Full Length Research Paper

Effect of cysteine supplementation on *in vitro* maturation of bovine oocyte

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This study was aimed at determining the effect of cysteine supplement during *in vitro* maturation of bovine oocytes. Cumulus-oocyte complexes (COCs) from abattoir 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 100 or 500 µM/ml of cysteine for 24 h. When COCs matured in TCM 199 media with 500 µM/ml cysteine, the rate of maturation were increased as compared to the control group (53.33 vs. 33.33%, respectively) ($P<0.05$). Also, the percentage of degenerated oocytes in the treatment groups was lower than that in the control group ($P<0.05$).

Key words: Bovine, cysteine, *in vitro* maturation (IVM), oocyte.

INTRODUCTION

The development of *in vitro* embryo production (IVEP) technology is very important for production of high quality embryos (Hansen and Block, 2004). The importance of *in vitro* embryo production (IVEP) technology has been established in accelerating the genetic improvement of herds and this process is the base of other technologies Such as somatic cell nuclear transfer technology (Feugang et al., 2009).

The *in vitro* maturation (IVM) conditions are simpler than *in vivo* maturation condition and limited materials are used for IVM process which can seriously affect the maturation status of oocyte (Vahedi et al., 2009). Addition of useful materials such as gonadotropins, estradiol, growth factors (Vahedi et al., 2009) and antioxidants (Balasubramanian and Rhob, 2007) are necessary for improvement of bovine oocytes IVM.

A major factor affecting *in vitro* mammalian embryo development is increased oxidative stress (Gasparrini et al., 2000). Glutathione (L-γ-glutamyl-L-cysteinyl-glycine; GSH) is the major non-protein sulphydryl compound in mammalian cells that plays a critical role in protecting the cell from oxidative damages, improving formation of male pronucleus and chromatin decondensation, regulation of protein and DNA synthesis and preservation of meiotic spindle via altering redox status (Bai et al., 2008; (Kim et al., 2004; Zhou et al., 2008).

At the time of ovulation, GSH content of oocytes increases and builds a pool that protects the cell in the later stages of development (Telford et al., 1990).

In the studies of de Matos et al. (1997) and Miyamura et al. (1995), GSH synthesis occurred during bovine oocyte maturation and it is one of the indices of cytoplasmic maturation (Yi et al., 2003; Zhou et al., 2008).

Addition of Thiol containing precursors of GSH such as cysteine (CySH), cysteamine, 2-mercaptorthanol or use of a cysteine-rich medium (TCM 199 or Waymouth MB 75211) increased GSH content of oocytes after maturation (Bai et al., 2008; de Matos et al., 2002). de Matos and Furnus (2000) reported that addition of thiol compounds (cysteamine) during bovine IVM caused the high intracellular GSH level and improved bovine embryo development and quality.

A thiol is an organosulfur compound that contains a carbon-bonded sulfhydryl (-C-SH or R-SH) group. Cysteine is a very unstable critical component amino acid of GSH, a thiol tripeptide synthesized by the y-glutamyl cycle. This amino acid can transfer from extracellular environment into oocyte (Bai et al., 2008). It is is rapidly

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Table 1. Maturation rate of bovine oocytes following CySH supplementation during *in vitro* maturation

Treatment	Oocyte used	Unmatured oocyte (% mean ± S.E)	Matured oocyte (% mean ± S.E)	Oocyte degenerated (% mean ± S.E)
IVM − CySH	45	23 (51.11 ± 2.22)	15 (33.33 ± 3.84)[c]	7 (15.55 ± 2.22)[a]
IVM + CySH (µM)				
100	45	23 (51.11 ± 4.44)	19 (42.22 ± 5.87)[bc]	3 (6.66 ± 3.84)[b]
500	45	19 (42.22 ± 4.44)	24 (53.33 ± 3.84)[a]	2 (4.44 ± 2.22)[b]

Superscripts within columns differ at P < 0.05. *IVM − CySH, oocytes matured in HEPES buffered TCM199 in the absence of cysteine; IVM + CySH, oocytes matured in TCM199.

oxidized to cystine in culture medium and it's deficiency in medium due to autoxidation to cystine may result in GSH synthesis failure *in vitro* (Sagara et al., 1993).

Consequently, this study investigates the effect of supplementation of medium with cysteine on IVM of bovine oocytes.

MATERIALS AND METHODS

All chemicals used in this study were obtained from Sigma–Aldrich Company.

Aspiration of oocytes from ovaries

Ovaries were obtained from local abattoir (Tabriz abattoir, East Azarbaijan, Iran) shortly after slaughter and transported to the laboratory in 0.9% NaCl solution plus 100 IU/ml potassium penicillin G, and 100 µg/ml stereptomycin sulfate at 35°C, within 2 to 4 h from slaughter (Ball et al., 1983).

Bovine COCs were aspirated from small antral follicles (2 to 8 mm) using a 18-guage needle connected to a 10 ml sterile syringe that contained 1 ml oocyte collection medium [HEPES- buffer 199 (TCM199; Sigma-Aldrich Chemie GmbH, Riedstrasse 2, 89555, Steinheim, Germany) supplemented with 10% fetal bovine serum (Leonorenstr. 2-6. D-12247 Berlin) and 2 IU/ml of heparin], and the contents recovered were placed into a 15 ml conical tube and allowed to settle for 10 min.

Quality assessment of oocytes

COCs were assessed morphologically and only those that had two or more layer of cumulus cells and homogeneous granular ooplasm were selected for IVM procedures (Badr, 2009).

In vitro maturation of oocytes

The basic medium for IVM was HEPES-buffered tissue culture medium 199 supplemented with 0.2 mM sodium pyruvate, 1 µg/ml 17-β-estradiol, 10% fetal calf serum, and 0.5 µg/ml bFSH. Assessed levels of cysteine were 0 (control), 100 and 500 µM to culture media over three replicates.

The COCs selected were washed three times thoroughly in Hepes-buffered TCM 199 medium, supplemented with 10% FCS, washed once in IVM medium, placed in 50 µl drops (10 oocytes/drop) of the same medium under sterile silicone oil (Sigma-Aldrich Chemie GmbH, Riedstrasse 2, D-89555, Steinheim, Germany) and matured for 22 to 24 h at 38.5°C in an atmosphere of 5% CO_2 in humidified air.

Statistical analysis

Oocytes from ovaries obtained at the slaughterhouse were pooled. All experiments were carried out in three replicates (one replicate per day). Differences among treatments in each experiment were made by one-way analysis of variance (ANOVA) after arcsine transformation of the *in vitro* maturation data. Comparison of means among treatments was performed using Tukey-HSD comparisons test. A probability of P < 0.05 was considered to be statistically significant.

RESULTS

Cysteine supplementation to IVM medium

The results of supplementation of cysteine are shown in Table 1. In a treatment with 100 µM CySH, 42.2% oocytes were matured and 51.1% remained unmatured. Between treatments, rate of oocyte maturation in the control group was lower than for the other treatments and the differences between control and 500 mM CySH were significant (P < 0.05). Percentage of unmatured oocytes in treatment with 500 mM CySH was lower than that of the control group (P < 0.05), but differences with 100 mM CySH treatment was not significant. The percentage of degenerated oocytes in the control group was higher than that of the CySH treatments (P < 0.05). The MII rate of oocytes IVM in 500 µM cysteine was significantly higher than that in both the control and 100 µM treatment groups.

DISCUSSION

Previous studies of mammalian oocyte IVM have been reported for more than 70 years. Pincus and Enzmann, (1935) observed that some of the oocytes from human and rabbit spontaneously resumed meoisis when released from follicles and cultured in a medium.

Studies on the environmental factors during IVP (oocyte maturation, fertilization and embryo culture)

provide the basis for defining conditions for the production of embryos *in vitro* (Yoshida et al., 1993). Reactive oxygen species (ROS) production is one of the regular processes of cellular metabolism (Gordon, 2003).

There is evidence that the ROS in *in vitro* oocyte maturation affect IVP of bovine embryos (Geshi et al., 2000). Oxidative damage to cellular elements through the ROS is one of the important processes which cause damage to appropriate cell function (Del Corso et al., 1994).

There are different mechanisms for controlling cellular ROS levels such as GSH and superoxide dismutase. GSH is a non-protein sulphydryl compound in cattle cells. It serves as a reservoir for CySH and plays an important role in protecting mammalian cells from oxidative stress and it's intracellular synthesis is very important in oocyte cytoplasmic maturation (Gasparrini et al., 2008; Gordon, 2003; Luberda, 2005). Some previous studies have indicated that aggregation of GSH during IVM has in some species such as ewe, goat and cow (Kochhar et al., 2002; Salamone et al., 2001 and Urdaneta et al., 2003).

It was demonstrated that addition of low molecular weight thiol compounds such as β-mercaptoethanol and CySH to IVM media, promotes synthesis of GSH in pig oocytes (Abeydeera et al., 1999).

Gasparrini et al. (2000) and Bai et al. (2009) reported that addition of thiol components such as cysteamine cystine, cysteine to IVM medium stimulated synthesis of GSH improved embryo production.

In a study, Sagara et al. (1993) demonstrated that the presence of CySH in the extracellular environment is necessary for GSH biosynthesis.

In this study, the effects of CySH on bovine *in vitro* oocyte maturation were evaluated and the *in vitro* system was improved by supplementation of CySH in the maturation medium. The higher rate of oocyte maturation and lower rate of degenerated oocyte in the 500 mM CySH treatment (53.33 and 0.67%, respectively) may be due the higher level of GSH in this group. It was observed that the high intracellular GSH level in bovine oocytes, obtained after addition of thiol compounds to IVM media was maintained through IVF and early development, and decreasing to control level in the six to eight-cell embryos (Gasparrini et al., 2003).

Yoshida et al. (1993) reported that the composition of pig oocytes maturation medium affects the GSH concentration and the supplementation of CySH to IVM medium is associated with increased GSH synthesis and redound levels of GSH which is comparable to those found in matured oocytes *in vivo*. The findings of Gasparrini et al. (2006) demonstrated that addition of IVM medium with either CySH or cysteamine caused increases of oocytes GSH content.

Considering these facts, the efficiency of GSH synthesis in COCs should be best when the environment is rich in CySH, because both oocytes and attached cumulus cells can uptake CySH by system alanine–serine– cysteine transport system (Nagai, 2001). It is

likely that the cysteine-induced GSH synthesis occurs during the first hours of IVM, before the amino acid was oxidized (Gasparrini et al., 2006). Consequently, thiol compounds such as CySH can be used as tools to enhance the efficiency of bovine oocytes *in vitro* maturation.

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