

A COMPARATIVE STUDY ON CLEAVAGE AND MORULA DEVELOPMENT IN SYNTHETIC OVIDUCTAL FLUID MEDIA (mSOF) WITH OR WITHOUT CITRATE AT TWENTY PER CENT OXYGEN LEVEL

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SUMMARY

The efficiency of buffalo embryo production is much lower than that of cattle. The culture system employed for performing fertilization and subsequent embryo culture up to the transferable stage are sub optimal and need substantial improvement. The present study was carried out with the aim to increase the production of the number of transferable buffalo embryos by adding citrate in embryo development media (mSOF). Follicle of slaughter house ovaries were aspirated and the collected cumulus oophorus complexes (COCS) were cultured in TCM-199 medium supplemented with 10% fetal calf serum, 20% buffalo follicular fluid and 0.5 g / ml FSH for 24 h. The matured oocytes were then inseminated with frozen thawed buffalo semen processed in BO medium. After 18 h of post insemination, the oocytes were cultured in modified synthetic oviductal fluid containing 0.8% BSA and amino acids without (mSOFaa) or with citrate (mSOFaac). The developmental stages of embryos were assessed at every 48 h interval. Cleavage and morula development rate in mSOFaac under 20% O₂ tension were 6.11% and 1.53%, while under same O₂ tension and cultured in mSOFaa, the cleavage and morula development rate were 9% and 4%, respectively. It may be concluded that citrate at 20% O₂ level reduces cleavage and morula development in buffalo.

Key words: *In-vitro* embryo culture, buffalo, synthetic oviductal fluid media

The efficiency of buffalo embryo production is much lower than that of cattle (Palta and Chauhan, 1998). The culture system employed for performing fertilization and subsequent embryo culture up to the transferable stage are sub optimal and need substantial improvement. Citrate stimulates fatty acids synthesis (Goodridge, 1973) and is a chelator of calcium ions. This feature may be of importance for maintaining junctional integrity and thus important for compaction of cells (Gray *et al.*, 1992) in growing embryos under 5 % O₂ tension. Better embryonic development was noticed in mouse in presence of citrate in mSOFaa under 5 % O₂ tension (Keskinetepe *et al.*, 1998). The present study compares cleavage and morula development following IVF in mSOFaa and mSOFaac under 20 % O₂ tension in buffalo.

Follicles of slaughter house buffalo ovaries

were aspirated and collected cumulus oophorus complexes (COCS) were cultured in TCM-199 medium supplemented with 10 % fetal calf serum, 20 % buffalo follicular fluid and 0.5 mg / ml FSH for 24 h. The matured oocytes were then inseminated with frozen thawed buffalo semen processed in BO medium. On 18 h of post insemination, the oocytes were cultured in modified synthetic oviductal fluid containing 0.8 % BSA and amino acids (mSOFaa) or mSOF containing amino acid and sodium citrate (mSOFaac). The developmental stages of embryos were assessed at every 48 h interval.

Cleavage and morulae development rate in mSOFaac was 6.11 % and 1.53 %, while in mSOFaa was 9 % and 4 %, respectively (Table 1). Better embryonic development had been noticed in mouse in presence of citrate in mSOFaa under 5 % O₂ tension (Keskinetepe *et al.*, 1998). But in this study, under 20 % O₂ tension, the results were poor in mSOFaac as compared to sodium citrate, which by interacting with high level of O₂ tension might be producing some toxic

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Table 1
Comparative study on cleavage and morula
development of buffalo embryos in synthetic
oviductal fluid under 20% O₂ tension

Gp.	Media used for IVC	No. of culturable oocytes	No. of matured oocytes	No. of cleaved oocytes	No. of morula
I	mSOFaac	131	108	8 (6.11 %)	2 (1.53 %)
II	mSOFaa	128	118	11 (9 %)	5 (4 %)

compounds, which may be detrimental to embryo. While, under 5% O₂ tension better embryonic growth has been noticed in presence of citrate in another study of our laboratory.

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