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Improvement in Bovine Embryo Production In Vitro by Glutathione-Containing Culture Media

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ABSTRACT Bovine oocytes were matured, fertilized, and cultured (TCM 199 with serum and co-culture) in vitro (IVMFC) with addition, during different phases of the procedure, of antioxidants: superoxide dismutase (SOD) and reduced glutathione (GSH). The addition of SOD (1,500 or 3,000 IU/ml) did not improve proportions of oocytes undergoing cleavage or the development of embryos to morula and blastocyst stages. The cleavage rates were significantly lower than in the control group (CTR 57.5%) when SOD was present during the insemination interval (IVF) or throughout the entire procedure (IVMFC). Thus when the lower concentration was present for IVF and IVMFC, 35.1% and 36.4% of inseminated oocytes cleaved ($P < 0.01$ compared to CTR) and cleavage results with the higher concentration during IVF and IVMFC were 38.5% and 29.2% ($P < 0.025$ and $P < 0.001$ compared to CTR, respectively). Significant improvements in proportions of oocytes undergoing cleavage (84.5% vs. 57.0%, $P < 0.001$) and morula/blastocyst development (33.3% vs. 13.9%, $P < 0.005$) were achieved when GSH (1 mM) was added to the culture medium. In a defined medium for culture (mSOF and BSA) the presence of SOD (3,000 IU/ml) was ineffective, but in a defined medium supplemented with GSH (1 mM) at day 6 postinsemination (i.e., when 90% of developing embryos were in 8–16 cell stages), development to the morula and blastocyst stages was supported for 35.5% of cultured oocytes ($P < 0.005$ compared to 19.2% for CTR). These data suggest that bovine embryos are sensitive to oxidative stress and that medium supplementation with the radical scavenger glutathione can improve embryo development in vitro.

Key Words: Bovine embryos, Superoxide dismutase, Glutathione, Development, In vitro

INTRODUCTION

In vitro culture of mammalian embryos has been widely investigated in efforts to overcome the developmental retardation occurring in vitro and to approximate efficiency provided in vivo. One of the major differences between the in vivo and the in vitro environment for the embryo is the oxygen concentration (Bishop, 1956; Mastroianni and Jones, 1965; Maas et al., 1976). Lowering the oxygen concentration in vitro may enhance embryonic development (mouse: Quinn and Harlow, 1978; Pabon et al., 1989; sheep and cow: Tervit et al., 1972; Thompson et al., 1990; goat: Batt et al., 1991). Reactive oxygen species, derived from oxygen metabolism, are apparently involved in the "two-cell block" of mouse embryos (Nasr-Esfahani et al., 1990; Nasr-Esfahani and Johnson, 1991, 1992). Generation of free radicals is an inevitable consequence of oxidative reactions; cellular protective processes have evolved to include "antioxidants" or "free radical scavengers." The latter prevent cellular damage caused by free radical reactions with essential biochemical components by interfering with inactivation of enzymes and alterations of membranes and DNA (Yu, 1994).

Several studies with mouse embryos (Noda et al., 1989, 1991; Nonogaky et al., 1991; Umaoka et al., 1991, 1992; Chun et al., 1994) indicate that the two-cell block occurring in vitro could be alleviated by protection against oxidative stress mediated via a potent scavenger of oxygen radicals, superoxide dismutase (SOD). Moreover, Legge and Sellens (1991) reported that when reduced glutathione (GSH) was added to a serum-free medium for the culture of mouse embryos, development to the morula or blastocyst stage was promoted, but neither SOD nor catalase had any effect on the embryonic development. In contrast, Payne et al. (1992) observed different effects on development in vitro for one-cell mouse embryos of different strains after adding SOD or catalase to the culture medium, and concluded that the developmental block may not be overcome simply by addition of the antioxidant enzymes. Lauria et al. (1994) observed that addition of SOD from oocyte collection to day 8 of culture under 5% CO_2 in air improved the cleavage rate of in vitro–inseminated bovine oocytes without further development.

These conflicting data and the need for studies regarding the effect of antioxidants on in vitro production of bovine embryos prompted our investigation. Putative beneficial effects of SOD and GSH for in vitro production of bovine embryos were assessed. Each oxygen toxicity protecting agent was included in incubation under lowered (5%) oxygen concentration during in vitro maturation (IVM), fertilization (IVF), and culture

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(IVC), and during the entire procedure (IVMFC). To further delineate the period of greatest influence, presumptive zygotes or embryos were cultured in SOD- or GSH-supplemented defined medium.

MATERIALS AND METHODS

Each experiment was performed at 39°C in media under paraffin oil and a humidified atmosphere of 5% CO_2, 5% O_2, and 90% N_2. Superoxide dismutase (CuZn SOD from bovine erythrocytes: S-5395) and reduced glutathione (G-6013) were purchased from Sigma Chemical Co. (St. Louis, MO).

In Vitro Maturation

Immature bovine cumulus–oocyte complexes (COCs) were aspirated from small (2–5 mm) antral follicles within 15 min of slaughter, using an 18 gauge needle attached to a 10 ml syringe. Aspirated follicular fluids were pooled in a 50 ml tissue culture flask and maintained between 30°C and 33°C for 2 hr during transit. Subsequent oocyte handling was performed at 39°C. The pooled follicular fluid was poured into 15 ml conical tubes, and COCs were allowed to gravitate during a 10–15 min interval. Oocytes were selected as previously described (Younis et al., 1989; Kastrop et al., 1990), and then randomly assigned to individual treatment drops (100 μl, 15–20 COCs/drop) and incubated for 24–25 hr.

Undefined conditions (experiments I and II). The IVM medium consisted of TCM 199 with Earle's salts (No. M-5017, Sigma Chemical Co.) supplemented with sodium bicarbonate (2.6 mg/ml), sodium pyruvate (50 μg/ml), gentamicin sulfate (50 μg/ml), o-LH (50 μg/ml; NIDDK-oLH-26), and proestrous (day 20) cow serum (20% v:v).

Defined conditions (experiments III and IV). The IVM medium consisted of modified TCM 199 (No. M-3769, Sigma Chemical Co.) supplemented as above but with 100 μg o-LH/ml and without serum.

In Vitro Fertilization

Frozen–thawed bull semen was prepared for IVF in a similar manner to the method of Parrish et al. (1986) as modified in our laboratory (Younis et al., 1989). Briefly, in each of six test tubes, 0.1 ml of thawed semen was layered under 1.5 ml HEPES-TALP, and sperm cells were allowed to swim up for 1 hr at 39°C. The top 1 ml from each tube was then removed and pooled in a 15 ml conical tube, and centrifuged (350 × *g*, 10 min). After the supernatant was discarded, the sperm pellet was resuspended with 5.0 ml HEPES-TALP and recentrifuged as before. The supernatant was again discarded, and the sperm pellet (100 μl) was added to 100 μl heparin-containing (heparin, sodium salt, Sigma H-7005, 200 μg/ml) HEPES-TALP in a microcentrifuge tube, for a 15 min incubation at a final concentration of 100 μg heparin/ml. Matured oocytes were washed once in IVF-TALP (Younis and Brackett, 1992) under paraffin oil. Oocytes were transferred to 50 μl drops of IVF-TALP (10–15 COCs/drop) and coincubated with approximately 10^5 motile sperm cells per drop (yielding final concentrations of 10^6 sperm and approximately 6 μg heparin per ml) for 24 hr.

In Vitro Culture

The cumulus cells still surrounding the inseminated oocytes were mechanically removed by gentle aspiration with a glass pipette and oocytes were transferred to each of several 100 μl culture drops under oil. In all IVC trials, using undefined and defined conditions, 10 embryos were cultured in each drop. Medium (50 μl) was removed from each culture drop and replaced with 50 μl of fresh medium daily.

Undefined conditions (experiments I and II). Drops for IVC contained a cumulus cell monolayer in HEPES-buffered TCM 199 (No. M2520, Sigma Chemical Co.) supplemented with sodium bicarbonate (2.2 mg/ml), sodium pyruvate (50 μg/ml), gentamicin sulfate (50 μg/ml), and proestrus (day 20) cow serum (10% v:v).

Cumulus cell cultures were initiated at time of oocyte recovery, and the medium (50 μl) was replaced with fresh medium before introducing the oocytes (i.e., presumptive zygotes).

Defined conditions (experiments III and IV). The IVC medium consisted of synthetic oviduct fluid (SOF, Tervit et al., 1972) modified by the addition of MEM nonessential amino acids (10 μl/ml; Sigma Chemical Co.), HEPES (25 mM), sodium citrate (0.5 mM), and BSA (8 mg/ml) (Brackett and Keskintepe, 1994). The culture was performed without a somatic cell monolayer.

Design and Analysis

Four separate experiments, each using a completely randomized design, were performed to evaluate the effect of the addition of SOD or GSH on different phases of the in vitro procedure for obtaining bovine embryos from immature oocytes.

In experiment I the effects of the addition of 1,500 or 3,000 IU SOD/ml, and in experiment II the effects of the addition of 1 mM GSH to the IVM, IVF, IVC or IVMFC media in undefined conditions, were compared to the control (CTR) group. Effects were reflected by data on cleavage (taken as evidence for fertilization) recorded at 46–48 hr after oocyte insemination and by data on embryonic development to morula and blastocyst stages recorded at 8 days after insemination.

The effects of the additions of 3,000 IU SOD/ml (experiment III) and 1 mM GSH (experiment IV) to defined culture medium at different time intervals after insemination (IVC day 2–10; IVC day 2–5; IVC day 6–10) were compared to the control group. Effects were reflected by data on embryos developing to morula and blastocyst stages recorded at 8 days after insemination and by data on embryos reaching the expanded blastocyst stage by 10 days after insemination.

Statistical differences between proportions reflecting various treatments were ascertained by χ^2 test. A P

TABLE 1. Effects of Added Superoxide Dismutase (SOD) for IVM, IVF, IVC, and IVMFC on the Development of In Vitro–Inseminated Bovine Oocytes (Experiment I)*

Treatment SOD (IU/ml)	Interval	No. of oocytes/embryos			
		Inseminated	Cleaved (%)	Morulae and blastocysts (%)	% Cleaved reaching morulae and blastocysts
0 (Control)	IVMFC	73	42 (57.5)^{a,A,C}	19 (26.0)^{d,e,E,F}	45.2^{h,H}
1,500	IVM	74	40 (54.1)^{a}	27 (36.5)^{e}	67.5^{i}
	IVF	74	26 (35.1)^{b}	12 (16.2)^{d,f}	46.1^{h}
	IVC	65	34 (52.3)^{a,c}	8 (12.3)^{f}	23.5^{j}
	IVMFC	77	28 (36.4)^{d,f}	13 (16.9)^{d,f}	46.4^{h}
3,000	IVM	67	35 (52.2)^{A,B}	17 (25.4)^{E,F}	48.6^{H}
	IVF	65	25 (38.5)^{B,D}	10 (15.4)^{E,F}	40.0^{H}
	IVC	73	51 (69.9)^{C}	24 (32.9)^{F}	47.0^{H}
	IVMFC	72	21 (29.2)^{D}	7 (9.7)^{G}	33.3^{H}

*Pooled data from 3 replicates.
[a]Lower case superscripts used for comparison within lower, and upper case superscripts used for comparison within higher SOD concentration and with untreated control. Different superscripts within treatment concentrations and with *control values* denote significant differences; also, underlined entries within columns are significantly different ($P < 0.05$).

TABLE 2. Effect of Adding GSH (1 mM) for IVM, IVF, IVC, and IVMFC on the Development of In Vitro–Inseminated Bovine Oocytes (Experiment II)*

Treatment GSH (1 mM)	No. of oocytes/embryos			
	Inseminated	Cleaved (%)	Morulae and blastocysts (%)	% Cleaved reaching morulae and blastocysts
Control	79	45 (57.0)^{a}	11 (13.9)^{c}	24.4^{e,f}
IVM	77	51 (66.2)^{a}	14 (18.2)^{c}	27.4^{e,f}
IVF	81	49 (60.5)^{a}	9 (11.1)^{c}	18.4^{e}
IVC	84	71 (84.5)^{b}	28 (33.3)^{d}	39.4^{f}
IVMFC	68	36 (52.9)^{a}	11 (16.2)^{c}	30.5^{e,f}

*Pooled data from 3 replicates
[a]Different superscripts within columns denote significant differences ($P < 0.05$).

value of less than 0.05 was considered statistically significant.

RESULTS

Experiment I: Effect of Superoxide Dismutase (SOD) on IVM, IVF, IVC, and IVMFC in Undefined Conditions

Results of experiment I revealed no significant increases in the proportions of oocytes that cleaved when SOD (1,500 or 3,000 IU/ml) was added to media for IVM or IVC (Table 1). However, inclusion of 1,500 IU SOD/ml during IVM apparently improved quality of oocytes since 67.5% of resulting 2–4-cell embryos reached morulae and blastocysts documenting significant improvement ($P < 0.05$) in developmental potential after this treatment. Cleavage rates were significantly lower than in the control group (57.5%) when SOD (either 1,500 or 3,000 IU/ml) was present during the insemination interval (IVF) or throughout (IVMFC). Thus, when the lower concentrations was present for IVF and IVMFC, 35.1% and 36.4% of inseminated oocytes cleaved ($P < 0.01$ compared to CTR). Cleavage results with the higher concentration during IVF and IVMFC were 38.5% and 29.2% ($P < 0.025$ and $P < 0.001$ when compared to CTR, respectively). The development of embryos to morula and blastocyst stages was not improved by the addition of 1,500 or 3,000 IU SOD/ml to media for IVF, IVC, or IVMFC. A beneficial effect was seen when both cleavage ($P < 0.05$) and advanced development ($P < 0.005$; morula and blastocyst stages) resulting from the higher SOD concentration during IVC were compared with those from the lower concentration (underlined in Table 1); however, the highest values for IVC, i.e., those resulting from addition of 3,000 IU SOD, were not significantly improved over untreated control cleavage and uterine stage embryonic development.

Experiment II: Effect of GSH on IVM, IVF, IVC, and IVMFC in Undefined Conditions

As shown in Table 2, when compared to control values significantly higher proportions of oocytes cleaved (84.5% vs. 57.0%, $P < 0.001$) and reached the morula and blastocyst stages (33.3% vs. 13.9%, $P < 0.005$) when GSH (1 mM) was included in culture medium.

Experiment III: Effect of SOD on IVC (d2–10, d2–5, d6–10) in Defined Conditions

The development of inseminated oocytes to the morula and blastocyst, and to expanded blastocyst stages was not improved when SOD was added to the defined conditions of culture throughout or for different time intervals (Table 3). Although the highest values did not significantly exceed those of the untreated con-

TABLE 3. Effect of the Addition of 3,000 IU/ml of SOD for In Vitro Culture on the Development of In Vitro–Inseminated Bovine Oocytes (Experiment III)*

Treatment SOD (IU/ml)	Interval	No. of oocytes/embryos		
		Inseminated	Morulae and blastocysts (%)	Expanded blastocysts (%)
0 (Control)	IVMFC	103	18 (17.5)[a]	6 (5.8)[c,d]
3,000	IVC (d2–10)	130	19 (14.6)[a,b]	9 (6.9)[c,d]
	IVC (d2–5)	126	11 (8.7)[b]	3 (2.4)[c]
	IVC (d6–10)	113	19 (16.8)[a,b]	9 (8.0)[d]

*Pooled data from 3 replicates.
[a]Different superscripts within columns denote significant differences ($P < 0.05$).

TABLE 4. Effect of Adding GSH (1 mM) During Culture on the Development of In Vitro–Inseminated Bovine Oocytes (Experiment IV)*

Treatment GSH (1 mM)	No. of oocytes/embryos		
	Inseminated	Morulae and blastocysts (%)	Expanded blastocysts (%)
Control	125	24 (19.2)[a]	6 (4.8)[c]
IVC (d2–10)	130	17 (13.1)[a]	0 (0.0)[d]
IVC (d2–5)	124	21 (16.9)[a]	0 (0.0)[d]
IVC (d6–10)	110	39 (35.5)[b]	8 (7.3)[c]

*Pooled data from three replicates.
[a]Different superscripts within columns denote significant differences ($P < 0.05$).

trol, the presence of SOD during the last half of the IVC interval (i.e., d6–10) enabled significantly ($P < 0.025$) better advanced embryonic development than when SOD was present during the first part of IVC (Table 3).

Experiment IV: Effect of GSH on IVC (d2–10, d2–5, d6–10) in Defined Conditions

Data shown in Table 4 delineates the interval during which GSH exerted a beneficial effect. By day 6 postinsemination 90% of the fertilized (cleaved) oocytes (in conditions for Table 4) reached 8–16-cell stage development. A significant improvement in the proportions of inseminated oocytes that reached morula and blastocyst stages was observed after adding GSH for days 6–10 of IVC in defined conditions (35.5% vs. 19.2%, $P < 0.005$). The presence of GSH from day 2 to 5 of culture (i.e., d2–10 and d2–5) significantly depressed to zero the development to the expanded blastocyst stage.

DISCUSSION

Active oxygen-derived intermediates (superoxide radicals $O_2^{-\cdot}$, hydroxyl radicals $\cdot OH$, and hydrogen peroxide H_2O_2) react with proteins, lipids, and DNA, resulting in inactivation of enzymes, membrane lipid peroxidation, and DNA alterations. Cells have evolved their own protective system against the products of oxygen metabolism. Eucaryotic cells contain Cu/Zn SOD in the cytosolic compartment and GSH in high intracellular concentrations in a variety of tissues (Yu, 1994).

A potent scavenger of superoxide radicals, SOD catalyzes their dismutation as follows: $O_2^{-\cdot} + O_2^{-\cdot} + 2H^+ \rightarrow H_2O_2 + O_2$ (McCord and Fridovich, 1969). The presence of Cu/Zn SOD has been detected in the nucleus and cytoplasm of the epithelial cells of human fallopian tube (Narimoto et al., 1991), in rabbit oviductal fluid (Noda et al., 1991), and in follicular, oviductal, and uterine fluids in the mouse (Chun et al., 1994), suggesting a protective role of this enzyme in combating oxidative stress of oocytes and developing embryos.

Reduced GSH, a tripeptide, plays an important role as a cellular antioxidant interacting with superoxide and hydroxyl radicals. As the substrate in the reduction of H_2O_2 catalyzed by glutathione peroxidase, GSH detoxifies intracellular peroxides. It is synthesized intracellularly and transported across cell membranes (Meister, 1983). An extracellular role of GSH in the prevention of lipid peroxidation of cellular membranes by extracellular active oxygen species has also been reported (Thomas et al., 1988; Avissar et al., 1989).

Although a beneficial effect of low oxygen concentration on embryonic development in vitro has been widely demonstrated (Quinn and Harlow, 1978; Pabon et al., 1989; Tervit et al., 1972; Thompson et al., 1990; Batt et al., 1991), oocytes and embryos are inevitably more exposed to oxygen and other insults in vitro than in vivo because of the necessary manipulations with transient exposure to atmospheric oxygen and visible light. Illumination is known to enhance the generation of oxygen radicals (Halliwell and Gutteridge, 1989), and effects of visible light in the production of oxygen radicals in the culture of hamster and mouse embryos have been reported (Goto et al., 1993; Nakayama et al., 1994).

Effect of SOD and GSH During IVM

In spite of the above rationale, addition of exogenous Cu/Zn SOD or GSH to the maturation medium did not increase proportions of oocytes that cleaved and developed further in vitro (Tables 1 and 2). However, the percentage of cleaved oocytes reaching morula and blastocyst stages was higher ($P < 0.05$), suggesting improved quality of oocytes matured in vitro with 1,500 IU SOD per ml (Table 1). Developmental competence of oocytes is strictly dependent on their (nuclear, cytoplasmic, and membrane) maturation. This SOD treatment was apparently effective in protecting oocytes from superoxide radicals during maturation.

The ineffective addition of GSH might be explained by the fact that the cumulus cells surrounding the oocytes are GSH rich, as already shown in the hamster (Zuelke and Perreault, 1994). This should sufficiently protect oocytes from free radical damage, as previously reported in the mouse (Legge, 1989). In the latter work, hydrogen peroxide production was detected in oocytes and with increased levels in zygotes, but not in the cumulus oophorus. Moreover, experiments I and II were carried out in commercially obtained TCM 199 already containing a very small amount of GSH (0.05 mg/l) and its precursor cysteine (0.11 mg/ml), to which serum with many unknown components was added. The presence of GSH, even in the control group, could be sufficient to protect oocytes from oxidative stress and the cysteine could support a good level of GSH synthesis during in vitro maturation to decrease chances of finding expected differences between untreated and treated groups.

Effect of SOD and GSH During the Insemination Interval (IVF)

Reactive oxygen species are deleterious to sperm cells, which are particularly sensitive to lipid peroxidation (Lardy and Phillips, 1941; McLeod, 1943; Holland and Storey, 1981). Vandermark et al. (1949) postulated that part of the deleterious effect of high oxygen tension on bull sperm motility is caused by increased H_2O_2 production, and that is prevented by the addition of catalase, in which bovine sperm is particularly deficient (Mann, 1964).

Additions of SOD and/or GSH for protection of spermatozoa of different species have been reported (SOD: human: Alvarez et al., 1987; Kobayashi et al., 1991; rabbit: Holland et al., 1982; bull: Mennella and Jones, 1980; Magnes and Li, 1980; GSH: mouse: Alvarez and Storey, 1984; dog, ram, and goat: Li, 1975). A physiological role of reactive oxygen species in mediating sperm–zona interaction by the induction of lipid peroxidation has been suggested (Aitken et al., 1989), and positive effects of superoxide radicals in the hyperactivation and capacitation in vitro of human (de Lamirande and Gagnon, 1993) and hamster (Bize et al., 1991) sperm cells have been noted. Diminished generation of superoxide during the interaction of human gametes has been demonstrated and attributed to the release of SOD during this phase (Miesel et al., 1993). Our results (Table 1) showed a significant depressing effect of SOD during the insemination interval in contrast with findings of no effects on mouse fertilization reported by Nonogaky et al. (1992). An explanation for the negative effect of SOD in our experiment could be compromise of a positive role of active oxygen species during fertilization. An alternative explanation might include toxicity of hydrogen peroxide, rather than superoxide radical, as reported for human sperm movement and for the capacity of human sperm cells to acrosome react and fuse with the egg membrane (Aitken et al., 1993). Thus, Aitken et al. (1993) concluded that the presence of SOD might be deleterious since the product of dismutation (H_2O_2) is more harmful than $O_2^{-\cdot}$. Toxicity affecting the oocyte, or sperm quality as reflected by decreased embryonic development represent possibilities since SOD treatment of sperm prior to insemination did not decrease fertilization rates (unpublished data).

Effect of SOD and GSH During IVC

Protection of embryos from oxidative stress may be a prerequisite for development in vitro because of extreme sensitivity to superoxide anions. Addition of SOD to the culture medium has facilitated development of mouse embryos through the two-cell block and culture of rabbit embryos under either high (Noda et al., 1991; Chun et al., 1994; Li et al., 1993) or low oxygen conditions (Umaoka et al., 1991, 1992) without somatic cell monolayer co-culture support. Nonogaky et al. (1991) demonstrated that SOD is most effective when applied 7–30 hr after insemination in the mouse, approximately the time of the developmental block. The positive effect of SOD (Cu/Zn SOD from bovine erythrocytes or recombinant human) has been obtained with concentrations around 1,500 IU/ml or higher. In our system of bovine in vitro–derived embryos cultured under low oxygen tension and with coculture of cumulus cells or in defined medium (Tables 1, 3), the addition of SOD (1,500–3,000 IU/ml) did not improve the development to morula and blastocyst stages. This might be due to the undefined conditions including the monolayer that lowers the oxygen tension in the immediate vicinity of the embryos as proposed by Bavister (1988). In the defined conditions the antioxidant role of BSA suggested by Natsuyama et al. (1993) might have the same effect. Furthermore, hydrogen peroxide is highly involved in blocking development. The period of in vitro development at which this block occurs in the mouse embryo (the mid-two-cell stage to the early four-cell stage) is associated with a rise in reactive oxygen species such as H_2O_2 (Nasr-Esfahani et al., 1990; Noda et al., 1991). The ineffective action of SOD and the significantly improved development in the culture medium for bovine embryos supplemented with GSH (Table 2) are in agreement with findings for mouse embryos cultured under high oxygen concentration in serum-free medium (Legge and Sellens, 1991; Gardiner

and Reed, 1994). GSH showed a beneficial effect on development, and the greatest influence was with addition on day 6 postinsemination in the present work. One of the effects of GSH, as previously mentioned, is the reduction of H_2O_2 acting as a substrate of GSH peroxidase; the importance of H_2O_2 in the block of development was described above. In vitro development is usually retarded; thus, day 6 postinsemination, corresponding to the time that embryos progress from 8–16 cells to the morula stage, is a phase in which they could be more sensitive to the oxidative stress. Our finding of a protective role for extracellular GSH in embryo culture is compatible with the findings of Takahashi et al. (1993), who added low-molecular-weight thiol compounds to improve bovine blastocyst development in the presence of serum, and the work of Gardiner and Reed (1994), who reported lowered intracellular GSH content with mouse embryo development up to the blastocyst stage.

Proportions of oocytes developing to morula and blastocyst stages were not increased by the presence of SOD in maturation and culture medium, and SOD was deleterious when present during the insemination interval. Interestingly, cleaved oocytes after IVM with an appropriate concentration of SOD demonstrated greater viability in vitro. The addition of GSH for culture significantly improved the development of bovine embryos. The efficacy of exogenous GSH in enhancing development emphasizes a possible extracellular role since intracellular concentrations of GSH are generally adequate and GSH is not readily transported into cells (Meister, 1983). Several factors are involved in the retardation of in vitro development, and one most likely is oxidative stress. Supplementation of culture media for bovine embryos with antioxidants seems worthy of consideration.

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