Springfield Publishers

Current Trends in Veterinary and Dairy Research

Open access

Mini Review

Submission: February 25, 2020 | **Published:** March 13, 2020

Biochemical Events During Bovine Oocyte Maturation: What Do We Know So Far?

S Amsaveni*

National Institute of Research on Tuberculosis, Chennai, India

***Corresponding author:** S Amsaveni, National Institute of Research on Tuberculosis, No.1, Mayor Sathiyamoorthy Road, Chetpet, Chennai - 600 031, India

Abstract

Oocyte maturation and developmental competence are the words that are closely related and used interchangeably in the field of reproductive biology. Assisted Reproductive technology is a boon to the cattle industry to obtain offspring of genetically high-quality in a short period of time from elite donor cows. In-vitro fertilization is superior to Embryo transfer, which allows the breeders to maximize the use of valuable and sexed semen. The oocyte performance does not always remain the same as it gets influenced by several environmental and physiological factors once it comes out of the live animal. So, the complete understanding of these factors and the process that oocytes undergo from immature stage to reach mature status is essential. The optimum culture conditions required supporting the growth and differentiation is must to obtain competent oocytes. This review will focus on the biochemical events that have been recorded during in-vitro maturation of bovine oocytes.

Keywords: Bovine oocyte; Meiotic arrest; Developmental competence; Meiotic resumption

Abbreviations: ART: Assisted Reproductive Techniques; MP: Meiotic Prophase; LH: Luteinizing Hormone; MII: Metaphase Phase II; IVF: *In Vitro* Fertilization; GV: Germinal Vesicle; GVBD: Germinal Vesicle Breakd; OSF: Oocyte-Secreted Factor; TGFB: Transforming Growth Factor-B; BPM: Bone Morphogenetic Protein; GDF9: Growth Differentiation Factor-9; FGF: Fibroblast Growth Factor; CTSB: Cathepsin B; IGFBP4: Insulin Like Growth Factor Binding Protein 4; FGF11: Fibroblast Growth Factor 11; COL18A1: Collagen Type Xviii Alpha 1 chain; MPF: Maturation-Promoting Factor; MAPK: Mitogen-Activated Protein Kinase; HH1K: Histone H1 Kinase; MI: Metaphase I; cAMP: Cyclic AMP; AC: Adenylyl Cyclases; ROS: Reactive Oxygen Species; IVC: In-Vitro Culture; OS: Oxidative Stress; mtDNA: mitochondrial DNA; SOD: Super-Oxide Dismutase; GSH: Glutathione Peroxidase

Introduction

Oocyte maturation is a composite series of events that results in growth and differentiation of oocytes from meiotic arrest to meiotic resumption [1,2]. The primordial follicles, fundamental developmental unit of the mammalian ovary are the oocytes covered by single layer of flattened granulosa cells [3]. The size of this primordial follicle pool determines the reproductive capability in females [1]. "Developmental competence is the inherent capacity of the immature oocyte to successfully mature, upon fertilization can develop into a viable blastocyst followed by a healthy progeny [4]. Acquisition of this developmental competence is the major challenge for Assisted Reproductive Techniques (ART) [5].

During the fetal development primordial germ cells gets blocked at the (G2/M border) diplotene stage of the first Meiotic Prophase (MI), primordial follicle stage. Once the oestrus cycle begins in bovines these primordial follicles undergo sequence of events from pre-antral follicle towards pre-ovulatory follicle under the influence of Luteinizing Hormone (LH) surge that facilitates the capacity of resuming the arrested meiosis. After meiotic resumption from prophase of MI, the oocyte development once again get arrested at Metaphase Phase II (MII) till fertilization-'Meiotic Arrest'[1,6,7]

Sometimes the ovulated metaphase II oocyte might resist fertilization or may not be highly competent to progress into a fertilizable egg [8]. The successful oocyte maturation occurs between the first and the second meiotic block of the folliculogenesis followed by oogenesis. In-vitro maturation of immature oocytes from antral follicle/prior stages of follicle would increase the

number of fertilizable oocytes during In-Vitro Fertilization (IVF) techniques for successful embryo implantation. High demand of transferable embryos from elite cattle is increasing day by day across the globe when used in conjunction with the sexed embryos so that predetermined numbers of pregnancies can be achieved within a short period of time. IVF could effectively replace conventional embryo production methods [9]. This review is aimed at providing an overview about the dynamic events that occur during the sequence of stages from immature to fully matured bovine oocyte.

The cell organelles during the meiotic resumption acquire different positions in the cytoplasm of the cell during the transitional stages. The cytoskeletal microfilaments and microtubules aids in the movement of these organelles and acts on chromosome segregation. Distribution of Golgi and Endoplasmic reticulum distribution until MII arrest are same as that of human oocyte competence [10]. Bovine Germinal Vesicle (GV) chromatin condenses into a peri nucleolar ring during follicular growth and less condensed pattern of GV chromatin is observed before Germinal Vesicle Breakdown (GVBD) in bovine oocytes [11]. Independent Golgi structures vesiculate following GVBD, Matrix fraction disperses in MII oocytes and redistribution of Golgi matrix are notable events. Microtubule-dependent movement of these organelles is also essential during in vitro oocyte maturation [6].

Energy metabolism

In bovine oocytes oxidative metabolism is the primary way of energy generation [12]. Bovine cumulus cells exhibit high concentrations of enzymes, alanine aminotransferase and aspartate aminotransferase. Aspartate supplements tri-carboxylic acid cycle by contributing oxaloacetate. Glucose present in the invitro maturation medium was extensively utilized by cumulus cells and converted into pyruvate which is the primary end product of glycolysis that can enter the tri-carboxylic acid cycle serving as an important route for ATP production and serving as preferred energy substrate of the oocyte. Energy production, in terms of ATP generation, is much higher for β-oxidation than glycolysis. Lipid found in cumulus cells plays a major role of oocyte maturation and attaining developmental competence. Carnitine and creatinine are two biochemicals that were observed during oocyte maturation. Within 8hrs of oocyte maturation though creatinine is found in impressive quantities carnitine metabolites takes an upper role because of its high concentration in maturation medium suggesting that this pathway is very important for energy production [13]

The cumulus cells surrounding the oocyte in ovarian follicles nourish them with small molecules that permit growth and control maturation. Multiple connexins are found in the gap junctions between the cumulus cells and oocytes. The nutrients reach the germinal cell through gap junction channels. This Gap junctional coupling between cumulus cells is required for oocytes to reach developmental competence [14].

Oocyte-Secreted Factors (OSFs) help in the regulation of cumulus cells differentiation. Relevant OSF related to fertility that belong to the Transforming Growth Factor-B (TGFB) superfamily of paracrine factors are Bone Morphogenetic Protein (BMP15) and Growth Differentiation Factor-9 (GDF9), that are exclusively expressed by ruminants in oocytes (6) are produced within the oocyte and act on cumulus and granulosa cells to regulate folliculogenesis and oogenesis. Fibroblast Growth Factor 10 (FGF 10) enhances cumulus expansion in bovine oocytes [15]. BMP15 and FGF10 increase glucose uptake by cumulus cells and are associated with up-regulation of expression of key genes involved in hyaluronic acid production [16-19]. FGF10 influenced the expression of Cathepsin B (CTSB) and Sprout RTK Signaling Antagonist (SPRY2) in cumulus cells and BMP15 in oocytes. Also, the expression of three up-regulated Sprout RTK signaling antagonist 1 (SPRY1), Insulin like Growth Factor Binding Protein 4 (IGFBP4), Fibroblast Growth Factor 11 (FGF11) and down regulated genes Rho GTPase activating protein 22 gene(ARHGAP22), Collagen Type Xviii Alpha 1 Chain (COL18A1) and glypican 4 (GPC4) genes were shown as candidate markers of oocyte developmental competence in bovines [20].

Events of Nuclear and Chromosome Maturation

The resumption of meiosis is induced by the pre-ovulatory gonadotropin surge in-vivo. The nuclear and cytoplasmic events occurring during the process of meiotic resumption are called maturation and are the prerequisites for fertilization and early embryonic development. The increased activities of Maturation-Promoting Factor (MPF) and Mitogen-Activated Protein Kinase (MAPK) are necessary for onset of GVBD and metaphase progression during oocyte maturation and meiotic arrest [21,22]. As like other mammals the M-Phase/Maturation Promoting Factor (MPF) plays a centric role in the oocyte maturation process in bovines. MPF is a heterodimer, consisting of a catalytic subunit CDK1 (p34cdc2) and a regulatory subunit Cyclin B. Bovine immature oocyte lack cyclin B and that cyclin B synthesis is the trigger for GVBD, but in other mammal's protein synthesis requirement is important for GVBD. Though there are high amounts of cyclin B1 mRNA that are present in bovine oocytes that were arrested at the GV stage, the cyclin B1 protein remains undetectable at that stage which is required for forming pre-MPF complex. So that this lack of cyclin B1 protein results in the arrest of the fully grown bovine oocyte at the G2/M transition [6,23-25]. De novo synthesis of cyclin B2 occurs during the process of maturation [21]. Bovine oocytes display a biphasic pattern of expression by synthesizing cyclin B2 before MI and before MII. The newly synthesized cyclin B2 is required for pre-MPF that gets activated during M1. Bovine oocytes displayed a second round of cyclin B2 synthesis during which parallel increase in Histone H1 Kinase (HH1K) activity has been observed. HH1K is widely accepted as a biochemical indicator of p34Cdc2 protein kinase complex activity and therefore MPF activity. The quantum of cyclin present in bovine oocytes is critical to meiotic resumption.

Inactivation of MPF is associated with cyclin degradation and is closely linked to the meiotic release [6,22,26]. In general, p34cdc2 is phosphorylated at Thr-14 and Tyr-15 after association with cyclin B to prevent the premature activation of p34cdc2/cyclin B. Activated MPF is composed of certain amount of cyclin B, dephosphorylated Tyr15 and Thr14 residues on p34cdc2 and phosphorylation of Thr161 of cdc2 subunit. Activation of cyclin-dependent kinases in higher eukaryotic cells can be achieved through dephosphorylation by members of the Cdc25 phosphatase family [27].

This protein can be detected only at a certain time before mitotic entry. Cdc25 initiates mitosis by dephosphorylating cdc2 on Tyr15 and Thr14, thus activating MPF at the G2 to M transition in bovine oocytes during maturation process is accompanied by the Cdc25C activation due to hyperphosphorylation and was exclusively found in the nucleus at the germinal vesicle stage and during the early embryonic development until the blastocyst stage. Cdc25C was expressed throughout the maturation process and the early development [28,29]. The accumulation of cyclin B1 and the phosphorylation state of cdc2 are important in determining the timing of entry into mitosis in bovine oocytes. Also, mRNA molecules coding for proteins required for chromatin condensation and GVBD, spindle assembly of Metaphase I (MI), MPF and MAP kinase become polyadenylated during the meiotic maturation process resulting in molecular maturation [30,31].

The c-mos proto-oncogene product (Mos) is believed to be an active component of the cytostatic factor that stabilizes and sustains the activity of MPF. It has been demonstrated that Mos is present and actively synthesized in mature bovine oocytes and has association with β-Tubulin (cytoskeletal protein) in mature bovine oocytes that is necessary for maintaining the meiotic arrest [20,32].

In bovine somatic cells, where positive correlation between the translation initiation factor eIF4E (the cap binding protein) phosphorylation (correlated with the activation of Map kinase) and translation rate exists, whereas only a partial effect has been observed in case of bovine oocytes. This substantial phosphorylation begins at the time of GVBD and drops down sharply as it progresses to the metaphase II stage to the levels similar to those found in GV-stage oocytes. But, a specific repressor of eIF4E, the binding protein 4E-BP1 which forms a complex with eIF4E, is present in bovine oocytes and could be involved in preventing eIF4E function in metaphase II stage oocytes, resulting in decreased translation rates in metaphase II oocytes [23] Inactivation of MAPK is closely associated with arrest of mammalian oocytes in the GV stage and also with pronuclear development [33,34]. Prolactin prolonged the time of meiotic maturation of bovine oocytes [35].

In bovine oocytes activation of both the enzymes MPF and MAPK occurred simultaneously and was associated with GVBD. It is proposed that MAP kinases take part in the regulation of microtubule and chromatin organization during oocyte maturation, MPF and MAP kinase seem to be activated with different kinetics in different species [36].

In bovine oocytes, Calcium (Ca) appears to be necessary for GVBD and progression of meiosis. Protein Kinase C (PKC) activation accelerated GVDB while PKC inhibition prevented GVBD. The synthesis and degradation of cyclic AMP (cAMP) is regulated by Adenylyl Cyclases (AC) and Phosphodiesterase's (PDE), respectively has an important role in the maintenance of meiotic arrest of oocytes but the level of arrest is not appreciable as other mechanisms of meiotic arrest dominates in bovine oocytes [37,38].

The standard In-Vitro Culture (IVC) conditions usually increase Reactive Oxygen Species (ROS) because oocytes use oxygen to produce energy through mitochondrial oxidative phosphorylation and ROS production is increased during IVM, which have been implicated as one of the major causes for reduced embryonic development [21]. Reactive Oxygen Species (ROS) production are a normal process of cellular metabolism. The micromolecular environment of the oocytes in the culture medium may increase the ROS thereby resulting in oxidative cell damage. Oocytes with their own enzymatic antioxidant system could be capable of controlling the increase in ROS thereby maintaining a balance. Oxidative Stress (OS) is caused by an imbalance between pro-oxidants and antioxidants. This ratio could change with increased levels of pro-oxidants, such as ROS, or a decrease in antioxidant defense mechanisms [39]. The mechanisms of antioxidants can be divided into nonenzymatic and enzymatic [40]. Mitochondrial DNA (mtDNA) as it lacks histones that should protect them from damage are highly susceptible when compared to nuclear DNA to the oxidative stress due to ROS. 23 Antioxidant Enzymes includes Super-Oxide Dismutase (SOD), Glutathione Peroxidase (GSH), catalase and non-enzymatic antioxidant agents like ascorbic acid, glutathione etc. Among the important enzymatic antioxidant SOD been established exerting the highest with respect to cumulus cells [41] Also in bovine oocytes GSH is considered as a vital biochemical marker of oocyte viability and quality [42].

Conclusion

Agents that determine the developmental competence have been employed in invitro-maturation studies of bovine oocytes. The biochemical, physiological and molecular events that encompass the mystery of meiotic arrest and resumption in bovine oocytes have been unraveled by various researchers. Attempts to understand these events have helped the community involved in assisted reproductive techniques to optimize the culture conditions of oocyte maturation, fertilization and embryo production to obtain good quality offspring of genetically improved traits in a short period of time. More in depth multi-omics approaches employing recent technologies will help us to understand the micro-molecular

changes at nano scale levels that could act as potent biomarkers for bovine oocyte developmental competence.

References

1. Pan B, Li J (2019) The art of oocyte meiotic arrest regulation. Reprod Biol Endocrinol 17(1): 8.
2. Downs SM (2002) The Biochemistry of Oocyte Maturation. The Future of the Oocyte 2002(41): 81–99.
3. Cordeiro MH, Kim SY, Woodruff TK (2015) Ovarian Follicle Biology and the Basis for Gonadotoxicity. Cancer Treatment and the Ovary 3-20.
4. Cetica PD, Pintos LN, Dalvit GC, Beconi MT (2001) Antioxidant enzyme activity and oxidative stress in bovine oocyte *in vitro* maturation. IUBMB Life 51(1): 57-64.
5. Kempisty B, Zawierucha P, Ciesiolka S, Piotrowska H, Antosik P, et al. (2015) Developmental competence of mammalian oocytes-insights into molecular research and the promise of microfluidic technology: a review. Vet Med 60: 663– 674.
6. Levesque JT, Sirard MA (1996) Resumption of meiosis is initiated by the accumulation of cyclin B in bovine oocytes. Biol Reprod 55: 1427–1436.
7. Gordo AC, He CL, Smith S, Fissore RA (2001) Mitogen activated protein kinase plays a significant role in metaphase II arrest, spindle morphology, and maintenance of maturation promoting factor activity in bovine oocytes. Mol Reprod Dev 59(1): 106-114.
8. Oulhen N, Mori M, Dumollard R (2013) Meeting report - oocyte maturation and fertilization: lessons from canonical and emerging models. J Cell Sci 126: 4321-4324.
9. Bousquet D, Twagiramungu H, Morin N, Brisson C, Carboneau G, et al. (1999) *In vitro* embryo production in the cow: An effective alternative to the conventional embryo production approach. Theriogenology 51(1): 59-70.
10. Rawe VY, Espanol AJ, Nodar F, Olmedo B (2005) Mammalian Oocyte *in Vitro* Maturation: The Endomembrane System Dynamics. S420 Abstracts 84(Supp 1): S420.
11. Buccione R, Schroeder AC, Eppig JJ (1990) Interactions between somatic cells and germ cells throughout mammalian oogenesis. Biol Reprod 43(4): 543–547.
12. Uhde K, Van Tol HTA, Stout TAE, et al. (2018). Metabolomic profiles of bovine cumulus cells and cumulus-oocyte-complex-conditioned medium during maturation *in vitro.* Sci Rep 8, 9477.
13. Vozzi C, Formenton A, Chanson A, Senn A, Sahli R, et al. (2001) Involvement of connexin 43 in meiotic maturation of bovine oocytes. Reproduction 122(4): 619-628.
14. Zhang K, Hansen PJ, Ealy AD (2010) Fibroblast growth factor 10 enhances bovine oocyte maturation and developmental competence *in vitro*. Reproduction 140(6), 815-826.
15. Araújo VR, Almeida AP, Magalhães DM, Matos MHT, Tavares LMT, et al. (2010) Role of Bone Morphogenetic Proteins-6 and -7 (BMP-6 and -7) in the regulation of early foliculogenesis in mammals. Rev Bras Reprodução Anim 34: 69–78.
16. Castro FC, Cruz MH, Leal CL (2015) Role of Growth Differentiation Factor 9 and Bone Morphogenetic Protein 15 in Ovarian Function and Their Importance in Mammalian Female Fertility - A Review. Asian Australas J Anim Sci 29: 1065-1074.
17. Su YQ, Sugiura K, Wigglesworth K, O Brien MJ, Affourtit JP et al. (2008) Oocyte regulation of metabolic cooperativity between mouse cumulus cells and oocytes: BMP15 and GDF9 control cholesterol biosynthesis in cumulus cells. Development 135(1): 111-121.
18. Moore RK, Erickson GF, Shimasaki S (2004) Are BMP-15 and GDF-9 primary determinants of ovulation quota in mammals? Trends Endocrinol Metab15(8): 356-361.
19. Melo EO, Cordeiro DM, Pellegrino R, Wei Z, Daye ZJ, et al. (2017) Identification of molecular markers for oocyte competence in bovine cumulus cells. Anim Genet 48: 19–29.
20. Wu B, Ignotz G, Currie WB, Yang X (1997) Dynamics of maturation promoting factor and its constituent proteins during *in vitro* maturation of bovine oocytes. Biol Reprod 56: 253–259.
21. Khazaei M, Aghaz F (2017) Reactive Oxygen Species Generation and Use of Antioxidants during *In Vitro* Maturation of Oocytes. Int J Fertil Steril 11(2): 63-70.
22. Bilodeau Goeseels S (2008) Review: The regulation of meiotic maturation in bovine oocytes. Can Vet J 88(3): 343-349.
23. Kanitz W (2002) Quantification of cyclin B1 and p34(cdc2) in bovine cumulus-oocyte complexes and expression mapping of genes involved in the cell cycle by complementary DNA macroarrays. Biology of Reproduction 67(5): 1456-1464.
24. Shimaoka T, Nishimura T, Kano K, Naito K (2009) Critical effect of pigWee1B on the regulation of meiotic resumption in porcine immature oocytes. Cell Cycle 8(15): 2375-2384.
25. Jones KT (2004) Turning it on and off: M-phase promoting factor during meiotic maturation and fertilization. Mol Hum Reprod, 10: 1–5.
26. Murray AW, Solomon MJ, Kirschner MW (1989) The role of cyclin synthesis and degradation in the control of maturation promoting factor activity. Nature 339: 280-286.
27. Galaktionov K, Jesus C, Beach D (1995) Raf1 interaction with cdc25 phosphatase ties mitogenic signal transduction to cell cycle activation. Genes Dev 9(9): 1046–1058.
28. Nilsson I, Hoffmann I (2000) Cell cycle regulation by the Cdc25 phosphatase family. Prog Cell Cycle Res. 4: 107-114.
29. Gall L, Bourhis DL, Ruffini S, Boulesteix C, Vignon X (2008) Unexpected nuclear localization of Cdc25C in bovine oocytes, early embryos, and nuclear-transferred embryos. Reproduction 135(4): 431–438.
30. Wehrend A, Meinecke B (2001) Kinetics of meiotic progression, M-phase promoting factor (MPF) and mitogen-activated protein kinase (MAP kinase) activities during *in vitro* maturation of porcine and bovine oocytes: species specific differences in the length of the meiotic stages. Anim Reprod Sci 66(3-4): 175–184.
31. Liu L, Yang X (1999) Interplay of Maturation-Promoting Factor and Mitogen-Activated Protein Kinase Inactivation during Metaphase-to-Interphase Transition of Activated Bovine Oocytes. Biol Reprod 61(1): 1–7.
32. Wu B, Ignotz G, Currie WB, Yang X (1997) Expression of Mos proto-oncoprotein in bovine oocytes during maturation *in vitro*. Biol Reprod 56: 260–265.
33. Krischek C, Meinecke B (2002) *In vitro* maturation of bovine oocytes requires polyadenylation of mRNAs coding proteins for chromatin condensation, spindle assembly, MPF and MAP kinase activation. Anim Reprod Sci 73(3-4): 129-140.
34. Tomek W, Sterza Melo FA, Kubelka M, Wollenhaupt K, Torner H (2002) Regulation of translation during *in vitro* maturation of bovine oocytes: the role of MAP kinase, eIF4E (Cap binding protein) phosphorylation and eIF4E-BP1. Biol Reprod 66 1274–1282.
35. Torner H, Kubelka M, Heleil B, Tomek W, Alm H, et al. (2001). Dynamics of meiosis and protein kinase activities in bovine oocytes correlated to prolactin treatment and follicle size. Theriogenology 55(4): 885-899.
36. Fissore RA, He CL, Vande Woude GF (1996) Potential role of mitogen-activated protein kinase during meiosis resumption in bovine oocytes. Biol Reprod 55(6): 1261–1270.
37. Lefèvre Brigitte, Pesty Arlette, Koziak Katarzyna, Testart Jacques (1992) Protein kinase C modulators influence meiosis kinetics by not fertilizability of mouse oocytes. J Exp Zool 264(2): 206-213.

38. Aktas H, Leibfried Rutledge ML, First N (2003) Meiotic state of bovine oocytes is regulated by interactions between cAMP, cumulus and granulosa. Mol Reprod Dev 65(3): 336-343.

39. Burton GJ, E Jauniaux (2011) Oxidative stress. Best Pract Res Clin Obstet Gynaecol 25(3): 287-299.

40. Wang S, He G, Chen M, Zuo T, Xu W, et al. (2017) The role of antioxidant enzymes in the ovaries. Oxid Med Cell Longev. 2017: 4371714.

41. Torres Osorio V, Urrego R, Echeverri Zuluaga JJ, López Herrera A (2019) Oxidative stress and antioxidant use during *in vitro* mammal embryo production. Review 10(2): 433-459.

42. Ashibe S, Miyamoto R, Kato Y, Nagao Y (2019) Detrimental effects of oxidative stress in bovine oocytes during intracytoplasmic sperm injection (ICSI). Theriogenology 133: 71-78.

-- *** --