Advances in Environmental Biology, 5(7): 1473-1475, 2011
ISSN 1995-0756

ORIGINAL ARTICLE

Effect of Antioxidant Supplements on in Vitro Maturation of Bovine Oocyte

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Beheshti R, Mohammadi-Roshandeh A, Giasi Ghalehkandi J., Ghaemmaghami S, Houshangi A.F.: Effect of Antioxidant Supplements on in Vitro Maturation of Bovine Oocyte

ABSTRACT

This study was aimed at determining the effect of acysteamine 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-ß-estradiol, 10% fetal calf serum (FCS), 0.5 µg/ml FSH and 0 (control), 100 or 500 µmol/ml of cysteamine for 24 hours. When COCs matured in TCM 199 media with 500 µmol/ml cysteamine, The rate of maturation were increased as compared with control group ($P < 0.05$). Also, the rates of degenerated oocytes in treatment groups lower than control group that it is not significant.

Key words: bovine, cysteamine, IVM, oocyte.

Introduction

The *in vitro* maturation (IVM) conditions are simpler than *in vivo* maturation condition and limited materials are using for IVM process which can seriously affect the maturation status of oocyte [13]. Addition of usfule materials such as gonadotropins, estradiol, growth factors [13] and antioxidants [2] are necessary for improvement of bovine oocytes IVM.

In vitro maturation of sheep and bovine oocytes can be assisted by co-culture with cumulus cells or follicular shell pieces [6]. A major factor affecting *in vitro* mammalian embryo development is increased oxidative stress [9]. Glutathione (GSH) is the major non-protein sulphydryl compound in mammalian cells which plays a critical role in protecting the cell from oxidative damages [12]. In The studies of de Matos *et al.* (1996) and Miyamura *et al.* (1995) were found that GSH synthesis occures during bovine oocyte maturation [14].

Low molecular weight thiol compounds such as ß-mercaptoethanol and cysteamine, added during bovine and ovine oocytes IVM, promoting cysteine uptake in mammalian cells and enhancing intracellular glutathione (GSH) synthesis [9]. de Matos and Furnus [6] reported that addition of tiol compounds such as cysteamine during bovine IVM caused the high intracellular GSH level and improved bovine embryo development and quality. Cysteamine is a thiol that increased GSH and promoted male pronucleus formation in porcine oocyte. In TCM-199, a cysteine-rich medium, cysteamine converted cystine to cysteine [14]. Consequently, this study investigates the effect of ovarian supplementation of medium with cysteamine on IVM of bovine oocytes.

Materials and methods

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

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2.1. 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 hours from slaughter [3].

Bovine cumulus oocyte complexs (CoCs) were aspirated from small antral follicles (2-8 mm) using a 18-guage needle connected to a 10 ml strile syring that conatien 1 ml Oocyte Collection Medium (HEPES-TCM 199 (M7528) supplemented with 10% FBS (F6178), and 2 IU/ml of heparin), and the contents recovered into a 15 ml conical tube and allowed to settle for 10 minute. CoCs were washed three times with maturation medium.

2.2. Quality Assessment of Oocytes:

CoCs with two or more layer of granulosa cells and homogeneous granular ooplasm were selected to IVM procedure [1].

2.3. in Vitro Maturation of Oocytes:

The basic medium for IVM was Hepes-buffered tissue culture medium199 supplemented with 0.2 mM sodium pyruvate, 1 µg/ml 17-ß-estradiol, 10% fetal calf serum (FCS) and 0.5 µg/ml FSH. In treatment groups cystamine 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.

2.4. Statistical Analysis:

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

Results:

3.1. Cysteamine Supplementation to Ivm Medium:

The result of supplementation of cysteamine domenested at table 1.

In a treatment with 100 mM cystaemine, 33.33% oocytes were matured and 51.11% remained unmatured. Between treatments rate of oocyte maturation in control group lower than other treatments ($P < 0.05$). Rate of unmatured oocytes in treatment with 500 mM cystaemine lower than treatment with 100 mM cystaemine ($P < 0.05$), but differences with control group are not significant.

Table 1: Effect of cysteamine supplementation during *in vitro* maturation.

Treatments	Oocytes used	Unmatured Oocyte (%)	Matured oocyte (%)	Degenerated Oocyte (%)
IVM –Cys	45	23 (51.11%)[a,b]	15 (15.56%)	7 (33.33%)[b]
100 µM	45	19 (42.22%)[b]	21 (46.66%)[a]	5 (11.11%)
IVM + Cys				
500 µM	45	14 (31.11%)[a]	25 (55.56%)[a]	6 (13.33%)

Superscripts within columns differ; $P < 0.05$.

*IVM–Cys, oocytes matured in TCM199 in the absence of cysteamine; IVM + Cys, oocytes matured in TCM199.

Discussion:

Previous studies of mammalian oocytes IVM has been reported in over 70 years that Pincus and his associates observed, some of oocytes from human and rabbit spontaneously resumed meoisis when released from follicles and cultured in a medium.

Reactive oxygen species (ROS) production is one of the regular processes of cellular metabolism [11].

There is evidence that the ROS in *in vitro* oocyte maturation affect IVP of bovine embryos [10]. Oxidative damage to cellular elements through the ROS is one of the important proces which causes damage to appropriate cell function [4]. There are different mechanisms for controlling cellular ROS levels as GSH, superoxide dismutase. Glutathione (GSH) is a non-protein sulphydryl compound in cattle cells. It serves as a reservoir for cysteine and which plays a important role in protecting mammalian cells from oxidative stress [7,11]. Gasparrini *et al.* [9] reported that adition of thiol components such as cysteamine to IVM medium improved embryo production.

In a study De Matos *et al*. (1997) demonstrated that cysteamine promoting bovine and ovine embryo development which it is mediated by GSH. [9,15]. In this study, the effects of cysteamine on bovine *in vitro* oocyte maturation were evaluated and the *in vitro* system was improved by addition of cysteamine in the maturation medium. The higher rate of oocyte maturation in the 500 mM cysteamine treatment (55.56%) may be due the higher level of GSH in this group. The findings of Gasparrini *et al.* (2003) domenstaret that the cysteamine-induced stimulation during IVM may defeat the problem due the low cytoplasmic GSH concentration in the critical stage of development. Consequently thiol compounds such as cysteamine can be used as tools to enhance the efficiency of bovine oocytes *in vitro* maturation.

Acknowledgements

This article is summarized from research project No. 51955880614006, supported by Islamic Azad University, Shabestar Branch, Shabestar, East Azarbaijan, Iran.

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