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T1 Cysteamine, glutathione and ionomycin treatments improve *in vitro* fertilization of prepubertal goat oocytes

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Date submitted: 7.5.04. Date accepted: 8.7.04

Summary

The aim of this study was to improve *in vitro* embryo development of prepubertal goat oocytes by studying the effect of adding cysteamine to *in vitro* maturation medium, glutathione (GSH) to *in vitro* fertilization medium and ionomycin to the sperm capacitation medium. In experiment 1, we analysed the effect of 1 mM GSH added to fertilization medium of oocytes matured with 400 µM cysteamine. The control group were oocytes without cysteamine and GSH. In experiment 2, oocytes matured and fertilized in the presence of 400 µM cysteamine and 1 mM GSH, respectively, were inseminated with spermatozoa treated with ionomycin or heparin. In experiment 1, the percentages of total and normal fertilized oocytes were significantly higher for oocytes supplemented with cysteamine and GSH (40.26% and 30.20%, respectively) than for oocytes from the control group (16.66%, and 10.61%, respectively). The percentage of total embryos obtained after 7 days of culture was significantly higher in the group supplemented with cysteamine and GSH (30.62%) than in the control group (8.09%). In experiment 2, percentages of total and normal fertilized oocytes were significantly higher for the group of spermatozoa capacitated with ionomycin (52.21% and 37.17%, respectively) than with heparin (38.62% and 28.35%, respectively). After 7 days of culture, total embryo rate was significantly higher in the group of sperm capacitated with ionomycin (44.91%) than with heparin (38.69%). However, the percentage of embryos developed to the blastocyst stage was not affected by any of the treatments studied.

Keywords: Cysteamine, Glutathione, Goat, Ionomycin, Prepubertal

Introduction

Q1 *In vitro* matured prepubertal goat oocytes often show deficiencies after their *in vitro* fertilization (IVF) as indicated by a low incidence of male pronuclear formation (Martino *et al.*, 1995; Mogas *et al.*, 1997), which would cause a high number of haploid embryos (Villamediana *et al.*, 2001) and a low developmental competence to the blastocyst stage (Izquierdo *et al.*, 1999).

During fertilization, the sperm nucleus is decondensed and transformed into a male pronucleus (MPN). This transformation of the sperm nucleus during IVF has been related to the levels of intracellular

glutathione (GSH) (Sutovsky & Schatten, 1997). The tripeptide glutathione (γ -glutamyl-cysteinyl-glycine) is a reducing agent that plays a number of important roles in cellular metabolism (reviewed by Knäpen *et al.*, 1999), participating in various mechanisms such as amino acid transport, DNA and protein synthesis, reduction of disulphide bonds and protection of cells against oxidative damage. During the development and maturation of the oocyte in the ovary, GSH content increases as the oocyte approaches the time of ovulation (Perrault *et al.*, 1988). When the oocytes are *in vitro* matured, GSH synthesis can be stimulated by the addition of low-molecular-weight thiol compounds.

Cysteamine is a low-molecular-weight thiol which when present during *in vitro* maturation (IVM) increases the intracytoplasmic oocyte GSH concentration in cattle (De Matos *et al.*, 1995; Luvoni *et al.*, 1996; De Matos & Furnus, 2000), goat (Rodríguez *et al.*, 2003), buffalo (Gasparrini *et al.*, 2003) and ewes (De Matos *et al.*, 2002). Addition of cysteamine to the IVM

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medium of cattle oocytes improves *in vitro* blastocyst development (De Matos *et al.*, 1995, 1996). Similar results were found in sheep (De Matos *et al.*, 2002), buffalo (Gasparrini *et al.*, 2000), pig (Gruppen *et al.*, 1995; Yamauchi & Nagai, 1999) and mouse (De Matos *et al.*, 2003). Previous studies in our laboratory (Rodríguez-González *et al.*, 2003) showed that 100 μM of cysteamine added to the IVM medium of prepubertal goat oocytes increased intracellular GSH levels and significantly improved the percentages MPN formation after IVF and embryo development. Later, Urdaneta *et al.* (2003) concluded that the addition of 400 μM of cysteamine to the IVM medium improved embryo development of prepubertal goat oocytes compared with 100 μM . However, the percentage of blastocysts obtained in both studies was low.

During fertilization, several researchers add GSH to the sperm preparation medium (pig: Jeong & Yang, 2001; cattle: Earl *et al.*, 1997; Taneja *et al.*, 1998) and/or IVF medium (pig: Boquest *et al.*, 1999; cattle: Van Soom *et al.*, 1998), obtaining an improvement in the blastocyst formation rate. The use of GSH during sperm preparation could be beneficial to membrane stabilization of the spermatozoa and the protection of sperm from free radical damage, and could thus help subsequent fertilization and development.

Goat oocytes have significantly improved IVF rates when spermatozoa are capacitated with ionomycin (Wang *et al.*, 2002). Ionomycin is a calcium ionophore that can increase Ca^{2+} cycling across phospholipid membranes and induce the acrosome reaction (Ball *et al.*, 1983). Wang *et al.* (2002), studying various factors affecting the effectiveness of the IVM-IVF system of goat oocytes, concluded that spermatozoa treated with heparin plus 100–200 nM of ionomycin gave significantly higher fertilization rates compared with the conventional goat sperm treatment of heparin alone.

The aim of this study was to improve *in vitro* embryo development of prepubertal goat oocytes by studying the effect of adding 400 μM cysteamine to IVM medium, 1 mM GSH to the IVF medium and 200 nM ionomycin to the sperm capacitation medium.

Materials and methods

Oocyte collection

We obtained ovaries from prepubertal (approximately 2 months old) goats from a local slaughterhouse and transported them at 37 °C in Dulbecco's phosphate-buffered saline (PBS; P-4417, Sigma Chemical, St Louis, MO, USA) supplemented with 50 $\mu\text{g}/\text{ml}$ gentamicin. Oocytes were recovered by cutting the surface of ovaries with a razor blade in a 60 mm culture dish

with TCM199 (M-2520; Sigma, USA), supplemented with 2.2 mg/ml NaHCO_3 and 50 $\mu\text{g}/\text{ml}$ gentamicin. We selected only oocytes with one or more complete layers of unexpanded cumulus cells and homogeneous cytoplasm.

In vitro maturation of oocytes

The oocyte maturation medium was TCM199 (M-7528; Sigma, USA) supplemented with 275 $\mu\text{g}/\text{ml}$ sodium pyruvate (P-3662; Sigma, USA), 146 $\mu\text{g}/\text{ml}$ L-glutamine (G-5763; Sigma, USA), 10% (v/v) steer serum, 10 $\mu\text{g}/\text{ml}$ o-LH (L-5269; Sigma, USA), 10 $\mu\text{g}/\text{ml}$ o-FSH (Ovagen, Immuno Chemicals Products, Auckland, New Zealand), 1 $\mu\text{g}/\text{ml}$ 17 β -estradiol (E-2257; Sigma, USA) and 50 $\mu\text{g}/\text{ml}$ gentamicin. A group of oocytes were supplemented with 400 μM cysteamine (M-9768; Sigma, USA). The cumulus-oocyte complexes were culture in groups of 20–25 in 100 μl microdrops of maturation medium and incubated for 27 h at 38.5 °C in a humidified atmosphere of 5% CO_2 in air under mineral oil (M-8410; Sigma, USA).

Sperm preparation and capacitation

The oocytes were inseminated with fresh semen, at the end of the maturation period. We collected ejaculates from two Murciano-Granadino bucks of proven fertility into artificial vaginas and transported them at 37–38 °C within 30 min to the laboratory. The motility of sperm cells was evaluated under an inverted microscope and the motile sperm fraction was separated by swim-up (Parrish *et al.*, 1986). Seventy microlitres of semen was placed in each of several conical tubes under 2 ml Defined Medium (Brackett & Oliphant, 1975) as modified by Younis *et al.* (1991) and referred to here as mDM, and incubated for 45–60 min in a humidified atmosphere of 5% CO_2 in air at 38.5 °C. After incubation, 600 μl from the top of each tube containing highly motile spermatozoa was removed and pooled in a sterile 15 ml centrifuge tube and centrifuged at 200 g for 10 min.

After discarding the supernatant, the resulting sperm pellet was treated in one of two ways: (1) the sperm pellet was resuspended 1:1 (v:v) with mDM medium containing heparin (heparin-sodium salt; 170 UI/mg; final concentration 50 $\mu\text{g}/\text{ml}$ of heparin) and incubated in a humidified air atmosphere of 5% CO_2 at 38.5 °C (final concentration approximately 84×10^6 sperm/ml) for 45–60 min (heparin group) or (2) the sperm pellet was resuspended 1:1 (v:v) with mDM medium containing 20 $\mu\text{g}/\text{ml}$ heparin, 1 mM 8-Br-cAMP and 400 nM ionomycin for 15 min (ionomycin group; final concentration 10 $\mu\text{g}/\text{ml}$ heparin, 0.5 mM 8-Br-cAMP and 200 nM ionomycin).

***In vitro* fertilization of oocytes**

After maturation, groups of 20 oocytes were transferred into 80 μ l fertilization microdrops of modified Tyrode's medium (TALP), as described by Parrish *et al.* (1986), supplemented with 1 μ g/ml hypotaurine (H-1384; Sigma, USA). After capacitation, sperm concentration was assessed with a haemocytometer, and an aliquot (5 μ l) of the sperm suspension added to the fertilization microdrops (final concentration 3.5×10^6 sperm/ml). Oocytes and sperm were incubated for 24 h in a humidified atmosphere of 5% CO₂ in air at 38.5 °C.

Evaluation of oocytes after IVM and IVF

To evaluate the nuclear stage after IVM, a sample of oocytes was fixed in ethanol (90%):acetic acid (3:1 v/v) after mechanically denuding the oocytes in a 3% sodium citrate solution. After 24–48 h, fixed oocytes were stained with 1% lacmoid (L-7512; Sigma, USA) in 45% acetic acid solution. The oocytes were examined by phase-contrast microscopy. We measured oocyte maturation by the percentage of oocytes reaching the metaphase II (MII) stage.

To evaluate the pronuclear stage after 17 h of IVF a sample of oocytes was processed in the same way as the oocytes fixed after IVM. Oocytes with a sperm tail in the cytoplasm were considered to be fertilized and classified into one of three groups: 2PN (female pronucleus, male pronucleus and one sperm tail; normal fertilization), polyspermy (two or more sperm tails in the cytoplasm with condensed heads or two or more decondensed heads in the cytoplasm) and asynchrony (female pronucleus and a condensed sperm head).

***In vitro* embryo culture**

At 24 h after insemination, the oocytes were washed in the culture medium with the aid of a fine pipette to separate oocytes from any sperm cells. Groups of 20–25 presumptive zygotes were cultured *in vitro* for 7 days (8 days post-insemination; day 0 = the day of insemination) in 20–25 μ l microdrops (1 μ l culture medium per embryo) under mineral oil in a humidified atmosphere of 5% CO₂, 5% O₂ and 90% N₂ at 38.5 °C. A semi-defined two-step culture system with G1.2/G2.2 media (Gardner & Lane, 1997) was used for embryo culture. Embryos were placed in G1.2 for 3 days and then moved to G2.2 for another 4 days. After 24 h in culture (that is, 48 h post-insemination), 10% (v/v) fetal bovine serum (FBS, Life Technologies) was added to the microdrops (0.1 μ l serum per embryo).

At the end of the culture period, we assessed total cell number of embryos with fluorescence microscopy after Hoechst staining and recorded the percentage of total embryos, morulae (embryos with 16 or more cells

and without a blastocoele) and blastocysts (embryos with 60 or more cells with blastocoele formation).

Statistical analysis

We calculated differences among treatment groups by means of chi-square analysis or Fisher's exact test where appropriate. We calculated overall chi-square and found it significant before performing the Fisher's exact test to detect differences between treatment groups, using Graph Pad In Stat (version 3.01 for Windows 95, Graph Pad Software, San Diego, CA, USA). Differences with a probability value of 0.05 or less were considered significant.

Experimental design

Experiment 1

We analysed the effect of adding 1 mM (0.3 mg/ml) GSH to the IVF medium of oocytes matured with 400 μ M cysteamine compared with oocytes matured with cysteamine but without GSH supplementation and a control group. The control group was a sample of oocytes matured and fertilized in the absence of both cysteamine and GSH. In this experiment spermatozoa were capacitated with our conventional protocol (heparin).

Experiment 2

Experiment 2 was designed based on the results obtained in experiment 1. We analysed the effect of sperm capacitation with ionomycin or heparin on IVF and embryo development of prepubertal goat oocytes. According to the results of experiment 1, the oocytes were matured and fertilized in the presence of 400 μ M cysteamine and 1 mM GSH, respectively.

Results

Experiment 1

Table 1 shows the nuclear stage after IVM of 220 prepubertal goat oocytes. The percentage of oocytes reaching the MII stage was significantly higher when cysteamine was added to IVM medium (82.72%) compared with oocytes matured in a conventional IVM medium (53.63%; $p < 0.0001$).

Table 2 shows the IVF parameters of 424 IVM oocytes. There were significant differences in the total fertilized oocytes and oocytes with 2 PN among treatments. The percentages of total fertilized oocytes and oocytes with 2PN were significantly higher in oocytes supplemented with cysteamine and GSH (40.26% and 30.20%, respectively) than in oocytes with cysteamine (27.97%, $p < 0.04$, and 16.08%, $p < 0.007$,

Table 1 Effect of cysteamine addition to the IVM medium on nuclear maturation of prepubertal goat oocytes (seven replicates)

Treatment	Total oocytes	GV n (%)	GVBD n (%)	MI n (%)	A-TI n (%)	MII n (%)
0 μ M cysteamine	110	9 (8.18) ^a	4 (3.63)	38 (34.54) ^a	0	59 (53.63) ^a
400 μ M cysteamine	110	0 ^b	0	19 (17.27) ^a	0	91 (82.72) ^b

GV, germinal vesicle; GVBD, germinal vesicle breakdown; MI, metaphase I; A-TI, anaphase–telophase I transition; MII, metaphase II.

^{a,b}Values in the same column with different letters differ significantly ($p < 0.05$).

Table 2 Effect of cysteamine addition to the IVM medium and GSH to the IVF medium on *in vitro* fertilization of prepubertal goat oocytes (seven replicates)

Treatment	Total oocytes	Fertilized oocytes			
		Total n(%)	2PN n (%)	Asynchronous n (%)	Polyspermic n (%)
Control group	132	22 (16.66) ^a	14 (10.61) ^a	1 (0.76)	7 (5.30)
400 μ M cysteamine GSH–	143	40 (27.97) ^c	23 (16.08) ^a	0	17 (11.89)
400 μ M cysteamine GSH+	149	60 (40.26) ^b	45 (30.20) ^b	1 (0.67)	14 (9.40)

PN, pronucleus.

Control group: oocytes matured without cysteamine and without GSH on IVF medium.

^{a,b,c}Values in the same column with different letters differ significantly ($p < 0.05$).

Table 3 Effect of cysteamine addition to the IVM medium and GSH to the IVF medium on embryo development of prepubertal goat oocytes (seven replicates)

Treatment	Inseminated oocytes	Embryo development at day 8 post-insemination				
		Total embryos n (%)	>8-cell embryos n (%) [*]	Morulae (M) n (%) [*]	Blastocysts (B) n (%) [*]	M + B n (%) [*]
Control group	321	26 (8.09) ^a	1 (3.84) ^a	0	0	0
400 μ M cysteamine GSH–	254	43 (16.92) ^c	3 (6.98) ^a	2 (4.65)	0	2 (4.65)
400 μ M cysteamine GSH+	320	98 (30.62) ^b	22 (22.45) ^b	3 (3.06)	1 (1.02)	4 (4.08)

Control group: oocytes matured without cysteamine and without GSH on IVF medium.

^{*}Percentages calculated from total embryos.

^{a,b,c}Values in the same column with different letters differ significantly ($p < 0.05$).

respectively) and oocytes in the control group (16.66%, $p < 0.0001$, and 10.61%, $p < 0.0002$, respectively).

Table 3 shows the rates of cleavage and development to the blastocyst stage of oocytes in the control group, oocytes supplemented with cysteamine and GSH, and oocytes matured with cysteamine. The percentage of total embryos obtained after 8 days of culture was significantly higher in the group supplemented with cysteamine and GSH (30.62%) than in oocytes matured with cysteamine (16.92%; $p < 0.0003$) and oocytes in the control group (8.09%, $p < 0.0001$). Also the percentage of embryos developed beyond the 8-cell stage was significantly higher in oocytes of the cysteamine + GSH group (22.45%) than in those from the other two groups (6.98%, $p < 0.002$, and 3.84%, $p < 0.0001$ for oocytes matured with cysteamine and the control group, respectively).

Experiment 2

Table 4 shows that the percentage of total fertilized oocytes was significantly higher in the group of spermatozoa capacitated with ionomycin (52.21%) than with heparin (38.62%; $p < 0.0007$). Also the percentage of oocytes with 2PN was higher in the ionomycin group (37.17%) than in the heparin group (28.35%, $p < 0.02$).

Table 5 shows the embryo development of 845 oocytes inseminated with sperm capacitated with ionomycin and 668 oocytes inseminated with heparin-capacitated-sperm. After 7 days of culture, the percentage of inseminated oocytes that developed to embryos was 38.69% and 44.91% ($p < 0.02$) for the ionomycin and heparin groups, respectively. However, the percentage of embryos developed beyond the >8-cell stage did not differ between the groups.

Table 4 Effect of sperm treatment on the *in vitro* fertilization of *in vitro* matured prepubertal goat oocytes (nine replicates)

Sperm treatment	Total oocytes	Fertilized oocytes			
		Total <i>n</i> (%)	2PN <i>n</i> (%)	Asynchronous <i>n</i> (%)	Polyspermic <i>n</i> (%)
Ionomycin	339	177 (52.21) ^a	126 (37.17) ^a	11 (3.24)	40 (11.80)
Heparin	321	124 (38.62) ^b	91 (28.35) ^b	5 (1.56)	28 (8.72)

PN, pronucleus.

^{a,b}Values in the same column with different letters differ significantly ($p < 0.05$).

Table 5 Effect of sperm treatment on the embryo development of *in vitro* matured prepubertal goat oocytes (nine replicates)

Treatment	Inseminated oocytes	Embryo development at day 8 post-insemination				
		Total embryos <i>n</i> (%)	>8-cell embryos <i>n</i> (%) [*]	Morulae (M) <i>n</i> (%) [*]	Blastocysts (B) <i>n</i> (%) [*]	M + B <i>n</i> (%) [*]
Ionomycin	845	327 (38.69) ^a	40 (12.23)	5 (1.52)	7 (2.14)	12 (3.66)
Heparin	668	300 (44.91) ^b	43 (14.33)	1 (0.33)	5 (1.66)	6 (2.0)

^{*}Percentages calculated from total embryos.

^{a,b}Values in the same column with different letters differ significantly ($p < 0.05$).

Discussion

In experiment 1, our results showed that supplementing the IVM medium with 400 μ M of cysteamine significantly improved nuclear maturation, total fertilization and total embryos obtained for prepubertal goat oocytes compared with maturation without cysteamine, but these differences disappeared when we compared the percentage of embryos with more than 8 cells. These results agree with previous results obtained in our laboratory. Thus, Rodríguez-González *et al.* (2003) observed that 100 μ M of cysteamine added to the IVM medium increased intracellular GSH levels and significantly improved the percentages of MPN and embryo development. Urdaneta *et al.* (2003) concluded when adding 100 and 400 μ M of cysteamine to the IVM medium that the higher concentration improved embryo development of prepubertal goat oocytes.

Intracytoplasmic GSH plays a central role in the protection of cells against oxidative and electrophilic stress (Knapen *et al.*, 1999). According to De Matos & Furnus (2000), the high intracytoplasmic bovine oocyte GSH levels obtained after IVM with thiol compounds remain constant after fertilization and disappear later at the 6- to 8-cell embryo stage. In our study, reduction of reactive oxygen species and the presence of a high intracellular content of GSH until the beginning of embryo development could be responsible, at least in part, for the improvement observed in maturation, total fertilization and cleavage rates of oocytes matured in the presence of 400 μ M cysteamine. Moreover, when we added GSH to the IVF medium of IVM oocytes

with cysteamine, the percentage of total fertilization, zygotes with 2 pronuclei, total embryos and embryos developed beyond 8-cell stage were significantly improved, although these treatments did not affect blastocyst rate. In contrast, some studies have indicated no effect on fertilization (Boquest *et al.*, 1999; Kim *et al.*, 1999) or cleavage (Earl *et al.*, 1997; Taneja *et al.*, 1998; Boquest *et al.*, 1999; Kim *et al.*, 1999) rates with the presence of GSH during sperm–oocyte co-incubation, but all of them observed improvements in blastocyst rates.

In cattle, Kim *et al.* (1999) have observed that the effect of GSH (1 mM) during IVF on embryo development was bull-dependent. Thus, there was an adverse effect, no effect or a beneficial effect on blastocyst formation depending on the bull used. They hypothesize that the male-dependent effect of GSH during IVF is related to differences in the amounts of reactive oxygen species produced by spermatozoa of particular males. These authors also studied the effect of different concentrations of GSH. They found that 1 mM GSH was the best dose to improve blastocyst production and higher doses (10 mM) adversely affected embryo development. The 1 mM GSH concentration also improved bovine blastocyst rates according to Earl *et al.* (1997). Nevertheless, in pigs 1 mM could be an excessive dose. Thus, Boquest *et al.* (1999), using 0.5 mM of GSH, and Jeong & Yang (2001) using 0.98 mM, observed a negative effect on blastocyst development.

Extracellularly, GSH prevents lipid peroxidation of cellular membranes by extracellular reactive oxygen species. Acting as an antioxidant, GSH may have reduced the exposure of both oocytes and spermatozoa

to oxidative stress at or just prior to fertilization, by removing excessive reactive oxygen species present in the insemination medium. Thus, the addition of GSH to IVF medium could be beneficial for membrane stabilization of sperm, oocytes and early embryos. GSH has been detected in the oviductal fluid protecting gametes and embryos (Gardiner *et al.*, 1998).

In our study, supplementation with cysteamine and GSH for IVM and IVF culture media improved fertilization and embryo development. The effect of these compounds could be to increase intracellular GSH levels and also to protect oocytes, zygotes and early embryos against reactive oxygen species. According to Armstrong (2001), oocytes from juvenile donors and the embryos derived from them appear less robust and may be less tolerant to suboptimal handling and *in vitro* culture conditions than are oocytes from adult females. Thus, developmental deficiencies of the oocytes of prepubertal females may be exacerbated under suboptimal culture conditions. However, embryo development up to blastocyst stage has not been improved by the culture media analysed in this study. Rizos *et al.* (2002) showed that the percentage of blastocysts obtained is related to the quality of oocytes, and the blastocyst quality is regulated by the culture conditions.

In experiment 2, our data showed that using fresh ejaculates, oocytes inseminated with sperm treated with ionomycin have significantly improved percentages of total and normal fertilization compared with sperm treated with heparin. Ionomycin also improved fertilization rates in the horse (Alm *et al.*, 2001) and adult goats (Wang *et al.*, 2002). As is well known, ionomycin belongs to the calcium ionophore molecule family, and they have been described as potent sperm capacitors. Moreover, it should be pointed out that in our study the group of oocytes inseminated with sperm treated with 50 µg/ml of heparin gave a greater percentage of total embryos than normal fertilized oocytes while in the group of oocytes inseminated with sperm treated with ionomycin the two rates were similar. This fact may be due to the cleavage of abnormal fertilized oocytes in the group inseminated with sperm treated with heparin.

In our study, the oocytes obtained from prepubertal goat ovaries recovered from a commercial slaughterhouse, independently of the oocyte and sperm treatment used, showed a low embryo developmental competence. Although the birth of live calves (Khatir *et al.*, 1998), lambs (O'Brien *et al.*, 1997; Dattena *et al.*, 2000) and piglets (Marchal *et al.*, 2001), has occurred following IVM, IVF and *in vitro* culture of oocytes obtained from non-stimulated prepubertal donors, it seems that these oocytes have cytoplasmic deficiencies that result in abnormal fertilization and/or their low developmental competence (reviewed by

Armstrong, 2001). In our laboratory, Villamediana *et al.* (2001) found a high number of haploid embryos after IVM and IVF oocytes from prepubertal goats, suggesting that it was due to a deficient cytoplasmic maturation of oocytes, which inhibits sperm head decondensation. In cattle, Salamone *et al.* (2001), studying cytoplasmic biochemical parameters, showed that the activities of MPF (maturation promoting factor) and MAPK (mitogen-activated protein kinase) and the relative amount of IP₃R (inositol 1,4,5-trisphosphate receptor), a mediator of calcium oscillations, were substantially lower in calf compared with adult oocytes. These findings suggest that the low developmental competence of prepubertal oocytes may be attributable to deficiencies in cytoplasmic mechanisms required for oocyte activation. Moreover, Oropeza *et al.* (2004) observed lower blastocyst development in oocytes recovered by OPU from 6- to 12-month-old calves compared with cow oocytes. These authors found differences in mRNA expression of developmentally important genes (RA for Glut-1; RA for eIF1A; and RA for IGF-1) between calf and adult 2- to 16-cell embryos. These authors concluded that the reduced developmental competence of oocytes from prepubertal calves is attributed to a deficient expression of facilitative glucose transporters and insufficient protein translation.

In conclusion, supplementation with cysteamine (400 µM) to IVM and GSH (1 mM) to IVF culture media improved normal fertilization and embryo development of prepubertal goat oocytes. Fresh goat spermatozoa treated with ionomycin increased the percentage of oocytes fertilized and zygotes with a male pronucleus. However, cysteamine, GSH and ionomycin did not improve blastocyst development.

Acknowledgement

This study was supported by MCYT (grant AGL2000-0353).

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