

Amino acid requirements for maturation of rhesus monkey (*Macacca mulatta*) oocytes in culture

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This study evaluated the effects of different amino acid formulations on supporting meiotic and cytoplasmic maturation of rhesus monkey (*Macacca mulatta*) oocytes *in vitro*. Five hundred and forty-six cumulus–oocyte complexes (COCs) aspirated from unstimulated adult monkey follicles ($\geq 1000 \mu\text{m}$ in diameter) were cultured in either modified Connaught Medical Research Laboratories 1066 medium (mCMRL-1066) or in one of eight chemically defined media (modified basic medium 5 supplemented with 5.5 mmol glucose l^{-1} , 0.003 mmol pantothenic acid l^{-1} and different amino acid formulations) as below: (1) modified basic medium 5 (mBM5) containing no amino acid; (2) mBM5 + 0.2 mmol glutamine l^{-1} ; (3) mBM5 + 11 amino acids from hamster embryo culture medium 6 (HECM-6) (11 AA); (4) mBM5 + Eagle's non-essential amino acids (NEA); (5) mBM5 + NEA + 0.2 mmol glutamine l^{-1} ; (6) mBM5 + Eagle's essential amino acids (EA) without glutamine; (7) mBM5 + EA + 0.2 mmol glutamine l^{-1} ; (8) mBM5 + Eagle's 20 amino acids (20 AA) + 0.2 mmol

glutamine l^{-1} ; and (9) mCMRL-1066 (control). All media contained FSH, LH, oestradiol and progesterone. After maturation, mature oocytes were subjected to the same fertilization and embryo culture procedures. COCs matured in treatment 5 had greater potential to progress to metaphase II (66%; $P < 0.05$) than did those in treatments 1 (37.3%), 2 (48.3%), 3 (41%), 6 (41%) and 9 (43%). Oocytes matured in treatment 8 had the best morula (53%) and blastocyst (18%) developmental responses ($P < 0.05$). The lowest ($P < 0.05$) morula and blastocyst developmental responses were obtained from COCs matured in treatments 1 (0%) and 6 (8%). The other media supported intermediate embryonic development (range 11–38% of morula and blastocyst). These results indicate that the choice of amino acids affects the competence of oocyte maturation and that Eagle's 20 AA with 0.2 mmol glutamine l^{-1} is more efficient than the other amino acid formulations for maturation of rhesus monkey oocytes.

Introduction

Successful oocyte maturation, defined as the potential of oocytes to undergo normal embryonic development, involves not only nuclear events (completion of meiotic maturation), but also cytoplasmic maturation, which is poorly understood (Eppig *et al.*, 1994). The importance of cytoplasmic maturation has been well recognized, but the underlying molecular, biochemical and physiological processes are far from clear, particularly in primates. The developmental potential of primate oocytes matured *in vitro* is remarkably inferior to that of *in vivo* matured oocytes (Wolf *et al.*, 1989; Cha *et al.*, 1991; Morgan *et al.*, 1991; Barnes *et al.*, 1995) and to that of *in vitro* matured oocytes from other species, including cows (Rose-Hellekant *et al.*, 1998), sheep (Guler *et al.*, 2000), pigs (Abeydeera *et al.*, 2000), rats (Vanderhyden and Armstrong, 1990) and mice

(De La Fuente *et al.*, 1999). This lack of developmental potential is primarily due to lack of knowledge about nutritional requirements of primate oocytes for supporting their maturation *in vitro*.

Studies have shown that the components of oocyte maturation medium involved in metabolism, such as energy substrates and nutrients, play important roles from oocyte maturation to preimplantation embryo development (Rose-Hellekant *et al.*, 1998; Watson *et al.*, 2000; Zheng *et al.*, 2001a). In rodents and cattle, oocyte maturation and embryo development could be influenced significantly by a different selection of amino acid components in the culture medium (Bae and Foote, 1975a; Bavister and Arlotto, 1990; Avery *et al.*, 1998; Rose-Hellekant *et al.*, 1998). However, the nuclear and developmental competences of oocytes matured with different selections of amino acids are totally unknown in primates.

In vitro maturation (IVM) of mammalian oocytes is generally performed in culture medium supplemented with serum, follicular fluid, conditioned culture medium or serum

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albumin. It is well known that these undefined components make it very difficult to determine the optimal metabolic and nutritional requirements of the oocytes during maturation. The use of chemically defined culture media that eliminate these components facilitates studies aimed at examining mechanisms involved in the initiation and completion of successful oocyte maturation. We have established a chemically defined culture system, hamster embryo culture medium 10 (HECM-10) supplemented with glucose, progesterone, oestradiol and gonadotrophins for IVM of oocytes from unstimulated rhesus monkeys (Zheng *et al.*, 2001a). This culture system was used in the present study to evaluate the ability of different combinations of amino acids to support maturation of rhesus monkey oocytes with the potential to undergo preimplantation embryonic development after *in vitro* fertilization (IVF).

Materials and Methods

Animals and oocyte recovery

Ovaries were collected on seven different days from healthy adult rhesus monkeys (*Macaca mulatta*) that were killed for industrial vaccine production. These animals had not been used for any experiment or received any treatment related to vaccine production. Ovaries collected on each day were used for one replicate of the study. The monkeys had normal menstrual cycles but the stages of the cycle were unknown at the time of use. Ovaries without apparent corpora lutea were excised and collected into Tyrode's albumin lactate pyruvate (TALP)-Hepes medium (Bavister, 1989) and transported to the laboratory at approximately 35°C within 1–2 h. Ovaries with active corpora lutea were excluded because cumulus–oocyte complexes (COCs) from these ovaries were of variable quality. Healthy follicles $\geq 1000\ \mu\text{m}$ in diameter were punctured to release oocytes into TALP-Hepes medium at 35°C.

In vitro maturation of oocytes

Oocytes that appeared normal (non-vesiculated, round, and medium to lightly pigmented), contained an intact nuclear membrane, and were surrounded by several layers of tightly condensed cumulus cells (COCs) were used in the study. COCs from each animal were assigned randomly and matured in the following media: (1) basic medium 5 (BM5) supplemented with 5.5 mmol glucose l^{-1} and 0.003 mmol pantothenic acid l^{-1} (modified BM5 (mBM5)) (Lane *et al.*, 1998; McKiernan and Bavister, 2000; Zheng *et al.*, 2001a) (Table 1); (2) mBM5 + 0.2 mmol glutamine l^{-1} ; (3) mBM5 + 11 amino acids as in hamster embryo culture medium 6 (HECM-6) (mBM5 + 11 AA) (McKiernan and Bavister, 2000) (Table 2); (4) mBM5 + non-essential amino acids as in Eagle's minimal essential medium (NEA) (Eagle, 1959) (Table 2); (5) mBM5 + NEA + 0.2 mmol glutamine l^{-1} ; (6) mBM5 + essential amino acids as in Eagle's minimal essential medium (EA) without 0.2 mmol glutamine l^{-1}

(Eagle, 1959) (Table 2); (7) mBM5 + EA with 0.2 mmol glutamine l^{-1} ; (8) mBM5 + Eagle's 20 amino acids (20 AA) with 0.2 mmol glutamine l^{-1} ; (9) control medium: modified Connaught Medical Research Laboratories 1066 medium (mCMRL-1066) (Boatman, 1987). Single-strength NEA and EA without glutamine were made from respective 100-strength and 50-strength concentrated stocks (Gibco, Grand Island, NY). The osmolarities of all the media were 286–289 mOsmol kg^{-1} . Medium mCMRL-1066 was used as the control because it could support IVM of rhesus monkey oocytes from non-stimulated females to produce blastocysts *in vitro* (Schramm and Bavister, 1996; Zheng *et al.*, 2001a).

Maturation medium droplets were covered with saline-equilibrated paraffin oil (Sigma, St Louis, MO) in a 60 mm plastic Petri dish (Falcon Plastics, Franklin Lakes, NY, No. 1007) and equilibrated for 4 h. The COCs were washed four times in the respective culture medium before IVM. Five to ten COCs were placed in 50 μl drops of maturation medium and cultured for 36 h at 37°C in 5% CO_2 and air with 100% humidity. All media were supplemented with 3 μg progesterone ml^{-1} (Sigma), 100 ng oestradiol ml^{-1} (Sigma), 5 μg ovine FSH ml^{-1} (oFSH-NIADDK-NIH, AFP55518) and 10 μg ovine LH ml^{-1} (oLH-NIADDK-NIH, AFP4117A) as reported by Schramm and Bavister (1996) and Si *et al.* (2000).

Evaluation of cumulus expansion and meiotic maturation

At the end of IVM culture, the extent of cumulus expansion was assessed subjectively under a stereomicroscope and classified into five categories: (i) category 0: no expansion; (ii) category 1: very slight expansion observed only in the outermost layer; (iii) category 2: slight expansion observed in two to three layers from the periphery; (iv) category 3: moderate expansion, observed on about 50% of the cumulus cell layers; and (v) category 4: full expansion except in the corona radiata layer. After removing some of the cumulus layer by drawing COCs through a narrow Pasteur pipette to facilitate morphological observation, oocytes were examined under the microscope for the status of nuclear maturation. Extrusion of the first polar body (metaphase II (MII)) was considered as indicating completion of nuclear maturation. Oocytes could not be stained to verify MII chromosome configurations because this would have precluded developmental studies.

IVF and embryo culture

Ova at MII in all groups were subjected to the same IVF and embryo culture procedures. Semen was collected by penile electroejaculation (Gould and Mann, 1988) under sedation with ketamine HCl (4–5 mg kg^{-1} , i.m.; Xingan, Shanghai). Approval for the electroejaculation process was obtained from the Laboratory Animal Service at Kunming Institute of Zoology, Chinese Academy of Sciences. Sperm capacitation and IVF were performed as described by

Table 1. Formulation of modified basic medium 5 (mBM5)

Component	Concentration (mmol l ⁻¹)
PVA	(0.1 mg ml ⁻¹)
NaCl	113.8
KCl	3.0
CaCl ₂	1.0
MgCl ₂	2.0
NaHCO ₃	25.0
1 mol HCl l ⁻¹	(140 µl per 100 ml)
Lactate	(64.8 µl per 100 ml)
Glucose	5.5
Pantothenic acid	0.003

PVA: polyvinyl alcohol.

Bavister *et al.* (1983). In brief, washed motile spermatozoa were resuspended at 20×10^6 ml⁻¹ in 200 µl drops of TALP medium (containing fraction V BSA), overlaid with paraffin oil in a 60 mm plastic Petri dish and incubated for > 4 h at 37°C in a humidified atmosphere of 5% CO₂ in air. Spermatozoa were incubated for an additional 1.0–1.5 h in the presence of 1.0 mmol l⁻¹ each of dibutyryl cyclic AMP (dbcAMP; Sigma) and caffeine (Sigma) for hyperactivation (Boatman and Bavister, 1984). Hyperactivated spermatozoa were then diluted to 1×10^5 spermatozoa ml⁻¹ in 100 µl drops of TALP medium containing 2% bovine calf serum (Hyclone Laboratories Inc, Logan, UT) and co-incubated with respective MII oocytes, segregated in the nine treatments, for 12–16 h at 37°C in a humidified atmosphere of 5% CO₂ in air. After co-incubation, spermatozoa were removed manually by aspirating ova through a pulled glass pipette. Oocytes were examined for evidence of activation (presence of two polar bodies or more than one pronucleus). All polyspermic ova containing three or more pronuclei were excluded from the study.

The nine treatments were kept separate, and normal putative embryos (activated ova) were washed and cultured in 50 µl drops of mCMRL-1066 containing 20% bovine calf serum at 37°C in a humidified atmosphere of 5% CO₂, 5% O₂ and 90% N₂. Embryos were placed into fresh culture medium every other day until developmental arrest or hatching, and examined daily under a Nikon Diaphot TMD microscope ($\times 200$ – 400 magnification). Embryos with a blastocoel were classified as blastocysts.

Statistical analysis

Values for MII were analysed as the percentages of total oocytes, and values for activated ova and all stages of embryos were analysed as the percentages of MII ova. All percentage data were subjected to arcsin (square root) transformation. The transformed data were analysed by ANOVA and Fisher's protected least significant difference (LSD) test. Values with $P \leq 0.05$ were considered significant.

Results

A total of 546 morphologically normal COCs were retrieved in seven replicates and used in this study.

Effect of maturation media on cumulus expansion and nuclear maturation of COCs

After 36 h of IVM, there were significant treatment effects on cumulus expansion. Most COCs matured in treatments containing no amino acid or EA with or without glutamine (treatments 1, 6 and 7, respectively) displayed minimal cumulus expansion of category 1. Those in treatments 3 (containing 11 AA) and 9 (control) displayed maximal cumulus expansion of category 3. The cumulus expansion of COCs matured in treatment 4 (containing NEA without glutamine) was category 2 (slight expression). COCs in treatments 2 (mBM5 + glutamine), 5 (mBM5 + NEA + glutamine) and 8 (mBM5 + 20 AA + glutamine) showed similar cumulus expansion of category 2–3 (slight to moderate expression).

Oocytes matured in treatment 5 (mBM5 + NEA + glutamine) displayed greater potential to progress to MII (66%, $P < 0.05$) than did oocytes matured in treatments 1 (mBM5 no amino acids, 37.3%), 2 (mBM5 + glutamine, 48.3%), 3 (mBM5 + 11 AA, 41%), 6 (mBM5 + EA, 41%) and 9 (mCMRL-1066, 43%). No other comparison was significant (treatments 4 (mBM5 + NEA): 61%; 7 (mBM5 + EA + glutamine): 59%; 8 (mBM5 + 20 AA + glutamine): 56%, respectively) (Table 3).

Effects of different IVM treatments on oocyte fertilization and embryo development

The abilities of oocytes matured in all treatments to undergo fertilization (range 64–94%) and cleavage (range 56–82%) were not significantly different (Table 3). However, the capabilities of ova to develop to more than the eight-cell, to at least the morula stage, and to blastocysts were significantly different ($P < 0.05$). In brief, ova in treatments 5 (mBM5 + NEA + glutamine, 56%) and 8 (mBM5 + 20 AA + glutamine, 65%) were more competent than those in treatments 1 (mBM5 no amino acids, 35%) and 7 (mBM5 + EA + glutamine, 31%) to develop beyond the eight-cell stage. Morula development in treatment 8 (mBM5 + 20 AA + glutamine, 53%) was better ($P < 0.05$) than development in treatments 1 (mBM5 no amino acids, 0%), 2 (mBM5 + glutamine, 34%), 4 (mBM5 + NEA, 30%), 5 (mBM5 + NEA + glutamine, 26%), 6 (mBM5 + EA, 8%), 7 (mBM5 + EA + glutamine, 11%) and 9 (mCMRL-1066, 38%), but did not significantly differ from that in treatment 3 (36%). COCs matured in treatment 8 (mBM5 + 20 AA + glutamine, 18%) had greater potential than those matured in the other eight treatments (range 0–4%) to progress to the blastocyst stage ($P < 0.05$).

The time interval for blastocyst formation was about 216 h in treatment 2, 180 h in treatment 3, 156–168 h in

Table 2. Compositions of 11 amino acids from hamster embryo culture medium 6 (HECM-6), Eagle's non-essential amino acids and Eagle's essential amino acids without glutamine

HECM-6 11 amino acids	Concentration (mmol l ⁻¹)	Eagle's non-essential amino acids	Concentration (mmol l ⁻¹)	Eagle's essential amino acids	Concentration (mmol l ⁻¹)
Taurine	0.50	Alanine	0.1	Arginine	0.6
Asparagine	0.01	Asparagine	0.1	Cysteine	0.1
Cysteine	0.01	Aspartic acid	0.1	Histidine	0.2
Histidine	0.01	Glutamic acid	0.1	Isoleucine	0.4
Lysine	0.01	Glycine	0.1	Leucine	0.4
Proline	0.01	Proline	0.1	Lysine	0.4
Serine	0.01	Serine	0.1	Methionine	0.1
Aspartic acid	0.01			Phenylalanine	0.2
Glycine	0.01			Threonine	0.4
Glutamic acid	0.01			Tryptophan	0.5
Glutamine	0.2			Tyrosine	0.2
				Valine	0.4

Table 3. Meiotic competence and developmental potential of unstimulated adult rhesus monkey cumulus–oocyte complexes matured with different amino acid treatments

Treatment	Composition	Number	MII (%) [*]	Developmental stage reached (%) [†]				
				Fertilized	> two-cell	> eight-cell	Morula	Blastocyst
1	mBM5	59	22/59 ^a (37.3 ± 14)	17/22 (77 ± 21)	13/17 (76 ± 13)	6/17 ^a (35 ± 23)	0/17 ^a (0 ± 0)	0/17 ^a (0 ± 0)
2	mBM5+GLN	60	29/60 ^a (48.3 ± 13)	26/29 (89.7 ± 16)	22/29 (76 ± 20)	16/29 ^{ab} (55 ± 14)	10/29 ^{be} (34 ± 15)	1/29 ^a (3.4 ± 13)
3	mBM5+11AA	61	25/61 ^a (41 ± 19)	16/25 (64 ± 20)	14/25 (56 ± 12)	11/25 ^{ab} (44 ± 10)	9/25 ^{bc} (36 ± 15)	1/25 ^a (4 ± 8)
4	mBM5+NEA	61	37/61 ^{ab} (61 ± 17)	33/37 (89 ± 13)	29/37 (78 ± 25)	22/37 ^{ab} (59 ± 15)	11/37 ^{bd} (30 ± 21)	0/37 ^a (0 ± 0)
5	mBM5+NEA+GLN	59	39/59 ^b (66 ± 21)	32/39 (82 ± 17)	27/39 (69 ± 15)	22/39 ^b (56 ± 20)	10/39 ^{bd} (26 ± 15)	0/39 ^a (0 ± 0)
6	mBM5+EA	63	26/63 ^a (41 ± 14)	20/26 (77 ± 19)	17/26 (65 ± 20)	10/26 ^{ab} (38 ± 24)	2/26 ^a (8 ± 8)	0/26 ^a (0 ± 0)
7	mBM5+EA+GLN	61	36/61 ^{ab} (59 ± 18)	28/36 (78 ± 21)	24/36 (67 ± 14)	11/36 ^a (31 ± 25)	4/36 ^{ade} (11 ± 11)	0/36 ^a (0 ± 0)
8	mBM5+20AA+GLN	61	34/61 ^{ab} (56 ± 19)	32/34 (94 ± 7)	28/34 (82 ± 11)	22/34 ^b (65 ± 16)	18/34 ^c (53 ± 21)	6/34 ^b (18 ± 13)
9	mCMRL-1066	61	26/61 ^a (43 ± 15)	22/26 (84 ± 14)	20/26 (77 ± 12)	16/26 ^{ab} (62 ± 25)	10/26 ^{be} (38 ± 24)	1/26 ^a (3.8 ± 13)

Data from seven replicates.

^{*}Percentage of the number of oocytes cultured.

[†]Percentage of the number of oocytes at metaphase II.

^{a–e}Within a column, values (mean ± SD) with different superscripts are significantly different ($P < 0.05$).

mBM5: modified basic medium 5; GLN: glutamine; 11 AA: 11 amino acids from hamster embryo culture medium 6; NEA: Eagles non-essential amino acids; EA: Eagle's essential amino acids; 20 AA: 20 amino acids; mCMRL: modified Connaught Medical Research Laboratories 1066 medium.

treatment eight (mBM5 + 20 AA + glutamine) and 156 h in treatment 9 (mCMRL-1066) after insemination. Two of six blastocysts obtained in treatment 8, and one of one in treatment 9 expanded fully. Because all blastocysts were used for other purposes, the hatching and number of cells of the blastocysts could not be determined in this study.

Discussion

The strategy for improving IVM and embryo development responses for mammalian oocytes is to customize the formulation of the culture medium according to the nutritional and metabolic requirements of the oocytes and embryos. Considerable progress has been made with

oocytes and embryos in some species, including hamsters (McKiernan *et al.*, 1991, 1995) and cows (Pinyopummintr and Bavister, 1996a,b; Rose-Hellekant *et al.*, 1998). For customizing a culture medium, it is helpful to start with a minimal, laboratory-prepared culture medium and add components shown to improve development (Bavister, 1995). Modified BM5, a simple chemically defined medium (McKiernan and Bavister, 2000) supplemented with glucose, pantothenic acid, gonadotrophins, oestradiol and progesterone could support IVM of rhesus oocytes (Si *et al.*, 2000; Zheng *et al.*, 2001a). Therefore, this medium was used in the present study to investigate the effects of various amino acid formulations on maturation and subsequent embryonic development of rhesus monkey oocytes. The present study demonstrates that certain amino acids are required for the nuclear and cytoplasmic maturation of rhesus monkey oocytes. This result is in agreement with previous studies on non-primate species, including mice (Gardner and Lane, 1993), cows (Avery *et al.*, 1998; Rose-Hellekant *et al.*, 1998) and pigs (Jeong and Yang, 2001), which indicate that the inclusion of certain amino acids in the medium stimulates oocyte maturation and subsequent embryo development.

In the present study, essential amino acids with or without glutamine had no effect on nuclear and cytoplasmic maturation of rhesus monkey oocytes (treatments 6 and 7 versus treatment 1). Similarly, poor embryonic development was obtained with bovine oocytes matured in modified basic medium 3 supplemented with essential amino acids from TCM-199 (Avery *et al.*, 1998). Culture in Eagle's essential amino acid in the absence of glutamine inhibited embryonic cleavage of mouse embryos (Gardner and Lane, 1993). This group of amino acids also contains all of the amino acids found to be most detrimental to hamster zygote development (Bavister and Arlotto, 1990; McKiernan *et al.*, 1995). In contrast, essential amino acids have beneficial effects on mouse embryos after the eight-cell stage of development *in vitro* (Lane and Gardner, 1997). Essential amino acids can be transported into the oocyte and embryo and are needed for protein synthesis and accumulation (Moor and Smith, 1979; Lane and Gardner, 1997; Van Winkle, 2001); some of them were effective signalling molecules (Patti *et al.*, 1998; Shigemitsu *et al.*, 1999). In the present study, essential amino acids stimulated cytoplasmic maturation of rhesus monkey oocytes when non-essential amino acids with glutamine were included in the medium (treatment 8 versus treatment 6). These differences could be attributed to differences in the types and concentrations of amino acids present, the developmental stage tested and the experimental design in different studies. It is noteworthy that when evaluating the action of certain amino acids, attention should be paid to the interactions among the components of a culture medium and the developmental stage examined (Partridge and Leese, 1996; Steeves and Gardner, 1999).

Inclusion of glutamine alone in the IVM medium could confer an intermediary benefit on the competence of rhesus

monkey oocytes. A stimulatory role for glutamine in oocyte maturation and embryo development has been reported in cows (Pinyopummintr and Bavister, 1996a,b; Avery *et al.*, 1998; Rose-Hellekant *et al.*, 1998), pigs (Petters *et al.*, 1990), rabbits (Bae and Foote, 1975a), mice (Chatot *et al.*, 1989), hamsters (McKiernan *et al.*, 1995) and humans (Devreker *et al.*, 2001). Glutamine is readily taken up and metabolized by COCs and embryos (Bae *et al.*, 1975b; Chatot *et al.*, 1990; Zuelke and Brackett, 1993) and can function both as an energy source and as a precursor for macromolecules (Zielke *et al.*, 1984; Newsholme *et al.*, 1985; Rieger *et al.*, 1992). Several studies on rabbit oocytes and bovine embryos demonstrate that glutamine is preferred, serving as an energy source to stimulate oocyte maturation and embryo development *in vitro* (Bae and Foote, 1975b; Rieger *et al.*, 1992). However, in the present study, it was not known how glutamine conferred its benefit on rhesus monkey oocytes. Inclusion of essential amino acids in mBM5 plus glutamine appeared to reduce embryo development (treatments 7 versus 2), although this reduction was not significant. It appeared that the action of glutamine could be counteracted by essential amino acids. This was confirmed by the observation that cumulus expansion of COCs in mBM5 plus essential amino acids plus glutamine (treatment 7) was much poorer than that of COCs in mBM5 plus glutamine (treatment 2). Glutamine and cysteine share the same transport systems (Christensen, 1990), so the competitive transportation for these amino acids might be partially responsible for the negative relationship between glutamine and essential amino acids.

The embryo developmental potential of oocytes matured in mBM5 plus non-essential amino acids (treatment 4) was significantly better than that of oocytes matured in mBM5 or mBM5 plus essential amino acids (treatments 1 and 6, respectively), inferring a stimulatory role for the non-essential amino acids in maturation of rhesus monkey oocytes. Other studies have reported the expression and regulation of transport systems for non-essential amino acid in oocytes and preimplantation embryos (for a review, see Van Winkle, 2001). The beneficial effects of non-essential amino acids on oocyte maturation and preimplantation embryo development *in vitro* were demonstrated in several species, including cows (Pinyopummintr and Bavister, 1996b; Avery *et al.*, 1998), mice (Gardner and Lane, 1993) and humans (Devreker *et al.*, 2001). *In vivo*, almost all of these non-essential amino acids are present at high concentrations in the endogenous amino acid pool of oocytes and oviduct fluid (Schultz *et al.*, 1981; Miller and Schultz, 1987). Taken together, these results indicate a common role for non-essential amino acids in mammalian oocyte maturation and early embryonic development. Non-essential amino acids are supposed to act through being utilized in protein synthesis, intracellular pH regulation (Bavister and McKiernan, 1993; Edwards *et al.*, 1998), osmoregulation and cellular signalling (Dawson and Baltz, 1997; Van Winkle, 2001). However, the mechanism of action of non-essential amino acids in the present study is

still unclear. The osmolalities of all the media were about 290 mOsmol kg⁻¹ (normal range). Thus, it seems that the non-essential amino acids did not function as an organic osmolyte to benefit maturation of rhesus monkey oocytes.

The medium conferring the highest morula and blastocyst developmental capacity on rhesus monkey oocytes in culture was mBM5 plus Eagle's 20 amino acids plus glutamine (treatment 8). However, in other studies on IVM of bovine oocytes (Avery *et al.*, 1998), and bovine and mouse embryo development *in vitro* (Gardner and Lane, 1993; Pinyopummintr and Bavister, 1996b), the effect of non-essential amino acids was similar or significantly better than that of non-essential amino acids plus essential amino acids. This discrepancy might be due to a species-specific or stage-specific requirement for amino acids during oocyte maturation and embryo development. Eagle's 20 amino acids with glutamine (treatment 8) functioned better than non-essential amino acids (treatment 4) or essential amino acids with glutamine (treatment 7) in IVM of rhesus monkey oocytes. Obviously, there were some positive interactions between the two groups. Interactions among amino acids have been reported (Bavister and Arlotto, 1990; Steeves and Gardner, 1999). However, it is difficult to deduce the mechanism responsible.

In the culture medium (containing gonadotrophins and glucose) used in the present study, inclusion of different groups of amino acids in IVM medium resulted in various extents of cumulus expansion. This finding implies a requirement for certain amino acids in cumulus expansion of rhesus monkey COCs. The COCs matured in mBM5 plus essential amino acids (treatment 6) displayed minimal cumulus expansion of category 1 as did those matured in mBM5 (treatment 1), indicating no action of essential amino acids on cumulus expansion of rhesus monkey COCs. Glutamine is a metabolic precursor for hexosamine synthesis and its inclusion in culture medium improves cumulus expansion of hamster and bovine COCs (Chen *et al.*, 1990; Kito and Bavister, 1997; Avery *et al.*, 1998; Rose-Hellekant *et al.*, 1998) as well as rhesus monkey COCs in the present study. Besides glutamine, non-essential amino acids were able to induce some degree of cumulus expansion in rhesus monkey COCs. Similarly, Avery *et al.* (1998) reported the effect of non-essential amino acids from TCM-199 on cumulus expansion of bovine COCs. Because the response of cumulus expansion was similar among mBM5 plus glutamine (treatment 2), mBM5 plus 11 amino acids from hamster embryo culture medium 6 (treatment 3), mBM5 plus non-essential amino acids plus glutamine (treatment 5) and mBM5 plus Eagle's 20 amino acids plus glutamine (treatment 8), it seems that cumulus expansion does not require non-essential amino acids in the presence of glutamine, so the beneficial effect of 11 amino acids as from hamster embryo culture medium as well as Eagle's 20 amino acids on cumulus expansion of rhesus monkey COCs can be attributed to glutamine. The COCs with very little cumulus expansion that were matured in treatments 1 (mBM5 no amino acids), 6 (mBM5 plus essential amino

acids) and 7 (mBM5 plus essential amino acids plus glutamine) had the poorest developmental competence, and COCs with better cumulus expansion showed improved developmental competence (treatments 2, 3, 4, 5, 8, and 9). However, the COCs with similar developmental competence that were matured in mBM5 containing glutamine, 11 amino acids from hamster embryo culture medium, non-essential amino acids, non-essential amino acids plus glutamine or mCMRL-1066 (treatment 2, 3, 4, 5, and 9, respectively) displayed different cumulus expansion, and vice versa. Thus, it can be concluded that although the acquisition of developmental competence of rhesus monkey COCs *in vitro* may require at least moderate cumulus expansion, these events are not closely correlated with each other. This result confirms the conclusion of Rose-Hellekant *et al.* (1998) and Zheng *et al.* (2001a). However, in our previous study with COCs from FSH-stimulated rhesus monkeys, the acquisition of developmental potential did not require moderate cumulus expansion (Zheng *et al.*, 2001b). This discrepancy might be due to the different animal treatment as well as to use of different culture systems in the two studies.

In summary, the results obtained from the present study demonstrate that the choice of amino acids included in IVM culture media affects developmental competence of oocytes. In our current culture medium, Eagle's 20 amino acids with 0.2 mmol glutamine l⁻¹ is more efficient than the other amino acid formulations for maturation of rhesus monkey oocyte. Further detailed investigations should be conducted to establish the optimal amino acid requirements for oocyte maturation. The results of these studies are anticipated to benefit assisted reproductive technology and conservation of endangered species as well as transgenic biotechnology and basic research.

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