EFFECT OF CYSTEAMINE SUPPLEMENTATION IN SEMI DEFINED MEDIA ON *IN VITRO* PRODUCTION OF BUFFALO EMBRYOS

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ABSTRACT

The present study was conducted to investigate the effect of cysteamine supplementation in semi defined media like synthetic oviductal fluid (SOF) media during various stages of *in vitro* culture on buffalo preimplantation embryo development. Six independent trials with a total of one hundred and seventy four oocytes were carried out with cysteamine supplementation at 100µM/ml during *in vitro* maturation (IVM), fertilization (IVF) and culture (IVC), one hundred twenty six oocytes without supplementation served as control. The mean cleavage rates were 24.1±1.67and 19.8±0.89 per cent in the treatment and control group, respectively. The percentage of morula that developed in treatment and control group were 15.1±1.35 and 7.9±1.47, respectively. The percentage of embryo that developed to the morula stage were significantly higher (P<0.01) in treatment group. Cysteamine supplementation during IVM, IVF and IVC improved the cleavage and morula development due to glutathione (GSH) synthesis stimulated by cysteamine.

Key words: Buffalo, Cysteamine, In *vitro* fertilization, Embryo.

INTRODUCTION

A major culture induced stress in IVF was enhanced oxidative damage, with increased reactive oxygen species (ROS) production (Orsi and Leese, 2001). Buffalo oocytes and embryos are likely to be more sensitive to oxidative stress due to higher lipid content. Antioxidants such as cysteamine and â-mercaptoethanol (thiol compounds) added during bovine and ovine IVM, increase intracellular glutathione (GSH) synthesis and consequently improve embryo development and quality. GSH (L-r-glutamyl-L-cysteinyl-glycine) is a tripeptide, major intracellular non protein sulphydryl, free thiol which protect cells against oxidation, amino acid transport, protein synthesis and reduction of disulfides.GSH plays an important role in protecting mammalian cells from oxidative stress and serve as oxygen species scavengers. Hence, the present study was conducted to determine the effect of cysteamine supplementation during IVM, IVF and IVC media on buffalo embryo development.

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MATERIALS AND METHODS

All chemicals were purchased from Sigma Chemicals Company (St.Louis, MO, USA) and disposable plastic wares from Nunc (Denmark), unless otherwise stated.

RETRIEVAL OF OOCYTES

Oocytes were retrieved either by slicing or aspiration method from the ovaries of buffalo collected from slaughter house and graded as A, B, C,D and E based on cellular investment and homogeneity of ooplasm. Only A and B graded oocytes were used for the experiments.

IN VITRO MATURATION OF OOCYTES

The IVM medium used was SOF supplemented with 10 per cent fetal calf serum (FCS), 2 per cent BME-essential amino acids, 1 per cent MEM- non essential amino acids, 1.0 ìg/ml follicle stimulating hormone (FSH), 0.02 ìg/ml luteinizing hormone (LH) and 1 ìg/ml 17-â estradiol at 38.5°C in an atmosphere of 5 per cent CO_2 incubator for 24 h. The maturation was assessed

based on the expansion of the cumulus cells and extrusion of the first polar body.

IN VITRO FERTILIZATION OF OOCYTES

Frozen semen from single buffalo bull was used for the entire study. For each trial four semen straws were thawed at 37°C for 30 second in a water bath, wiped with 70 per cent alcohol before being opened and processed by swim up method as described by Parrish *et al.* (1995) in SOF media with 20 per sent FBS was used to separate the motile sperms. The concentration of sperm was adjusted to 2 x 10^6 sperm / ml by diluting with SOF medium. The matured oocytes were denuded from the cumulus attachment by vortexing for 90 sec and transferred to 75 ml droplets of SOF medium supplemented with 20 per cent heat inactivated FBS, 2 per cent BME-essential amino acids and 1 per cent MEM- non essential amino acids at the rate of 15 oocytes per droplet for IVF. These droplets were inseminated with 2 ml of sperm suspension and co-incubated for 24 h at 38.5°C in 5 per cent CO_2 incubator.

IN VITRO CULTURE

The oocytes were cultured in 50 ml droplets of SOF with 2 per cent BME-essential amino acids and 1 per cent MEM- non essential amino acids for six days at 38.5° C in 5 per cent CO_2 incubator. The cleavage was assessed at 24 and 48 h post insemination and the developmental stages of cleaved embryos were monitored every 24 h up to 6 days. During the course of culture, the embryos were transferred to fresh SOF medium every 48 h. Data were analyzed by ANOVA (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

A total of two hundred and twenty two buffalo ovaries were utilized for oocytes recovery, of which 75 ovaries were subjected to slicing and 147 ovaries to follicle aspiration. Ovary slicing yielded a total of 314 oocytes with an average yield of 4.2±0.33 oocytes per ovary while, follicle aspiration yielded a total of 259 oocytes with an average yield of 1.9±0.25 oocytes per ovary. The total oocytes yield per ovary was significantly higher ($P<0.01$) by slicing than by follicle aspiration method. The recovery rate of culturable oocytes (A and B grade) by slicing method were (2.4±0.36) higher than by aspiration method (0.8±0.16).The poor rate of retrieval of oocytes by aspiration in comparison to slicing method may be attributed to the fact that slicing released oocytes from both surface follicles and those located in the deeper cortical stroma, while aspiration was restricted to only the surface follicles.

A total of one hundred and seventy four oocytes were carried out with cysteamine supplementation at 100µM/ml during *in vitro* maturation (IVM), fertilization (IVF) and culture (IVC), one hundred twenty six oocytes without supplementation served as control. The mean cleavage rates were 24.1±1.67and 19.8±0.89 per cent in the treatment and control group, respectively. The percentage of morula (mean±SE) that developed in treatment and control group were 15.1±1.35 and 7.9±1.47, respectively. The percentage of embryo that developed to the morula stage were significantly higher ($P<0.01$) in treatment group. This improvement could be probably related to GSH synthesis stimulation by cysteamine as reported by de Matos *et al.*(1996).The antioxidant effect of glutathione in protecting the sperm from free radical damage, sperm chromatin decondensation, oocytes activation and male pronucleus formation (Rangasamy,1996). The direct addition of GSH to the culture medium yielded contradictory results regarding its effect on embryo development and also there is no evidence that intact GSH could be taken up by mammalian cells. Considering these observations, in the present study, concluded that cysteamine was added during oocytes maturation, fertilization and embryo culture improved the cleavage and morula development due to glutathione synthesis stimulated by cysteamine.

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