

INCIDENCE OF PARTHENOGENETIC DEVELOPMENT OF BUFFALO (*BUBALUS BUBALIS*) OOCYTES

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ABSTRACT

The incidence of parthenogenetic development of oocytes in routine IVF practices i.e. without any chemical stimuli, was assessed. After 24 h of maturation while washing and transferring to fertilization medium nine (1.39%) oocytes out of 646 oocytes were found cleaved. All those parthenotes (cleaved oocytes) were transferred to embryo culture medium instead of fertilization medium for observing further development. It was found that three (33.33%) out of nine were arrested at the four-cell stage and the remaining (66.67%) were arrested at the two-cell stage itself.

Keywords: buffaloes, *Bubalus bubalis*, parthenogenesis, oocytes

INTRODUCTION

In buffalo the overall in vitro embryo production efficiency is lower than in cattle, mainly due to the lower cleavage rate (Gasparrini, 2002). *In vitro* fertilization procedures in water buffalo were derived from those in cattle. However, the success

rate is much lower in buffaloes, due to their inherent poor developmental competence resulting in poor cleavage and embryo development. Recent reports suggested that *in vitro* matured buffalo oocytes had better inherent developmental competence. Oocyte maturation can be defined as those events associated with the initiation of germinal vesicle breakdown (GVBD) and completion of the first meiotic division (Leibfried-Rutledge *et al.*, 1987). Maturation allows the oocyte to express its developmental potential after fertilization and is not merely confined to nuclear events or the ability to be fertilized (Gordon, 2003). Maturation media supplemented with hormones, serum, growth factors etc. improve the *in vitro* developmental competence of oocytes. Oocyte maturation triggered by hormone signaling alters membrane permeability, causes ion currents and increases cytosolic free calcium $[Ca^{2+}]_i$. At the end of maturation, the oocyte reaches an adequate Ca^{2+} store.

MATERIALS AND METHODS

Ovaries from sexually mature buffaloes (*Bubalus bubalis*) were collected irrespective of age,

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body condition, stage of oestrous cycle and season from the Chennai Corporation abattoir and utilized in this study. The ovaries were removed within 30 minutes of slaughter and washed in phosphate buffered saline (PBS) supplemented with 50 µg/ml gentamicin sulphate to remove blood and extraneous material. The washed ovaries were transported at 37°C in a thermos flask in the same media to the laboratory within 30 minutes. The extra-ovarian tissues were trimmed off and the ovaries were washed with PBS to remove blood clots and superficial bacterial contamination. The washed ovaries were kept in a sterile beaker containing PBS supplemented with 50 µg/ml gentamicin until oocyte retrieval by aspiration. The oocytes were screened under a stereozoom microscope, washed thrice in 35 mm petridishes and graded based on their cumulus mass investment and homogeneity of ooplasm as described by Nandi *et al.* (1998).

All the oocytes aspirated were subjected to in vitro maturation in TCM-199 supplemented with FCS and hormones FSH, LH and oestadiol. The cumulus oocyte complexes (COCs) were rinsed three times in maturation medium and were transferred to 100 µl of IVM droplets (15- 20 COCs per droplet). The oocytes were allowed to mature in these droplets at 38.5°C in an atmosphere of 5 percent CO₂ in air for 24 h in a CO₂ incubator. Observation for cleavage of oocytes, if any, was made after 24 h of maturation while washing and transferring to fertilization medium.

RESULTS AND DISCUSSION

By aspiration technique, 646 oocytes were retrieved from 344 buffalo ovaries, with recovery rate of 1.87 oocytes per ovary. 10.52 (68), 33.74 (218), 29.25 (189), 16.40 (106) and 10.06 (65)

percent of oocytes were classified as Grades A, B, C, D and E (expanded oocytes) respectively. After 24 h of maturation while washing and transferring to fertilization medium nine (1.39%) oocytes out of 646 oocytes were found cleaved. All those parthenotes (cleaved oocytes) were transferred to embryo culture medium instead of fertilization medium for observing further development. It was found that three (33.33%) out of nine were arrested at the four-cell stage and the remaining (66.67%) were arrested at the two-cell stage itself. It was also found that all the parthenotes were derived from culture grade oocytes ('A' and 'B' grade oocytes).

Chemical activation of in vitro matured buffalo oocytes successfully supported development to the blastocyst stage (Gasparrini, 2003). Sperm penetration provides the natural signal for oocyte activation. The sperm-oocyte interaction results in the release of Ca²⁺ from intracellular stores, in the form of repetitive waves or spikes that are responsible for meiotic progression and early development (Bootman and Berridge, 1995). All methods of chemical activation mimic this process by providing adequate but nontoxic calcium signals to the MII arrested oocytes with different mechanisms.

Ion currents and cytosolic free calcium ([Ca²⁺]_i) elevations are crucial events in triggering the complex machinery involved in both gamete maturation and fertilization. Oocyte maturation is triggered by hormone signaling which causes ion currents and [Ca²⁺]_i increase. Because extracellular Ca²⁺ is required for in vitro GVBD (DeFelici and Siracusa., 1982) and for first meiotic division (Paleos and Powers, 1981), Ca²⁺ ion transport through the plasma membrane seems to play a functional role in maturation. As a consequence of a net uptake of Ca²⁺ through the plasma membrane, at the end of maturation, the oocyte reaches an adequate

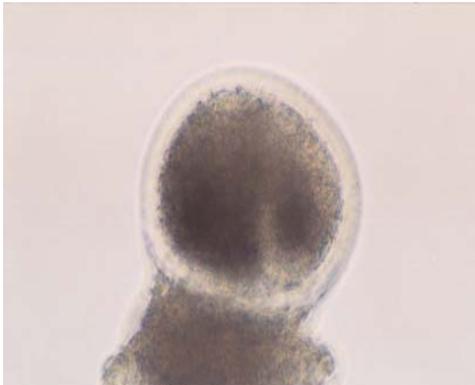


Figure 1. Parthenogenetic oocyte (2-cell) with intact cumulus mass (X 200).

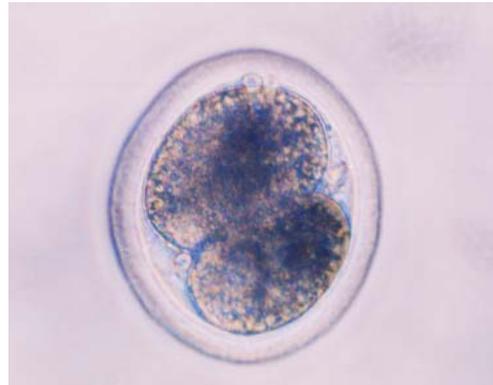


Figure 2. Parthenogenetic oocyte (2-cell) with out cumulus mass (X 200).

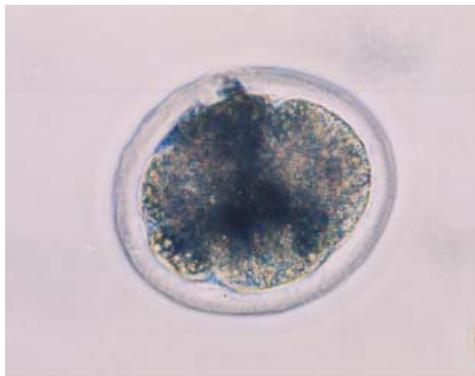


Figure 3. Parthenogenetic oocyte (4-cell) (X 200).

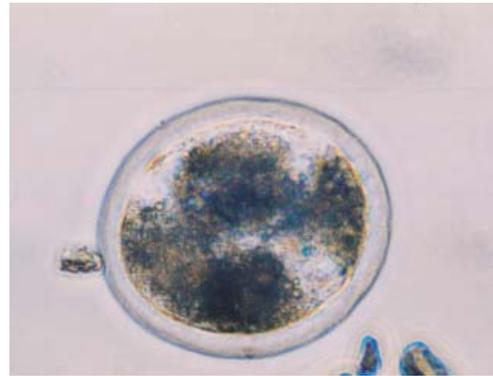


Figure 4. Degenerating parthenote (X 200).

Ca^{2+} store. This storage may occur via either gap junctions or specific channels. In the oocyte at fertilization also a dramatic ion flux occurs together with a temporally abrupt and/or spatially confined intracytoplasmic calcium [Ca^{2+}]_i increase (Boni *et al.*, 2007). Sperm-induced calcium (Ca) oscillations at the time of fertilization are a required signal for activation in most mammalian oocytes. A clear relationship between electrical properties of the oocyte plasma membrane and [Ca^{2+}]_i modifications has also been recorded following fertilization, as well as following chemical oocyte activation or after exposure to specific Ca^{2+} releasers (Tosti *et al.*, 2002).

Based on the results it was assumed that *in*

vitro maturation might have induced calcium (Ca) oscillations due to altered electrical properties of the oocyte plasma membrane as that of chemical oocyte activation by calcium ionophore resulting in cleavage, but to a lesser extent indicating that buffalo oocytes had better inherent developmental competence and that the poor cleavage and embryo development following IVF may be due partly to the poor quality of frozen/thawed sperm, improper sperm capacitation and/or fertilization as opined by Mishra *et al.* (2006). From this study, it was concluded that cleavage alone is not a reliable indicator of fertilized oocytes because oocytes matured *in vitro* may undergo parthenogenic development to the four-eight cell stage (Shioya *et*

al., 1988), where *in vitro* developmental block may or may not occur.

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