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In vitro maturation of bubaline oocytes with buffalo estrous serum in presence or absence of hormones

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ABSTRACT

The present investigation on *in vitro* maturation of buffalo oocytes was conducted on oocytes aspirated from buffalo ovaries obtained from slaughter house. The study revealed that although the buffalo oocytes can be matured in TCM-199 and Ham's F-10 medium but addition of BES and hormones significantly ($P<0.05$) improves the maturation of bubaline oocytes in TCM-199 (68.57%) and Ham's F-10 (61.11) medium.

Key words : Buffalo, oocytes, maturation

Recent advances in embryo biotechnology has allowed to produce offspring in farm animals through embryo transfer. *In vitro* maturation (IVM) and fertilization (IVF) are such biotechnological methods by which a substantial number of embryos can be produced in the laboratory where there is always a great demand in modern research. The matuuraion of oocytes requires serum as a protein supplement and hormones play an important role in maturation and subsequent fertilization of oocytes. Buffalo estrus serum can be obtained easily and be utilized as a cheap protein substitute for maturation of buffalo oocytes. Therefore the present investigation is an attempt to use buffalo estrus serum in presence or absence of hormones and evaluate the maturation rates *in vitro*.

MATERIALS AND METHODS

Buffalo ovaries were obtained immediately after slaughter from a local abattoir and transport to the laboratory in Dulbecco phosphate Buffer Saline (DPBS) at 25-30°C within one hour. The ovaries were washed thrice with Normal Saline Solution then two washings were given in DPBS at 37°C and stored in the same until processing.

The oocytes were aspirated from 2-8 mm follicles in TL-Hepes medium. The good quality cumulus oocytes complexes with uniform ooplasm were washed thrice in TL Hepes fortified with bovine serum albumin. Then the oocytes were cultured in TCM-199 or Ham's F-10 medium containing buffalo estrus serum (BES) 10% in presence or absence of hormones (oFSH 1 ìg/ml, oLH 10 IU/ml and E_2 1 µg/ml) for 26 hours. The maturation was assessed by expansion of cumulus mass, enlargement of perivitelline space and extrusion of Ist polar body.

Buffalo estrus serum was prepared by collecting the blood from estrus buffalo and harvesting the serum 12 hours post clotting and subsequently centrifuging the serum. The serum was heat inactivated at 56°C for 30 minutes and filtered through 0.22 µ millipore and stored until use. The data were analysed using 'Z' test as per Snedecor and Cochran(1967).

RESULTS AND DISCUSSION

The rates of *in vitro* maturation of bubaline oocytes in TCM-199 and Ham's F-10 are presented in Table 1. When buffalo estrus serum (BES) was added to TCM-199 it significantly ($P<0.05$) improved the maturation rates. Although the addition of hormone singly or in combination improved the maturation but the differences were statistically non-significant except with the addition of leutinizing hormone (LH), follicle stimulating hormone

Table 1. *In vitro* maturation of buffalo oocytes : effect of buffalo estrus serum (BES) and hormones in Ham's F-10 medium

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Table 1. *In vitro* maturation of buffalo oocytes : effect of buffalo estrus serum (BES) and hormones in Ham's F-10 medium

Treatment	Medium	Serum	Hormone	Oocytes Cultured	Oocytes Mature	Oocytes Maturation rate
1	Ham's F-10	–	–	150	40	26.66[a]
2	Ham's F-10	BES	–	788	322	40.86[b]
3	Ham's F-10	BES	LH	290	120	42.06[b]
4	Ham's F-10	BES	FSH	300	124	41.33[b]
5	Ham's F-10	BES	E_2	285	125	43.85[b]
6	Ham's F-10	BES	LH + FSH	260	120	46.15[b]
7	Ham's F-10	BES	LH + FSH + E_2	900	550	61.11[c]
8	Ham's F-10	BES	FSH + E_2	290	129	44.48[b]
9	Ham's F-10	BES	LH + E_2	295	136	46.10[b]

Values with unidentical superscripts differ significantly ($P < 0.05$)

(FSH) and estradiol (E_2) combined with BES which significantly improved the maturation rate.

The supplementation of BES in Ham's F-10 medium also improves the maturation rate significantly ($P<0.05$) but addition of hormones singly or in combination failed to exert a significant impact except the addition of gonadotropin and steroid (LH + FSH + E_2) where a maximum (61.11%) maturation was observed.

In almost all studies of mammalian *in vitro* maturation, the basic medium was supplemented with serum, crystallised albumin or estrus serum. In the present study buffalo estrus serum (BES) was used.

Our results indicate that buffalo oocytes could initiate maturation spontaneously after recovery from follicles. Similar findings have been reported by Totey *et al.* (1992, 1993); Madan *et al.*(1994) and Bhatt (1995). The medium supplement with BES to the medium during *in vitro* maturation of oocytes was responsible at least in part for the induction of maturation. Sanbuissho and Threlfall observed no significant difference where comparing the effects of FCS and ECS on bovine oocytes maturation. Similar findings were reported by Fukui and Ono (1989). In the present study, the rate of oocyte maturation in presence of BES were not as high as previously reported by Totey *et al.* (1993), Bhatt (1995) in buffalos the variation in maturation rates may be due to source of sera or the quality of oocytes used for maturation.

The findings suggest that sera may contain factors that promote the acquisition of development competence of oocytes during *in vitro* maturation. Beneficial role of serum source in culture medium has been also reported by Sanbuissho and Threlfall (1985), Downs *et al.* (1986), Totey *et al.* (1993) and Bhatt (1995). However, Suss *et al.* (1988) reported that completion of nuclear maturation of bovine oocytes is not depedent upon any supplement to the medium.

The effect of addition of LH, FSH or E_2 during *in vitro* maturation of oocytes has been studied in buffaloes by Totey *et al.*(1993) and Bhatt (1995). Our investigations showed that maturation rates of bubaline oocytes increased significantly ($P< 0.05$) in the presence of buffalo estrus serum (BES) in both single hormone or combination of FSH + LH or LH + E_2/ FSH + E_2 failed to exert a significant effect ($P<.05$) in both the media but addition of FSH + LH + E_2 significantly increased the

maturation rates and reached to a maximum of 68.57 and 61.11 per cent in TCM-199 and Ham's F-10 medium, respectively, in presence of BES. These findings corroborates with the findings of Totey *et al.*(1993) and Bhatt (1995) and Fukushima and Fukui (1985) who found that the maturation rate was significantly increased by the addition of LH or by the combination of LH, FSH and E_2 compared with that of controls.

Thus in the light of present findings it can be concluded that although buffalo oocytes can be matured in TCM - 199 and Ham's F-10 medium alone to some extent but addition of BES and hormones significantly improves the maturation of buffalo oocytes.

REFERENCES

Bhatt, S. (1995). *In vitro* maturation and fertilization of buffalo oocytes : Effect of media, sera and hormones. M.V.Sc. thesis submitted to G.B.P.U.A & T, Pantnagar.

Bhatt, S. and Maurya, S.N. (1995). *In vitro* maturation of buffalo oocytes. Scientific Compendium, National Symposium on Modern Trends in Reproductive Health Care. 5.1., held at Panjabrao Krishi Vidhypeeth, Akola, January, 13-15, 1995.

Downs, S. M.; Schroeder, A.C. and Eppig, J.J. (1986). Serum maintains the fertilizability of mouse oocytes matured *in-vitro* by preventing hardening of *Zona pellucida*. Gamete Res., **15** : 115-122.

Fukushima, M. and Fukui, Y. (1985). Effects of gonadotrophins and steroids on the subsequent fertilizability of extra-follicular bovine oocytes matured *in vitro*. Anim. Reprod. Sci., **9** : 323-332.

Madan, M.L., Chauhan, M.S., Singla, S.K. and Manik, R.S. (1994). Pregnancies established from water buffalo (*Bubalus bubalis*) blastocystes derived from *in-vitro* matured *in-vitro* fertilized oocytes and co-cultured with cumulus and oviductal cells. Theriogenology, **42**: 591-600.

Saha, S., Otoi, T. and Suzuki, T. (1996). The efficiency of ethylene glycol, trehlose and polyvinyl pyrrolidone for successful vitrification of IVF bovine embryos. J. Reprod. Develop., **42** : 163-169.

Sanbuissho, A. and Threlfall, W.R. (1985). The effect of estrous cow serum on the maturation and fertilization of bovine oocytes. Theriogenology, **23** : 226 (Abstr.).

Suss, I., Wuthrich, K. and Stranzinger, C. (1988). Chromosome configurations and time sequence of the first meiotic division in bovine oocytes matured *in vitro*. Biol. Reprod., **38** : 871-880.

Totey, S.M., Singh, G., Taneja, M., Pawshe, C.H. and Talwar, G.P. 1992. *In vitro* maturation, fertilization and development of follicular oocytes from buffalo (*Bubalus bubalis*). J. Reprod. Fert., **95** : 597-607.

Totey, S. M., Pawshe, C.H. and Singh, G. P. 1993b. *In-vitro* maturation and fertilization of buffalo oocytes (*Bubalus bubalis*) : Effect of media, hormone and sera. Theriogenology, **30** : 1153-1171.

Younis, A.I., Brackett, B. G. and Fayrer-Hosken, R.A. (1989). Influence of serum and hormones on bovine oocytes maturation and fertilization *in vitro*. Gamete Res., **23**: 189-201.