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Emerging biomarkers for assessment of oocyte quality in cattle

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Abstract

Oocyte quality is the primary determinant of success in bovine *in vitro* embryo production (IVEP), yet its accurate assessment remains a major limitation in assisted reproductive technologies. Conventional morphology-based evaluation of the cumulus–oocyte complex is subjective and poorly predictive of developmental competence, as it fails to capture the underlying molecular and functional status of the oocyte. Increasing evidence indicates that oocyte competence is governed by tightly coordinated nuclear, cytoplasmic, mitochondrial, and epigenetic processes, highlighting the need for precise and biologically relevant biomarkers. This review critically examines emerging molecular and non-invasive biomarkers for assessing oocyte quality in cattle. Key molecular indicators, including apoptosis regulators (BCL-2, BAX), oocyte-secreted factors (GDF9, BMP15), oxidative stress markers (SOD, GPX), and maternal effect genes, are discussed in relation to their roles in determining developmental competence. In parallel, non-invasive approaches based on cumulus cell transcriptomics, follicular fluid metabolite profiling, and extracellular vesicle-associated microRNAs are evaluated for their potential to predict oocyte quality without compromising viability. The integration of these biomarkers into OPU–IVF systems, embryo selection, and cryopreservation strategies offers a significant advancement toward improving reproductive efficiency. Importantly, the convergence of multi-omics technologies with artificial intelligence-driven analysis is redefining oocyte assessment from subjective evaluation to data-driven prediction. Collectively, these developments support a shift toward integrated, non-invasive, and precision-based approaches for optimizing bovine reproductive performance and accelerating genetic gain.

Keywords: Oocyte competence, bovine, IVEP, biomarkers, cumulus cells, follicular fluid, oxidative stress, miRNA

1. Introduction

The success of bovine reproductive technologies, particularly *in vitro* embryo production (IVEP), largely depends on the intrinsic quality of the oocyte (Molin *et al.*, 2025) [24]. Oocyte competence determines the ability to undergo successful maturation, fertilization, and subsequent embryonic development, thereby directly influencing breeding efficiency and genetic improvement programs (Conti & Franciosi, 2017) [7]. In addition, high-quality oocytes are essential for ensuring consistency in embryo yield and improving the overall success rates of advanced reproductive interventions. In cattle, variability in oocyte quality remains one of the major limiting factors affecting the overall efficiency and consistency of assisted reproductive technologies (Baruselli *et al.*, 2016) [4]. Traditionally, oocyte selection has relied on morphological evaluation of the cumulus–oocyte complex (COC), including parameters such as cumulus cell investment and cytoplasmic appearance (Ozturk, 2020) [25]. However, these criteria are inherently subjective and often fail to accurately reflect the underlying molecular and functional status of the oocyte. As a result, oocytes classified as morphologically “good quality” may still exhibit poor developmental competence, highlighting the limited predictive value of conventional assessment methods (Cimadomo *et al.*, 2023) [6].

In recent years, there has been increasing emphasis on the identification of reliable and objective biomarkers that can more precisely assess oocyte quality. This shift is driven by the need to move beyond visual assessment toward a more functional and mechanistic understanding of oocyte competence (Slezec-Frick *et al.*, 2026) ^[30]. Molecular, cellular, and metabolic indicators derived from the oocyte, surrounding cumulus cells, and follicular environment have shown significant potential in predicting developmental competence (Molin *et al.*, 2025) ^[24]. Importantly, the development of non-invasive or minimally invasive approaches, such as analysis of cumulus cell gene expression and follicular fluid composition, offers promising alternatives for real-time and practical application in reproductive programs. These approaches enable repeated assessment without compromising oocyte viability, making them highly suitable for routine use in IVEP systems (Anazawa *et al.*, 2024) ^[2]. Such advancements align with the broader objective of improving reproductive performance through integrated technological and management approaches in livestock systems (Upreti *et al.*, 2025) ^[35]. Therefore, the present review aims to critically evaluate emerging biomarkers associated with oocyte quality in cattle, with a focus on their biological relevance, predictive potential, and applicability in improving the efficiency of assisted reproductive technologies.

2. Biological Basis of Oocyte Competence

Oocyte competence refers to the intrinsic ability of an oocyte to complete maturation, undergo successful fertilization, and sustain early embryonic development. This competence is acquired progressively during folliculogenesis and is governed by tightly coordinated nuclear, cytoplasmic, metabolic, and epigenetic events. A clear understanding of these underlying mechanisms is essential for identifying biologically relevant biomarkers of oocyte quality (Yamada & Isaji, 2011; He *et al.*, 2021) ^[39, 14].

2.1. Nuclear and cytoplasmic maturation

Oocyte maturation comprises two interdependent processes: nuclear and cytoplasmic maturation. Nuclear maturation involves the resumption of meiosis from the germinal vesicle (GV) stage to metaphase II (MII), characterized by chromatin condensation, spindle formation, and extrusion of the first polar body (Torkashvand *et al.*, 2024) ^[31]. Although completion of nuclear maturation is necessary, it alone does not guarantee developmental competence. Cytoplasmic maturation encompasses a series of critical events, including redistribution of organelles, accumulation of maternal mRNAs and proteins, and proper cortical granule migration. These changes enable fertilization, prevent polyspermy, and support early embryogenesis prior to embryonic genome activation. Importantly, asynchrony between nuclear and cytoplasmic maturation is a major cause of reduced developmental potential in *in vitro* matured oocytes (Trebichalská *et al.*, 2020) ^[32].

2.2. Role of cumulus–oocyte communication

The oocyte develops within a specialized microenvironment formed by surrounding cumulus cells, collectively known as the cumulus–oocyte complex (COC) (Turathum *et al.*, 2021) ^[33]. Bidirectional communication between the oocyte and cumulus cells is essential for the acquisition of competence. This interaction is mediated through transzonal projections and gap junctions, which facilitate the transfer of ions, metabolites, amino acids, and signaling molecules. Cumulus

cells regulate meiotic arrest and resumption by modulating cyclic nucleotide levels within the oocyte and provide metabolic substrates such as pyruvate and amino acids that the oocyte cannot efficiently utilize on its own (Russell *et al.*, 2016) ^[27]. In turn, the oocyte secretes key growth factors, including GDF9 and BMP15, which regulate cumulus cell proliferation, differentiation, and expansion. Disruption of this tightly regulated communication impairs oocyte maturation and reduces developmental competence (Fountas *et al.*, 2024) ^[10].

2.3. Mitochondrial function and epigenetic regulation

Mitochondria play a central role in determining oocyte quality by regulating cellular energy metabolism and redox homeostasis. Adequate mitochondrial number, proper distribution, and high ATP production are essential for meiotic progression, spindle integrity, and early embryonic cleavage. Additionally, mitochondria are a major source of reactive oxygen species (ROS); therefore, a balanced redox state is critical, as excessive oxidative stress can damage lipids, proteins, and DNA, ultimately compromising oocyte competence (Kirillova *et al.*, 2021) ^[19].

Epigenetic regulation further contributes to the developmental potential of the oocyte. During oogenesis, dynamic modifications such as DNA methylation, histone modifications, and chromatin remodeling establish the maternal epigenetic landscape required for normal embryonic development and genomic imprinting. Aberrations in these epigenetic marks, often associated with suboptimal *in vitro* conditions, can lead to impaired gene expression and developmental failure (Voros *et al.*, 2025) ^[36]. Thus, mitochondrial integrity and epigenetic stability together represent fundamental determinants of oocyte competence and serve as important targets for emerging biomarker development.

3. Limitations of Conventional Assessment Methods

The evaluation of oocyte quality in cattle has traditionally relied on morphological grading of the cumulus–oocyte complex (COC). This approach primarily considers the degree of cumulus cell investment (number of cell layers and compactness) and the visual characteristics of the ooplasm, such as homogeneity and cytoplasmic granularity (Raes *et al.*, 2025) ^[26]. Oocytes surrounded by multiple layers of compact cumulus cells with evenly distributed cytoplasm are generally classified as high quality, whereas those with sparse or degenerated cumulus layers are considered inferior (Ozturk, 2020) ^[25]. Although widely used due to its simplicity and low cost, this method provides only a superficial assessment of oocyte status. A major limitation of morphological grading is its inherent subjectivity. The evaluation depends largely on the observer's experience and interpretation, leading to variability both within and between laboratories. Subtle differences in grading criteria, handling conditions, and timing of assessment can significantly influence classification outcomes (Balaban *et al.*, 2022) ^[3]. Moreover, morphological features do not capture the underlying molecular, metabolic, and epigenetic status of the oocyte, which are critical determinants of developmental competence (He *et al.*, 2021; Molin *et al.*, 2025) ^[14, 24].

Importantly, there is a well-recognized disconnect between morphological appearance and actual developmental potential. Oocytes deemed morphologically “good quality” may fail to mature properly, undergo fertilization, or develop into viable embryos, while some lower-grade oocytes can still

yield competent embryos under optimal conditions (Bartolacci *et al.*, 2022) ^[5]. This inconsistency highlights the poor predictive value of conventional assessment methods and underscores the need for more objective, reliable, and biologically relevant indicators. Consequently, the identification of precise biomarkers that reflect the functional competence of oocytes has become a key priority in improving the efficiency of bovine assisted reproductive technologies (Wrenzycki, 2021) ^[38].

4. Molecular Biomarkers of Oocyte Quality (Core Section)

Molecular biomarkers provide a more precise and mechanistic understanding of oocyte competence by reflecting the underlying cellular, metabolic, and regulatory status of the oocyte and its surrounding environment. Unlike morphological assessment, these biomarkers are closely associated with functional outcomes such as maturation, fertilization, and embryo development. Among the various categories, apoptosis-related genes, oocyte-secreted factors, oxidative stress markers, and maternal effect genes have

emerged as key indicators of oocyte quality in cattle ((Hussein *et al.*, 2006; Molin *et al.*, 2025) ^[15, 24].

4.1. Apoptosis-related markers (BCL-2, BAX)

The balance between pro-survival and pro-apoptotic signals within the oocyte and cumulus cells is a critical determinant of developmental competence. Members of the BCL-2 family regulate mitochondrial pathways of apoptosis, where BCL-2 functions as an anti-apoptotic protein, promoting cell survival, while BAX acts as a pro-apoptotic factor, facilitating programmed cell death (Lee *et al.*, 2014) ^[20]. A higher BCL-2/BAX ratio is generally associated with improved oocyte quality, enhanced resistance to cellular stress, and better embryonic development. In contrast, increased expression of BAX or a reduced BCL-2/BAX ratio reflects cellular damage, compromised mitochondrial integrity, and decreased developmental potential. Therefore, the relative expression of these genes is widely used as an indicator of oocyte viability and competence (Vucicevic *et al.*, 2016) ^[37] (Figure 1).

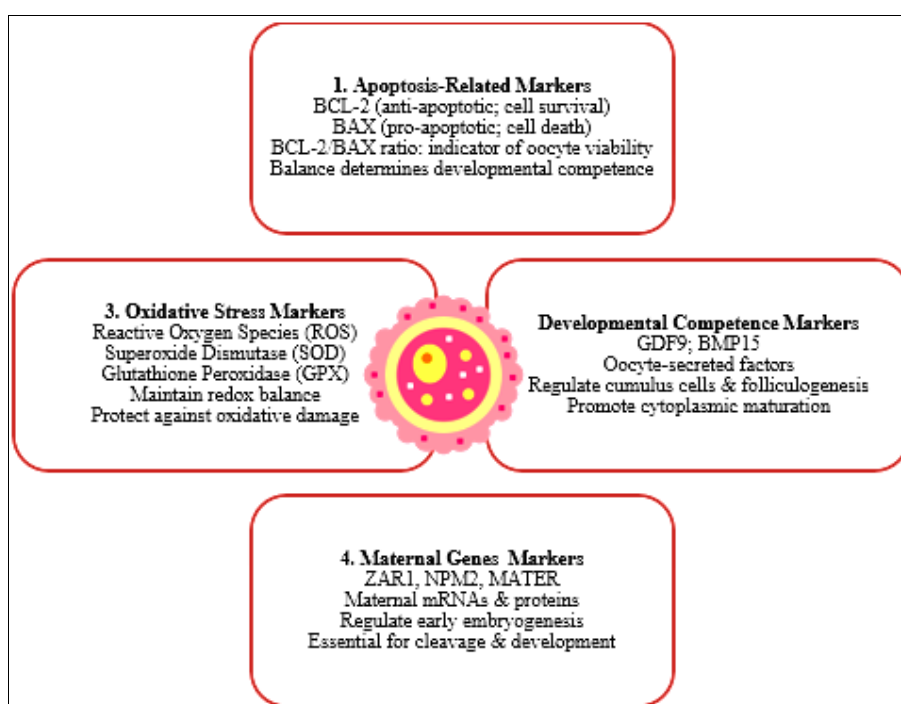


Fig 1: Molecular biomarkers regulating oocyte competence in cattle

4.2. Developmental competence markers (GDF9, BMP15)

Growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) are oocyte-secreted members of the transforming growth factor- β (TGF- β) superfamily and play indispensable roles in folliculogenesis and oocyte maturation (De Castro *et al.*, 2015) ^[8]. GDF9 is essential for the proliferation and differentiation of granulosa and cumulus cells, as well as for regulating metabolic cooperation within the follicle. Elevated expression of GDF9 is strongly associated with enhanced cytoplasmic maturation and improved developmental competence (Fountas *et al.*, 2024) ^[10]. Similarly, BMP15 works synergistically with GDF9 to promote cumulus cell expansion, regulate follicular development, and maintain oocyte–cumulus communication. Optimal expression levels of BMP15 are linked to better fertilization rates and embryo quality. Alterations or reduced expression of these factors can impair follicular dynamics and compromise oocyte competence, making them highly reliable molecular markers (Li *et al.*, 2014) ^[21].

4.3. Oxidative stress markers (SOD, GPX)

Oxidative stress plays a pivotal role in determining oocyte quality, particularly under *in vitro* conditions. Reactive oxygen species (ROS), when present in excess, can induce lipid peroxidation, DNA damage, and mitochondrial dysfunction (Agarwal *et al.*, 2022) ^[1]. Antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPX) form the primary defense system against oxidative damage. SOD catalyzes the dismutation of superoxide radicals into hydrogen peroxide, which is subsequently detoxified by GPX into water. Higher expression or activity of these enzymes reflects a robust antioxidant defense mechanism, contributing to improved oocyte survival, maturation, and embryonic development. Conversely, reduced antioxidant capacity leads to oxidative imbalance and diminished developmental competence (Ighodaro & Akinloye, 2017) ^[16].

4.4. Maternal effect genes

Maternal effect genes are transcribed and accumulated during oocyte growth and play a crucial role in regulating early embryonic development before activation of the embryonic genome. Genes such as ZAR1, NPM2, and MATER are involved in processes including chromatin organization, spindle formation, and early cleavage events. Adequate expression of these genes is essential for successful fertilization and progression beyond early embryonic stages (Innocenti *et al.*, 2022) ^[17]. Deficiencies or dysregulation in maternal transcripts often result in developmental arrest or poor embryo quality. As such, the expression profile of maternal effect genes serves as a strong indicator of intrinsic oocyte competence and developmental potential. Collectively, these molecular biomarkers provide valuable insights into the functional status of oocytes and offer a more reliable framework for assessing quality compared to conventional morphological methods (Jiao & Woodruff, 2013) ^[18].

5. Non-Invasive Biomarkers and Emerging Technologies

The increasing demand for objective and practical assessment of oocyte quality has driven the development of non-invasive and minimally invasive approaches. These strategies aim to evaluate the developmental competence of oocytes without compromising their viability, making them highly suitable for application in assisted reproductive technologies. Recent advances in molecular biology and computational tools have enabled the identification of surrogate biomarkers from the surrounding microenvironment, offering new avenues for precise and real-time evaluation (Zeng *et al.*, 2024; Molin *et al.*, 2025) ^[1, 24] (Figure 2).

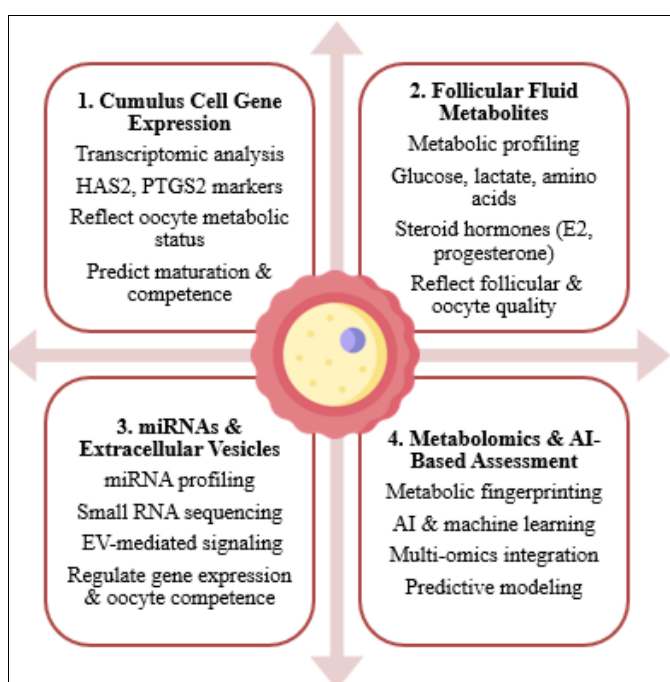


Fig 2: Emerging technologies for assessment of oocyte competence in cattle

5.1. Cumulus cell gene expression

Cumulus cells closely interact with the oocyte and reflect its physiological and metabolic status. Analysis of gene expression in cumulus cells has emerged as a reliable non-invasive approach to predict oocyte competence. Genes involved in cumulus expansion (e.g., HAS2, PTGS2), apoptosis regulation, and metabolic pathways have been shown to correlate with oocyte maturation and embryo

development. Since cumulus cells can be sampled without damaging the oocyte, their transcriptomic profiling serves as a valuable surrogate marker for assessing oocyte quality and has significant potential for routine use in IVEP systems (Shepel *et al.*, 2015; Anazawa *et al.*, 2024) ^[28, 2].

5.2. Follicular fluid metabolites

Follicular fluid provides a biochemical snapshot of the intrafollicular environment in which the oocyte develops. It contains a complex mixture of hormones, metabolites, proteins, and lipids that influence oocyte maturation and competence. Parameters such as glucose, lactate, amino acids, and steroid hormones (e.g., estradiol and progesterone) have been associated with oocyte developmental potential. Variations in the metabolic composition of follicular fluid can indicate differences in follicular health and oocyte quality, making it a valuable, non-invasive source of biomarkers (Zhang *et al.*, 2020) ^[40].

5.3. mi RNAs and extracellular vesicles

MicroRNAs (miRNAs) and extracellular vesicles (EVs) have gained considerable attention as novel regulators and indicators of oocyte competence. miRNAs are small non-coding RNAs that modulate gene expression at the post-transcriptional level and are involved in key processes such as folliculogenesis, apoptosis, and metabolism. Extracellular vesicles, including exosomes, facilitate intercellular communication by transporting miRNAs, proteins, and lipids between follicular cells and the oocyte. The profiling of miRNAs and EV-associated molecules in follicular fluid or culture media offers a promising, non-invasive strategy for predicting oocyte quality and developmental outcomes (Gad *et al.*, 2022; Fiorentino *et al.*, 2024) ^[11, 9].

5.4. Metabolomics and AI-based assessment

Advanced technologies such as metabolomics enable comprehensive analysis of small-molecule metabolites in oocyte culture media or follicular fluid, providing insights into the metabolic state of the oocyte. Specific metabolic signatures have been linked to high developmental competence and embryo viability (Gao & Xiao, 2023) ^[11]. In parallel, artificial intelligence (AI) and machine learning approaches are being increasingly applied to analyze complex datasets, including imaging and “omics” data, to improve the accuracy and consistency of oocyte assessment. AI-assisted models can integrate multiple biomarkers and morphological features to generate predictive algorithms, representing a significant step toward precision reproduction in cattle. Collectively, these non-invasive biomarkers and emerging technologies offer a powerful and practical framework for improving the selection of competent oocytes while preserving their developmental potential (Raes *et al.*, 2025) ^[26].

6. Integration into Assisted Reproductive Technologies

The translation of oocyte quality biomarkers into practical applications is essential for improving the efficiency and reliability of bovine assisted reproductive technologies. Integrating molecular and non-invasive indicators into existing systems enables more accurate selection of competent oocytes and embryos, ultimately enhancing reproductive outcomes and genetic gain (Mansour, 2025) ^[22] (Figure 3). The integration of advanced reproductive technologies with effective management and monitoring strategies has been shown to significantly enhance

reproductive efficiency and overall productivity in livestock systems (Upreti *et al.*, 2025) ^[35].

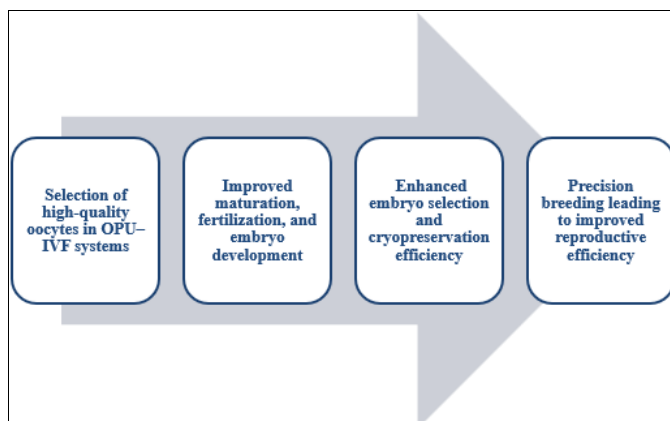


Fig 3: Integration of oocyte quality biomarkers into ARTs in cattle

6.1. Application in OPU–IVF systems

In ovum pick-up (OPU)–*in vitro* fertilization (IVF) programs, variability in oocyte quality remains a major constraint affecting embryo yield and consistency. Incorporation of biomarker-based assessment, particularly through cumulus cell gene expression and follicular fluid analysis, allows for the selection of oocytes with higher developmental competence prior to *in vitro* maturation. This approach can reduce the inclusion of suboptimal oocytes, thereby improving maturation rates, fertilization efficiency, and blastocyst yield. Additionally, biomarker-guided selection may help optimize donor management strategies by identifying animals with superior oocyte quality, leading to more efficient utilization of elite genetics (Sirait *et al.*, 2021) ^[29].

6.2. Embryo selection and cryopreservation

Beyond oocyte assessment, biomarkers also play a critical role in evaluating embryo quality and cryotolerance. Embryos derived from oocytes with favorable molecular profiles, such as optimal expression of developmental and antioxidant genes, exhibit improved viability and resistance to cryopreservation-induced stress. The integration of metabolic and molecular indicators, including culture media analysis and gene expression profiling, enables more precise selection of embryos with higher implantation potential and post-thaw survival. This is particularly important for large-scale embryo transfer programs, where maximizing the success rate of cryopreserved embryos is a key objective (Charpigny *et al.*, 2021; Molin *et al.*, 2025) ^[1, 24].

6.3. Potential for precision breeding

The application of biomarker-based selection strategies aligns with the emerging concept of precision breeding in cattle. By combining biomarker data with genomic selection and advanced reproductive technologies, it becomes possible to identify and propagate individuals with superior reproductive and genetic potential more efficiently (Mikkola *et al.*, 2024) ^[23]. This integrated approach facilitates informed decision-making at multiple stages, from donor selection to embryo transfer, ultimately accelerating genetic improvement while maintaining reproductive efficiency (Humboldt *et al.*, 2010; Upreti *et al.*, 2026) ^[1, 34]. In the long term, the incorporation of multi-omics biomarkers and predictive modeling tools is expected to transform conventional breeding programs into more targeted and data-driven systems. Overall, the

integration of emerging biomarkers into assisted reproductive technologies represents a significant advancement toward improving the accuracy, efficiency, and sustainability of bovine reproduction (Gu *et al.*, 2025) ^[13].

7. Conclusion

Oocyte quality is a key factor determining the success of bovine IVEP, yet traditional morphology-based assessment remains unreliable and subjective. Emerging molecular and non-invasive biomarkers provide a more accurate and objective evaluation by reflecting the true biological, metabolic, and functional status of the oocyte. Key indicators, like apoptosis regulators, oocyte secreted factors, oxidative stress markers, and maternal effect genes, along with cumulus cell analysis, and even follicular fluid profiling, together, provide valuable insights into developmental competence, without really compromising oocyte viability. The integration of these biomarkers with advanced technologies like metabolomics and artificial intelligence offers a powerful approach to improve oocyte selection, embryo development, and cryopreservation outcomes. Ultimately, adopting biomarker-based, precision-driven strategies will enhance reproductive efficiency, ensure better fertility outcomes, and accelerate genetic improvement in cattle.

Conflict of Interest

Not available

Financial Support

Not available

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