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

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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

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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

et al., 1996). ASC transporters have a high-affinity for alanine (Ala), serine (Ser), threonine (Thr) and Cys (Arriza et al., 1993; Shafqat et al., 1993; Kekuda et al., 1996; Utsunomiya-Tate et al., 1996).

The present study was conducted to investigate the effect of the availability of Cys, Gly and Glu on GSH synthesis in mammalian oocytes during *in vitro* maturation. The effect of the amino acid serine on intracellular GSH content in both oocytes and cumulus cells was also studied. Consequently, experiments were designed to evaluate the effect of Cys, Gly, Glu and Ser during IVM on intracellular GSH in cattle oocytes and cumulus cells, and subsequent embryo development up to blastocyst stage.

2. Materials and methods

2.1. Reagents and media

Cys, Gly, Glu, L-Glu, Ser, sodium pyruvate, bovine serum albumin-fatty acid-free (BSA-FAF), Percoll®, heparin sulfate, penicillamine, hypotaurine, BME essential amino acids, MEM-nonessential amino acid, polyvinylpyrrolidone (PVP, avg Mr 40,000), EDTA, 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), GSH, glutathione reductase, NADPH, 17β-estradiol, phosphate-buffered saline (PBS), kanamycin and antibiotics–antimicotics (AA); all reagents for media preparation were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All reagents were cell-culture tested. Porcine LH and FSH (Pluset) were from Biogenesis Bagó (Garín, Buenos Aires, Argentina).

IVM medium was bicarbonate-buffered synthetic oviduct fluid (SOF) (Tervit et al., 1972; Gardner et al., 1994; Gandhi et al., 2000) supplemented with 8 mg/ml BSA-FAF, 0.2 mM sodium pyruvate, 10 μg/ml LH, 1 μg/ml pFSH, 1 μg/ml 17 β-estradiol and 50 μg/ml kanamycin (control medium). IVM treatments were performed in SOF medium with or without Cys, Gly, Glu or Ser. *In vitro* fertilization (IVF) medium was TALP (Parrish et al., 1986) supplemented with 6 mg/ml BSA-FAF, 20 μM penicillamine, 10 μM hypotaurine and 10 μg/ml heparin sulfate. The medium for *in vitro* culture (IVC) was SOFm supplemented with 1 mM glutamine, 2% (v/v) BME-essential amino acids, 1% (v/v) MEM-nonessential amino acids and 8 mg/ml BSA-FAF (Gardner et al., 1994; Gandhi et al., 2000; Furnus et al., 2003).

2.2. Oocytes

Cattle ovaries were obtained from an abattoir and transported to the laboratory in sterile NaCl solution (9 g/l) with antibiotics at 37 °C within 3 h of slaughter. The ovaries were pooled regardless of stage of the estrus cycle of the donor. The COC were aspirated from 2 to 5 mm follicles, using an 18-G needle connected to a sterile test tube and to a vacuum line (50 mmHg). Only cumulus-intact complexes with evenly granulated cytoplasm were selected, using a low-power (20–30×) stereomicroscope, for IVM. Replicates of experiments (4–6) were performed on different days with different batches of COC.

2.3. IVM

The COC were washed twice in SOF buffered with 15 mM Hepes and twice in IVM medium (SOF) and transferred into 50 μl of IVM medium pre-equilibrated in a CO_2-incubator in groups of 10 for GSH/GSSG measurements. Incubations were conducted under mineral oil (Squibb,

USA) at 39 °C in an atmosphere of 5% CO_2 in air with saturated humidity for 24 h. Evaluation of GSH/GSSG measurements were performed in each replicate with the same batch of oocytes.

2.4. GSH/GSSG assay

After completion of IVM, the oocytes were stripped of surrounding cumulus cells by repeated pipetting with a narrow-bore glass pipette in Hepes–SOF, and washed three times in Mg^{2+}/Ca^{2+}-free PBS containing 1 mg/ml PVP. For each replicate, pools of ≥50 oocytes in 10 μl of PBS from each treatment were placed in microtubes, frozen at −20 °C and thawed at room temperature. This procedure was repeated three times. Complete oocyte disruption was achieved by repeated aspiration using a narrow-bore pipette. The cumulus cells from ≥50 COC were placed in Eppendorf tubes and washed twice by resuspension in PBS and centrifugation at 14,000 × *g* for 10 s. The pellets were resuspended in 500 μl of PBS and counted in a hemocytometer chamber. After centrifugation at 14,000 × *g* for 10 s, the pellets were resuspended in 40 μl of PBS, frozen at −20 °C and thawed at room temperature. Complete cell disruption was performed by addition of 400 μl of distilled water and repeated aspiration with a 26-G needle. The samples (either oocytes or cumulus cells) were added distilled water up to 1.2 ml and then mixed with 1.2 ml of 0.2 M phosphate buffer containing 10 mM EDTA. After rapid addition of 100 μl of 10 mM DTNB, 1 unit (in 50 μl) of glutathione reductase, and 50 μl of 4.3 mM NADPH, the increase in absorbance at 412 nm was measured, every 30 s up to 5 min, using a double-beam spectrophotometer (Beckman Mod. 35, Irvine, CA, USA). Blanks consisting of 10 μl of PBS or 10-μl aliquots of wash medium, instead of samples did not show detectable amounts of GSH/GSSG. Total GSH/GSSG content in oocytes and cumulus cells were calculated from a standard curve of GSH (Tietze, 1969; Takahashi et al., 1993; de Matos et al., 1995). Under these conditions, the assay limit is 25 pmol of GSH/GSSG.

2.5. Determination of cumulus cell number in COC

The COC, either compact or expanded, were dispersed by pipetting the cells up and down several times under stereomicroscope. The cell suspensions were transferred to Eppendorf tubes, and the number of cells in each suspension was estimated by counting in a hemocytometer chamber.

2.6. IVF

The expanded COC were washed twice in Hepes–TALP supplemented with 3 mg/ml BSA-FAF and placed into 50 μl drops of IVF medium under mineral oil. In all experiments, frozen semen from the same bull was used. Three 100-μl pellets, each containing 4×10^7 spermatozoa was thawed in a 37 °C water bath. Spermatozoa were washed in a discontinuous Percoll gradient prepared by depositing 2 ml of 90% Percoll under 2 ml of 45% Percoll in a 15-ml centrifuge tube. The semen samples were deposited on the top of the Percoll gradient and centrifuged for 20 min at 500 × *g*. The pellet was removed and re-suspended in 300 μl of Hepes–TALP solution and centrifuged at 300 × *g* for 10 min. After removal of the supernatant, spermatozoa were resuspended in IVF medium, counted in a hemocytometer chamber and further diluted. Final sperm concentration in IVF was 2×10^6 spermatozoa/ml. Incubations were conducted at 39 °C in 5% CO_2 in air with saturated humidity for 24 h.

2.7. IVC

After IVF the oocytes were washed twice in Hepes–SOFm and twice in SOFm, cultured without glucose during the first 24 h and then cultured for another 7 days with 1.5 mM glucose (Furnus et al., 1997). Embryo culture was carried out in 40 μl drops (10 resumptive zygotes/drop) of medium under mineral oil. The embryos were cultured at 39 °C in an atmosphere composed of 7% O_2, 5% CO_2, 88% N_2 with saturated humidity. The medium was changed every 48 h. At the end of incubations, the embryos were evaluated for the morphological stages of development with an inverted microscope (Nikon, Diaphot).

2.8. Blastocyst staining for total cell number

Day 8 blastocysts were fixed in 4% formaldehyde after washing three times in 1% polyvinylpyrrolidone (PVP) in PBS overnight. Embryos were placed in 1% Triton X-100 overnight and finally stained with Hoechst 33342. Embryos were then mounted on slides and covered with a cover slip. The total cell numbers of blastocysts from the groups of Experiment 5 were determined by counting the number of nuclei under an epifluorescent microscope. The total cell numbers of blastocysts were visualized by a Nikon Optiphot epifluorescent microscope with a 40× fluor objective (Tokyo, Japan) equipped with a 365-nm excitation filter, a 400-nm barrier filter, and a 400-nm emission filter.

2.9. Experimental design

2.9.1. Intracellular GSH/GSSG effects in oocytes and cumulus cells cultured in SOF medium

In Experiment 1, the effect of the addition of 1.5 mM or 5.6 mM glucose to maturation medium on GSH/GSSG concentrations, in both oocytes and cumulus cells was evaluated. These glucose concentrations were selected taken into account SOF (Tervit et al., 1972) formulation. SOF medium was used as IVM medium with the purpose of defining the trial conditions. The COC were matured during 24 h as described above. After this period, GSH/GSSG concentrations were evaluated.

2.9.2. Constitutive GSH amino acids effects during IVM on GSH/GSSG

In Experiment 2, the intracellular GSH/GSSG in COC matured in IVM medium supplemented with 0.6 mM Gly, 0.9 mM Glu (concentrations corresponding to TCM 199 formulation usually used for IVM maturation) and 0.6 mM Cys (de Matos et al., 1995) was investigated. In Experiment 3, COC were incubated in SOF with the omission of one of the constitutive amino acid for GSH synthesis. In Experiment 4, COC were incubated in SOF supplemented with constitutive amino acids and/or 5 mM Ser, and Cys with or without 5 mM Ser. GSH/GSSG content of oocytes and cumulus cells were determined 24 h after the start of incubation. Nuclear maturation was observed with Hoechst 33342.

2.9.3. Amino acids effects during IVM and subsequent embryo development

In Experiment 5, COC were cultured in SOF supplemented with constitutive amino acids (Gly + Glu + Cys) or Cys, with or without 5 mM Ser. Cleavage rates were recorded 48 h after insemination. Data reported for development to the blastocyst stage included those embryos that progressed to the expanded or hatched blastocyst stages.

Table 1
Intracellular GSH/GSSG in cattle oocytes and cumulus cells with different glucose concentrations during IVM

IVM Medium	Glucose (mM)	*n*	Oocyte GSH (pmol/oocyte)	Cumulus GSH (nmol/10^6 cells)
SOF	1.5	400	3.23 ± 0.5 a	0.41 ± 0.07 a
SOF	5.6	400	3.11 ± 0.6 a	0.40 ± 0.02 a

Values with different letters within each column differ ($p < 0.05$). Cattle COC were incubated as described in Section 2 in SOF medium supplemented with 1.5 mM or 5.6 mM glucose. All values (pmol GSH/oocyte and nmol GSH/106 cumulus cells) are expressed as mean ± S.E.M. (800 COC in four replicates).

2.10. Data analysis

Differences among treatments were analyzed by ANOVA and Student–Newman–Keuls Multiple Comparison post-test (CSS: Statistica, module C, Statsoft, Tulsa, OK), after logarithmic transformation of data for GSH/GSSG concentrations or angular transformation for embryo development data. Differences between treatments were analyzed by Student's *t*-test after angular transformation. Results are expressed as mean ± S.E.M.

3. Results

3.1. Intracellular GSH/GSSG in oocytes and cumulus cells cultured in SOF medium

Data included in Table 1 include the GSH/GSSG content of COC incubated in IVM media supplemented with different glucose concentrations (Exp. 1). No differences were found in the intracellular GSH/GSSG content at any glucose concentrations. Cumulus cell number per COC did not differ either before or after IVM at any glucose concentration (Before IVM: 13 000 ± 1100; after IVM: 14 023 ± 1180 and 14 167 ± 1207 cumulus cells/COC for SOF 1.5 mM glucose and SOF 5.6 mM glucose, respectively).

3.2. Constitutive GSH amino acid effect during IVM in oocytes and cumulus cells

In Experiment 2, 800 oocytes in six replicates were matured *in vitro* with constitutive amino acids for GSH synthesis. There was a decrease ($p < 0.05$) in GSH/GSSG when constitutive amino acids were absent in maturation medium (Table 2). GSH/GSSG content in oocytes matured in SOF alone was less compared with oocytes incubated with SOF supplemented with constitutive amino acids ($p < 0.01$). The intracellular GSH/GSSH concentrations in cumulus cells were greater

Table 2
Intracellular GSH/GSSG concentrations in cattle oocytes and cumulus cells matured in SOF medium with or without constitutive amino acids

Medium	Amino acids	*n*	Oocyte GSH (pmol/oocyte)	Cumulus GSH (nmol/10^6 cells)
SOF	+	404	6.03 ± 0.6 a	0.68 ± 0.05 a
SOF	–	396	3.14 ± 0.6 b	0.31 ± 0.03 b

Values with different letters within each column differ ($p < 0.05$). Bovine COC were incubated as described in Section 2 in SOF medium supplemented with (0.9 mM glutamic acid + 0.6 mM glycine + 0.6 mM cysteine) or without amino acids. All values (pmol GSH/GSSG oocyte and nmol GSH/GSSG/10^6 cumulus cells) are expressed as mean ± S.E.M. (800 COC in six replicates).

Table 3
Intracellular GSH/GSSG concentrations in cattle oocytes and cumulus cells matured with GSH synthesis precursors in SOF medium

IVM	*n*	Oocyte GSH (pmol/oocyte)	Cumulus GSH (nmol/10^6 cells)
SOF	200	3.2 ± 0.5 a	0.3 0 ± 0.10 a
SOF + aa	200	6.3 ± 0.6 b	0.5 9 ± 0.02 b
SOF + Gly + Glu	200	2.5 ± 0.2 a	0.2 0 ± 0.02 a
SOF + Cys + Glu	200	5.9 ± 0.6 b	0.5 3 ± 0.06 b
SOF + Cys + Gly	200	5.3 ± 0.4 b	0.5 0 ± 0.09 b

Values with different letters within each column differ ($p < 0.05$). Cattle COC were incubated as described in Materials and Methods, in SOF medium without amino acids (SOF); SOF with amino acid (0.6 mM Cys + 0.6 mM Gly + 0.9 mM Glu = SOF + aa); SOF with 0.9 mM Gly + 0.6 mM Glu (SOF + Gly + Glu); SOF with 0.6 mM Cys + 0.6 mM Glu (SOF + Cys + Glu) and SOF with 0.6 mM Cys + 0.9 mM Gly (SOF + Gys + Gly). All values for oocytes (pmol GSH/GSSG/oocyte) and cumulus cells (nmol GSH/GSSG/10^6 cumulus cells) are expressed as mean ± S.E.M. (1000 COC in 6 replicates for GSH).

in COC matured with SOF supplemented with Cys, Gly and Glu than in COC incubated without amino acids ($p < 0.01$).

In Experiment 3, COC were incubated in SOF with the omission of one of the constitutive amino acids for GSH synthesis. For this purpose, 1000 COC in six replicates were *in vitro* matured. Addition of Cys to maturation medium, either with or without Gly and Glu supplementation resulted in an increase of GSH/GSSG content (Table 3; $p < 0.01$). When Cys was omitted from the IVM medium intracellular GSH/GSSG content in oocytes or cumulus cells was less but not significantly altered compared to SOF alone.

3.3. Ser effects on intracellular GSH synthesis in oocytes and cumulus cells

Glutathione content in both oocytes and cumulus cells was reduced by incubation with 5 mM Ser (Exp.4), either in SOF with 0.6 mM Cys ($p < 0.001$) or in SOF supplemented with all constitutive amino acids for GSH synthesis ($p < 0.001$) (Table 4). No differences were found among treatments when Ser was present in maturation medium (Table 4).

In Experiment 5, 1200 oocytes in seven replicates were matured and fertilized *in vitro*. There was an increase in cleavage rate ($p < 0.05$) and blastocyst yield ($p < 0.05$) when 0.6 mM Cys was added to maturation medium (Table 5). No differences were found between SOF supplemented with constitutive amino acids and SOF with Cys in cleavage, morulae and blastocyst rates. How-

Table 4
Intracellular GSH/GSSG in cattle oocytes and cumulus cells matured in SOF medium supplemented with amino acids and/or serine

IVM	*n*	Oocyte GSH (pmol/oocyte)	Cumulus GSH (nmol/10^6 cells)
SOF aa	240	6.96 ± 0.40 a	0.61 ± 0.05 a
SOF aa + Ser	260	0.95 ± 0.17 b	0.22 ± 0.02 b
SOF + Cys	250	5.90 ± 0.09 a	0.58 ± 0.04 a
SOF + Cys + Ser	250	0.76 ± 0.02 b	0.19 ± 0.01 b

Values with different letters within each column differ ($p < 0.05$). Cattle COC were incubated as described in Section 2, in SOF medium supplemented with amino acids (0.6 mM Cys + 0.6 mM Gly + 0.9 mM Glu) (SOF aa); SOF aa + 5 mM Ser (SOF aa + Ser); SOF with 0.6 mM Cys (SOF + Cys) and SOF + 0.6 mM Cys + 5 mM Ser (SOF + Cys + Ser). All values (pmol GSH/oocyte and nmol GSH/10^6 cumulus cells) are expressed as mean ± S.E.M. (1000 COC in 4 replicates).

Table 5
Developmental capacities of cattle oocytes after different conditions of GSH synthesis during *in vitro* maturation

Oocyte treatment	Oocyte (*N*)	Cleaved (%)	Morula (%)	% Blastocyst from oocyte
SOF	270	53 ± 3.5 a	32 ± 4.3 a	18 ± 3.2 a
SOF aa	240	65 ± 3.1 b	43 ± 3.7 b	36 ± 3.1 b
SOF + Cys	250	66 ± 4.7 b	40 ± 2.8 b	32 ± 2.5 b
SOF + Cys + Ser	246	35 ± 4.6 c	20 ± 4.5 c	10 ± 2.7 c

Values with different letters within each column differ ($p < 0.05$). Developmental capacity of bovine oocytes matured *in vitro* with SOF without amino acids for GSH synthesis (SOF); SOF with amino acids (0.6 mM Cys + 0.6 mM Glu + 0.9 mM Gly) (SOF aa); SOF with 0.6 mM Cys (SOF + Cys); SOF medium supplemented with 0.6 mM Cys + 5 mM Ser (SOF + Cys + Glu). Cleavage rates were recorded 48 h after insemination. Data reported for development to the blastocyst stage included those embryos that progressed to the expanded or hatched blastocyst stages after 7 days in culture.

Table 6
Mean cell numbers of day 8 blastocysts developed from oocytes matured in three different maturation conditions

Oocyte treatment	Blastocyst (*n*)	Mean cell number ± S.E.M./blastocyst
SOF	15	100.3 ± 8.5 a
SOF aa	18	115.0 ± 5.0 b
SOF + Cys	21	118.4 ± 6.7 b
SOF + Cys + Ser	19	90.2 ± 5.5 a

Values with different letters within each column differ ($p < 0.05$). SOF alone (SOF), SOF with amino acids (0.6 mM Cys + 0.6 mM Glu + 0.9 mM Gly) (SOF aa); SOF with 0.6 mM Cys (SOF + Cys); SOF medium supplemented with 0.6 mM Cys + 5 mM Ser (SOF + Cys + Glu).

ever, the cleavage, morula and blastocyst rates were less ($p < 0.05$) (Table 5) when 5 mM Ser was added to maturation media compared with all treatments and SOF alone (control). There was a decrease ($p < 0.05$) in mean cell number per blastocyst obtained from oocytes matured with 5 mM Ser (Table 6).

In all experiments performed, the cell number per COC did not vary significantly with any treatment as well as the percentage of nuclear maturation (90–95%) evaluated by Hoechst 33342.

4. Discussion

The results of the present study indicate (1) maximal GSH/GSSG content was achieved by addition of 0.6 mM Cys to the maturation medium. In addition, GSH/GSSG concentrations were significantly decreased when Cys was omitted of the incubation medium; but the omission of Glu or Gly did not affect GSH/GSSG content in oocytes and cumulus cells. (2) The presence of Cys in IVM medium improves cleavage rate and embryo development up to blastocyst stage. (3) The supplementation of Ser at greater concentrations during *in vitro* maturation decreases intracellular GSH in both, oocytes and cumulus cells. (4) The lesser effect observed in GSH content when 5 mM Ser was added to IVM medium decreased the rate of embryo development compared with the developmental capacity of COC matured in SOF medium supplemented with Cys. (5) Embryo quality in terms of the number of cells per blastocyst was enhanced when Cys was added to IVM medium compared with blastocyst obtained from COC matured with Ser supplementation. Moreover, GSH content in oocytes and cumulus cells was not affected at any glucose concentrations.

Because of the metabolic and communication link between the cumulus and the oocyte, glucose availability and metabolism within the cumulus can have a significant impact on oocyte meiotic and developmental competence (Thompson et al., 2007). Glucose also contributes to the production of amino acids, glycosylated proteins and extracellular components (Zheng et al., 2007). In this previous study, the effect of two glucose concentrations (1.5 mM and 5.6 mM) was examined to define IVM conditions. The variables measured in this previous study, such as GSH content in oocyte and cumulus cells, and meiotic maturation (metaphase II + first polar body) were not affected at any glucose concentration.

In vitro maturation involves the removal of COC from antral follicles and culturing them in standard cell culture conditions until they reach metaphase II, but a small proportion of these mature oocytes have full developmental potential to term (Schroeder and Eppig, 1984; Gilchrist and Thompson, 2007). Funahashi and Day (1995) suggested that intracellular GSH content of porcine oocytes at the end of IVM appears to reflect the degree of cytoplasmic maturation. The constitutive amino acids involved in GSH synthesis are Gly, Glu and Cys. It could be argued that availability of Cys is the rate-limiting factor for GSH synthesis during *in vitro* maturation of cattle oocytes and that this might have consequences on subsequent embryo development. To address this question in the present study, the impact of GSH synthesis of COC matured without Gly or Glu was assessed. The absence of these amino acids during IVM did no affect the GSH content in both oocytes and cumulus cells. However, when Cys was present in IVM medium alone or with Glu and Gly, not only improved GSH content but also had a beneficial effect on cleavage, morula and blastocyst rates. In mice GSH content is highly correlated with rates of blastocyst formation and also number of cells per blastocyst (Maedomari et al., 2007). In agreement, in the present study when Ser was present during IVM, the detrimental effect on GSH in oocytes resulted in lesser percentages of embryo development and fewer cells per blastocyst compared with the other treatments.

The availability of the precursor amino acids is a regulatory factor for GSH synthesis, and it is likely that in mammalian cells amino acid supply from the outside of cells provides a control point in GSH synthesis (Bannai et al., 1989). Cys and Ser share the transport system ASC (Bannai et al., 1984; Bannai and Ishii, 1988), which is ubiquitous in mammalian cells, and especially reactive with such amino acids as Cys, Ser and Ala (Christensen, 1984). The greater Ser content of cell culture media acts competitively to limit a net influx of Cys via system ASC and, an external excess of Ser could also stimulate loss of cellular Cys via this transport system (Christensen, 1990). It may be possible that these hypotheses explain the lesser intracellular GSH in both, oocytes and cumulus cells, when Ser is present in the media at greater concentrations.

In a previous study, the addition of Cys to IVM medium exerted a stimulatory effect on GSH synthesis in denuded oocytes matured *in vitro*, suggesting that the transport system ASC is active in cattle oocytes (de Matos et al., 1997). Thus, it becomes important to know whether system ASC and other systems for Ser transport and exchange are actually present in cattle oocytes, and whether extracellular Ser depletes oocytes of amino acids in addition to Cys. Such studies are needed to fully understand the biochemical processes supporting optimum oocyte maturation.

In conclusion, the present study provides evidence that optimal embryo development *in vitro* is partially dependent on the presence of precursor amino acids for intracellular GSH synthesis. The incubation with Ser altered the intracellular GSH when this amino acid was added to IVM medium at greater concentrations, and this incubation also affected subsequent embryo development. GSH synthesis, however, was significantly decreased when Cys was absent in the incubation medium. The absence of Glu or Gly did not affect the intracellular GSH content in cattle oocytes. The production of GSH might be considered a metabolic marker to evaluate the oocyte's intrinsic

developmental competence, which refers to the biochemical and molecular state that allows a mature oocyte to be fertilized normally and develop to an embryo.

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