Global Veterinaria 11 (5): 497-510, 2013
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2013.11.5.76166

Factors Affecting Buffalo Oocytes Maturation

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Abstract: *in vitro* maturation, fertilization and culturing of buffalo oocytes were considered the most important application, to improve buffalo reproductivety and productivity. Oocyte maturation is the first and most critical step towards successful *in vitro* embryo production. There are many factors affect the maturation of buffalo oocytes as culture media, method of recovery, oocyte quality, season of collection and ovarian status and others. Proper oocytes selection in the laboratory is crucial for successful embryo production *in vitro*. Little information is available on *in vitro* maturation and fertilization of buffalo oocytes. *in vitro* fertilization results obtained by several workers were relatively poor when compared to cattle. So the present review aimed to throw some lights on the factors affecting oocytes *in vitro* maturation in buffalo.

Key words: *in vitro* Maturation · Buffalo · Oocytes

INTRODUCTION

Buffaloes are hardy dairy animals, resistant to climate, stress and diseases. Buffaloes represent an integral part of agricultural economy in Egypt. However, buffaloes have low reproductive potential which could be related to the low total number of follicles in the ovary [1]; poor super ovulatory response and high percentage of atretic follicles [2]. The poor reproductive ability of the buffalo has become the major impediment in multiplication and genetic improvement of this species, like delayed onset of reproductive maturity, seasonality of breeding, long calving intervals, latent oestrus, silent heat, low number of primordial follicles and poor superovulatory response [3]. So many efforts had been initiated to augment the reproductive potential of these animals using biotechnology [4].

The oocyte quality certainly also plays a key role in the acquisition of oocyte developmental competence *in vitro* [5]. Granulosa /cumulus cells play an essential role in promoting full oocyte maturation by mediating the positive effects of gonadotrophins [6]. The competence of buffalo oocytes is influenced by biological factors and environmental factors [7] such as follicle size, oocyte diameter, presence or absence of corpus luteum in the ovary and environmental temperature.

In vitro maturation (IVM) is one of the essential steps in the *in vitro* fertilization (IVF) process. Several workers have studied different aspects of IVM in mammalian oocytes [8-10]. In most of the studies, the basic medium is supplemented with hormones and different concentrations of serum. The maturation mediums with the selection of protein supplements and hormones for IVM play an important role in subsequent IVF and *in vitro* development [8]. Maturation of the oocytes included two aspects: nuclear and cytoplasm maturation. *in vitro* maturation oocyte provide an excellent opportunity for cheap and abundant embryo for carrying out basic research and for the application of emerging biotechnologies like cloning and transgenic. *in vitro* maturation of bovine oocytes is affected by several factors, such as transport time and temperature from the abbatoir to the laboratory, follicle size [11,12], developmental stage of oocyte [13], oocyte diameter [14], composition of media [15], hormones [16] and serum [17].

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Table 1: Lists of the meiotic stages after *in vitro* maturation of oocytes.

Stages	Events
1.Prophase I Leptotene	Chromosomes condense
Zygotene	Synapsis-Homologous chromosomes align (bivalents); synaptonemal complex formation.
Pachytene	Recombination nodules appear; crossing-over of genetic material occurs.
Diplotene	Chiasmata formation desynapsis; chromosomes decondense and engage in RNA synthesis.
Diakinesis	Chromosomes condense; appear tetravalent, RNA synthesis ceases.
GV stage	The nucleus of the oocyte is quite large in comparison to that of a somatic cell, called germinal vesicle.
GVBD stage	Germinal vesicle breaks down (disassembly of nuclear lamina and breakdown of the nuclear envelope).
2. Metaphase I	Bivalents line up on the metaphase plate of the spindle.
3. Anaphase I	Chromosomes move apart.
4. Telophase I	Formation of two daughter nuclei and extrusion of first polar body.
5. Prophase II	Nuclear envelope breaks down and the new spindle forms.
6. Metaphase II	Remaining chromatides (half the initial number) in each daughter nuclei) promptly align again on the second meiotic spindle.
7. Anaphase II	Chromatides start moving apart (subject to fertilization).
8. Telophase II	Formation of two daughter nuclei; extrusion of the second polar body.

Adapted from Karp [37] and Smiljakovic [38].

Oocyte Maturation: Oocyte maturation is long process during which oocytes acquire their intrinsic ability to support the subsequent stages of development in a stepwise manner, ultimately reaching activation of the embryonic genome. This process involves complex and distinct, although linked, events of nuclear and cytoplasmic maturation. Nuclear maturation mainly involves chromosomal segregation, whereas cytoplasmic maturation involves organelle reorganization and storage of mRNA, proteins and transcription factors that act in the overall maturation process, fertilization and early embryogenesis [18].

The maturation of the oocytes includes two aspects: Nuclear and cytoplasmic maturation [9].

Nuclear Maturation: Meiosis, a Greek word meaning reduction, consists of two successive cell divisions following one round of DNA replication. Meiosis gives rise to four haploid cells from a single diploid cell. This type of cell division is characteristic of germ cells. Meiosis, up to the diplotene stage, occurs in the fetal ovary. During the first meiotic division, maternal and paternal genes are exchanged before the pairs of chromosomes are divided into two daughter cells. The second meiotic division occurs without being preceded by DNA synthesis and nuclear reformation. The two meiotic divisions of the oocyte are asymmetrical, resulting in expulsion of polar bodies. Meiosis in each female germ cell results in a single egg and two polar bodies [19, 20]. In addition to observation of oocytes chromosomes at this stage is more reliable mean for defining the *in vitro* maturation progress [21-22].

The Mammalian oocytes begin meiosis during fetal life but don't complete meiosis-I until ovulation and meiosis-II was only completed upon fertilization [23]. The maturation of follicular oocytes is normally arrested at the prophase-I of the first meiotic division and the oocyte remain in the dormant stage, called a dictate nucleus. At this stage, nuclear material is enveloped and the resulting structure is called a germinal vesicle. The oocytes remain at this stage until the onset of puberty. Under the influence of gonadotropins and particularly in response to the LH surge, oocytes resume meiosis just before ovulation [18] and nuclear maturation refers to the progression of the oocyte nucleus from the germinal vesicle to the metaphase II stage in Table (1).

Nuclear maturation involves germinal vesicle breakdown (GVBD), condensation of chromosomes, metaphase I spindle formation, separation of the homologous chromosomes with extrusion of the first polar body and arrest at metaphase-II [24]. The nuclear membrane starts to fold, the nuclear pores disappear and then the nuclear membrane undergoes fragmentation and rapidly disappears [25-26]. It appears that nuclear maturation follows the same pattern *in vivo* and *in vitro* [27-3]. Nuclear maturation involves changes in protein synthesis patterns [28].

The ability of the oocyte to complete meiosis is known as meiotic competence. Meiotic competence is acquired gradually during follicular growth. Oocytes firstly acquire the capacity to undergo GVBD and chromosome condensation and then further follicular development is required to acquire the ability to progress to the metaphase I [29-26] and finally they acquire the

ability to reach metaphase II [30-18]. The ability to complete the MI to MII transition coincides with the achievement of full size and with the process of nucleolar compaction [31].

Meiotic competence is closely correlated with oocyte size, which in turn is correlated with follicle size [32]. The size of the antral follicle at which the oocyte acquires meiotic competence is species-specific [33]. Bovine oocytes acquire the ability to complete GVBD and meiosis by the time the antral follicle reaches 2-3 mm in diameter [34]. Meiotic competence is also related to oocyte diameter, since bovine oocytes must have a diameter of 110 ìm to complete nuclear maturation to the MII stage [35, 36].

Cytoplasmic Maturation: Cytoplasmic maturation could be defined as the unity of metabolic, molecular and ultra-structural processes that modifying the oocyte cytoplasm for normal fertilization [39-42] and acquisition of developmental competence [43-45]. It encompassed a wide array of synthesis for oocyte-specific developmentally regulated protein, relocation of cytoplasmic organelles and alteration to membrane transport system in the oocyte [46,47]. Moreover, ultra structural modification of Golgi complex, accumulation of ribosomes and increase in lattice like structures [48]. In addition to post transcriptional modification 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 [49,50].

Some elements of cytoplasmic maturation could be visualized as the line-up of cortical granules and increase in number and a change in the morphology and location of mitochondria [39], whereas, many other elements were molecular and very challenging to visualize or monitor. For that, the cytoplasmic maturation was often divided into three major processes: organelle redistribution, cytoskeleton dynamics and molecular maturation [48] and it could be used indirectly and retroactively to assess the ability of the mature oocyte to undergo normal fertilization, cleavage and blastocyst development [51]. Failure to complete cytoplasmic maturation could block the development at fertilization, embryonic genome activation, blastocyst formation or even post implantation [52]. Other indirect morphological parameters that could be taken into account to evaluation the cytoplasmic maturation were cumulus cell expansion, extrusion of the first polar body and an increased perivitelline space [53].

Glutathione (GSH) has been shown to play an important role in oocyte maturation. The process of oocyte cytoplasmic maturation involves numerous molecular events, including synthesis of biochemical compounds, protein phosphorylation and activation of particular metabolic pathways [54,55]. These changes are a prerequisite for normal fertilization and embryo development. The synthesis of intracellular glutathione is a critical part of oocyte cytoplasmic maturation [54]. The glutathione concentrations in oocytes matured *in vitro* are not relatively different among several mammalian species. However, GSH concentrations in matured *in vivo* oocytes are much higher than those matured *in vitro* [56].

Factors Affecting on *in vitro* Maturation

Media Additives: The culture medium and selection of protein supplements and hormones for IVM play an important role in the subsequent maturation rate and embryonic development following IVF [57]. Different culture media such as TCM-199 [4,58,59], minimum essential medium (MEM) [60] and Ham's F-10 [61-62] have been used for *in vitro* maturation of mammalian oocytes. TCM-199 is the most widely used culture medium for such purposes [63]. The beneficial effect of TCM-199 medium on IVM of animal oocytes may be related to some factors in its composition such as essential amino acids and glutamine that stimulate DNA and RNA synthesis and enhance cell division [8,64]. TCM199 improved the rate of *in vitro* maturation and oocyte maturation better than MEM [65], MEM has glucose and glutamine in high concentration compared to TCM199. It has been known for many years that glucose and glutamine are poor energy substrates for the cumulus cell- free rodent oocytes [66] and this is probably related to the low development of oocytes in MEM.

Serum may provide beneficial factors to the culture medium, including energy substrates, amino acids and vitamins. There may be specific effects of serum component possibly growth factors on oocyte maturation that are manifested as improved embryo development following IVF [67]. Also, Downs *et al*. [68] suggested that it may be important to include serum in the *in vitro* maturation medium to prevent hardening of zona pellucida which could adversely affect fertilization. In the same manner, Schroeder *et al*. [69] reported that fetuin, a major glycoprotein constituent of fetal calf serum, can prevent hardening of zona pellucida during *in vitro* maturation as it acts by preventing the action of proteolytic enzymes originating from precociously released cortical granules and improve the fertilization capacity of oocytes. Also,

Kan and Yamane [70] recorded another beneficial action of serum which is its antioxidant properties by reducing superoxide formation. In addition, serum added to the oocyte medium provides a source of albumin that balances the osmolarity [71]. There are different sources for supplemented sera such as fetal calf serum [72,73], oestrus buffalo serum [74], steer serum [7] and superovulated buffalo serum [75].

Addition of hormones such as follicle stimulating hormones [75], pregnant mare serum gonadotrophin [76], luteinizing hormone and estradiol [77] to culture media has been made to improve the developmental competence of *in vitro* matured oocytes. In many mammalian species, gonadotropins have been found to stimulate cumulus cells to synthesize molecules able to drive germinal vesicle breakdown GVBD as meiosis- activating sterols [78]. Estradiol has been found to improve the completion of maturational changes and also to support the synthesis of presumed male pronuclear growth factor [6,79]. The supplementation of maturation media with eCG resulted in a higher *in vitro* maturation rate of buffalo oocytes than FSH [80]. In addition, Alok *et al.* [81] showed that FSH is essential for cumulus cell expansion and maturation of buffalo oocytes *in vitro* as FSH enhances the expansion of cumulus cells surrounding the oocytes which in turn enhances sperm capacitation and the fertilization process.

When the IVM medium is supplemented with either cysteine or cysteamine, GSH content increases in the oocytes also in the absence of cumulus cells [82]. It is likely that the cysteine-induced GSH synthesis occurs during the first hours of IVM, before the amino acid is oxidized. It has also been proven that, in the presence of a thiol compound, such as b-mercaptoethanol, supplementation of IVM medium with cysteine increases the GSH level and improves the developmental competence of pig oocytes following fertilization [83].

Reactive oxygen species (ROS) production is one of the regular processes of cellular metabolism [64]. There is evidence that the ROS in *in-vitro* oocyte maturation affect IVP of bovine embryos [84]. Oxidative damage to cellular elements through the ROS is one of the important processes which cause damage to appropriate cell function [85]. There are different mechanisms for controlling cellular ROS levels as GSH, superoxide dismutase. Glutathione (GSH) is a non-protein sulphydryl compound in cattle cells. It serves as a reservoir for cysteine and which plays an important role in protecting mammalian cells from oxidative stress [86-88] indicating that the addition of thiol components such as cysteamine to IVM medium improved embryo production.

Season: In general, the reproductive traits of buffaloes are affected by climatic changes. However, although some degree of seasonal variation in breeding efficiency is usual with most domestic livestock, the variation is more marked in buffaloes. Particularly, ambient temperature and relative humidity showed a direct effect on breeding efficiency [89]. In some areas of the world as in Egypt and Iraq, Marai and Habeeb [90] reported that buffaloes breed throughout the year, but more so in the spring and a little less in autumn. When buffaloes subjected to heat stress yield fewer good quality oocytes than their unstressed counterparts [91].

There is relationship between season, quality of oocytes, cumulus cell expansion, maturation and developmental rates of *in vitro* maturation of buffalo oocytes [91,92]. Leibfried-Rutledge *et al.* [93] reported that high ambient temperature and humidity have deleterious effect on oocyte capability for maturation and fertilization *in vitro*. Moreover, Zeron *et al.* [94] mentioned that the heat stress can alter phospholipids composition of oocytes. While, Nandi *et al*. [7] stated that the external environmental conditions did not affect the fertilization if the aspirated oocytes successfully completed maturation. In contrast, Kadoom [95] recorded that the quality of oocytes was increased in summer season.

The hot climate was found to be adversely affecting the quality of oocytes [96]. In spite of the importance of the season on the quality of oocytes, few reports are available on this respect in Egyptian buffaloes [97, 92].

Follicular Size: Oocytes for IVM are generally selected using the following criteria: follicle size, cytoplasmic appearance, the appearance and number of cumulus cells around the oocytes (COC's). Cumulus expansion can importantly be used to microscopically assess the *in vitro* maturation rate of oocytes [98] and also Lonergan *et al*. [99] and Yang *et al*. [100] showed a relationship between follicle size and oocyte quality.

The stage of development of the follicle and growth of the oocyte go hand in hand. It has been reported that follicular size profoundly influences the quality of the oocyte obtained during ovulation and the quality of embryo obtained [101]. Follicle size thus affects the oocyte quality, potentially involving mRNA or protein reserves as factors involved in determining the oocyte competence [102]. This is a common problem that is associated with the use of non- ovulated immature oocytes collected from the ovary. This not only involves

the degree of oocyte maturation, but also the fact that many oocytes in the ovary are undergoing a process of apoptosis [103].

During the growth phase, oocytes increase in diameter to more than 120 µm [14]. Studies have shown that oocytes with a diameter of less than 110 µm may still be in the growth phase [35]. These oocytes are less capable of developing after fertilization and results with lower rates of blastocyst formation. Such small oocytes are also prone to undergo certain chromosome alterations during maturation, which impairs further development [32,104]. The oocyte diameter is directly proportional to the follicle diameter and oocytes continue to grow, even in follicles with a diameter of > 10 mm [105].

In sheep, this relationship of ovarian follicular size and oocyte diameter exists and has been shown to be influential in meiotic progression [106]. Not only is follicle size important in embryonic development, but the number of follicles on the ovarian surface also plays an important role. Sheep ovaries with 8 or more follicles on the surface have been shown to yield higher percentages of cleavage and blastocyst rates (94% and 52.4%, respectively), compared to 57% and 30.2% attained from ovaries having 4 or less follicles on the ovarian surface [107].

In buffalo, Yousaf and Chohan [108] found poor *in vitro* maturation rates (32% and 32.7%) to metaphase-²² stage for oocytes isolated from 2 to <3 and from 3 to <3 mm follicles, respectively, whereas significantly (P<0.05) more oocytes from 4 to<6 and from 6 to <8 mm follicles reached M II (67.1% and 79.1, respectively). In contrary, Leibfried and First [109] and Crister *et al*. [110] found no difference in nuclear maturation and fertilization when 1 to 5 mm follicles were cultured *in vitro*, but there were differences in the embryonic development.

It has been recorded 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 follicullogenesis [54].

The Existence of Cumulus Cells: The presence of cumulus cells was necessary for cytoplasmic and /or nuclear maturation of cattle [111,112] and buffalo [113,114] oocytes. Cumulus cells benefit oocytes development either by secreting soluble factors, which induced developmental competence, or by removing an embryo development suppressive component from the medium [115]. Cumulus cells supported IVM of oocytes to the MII stage and were involved in the cytoplasmic maturation needed for optimal developmental competence, such as male pronucleus formation and development to the blastocyst stage. Cumulus cells might be a good indicator for an oocytes ability to undergo meiosis I *in vitro* and that the developmental problems of denuded oocytes were due to deficient cytoplasmic maturation [116].

The oocyte granulosa cell gap junction is required for the coordination of nuclear and cytoplasmic meiotic competence and are vital for oocyte maturation and subsequent embryo development [117,118]. Moreover, Physical contact between oocytes and cumulus cells has been considered necessary for the transfer of nutrients and factors essential for oocytes development [119].

Various roles reported for cumulus cells include prevention of the hardening of zona pellucida [120], the provision of energy for oocyte maturation and production of cytoplasmic maturation factors and the uptake of nutrients for oocytes during maturation in culture medium. In addition, the cumulus cells are also important for fertilization, for example, such as the trapping of spermatozoa, guiding spermatozoa to the oocyte, protection of the oocytes against zona hardening and prevention of changes in the oocyte that are unfavorable for subsequent fertilization [121,122]. Moreover, factors (chemokines) secreted from COCs induce sperm capacitation and enhance fertilization, providing evidence for a regulatory loop between sperm and COCs during fertilization [123]. It has been demonstrated that chemokine signaling facilitates both sperm attraction to the COC and COC compaction by the cumulus extracellular matrix assembly [124].

Oocyte Quality: Naturally, the oocyte quality is determined by the oocyte's ability to mature, be fertilized and give rise to normal offspring [45,125]. The quality of the oocyte is also related to the oocytes' follicular environment [126], as well as several factors: such as age of the donor animal, stage of follicular development and the media used for maturing the oocytes [127].

A serious problem associated with the production of buffalo embryos through IVMFC is the very poor recovery of good quality immature oocytes [128]. Cumulus investment morphology and the microscopic aspect of the ooplasm are generally considered as the two main parameters to assess the quality of the cumulus oocyte complex (COC) [129,130]. The criteria employed by various authors for selection of oocytes for IVM include the presence of a multilayer compact cumulus oophorus

and homogeneous cytoplasm [131,132]. Some authors have attempted classification of oocytes for selection in IVM on the basis for the presence and appearance of the cumulus mass [133,74].

Ovarian Status: There were interactions between CL-bearing ovaries and each of seasons, physiological status and feeding system. It has been stated that buffalo ovaries bearing CL had significantly (P<0.05) lower mean number of oocytes following aspiration [5]. Also, Kumar *et al*. [134] recorded a low average yield from buffalo ovaries bearing CL (4.08) than that non-bearing CL (6.55). They demonstrated that this might be the result of a major portion of the ovary being occupied by the lutein cells, thereby restricting. However, Abdoon and Kandil [135] found that number of surface ovarian follicles were significantly (P<0.05) higher on buffalo ovaries bearing CL than those non-bearing CL. On the other hand, Das *et al*. [136]; Boediono *et al*. [137]; Shamiah [138] and El-Naby *et al.* [92] found a similar number of follicles and oocytes on ovaries with or without CL (3.55 and 2.68 vs.2.7/ovary, respectively).

In bovine, Varisanga *et al*. [139] found that oocyte yield per ovary from ovaries bearing CL was significantly higher (P<0.01) than ovaries without CL. Similar trend was reported by Dode *et al.* [140], who found a higher number of oocytes from pregnant than non-pregnant cow ovaries.

Recovery Methods: Many methods for oocytes recovery had been described in domestic animals these methods were: dissection of ovarian follicles [141], aspiration [142,143], slicing; where, the ovaries might be sliced with a razor blade and washed with phosphate buffer saline to collect the oocytes [144], or the follicles on the ovarian surface incised using blades [145]; finally, puncture of visible surface follicles [146].

Mermillod *et al.* [144] reported that all the contents of the follicles were aspirated by the aspiration method, the needles used as well as the aspiration vacuum were important factors in determining the number and quality of the oocytes collected, these parameters should be established for each species where, in cow good results obtained with 18 gauge needle connected to a 3cm Hg vacuum. The oocytes recovered by follicle aspiration method represent only 43.2% of the entire number of follicular oocytes within the ovary versus 100% using follicle dissection methods, this low recovery rate might be due to difficulties in separating the cumulus oophorus [147,148], but the processing of aspiration required less time than those of slicing and dissection methods [96]. In buffalo, Mistry and Dhami [149] and Rao and Mahesh [150] demonstrated that slicing was a simple effective method for collecting a high quality oocyte yield for *in vitro* culture, where the success rate was 5.7 oocytes per ovary. It has been shown that significantly more buffalo oocytes were recovered per ovary by dissection than aspiration, but the maturation rate in buffalo oocytes was higher in oocytes obtained from aspiration than oocytes obtained from dissection [151]. Wani *et al*. [152] observed that the slicing and puncture methods yielded better quality oocytes per ovary than aspiration method. While, Wang *et al*. [145] concluded that the recovery of oocytes using the slicing and puncture techniques yielded more oocytes per ovary than the aspiration methods. But, the rate of nuclear maturation of the oocytes was not affected by these different oocytes collection methods. The oocytes collection methods also showed no influence subsequent embryonic developmental competence after IVF using M II stage oocytes. However, Wang *et al.* [145] reported low number of oocytes recovery by slicing technique. He attributed this to the difference in slicing techniques that he used. On the other hand, Shirazi *et al.* [153] documented that the number of oocytes per ovary for slicing and aspiration didn't differ significantly in ewes. Furthermore, Iwasaki *et al.* [154] observed that mincing of the ovary was thought to be an effective method for obtaining large number of compact cumulus oocytes using razors and a food grater after aspiration of visible follicular oocytes. Even though, a significantly lower rate of oocytes reaching the metaphase II stage (MII) was observed when using slicing techniques if compared with the others. This lower maturation rate might be due to more preantral oocytes collected by slicing techniques if compared with the other techniques [145]. It was known that preantral oocytes had low maturation rate if compared with antral oocytes [155]. Moreover, Mehmood *et al*. [156] concluded that buffalo oocytes recovered from ovaries excised via the aspiration method had a better IVM rate, as compared to the slicing method. It is possible that slicing may release less developmentally competent oocytes from follicles deep in the cortex. It has been shown that oocytes released from embedded follicles in the ovarian cortex via slicing are not as meiotically competent as those from similarly sized follicles located on the bovine ovarian surface [157]. *in vitro* fertilization has been carried out in buffalo [77] and in cattle mostly with oocytes recovered via the aspiration method. In general, after aspiration or slicing, the collected fluid was screened under a stereomicroscope to select high quality oocytes [144].

Duration of Maturation: Oocytes remained in the germinal vesicle (GV) stage from the onset to 6-8 h of culture. The germinal vesicle breakdown (GVBD) occurred between 7-9 h and the metaphase-I became established within 12-18 h. Finally most oocytes reach the metaphase-II stage after 27 h (at 38.5°C) [18].

The most suitable maturation period in both cattle and buffaloes is 24h [158]. Gasparrini *et al.* [159] reported that the attainment of the MII stage commenced after 18 h maturation but the majority of oocytes completed nuclear maturation between 21 and 24 h. Also, Nandi *et al*. [3] found that cumulus expansion and extrusion of first polar body in buffalo oocytes commence at 16-17 h post-maturation to reach the maximum levels at 22-24 h. Other authors have also reported that the highest proportion of MII oocytes occurs after 24 h of IVM in the same species [160-163]. However, these results were in disagreement with previous findings of Neglia *et al*. [164], in which the majority of buffalo oocytes reached the MII stage between 15 and 19 h after the start of IVM and an increased incidence of degenerated oocytes was observed at later times. Large variations in the timing of the oocyte maturation process *in vitro* have also been reported in cattle [165,166].

CONCLUSION

Oocyte maturation is important step for success of *in vitro* fertilization in buffalo. Many factors affected the oocyte competence and development. The research must be directed towards the genetic factors affecting oocyte quality and maturation *in vitro.*

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