

## EFFECT OF FOLLICULAR FLUID SUPPLEMENTATION ON *IN-VITRO* MATURATION AND DEVELOPMENTAL RATE OF BUFFALO OOCYTES

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The effect of supplementation of follicular fluid (FF) at 5.0% concentration in *in vitro* maturation (IVM) media on *in vitro* production of buffalo embryos was studied. Oocytes were aspirated from slaughter house derived ovaries and graded. Culturable quality oocytes were randomly cultured in IVM media with FF (n = 334) and without FF (n = 228) and subjected to maturation. The maturation rate, cleavage rate and embryo developmental rate was recorded in both the groups. The maturation, cleavage, morula and blastocyst rates ( $78.2 \pm 1.8$ ,  $41.2 \pm 1.3$ ,  $15.2 \pm 0.7$  and  $7.7 \pm 0.9$  % respectively) were significantly ( $P < 0.01$ ) higher when oocytes were matured in media supplemented with FF than controls ( $55.3 \pm 1.9$ ,  $28.7 \pm 1.5$ ,  $6.7 \pm 0.8$  and  $2.2 \pm 0.8$  % respectively). Thus, it could be concluded that supplementation of FF (5.0 %) to IVM media would benefit the maturation, cleavage and embryo development in buffalo IVF programme.

**Key words:** Bubaline oocytes, Embryo development, IVM with or without follicular fluid

Follicle is an avascular compartment within the mammalian ovary, separated from the peri-follicular stroma by the follicular wall that constitutes a 'blood-follicle barrier'. The follicular fluid forms the biochemical environment of the oocyte before ovulation. Follicular fluid is in part an exudate of serum and is in addition partially composed

of locally produced substances, which are related to the metabolic activity of follicular cells (Gerard *et al.*, 2002). Since it has already been shown that changes in concentrations of gonadotropins, steroids and growth factors in follicular fluid of dairy cows were linked with alterations in oocyte quality (Driancourt and Thuel,

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1998), it is not unlikely that metabolites which are present in the follicular fluid can influence oocyte quality. Several *in vitro* studies showed that metabolites, such as glucose, urea etc., may influence the competence of bovine oocytes to mature and, after fertilization, to grow to the blastocyst stage (Armstrong *et al.*, 2001). The effect of supplementation of follicular fluid (FF) at 5.0 per cent concentration in *in vitro* maturation (IVM) media (Shang *et al.*, 2007) on *in vitro* production of buffalo embryos was studied.

## MATERIALS AND METHODS

*In vitro* embryo production of bubaline embryos were carried out with minor modifications in the protocol designed by Totey *et al.* (1992). A total of 35 trials were conducted (with FF: 19 and without FF: 16 trials).

**Oocyte retrieval :** Ovaries from apparently normal reproductive organs of adult buffaloes of unknown breeding history slaughtered in Chennai Corporation abattoir were collected within 10 min after slaughter. The oocytes were retrieved by aspiration technique as described by Prasad *et al.* (2010). The aspirated FF was screened under stereo zoom microscope (Nikon, Japan) and oocytes were recovered. After the oocyte recovery, the FF was transferred to 1.5mL micro-centrifuge tube and centrifuged at 10,000g for 10-15 min at 4°C. The supernatant FF was aliquoted in

separate micro-centrifuge tubes for supplementation in IVM media.

The recovered oocytes were graded based on their cumulus mass investment and homogeneity of ooplasm as described by Nandi *et al.* (1998). A and B grade oocytes were considered as culturable quality oocytes and were subjected for further processing.

***In vitro* maturation:** Tissue culture media based IVM media (TCM-199 + FBS 10% + FSH 0.5 µg / mL + LH 5.0 µg /mL + Oestradiol 1 µg / mL + Cysteamine 1 µM + Sodium Pyruvate 1 mM) was prepared freshly with and without FF supplementation (5%) on the day of oocyte collection. Selected oocytes were washed and randomly transferred into individual droplets (maximum 10-20 oocytes per 50µL droplet) of IVM media with FF (n = 334) and without FF (n = 228) and subjected to maturation at 38.5°C in a humidified atmosphere with 5% CO<sub>2</sub> in air for 20-22 hrs. After 20-22 hrs of incubation in IVM medium, the percentage of oocytes matured (maturation rate) was assessed based on the cumulus expansion.

***In vitro* fertilization and embryo culture:** The matured oocytes were washed and transferred to pre-equilibrated 50µL droplets (15 oocytes per droplet) of IVF media (TALP + Heparin 10 µg / mL + Caffeine 10 mM + Sodium pyruvate 0.6 mM + BSA FAF 0.6%). The oocytes were

co-incubated with two  $\mu\text{L}$  of sperm suspension at a concentration of  $2 \times 10^6/\text{mL}$  and co-incubated for 24 hrs. After 24 hrs of incubation in IVF media, presumptive zygotes were washed and transferred to embryo culture media (ECM) culture droplets. The cleavage rate (%) was assessed at 24 and 48 hrs post insemination and subsequently for the assessment of developmental stages, the cleaved embryos were monitored for every 24 hrs upto 10 days. The stages of embryos were assessed as described by Klumpp (2004) and percentage of morulae and blastocyst produced in each experimental group was calculated.

**Statistical analysis:** The data of maturation, cleavage and embryo development rates were analysed between

oocytes matured with and without supplementation of FF. All the data were analysed by student t-test (Snedecor and Cochran, 1994)

## RESULTS

**Recovery rate and quality of buffalo oocytes:** A total of 1420 oocytes were retrieved from 865 buffalo ovaries with a mean recovery rate of  $1.7 \pm 0.1$  oocytes per ovary, of which 234 (16.7 %), 328 (23.3 %), 528 (36.4 %), 190 (13.2 %) and 140 (10.5 %) oocytes were of A, B, C, D and E grades respectively.

The maturation rate, cleavage rate and embryo development rate of buffalo oocytes cultured in IVM media supplemented with and without FF were presented in Table 1.

**Table 1. Maturation rate, cleavage rate and embryo developmental rates of oocytes matured with and without follicular fluid supplementation**

Sl No	IVM Media		Significance	Overall (Mean $\pm$ SE)
	With FF (n=19 trials)	Without FF (n=16 trials)		
1	No. of oocytes cultured	334	228	562
2	No. of oocytes matured (78.2 $\pm$ 1.8 <sup>b</sup> )	127 (55.3 $\pm$ 1.9 <sup>a</sup> )	**	391 (67.7 $\pm$ 2.4)
3	No. of oocytes cleaved (41.2 $\pm$ 1.2 <sup>b</sup> )	65 (28.7 $\pm$ 1.5 <sup>a</sup> )	**	203 (35.5 $\pm$ 1.4)
4	No. of Morulae (15.2 $\pm$ 0.7 <sup>b</sup> )	14 (6.0 $\pm$ 0.1 <sup>a</sup> )	**	65 (10.9 $\pm$ 0.9)
5	No. of Blastocysts (7.7 $\pm$ 0.9 <sup>b</sup> )	5 (2.2 $\pm$ 0.8 <sup>a</sup> )	**	31 (5.2 $\pm$ 0.8)

Percentage (Mean  $\pm$  SE) in parenthesis

Values within rows with different superscripts differ significantly

\*\* Significant ( $P \leq 0.01$ )

**Maturation rate:** The maturation rate was significantly ( $P \leq 0.01$ ) higher in oocytes cultured in IVM media supplemented with FF when compared to oocytes cultured without FF. In total, out of the 562 oocytes cultured, maturation was observed in 391 ( $67.7 \pm 2.4 \%$ ) oocytes.

**Cleavage rate:** There was a significant increase ( $P \leq 0.01$ ) in cleavage rate when buffalo oocytes were matured with FF. In this study, a total of 203 ( $35.5 \pm 1.4 \%$ ) oocytes were found to be cleaved.

**Developmental rate:** Significantly ( $P \leq 0.01$ ) higher percentage of morulae and blastocysts were produced from oocytes matured in IVM media supplemented with FF than without FF. A cumulative total of 65 ( $10.9 \pm 0.9 \%$ ) morulae and 31 ( $5.2 \pm 0.8 \%$ ) blastocysts were produced.

## DISCUSSION

In the present study, the mean oocyte recovery rate was  $1.7 \pm 0.1$  per ovary using the aspiration technique. This was in accordance with the findings of Das *et al.* (1996), but higher than (0.73-1.3) reported by Totey *et al.* (1992).

In a typical IVF procedure the IVM of oocytes presents the most challenging step because events during IVM have been demonstrated to affect not only the process of fertilization but the subsequent stages of early cleavage divisions, blastocyst formation and successful implantation which are also thought to be dependent on the genes expressed during this time (Knijn

*et al.*, 2002). A number of ultrastructural and molecular changes occurring during oocyte development are linked to the developmental competence of the gamete (Hyttel, 1997). Therefore, development of a suitable culture system for IVM of oocytes is a major component of *in vitro* embryo production procedures especially for buffaloes because oocyte maturation and cleavage rates in buffalo were lower than those for cattle (Chauhan *et al.*, 1997).

In this study, when oocytes were cultured in conventional IVM media without FF the maturation and cleavage rates were  $55.3 \pm 1.9$  per cent and  $28.7 \pm 1.50$  per cent respectively. In corroboration with our findings, Hegab *et al.* (2009) also achieved 68.0 per cent maturation rate and 25.0 per cent cleavage rate when oocytes were matured with conventional TCM199 based media. But, when oocytes were cultured with FF the mean percentage of oocytes matured ( $78.2 \pm 1.8 \%$ ) and cleaved ( $41.2 \pm 1.3 \%$ ) was significantly ( $P \leq 0.01$ ) increased. The present study supported the observation of Chauhan *et al.* (1997) and Aquino and Ocampo (2014), who suggested that the buffalo FF promoted nuclear and cytoplasmic maturation. Somfai *et al.* (2012) opined that supplementation of IVM medium with FF promoted sperm penetration both by the improvement of cumulus expansion and by enhancing ATP levels in oocytes.

Similarly, it was found that the percentage of morulae and blastocysts were significantly higher in the FF supplemented group than

without FF supplementation. The culture medium employed for IVM of oocytes not only affect the proportion of oocytes that reach the metaphase II and become capable of undergoing fertilization, but also influenced subsequent embryonic development (Rizos *et al.*, 2002 and Elmileik *et al.*, 1995). Chi *et al.* (1998) suggested that the FF stimulated the synthesis of cell cycle protein in embryos which in turn supported their development.

The presence of enzymes like lactate dehydrogenase, ATPase, transaminase and alkaline phosphatase, glycosaminoglycans, proteins and steroids, gonadotrophins

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- (Caucig *et al.*, 1971) and antioxidants (Hussein *et al.*, 2013) might have enhanced the maturation, cleavage rates and embryo development *in vitro*.
- Thus it could be concluded that supplementation of FF (5.0 %) to IVM media would benefit the maturation, cleavage and embryo development in buffalo IVF programme.

## CONFLICT OF INTEREST

Authors declare that there is no conflict of interest regarding the present research work.

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