

IN VITRO MATURATION AND VITRIFICATION OF CATTLE AND BUFFALO OOCYTES IN DIFFERENT MEDIA

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ABSTRACT

The immature oocytes have long been used for experimental trials for betterment of production of genetically superior breed both in terms of meat and milk production. In these experiments immature oocyte were harvested from the buffalo (*Bubalus bubalis*) and cattle ovaries (obtained from slaughter house) and transported to the laboratory at 28 °C in phosphate buffer saline solution (PBS) within two hours of slaughtering. The ovaries were sliced with a ten unit blade slicer in PBS supplemented with 0.4% bovine serum albumin (BSA) and 250 iu/L heparin. Good quality oocytes were incubated in four different media i.e. TCM199, Ham's F10, MEM, and IVF Universal medium (medicult) supplemented with 10 iu/ml follicle stimulating hormone (FSH), 10 iu/ml Leutinizing hormone (LH) and 1.0 µg/ml estradiol 17-β. The average maturation rates for cattle and buffalo oocytes were 56.0±1.9 % and 55.4±0.8 % respectively. There was no significant difference in maturation rates of oocytes among these media. The immature vitrified oocytes were incubated after thawing at 37 °C for maturation rates. No difference could be detected in the maturation rates between cattle (52.2±0.13 %) and buffalo oocytes (47.85±0.2 %). The maturation rates of vitrified COCs were significantly lower after vitrification than untreated COCs, both in cattle and buffalo.

Key-words: Biological study, oocytes, cattle, media, *in vitro* study

INTRODUCTION

The use of available reproductive bio-techniques in livestock like artificial insemination, embryo transfer and *in vitro* production of embryo can result in enhanced reproductive rates. Among the few reproductive techniques the use of *in vitro* matured oocytes for the production of livestock embryos is rapidly increasing (Hasler *et al.*, 1995).

The majority of immature bovine oocytes fail to develop to the blastocyst stage following maturation, fertilization and culture *in vitro*. The evidence suggests that while culture conditions during *in vitro* embryo production can impact on the developmental potential of the early embryo, the intrinsic quality of the oocyte is the key factor determining the proportion of oocytes developing to the blastocyst stage (Lonergan *et al.*, 2003). The improvement in maturation rate of oocytes in cattle and buffalo is the first step in this direction.

The interest in the development of gamete bank is increasing with the advancement in the knowledge of conservation of endangered species and also in the field genetic engineering. The development of a repeatable method for the long-term preservation of gametes or embryos would provide numerous applications, including: transport of paternal and/or maternal germplasm, rapid regeneration or expansion of new and existing lines, extraction or rescue of healthy stock from diseased herds, improve or eliminate quarantine conditions and provide a method for the international export/import of potential breeding stock. While maintaining genetic resources through oocytes and embryo banking, successful preservation would enhance further development of other animal production technologies such as sperm sexing, artificial insemination, *in vitro* fertilization and non-surgical embryo transfer. There are different methods for the cryopreservation of oocytes and embryo. Vitrification is one such method, for the cryopreservation of oocytes. It is the solidification of a liquid by an extreme increase in the viscosity during very rapid cooling. The solid, called glass has the molecular and ionic distribution of the liquid state, and thus avoids the potentially detrimental effects of intra-cellular and extra-cellular crystallization (Arav *et al.*, 1993). The present experiment is designed to evolve methodologies by comparing upon the maturation rates of cattle and buffalo oocytes in different media along with the vitrification success rate in immature oocytes.

MATERIALS AND METHODS

Ovaries were collected from the slaughterhouse within 45 minutes of the slaughter of animals and brought to the laboratory in Phosphate buffer saline (PBS) at a temperature ranging between 28-30°C (Otoi *et al.*, 1993). In the laboratory, the ovaries were washed two to three times with fresh PBS, supplemented with 0.4% BSA and 250 iu/L heparin at 28° C. Ovaries were sliced in the same solution with the help of a pair of forceps and ten unit blade slicer. The follicles in the size range of 2-6 mm diameter were punctured to harvest the immature COCs. Oocytes having

characters: surrounded by more than six layers, evenly granulated, round in shape, no apparent sign of degeneration and injury on the ooplasm were isolated under stereomicroscope as done by Hamano and Kuwayama (1993). Four media were used for maturation i.e. TCM199, MEM, Ham's F10, and IVF universal medium. COCs were washed with maturation drops of each media supplemented with 10 iu/ml FSH, 10 iu/ml LH and 1 mg/ml estradiol 17-B. Finally, they were incubated at 38.5°C, 5% CO₂ and humidified atmosphere for 24 to 26 hours in maturation drops under paraffin oil. The maturation was recognized by the expansion of granulosa cells. A group of immature oocytes were exposed to vitrification solution (5.5M ethylene glycol + 1M sucrose), after thawing at 37° C they were incubated under the same conditions as described above and their viability was assessed with maturation rate results. The data were analysed by applying ANOVA and paired t-test using Systat Statistical Package (10.2 version, USA).

RESULTS AND DISCUSSION

Oocytes were matured in different media. The average maturation rates recorded in cattle were 51.92%, 55.9%, 54.19%, and 59.50% using the culture media TCM199, Ham's F10, MEM, and IVF universal medium, respectively (Fig. 1). No media dependent difference could be found during maturation of cattle oocytes. The oocyte maturation rate recorded in M199 in this study were same as observed by Gliedt *et al* (1996), who recorded a maturation rate of 53.7 %. The maturation rates quoted by Sahoo *et al.* (1998) were however, far greater in case of TCM 199 (85.7%) as compared to this study. The observation recorded in case of MEM was slightly greater (60.2% vs 54.19%). In TCM 199 after culture 69.4% (77/111) of oocytes reached full maturation showing cumulus expansion (Barile *et al.*, 1990). In comparison to above mentioned studies low maturation rate of our studies may be due to slight differences in laboratory condition and expertise.

Table 1. Maturation rate in cattle and buffalo.

Species	No. of oocytes cultured	No. of oocyte matured	Maturation rate (%)
			(Mean+SE)
Cattle	508	284	56.0+1.9
Buffalo	182	101	55.4+0.8

In buffalo, the average maturation rate observed was 55.4±0.8%. The maturation rates in M199, Ham's F10, MEM and IVF universal medium were 56.4 %, 53.6 %, 55.0 %, and 54.2 %. respectively. There was no significant difference in maturation rates of oocytes cultured in these media (Fig. 2). Totey *et al.* (1992) observed 47.4 % and 44.8 % *in vitro* maturation rates in buffalo oocytes cultured in M199 and Ham's F10 media respectively. So these results are in line with our study.

The immature oocytes were vitrified with ethylene glycol based vitrification solution. The maturation rate after vitrification was 47.85± 0.2 % and 52.22±0.13 % in cattle and buffalo respectively (Fig 3). There was no significant difference ($P>0.05$) in maturation rate after vitrification in both species. But, in both species the maturation rate after vitrification (47.85± 0.2 % and 52.22±0.13 % in cattle and buffalo) remained significantly lower ($p<0.05$) than the normal maturation rates (56.0% and 55.4 in cattle and buffalo respectively). The low maturation rate after vitrification may be due to destruction of some DNA content of nucleus of cell as described by Men *et al.* (2001).

It has also been documented that the vitrification of immature oocytes disturbs the gap junctions of COC. All the nutrition to the cell is provided by the granulosa cells through these gap junctions; even that receptors of hormones are located on granulosa cells (Xie *et al.*, 1990). Therefore, due to this damage the decrease in viability is understandable.

The vitrification at M II stage is more suitable but the immature state of egg development might prove to be a more successful approach for cryopreservation because its oolemma is more permeable to cryoprotectant, and its chromatin is more conveniently and safely packaged in the nucleus (Blerkom and Davis, 1994). In conclusion, there was no significant difference among the media used for maturation of oocytes, but vitrification significantly reduced the maturation rate in both species.

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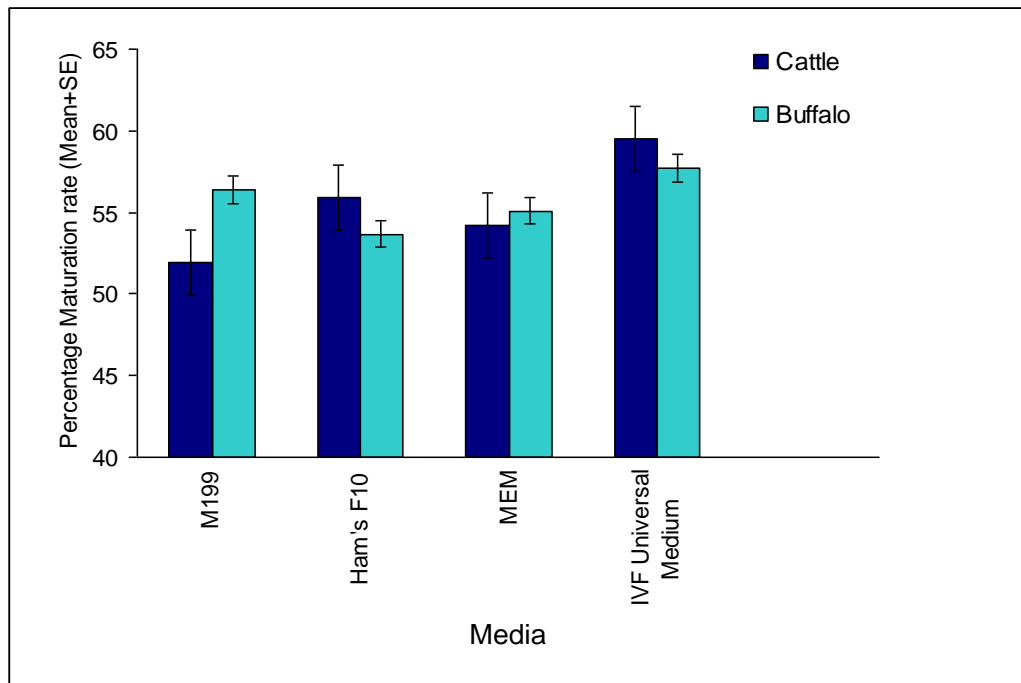


Fig 1. Maturation rate of cattle and buffalo oocytes in different media.

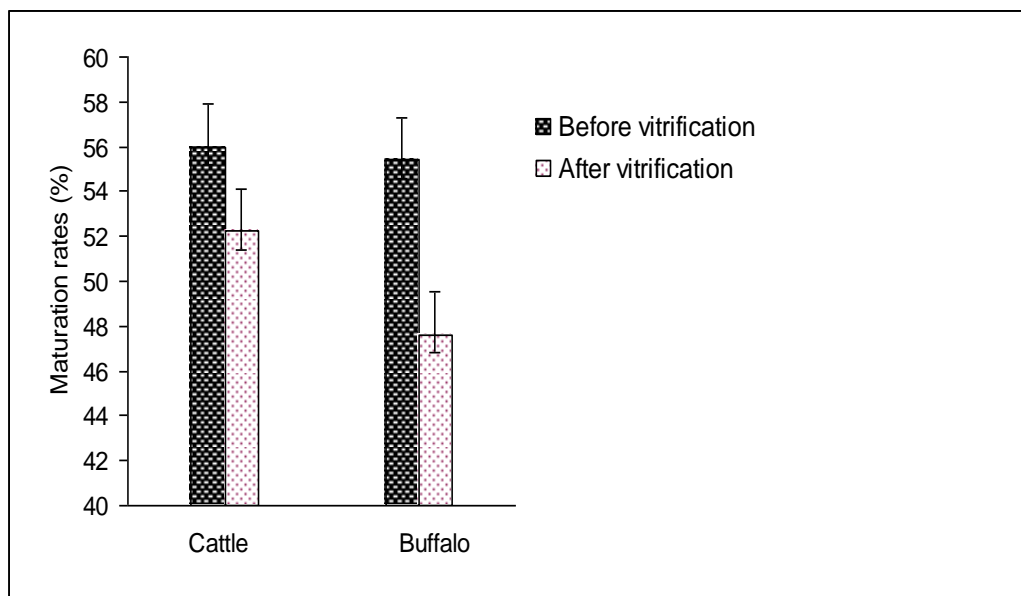


Fig. 2 . Effect of vitrification on maturation rates of immature oocytes.

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