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Precursors of taurine in female genital tract: Effects on developmental capacity of bovine embryo produced *in vitro*

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Summary. Two precursors of taurine have been studied: cysteamine and hypotaurine. Cysteamine has been quantified in genital secretions and found in follicular fluids of all species tested. On the contrary cysteamine was not detected (or traces) in tubal fluids of the same species. Addition of 50, 100 or 250 μM of cysteamine to the maturation medium used in the culturing of bovine oocytes did not improve the cleavage rate nor the embryo's developmental potential *in vitro*. Furthermore, at 250 μM, cysteamine seems to be toxic to the embryo. Addition of 0.5–1 mM hypotaurine to the bovine embryo culture medium improved significantly blastocyst production and quality. The respective roles of these 2 taurine precursors on maturation and embryo development are discussed.

Keywords: Amino acids – Cysteamine – Cystamine – Hypotaurine –Taurine – Bovine *in vitro* embryo production – Follicular fluid – Oviduct fluid

Introduction

Despite the efforts made to improve the efficiency of culture media for *in vitro* maturation (IVM), *in vitro* fertilization (IVF) and *in vitro* embryo culture (IVEC), delayed development is observed and a low percentage of embryos reach the blastocyst stage and thus are suitable for transfer. *In vitro* matured mammalian oocytes probably have cytoplasm deficiencies as indicated by their low frequency of male pronuclear formation and low embryonic development rate. For effective development of *in vitro* matured *in vitro* fertilized bovine embryos it is necessary to overcome the "8 cell block" developmental arrest. Reactive oxygen species induce lipid peroxidation and are implicated in this embryo cell block observed *in vitro* (Nasr-Esfahani et al., 1990; Noda et

al., 1991; Goto et al., 1993). Co-culture systems with somatic cells permit to overcome the "8 cell block" of bovine embryo (Heyman et al., 1987; Goto et al., 1988; Eyestone et al., 1989). However, the development of more simple culture methods is necessary. For this purpose, several culture media supplemented with various antioxidant compounds have been tested (Vermeiden and Bast, 1995; Johnson and Nasr-Esfahani, 1994), in particular low-molecular-weight thiol compounds (glutathione, cysteine, cysteamine, N-acetylcysteine, β-mercaptoethanol etc.) and thiol derivatives (taurine, hypotaurine).

Glutathione (GSH) improves bovine embryo production *in vitro* (Luvoni et al., 1996). Positive effects of cysteine during oocyte *in vitro* maturation has been demonstrated in pig (Yoshida et al., 1993; Sawai et al., 1997). GSH concentration in oocyte is related to its ability to decondense the sperm nucleus (Yoshida et al., 1993; Funahashi et al., 1994). GSH may reduce S-S bridges in sperm chromatin and could be involved in male pronuclear swelling.

Cysteamine (mercaptoethylamine) a β-aminothiol is a precursor of hypotaurine and taurine (Fig. 1). It is converted to hypotaurine via enzymatic oxidation (Cavallini et al., 1966). Visible light induces the production of oxygen radicals by embryo (Nakayama et al., 1994), and light (ultraviolet) can induce oxidation of hypotaurine to taurine (Ricci et al., 1978). Cysteamine provides protection against ionizing radiation both *in vitro* and *in vivo* (Bird, 1980; Zheng et al., 1988; Rao et al., 1990). GSH and cysteamine could also be involved in the formation of male pronucleus growth factor (MPGF, Thibault, 1973). Cysteamine enhances the decondensation of spermatozoa in hamster oocytes matured *in vitro* (Kito and Bavister, 1997). Neutralization of reactive oxygen species by cysteamine could produce hypotaurine and taurine which themselves have antioxidant activity. Consequently cysteamine appears to be a good candidate to be added to the culture media for *in vitro* embryo production. Cysteamine has beneficial effects on cell viability in culture: it improves the rate of bovine (De Matos et al., 1995) and porcine (Grupen et al., 1995) embryos development when added to the *in vitro* maturation medium. The positive effect of cysteamine can be direct by its antioxidant (and anti-radiation) action and indirect by its ability to increase the endogenous pool of glutathione (Issel et al., 1988). This indirect effect of cysteamine has been demonstrated in bovine embryo (Takahashi et al., 1993; De Matos et al., 1995). The results obtained during IVM/IVF/IVEC are influenced by numerous factors gathered under the term "conditions of culture". Then, the effects of compounds added to the culture media can be modulated by these conditions. De Matos used a cell-free culture system (De Matos et al., 1995). On the contrary we used co-culture conditions for IVM/IVEC. For these reasons it seems important to confirm the beneficial effects of cysteamine in our *in vitro* culture system.

Hypotaurine and taurine are major constituents of the free amino acid pool in oviduct fluid (Guérin et al., 1995a). The ratio hypotaurine/taurine is elevated in tubal fluid of several species compared with serum (Guérin et al., 1995b) suggesting that the concentration of hypotaurine is particularly regu-

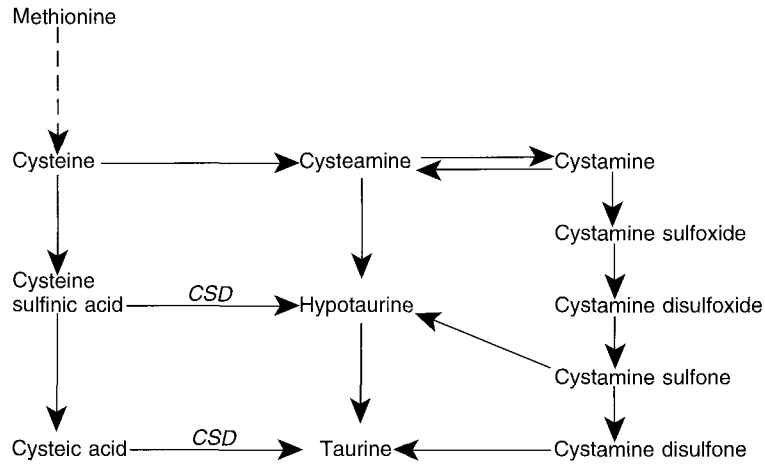

Fig. 1. Biosynthetic pathways of cysteamine, hypotaurine and taurine. *CSD* Cysteine sulfinate decarboxylase

lated in the oviduct. Hypotaurine has specific antioxidant activity (Fellman et al., 1987). Actions of taurine include the neutralization of cytotoxic aldehydes produced during lipid peroxidation reactions, calcium modulation, phospholipid interactions and membrane protein receptor interactions (Boatman, 1997). Furthermore taurine acts as an osmoregulator for the mammalian embryo (Van Winkle et al., 1994) and is a membrane protector (Huxtable, 1992). Hypotaurine and taurine are considered as house-keeping compounds. Supplementation of the culture medium with taurine or hypotaurine improves the development of hamster (Petters and Reed, 1991) and pig (Reed et al., 1992) embryos *in vitro*. Taurine has a beneficial effect on preimplantation mouse embryo *in vitro* (Dumoulin et al., 1992). The positive effect of hypotaurine on bovine *in vitro* fertilization (Susko-Parrish et al., 1990) and hamster embryo development (Barnett and Bavister, 1992) have been reported.

In our previous work we demonstrated the presence of significant amounts of hypotaurine and taurine in female genital secretions of mammals (Guérin et al., 1995a). We also demonstrated the synthesis of hypotaurine via the cysteine sulfinic acid pathway in oviduct epithelial cells (Guérin et al., 1995b).

Hypotaurine has not yet been tested on bovine embryo development, and cysteamine has never been quantified in gamete and embryo environment in mammals. For these reasons we conducted experiments in order to:

(1) quantify cysteamine in oviduct and follicular fluids of mammals,

(2) assess the effect of cysteamine addition to the maturation medium of bovine oocytes, on bovine embryo development *in vitro*,

and (3) assess the effect of cysteamine and hypotaurine addition to the embryo culture medium on *in vitro* production of bovine embryos.

Material and methods

I. Cysteamine in genital secretions

1. Collection of oviduct fluids

Surgical procedures have been detailed previously (Guérin et al., 1995c).

*Minipigs Yucatan Sows (4 years aged), and New Zealand rabbits (adults) were anesthetized. After a mid-ventral laparotomy oviducts were tied at the two ends by irresorbable thread. Three days later the females were anesthetized and tubal fluid collected by puncture of the dilated oviduct with a sterile needle (David et al., 1969).

*For cow and ewe we used continuous volumetric collection after catheterization (Clewe and Mastroianni, 1960; Stanke et al., 1973). Charolaises ewes (adults) and Montbéliardes and Françaises frisonnes cows (adults) were anesthetized. The abdominal ostium of the oviduct was exposed at flank laparotomy and catheterized with a polyethylene tube (0.9 mm ID; 1.3 mm OD and 1 mm ID; 2 mm OD for ewe and cow respectively). The widened end of the catheter was fixed with a ligature passed around the infundibulum. The cannulae was fitted with a 2 or 5 ml collecting flask. Oviduct fluid was collected by puncture of the container 48 hours later.

Animals received, after surgery, an intramuscular injection of estradiol benzoate (60 mg, 45 mg, 10 mg and 60 mg for cow, sow, rabbit and ewes respectively).

*Bitches (1 Labrador and 1 Golden Retriever, 4 and 5 years old respectively) were anesthetized and ovariectomized. The oviducts were dissected and flushed with 1 ml of sterile phosphate buffer saline.

Veinous blood was collected in every female and serum was stored at −25°C until use.

2. Collection of follicular fluids

Follicular fluids were collected either at the slaughter house (sows) or in vivo by surgical (goats and rabbits) or non-surgical (cows) puncture. Follicle size of cows, goats and sows were measured with a caliper and follicular fluid was aspirated with a 25 gauge needle.

*Cows: (Montbéliardes and Françaises frisonnes, cyclic adults). Estrus were synchronized by cloprostenol (Estrumate, Coopers). Follicular growth was daily visualised by ovarian trans-rectal ultrasonographic examination using a real time, B-mode scanner equipped with a 5 MHz linear-array transducer (Aloka SSD500). Only cows exhibiting standing estrus were used in the experiment. Standing animals were anesthetized. Follicles ranging from 12 to 20 mm diameter were transvaginally aspirated with a 21 gauge sterile needle connected with a polyethylene tube.

*Goats: (Alpines, adults). Estrus were induced by fluorogestone acetate vaginal sponges (Chrono-gest, Intervet). Forty eight hours before the sponges were removed the animals received an intramuscular injection of 400 IU of eCG (Folligon, Intervet) and 100 μg cloprostenol. Twenty four hours after sponge removal the females were anesthetized. Ovaries were exposed at flank laparotomy and follicular fluid of follicles over 5 mm diameter was aspirated with a sterile 25 gauge sterile needle.

*Sows: (misceleanous races, adults). Follicles of 6 to 10 mm diameter were punctured at slaughterhouse within 15 min of death.

*Rabbits (New Zealand, adults) received an intramuscular injection of 400 IU of eCG (Folligon, Intervet) and were anesthetized three days later. Ovaries were exposed at mid-ventral laparotomy and follicular fluid of follicles over 1 mm diameter was aspirated with a microcapillary tube.

Samples containing traces of blood were discarded. The others were transported to the laboratory within one hour at 0°C, centrifuged (1,200 × g at +4°C) and stored at −25°C until analysis.

3. Dosage of cysteamine

*Elimination of proteins from the samples was performed by filtration on millipore filters (ref. UFC3 LGC 00), and not by acid precipitation. This procedure was used in order to avoid the possible spontaneous oxidation of cysteamine to cystamine at acidic pH.

*Amino acids were separated by ion exchange chromatography (Amino acid Analyzer Beckman 6300). Glucosaminic acid (10 nM) was used as an internal standard. Amino acids were detected by ninhydrin coloration and absorbance at 440 and 570 nm.
*In order to confirm identification of the peaks, cysteamine and cystamine (100 nM) were incubated in M_{16} medium with H_2O_2 (100 μM) and dithiothreitol (1 mM), respectively. Incubations were performed at room temperature for 30 min.

II. In vitro production of embryos

1. Collection and *in vitro* maturation of oocytes

Bovine ovaries were collected from a local slaughterhouse and transported to the laboratory in a phosphate-buffered saline solution at 30–35°C within 3 h of collection. Cumulus-oocyte complexes were aspirated from antral follicles 1–6 mm in diameter using an air pumping system. Immature oocytes surrounded by compact cumulus cells were selected, washed and matured on a confluent layer of preovulatory granulosa cells in bicarbonate buffered tissue culture medium 199 with Earle's salts (Life Technologies) supplemented with 20% (v/v) fetal bovine serum (FBS, Hyclone), 10 μg/mL FSH/LH (Stimufol, Mérial), 1 μg/mL 17β-estradiol (E2257, Sigma) and 0.5 ng/mL epidermal growth factor (E6135, Sigma).

Cumulus-oocyte complexes were cultured at 39°C in an humidified atmosphere of 5% CO_2 in air for 24 h. At the end of the culture, oocytes showing expanded cumulus were morphologically judged as matured and were selected for *in vitro* fertilization.

2. Preparation of spermatozoa and *in vitro* fertilization

Frozen semen from a single bull was thawed at 37°C and layered under 1 mL of modified Tyrode's calcium free solution supplemented with 10 mM Hepes. After a 45 min swim-up period, spermatozoa were pelleted by centrifugation (200 × g, 10 min), counted and added to each fertilization tube at a final concentration of 10^6 cells/mL.

The fertilization medium was modified Tyrode's solution supplemented with 10 μg/mL heparin (sodium salt) (H 3125, Sigma), 20 μM penicillamine (P 4875, Sigma), 10 μM hypotaurine (H 1384, Sigma) and 1 μM epinephrine (E 4250, Sigma). The expanded cumulus-oocyte complexes (groups of 20–25) were washed and incubated into tubes containing 500 μL of fertilization medium for 18 h at 39°C in an humidified atmosphere of 5% CO_2 in air.

3. *In vitro* culture of embryos

After removal of the cumulus cells by vortexing, zygotes were washed once in fertilization medium and twice in culture medium. Groups of up to 20–25 zygotes were cultured on a monolayer of Vero cells in 50 μL droplets of B_2 medium (Ménézo, 1976) supplemented with 10% (v/v) FBS. The Vero cell culture was prepared according to a protocol already described (Ménézo et al., 1990). Co-culture droplets were placed under mineral oil. Embryo culture was carried out at 39°C in an humidified atmosphere of 5% CO_2 in air for 7 days (day 0 = day of insemination). On day 2 embryos were examined for cleavage to 2–8 cells. On day 7, embryo development to the morula and blastocyst stages, and embryo quality were scored according to Hasler (Hasler et al., 1995). Grade 1 blastocysts were characterized by a compact inner cell mass and a continuous trophoblastic layer.

4. Cysteamine addition to maturation medium

The aim of this experiment was to determine whether or not cysteamine, added to the maturation medium, increases the maturation of bovine oocytes and/or the development competence of *in vitro* produced bovine embryos. A total of 715 (175 to 195 per group) cumulus oocyte complexes (COCs) were *in vitro* matured, fertilized and cultured (for 7 days). COCs were pooled and randomly allocated to one of 4 groups: maturation medium (control), maturation medium + cysteamine (M 9768, Sigma) at the following concentrations: 50, 100 or 250 μM. These concentrations (50–100 μM) correspond to the one found in bovine follicular fluid (see results).

5. Cysteamine addition to embryo culture medium

The aim of this experiment was to determine whether or not cysteamine increases the cleavage rate and/or the development competence of *in vitro* produced bovine embryos. After *in vitro* fertilization, zygotes were pooled and randomly allocated to one of 2 treatments: culture medium, culture medium + cysteamine at a concentration of 100 μM. A total of 104 *in vitro* matured oocytes were inseminated and cultured (56 and 48 per group).

6. Hypotaurine addition to embryo culture medium

The aim of this experiment was to determine whether or not hypotaurine increases the cleavage rate and/or the development competence of *in vitro* produced bovine embryos. After *in vitro* fertilization, zygotes were pooled and randomly allocated to one of 8 groups: culture medium (control), culture medium + hypotaurine (H 1384, Sigma) at the following concentrations: 0.2, 0.5, 1, 2, 10, 25 and 50 mM. The concentration of 0.2 mM corresponds to the one found in bovine oviduct fluid (Guérin et al., 1995a). A total of 3,271 *in vitro* matured oocytes were inseminated and cultured (227 to 519 per group).

7. Statistical analysis

Differences between groups were assessed by Chi square test.

Results

1. Analytical process

Ion exchange chromatography allowed the separation and the quantification of cysteamine and cystamine. The retention times were 111.5 and 122.8 min respectively. The coloration ratio at 570 and 440 nm for cysteamine and cystamine was higher compared with that of the classical amino acids (0.79 for cysteamine). A chromatogram corresponding to bovine follicular fluid is shown in Fig. 2.

Detailed quantification results are presented in Table 1. Cysteamine is present in follicular fluids of all species tested. The concentration range for various species was 45–80 μM. This compound was not detected (or traces) in oviduct fluids of the corresponding species.

2. Cysteamine addition to maturation medium

Detailed results are presented in Table 2.

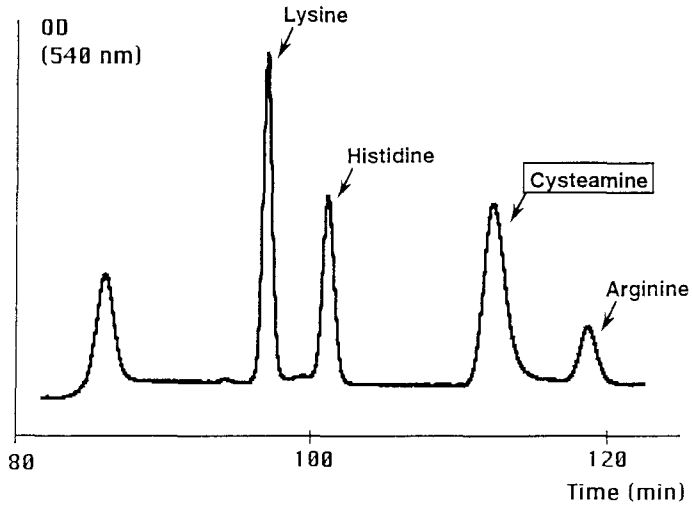

Fig. 2. Cation exchange chromatogram corresponding to bovine follicular fluid. The terminal portion of the chromatogram is shown

Table 1. Mean cysteamine concentration in rabbit, cow, sow, ewe, goat and dog genital secretions (nmol/mL; mean ± SEM)

	Rabbit (n = 8)	Cow (n = 10)	Sow (n = 7)	Ewe (n = 4)	Goat (n = 6)	Dog[1] (n = 2)
Follicular fluid	79.0 ± 11.2	53.7 ± 4.0	45.4 ± 10.4	Not determined	58.5 ± 10.2	Presence
Oviduct fluid	ND[2]	Traces	ND	Traces	Not determined	ND

[1] Flushing, [2] Not detected.

The mean maturation rate in treatment groups was 87.1% (88.7% in control). The mean cleavage rate in treatment groups was 77.0% (78.7% in control). No significant differences in maturation and cleavage rates were detected among groups ($p > 0.05$). After culture, the mean development rate (morula/blastocyst) was 41.1% for oocytes matured in the presence of cysteamine versus 43.3% for those matured in the control medium. A negative effect of cysteamine on the development rates was observed ($p < 0.05$). Value obtained with 250μM was 31% only.

Moreover a negative effect of cysteamine on the grade 1 blastocysts rates was observed ($p < 0.01$) for oocytes matured in the presence of 250μM cysteamine.

3. Cysteamine addition to embryo culture medium

Detailed results are shown in Table 3.

The mean cleavage rate in treatment groups was 88.0% (94.6% in control). No significant difference was observed between treatment groups ($p > 0.05$).

Table 2. Effect of addition of cysteamine to the *maturation medium* on oocyte maturation, embryo cleavage, morula/blastocyst formation and blastocyst quality. A significantly negative effect of cysteamine is observed for 2 parameters: developed embryos ($p < 0.05$) and grade 1 blastocysts ($p < 0.01$)

	No. of oocytes and embryos (%)			
	Control	+Cysteamine		
		50μM	100μM	250μM
Total cultured oocytes	150	195	195	175
Matured oocytes	133 (88.7)	170 (87.2)	172 (88.2)	148 (84.6)
Cleaved embryos	118 (78.7)	152 (77.9)	149 (76.4)	132 (75.4)
Developed embryos (≥morula)	65 (43.3)	88 (45.1)	86 (44.1)	55 (31.4)
Grade 1 blastocysts	28 (18.7)	37 (19.0)	35 (17.9)	14 (8.0)

Table 3. Effect of addition of cysteamine to the *embryo culture medium* on embryo cleavage, morula/blastocyst formation and blastocyst quality. A significantly negative effect of cysteamine is observed for 2 parameters: developed embryos ($p < 0.001$) and grade 1 blastocysts ($p < 0.001$)

	No of oocytes/embryos (%)	
	Control	+Cysteamine 100μM
Total inseminated oocytes	56	48
Cleaved embryos	53 (94.6)	42 (87.5)
Developed embryos (≥morula)	28 (50)	5 (10.4)
Grade 1 blastocysts	14 (25.0)	0 (0)

A negative effect of cysteamine on the development rate was observed: 50.0% and 10.4% of the inseminated oocytes reached the morula/blastocyst stage in control medium and in cysteamine supplemented medium, respectively ($p < 0.001$). Moreover, all developed embryos degenerated rapidly after 7 days of culture in medium supplemented with 100μM of cysteamine whereas 25% of the produced blastocyst were judged grade 1 in the control medium.

A negative effect of cysteamine on the grade 1 blastocyst rate was observed ($p < 0.001$).

4. Hypotaurine addition to embryo culture medium

Detailed results are shown in Table 4.

The mean cleavage rate was 81.7% for embryos cultured in the presence of hypotaurine versus 79.7% for those cultured in the control medium. A positive effect of hypotaurine on the cleavage rate was observed ($p < 0.001$).

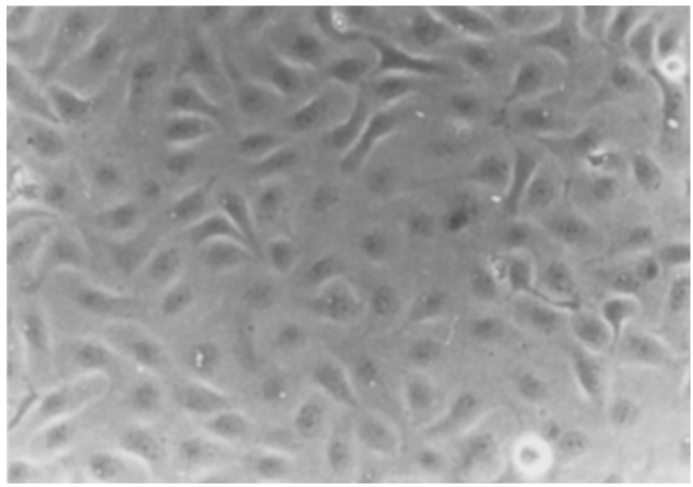

Fig. 3. Bovine granulosa cell monolayer observed with a contrast phase microscope (×875)

The mean blastocyst development and grade 1 blastocyst rates were respectively 27.2% and 17.4% in treatment groups (versus 23.8% and 15.6% respectively in control medium). We observed a positive effect of hypotaurine on blastocyst and grade 1 blastocyst percentages which was significant at 0.5 mM, 1 mM and 2 mM ($p < 0.001$).

5. Complementary results

We obtained granulosa cell monolayers (Fig. 3). Cysteamine has been detected in human and bovine granulosa cell extracts. Nevertheless it was not found in conditioned media.

Incubation of cystamine (100 nM) with dithiotreitol in M_{16} culture medium (30 min at room temperature) led to the formation of cysteamine.

The incubation of cysteamine with H_2O_2 (in M_{16} culture medium, 30 min at room temperature) led to the formation of hypotaurine and taurine.

Discussion

1. Cysteamine in genital tract

Cysteamine is present in follicular fluid, and has been detected in serum of the same animals at equivalent concentrations (results not shown). It has been detected in cow granulosa cell extracts but not in conditioned media of granulosa cells monolayers. The absence of detectable levels of cysteamine in this conditioned medium and its presence in serum suggest that cysteamine in follicular fluid could originate either from serum and/or from the synthesis by theca. Cysteamine present in granulosa cells could be transported into the

Table 4. Effect of addition of hypotaurine to the *embryo culture medium* on embryo cleavage, blastocyst formation and blastocyst quality. For each of the parameters tested, a significant positive effect of hypotaurine addition is observed: cleaved embryos ($p < 0.001$); blastocysts ($p < 0.001$); grade 1 blastocysts ($p < 0.001$)

	No of oocytes/embryos (%)							
	Control	+Hypotaurine						
		0.2 mM	0.5 mM	1 mM	2 mM	10 mM	25 mM	50 mM
Inseminated oocytes	454	505	354	336	366	510	519	227
Cleaved embryos (48 h)	362 (79.7)	399 (79.0)	301 (85.0)	304 (90.5)	302 (82.5)	404 (79.2)	427 (82.3)	173 (76.2)
Blastocysts (day 7)	108 (23.8)	129.(25.5)	123 (34.7)	125 (37.2)	106 (29.0)	125 (24.5)	122 (23.5)	53 (23.3)
Grade 1 blastocysts	71 (15.6)	86 (17.0)	76 (21.5)	75 (22.3)	78 (21.3)	88 (17.3)	76 (14.6)	19 (8.4)

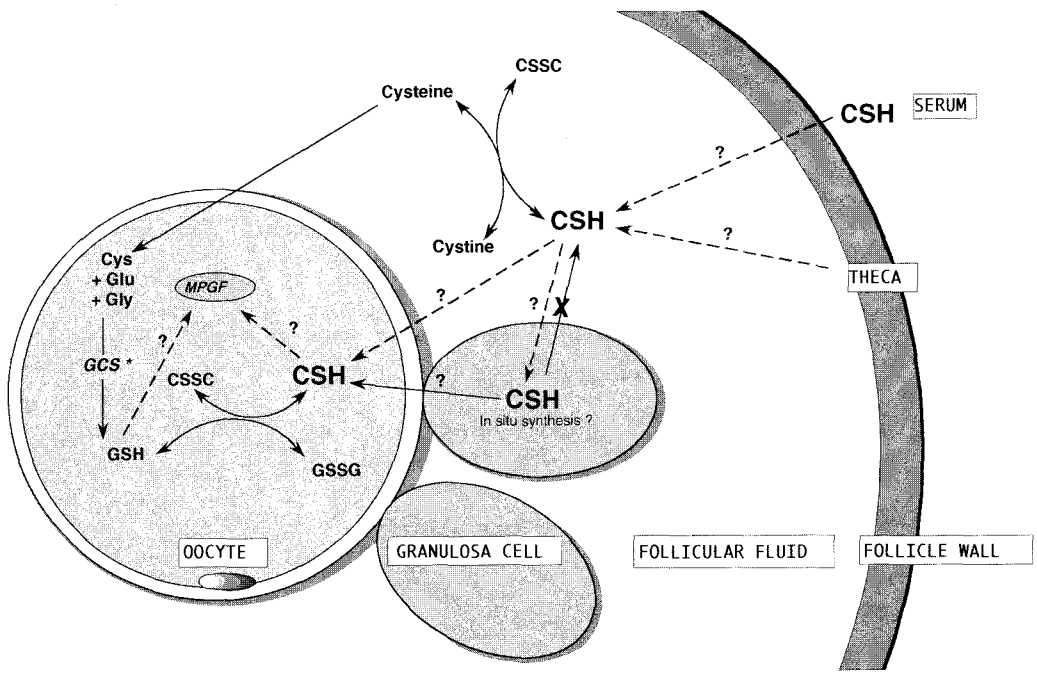

Fig. 4. Hypothetical model of cysteamine metabolism in follicle and oocyte, schematic representation. *CSH* cysteamine; *CSSC* cystamine; *GCS* Glutamylcysteine synthetase; *MPGF* Male pronucleus growth factor; *Harvey (1995) Biol. Reprod. 53: 532

oocyte. A possible model of cysteamine metabolism in follicular fluid and oocyte is presented in Fig. 4.

Using pulse labelling with ^{35}S-methionine, we were never able to detect any cysteamine synthesis by bovine granulosa and oviduct epithelial cells (results not shown). The same technique permitted us to detect hypotaurine and taurine synthesis under identical conditions (Guérin et al., 1995a). This observation and the absence (or traces) of cysteamine in oviduct fluid of all species tested suggest that the synthesis of hypotaurine/taurine by oviduct epithelial cells occurs only via the cysteinesulfinic acid pathway (Guérin et al., 1995b) and not by the cysteamine pathway (Fig. 1).

2. *Cysteamine effect on oocyte maturation and embryo development*

The presence of significant, but quite moderate, amounts of cysteamine in follicular fluid of all species tested, suggests that this compound could have a role on oocyte maturation and/or embryo development. De Matos observed a beneficial effect of cysteamine when added to the maturation medium on the subsequent development of bovine embryos to the blastocyst stage (De Matos et al., 1995). The same observation was made by Grupen on *in vitro* development of pig embryos (Grupen et al., 1995). Nevertheless, our results indicate that the addition of cysteamine either to maturation or to culture medium did not induce an improvement of bovine embryo development in our *in vitro*

culture system. On the contrary, after addition of cysteamine to the embryo culture medium, a negative effect on blastocyst development rates is observed: no cleaved embryo reaches the blastocyst stage in medium supplemented with cysteamine. Furthermore, the developed embryos are dark colored and contain numerous swollen cells. Thus in the *in vitro* culture system used in our study, cysteamine seems to be toxic for bovine embryos.

The positive effects of cysteamine observed previously on oocyte maturation/embryo production presumably occurs because of an increase in the GSH pool of oocyte/embryo. GSH is synthesized by the γ-glutamyl cycle in embryo (Harvey et al., 1995). This synthesis is dependent on the availability of cysteine in the medium (Meister, 1983). However cysteine is rapidly oxidized in culture media to form cystine (Mohindru et al., 1985) and/or cysteine sulfinic acid (personal observation). Lymphocytes (and probably embryo) cannot use cystine for GSH synthesis (Ishii et al., 1981). When compared with our protocol, De Matos and Grupen used the same basic medium (TCM 199) and similar cysteamine concentrations (range from 100 to 500μM). But in our study, oocytes and embryos were co-cultured with granulosa cells and Vero cells respectively. TCM 199 contains 0.6μM cysteine and 83.2μM cystine. Cysteine present in TCM 199 and in fetal calf serum may be oxidized completely to cystine. Consequently GSH synthesis could be inhibited and embryo development impaired. Low-molecular-weight thiol compounds such as cysteamine can reduce cystine to cysteine and consequently facilitate glutathione synthesis (Fig. 4) and embryo development. In co-culture conditions, cysteamine could be transported into granulosa cells, and consequently it may be no more disposable for this effect.

Furthermore cysteamine is a cationic thiol that condenses near DNA at pH 7, leading to high concentrations of cations near DNA, which allow them to scavenge hydroxyl radicals and repair DNA radicals (Zheng et al., 1988; Smoluk et al., 1988). Then, even at very low concentrations, this compound is able to protect DNA against ionizing radiations and possibly reactive oxygen species. This could explain the beneficial effects observed at 50–100μM by De Matos. The concentrations of cysteamine in the maturation medium at the end of the incubation have not been determined in our work nor in De Matos one's. Comparison of these concentrations would be of great interest.

It is obvious that the conditions of oocyte maturation and embryo culture influence the effects of cysteamine supplementation. In our work the percentage of developed morula/blastocyst in the control group is 5 fold higher as compared with those of Takahashi and 2 fold higher as compared with those of De Matos. Even the number of nuclei per blastocyst observed by De Matos are much lower than the one we have previously described: 154 $\pm$ 25 cells at day 7 (Guyader-Joly et al., 1996) versus 101–105 cells at day 8 (De Matos et al., 1995).

Embryos are cultured in a complex medium (B_2) in our study. The presence of the feeder cells during *in vitro* maturation and the use of a complex culture medium in the co-culture system during *in vitro* embryo culture could partly explain the divergence between our results and others. Cysteamine may have beneficial effects when oocytes and embryos are cultured in suboptimal

conditions. The feeder cells may modulate the effect of maturation conditions. Furthermore, our results demonstrate that cysteamine can be chemically oxidized to hypotaurine. In maturation medium without granulosa cells, partial oxidation of cysteamine may occur. On the one hand, the hypothetical conversion of cysteamine to hypotaurine could permit the neutralization of its deleterious effects in TCM 199 without co-culture. On the other hand, the production of hypotaurine by this way could explain its positive effect on embryo development observed by De Matos and by Grupen.

Our results show that cysteamine can be toxic to oocyte and embryo. The mechanism of this toxicity is not understood. It is possible that this thiol compound reduces the S-S bridges of active peptides and/or proteins present in the maturation and culture medium (FSH/LH, BSA). Denaturation of these proteins and/or peptides could severely affect further embryonic development.

Further studies are necessary to explain these observations. The metabolism of cysteamine in culture media and embryo *in vitro* are under investigation.

3. Hypotaurine assay

The positive effects of hypotaurine on embryo development can be explained by its antioxidant properties. Hypotaurine is able to neutralize hydroxyl radicals (Fellman and Roth, 1985). Furthermore, the neutralization of this oxygen radical by hypotaurine leads to the formation of taurine (Fellman et al., 1987) which has itself others beneficial effects (Aruoma et al., 1988).

Our data suggest that the addition of hypotaurine to culture media can improve embryo production *in vitro*. The beneficial effects of hypotaurine in culture media on embryo development and quality are obvious. The benefit is maximum for 0.5 to 1 mM hypotaurine. This concentration corresponds to that found in mammalian oviduct fluid (Guérin et al., 1995a). Consequently, hypotaurine should be systematically added in culture media used for *in vitro* fertilization and embryo culture.

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