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Effect of different maturation media on *in vitro* maturation of buffalo oocytes (*Bubalus bubalis*)

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Abstract
Buffalo is an integral part of livestock agriculture in Asia since many centuries, because they provide draught power, milk, meat and hide to millions of people, particularly small-scale farmers. The low production efficiency can be attributed to delayed puberty, higher age at calving, long postpartum anestrus period, lack of overt sign of heat and low conception rate. In the present study, three different maturation media were used for in vitro maturation. A total of 450 culture grade oocytes (replicates: 6) and three different maturation media i.e. IVM-I (TCM-199 + hormones + 10% EBS + Gentamycin), IVM-II [(TCM-199 + hormones + gentamycin) and IVM-III [(TCM-199 + Gentamycin) respectively. Results indicated that among three treatments, highest proportion (66.64±.57) of oocytes extruded PB in medium with TCM 199H + 10% EBS + hormones followed by 52.04±0.72 in medium with TCM 199H + hormones and the lowest proportion was observed in medium with TCM 199H + Gentamicin (7.39±0.23). The proportion of MII chromosomes in IVM-I was significantly (P≤0.05) higher than IVM-II (52.83±0.69) and IVM-III (8.70±1.00).

Keywords: Buffalo, *In vitro* maturation, oocyte, polar body, cumulus cell expansion

1. Introduction
Buffalo is an integral part of livestock agriculture in Asia since many centuries, because they provide draught power, milk, meat and hide to millions of people, particularly small-scale farmers. The low production efficiency can be attributed to delayed puberty, higher age at calving, long postpartum anestrus period, lack of overt sign of heat and low conception rate. Therefore, the emphasis has now shifted to *in vitro* embryo production (IVEP). *In vitro* maturation and *in vitro* fertilization procedures performed on oocytes obtained from slaughter house derived ovaries have recently provided a practical means for producing large number of bovine zygotes at low cost for research and commercial settings [1]. The culture medium and selection of protein supplements and hormones for IVM play an important role in subsequent maturation rate and embryonic development following IVF [2]. Therefore, the present work was undertaken to identify the best *in vitro* maturation media.

2. Materials and Methods
Ovaries from buffaloes were recovered at local slaughter house at Bahadurpura, Hyderabad after slaughter. The oocytes were aspirated aseptically from the follicles of >4mm diameter by using 20 G needle attached to 5 ml disposable syringe containing collection medium and observed under stereozoom microscope (SZX-ILLK200, Olympus, Japan). The COC's having homogenous cytoplasm and surrounded by more than three layers of compact cumulus cells were considered as good quality oocytes (Fig. 1). The oocytes were washed thrice in respective IVM medium and randomly allotted to three different *in vitro* maturation media (IVM-I, IVM-II and IVM-III). IVM-I was prepared by supplementing TCM 199H with 10 µg/ml FSH, 10 µg/ml LH, 1 µg/ml Estradiol 17β, 50 µg/ml gentamycin sulphate, 10 µg/ml of Bovine serum albumin (FAF) and 10% estrus buffalo serum (EBS), IVM-II was prepared by supplementing TCM 199H with 10µg/ml FH, 10 µg/ml Lutenizing hormone (L9773, Sigma, USA) 1 µg/ml estradiol -17β (E4389, Sigma, USA) 50 µg/ml gentamicin sulphate and IVM-III was prepared by supplementing TCM 199H with 50 µg/ml gentamicin sulphate. Oocytes were kept individually in 50 µl droplets of IVM medium in 35 mm tissue culture dishes (Cat no-460035, Tarsons, India). The droplets were overlaid with autoclaved light weight mineral and incubated at 39^0C in 5%CO_2 for 22 hrs.

In vitro matured oocytes were evaluated for CCE, extrusion of first PB and presence of MII stage chromosomes by staining with propidium iodide.

3. Results and Discussion

Oocyte maturation is the process of complex changes in the protein phosphorylation which transform the primary oocyte into a mature secondary oocyte [3]. Among total of 450 culture grade oocytes, Cumulus cell expansion (Fig. 2) was found highest in IVM-I (87.85±0.58), followed by IVM-II (74.06±0.80), while it was lowest in IVM-III (50.96±1.32). There was significant (P≤0.05) difference among the three treatment groups. Mean% ± SE values of cumulus expansion for IVM I, II and III treatment groups were 87.85±0.58, 74.06±0.80 and 50.96±1.32, respectively (Table. 1). CCE in IVM-I (87.85±0.58) was similar to the reports of (80-83%) [4] using 10% steer serum, (86%) [5] using EBS/FCS and (91.55±0.93 to 92.11±0.60) [6]. CCE in IVM-III (50.96±1.32) was similar to the findings of [1] Singh *et al.* 2012 (50.30%) [7] reported maturation rate basing on cumulus expansion as 77.44±0.68, 55.17±2.7 and 26.62±0.75 using EBS + Hormones, hormones without EBS and TCM199H without EBS and Hormones, respectively [8] and [9] reported that oocytes matured *in vitro* in the presence of gonadotropins and estradiol had higher maturation rates compared with medium without hormones which supports the present observations and moreover the gonadotropins and estradiol cause synergistic enhancement of nuclear maturation in mammalian oocytes *in vitro*.

All the three *in vitro* maturation media observed to extrude polar body (Fig. 3). Among three treatments, highest proportion (66.64±.57) of oocytes extruded PB when COC's were cultured in the medium supplemented with TCM 199H and hormones with EBS (IVM-I) and the lowest proportion was observed in TCM 199 H + Gentamicin (7.39±0.23). Among the treatment groups, the proportion of oocytes extruded PB in IVM-I was significantly (P≤0.05) higher than IVM-II (52.04±0.72) and IVM-III (7.39±0.23). The mean%±SE values of extrusion of polar body in IVM-I, IVM-II and IVM-III were 66.64±0.57, 52.04±0.72 and 7.39±0.23, respectively (Table.1). In the present study, the proportion of extrusion of PB in IVM-I (66.64±.57) were in accordance with the findings of (70.01%) [10]. The proportion of MII chromosomes (Fig. 4) in IVM-I (TCM 199H + EBS) was significantly (P≤0.05) higher than IVM-II (52.83±0.69) and IVM-III (8.70±1.00). The mean% ± SE values of MII chromosomes in IVM-I, IVM-II and IVM-III were 70.06±0. 39, 52.83±0.69 and 8.70±1.00, respectively (Table. 1). The MII stage chromosomes using serum (IVM-I) in the present study was in agreement to the findings of [11-13], who reported 68-71%, 65% and 58.07±2.08 to 68.10±0.75, respectively. On contrary, the MII stage chromosomes in the present study was less than observations of [14], who reported 82.4-91.5%. It is known that the ability of buffalo oocytes to undergo IVM is affected by biological and environmental factors [15]. TCM 199H supplemented with hormones and 10% EBS identified to be the best to support oocytes maturation in terms of cumulus cell expansion (CCE), extrusion of 1st polar body (PB) and presence of MII stage chromosomes.

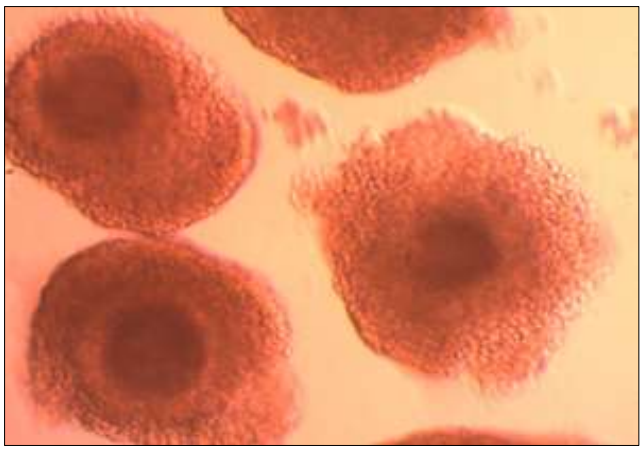

Fig 1: Culturable oocytes

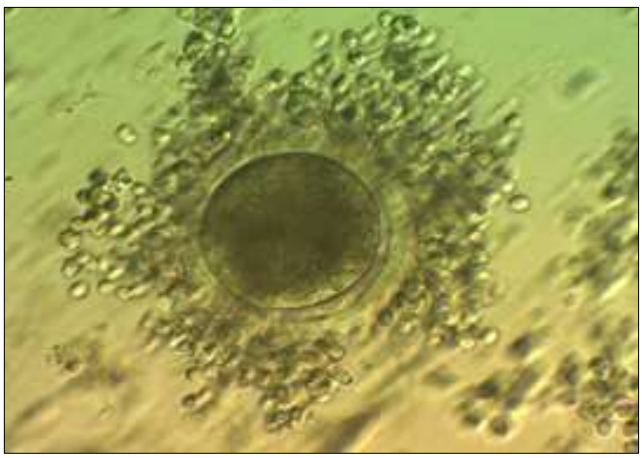

Fig 2: Cumulus cell expansion with PB

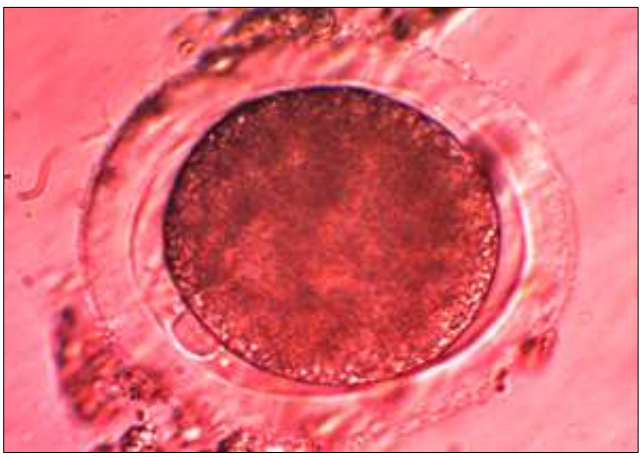

Fig 3: Extrusion of 1st polar body

Fig 4: MII stage chromosome

Table 1: Effect of different *in vitro* maturation media on *in vitro* maturation rate

Treatments	No. of immature oocytes/replicates	Oocyte maturation rate		
		Cumulus cell expansion*	Extrusion of polar body*	MII stage chromosomes*
IVM-I	150/6	132 (87.85±0.58)^{c}	100 66.64±0.57)^{c}	105 (70.06±0. 39)^{c}
IVM-II	150/6	111 (74.06±0.80)^{b}	78 (52.04±0.72)^{b}	79 (52.83±0.69)^{b}
IVM-III (Control)	150/6	76 (50.96±1.32)^{a}	11 (7.39±0.23)^{a}	13 (8.70±1.00)^{a}

*Figures in paranthesis are mean% ± SE. Figures with different superscripts with in the column are significantly different. One way ANOVA followed by Duncan's multiple range test (P≤0.05).

4. Conclusions

In the present study it was revealed that the addition of serum to the TCM-199 medium enhanced the maturation rate of follicular oocytes. Beneficial action of serum may be due to its antioxidant properties and it contains a number of known growth factors that have an important role in the regulation of oocyte maturation, particularly through cumulus cells which prevents the hardening of zona pellucida [16] (Mahmoud and Nawito, 2003) [12].

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