical in assisted reproduction techniques and research related to fertility and developmental biology.

Studying oocyte maturation biomarkers is of significant importance in the field of reproductive biology and assisted reproductive technologies. These biomarkers provide valuable insights into the complex processes that occur during oocyte maturation and can be used for various purposes, including [12]:

3.5.3. Assessing Oocyte Quality

Oocyte quality is a crucial factor affecting the success of fertilization, embryo development, and subsequent pregnancy rates. Maturation biomarkers can serve as indicators of oocyte quality, allowing researchers and clinicians to evaluate the developmental competence of oocytes. By studying specific biomarkers, such as gene expression patterns, morphological characteristics, or metabolic activity, it becomes possible to identify oocytes with a higher likelihood of successful fertilization and subsequent embryo development.

3.5.4. Optimizing Culture Conditions

Understanding the biomarkers associated with oocyte maturation helps in optimizing *in vitro* culture conditions. By studying specific biomarkers' expression or activity levels, researchers can determine the most suitable culture media composition, growth factors, or environmental parameters for promoting optimal oocyte maturation. This knowledge contributes to enhancing the efficiency and success rates of assisted reproductive technologies, such as *in vitro* fertilization (IVF) or *in vitro* embryo production (IVEP).

3.5.5. Developing Non-invasive Assessment Methods

Traditional methods for assessing oocyte maturation often involve invasive procedures, such as oocyte collection and evaluation under a microscope. Studying maturation biomarkers provides the potential for developing non-invasive assessment methods. For instance, biomarkers present in the surrounding follicular fluid or the cumulus-oocyte complex (COC) can be analyzed to assess the oocyte maturation stage without the need for invasive procedures. Non-invasive assessment methods improve animal welfare and reduce the stress associated with oocyte collection procedures [13].

Predicting Oocyte Developmental Potential

Biomarkers associated with oocyte maturation can serve as predictors of oocyte developmental potential. By identifying specific molecular markers or morphological characteristics, it becomes possible to distinguish oocytes with a higher chance of developing into high-quality embryos and subsequently achieving successful pregnancies. This information is valuable for optimizing embryo selection methods and improving the overall efficiency of assisted reproductive technologies.

3.5.6. Advancing Research and Knowledge

Studying oocyte maturation biomarkers contributes to the overall understanding of the molecular and cellular processes underlying oocyte development. It helps in unraveling the intricate regulatory mechanisms involved in oocyte maturation, identifying key signaling pathways, and discovering new molecular targets for improving reproductive outcomes. The knowledge gained from studying these biomarkers has broader implications for reproductive biology and can contribute to advancements in both animal and human reproductive medicine.

4. History and Recent Studies of Oocyte Biomarkers in Bovine Species

Oocyte biomarkers have been extensively studied in bovine species to understand the complex processes of oocyte maturation, evaluate oocyte quality, and improve reproductive technologies. This short review provides a brief overview of the historical developments and recent studies on oocyte biomarkers in bovine reproductive biology.

4.1. Historical Perspective

The investigation of oocyte biomarkers in bovine species has evolved over the years with advancements in molecular and cellular techniques. Initially, morphological characteristics, such as cumulus cell expansion, nuclear maturation, and cytoplasmic features, were used to evaluate oocyte quality [13]. Subsequently, studies focused on identifying specific molecular markers and gene expression patterns associated with oocyte maturation and developmental competence. These markers included growth factors, receptors, gap junction proteins, and genes involved in metabolism and chromosomal segregation [12].

4.2. Recent Studies

Recent studies have expanded the understanding of oocyte biomarkers in bovine species, employing advanced molecular techniques and omics approaches. Here are some notable recent findings:

4.2.1. Transcriptomic Biomarkers

Transcriptomic analysis has identified specific genes and expression patterns associated with oocyte maturation and developmental competence. For example, studies have identified differential expression of genes involved in cell cycle regulation, DNA repair, mitochondrial function, and stress response between competent and incompetent oocytes. These biomarkers have the potential for predicting oocyte quality and selecting embryos with higher developmental potential [14].

4.2.2. Epigenetic Biomarkers

Epigenetic modifications, such as DNA methylation and histone modifications, have been studied as potential biomarkers of oocyte quality. Recent studies have identified differential methylation patterns in specific genomic regions of oocytes associated with their developmental competence. Epigenetic biomarkers offer insights into the long-term effects of maternal factors on embryo development and can help predict the developmental potential of oocytes [15].

4.2.3. Metabolic Biomarkers

Metabolomic profiling has revealed distinct metabolic signatures in oocytes of different quality. Studies have identified alterations in energy metabolism, lipid metabolism, and amino acid metabolism in competent and incompetent oocytes. Metabolic biomarkers provide valuable information on the physiological state of oocytes and can aid in selecting oocytes with higher developmental competence [16].

4.2.4. Non-coding RNA Biomarkers

Non-coding RNAs, such as microRNAs and long non-coding RNAs, have emerged as potential biomarkers of oocyte quality. Studies have demonstrated differential expression of specific non-coding RNAs incompetent and incompetent oocytes. These biomarkers regulate gene expression and cellular processes crucial for oocyte maturation and embryo development.

5. Potential Genetic Markers that Predict Maturation of Oocyte

Identifying genetic markers that predict oocyte maturation is an area of active research in the field of reproductive biology. While our understanding of the genetic basis of oocyte maturation is continuously evolving, here are some potential genetic markers that have been implicated in predicting oocyte maturation [17]:

5.1. BCL2L10

This gene encodes a protein involved in regulating apoptosis (programmed cell death). Studies have suggested that BCL2L10 may play a role in oocyte maturation and its expression levels could be indicative of maturation status.

5.2. MOS

MOS (Proto-oncogene serine/threonine-protein kinase mos) is a gene involved in the regulation of meiosis. It is essential for the resumption of meiosis during oocyte maturation, and its expression and activity have been linked to oocyte maturation.

5.3. CCNB1

Cyclin B1 (encoded by CCNB1) is a key regulator of the cell cycle and is involved in the meiotic progression of oocytes. Its expression level has been associated with oocyte maturation.

5.4. GDF9

Growth differentiation factor 9 (GDF9) is a gene that plays a critical role in folliculogenesis and oocyte development. Variations in the GDF9 gene have been linked to differences in oocyte maturation potential.

5.5. BMP15

Bone morphogenetic protein 15 (BMP15) is another gene that is crucial for follicular development and oocyte maturation. Mutations in BMP15 have been associated with altered oocyte maturation and fertility outcomes.

5.6. FIGLA

Folliculogenesis-specific basic helix-loop-helix transcription factor (FIGLA) is a gene involved in early oocyte development. Its expression is restricted to oocytes and has been linked to oocyte growth and maturation.

5.7. NLRP5

Nucleotide-binding oligomerization domain-like receptor family, pyrin domain-containing 5 (NLRP5), is a gene involved in oocyte and early embryo development. Variants in this gene have been associated with defects in oocyte maturation and early embryonic development.

5.8. ZP3

Zona pellucida glycoprotein 3 (ZP3) is a gene encoding a protein that plays a role in sperm egg binding during fertilization. Changes in ZP3 expression have been linked to oocyte maturation.

5.9. CEP55

Centrosomal protein 55 (CEP55) is a gene that regulates cell division. Studies have suggested its involvement in oocyte maturation and early embryonic development.

6. Conclusion

It's important to note that genetic markers alone may not provide a complete picture of oocyte maturation, and they are often used in conjunction with other assessments, such as morphological and biochemical indicators, to better predict oocyte development and fertility outcomes. Additionally, as the field of genetics advances, more genetic markers related to oocyte maturation may be identified and characterized.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Moawad, A., Ghoneim, I., Darwish, G., Badr, M. and El-Badry, D., and El-Wishy, A. B. Factors affecting *in vitro* embryo production: insights into dromedary camel. Journal of Animal Reproduction and Biotechnology, 2020, 35.

[2] Ferré, L. B., Kjelland, M. E., Strøbech, L. B., Hyttel, P., Mermillod, P., and Ross, P. J. Review: Recent advances in bovine *in vitro* embryo production: reproductive biotechnology history and methods. Animal : an international journal of animal bioscience, 2020, 14(5): 991–1004.

[3] Baldassarre, H. Laparoscopic Ovum Pick-Up Followed by *In Vitro* Embryo Production and Transfer in Assisted Breeding Programs for Ruminants. Animals, 2021, 11(1): 216.

[4] Souza-Fabjan, J. M. G., Leal, G. R., Monteiro, C. A. S., Batista, R. I. T. P., Barbosa, N. O., and Freitas, V. J. F. *In vitro* embryo production in small ruminants: what is still missing?. Animal reproduction, 2023, 20(3): e20230055.

[5] Falchi, L., Ledda, S., and Zedda, M. T. Embryo biotechnologies in sheep: Achievements and new improvements. Reproduction in domestic animals = Zuchthygiene, 2022, 57 (Suppl 5): 22–33.

[6] Lonergan, P., and Fair, T. Maturation of Oocytes *in Vitro*. Annual Review of Animal Bioscience, 2016, 4: 255-68.

[7] Naspinska, R., Moreira da Silva, M.H., and Moreira da Silva, F. Current Advances in Bovine *In Vitro* Maturation and Embryo Production Using Different Antioxidants: A Review. Journal of Developmental Biology, 2023, 11: 36.

[8] Walter, J., Monthoux, C., and Fortes, C. The bovine cumulus proteome is influenced by maturation condition and maturational competence of the oocyte. Scientific Reports, 2020, 10: 9880.

[9] Salhab, M., Dhorne-Pollet, S., Auclair, S., Guyader-Joly, C., Brisard, D., Dalbies-Tran, R., Dupont, J., Ponsart, C., Mermillod, P., and Uzbekova, S. *In vitro* maturation of oocytes alters gene expression and signaling pathways in bovine cumulus cells. Molecular reproduction and development, 2013, 80(2): 166–182.

[10] Chanakarn, S., Elnur, B., Luhan, T., Z, H., Chang, L. and Francesca E. D. Quantitative morphokinetic parameters identify novel dynamics of oocyte meiotic maturation and cumulus expansion, Biology of Reproduction, 2022, 107(4): 1097–1112.

[11] Lemseffer Y., Terret, M.E., Campillo, C., and Labrune, E. Methods for Assessing Oocyte Quality: A Review of Literature. Biomedicines. 2022, 4;10(9):2184.

[12] Sirait, B., Wiweko, B., Jusuf, A. A., Iftitah, D., and Muharam, R. Oocyte Competence Biomarkers Associated With Oocyte Maturation: A Review. Frontiers in cell and developmental biology, 2021, 9: 710292.

[13] Melo, E.O., Cordeiro, D.M., Pellegrino, R., Wei, Z., Daye, Z.J., Nishimura, R.C., and Dode MA. Identification of molecular markers for oocyte competence in bovine cumulus cells. Animal Genetics, 2017, 48(1):19-29.

[14] Fair T., Carter F., Park S., Evans A.C. and Lonergan P. Global gene expression analysis during bovine oocyte *in vitro* maturation. Theriogenology, 2007, 68(Suppl 1):7.

[15] Regassa A., Rings F., Hoelker M., Cinar U., Tholen E., Looft C., Schellander K. and Tesfaye D. Transcriptome dynamics and molecular cross-talk between bovine oocyte and its companion cumulus cells. BMC Genomics, 2011, 12: 57.

[16] Thompson J.G., Lane M. and Gilchrist R.B. Metabolism of the bovine cumulus–oocyte complex and influence on subsequent developmental competence. Society of Reproduction and Fertility, 2006, 64: 179–90.

[17] Assidi, M., Dufort, I., Ali, A., Hamel, M., Algriany, O., Dielemann, S. and Sirard, M.A. Identification of potential markers of oocyte competence expressed in bovine cumulus cells matured with follicle-stimulating hormone and/or phorbol myristate acetate *in vitro*. Biology of Reproduction, 2008, 79(2):209-22.