ELSEVIER

Animal Reproduction Science 109 (2008) 88–99

ANIMAL REPRODUCTION SCIENCE

www.elsevier.com/locate/anireprosci

Metabolic requirements associated with GSH synthesis during *in vitro* maturation of cattle oocytes

C.C. Furnus[a,b,d,*], D.G. de Matos[c], S. Picco[a,d], P. Peral García[a], A.M. Inda[b,e], G. Mattioli[f], A.L. Errecalde[b]

[a] *Centro de Investigaciones en Genética Básica y Aplicada (CIGEBA), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata (CP 1900), Buenos Aires, Argentina*

[b] *Cátedra de Citología, Histología y Embriología "A", Facultad de Ciencias Médicas, Argentina*

[c] *Reproductive Medicines Associates, New Jersey, USA*

[d] *CONICET, Argentina*

[e] *CIC, Argentina*

[f] *Cátedra de Fisiología, Facultad de Ciencias Veterinarias, UNLP, Argentina*

Received 10 May 2007; accepted 13 December 2007

Available online 23 December 2007

Abstract

Glutathione (GSH) concentration increases in bovine oocytes during *in vitro* maturation (IVM). The constitutive amino acids involved in GSH synthesis are glycine (Gly), glutamate (Glu) and cysteine (Cys). The present study was conducted to investigate the effect of the availability of glucose, Cys, Gly and Glu on GSH synthesis during IVM. The effect of the amino acid serine (Ser) on intracellular reduced/oxidized glutathione (GSH/GSSG) content in both oocytes and cumulus cells was also studied. Cumulus–oocyte complexes (COC) of cattle obtained from ovaries collected from an abattoir were matured in synthetic oviduct fluid (SOF) medium containing 8 mg/ml bovine serum albumin-fatty acid-free (BSA-FAF), 10 μg/ml LH, 1 μg/ml porcine FSH (pFSH) and 1 μg/ml 17 beta-estradiol (17β-E2). GSH/GSSG content was measured using a double-beam spectrophotometer. The COC were cultured in SOF supplemented with 1.5 mM or 5.6 mM glucose (Exp. 1); with or without Cys + Glu + Gly (Exp. 2); with the omission of one constitutive GSH amino acid (Exp. 3); with 0.6 mM Cys or Cys + Ser (Exp. 4). The developmental capacity of oocytes matured in IVM medium supplemented with Cys and the cell number per blastocyst were determined (Exp. 5). The results reported here indicate (1) no differences in the intracellular GSH/GSSG content at any glucose concentrations. Also, cumulus cell number per COC did not differ either before or after IVM (Exp. 1). (2) Glutathione content in oocytes matured in SOF alone were significantly different from oocytes incubated with SOF supplemented with Cys + Glu + Gly (Exp. 2). (3) Addition of Cys to maturation medium, either

* Corresponding author at: CIGEBA, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Calle 60 y 118 s/n, La Plata (1900), Buenos Aires, Argentina. Tel.: +54 221 421 1799; fax: +54 221 425 7980.

E-mail address: cfurnus@fcv.unlp.edu.ar (C.C. Furnus).

0378-4320/$ – see front matter
doi:10.1016/j.anireprosci.2007.12.003

with or without Gly and Glu supplementation resulted in an increase of GSH/GSSG content. However, when Cys was omitted from the IVM medium intracellular GSH in oocytes or cumulus cells was less but not significantly altered compared to SOF alone (Exp. 3). (4) Glutathione content in both oocytes and cumulus cells was significantly reduced by incubation with 5 mM Ser (Exp.4). (5) There was a significant increase in cleavage and blastocyst rates when Cys was added to maturation medium. In contrast, the cleavage, morula and blastocyst rates were significantly different when 5 mM Ser was added to maturation media. There was also a significant difference in mean cell number per blastocyst, obtained from oocytes matured with 5 mM Ser (Exp. 5). This study provides evidence that optimal embryo development *in vitro* is partially dependent on the presence of precursor amino acids for intracellular GSH production. Moreover, the availability of Cys might be a critical factor for GSH synthesis during IVM in cattle oocytes. Greater Ser concentration in IVM medium altered "normal" intracellular GSH in both oocytes and cumulus cells with negative consequences for subsequent developmental capacity.

Keywords: Oocyte; Bovine embryo; Glutathione (GSH); *In vitro* maturation; Embryo metabolism

1. Introduction

Glutathione (GSH) synthesis during *in vitro* maturation (IVM) has an important role in embryo development. The increase in GSH concentrations during IVM of cattle oocytes (Miyamura et al., 1995) improved subsequent embryo development to blastocyst stage (de Matos et al., 1995, 1996; Furnus et al., 1998).

GSH is the major non-protein sulphydryl compound in mammalian cells and protects cells from oxidative damage (Pastore et al., 2003). Multiple actions have been described for this compound, including an effect on amino acid transport, DNA and protein synthesis and reduction of disulfides (Lafleur et al., 1994). GSH has an important role in cellular defense against hazardous agents of endogenous and exogenous origin (Meister and Anderson, 1983; Lafleur et al., 1994).

GSH is also important for sperm function, chromatin decondensation and hence for male pronuclear formation, following sperm penetration (Perreault et al., 1988; Yoshida et al., 1992; Yoshida, 1993; Grupen et al., 1995; Williams and Ford, 2005). Greater concentrations of intracellular GSH enhance *in vitro* production of pig embryos (Whitaker and Knight, 2004) and *in vitro* maturation of buffalo oocytes (Gasparrini et al., 2006). An improvement in mouse embryo development was observed when cysteamine was added to the IVM medium of oocytes from adult mice (de Matos et al., 2003).

The increase in GSH content provides oocytes with large stores of GSH available for protection during subsequent embryo development until blastocyst stage (de Matos et al., 1995, 1996; Gardiner and Reed, 1995a,b). Moreover, there are effects of green tea polyphenols (GTP) during IVM of cattle oocytes enhancing intracellular GSH concentration and subsequent embryo development (Wang et al., 2006). However, in horse oocytes, GSH increases during IVM but the relative intra-oocyte content of this thiol does not affect maturation and early development efficiency after fertilization (Luciano et al., 2006).

The constitutive amino acids involved in GSH synthesis are glycine (Gly), glutamine (Glu) and cysteine (Cys). Cys is a rate-limiting step in GSH synthesis by the γ-glutamyl cycle (Meister and Tate, 1976; Chance et al., 1979; Meister, 1983; de Matos et al., 1996) and is transported into cells via transport system alanine-serine-cysteine (ASC). The ASC neutral amino acid transporters (Kanai and Hediger, 2003) exhibit the properties of the classical Na^+-dependent amino acid transport system (Arriza et al., 1993; Shafqat et al., 1993; Kekuda et al., 1996; Utsunomiya-Tate