Chapter

Mechanistic Advances on Developmental Initiation, Maturation, and Ovulation of Oocytes in Domestic Cattle

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Abstract

With the continuous development of society, people's demand for meat and milk continues to expand, especially beef and milk consumption. In order to improve the production efficiency of domestic cattle, frozen sperm, artificial fertilization, embryo transfer, and other reproductive technologies have been deeply studied and widely used, but the problem of beef and milk supply has not been fundamentally solved. The quality of oocytes is directly related to fertilization, embryo development, growth of fetal calf before and after birth, and its production performance. A thorough understanding of the mechanism of oocyte development initiation, maturation, and ovulation in domestic cattle will lay a theoretical foundation for us to further improve its production efficiency. This chapter focuses on summarizing and analyzing the recent progress of domestic cattle raising on the above issues and puts forward suggestions and prospects for future related work.

Keywords: oogenesis, oocyte maturation, ovulation, oocyte quality, assessing techniques, domestic cattle

1. Introduction

Cattle are domesticated farm animals that are raised for meat, milk, leather, or labor. Generally, cattle refers to domesticated cattle in the Western or European regions, as well as in India and Africa. All modern domesticated cattle are believed to belong to either *Bos taurus* (European breeds, such as Shorthorn and Jersey) or Indian cattle (zebu breeds, such as Brahman), or hybrids of these two breeds (such as Santa Gertrudis). Other cattle, such as Asian water buffalo, Tibetan yaks, Southeast Asian Gayal and Banteng cattle, and North American plains bison, have also been domesticated or semi-domesticated and are considered to be cattle. According to Foreign Agricultural Service (FAS/USDA) data for 2023, the largest stocks of cattle in the world are found in India, followed by Brazil and China. India's cattle inventory is the largest in the world, accounting for approximately 33% of the world's cattle stock.

The total number of cattle in India, Brazil, and China accounts for about 64% of the world's livestock inventory (**Figure 1**) [1].

The estrous cycle is a series of physiological events that occur in female mammals in preparation for pregnancy. The estrous cycle of cows lasts an average of 21 days, usually between 18 and 24 days [2]. Based on the sexual behavior of animals and the corresponding changes in their bodies and reproductive organs, the estrous cycle can be divided into four stages: proestrus, estrus, metestrus, and diestrus. Furthermore, based on the development, maturation, and ovulation of follicles on the ovaries, as well as the formation and degradation of corpora lutea, the estrous cycle can also be divided into follicular and luteal phases.

Proestrus is the first stage of the estrous cycle, usually lasting one to 3 days before the onset of estrus. During this stage, the corpus luteum (CL) regresses, leading to a decrease in progesterone and an increase in estrogen levels as the follicles start to develop on the ovaries. These follicles contain immature oocytes, and the dominant follicle will mature and release the oocyte during ovulation. During estrus, the follicles continue to grow and mature. During this stage, the oocyte undergoes meiosis and stops at the metaphase of the second meiosis. Ovulation occurs during metestrus, where the mature follicle ruptures and releases the oocyte into the oviduct. The oocyte travels down the oviduct, where it may be fertilized by sperm. If fertilization occurs, the oocyte will continue to develop into an embryo.

The development, maturation, and ovulation of oocytes are critical processes in the estrous cycle of domesticated cows. Exploring and investigating the development, maturation, and ovulation processes of bovine oocytes in domesticated cows contributes to improving the utilization rate of ovarian follicles, accelerating the breeding of superior cattle, and safeguarding valuable genetic resources. It is worth noting that according to data from the International Embryo Technology Society (IETS), the number of bovine embryos produced continues to rise, reaching 1.5 million annually. Furthermore, since 2015, the quantity of bovine embryos produced through in vitro fertilization (IVF) has surpassed the number of embryos produced in vivo (IVP), with over one million bovine IVF embryos produced per year [source:

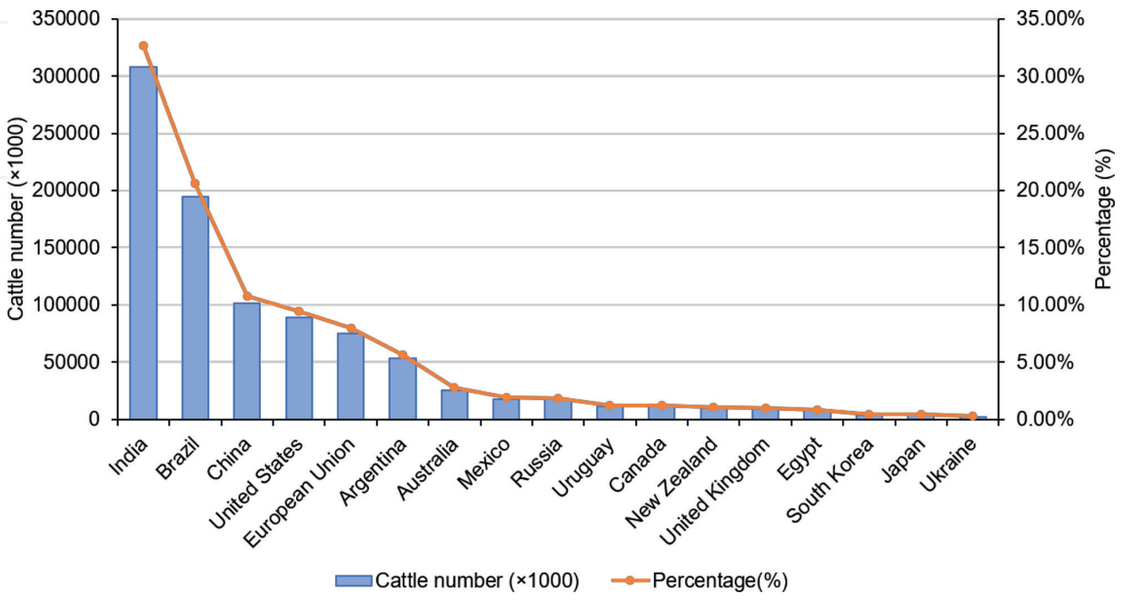

Figure 1.
Proportional distribution of cattle worldwide. (Data from FAS/USDA, 2023).

https://www.iets.org/]. However, the efficiency of bovine IVF remains low, with the number of transplantable embryos not exceeding 40%. Moreover, the pregnancy rate of bovine IVF embryos is relatively low, accompanied by a higher abortion rate. Only approximately 20–50% of cows that undergo IVF embryo transfer successfully give birth to calves, and some IVF calves exhibit a series of postnatal diseases, indicating the urgent need to improve the quality of bovine IVF embryos [3]. Therefore, exploring the mechanisms of development, maturation, and ovulation of domesticated bovine oocytes can contribute to improving the efficiency and quality of bovine IVF embryos, further enhancing the efficiency of breeding superior cattle in domestic settings, and increasing industrial benefits.

Based on this, this chapter primarily aims to provide support for improving the breeding efficiency of superior cattle and gaining a better understanding of the reproductive mechanisms in female mammals by reviewing the recent research on the development, maturation, and ovulation mechanisms of *in vitro* cultured bovine oocytes (follicles).

2. Oocyte developmental initiation

During embryonic development, the inner cell mass, which possesses pluripotency, can be isolated and cultivated *in vitro* to form a stem cell line. These stem cells can differentiate into various tissue-specific cells, with some pluripotent cells differentiating into primordial germ cells, the precursors of female oogonia and male spermatogonia. In bovine female embryos, primordial germ cells differentiate into oogonia during the first 3 months of gestation. During mid-gestation, oogonia migrate to the ovarian cortex, undergo the premeiotic phase of the first meiosis, and pause at the diploid stage. During this process, oogonia proliferate and around 2.7 million germ cells are produced at around 110 days of gestation, reaching their peak. Concurrently, somatic cells from the ovarian surface epithelium invade the ovary and form a flattened layer of cells, called follicular cells, around each oocyte, thus forming the primordial follicle [4].

In domestic cattle, some primordial follicles begin to grow during mid-gestation, while most remain quiescent, which can last for months to years. The growth of primordial follicles, including the proliferation of granulosa cells and the increase in oocyte volume, suggests that primary oocytes paused at the premeiotic phase of the first meiosis will be reactivated and prepared for restarting meiosis. The characteristics of growing primordial follicles are that the flattened follicular cells surrounding the oocyte increase in size and differentiate into cuboidal granulosa cells, forming primary follicles. Then, the paused primary oocytes increase in volume, and the surrounding granulosa cells divide to form multiple layers, forming secondary follicles. Primordial, primary, and secondary follicles collectively are called preantral follicles [5].

2.1 Signal and factors involved in preantral follicle activation and growth in domestic cattle

Numerous experimental results have demonstrated the importance of several pathways, including the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT), mammalian target of rapamycin 1 (mTORC1), and drosophila mothers against decapentaplegic protein 6 (SMAD6), as well as factors such as bone morphogenetic

proteins (BMPs), growth and differentiation factors (GDFs), anti-Mullerian hormone (AMH), insulin, basic fibroblast growth factor (bFGF), and epidermal growth factor (EGF), in the regulation of preantral follicle activation and growth in domestic cattle.

Many studies have shown that the PI3K signal participates in various life processes, such as cell proliferation, apoptosis, and differentiation [6, 7]. PI3K can activate or inhibit downstream target proteins (glycogen synthase kinase 3, GSK3; Bcl2 antagonist of cell death, BAD; cysteinyl aspartate specific proteinase, CASPASE9; p27 (Kip1), p27, etc.) by phosphorylating its downstream molecule AKT, leading to transcriptional regulation and thus regulating the aforementioned life processes in cells. Maidarti et al. have demonstrated that inhibiting PTEN (phosphatase and tensin homolog deleted on chromosome 10) to activate the PI3K-AKT pathway helps promote preantral follicle growth in domestic cattle ovaries [8]. Some upstream regulatory factors that can activate the PI3K-ATK pathway, such as insulin, FGF, VEGF (vascular endothelial-derived growth factor), and EGF, have been shown to increase the growth and development efficiency of primordial, primary, or stimulated follicles *in vitro* [9, 10]. These results indicate that the PI3K-AKT pathway mediates the proliferation and differentiation of oocytes and granulosa cells in preantral follicles by activating downstream target genes and promoting follicle development.

PI3K-AKT can also activate the downstream mTOR pathway through phosphorylation modification. The mTOR pathway can form two different protein complexes, called mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). The mTOR pathway also regulates cell survival, growth, and proliferation and can regulate preantral follicle development [11], participating in the regulation of granulosa cell proliferation and autophagy in domestic cattle ovaries [12].

BMPs, GDFs, and AMH are members of the TGFβ superfamily, which regulate the process of preantral follicle development through the activation of the SMAD6 pathway, among other mechanisms [13]. Studies have shown that BMP2 [14] and BMP15 [15] increase the number of secondary follicles *in vitro*, but continuous addition of BMP15 can alter the ultrastructure of follicles and even cause follicular atresia [16]. The addition of GDF-9 to the ovarian cortex culture system of fetal cattle significantly improves the survival rate and growth of follicles, while the rate of primary and secondary follicle formation also increases significantly [15]. In contrast to insulin, which promotes the development of preantral follicles in domestic cattle, AMH inhibits the activation of ovarian follicles and hinders follicle growth [17]. Furthermore, growth factors and cytokines such as bFGF [18], EGF [19], and Activin [20] have been found to promote preantral follicle survival and development in domestic cattle through autocrine and/or paracrine mechanisms. Also, bFGF and EGF could enhance the antrum formation rate to 33.3% in 21 days of *in vitro* culture [18].

Although it is generally believed that the development of preantral follicles in normal conditions is gonadotropin-independent, *in vitro* culture experiments of cattle follicles have shown that some hormones such as follicle stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E2) can regulate preantral follicle development under *in vitro* conditions [18, 20, 21]. Sun et al. found that the addition of FSH alone or in combination with LH and E2 significantly increased the diameter of primary follicles in cattle, with the combination of the three hormones showing the best effect [18]. Vasconcelos et al.'s study suggested that FSH can promote the diameter and cavity formation rate of secondary follicles in cattle, which is consistent with the aforementioned findings [22]. However, not all female hormones can improve the *in vitro* culture outcomes of follicles. The results of Paulino et al. indicated that

progesterone cannot synergize with EGF to enhance the growth of secondary follicles in cattle *in vitro* [23]. In fact, many bovine follicle culture systems incorporate gonadal hormones to improve their growth and developmental capabilities *in vitro*, but their underlying molecular mechanisms still require further investigation.

2.2 Progresses in *in vitro* culture of preantral follicles in domestic cattle

As mentioned earlier, preantral follicles in cattle can be classified into primordial follicles, primary follicles, and secondary follicles. Based on this classification, researchers have successfully achieved the precise *in vitro* isolation of preantral follicles from bovine ovaries and cultured them using different methods. In addition to isolating and culturing follicles of different hierarchical levels, some studies have also isolated the cortical portion of bovine ovaries and directly cultured them after cutting them into strip-like cortical strips [24]. Therefore, the *in vitro* culture of bovine ovaries can be categorized into primordial follicle culture, primary follicle culture, secondary follicle culture, preantral follicle culture (including the previous three categories), and ovarian cortical culture (see **Table 1**).

At present, there are two methods for cultivating preantral follicles of domestic calves: the 2D (two-dimensional) and 3D (three-dimensional) culture systems, with the latter using a matrix to support follicle growth. The basic culture media mainly used in these two cultivation systems include TCM199, McCoy, and αMEM. Jimenez et al. found that, compared to the basic culture media TCM199 and McCoy, α-MEM can better maintain follicle survival and promote follicle growth [25]. However, other research suggests that all three kinds of culture media can support the development of domestic cow preantral follicles and that there is no significant difference between α-MEM and TCM199 [19]. In addition, some studies have shown that personalized base media can also maintain follicle development (see **Table 1** for details).

3. Oocyte maturation

The process of producing mature oocytes in female mammals is a prolonged, complex, and discontinuous meiotic division process. Mammalian oocytes enter the early meiotic division stage during fetal development and are arrested at the prophase of the first meiotic division, namely the germinal vesicle (GV) stage. In post-pubertal cows, the elevation of follicle-stimulating hormone (FSH) concentration leads to the formation of dominant follicles on the ovary [31], and the pre-ovulatory surge of luteinizing hormone (LH) triggers the wall granulosa cells of dominant follicles to initiate the resumption of meiotic division in oocytes and progress to the second meiotic division (MII) stage, where oocytes are arrested at MII stage until ovulation. After fertilization, meiotic division is completed, forming male and female pronuclei, which then combine to form a diploid zygote. Oocyte maturation includes three developmental processes that are crucial for producing fertilizable and embryo-receptive oocytes: (1) nuclear maturation, which includes the resumption and completion of the first meiotic division and the maintenance of stable MII arrest; (2) epigenetic maturation, which occurs during oocyte growth and results in genomic modifications that regulate gene expression; and (3) cytoplasmic maturation, which usually refers to the processes occurring in the developing oocyte cytoplasm and is critical for fertilization and early embryonic development.

Follicle category	Base medium	Supplementary	Culture system	Status of follicles	References
Primordial follicle	α-MEM	BMP2, FSH	2D	Follicular diameter (+); Primordial follicles survival (+); Ultrastructure maintenance (+)	[14]
	α-MEM	GDF9, bFGF	2D	Primordial and primary follicles survival (+); Follicular diameter (+); Follicular activation (+)	[16]
Primary follicle	α-MEM	FSH, LH, E2, bFGF, EGF	3D	Follicular diameter (+); Antrum formation (+); Follicular maturation markers (+)	[18]
Secondary follicle	TCM199	P, EGF	2D	Follicular diameter (+); Ultrastructure maintenance (+)	[23]
	TCM199	KSR, FBS	2D	Follicles survival (+); Histological structure (+)	[26]
	TCM199	NAC	2D	Follicles survival (+); Ultrastructure maintenance (+); Mitochondrial cristae (−)	[27]
	TCM199	AVE	2D	Antrum formation (+); Follicles survival (+); Ultrastructure maintenance (+);	[28]
	TCM199	MLT	2D	Follicles survival (+); Follicular diameter (+); Antrum formation (+);	[29]
Preantral follicle	α-MEM	IL-1β	2D	Follicles survival (+); Follicular activation (+)	[30]
Ovarian cortex	WM	AMH	2D	Follicular activation (−); Follicular diameter (−);	[17]
	TCM199	FSH, ActA	2D	Follicular activation (+); Primordial follicles survival (−); Secondary follicles survival (+)	[20]

Abbreviations: 2D, two-dimensional culture system; 3D, three-dimensional culture system; KSR, knockout serum replacement; FBS, fetal bovine serum; NAC, N-acetylcysteine; AVE, Aloe vera extract; MLT, melatonin; IL-1, interleukin-1; T, testosterone; WM, Waymouth's medium MB 752/1; ActA, activin A; FSH, follicle-stimulating hormone; LH, luteinizing hormone; P, progesterone; bFGF, basic fibroblast growth factor; EGF, epidermal growth factor; BMP, bone morphogenetic protein; GDF, growth differentiation factor.+ means positive effects; − means negative effects.

Table 1.
Recent progress in in vitro culture of preantral follicles of domestic cattle in the past decade.

3.1 Maturation of bovine oocyte nucleus

The maturation of the bovine oocyte nucleus, from the GV stage to the MII stage, involves multiple processes such as germinal vesicle breakdown (GVBD), chromosome condensation, development to the first meiotic division (MI), spindle

formation, homologous chromosome separation, and extrusion of polar bodies before entering the MII phase [32].

3.1.1 Maintenance of meiotic arrest

cAMP is a critical molecule that regulates the meiotic division of bovine oocytes. The cAMP in the oocyte is produced through the synergistic action of the oocyte itself and the cumulus cells. First, the oocyte has a continuously active G protein-coupled receptor (GPR3 or GPR12), which activates Gas protein and then adenylate cyclase (ADCY) to synthesize the cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP), resulting in high levels of cAMP to maintain meiotic arrest [33, 34]. Second, the cyclic guanosine monophosphate (cGMP) in cumulus cells diffuses rapidly into the oocyte through gap junctions, inhibiting the activity of phosphodiesterase 3A (PDE3A) in the oocyte, an enzyme that catalyzes cAMP degradation to AMP, thus maintaining a high level of cAMP in the oocyte [35].

The high concentration of cAMP activates Protein kinase A (PKA) to maintain meiotic arrest in two ways. First, PKA acts on the cell cycle protein-cell division cycle 25C (CDC25), causing its phosphorylation and inactivation, leading to its retention in the cytoplasm, thereby blocking the dephosphorylation of cyclin-dependent kinase 1 (CDK1), a catalytic subunit of maturation promoting factor (MPF), by CDC25, resulting in the inactivation of CDK1. Second, PKA-mediated phosphorylation of nuclear protein Wee1B enhances its ability to inhibit CDK1 activity. PKA inhibits CDK1 activity through these two pathways, thereby suppressing MPF activity and maintaining meiotic arrest in the GV stage [36].

Natriuretic peptide C (NPPC, also known as CNP) secreted by mural granulose cells (MGCs) activates cGMP synthesis in bovine oocytes via its specific receptor, natriuretic peptide receptor 2 (NPR2), thereby inhibiting meiotic division [37]. NPR2 also exists in the bovine oocyte membrane, and CNP can directly activate cGMP synthesis in oocytes through NPR2 in the oocyte membrane [38]. Estradiol (E2) regulates the expression of NPPC/NPR2 by binding to the estrogen receptor (ER) ERα and ERβ [39]. Recent studies in cattle revealed that a tyrosine phosphatase—Src homology containing protein tyrosine phosphatase 2 (SHP2)—is involved in ERα transcriptional activity, promoting the expression of NPPC and NPR2 genes associated with meiotic arrest in the oocyte [40]. In addition, in mice, transforming growth factor β (TGF-β) was found to regulate NPPC expression in MGCs through SMAD Family Member 3 (SMAD3). In the presence of FSH, TGF-β further increased the NPPC level and inhibited the meiotic division recovery of cumulus-oocyte complexes [41]. The regulation of NPPC/NPR2 in the oocyte is reversible, as the LH peak can inhibit the activity of NPR2, reducing cGMP levels, and releasing the meiotic arrest of the oocyte [42].

3.1.2 Meiotic division resumption

When the follicle is stimulated by luteinizing hormone (LH), the cAMP levels in the oocyte and cumulus cells increase, interrupting the communication between the cumulus-oocyte complex and reducing cAMP flow into the oocyte, releasing the inhibition and promoting the resumption of meiotic division. The LH surge also promotes the release of epidermal growth factor (EGF) from the cumulus cells, activating the EGF receptor in the cumulus cells and leading to an increase in intracellular calcium ions. This elevation of calcium ions inhibits the activity of NPR2, reduces cGMP levels, and releases the meiotic arrest of the oocyte [42]. When the oocyte is isolated

from the follicle for in vitro maturation (IVM), the concentration of cAMP in the oocyte will decrease spontaneously due to the absence of inhibitory factors, leading to the spontaneous resumption of meiotic division [43]. Therefore, maintaining a high level of cAMP in the oocyte is an important strategy for maintaining meiotic arrest and improving IVM efficiency [44].

3.2 Maturation of bovine oocyte cytoplasm

Cytoplasmic maturation of the oocyte affects its quality and function and therefore determines the success rate of fertilization and embryo development [45]. Cytoplasmic maturation includes the synthesis, activation, and degradation of maternal mRNA, as well as the orderly arrangement of organelles.

During the growth phase, the oocyte has high transcriptional activity and accumulates a large amount of maternal mRNA and proteins, which control the initial stages of development after fertilization [46], but stop as the germinal vesicle breaks during meiotic division. Subsequently, as the oocyte matures, maternal mRNA storage begins to degrade, albeit at a slow rate [47]. After fertilization, the degradation of maternal mRNA accelerates, creating space for zygotic genome activation (maternal-to-zygotic transition, MZT) and the synthesis of new mRNA [48]. The degradation of maternal mRNA is regulated by various factors, including specialized RNA-binding proteins (such as Argonaute2), microRNA, poly(A) tail, transcription factors, and environmental factors [48]. The degradation of maternal mRNA has a significant impact on the quality and success rate of oocyte and embryo development. Abnormal maternal mRNA degradation may lead to a decline in oocyte quality, fertilization disorders, early miscarriage, and other issues [48].

In the process of oocyte maturation, ultrastructural changes, and redistribution can be observed in the cytoplasmic organelles, including maturation of cortical granules, mitochondria, endoplasmic reticulum (ER), and cytoskeleton. In the germinal vesicle (GV) stage, the organelles are unevenly distributed in the cytoplasm, mainly concentrated around the nucleus, while the cytoplasm beneath the zona pellucida contains fewer organelles [49]. The distribution of organelles remains uneven in MI stage, but changes do occur, such as an increase in the density of mitochondria and ER and a decrease in the density of Golgi apparatus and lysosomes. During the second meiotic division, the oocyte stops at MII stage and waits for fertilization, during which the distribution of organelles undergoes dynamic changes, such as the movement of mitochondria and ER from around the nucleus toward the periphery, forming a ring structure. This redistribution of organelles helps to form an eccentric spindle and asymmetric cytoplasm distribution [50]. These changes are mainly regulated by two types of cytoskeletons, microtubules, and actin, which interact with small GTPases, actin-nucleating factors, microtubule stabilizers, signal transduction pathways, and other factors to affect the developmental potential and fertilization ability of the oocyte [51].

4. Epigenetic maturation of bovine oocytes

Epigenetic maturation of oocytes refers to a series of genome modifications occurring at a critical stage during oocyte growth, regulating gene expression, nuclear structure, and chromosome stability in oocyte development and post-fertilization.

This process involves DNA methylation, histone modification, X-chromosome inactivation, and nucleolar reprogramming.

4.1 DNA methylation

DNA methylation is catalyzed by DNA methyltransferases (DNMT1, DNMT3A, and DNMT3B), which transfer the methyl group from S-adenosylmethionine (SAM) to the fifth carbon atom of cytosine residues, forming 5-methylcytosine (5mC) [52]. This modification is mainly present in CpG dinucleotides, while its presence in non-CpG environments is lower [53]. The process of DNA methylation is associated with the expression of the DNMT3 family of proteins [54] and is the basic mechanism for phenomena such as transposon silencing, X chromosome inactivation, and genomic imprinting [53]. DNA methylation is an important epigenetic modification that can affect gene expression without altering DNA sequences. In mammals, the state of DNA methylation is inherited from gametes, thus determining gene imprinting [55, 56]. Some genes in bovine oocytes, such as insulin like growth factor 2 receptor (IGF2R), potassium voltage-gated channel subfamily Q member 1 (KCNQ1), pleomorphic adenoma gene-like 1 (PLAGL1), and small nuclear ribonucleoprotein polypeptide N (SNRPN), are imprinted genes that undergo DNA methylation modification [57]. In addition to DNA methylation, chromatin status in oocytes can also affect gene imprinting, such as through histone modifications [55].

4.2 Histone modifications

During oocyte development, there are various histone modifications, including acetylation and methylation, which can regulate nuclear-cytoplasmic balance, gene expression, and developmental potential [58–60]. Histone methylation refers to the transfer of methyl groups to lysine and arginine residues on the extended histone tails of nucleosomes. Different states of histone methylation can affect chromatin structure and function and thus influence oocyte quality and development. Histone H3 lysine K4 trimethylated (H3K4me3) is an activating modification of gene transcription, which is widely present in bovine oocytes, especially on the promoters and gene bodies of development-related genes [58]. Another modification, histone H3 lysine K27 trimethylated (H3K27me3), is a suppressive modification of gene transcription which, together with histone H3 lysine K9 trimethylated (H3K9me3), occupies gene bodies to form bivalent regions in bovine oocytes [59]. These modifications decrease before embryonic genome activation and then increase with cell division [60].

Histone acetylation refers to the addition of acetyl groups to lysine residues of histones. It is a dynamic modification regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs). Histone acetylation increases during the maturation of bovine oocytes, particularly at H4K5 and H4K8 sites, which may be related to chromatin remodeling and transcriptional activation in oocyte nuclei [61]. HDACs can remove acetyl groups from histones and inhibit gene transcription. The expression of HDACs in bovine oocytes is influenced by nutrients and metabolic pathways, which in turn affect oocyte quality and developmental capacity [62]. Studies have analyzed changes in histone acetylation status and expression of related genes in bovine oocytes of different abilities during *in vitro* maturation and found that oocytes of higher competence have higher levels of histone acetylation and lower expression of histone deacetylases [63]. Therefore, the addition of HDAC inhibitors (such as

scriptaid) during *in vitro* maturation can improve the maturation, fertilization, and embryo development rates of bovine oocytes [64].

5. Ovulation

Ovulation in bovine oocytes refers to the process where the dominant follicle in the ovary ruptures and releases the oocyte, marking the transition from the follicular phase to the luteal phase. This process is primarily regulated by the ovarian cycle and hormones, with a cycle range of 18–24 days, and ovulation usually occurs in the middle to late phase of the cycle. Prior to ovulation, the luteinizing hormone (LH) increases, stimulating the development of the follicle and the maturation of granulosa cells [65]. When the LH peak is reached, it induces the release of the oocyte from the first meiotic division, marking the imminent ovulation.

It takes about 100 days for a follicle and its oocyte to mature to the ovulation stage [66]. The bovine oocyte reaches its maximum size when the follicle develops to a diameter of about 3 millimeters. After ovulation, the cumulus cells expand and shed, leaving the oocyte completely exposed on the surface of the ovary, close to the oviduct. The oviduct fimbria responsible for capturing the oocyte expands and folds to help draw the oocyte into the oviduct.

Once in the oviduct, the oocyte completes the second meiotic division within 4–6 hours, forming a two-cell embryo with two chromosomes and two cytoplasms. These two cells further divide and eventually develop into a blastocyst in the oviduct, which enters the uterus about 3–4 days later.

Ovulation is a complex process regulated by LH, which can activate a series of inflammation-related signaling pathways such as prostaglandins, interleukins, and chemokines [67]. However, ovulation also involves some processes that are different from inflammation, such as the effects of steroid hormones, oocyte maturation, and final release. In addition, ovulation is influenced by other factors such as sexual activity, nutrition, and the immune system [68]. The characteristic of bovine ovulation is the presence of neutrophils, macrophages, and dendritic cells in the ovulatory follicles, reflected in compartmentalized expression of cytokines and growth factors [69].

6. Techniques for assessing oocyte quality

The blastocyst rate in bovine *in vitro* embryo production is only 20–40% [70], and the key to improving blastocyst rate lies in enhancing the quality of oocytes [71]. Oocyte quality refers to the ability of oocytes to develop into blastocysts *in vitro*. Ideally, an oocyte quality assessment method should be simple, rapid, cost-effective, reasonably reliable, and most importantly, non-invasive. However, the current evaluation methods do not fully meet all these requirements.

Morphological analysis under an optical microscope is the most commonly used non-invasive quality assessment method. It involves observing the appearance of surrounding cells, nuclear maturation, cytoplasmic characteristics, and extracellular structures of oocytes to determine their quality and developmental potential. An oocyte with an "ideal" morphology should exhibit criteria such as matured nucleus, normal size, cytoplasmic appearance, zona pellucida thickness, perivitelline space, and first polar body morphology [72]. Artificial intelligence (AI) has been incorporated into oocyte morphological research in human-assisted reproduction, utilizing

digital image analysis techniques to identify and classify oocytes with the highest developmental potential [73]. AI can enhance the accuracy and consistency of oocyte morphological evaluation [74].

Brilliant cresyl blue (BCB) staining is a non-invasive method for assessing oocyte quality, which reflects the activity of glucose-6-phosphate dehydrogenase (G6PDH) in oocytes. This enzyme exhibits high activity in growing oocytes but decreases in mature oocytes [75].

Oocyte quality can also be indirectly assessed by examining the surrounding environment. Follicular fluid is the only biological fluid during oocyte development and contains metabolites crucial for oocyte growth and development [76]. Therefore, follicular fluid can reflect embryo viability and oocyte quality [76]. Through the analysis of follicular fluid, follicular development, and oocyte quality can be assessed non-invasively. Raman spectroscopy can be used for biological diagnostics and chemical identification of follicular fluid [77]. Metabolomic analysis can investigate the composition and changes of metabolites in follicular fluid and their relationship with oocyte quality and IVF outcomes [78]. Proteomic analysis can examine protein expression and function in follicular fluid, as well as their relationship with oocyte maturation and IVF success rates [79, 80].

Granulosa cells, the layer of cells closely associated with oocytes, play a vital role in oocyte development and function. The morphological characteristics of granulosa cells are observed to assess oocyte quality. For example, the quantity, size, shape, distribution, proliferative capacity, and apoptotic rate of granulosa cells can influence the supply of nutrients and signaling molecules in follicular fluid, thereby affecting oocyte growth and maturation [81]. By detecting mRNA transcripts in granulosa cells, biomarkers related to oocyte and embryo developmental competence can be identified. Genes such as HAS2, GREM1, PTGS2, and others have been found to be associated with oocyte maturity and embryo implantation rate based on their expression levels in granulosa cells [82]. By analyzing proteins or metabolites in granulosa cells, biomarkers related to oocyte and embryo developmental competence can be identified. For instance, antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX)) [83], growth factors (bone morphogenetic protein 4 (BMP4), gremlin (GREM), connective tissue growth factor (CTGF)) [84], and hormones (luteinizing hormone (LH), estradiol (E2), and progesterone (P4)) [85] found in granulosa cells can reflect oocyte stress response, metabolic status, and signal transduction.

There are also invasive techniques for assessing oocyte quality, although they may not be feasible for clinical applications. However, these techniques can provide a deeper understanding of the structure, function, and metabolic characteristics of oocytes, as well as predict non-invasive biomarkers for oocyte *in vitro* culture.

Genetic assessment is the process of using molecular biology techniques to test the chromosomes, genes, or epigenetic features of oocytes in order to evaluate their quality and developmental potential. Research has already been conducted on transcriptome data analysis of bovine oocytes [86], and in the future, analyses such as genome sequencing, whole exome sequencing, and single nucleotide polymorphism can also be performed.

Metabolic testing is a method of evaluating the quality of oocytes by analyzing their metabolites or energy levels. This method can detect various indicators such as glucose uptake [71], intracellular oxygen metabolism [87], lactate production [88], ATP content [89], mitochondrial function [90, 91], and epigenetic changes [92], which may reflect the vitality and maturity of oocytes.

7. Conclusion

Although many aspects of bovine oocyte development have been well studied and related molecular mechanisms have been identified, oocyte development remains quite mysterious. It is currently unclear why less than 50% of oocytes can develop into blastocysts after fertilization. Focusing on the study of oocyte maturation conditions and the identification of competent oocytes should help improve reproductive success rates.

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