Influence of epidermal growth factor and transforming growth factor- α on *in vitro* maturation of cumulus cell-enclosed bovine oocytes in a defined medium

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This study was carried out to determine the effects of growth factors (epidermal growth factor, transforming growth factors- α and - β 1, basic fibroblast growth factor, insulin), gonadotrophins (LH, FSH), and fetal bovine serum added to TCM199 medium on cumulus expansion and fertilization during *in vitro* maturation, and on subsequent embryonic development of bovine cumulus cell-enclosed oocytes. Epidermal growth factor, transforming growth factor- α , LH and FSH enhanced cumulus expansion and oocyte fertilizability. No significant effect was achieved with transforming growth factor- β 1 nor with basic fibroblast growth factor. No additive stimulation on cumulus expansion and oocyte fertilizability was observed when epidermal growth factor was combined with LH or FSH. The addition of either epidermal growth factor or transforming growth factor- α to the maturation medium increased the number of fertilized ova that developed to the blastocyst stage. These results demonstrate the potential use of epidermal growth factor and transforming growth factor- α in obtaining high quality mature bovine oocytes for *in vitro* fertilization.

Introduction

It is generally accepted that gonadotrophins play a major role in stimulating the resumption of meiosis in mammalian oocytes and in the expansion of the cumulus oophorus *in vivo* (Lindner *et al.*, 1974; Eppig, 1980; Tsafiri *et al.*, 1982; Eppig and Downs, 1984). However, it is possible that other hormonal factors influence meiotic maturation of mammalian oocytes during the preovulatory period. There have been many studies on the intraovarian regulatory role of growth factors in recent years and there is increasing evidence from these studies that growth factors play an important role in the *in vitro* maturation of rodent oocytes. Dekel and Sherizly (1985) demonstrated that epidermal growth factor (EGF) induced maturation of rat oocytes in the presence of follicle cells. Similarly, EGF has been shown to induce germinal vesicle breakdown specifically in cumulus cell-enclosed mouse oocytes maintained in meiotic arrest (Downs *et al.*, 1988; Downs, 1989). Transforming growth factor- α (TGF- α), structurally and functionally related to EGF (Marquardt *et al.*, 1983), also stimulates the meiotic maturation of cumulus cell-enclosed mouse oocytes (Brucker *et al.*, 1991). Feng *et al.* (1988) found that TGF- β stimulates the maturation of follicle-enclosed oocytes and cumulus-oocyte complexes in rats. However, there have been only a few studies on the physiological role of growth factors in bovine oocyte maturation when the cumulus cell-enclosed oocytes are cultured in chemically defined medium.

Several reports indicate that cumulus cells are closely involved in the acquisition of full developmental competence

during oocyte maturation (Leibfried and First, 1979; Staigmiller and Moor, 1984; Crister *et al.*, 1986). Bovine oocytes failed to mature or had a low maturation rate when oocytes were cultured in the absence of cumulus cells (Leibfried and First, 1979; Fukui and Sakuma, 1980). Cumulus cells that tightly adhered to oocytes become dispersed (that is, undergo cumulus expansion) in response to the endogenous surge of gonadotrophins (Dekel and Phillips, 1979; Eppig, 1980; Leibfried and First, 1982). The expansion of cumulus-oocyte complexes is possibly related to the fertilization rate and to the developmental potential of mammalian oocytes (Schroeder and Eppig, 1984; Vanderhyden and Armstrong, 1989; Chen *et al.*, 1993). Factors inducing cumulus expansion may therefore be of great importance in achieving complete oocyte maturation, which is required for fertilization and subsequent normal embryonic development.

The present study was designed to clarify the positive effect of certain growth factors on the cumulus expansion of cumulus cell-enclosed bovine oocytes in a defined medium. The effect of growth factors on complete oocyte maturation and on the potential for embryonic development following *in vitro* fertilization was also investigated.

Materials and Methods

Materials

Recombinant human EGF was purchased from Wakunaga Pharmaceutical Co., Osaka; recombinant human transforming growth factor- α (TGF- α) from Earth Pharmaceutical Co., Ltd, Ako, Hyogo; transforming growth factor- β 1 (TGF- β 1), purified

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Fig. 1. Bovine cumulus–oocyte complexes exhibiting three degrees of cumulus expansion. (a) Degree 0: no morphological change was observed compared with freshly recovered cumulus–oocyte complexes; (b) degree 1: cumulus oophorus slightly expanded but cell to cell binding was still relatively tight; clustered cells were still observed; (c) degree 2: cumulus oophorus was extensively expanded and cumulus cells were homogeneously spread. Differential interference contrast, Nomarski optics. Scale bar represents 200 μm .

from platelets, and basic fibroblast growth factor (bFGF) from bovine brains from R&D Systems, Inc., Minneapolis, MN; bovine LH (iodination grade) and FSH (iodination grade) from UCB-Bioproducts SA, Belgium. TCM199 was from Nissui Pharmaceutical Co., Tokyo. Fetal bovine serum (FBS) was from Flow Laboratories, North Ryde. Bovine insulin, fatty acid-free BSA and heparin sodium salt, from porcine intestinal mucosa, were from Sigma Chemical Co., St Louis, MO. Mineral oil was from Squibb and Sons, Inc., Princeton, NJ and type I collagen was from Koken Co., Tokyo. Sixty millimetre tissue culture dishes were from Nunc, Roskilde.

Oocyte recovery and in vitro maturation (IVM)

Cumulus–oocyte complexes were recovered as described by Kobayashi *et al.* (1992). Briefly, ovaries from Holstein or Japanese Black cows and heifers were collected at a local abattoir. They were minced with surgical blades in TCM199 supplemented with 25 mmol Hepes l^{-1} , 15 μg heparin ml^{-1} , 1.25 mmol sodium pyruvate l^{-1} and 10 μg gentamycin ml^{-1} (IBL, Fujioka) to release the cumulus–oocyte complexes from small antral follicles. Only oocytes with an unexpected cumulus and homogeneous cytoplasmic granulation were selected. They were placed in 350 μl droplets of the maturation media in 60 mm tissue culture dishes and covered with mineral oil. Each droplet contained approximately 30 cumulus–oocyte complexes. The cumulus–oocyte complexes were then incubated for 24 h at 38.5°C in a humidified atmosphere of 5% CO_2 :95% air.

Sperm preparation and in vitro fertilization (IVF)

Sperm preparation and *in vitro* fertilization were performed as described by Kobayashi *et al.* (1992). Briefly, commercially

distributed, frozen semen from a single Japanese Black bull preserved in 0.5 ml straws was used. A 0.5 ml straw containing approximately 2×10^7 spermatozoa was rapidly thawed at 34°C and spermatozoa were washed twice with BO medium (Brackett and Oliphant, 1975) containing 5 mmol caffeine l^{-1} and 15 μg heparin ml^{-1} by centrifugation at 700 g for 7 min. The resulting spermatozoa pellet was resuspended in 1 ml of the same medium. The concentration of spermatozoa was determined by a haemocytometer and adjusted to 10^7 spermatozoa ml^{-1} by further dilution. A 50 μl aliquot of the spermatozoa suspension was added to 50 μl droplets of the BO medium containing 5 mmol caffeine l^{-1} and 10 mg BSA ml^{-1} placed under mineral oil. After *in vitro* maturation, the cumulus–oocyte complexes were washed three times with the same medium, transferred to the droplets of spermatozoa suspension and incubated for 6 h at 38.5°C in a humidified atmosphere of 5% CO_2 :95% air.

Embryo culture

After *in vitro* fertilization, the cumulus–oocyte complexes were washed three times with TCM199 supplemented with 5 μg insulin ml^{-1} , and were transferred to 350 μl droplets of the same medium placed under mineral oil. Tissue culture dishes used for embryo cultures were precoated with 100 μg Type 1 collagen ml^{-1} to enhance the attachment of cumulus cells to culture dishes. Fertilized ova were cocultured with cumulus cells at 38.5°C in a humidified atmosphere of 5% CO_2 :95% air.

Evaluation of cumulus expansion

The proportions of oocytes exhibiting cumulus expansion were determined at the endpoint of *in vitro* maturation. The degree of cumulus expansion was assessed by the morphology of the cumulus oophorus as follows: degree 0: no expansion

Table 1. Effects of growth factors and gonadotrophins on cumulus expansion and cleavage of bovine oocytes during *in vitro* maturation

Treatment	Number of cumulus-oocyte complexes examined	Number (%) of cumulus-oocyte complexes expanded			Number (%) of fertilized ova cleaved
		Degree 0	Degree 1	Degree 2	
None	127	114 (89.8 ± 2.7)	13 (10.2 ± 2.7)	0	62 (48.8 ± 7.0)
EGF	118	0 ^a	10 (8.5 ± 5.2)	108 (91.5 ± 5.2) ^a	83 (70.3 ± 4.5) ^a
TGF- α	121	0 ^a	7 (5.8 ± 0.0)	114 (94.2 ± 0.0) ^a	89 (73.6 ± 6.1) ^a
TGF- β 1	126	119 (94.4 ± 3.8)	7 (5.6 ± 3.8)	0	38 (30.2 ± 5.0) ^b
bFGF	125	108 (86.4 ± 4.1)	17 (13.6 ± 4.1)	0	50 (40.0 ± 3.6)
Insulin	131	101 (77.1 ± 5.8) ^b	30 (22.9 ± 5.8) ^b	0	71 (54.2 ± 7.0)
LH	119	0 ^a	0 ^a	119 (100.0 ± 0.0) ^a	89 (74.8 ± 6.2) ^a
FSH	117	0 ^a	0 ^a	117 (100.0 ± 0.0) ^a	99 (84.6 ± 3.4) ^a
LH plus FSH	119	0 ^a	1 (0.8 ± 1.0) ^b	118 (99.2 ± 1.0) ^a	80 (67.2 ± 3.3)
LH plus EGF	119	0 ^a	0 ^a	119 (100.0 ± 0.0) ^a	87 (73.1 ± 10.9) ^a
FSH plus EGF	116	0 ^a	0 ^a	116 (100.0 ± 0.0) ^a	79 (68.1 ± 9.0) ^b
LH plus FSH plus EGF	119	0 ^a	0 ^a	119 (100.0 ± 0.0) ^a	88 (73.9 ± 5.6) ^a
FBS	119	2 (1.7 ± 2.9) ^a	74 (62.2 ± 7.6) ^a	43 (36.1 ± 7.1) ^a	78 (65.5 ± 5.6) ^b

EGF: epidermal growth factor; TGF- α : transforming growth factor- α ; TGF- β 1: transforming growth factor- β 1; bFGF: basic fibroblast growth factor; FBS: fetal bovine serum. Percentages are expressed as means ± SEM of four replicates. ^{a,b}Percentages with a superscript differ from the percentage of the control in each column at ^a $P < 0.001$ and at ^b $P < 0.01$. Data were analysed by χ^2 test. Degree of expansion: 0: no expansion; 1: cumulus cells nonhomogeneously spread and cells still clustered; 2: cumulus cells homogeneously spread and cells no longer clustered.

was seen; degree 1: cumulus cells were nonhomogeneously spread and clustered cells were still observed; degree 2: cumulus cells were homogeneously spread and clustered cells were no longer present. Typical morphology of *in vitro* matured cumulus-oocyte complexes in these three categories are shown (Fig. 1).

Experiment 1

The effects of growth factors, gonadotrophins and fetal bovine serum (FBS) added to the maturation medium on cumulus expansion and cleavage were examined. Selected cumulus-oocyte complexes were matured with TCM199 containing EGF (10 ng ml⁻¹), TGF- α (10 ng ml⁻¹), TGF- β 1 (10 ng ml⁻¹), bFGF (10 ng ml⁻¹), insulin (5 µg ml⁻¹), LH (5 µg ml⁻¹), FSH (500 ng ml⁻¹), combinations of LH, FSH, and EGF (LH plus FSH, LH plus EGF, FSH plus EGF, and LH plus FSH plus EGF, at the same concentrations as indicated above), or FBS (10%). TCM199 alone served as control medium. After incubation for 24 h, the cumulus-oocyte complexes were observed to determine the degree of cumulus expansion and the ability of oocytes to be fertilized. Forty-eight hours after *in vitro* fertilization, ova were denuded from the surrounding cumulus cells by pipetting, and observed for the occurrence of cleavage.

Experiment 2

Dose-dependent stimulation by EGF and TGF- α on cumulus expansion and fertilization was examined. EGF and TGF- α were added to the maturation medium at concentrations of 0, 0.01, 0.1, 1, 10 and 100 ng ml⁻¹. The degree of cumulus expansion and cleavage rates were determined as described in Expt 1.

Experiment 3

The influence of EGF, TGF- α , LH, FSH, and combinations of LH, FSH and EGF during *in vitro* maturation were tested on cleavage and subsequent embryonic development. The cumulus-oocyte complexes were matured with TCM199 alone or with TCM199 containing EGF, TGF- α , LH plus FSH, LH plus FSH plus EGF, or FBS and fertilized. Fertilized cumulus-oocyte complexes were then transferred to the development medium (TCM199 supplemented with 5 µg insulin ml⁻¹) as described above and cocultured with cumulus cells for 9 days without change of medium. Embryos were denuded mechanically, by pipetting, 48 h after IVF. The concentrations of growth factors, gonadotrophins and serum used in Expt 3 were the same as in Expt 1. The developmental capacity of the fertilized ova was calculated from the proportion of fertilized ova that developed to the blastocyst stage within 9 days after IVF.

Statistical analysis

The proportion of cumulus-oocyte complexes expanded to the different degrees, the proportion of fertilized ova that cleaved, and the proportion of fertilized ova that developed to the blastocyst stage were compared by χ^2 test. In Expts 1, 2 and 3, a comparison was made between each treatment and the corresponding control.

Results

Experiment 1

The effects on cumulus expansion and cleavage of adding the different growth factors, gonadotrophins and serum to the

Fig. 2. Bovine cumulus–oocyte complexes cultured for 24 h in different maturation media. (a) TCM199 alone; (b) epidermal growth factor (10 ng ml^{-1}); (c) transforming growth factor- α (10 ng ml^{-1}); (d) transforming growth factor- $\beta 1$ (10 ng ml^{-1}); (e) basic fibroblast growth factor (10 ng ml^{-1}); (f) LH ($5 \text{ } \mu\text{g ml}^{-1}$) plus FSH ($0.5 \text{ } \mu\text{g ml}^{-1}$); (g) insulin ($5 \text{ } \mu\text{g ml}^{-1}$); (h) fetal bovine serum (10%). Phase contrast. Scale bar represents $400 \text{ } \mu\text{m}$.

maturation medium are summarized (Table 1). The data are from four replicates. Microscope observations of cumulus–oocyte complexes cultured for 24 h are shown (Fig. 2).

When cumulus–oocyte complexes were incubated in TCM199 alone, only 10.2% (13 of 127) of the cumulus–oocyte complexes showed any expansion (degree 1) and no fully

Fig. 3. Effect of epidermal growth factor (EGF) on cumulus expansion and cleavage of bovine oocytes during *in vitro* maturation. (a) Percentage of cumulus-oocyte complexes expanded to degree 0 (□), degree 1 (▨), and degree 2 (■). Degree 0: no expansion; degree 1: cumulus cells nonhomogeneously spread and cells still clustered; degree 2: cumulus cells homogeneously spread and cells no longer clustered. (b) Percentages of fertilized ova undergoing cleavage. Values are expressed as means $\pm$ SEM from four replicates. Total numbers of oocytes examined at the EGF concentrations of 0, 0.01, 0.1, 1, 10, and 100 ng ml⁻¹ were 116, 117, 116, 116, 118 and 116, respectively. *Percentages were significantly different from control ($P < 0.001$; χ^2 test).

expanded cumulus-oocyte complexes (degree 2) were observed. Most (89.8%; 114 of 127) of the cumulus-oocyte complexes remained unexpanded with the cumulus cells still tightly bound to oocytes (Table 1, Fig. 2a).

EGF and TGF- α greatly increased ($P < 0.001$) the proportion of cumulus-oocyte complexes with cumulus expansion. Cumulus-oocyte complexes incubated in the media containing EGF or TGF- α all exhibited cumulus expansion, and more than 90% reached degree 2 (91.5%; 108 of 118 and 94.2%; 114 of 121, respectively). No significant difference on cumulus expansion was observed between EGF and TGF- α . Enhancement of cumulus expansion by these two growth factors was comparable to the effects of LH and FSH which also stimulated cumulus expansion intensively with all the cumulus-oocyte complexes reaching degree 2 (Fig. 2f). Cumulus expansion was also induced in the presence of FBS (98.3%; 117 of 119); although a high percentage (62.2%; 74 of 119) then remained at degree 1. The binding of cumulus cells surrounding the oocytes appeared looser; however, the cells did not spread as exten-

Fig. 4. Effect of transforming growth factor- α (TGF- α) on cumulus expansion and cleavage of bovine oocytes during *in vitro* maturation. (a) Percentages of cumulus-oocyte complexes expanded to degree 0 (□), degree 1 (▨), and degree 2 (■). Degree 0: no expansion; degree 1: cumulus cells nonhomogeneously spread and cells still clustered; degree 2: cumulus cells homogeneously spread and cells no longer clustered. (b) Percentages of fertilized ova undergoing cleavage. Values are expressed as means $\pm$ SEM from four replicates. Total numbers of oocytes examined at the TGF- α concentrations of 0, 0.01, 0.1, 1, 10, and 100 ng ml⁻¹ were 123, 121, 122, 123, 124 and 122, respectively. *Percentages were significantly different from control ($P < 0.001$; χ^2 test).

sively compared with complexes stimulated by EGF, TGF- α , LH or FSH.

A significant ($P < 0.01$) increase in fertilization rate was also observed with EGF (70.3%; 83 of 118) or TGF- α (73.6%; 89 of 121) compared with control medium (48.8%; 62 of 127). Similarly, higher proportions of cumulus-oocyte complexes cleaved in the presence of LH (74.8%; 89 of 119), FSH (84.6%; 99 of 117) or FBS (65.5%; 78 of 119). However, there were no significant differences ($P > 0.05$) in cleavage rate among media containing EGF, TGF- α , LH, FSH or FBS. No additive effect was observed on the number of ova that cleaved during IVM when EGF was combined with LH or FSH.

TGF- β 1 and bFGF had no significant effect on cumulus expansion and slightly reduced cleavage rates (30.2%; 38 of 126 and 40.0%; 50 of 125, respectively).

The addition of insulin resulted in a higher proportion (22.9%; 30 of 131) of cumulus-oocyte complexes expanding to

Table 2. Effect of EGF and TGF- α on *in vitro* development of bovine oocytes during *in vitro* maturation

Treatment	Number of ova	Number (%) of ova	
		Undergoing cleavage	Developing to blastocyst
None	194	98 (50.5 $\pm$ 4.6) ^a	19 (9.8 $\pm$ 1.9) ^c
EGF	196	152 (77.6 $\pm$ 5.2) ^b	35 (17.9 $\pm$ 3.7) ^d
TGF- α	198	156 (78.8 $\pm$ 4.5) ^b	42 (21.2 $\pm$ 4.6) ^d
LH plus FSH	195	136 (69.7 $\pm$ 2.7) ^b	31 (15.9 $\pm$ 3.5) ^{c,d}
LH plus FSH plus EGF	198	155 (78.3 $\pm$ 3.8) ^b	38 (19.2 $\pm$ 3.2) ^d
FBS	198	156 (78.8 $\pm$ 1.7) ^b	38 (19.2 $\pm$ 0.7) ^d

Percentages are expressed as means $\pm$ SEM of seven replicates.

Percentages with different superscripts are significantly different ^{a,b} $P < 0.001$; ^{c,d} $P < 0.05$.

EGF: epidermal growth factor; TGF- α : transforming growth factor- α ; FBS: fetal bovine serum.

Data were analysed by χ^2 test.

degree 1 ($P < 0.01$). Insulin slightly increased the fertilization rate, but the difference from controls was not statistically significant ($P > 0.05$).

Experiment 2

Dose-dependent stimulation by EGF or TGF- α of cumulus expansion and cleavage rate was determined. Data are from four replicates. The proportion of cumulus-oocyte complexes exhibiting cumulus expansion (degree 1 plus degree 2) was increased significantly ($P < 0.001$) by the addition of EGF at a concentration as low as 0.01 ng ml⁻¹ with all cumulus-oocyte complexes exhibiting cumulus expansion at 1 ng ml⁻¹ (Fig. 3a). The maximum effect of EGF was observed at 100 ng ml⁻¹; however, differences were not significant among the doses used (1 to 100 ng ml⁻¹; $P > 0.05$). EGF also increased the fertilization rate in a dose-dependent manner (Fig. 3b). Maximum stimulation was observed at 10 ng ml⁻¹, but there were no significant differences ($P > 0.05$) among the doses of 1–100 ng ml⁻¹.

TGF- α enhanced cumulus expansion and rate of fertilization in a dose-dependent manner (Fig. 4a, b). The proportion of cumulus-oocyte complexes exhibiting cumulus expansion (degree 1 plus degree 2) was significantly increased ($P < 0.001$) at 0.01 ng ml⁻¹ and nearly all complexes had expanded at doses of 0.1 to 100 ng ml⁻¹. The maximum effect on cumulus expansion to degree 2 was observed at 10 ng ml⁻¹. The highest rate of cleavage was obtained at 10 ng ml⁻¹, but differences were not significant among doses of 0.01–100 ng ml⁻¹ ($P > 0.05$).

Experiment 3

The effects of growth factors, gonadotrophins or serum added to the maturation medium on cleavage and subsequent embryo development are summarized (Table 2). The data are from seven replicates.

The cleavage rate of cumulus-oocyte complexes incubated in TCM199 alone was 50.5% (98 of 194) and the

proportion of fertilized ova developing to blastocyst was 9.8% (19 of 194).

Consistent with the results of Expts 1 and 2, cleavage rates were significantly increased ($P < 0.001$) by the addition of EGF (77.6%; 152 of 196) or TGF- α (78.8%; 156 of 198). The proportion of fertilized ova that developed to blastocysts was also significantly increased ($P < 0.05$) by the addition of EGF (17.9%; 35 of 196) or TGF- α (21.2%; 42 of 198). The effects of EGF on fertilization rate and blastocyst formation were unchanged by further supplementation with gonadotrophins ($P > 0.05$).

Other treatments (LH plus FSH or FBS) also had a positive effect on cleavage rate and subsequent embryo development. However, cleavage rate and blastocyst formation with these treatments were not significantly different from those in EGF-containing medium.

Discussion

The present study demonstrates that exposure of cumulus-enclosed bovine oocytes during *in vitro* maturation to EGF and TGF- α stimulated cumulus expansion and oocyte fertilizability when cultured in defined medium. Consistent with previous findings (Ball *et al.*, 1983; Brackett *et al.*, 1989; Younis *et al.*, 1989; Zuelke and Brackett, 1990), LH or FSH, alone and in combination, also stimulated cumulus expansion and oocyte fertilizability. However, combinations of EGF and gonadotrophins had no additive or synergistic effect on oocyte fertilizability and subsequent development to the blastocyst stage.

These results suggest that the stimulatory mechanisms of EGF and gonadotrophins are mediated by the same physiological process. Downs *et al.* (1988) reported that FSH and EGF stimulated cAMP production and germinal vesicle breakdown in mouse cumulus-oocyte complexes. As dibutyryl cAMP also stimulates germinal vesicle breakdown, they proposed that FSH and EGF act via a cAMP-dependent process. Moreover, cumulus expansion of mouse cumulus-oocyte

complexes occurs after the increase in cAMP (Buccione *et al.*, 1990). It is therefore possible that in bovine cumulus–oocyte complexes EGF and gonadotrophins act via a cAMP-dependent process.

Ova, matured in medium with added EGF or TGF- α , developed to the blastocyst stage at as high a rate as ova matured in the presence of FBS, when these ova were fertilized and cocultured with cumulus or granulosa cells in insulin-containing medium. In our previous study (Hoshi *et al.*, 1991), the addition of insulin during embryo culture potentiated the rate of blastocyst formation with cumulus or granulosa cell monolayer cultures in a chemically defined medium after the cumulus–oocyte complexes were matured in medium containing FBS. The result that EGF alone improved fertilizability and developmental capacity is in agreement with the results of Coskun *et al.* (1991) who assessed the development to the 4- to 8-cell stage. Harper and Brackett (1993) reported that during IVM EGF, in a defined medium, improved the number of bovine ova developing to blastocysts after IVF, although the requirement for gonadotrophins (FSH or LH) in the action of EGF in their study differed from the present study.

The stimulatory action of EGF on oocyte maturation was observed in mice (Downs *et al.*, 1988), mice and humans (Das *et al.*, 1991) and rats (Dekel and Sherizly, 1985). Downs (1989) examined a number of growth factors (TGF- β , platelet-derived growth factor, nerve growth factor, insulin-like growth factor-I and II, insulin, and FGF) and demonstrated that the stimulatory action was specific to EGF. Similar results were obtained in this study. No positive effects on cumulus expansion nor on bovine oocyte fertilizability were observed with insulin, bFGF or TGF- β 1. Basic FGF and TGF- β 1 slightly inhibited cleavage rate. TGF- α , which has similar biological functions to EGF and binds to the same receptors, also stimulates meiotic maturation of cumulus-enclosed mouse oocytes (Brucker *et al.*, 1991). TGF- α also mimics the action of LH on oocyte maturation by inducing meiotic resumption in follicle-enclosed rat oocytes (Tsafiriri *et al.*, 1989).

It is of interest that although the number of cleaved ova that matured in TCM199 medium alone was significantly lower than in the presence of EGF (77.6%) and TGF- α (78.8%), 50.5% of control cumulus–oocyte complexes underwent fertilization and cleavage and a small number (9.8%) developed to the blastocyst stage. This result is in agreement with the findings of Zuelke and Brackett (1990) who found that almost 50% of bovine oocytes matured in TCM199 medium alone could be fertilized and cleaved to two-cell and four-cell stages by 48 h after IVF. In a study of different nutrient media used for maturation of bovine oocytes, Rose and Bavister (1992) reported that TCM199 is one of the best nutrient media for normal oocyte maturation. The choice of TCM199 as basal medium for IVM may therefore facilitate fertilization. With regard to *in vitro* development, Takagi *et al.* (1991) found that the use of Type 1 collagen-precoated dishes during embryo culture after IVF resulted in improved cumulus cell proliferation and embryo development in a serum-free medium. Since IVF and embryonic development were successful in TCM199 medium alone during IVM, it remains unclear whether the cumulus expansion induced by EGF-like molecules and gonadotrophins is directly related to the increased fertilizability and subsequent embryonic development.

In conclusion, EGF-like molecules enhance bovine oocyte maturation *in vitro* in a chemically defined medium as reflected in improved fertilization and subsequent embryonic development *in vitro*. The IVM culture system with defined medium is useful for investigating specific molecular mechanisms by which EGF-like molecules stimulate oocyte maturation and improve the overall efficiency of embryo production.

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