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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Effect of β-mercaptoethanol on *in vitro* maturation on oocyte of Murrah buffalo

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Abstract

This study was conducted to determine the effect of supplementing maturation medium with β-mercaptoethanol on *in vitro* maturation on oocytes of Murrah buffalo. Buffalo cumulus-oocyte complexes (COCs) from ovaries were matured *in vitro* in Hepes-TCM 199 supplemented with 0.2 mM sodium pyruvate, 1 µg/ml 17-β-estradiol, 10% fetal calf serum (FCS), 0.5 µg/ml bFSH and 0 (control) and 0.1 or 0.5 mM/ml of β-mercaptoethanol for 24 h. When COCs matured in TCM 199 media with 500 µM/ml β-mercaptoethanol , the rate of maturation increased significantly as compared to the control group (53.33 vs. 33.33%, respectively). Also, the percentage of degenerated oocytes in the treatment groups was lower than that in the control group ($P<0.05$).

Keywords: Buffalo; β-mercaptoethanol; Oocyte development; Oxidative stress

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Introduction

The improvement of *in vitro* embryo production (IVEP) has a critical role in maternal contribution to genetic improvement in buffalo because in this species the multiple ovulation programs have limitations (Zicarelli, 1997). In fact IVEP improvement is important for production of embryos with high quality for use in animal biotechnology and biomedical research (Feugang et al., 2009).

In mammals, oocyte and embryo *in vitro* culture is negatively affected by the increased oxidative stress. It is known that reactive oxygen species (ROS) cause damage to cell functions and mammalian cells neutralize ROS via antioxidant systems such as catalase or superoxide dismutase and thiol compounds (Del Corso et al., 1994). However buffalo oocytes have high lipid reservoir and are very sensitive to oxidative stress, due to their high lipid content (Boni et al., 1992).

Glutathione (L-γ-glutamyl-L-cysteinyl-glycine; GSH) is a tripeptide thiol compound that has many important functions such as maintaining the redox state in cells, improving formation of male pronucleus and chromatin decondensation, protein and DNA synthesis and reduction of disulfides (Lafleur et al., 1994; Kim et al., 2004). The GSH content of oocytes increases during maturation of oocyte in the ovary that is used for cells protection in later steps (Beheshti et al., 2011).

It was reported that in the presence of low molecular weight, thiol containing precursors of GSH such as cysteamine and β-mercaptoethanol in IVM medium, increases the GSH content of oocytes after maturation that has beneficial effect on development of 6–8-cells stage bovine embryos to the blastocyst stage (de Matos et al., 2002; Gasparrini et al., 2003; Beheshti et al., 2011).

It has also been shown that supplementation of thiol compounds, such as cysteamine and β-mercaptoethanol increased GSH synthesis during bovine and ovine IVM which caused embryo development and quality (de Matos et al., 2002; de Matos et al., 2000). Songsasen et al. (2002) demonstrated that the rate of oocytes exhibiting pronuclei formation in buffaloes increased when the IVM medium supplemented of with β-mercaptoethanol.

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The aim of this study was to evaluate the effect of supplementation of IVM medium with β-mercaptoethanol on IVM of buffalo oocytes.

Materials and Methods

All reagents used in this study were obtained from Sigma Company.

Oocyte collection

Buffalo ovaries were collected from local abattoir (Tabriz abattoir, East Azerbaijan, Iran), shortly after slaughter of buffalo and transported to the laboratory in 0.9% NaCl solution with 100 IU/ml potassium penicillin G, 100 µg/ml streptomycin sulfate at 35°C, within 2 to 4 hours from slaughter house. In the laboratory, ovaries were washed three times in modified Dulbecco's phosphate buffered saline (mPBS) containing 100 IU/ml penicillin and 100 µg/ml streptomycin (Abdoon et al., 2001).

Buffalo cumulus-oocyte complexes (COCs) were aspirated from small antral follicles (2-8 mm) using a 18-guage needle connected to a 10 ml sterile disposal syringe that contain 1 ml oocyte collection medium (HEPES-TCM 199 supplemented with 10% FBS, and 2 IU/ml of heparin), and the contents were recovered into a 15 ml conical tube and allowed to settle for 10 minute. Only oocytes with unexpanded cumulus oophorus and evenly granulated cytoplasm were cultured in a 30 mm plastic dish (Sterilin, Middlesex, UK; 20-30 oocytes/dish) containing 2 ml TCM 199 (Whittaker M. A. Bioproducts, Walkersville, MD, USA; pH 7-4 (Fukui and One, 1989). The cells were collected following Moor and Trounson (1977). The COCs with multi-layers of compact investment with a homogeneous granular ooplasm were selected to IVM procedure (Badr, 2009). COCs selected were washed thoroughly three times with maturation medium.

***In vitro* maturation of oocytes**

The basic IVM medium was Hepes-buffered tissue culture medium199 supplemented with 10% fetal calf serum (FCS), 0.5 µg/ml FSH, 0.2 mM sodium pyruvate, 1 µg/ml 17-ß-estradiol,. In treatment groups, β-mercaptoethanol was supplemented at three levels (0, 100, 500 µM). To accomplish IVM, 10 CoCs were cultured in 50 µl drops for 24 hr at 38 and 5% CO_2 atmosphere.

Statistical analysis

Proportional data for *in vitro* development of embryos were analyzed (by ANOVA) and comparison of means among treatments was performed using Tukey test. A significance level of P<0.05 was used.

Results

The results of addition of β-mercaptoethanol on COCs expansion are shown in Table 1. The expansion rate in treatment with 500 µM β-mercaptoethanol (41.65±17.88) was significantly higher than other groups (P<0.05). In the treatment with 100 µM β-mercaptoethanol, 73% of oocytes were matured and 12% remained immature. Between treatments, rate of oocyte maturation in the control group was lower than for the other treatments. The percentage of degenerated oocytes in the 100 µM β-mercaptoethanol group was higher than other treatments. The rate of unmatured oocytes in treatment with 500 µM β-mercaptoethanol was lower than that of the control group (Fig. 1).

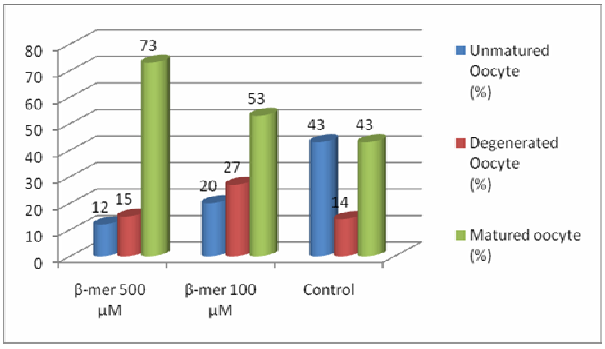

Figure 1: Effect of β-mercaptoethanol on buffalo oocyte IVM.

Table 1: Effect of β-mercaptoethanol supplementation on COCs expansion

Treatments		Oocytes used	Mean COCs expansion
Control	0 µM	14	21.86±12.5[b]
IVM + β-mercaptoethanol	100 µM	25	22.28±4.79[b]
	500 µM	14	41.65±17.88[a]

[a,b] Superscripts within columns differ (P<0.05)

Discussion

Buffaloes are important livestock specie, because of their ability to live in ecologically disadvantaged agricultural area and production of high quality milk (Gasparrini et al., 2006). In this study, supplementation of β-mercaptoethanol improved buffalo oocyte *in vitro* maturation. The higher rate of oocyte IVM was in the 500 mM β-mercaptoethanol treatment. There is evidence that the oxidative stress had negative effects on *in vitro* mammalian embryo development. Antioxidant systems such as superoxide dismutase, catalase, and thiol compounds, in mammalian cells scavenge ROS (Beheshti et al., 2011). Buffalo oocytes are capable to synthesize GSH *in vitro* (Gasparrini et al., 2003). It is shown that adequate synthesis of GSH

in maturation period is very important for later embryo development (de Matos et al., 2002). GSH is a non-protein sulphydryl compound and plays important role in protecting mammalian cells from oxidative stress and its intracellular synthesis is very important in oocyte cytoplasmic maturation (Gordon, 2003; Gasparrini et al., 2008; Ruder, 2008). Some studies reported that addition of β-mercaptoethanol in bovine and ovine IVM medium, increased GSH synthesis and improved embryo development and quality (de Matos et al., 1996, 2002).

Kobayashi et al. (2006) demonstrated that β-mercaptoethanol added during porcine IVM; improved blastocyst formation, by increasing intracellular GSH synthesis. Previous reports indicated that addition of 100 μM β-mercaptoethanol improved the blastocyst formation and their cell numbers of bovine oocytes (Kobayashi et al., 2006). Dea et al. (2011) reported that addition of low molecular weight thiol compounds e.g. cysteamine and β-mercaptoethanol led to low oxidative stress in many species.

In conclusion, the addition of β-mercaptoethanol to a TCM-199 medium for buffalo IVM culture enhanced expansion of COCs and maturation rate of oocytes.

References

Abdoon, A.S.S., Kandila, O.M., Otoib, T. and Suzukib, T. 2001. Influence of oocyte quality, culture media and gonadotropins on cleavage rate and development of *in vitro* fertilized buffalo embryos. *Animal Reproduction Science*, 65:215-223.

Badr, M.R. 2009. Effects of supplementation with amino acids on *in vitro* buffalo embryo development in defined culture media. *Global Veterinaria*, 3:407-413.

Beheshti, R., Mohammadi-Roshandeh, A., Giasi Ghalehkandi, J., Ghaemmaghami, S. and Houshangi, A.F. 2011. Effect of antioxidant supplements *on in vitro* maturation of bovine oocyte. *Advanced Environmental Biology*, 5: 1473-1475.

Boni, R., Sangella, L., Dale. B., Rovello, S., Di Palo, R. and Barbieri, V. 1992. Maturazione in vitro di oociti bufalini: indagine ultrastrutturale, *Acta Medica Veterinaria*, 38:153-161.

Dea, A.K., Malakar, D., Aksheya, Y.S., Jenaa, M.K., Garga, S., Duttaa, R. and Sahua, S. 2011. *In vitro* development of goat (*Capra hircus*) embryos following cysteamine supplementation of the *in vitro* maturation and *in vitro* culture media. *Small Ruminant Research,* 96:185-190.

de Matos, D.G., Furnus, C.C., Moses, D.F., Martinez, A.G. and Matkovic, M. 1996. Stimulation of glutathione synthesis of *in vitro* matured bovine oocytes and its effect on embryo development and freezability. *Molecular Reproduction and Development*, 45:451-457.

de Matos, D.G. and Furnus, C.C. 2000. The importance of having high glutathione level after bovine *in vitro* maturation on embryo development: effect of b-mercaptoethanol, cysteine and cystine. *Theriogenology*, 53:761-771.

de Matos, D.G., Gasparrini, B., Pasqualini, S.R. and Thompson, J.G. 2002. Effect of glutathione synthesis stimulation during *in vitro* maturation of ovine oocytes on embryo development and intracellular peroxide content. *Theriogenology*. 57: 1443-1451.

Del Corso, A., Cappiello, M. and Mura, U. 1994. Thiol-dependent oxidation of enzymes: the last chance against oxidative stress. *International Journal of Biochemistry*, 26:745-750.

Feugang, J.M., Camargo-Rodríguez, O. and Erdogan, M. 2009. Culture systems for bovine embryos. *Livestock Science*, 121:141-149.

Fukui, Y. and One, H. 1989. Effects of sera, hormones and granulosa cells added to culture medium for in-vitro maturation, fertilization, cleavage and development of bovine oocytes. *Journal of Reproduction and Fertility,* 86:501-506.

Kim, M.K., Fibrianto, H.Y., Oh, H.J., Jang, G., Kim, H.J. and Lee, K.S. 2004. Effect of ß-mercaptoethanol or epidermal growth factor supplementation on *in vitro* maturation of canine oocytes collected from dogs with different stages of the estrus cycle. *Journal of Veterinary Science,* 5:253-258.

Moor, R.M. and Trounson, A.O. 1977. Hormonal and follicular factors affecting maturation of sheep oocytes in vitro and their subsequent developmental capacity. *Journal of Reproduction and Fertility,* 49:101-109.

Lafleur, M.V.M., Hoorweg, J.J., Joenje, H., Westmijze, E.J. and Retel, J. 1994. The ambivalent role of glutathione in the protection of DNA against singlet oxygen. *Free Radical Research,* 21:9-17.

Gasparrini, B., Sayoud, H., Neglia, G., de Matos, D.G., Donnay, I. and Zicarelli, L. 2003. Glutathione synthesis during *in vitro* maturation of buffalo (*Bubalus bubalis*) oocytes: effects of cysteamine on embryo development. *Theriogenology,* 60**:**943-952.

Gasparrini, B., Boccia, L., Marchandise, J., Di Palo, R., George, F., Donnay, I. and Zicarellia, L. 2006. Enrichment of *in vitro* maturation medium for buffalo (*Bubalus bubalis*) oocytes with thiol compounds: effects of cystine on glutathione synthesis and embryo development. *Theriogenology*, 65:275-287

Gasparrini, B., Rosa, A.D., Attanasio, L., Boccia, L., Di Palo, R., Campanile G. and Zicarelli, L. 2008.

Influence of the duration of *in vitro* maturation and gamete co-incubation on the efficiency of *in vitro* embryo development in Italian Mediterranean buffalo (*Bubalus bubalis*). *Animal Reproduction Science*, 105:354-364.

Gordon, I. 2003. Laboratory production of cattle embryos: Maturing the oocyte, 2nd edition, Cambridge, MA 02138, CABI Publishing.

Kobayashi, M., Lee, E.S. and Fukui, Y. 2006. Cysteamine or β-mercaptoethanol added to a defined maturation medium improves blastocyst formation of porcine oocytes after intracytoplasmic sperm injection. *Theriogenology*, 65:1191-1199.

Ruder, E.H., Hartman, T.J., Blumberg, J. and Goldman, M.B. 2008. Oxidative stress and antioxidants: exposure and impact on female fertility**.** *Human Reproduction Update,* 14:345-357.

Songsasen, N. and Apimeteetumrong, M. 2002. Effects of β-mercaptoethanol on formation of pronuclei and developmental competence of swamp buffalo oocytes. *Animal Reproduction Science*, 71:193-202.

Zicarelli, L. 1997. Superovulatory response in buffaloes bred in Italy, Third Course on Biotechnology of Reproduction in Buffaloes, Caserta, Italy, October 6-10: 29-52.