Different Aspects of Cattle Oocyte in vitro Maturation: Review

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Abstract: Cattle are considered one of the most important pillars of animal production in most countries of the world. As it being a building block in their national security strategies; endeavoring them to increase this animal population by all possible means. During the last three decades; it was clear that in vitro maturation, fertilization and culturing of bovine oocytes are the most important of these applications, which intended to improve this animal productivity and reproductivity. For that, the goals of this review are to discuss the different aspects of cattle oocyte maturation from the different angles.

Key words: Bovine • Oocyte • in vitro Maturation • Cytoplasmic • Nuclear

INTRODUCTION

in vitro embryo production (IVEP) using slaughterhouse ovaries as a source of oocytes is of a great importance not only for the inexpensive mass production of cattle embryos for agriculture purposes but also as a research tool for improving cattle genetic merits at high rate as well as understanding this animal embryology that help improving its population [1-3]. Moreover, the emerging embryo technologies are offering new opportunities in reproductive medicine and infertility treatment [4]. A constant and high rate of blastocyst development requires strict precautions and precise standards in every aspect of the IVEP system [3]. Even though, the success of bovine IVEP is still low (10-30%) [5, 6], if compared with in vivo results (50-80%) [6, 7] Moreover, there is a huge difference in blastocyst rate and quality obtained through IVEP between laboratories even they used the same techniques and media [8, 9]. These huge discrepancies between in vitro and in vivo signify that the new technique remains challenging and in many areas the standard operating procedures need to be optimized [10, 12].

Upon that, the key element for IVEP success of mammalian embryos has been widely investigated in efforts to approximate the efficiency and quality provided in vivo[4]. Moreover, the recent intentions are directed to mimic in vivo environment to avoid IVEP disorders at the level of embryo morphology and gene expression or even at the level of gestation and offspring [1].

Oocyte Maturation

Oocytes Recovery from the Slaughtered Animals: Mammalian ovaries contain large number of immature oocytes which can be utilized for the reproductive technologies such as in vitro fertilization (IVF) and nuclear transfer, to increase the endangered and genetically superior animals’ populations. Furthermore, these oocytes are considered as a vast resource and a potential source for the female gametes to produce live offspring that can be used not only for domestic and zoological researches but also for the agriculture procedures [13]. Upon that, large-scale production of average genetic merit embryos and large quantities of material can be obtained by collecting such ovaries at slaughterhouses then shipping them to the laboratory.
either dry or in warmed saline [14, 15]. From these collected ovaries, oocytes could be obtained easily and inexpensively [16]. Successful in vitro production of high quality blastocysts depends not only on the culture system but also on the oocyte recovery technique that determine the acquisition of the oocyte developmental competence [17].

Oocytes recovery can be achieved by four methods. These methods are dissection of the ovarian follicles [18], aspiration [19-22], slicing [15, 19, 23], finally, puncture of visible surface follicles [19, 20]. By aspiration method the needles used as well as the aspiration vacuum were important factors in determining the number and quality of the oocytes, these parameters should be established for each species where in cow good results obtained with 18 gauge needle connected to a 3 cm Hg vacuum [15]. The oocytes recovered by aspiration method represent only 43.2% of the entire number of oocytes; this low recovery rate might be due to difficulties in separating the cumulus oophorus [24, 25]. In buffalo, El-Gaafary and Abdel-Ghaffar [26] showed that significantly more oocytes were recovered per ovary by dissection than aspiration. Das et al. [27, 28] demonstrated that slicing was a simple and effective method for collecting a high quality buffalo oocytes yield for in vitro culture, where the success rate was 5.7 oocytes per ovary. Even though, a significantly lower rate of oocytes reaching the metaphase-II stage was observed when using slicing techniques; this lower maturation rate might be due to more preantral oocytes collected [23]. It was known that preantral oocytes had low maturation rate if compared with antral oocytes [29, 30]. Moreover, Takagi et al. [24] observed a significant difference in the developmental competence between the oocytes collected by aspiration and mincing techniques. On contrary, Alm et al. [17], Pawshe et al. [20] and Martino et al. [31] noticed that there was non-significant difference in the blastocyst developmental rate between aspiration and slicing methods depending upon the culture system used in their experiments. Additionally, Carolan et al. [32] found that, oocytes recovery by slicing rather than aspiration method can result in marked increase in blastocysts yield after IVM/IVF/IVC.

**Oocyte Selection for in vitro Maturation (IVM):** For the most successful species, it was only possible to obtain development of a maximum of 40 to 50% of the zygotes to the blastocyst stage [33, 34]. This limited success had been attributed to: the reduced oocyte developmental competence which was suggested to be the primary reason for the reduced potential of in vitro produced embryos [35, 36], or due to the heterogeneous population of oocytes obtained from the follicles [37]. For these reasons great efforts had been devoted for the establishment of new non-invasive and non-perturbing means for classifying and selecting the most competent oocytes.

Bovine oocyte selection for IVM was performed on the basis of visual assessment for the morphological features [38]. Cumulus cells establish intimate contacts together as well as with the oocyte to allow cell communication through gap junctions, which were necessary for efficient maturation of the oocyte [39]. It had been reported that, the surrounding cumulus cells had an essential role not only in nuclear maturation but also in cytoplasmic maturation which was needed for male pronuclear formation [40, 41]. In mouse, even if cumulus cells were not essential for normal oocyte fertilization [42]; their presence during meiotic maturation (in particular the maintenance of efficient gap junctions) could influence the oocyte competence to undergo normal fertilization and the acquisition of the capacity to transform the sperm nucleus into male pronucleus [43, 44]. It was interesting to know that when the granulosa cells differentiated into cumulus cells they produced a factor(s), which via paracrine signals could induce nuclear and cytoplasmic maturation in the oocytes derived from early antral or preovulatory follicles [43, 45]. Moreover, it had been reported that the secretory products of cumulus cells had fundamental effects on the oocyte developmental competence [46]. de Loos et al. [47] described that the presence of an intact complement of cumulus cells surrounding the oocyte with a homogenous appearance of the ooplasm were the best predictive morphological criteria of immature oocyte ability to undergo maturation and high embryonic development (Image 1). Upon that, when oocytes exhibiting cumulus oocyte complexes (COCs) with complete and compact cumulus investment were selected an average of 30 to 40% of them will eventually develop to the morula and blastocysts stages [38, 48, 49].

Cumulus cell expansion observed at the time of oocyte collection might reflect follicular atresia or premature meiotic resumption in the oocyte, there for; Cresylbrilliant blue vital staining could be used for better selection of COCs according to their glucose-6-phosphate dehydrogenase activity that reflected their vitality and functionality [50]. Additionally, Laurincik et al. [51] categorized the oocytes into two groups: those in which a dark rim of corona cells was visible around the zonapellucida (ZP) and those in which the corona...
the bovine oocyte derived from different follicular sizes to undergo normal fertilization and early embryonic development in vitro and reported that the follicles could categorized into three groups: > 4 to 8 mm (large), > 2 to 4 mm (medium) and < 2 to 1 mm in diameter (small) where, there were high percentages of embryonic development and blastocyst rate recovered from the large follicles. Similarly, Mate and Rodgers[55] stated that there was an effect for the follicular size on the oocyte developmental competence where, the percentage of the oocytes reached M-II from large follicles (=1.5 mm) was 55% compared to 20% recovered from small follicles (<1.5 mm). Lonergan et al. [56] added that the oocytes obtained from 6-7mm follicles usually had many layers of cumulus cells and significantly produced higher proportion of morula/blastocyst stage embryos (65.9%) compared to those obtained from 2-6 mm follicles (34.3%).

On contrary, Leibfried and First [38] and Critser et al. [57] found no differences in nuclear maturation and fertilization when 1 to 5 mm follicles were cultured in vitro, but there were differences in the embryonic development.

Even thought, First et al. [58] and Eppig [59] reported that the follicular size wasn’t the only criterion determined the oocyte competence to develop, as there were some oocytes originating from large follicles failed to produce embryos, while other from small ones already have this capacity; this simply because the oocyte capacity to mature, fertilize and to develop into blastocysts was acquired in a step wise fashion during oogenesis and folliculogenesis. Anyway, the morphological criteria still routinely used for a rough selection of the oocytes in most laboratories as there was no any real breakthrough with respect to increased blastocyst rates had been achieved yet [60].

Phases of Oocyte Maturation: Oocyte maturation was a complex long cellular process involving both meiotic cycle progression (from diplotene to Metaphase-II) and cytoplasmic events reprogramming to enable the oocyte to attain its developmental competence to be fertilized and undergo embryogenesis [2,59,61,62]. During folliculogenesis, the oocyte was grew, matured and attained the competence to be fertilized and undergo normal embryogenesis [59]; while, in vitro matured oocytes should bypass what was called the oocyte capacitation (the ultra-structural modifications took place in the oocyte of dominant follicles before the LH peak) [60]; and other substantial changes that ordinary took place in vivo under the influence of LH and the follicular environment [63].
These modifications that permit the oocytes to attain its full developmental competence [60]; could be divided into two categories, those involved in the resumption and completion of the first meiotic division referred to as nuclear maturation and those occurring in the cytoplasm referred as cytoplasmic maturation [13,59].

**Nuclear Maturation:** Meiosis from the Greek word Meiosis defined as the unique cell division, that produces gametes or two cell divisions with no intervening with DNA replication resulting in halving of the chromosome complement [2]. So, the main aim of meiosis was to create haploid gametes and to allow genetic recombination resulting in generation of genetically unique gametes [64].

Male and female gametogenesis was remarkably different; male meiosis begins at puberty and runs as a continuous process through meiosis-I and II in less than a week however the final specialization to a full fertilizable and mobile gamete took additional several weeks; while, the mammalian oocyte begins meiosis during fetal life but doesn’t complete meiosis-I until ovulation and meiosis-II was only completed upon fertilization [65].

The first meiotic division in the oocyte is of a particular importance because if that division occurred correctly, the mature metaphase-II oocyte will have a good chance to be fertilized and go on to a normal development; while if meiosis-I division goes wrong, the result will be usually an error-prone embryo that either is unable to complete development or occasionally gives rise to individuals with chromosomal alterations [64].

**in vitro**, meiotic maturation of mammalian oocytes was firstly described in rabbits by Pincus and Enzmann [66]. In the ensuing 60 years, numerous papers had been published on oocytes maturation in vitro [67]. Sirard et al. [36]; Duranthon and Renard [68] and Marteil et al. [69] documented that oocyte nuclear maturation could be defined as series of nuclear modifications that took place during resumption and continuation of meiosis producing a haploid chromosome complement from the previous diploid state.

It was seemed that nuclear maturation followed the same patterns in vivo and in vitro [2, 13,70]. Where, the competence to complete nuclear maturation was acquired in at least two steps: firstly, the oocyte become competent to reinitiate meiosis, undergo germinal vesicles breakdown (GVBD) and progress to metaphase-I; secondly, the oocyte become competent to advance beyond metaphase-I, enter anaphase and proceed to metaphase-II [59] In vivo the oocyte resume meiosis in response to the ovulatory LH surge [66,71,72] while, in vitro after removal from the follicle [66].

When meiosis resume, the arrested oocytes at the prophase I displaying a visible nucleus referred to as germinal vesicle (GV) [64]; this GV undergo germinal vesicle breakdown (GVBD) (i.e. dissolution of the nuclear envelope) [2,64]. The nucleolus disappears rapidly after coming in contact with the cytoplasm and chromatids condenses in what was called diakinesis phase [2]. During diakinesis, the nuclear membrane starts to fold, the nuclear pores disappear then the nuclear membrane fragments before disappearing rapidly to leave only small sacs with double walls [73,74]. Where then, the kinetochores appear and the microtubules pull the homologues chromosomes [2]; that were paired aligning on the meiotic spindle at metaphase-I (M-I) [64]. This phase was accompanied by dramatic decrease of mRNA transcription and characteristic alterations in the protein synthesis [75,76]. During in vitro maturation RNA synthesis strongly decreased because the condensed DNA present in oocytes from GVBD stage was practically disable for transcription, although, the translation increased only from GV to GVBD then decreased up to metaphase-II [77]. After M-I phase the oocytes should pass through short anaphase I (i.e. migration of the homologues chromosomes to their respective poles) and Telophase-I (i.e. the chromosomes found at each pole are surrounded by a nuclear membrane). The oocytes become arrested at metaphase-I are referred to as ‘partially competent’ to undergo nuclear maturation [59]. Separation was completed when metaphase-II (M-II) occurred, recognizable by the presence of 1st polar body (PB) [2,64,69]. Now the oocyte remains arrested at the metaphase-II stage until fertilization [2,73]. Meiosis was completed following successful fertilization and could be confirmed by the presence of the 2nd PB [64,69]. The legal nomenclature in several countries defined the resulting structure after the 2 pronuclei dissolving as zygote (i.e. the chromosomes become localized centrally at syngamy or synkaryosis) [64]. Stages of meiotic maturation summarized according to Smiljakovic [78] in Table 2 and Image 2.

Trimarchi and Keefe[12]stated that cellular systems within oocytes were dynamic and diverse. For example, progression of the nucleus from the germinal vesicle stage through meiosis I and its arrest at metaphase-II occurs with precise timing [79]and any disruption in this dynamics presumably underlies the majority of aberration observed in the embryos that consequently lead to spontaneous abortions [80]. The average time that bovine oocytes spend at each nuclear stage was represented in the table (2). Time zero represented the beginning of in vitro maturation, according to Sirard et al. [81].
Image 2: Stages of oocyte nuclear maturation determined by aceto-orcein staining of the oocytes after removal of the cumulus cells: (See table 1&2 for the characteristics of each phase) (A) GV  (B) GVBD  (C) Diakinesis  (D) Metaphase-I  (E) Anaphase-I  (F) Telophase-I  (G) Metaphase-II

Table 1: Stages of meiotic maturation summarized according to Smiljakovic [78]:

<table>
<thead>
<tr>
<th>Stages</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Prophase</td>
<td>Leptotene: Chromosomes condense</td>
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<tr>
<td></td>
<td>Zygote: Synapsis-Homologous chromosomes align (bivalents); synaptonemal complex formation</td>
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<tr>
<td></td>
<td>Pachytene: Recombination nodules appear; crossing-over of genetic material occurs</td>
</tr>
<tr>
<td></td>
<td>Diplotene: Chiasmata formation, desynapsis; chromosomes decondense and engage in RNA synthesis</td>
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<td></td>
<td>GV: The nucleus of the oocyte is quite large in comparison to that of a somatic cell, called germinal vesicle</td>
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<td></td>
<td>GVBD: Germinal vesicle break down, breaks down(disassembly of nuclear lamina and breakdown of the nuclear envelope)</td>
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<tr>
<td></td>
<td>Diakinesis: Chromosomes condense, appear tetravalent, RNA synthesis ceases</td>
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<tr>
<td></td>
<td>Metaphase-I: Bivalents line up on the metaphase plate of the spindle</td>
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<tr>
<td></td>
<td>Anaphase-I: Chromosomes move apart</td>
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<td></td>
<td>Telophase-I: Formation of two daughter nuclei and extrusion of first polar body</td>
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<tr>
<td></td>
<td>Metaphase-II: Remaining chromatids (half the initial number) in each daughter nuclei) promptly align again on the second meiotic spindle</td>
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Table 2: The average time that bovine oocytes spend at each nuclear stage

<table>
<thead>
<tr>
<th>Nuclear stages</th>
<th>Time interval (h)</th>
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</thead>
<tbody>
<tr>
<td>Germinal vesicle (GV)</td>
<td>0-6.6</td>
</tr>
<tr>
<td>Germinal vesicle breakdown (GVBD)</td>
<td>6.6-8.0</td>
</tr>
<tr>
<td>Condensed</td>
<td>8.0-10.3</td>
</tr>
<tr>
<td>Metaphase-I (M-I)</td>
<td>10.3-15.4</td>
</tr>
<tr>
<td>Anaphase-I (A-I)</td>
<td>15.4-16.6</td>
</tr>
<tr>
<td>Telophase-I (T-I)</td>
<td>16.6-18.0</td>
</tr>
<tr>
<td>Metaphase-II (M-II)</td>
<td>18.0-24.0</td>
</tr>
</tbody>
</table>

Cytoplasmic Maturation: The endpoint of in vivo maturation was the release of a mature metaphase-II oocyte from the follicle, which upon sperm penetration, activation and genomic activation could support normal embryonic and fetal development to term and the birth of live offspring [64]. On the other hand, the immature mammalian oocytes recovered from follicles were able to undergo spontaneous hormone-independent resumption and completion of meiosis [82]; but they didn’t appear to have full developmental competence under common in vitro maturation conditions as that evidenced by low morula and blastocyst rates of development [83]. The main reason for this incompetence was attributed to the deficient cytoplasmic maturation to enhance complete functional maturation of cow COCs in vitro [84].

Cytoplasmic maturation could be defined as the unity of metabolic, molecular and ultra-structural processes that modifying the oocyte cytoplasm for normal fertilization [2, 33, 36, 64, 85-88], and acquisition of developmental competence [62, 68, 70, 89, 90]. It includes a wide array of synthesis for oocyte-specific developmentally regulated protein, relocation of cytoplasmic organelles and alteration to membrane transport system in the oocyte [13, 33, 36, 69, 86, 87, 91-94]. In addition to, post-transcriptional modifications of mRNAs, proteins translation, post translational modification of proteins, substrates and nutrients that were accumulated during oogenesis and required to achieve oocyte developmental competence that fosters embryonic developmental
competence [33,81,95, 96]. Moreover, ultra structural modification of the Golgi complex, accumulation of ribosomes and increase in lattice like structures [70, 88, 97].

Thus, some elements of cytoplasmic maturation could be visualized [88]; whereas, many other elements were molecular and very challenging to visualize [67,92]. For that, the cytoplasmic maturation was often divided into three major processes: organelle redistribution, cytoskeleton dynamics and molecular maturation [98] and it could be used indirectly and retroactively to assess the ability of the mature oocyte to undergo normal fertilization, cleavage and blastocyst development [2]. Failure to complete cytoplasmic maturation could block the development at fertilization, embryonic genome activation, blastocyst formation or even post-implantation [70, 88, 97].

The oocytes developmental programs that regulate the acquisition of the competence to undergo nuclear and cytoplasmic maturation weren’t always coordinated [59]. Although, it was clear that some aspects of cytoplasmic maturation couldn’t begin until the initiation of nuclear maturation and mixing GV contents with the ooplasm as for example happened with male pronucleus formation and activation [59,105]; and the production of oocyte maternal mRNAs and proteins [59]. In addition, the maturation specific deadenylation of mRNAs in frog oocytes required an activity derived from the GV and this didn’t occur in a nucleate fragment [106]. Even though, the developmental program of the GV stage didn’t appear to be linked to the acquisition of competence to complete nuclear maturation [59].

Other indirect morphological parameters that could be taken into account to evaluate the cytoplasmic maturation were cumulus cell expansion, extrusion of the 1st PB and an increased perivitelline space [97].

In general, nuclear maturation was viewed only morphologically and for this reason it might be not consider as an absolute indicator for the normality after IVM, while, cytoplasmic maturation provides unequivocal information about the success of oocyte maturation [94,100]. Ultimately, the quality and completeness of cytoplasmic maturation played an important role in the development of oocyte competence [64; 69,87]. Where, it had been reported that the cytoplasmic maturation was crucial for male pronuclear formation (cytoplasmic maturation could be determined by the incidence of male pronuclear formation) [85], meiotic to mitotic cell cycle progression, activation pathways required for genetic and epigenetic programs and pre-implantation embryonic developmental competence [2,59,92, 01,102]; furthermore, it providing a foundation for implantation, initiation of pregnancy and normal fetal development [36,93]; so, for these reasons the oocyte cytoplasmic maturation attained much more concern for studying in mammals [64, 69, 87].

**Synchronization Between Nuclear and Cytoplasmic Maturation:** As follicular development progresses, the proportion of oocytes competent to complete nuclear and cytoplasmic maturation increased [54, 56, 103, 104]. Nevertheless, some oocytes that were only partially competent to undergo nuclear maturation could undergo fertilization and pre-implantation development [59]. Moreover, some oocytes competent to complete nuclear maturation weren’t able to develop to the blastocyst stage indicating deficient or defective cytoplasmic maturation [101]. Upon this, the ability to develop to the blastocyst stage was an important indicator for the completion of cytoplasmic maturation [59].

The oocytes developmental programs that regulate the acquisition of the competence to undergo nuclear and cytoplasmic maturation weren’t always coordinated [59]. Although, it was clear that some aspects of cytoplasmic maturation couldn’t begin until the initiation of nuclear maturation and mixing GV contents with the ooplasm as for example happened with male pronucleus formation and activation [59,105]; and the production of oocyte maternal mRNAs and proteins [59]. In addition, the maturation specific deadenylation of mRNAs in frog oocytes required an activity derived from the GV and this didn’t occur in a nucleate fragment [106]. Even though, the developmental program of the GV stage didn’t appear to be linked to the acquisition of competence to complete nuclear maturation [59].

Anyway, continued differentiation of GV stage oocytes was necessary even after the acquisition of competence to undergo nuclear maturation to allow the deposition of maternal factors required for the pre-implantation embryos development beyond the 2-cell stage [59]. On the other hand, some processes of cytoplasmic maturation related to successful pre-implantation development probably still occur without coordination with nuclear maturation [59]; Or, even progressed to completion after removal of the nucleus reflecting both its independence from the meiotic maturation and by extension its regulation through post-translational mechanisms [107]. For that, Eppig et al. [101, 108] and Chesnel et al. [109] concluded that the completion of oocyte growth wasn’t an absolute indicator for the termination of the nuclear maturation simply because even fully grown oocytes could suffer from a partial cytoplasmic maturation that might impair nuclear maturation. Moreover, completion of the nuclear maturation in isolation from the cytoplasmic maturation was insufficient to endow the oocyte with the capacity to support fertilization and subsequent development to term as the acquisition of full developmental competence required very complex interplay between both nuclear and cytoplasmic maturation [81, 92,101, 108, 110, 111].

**Developmental Competence:** The ability to obtain a large number of competent oocytes was one of the main goals of assisted reproductive technologies (ARTs) that use extracorporeal fertilization [110]. In the ensuing 40 years much effort had been applied to enhance in vitro matured
oocyte capacity; even though, there was still difference between *in vivo* and *in vitro* matured oocytes where, *in vivo* matured oocyte shown higher potential of development competence [112-115]. Although, no apparent difference seen at the level of the nuclear maturation, fertilization and cleavage rates, but rather in the developmental competence of this oocyte indicating that the cytoplasmic competence might differ between *in vitro* and *in vivo* matured oocytes [116]. Furthermore, recent studies revealed that there were additional discrepancies between *in vitro* and *in vivo* maturation at the ultra-structural level [70,90,117,118]. In this respect, King et al. [119] observed differences at the chromatin level in morulae/blastocyst obtained from either procedure. Many explanations stated for this dissimilarity; from between were the initial oocyte intrinsic competence and different follicular conditions that might lead to a difference in the developmental competence [116].

Developmental competence could be defined as the ability of the oocyte to produce normal viable and fertile offspring after fertilization [2]. The oocyte developmental competence was acquired within the ovary during the stages preceding ovulation (The acquisition of developmental competence was a gradual process during follicular development; the gradual increase referred to the percentage of competent oocytes not to the highest competence of a single oocyte), but in case of *in vitro* maturation it preceded the oocyte isolation from its follicle [120].

The developmental competence was a difficult parameter to assess since embryonic development might failed due to many reasons independent from the oocyte quality, but could be expressed as the percentage of oocytes develop to the blastocyst stage [120]; However, development to the blastocyst stage didn’t guarantee that the embryo would develop to term [2].

Other aspects used to evaluate developmental competence included morphological evaluations such as the number of blastomeres, the ratio between inner cell mass and trophoectoderm cell numbers; and functional evaluations such as metabolic rates and the ability to resume development after freezing should also be considered to provide more complete idea about the developmental potential of the oocyte [2].

Multiple studies had been carried out to investigate the basic factors affecting the developmental competence of the oocyte. These studies concluded that; the follicular size [53,54] and the quality of the follicle origin [121,122] could influence the developmental capacity of bovine oocytes. A study followed the fate of individual oocytes according to its origin had corroborated that developmental competence of the oocyte increases with follicular size [123]. Moreover, Lonergan et al. [55] reported that oocytes from bovine follicles greater than 6 mm in diameter produced blastocysts *in vitro* at substantially greater rates than those from 2 to 6 mm follicles. Additionally, Pavlok et al. [54] stated that follicles smaller than 2 mm in diameter yielded oocytes capable of fertilization but lack the ability to cleave beyond the 8-cell stage, so the follicles should reach a diameter of at least 2-3 mm before the oocyte reaches a satisfactory developmental competence. In this respect, it had been observed that some oocytes acquired its intrinsic capacity to develop into an embryo after IVM-IVF-IVC at the follicular stage of 3 mm, but the proportion of competent oocytes didn’t increased during development up to 7 mm [124]. Even though, there were many factors besides follicular size might be critical for the oocyte to acquire its developmental competence, as some of large sized follicles contain developmentally incompetent oocytes while, some of medium size follicles contain competent oocytes [123]. The oocyte diameter was another factor that could be correlated with follicular size and the IVF outcome [13,125].

Congruently, positive relationship had been observed between the bovine oocytes diameter, follicle size and RNA transcriptional activity [126]. The oocyte diameter varied from <100 µm for oocytes recovered from antral follicles <1 mm to >120 µm for oocytes from follicles reaching 4 mm in diameter [139]; and it was observed that the oocytes gradually acquire competence to undergo meiotic maturation and sustain embryonic development after reaching a diameter between 110 and 120 µm [60,99,126,127].

Additionally, the composition of the follicular fluid might also play a role in the oocyte developmental competence [54,122,128,129]. For example, melatonin concentrations in human ovarian follicular fluid (FF) obtained from Graafian follicles was significantly higher than those in simultaneously collected plasma samples [130]. Among the proposed functions of melatonin in the FF were the antioxidative action, reduction of critical cells apoptosis, allowing the preovulatory follicles to fully develop, enhanced mature oocytes quality, ovulation and fertilization rate [131,132].

The developmental competence of the oocyte might be lost also during *in vitro* maturation [2]. Where, extended incubation during oocyte maturation led to decreased developmental competence that could be the result of oocyte aging [2]. Moreover, the time at which the
oocytes were aspirated from the post-mortem ovaries affected the developmental rates [79]. An interval of 4 h between the slaughter of the cow and oocyte aspiration had been reported to yield the highest rates of development after 5 days of incubation [133]. Furthermore, cumulus cells were sometimes lost during the oocyte aspiration, the number and qualities of cumulus cells surrounding the oocyte were important in the developmental competence [123, 134]. Additionally, the developmental competence of bovine oocytes with corona cells only (corona-enclosed oocytes) was not comparable with that of COCs [135].

On conclusion, oocyte maturation is a complex process that is affected by many factors starting from its recovery process and ending by its culture. So a great attention should be paid to all of these preceding factors to ensure efficient oocyte with high developmental competence. In between these factors that could be detrimental to the oocyte developmental competence is the antioxidant state of the in vitro maturation and culturing surrounding the oocyte. Moreover, more recent non-invasive techniques should be developed to assess the oocyte developmental competence since recovery to its transfer to the uterus to ensure efficient pregnancy rate after embryo transfer process.

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REFERENCES


