韓國家畜繁殖學會誌 21(1) : 61~69(1997)
Korean J. Animal Reprod.

Effect of Epidermal Growth Factor (EGF) and anti-EGF on Early Embryonic Development in Mice

Byun, H. K. and H. J. Lee

Infertility Research Laboratory, Cheil Medical Research Institute

Samsung Cheil Hospital and Women's Healthcare Center

Seoul, Korea

Epidermal Growth Factor (EGF)와 anti-EGF가 생쥐배아의 발생에 미치는 영향

변 혜 경 · 이 호 준

삼성제일병원 제일의학연구소 불임연구실

요 약

본 연구는 EGF와 anti-EGF가 초기 생쥐배아의 발생 및 부화에 미치는 영향을 알아보고자 실행되었다. 초기 2세포기부터 상실배까지의 배아를 EGF와 anti-EGF를 각각 처리한 Ham's F10 배양액에서 배양하여 그 발생률과 부화율을 대조군과 비교하였다. EGF 처리시 배양시간에 따른 발생률은 증진되었으나 통계학적 유의성은 없었다. EGF 처리군에서의 부화율(57.5, 62.5, 65.0, 62.5%)은 대조군(35%)에 비하여 유의하게 ($p<0.01$) 높았다. Anti-EGF 처리시 각 발생시기별 1:1000 실험군의 발생률은 대조군과 차이가 없었다. 그러나, 1:100 실험군의 경우, 2~4세포기의 배아는 모두 4~8세포기에서 정지되었고, 8세포기와 상실배의 포배형성은 48 시간 이상 지연되었으며 부화 역시 대조군에 비해 억제되었다(8세포기; 2%, 44%, 상실배; 6.2%, 58.3%). 이 실험에서, EGF는 생쥐 배아의 포배형성과 부화를 증진시키는 반면, anti-EGF는 이를 억제하였다. Anti-EGF 처리시 나타나는 발생정지 현상은 anti-EGF가 배아에서 만들어지는 EGF와 반응하여 EGF의 작용을 억제시키기 때문으로 사료된다. 그러므로 EGF는 paracrine mode로서 뿐만 아니라 autocrine mode로서 생쥐 초기배아의 발생에 중요한 인자로 작용함을 알 수 있었다.

(Key words : EGF, Anti-EGF, Blocking, Blastulation, Hatching)

I. INTRODUCTION

Preimplantation embryo development and differentiation in mammals have been known to be regulated by specific gene expression (Wiley and Obasaju, 1989; Telford et al., 1990; Schultz et al., 1995), hormones (Roblero and Izquierdo, 1976; Rosenblum et al., 1989), energy sources (Brinster, 1965; Biggers et al., 1989; Kane et al., 1992; Takahashi and First, 1992) and growth factors (Wales, 1992; Yang et al., 1993; Babalola and Schultz, 1995; Erickson, 1995; Moesner and Dodson, 1995). Among these, growth factors provided by the maternal reproductive tracts (Brigstock et al., 1989; Tamada et al., 1991; Chegini et al., 1994; Viuff et al., 1995) or produced by the embryo (Rappolee et al., 1988; Werb, 1990; Hemmings et al., 1992; Doherty et al., 1994; Chia et al., 1995) have

been reported to regulate embryonic development and differentiation.

Different growth factors may express and act at various stages of development in mouse embryos. Transforming growth factor-α (TGF-α) and insulin-like growth factor-I (IGF-I) are expressed from oocyte to blastocyst stage and act very early in development (Werb, 1990; Doherty et al., 1994). Insulin-like growth factor-II (IGF-II) is expressed at the 4-cell stage and transforming growth factor-α (TGF-α) is after the morular stage (Rappolee et al., 1988; Werb, 1990). Werb (1990) reported that the stage of EGF expression is after postimplantation stage. EGF, fibroblast growth factor(FGF), platelet-derived growth factor (PDGF) and IGFs influence the development of mouse embryos (Wood and Kaye, 1989; Paria and Dey, 1990).

EGF, as a potent mitogen (Hill, 1989), has been detected in the oviduct (Lei and Rao, 1992; Morishige et al., 1993); In addition, EGF and the receptor have been detected in the endometrium (Chegini et al., 1986; Berchuck et al., 1989; Imai et al., 1995) of mammals, suggesting that EGF participates in embryonic development. EGF plays a role in mitosis and functional differentiation of trophectoderm cells in mouse embryos. EGF can elevate intercellular cAMP levels which stimulates blastocoel expansion in mouse embryos (Dardik and Schultz, 1991) and induces implantation in mammals (Paria et al., 1993, 1994; Tamada et al., 1994).

It is known that EGF binds to a plasma membrane receptor and initiates a number of cellular responses, such as protein phosphorylation, nutrient transport and macromolecular synthesis, which requires nuclear activity and communication between the cell surface and the nucleus (Donaldson et al., 1989). The mechanism by which these responses are initiated, however, is not yet understood.

In this study, we determined the paracrine mode of EGF on the blastulation and hatching in the mouse preimplantation embryo. Furthermore, with anti-EGF, we studied the biological significance of the autocrine mode of EGF on the early development in the mouse embryo.

II. MATERIALS AND METHODS

1. Embryo collection

EGF (Sigma, St. Louis, MO) was prepared as 1 μg/ml stock solutions and stored at -20 ℃ until use. For culture, individual aliquots of the stock solutions of EGF were thawed and diluted as 1~1,000 ng/ml with 0.4% BSA-Ham's F10 culture medium (Gibco) before use. Anti-EGF (Sigma) was diluted from 1:10 to 1:1,000 as serial dilution with 0.4% BSA-Ham's F10 culture medium.

2. Embryo culture in the medium treated with EGF or anti-EGF

To investigate the effect of EGF on the development of mouse embryo, early 2-cell stage embryos were collected from the superovulated C57BL×CBA F1 hybrid females (6 weeks old) that were mated with males of the same strain (10~12 week old). Pooled embryos were washed through 0.4% BSA-Ham's F10 medium and randomly allocated into control and experimental groups (EGF, 1~1,000 ng/ml). These embryos were cultured in the 20-μl drops under autoclaved mineral oil (10 embryos/drop) (Sigma) at 37 ℃, 5% CO_2 in humidified air for 72 hrs in an incubator (Queue, 2700).

To investigate the effect of anti-EGF on the mouse embryo, from 2-cell to morular stage embryos were collected from the females with the same stimulation method. Pooled embryos were washed and randomly allocated into control and

experimental groups (anti-EGF, 1:10~1:1,000). These embryos were cultured in the 20-㎕ drops under autoclaved mineral oil (10 embryos /drop) (Sigma) at 37℃, 5% CO_2 in humidified air for 48~72 hrs in an incubator (Queue, 2700). The embryos were observed every 24 hr to monitor their development.

3. Statical analysis

To compare the percentages of embryos that developed to specific stages in control with those in the anti-EGF or in the EGF-supplemented groups, Fisher's exact test and Chi-square test were used. Values were evaluated as significant when p was smaller than 0.05.

Ⅲ. RESULTS

When mouse early 2-cell embryos were cultured in the EGF supplemented medium, hatching rates (57.5, 62.5, 65.0, 62.5%, respectively) were significantly ($p<0.01$) higher than that of control (35%) (Fig. 1). According to time, blastulation was significantly enhanced in over 10 ng /ml-EGF group. It seemed that blastulation was EGF conecntration-dependent.

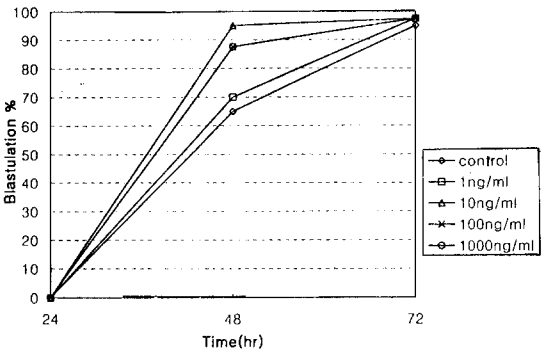

Fig. 1. Blastulation rate according to the treatment of EGF

As shown in Table 1-to-4, there is no significance in embryonic development between control and the anti-EGF supplemented group (1:1,000) from 2-cell to morular stage. However, when the embryos were cultured in the anti-EGF supplemented medium (1:100), in the

Table 1. Development of mouse 2-cell embryos treated with anti-EGF

Groups	Total No. of embryos	24hr		48hr				72hr				
		2~4	8	2~4	8	M	B	2~4	8	M	B	D
Control	90	18	72	16	10	38	26	12	2	0	76	0
1:1,000	80	15	65	11	8	52	9	7	1	2	70	0
1: 100	80	72	8	65	15	0	0	0	0	0	0	80

Control : 0.4% BSA-Ham's F10, n=8

2~4: 2~4-cell, 8: 8-cell, M: morula, B: blastocyst, D: degeneration

Table 2. Development of mouse 4-cell embryos treated with anti-EGF

Groups	Total No. of embryos	24hr			48hr						72hr		
		4	8	C	4	8	C	M	B	D	B	H	D
Control	80	0	40	40	0	0	0	48	32	0	24	56	0
1:1,000	80	3	49	28	0	0	0	40	37	3	34	43	3
1: 100	80	5	57	18	5	57	18	0	0	0	0	0	80

Control : 0.4% BSA-Ham's F10, n=8

4: 4-cell, 8: 8-cell, C: compaction, M: morula, B: blastocyst, H: hatching, D: degeneration

Table 3. Development of mouse 8-cell embryos treated with anti-EGF

Groups	Total No. of embryos	24hr				48hr			
		8	M	B	D	8	B	H	D
Control	50	2	25	23	0	0	28	22	0
1:1,000	50	4	28	18	0	2	20	27	1
1: 100	50	29	21	0	5	25	0	0	25

Control : 0.4% BSA-Ham's F10, n=5

8: 8-cell, M: morula, B: blastocyst, H: hatching, D: degeneration

Table 4. Development of mouse morular embryos treated with anti-EGF

Groups	Total No. of embryos	24hr			48hr			
		M	B	D	M	B	H	D
Control	60	30	30	0	0	25	35	0
1:1,000	65	35	30	0	0	39	26	0
1: 100	65	30	35	0	15	30	4	16

Control : 0.4% BSA-Ham's F10, n=6

M: morula, B: blastocyst, H: hatching, D: degeneration

2 and 4-cell stage groups, all embryos were arrested at the 4~8-cell stages and degeneration was observed (Table 1 and 2); in the 8-cell and morular stage groups, blastulation was delayed over 48 hr and hatching was inhibited compared with control (Table 3 and 4). When the morulae were cultured in the anti-EGF supplemented medium (1:10), all the embryos were arrested and degenerated (data were not shown). Fig. 2 shows that there is a significant ($p<0.01$) inhibiting effect of anti-EGF in the anti-EGF supplemented groups (1:100) according to the embryo stage.

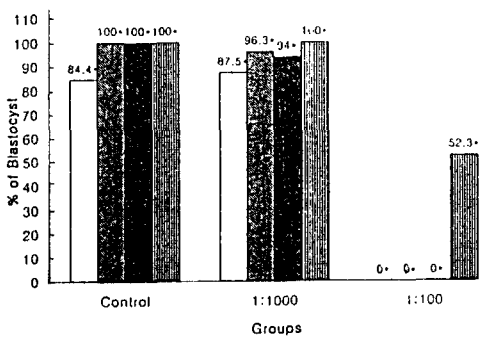

Fig. 2. Comparison of blastulation with control group and anti-EGF treated group according to embryo stage. *$p<0.01$

□2-cell stage ▨4-cell stage

▩8-cell stage ■Morular

Ⅳ. DISCUSSION

The major modes of growth factor action have been classified into four: endocrine, paracrine, autocrine and juxtacrine (Sporn and Todaro, 1980; James and Bradshaw, 1984; Massague, 1990). An intracrine mode of action has been suggested that growth factors which are made in cells but are incapable of being secreted or of being inserted into cell plasma membranes nevertheless induce observable phenotypic changes in those cells (Re, 1989).

Both EGF and TGF-α bind to EGF receptor and activate the receptor to stimulate blastocoel expansion in the mouse embryos (Dardik and Schultz, 1991). Our study shows that EGF enhances development and blastulation in the mouse embryos, suggesting that EGF participa-

tes in the blastulation as a paracrine mode. When the mouse embryos were cultured in the presence of the antibody of EGF, they were arrested to degeneration (unpublished our data). However, when the embryos were cocultured with human oviductal epithelial cells in the culture medium supplemented with anti-EGF, the phenomenon of developmental arrest was not observed (Morishige et al., 1993). This shows that the major modes of EGF action in the mouse embryo are not intracrine but autocrine by the embryo and paracrine by the reproductive tract.

EGF plays a role in early development in the mouse embryo. EGF has a significant mitogenic effect on the 2-cell stage embryo (Paria and Dey, 1990). During the morula-blastocyst transition, EGF stimulates the trophectoderm cells to synthesize proteins (Nielson et al., 1986; Wood and Kaye, 1989). EGF stimulates blastocoel expansion to improve hatching and implantation (Dardik and Schultz, 1991; Paria et al., 1991) and the stimulatory effect of EGF is concentration-dependent. Of our findings with anti-EGF, anti-EGF has an inhibiting effect on the development and the blastocyst hatching of mouse embryos. It may be postulated that anti-EGF neutralizes the biological effect of EGF and that EGF is an essential factor in the development and hatching process in mouse embryos. Although the concentration of the anti-EGF was indeterminable, it is thought that anti-EGF in the culture medium binds to EGF secreted from the embryo by itself and neutralizes the EGF activity during embryonic development and hatching. However, in the low concentration of anti-EGF supplemented group (1:1000), the embryos showed the same pattern of development as those of control, suggesting that the embryonic EGF overcomes the developmental arrest which is caused by anti-EGF in the culture medium.

Werb (1990) reported that mouse early embryo responded to the maternal EGF until implantation and EGF might be expressed in postimplantation embryo (day 9) during the organogenesis. The mRNA transcripts of EGF receptor were detected in unfertilized oocytes and 2-cell stage embryos, which was regulated by the paracrine function of the maternal EGF in the reproductive tracts (Hofmann et al., 1992; Wiley et al., 1992). However the mRNA transcripts of EGF were undetected in any stage of preimplantation embryo (Rappolee et al., 1988; Werb, 1992). Using the immunocytochemistry with anti-EGF, we have found that EGF was localized in the late 4-cell stage and the further stage embryos, especially intensely at the trophoblast in the blastocyst and the hatched blastocyst, suggesting that mouse preimplantation embryo may produce EGF molecules after the late 4-cell stage (Byun et al., 1995; Byun and Lee, 1995). Since the increase of the mRNAs of EGF receptor after the 4-cell stage may be related to the up-regulating function of EGF (Hofmann et al., 1992), embryonic EGF is expressed after the late 4-cell stage and activates EGF receptor as an autocrine mode.

It is concluded that EGF is essential on the development after the 4-cell stage and blastulation to hatching in mouse embryos, and that anti-EGF inhibits the EGF activity in mouse embryos. Therefore, it is suggested that EGF is one of the most important factors in the early embryonic development of the mouse embryo as autocrine and paracrine mechanism.

V. SUMMARY

The present study was carried out to investigate the effects of EGF and anti-EGF on early embryonic development and hatching in mice. Developmental and hatching rates of mouse em-

bryos from 2-cell to morular stage which were cultured in Ham's F10 medium supplernented with EGF (1~1,000 ng/ml) or anti-EGF (whole serum diluted from 1:10 to 1:1,000) were compared to those of control.

When mouse early 2-cell embryos were cultured in the EGF supplemented medium, blastulation was accelerated compared with control. Hatching rate was also significantly ($p<0.01$) higher than that of control. There is no significance in embryonic development between control and the anti-EGF supplemented group (1:1,000) from 2-cell to morular stage. But when the embryos were cultured in the anti-EGF supplemented medium (1:100), in 2 and 4-cell stage groups, all embryos were blocked at the 4~8 cell stage and degeneration was obscrved; in 8-cell and morular stage groups, blastulation was delayed over 48 hrs and hatching was inhibited compared with control.

EGF enhanced blastulation and hatching of mouse embryos. On the other hand, anti-EGF inhibited development and hatching of the cmbryos. Although the concentration of the anti-EGF was indeterminable, it is though that anti-EGF in the culture medium bound EGF secreted from the embryo by itself and neutralized the EGF activiy during embryonic development and hatching. But in the low concentration of anti-EGF supplemented group (1:1,000), endogenous EGF overcame the developmental blocking which was caused by anti-EGF. It is concluded that EGF is essential on development and hatching in mouse embryos and that anti-EGF inhibits EGF activity in mouse embryos. Therefore, it is suggested that EGF is one of the most important factors in the early embryonic development of mouse as autocrine and paracrine mechanism.

Ⅵ. REFERENCES

1. Babalola, G. O. and R. M. Schultz. 1995. Modulation of gene expression in the preimplantation mouse embryo by TGF-α and TGF-β. Mol. Reprod. Devel., 41:133-139.
2. Berchuck, A., A. P. Soisson, G. J. Olt, J. T. Soper, D. L. Clarke-Pearson, R. C. Bast and K. S. McCarty. 1989. Epidermal growth factor receptor expression in normal and malignant endometrium. Ami. J. Obstet. Gynecol., 161:1247-1252.
3. Biggers, J. D., D. K. Gardner and H. J. Leese. 1989. Control of carbohydrate metabolism in preimplantation mammalian embryos. In: growth factors in mammalian development. (Rosenblum IY, Heyner S eds) CRC Press, New York, 19-32.
4. Brigstock, D. R., R. B. Heap and K. D. Brown. 1989. Polypeptide growth factors in uterine tissues and secretions. J. Reprod. Fert., 85:747-758.
5. Brinster, R. L. 1965. Studies on the development of mouse embryos *in vitro*. Ⅱ. the effect of energy source. J. Reprod. Fert., 158:59-68.
6. Byun, H. K., H. J. Lee, S. R. Kim, H. K. Kim and M. K. Kim. 1995. Effect of epidermal growth factor (EGF) on early embryonic development in mouse. Kor. J. Fertil. Steril., 22:163-170.
7. Byun, H. K. and H. J. Lee. 1995. The expression of epidermal growth factor (EGF) in the mouse and human oocytes and embryos. European society of human reproduction and embryology, Abstract 297.
8. Chegini, N., C. V. Rao, N. Wakim and J. Sanfilippo. 1986. Binding of 125I-epidermal growth factor in human uterus. Cell Tissue Res., 246:543-548.
9. Chegini, N., Y. Zhao and F. W. Mclean. 1994. Expression of messenger ribonucleic

acid and presence of immunoreactive proteins for epidermal growth factor (EGF), transforming growth factor alpha (TGFα) and EGF/TGFα receptors and 125I-EGF binding sites in human fallopian tube. Biol. Reprod., 50:1049-1058.

10. Chia, C. M., R. M. Winston and A. H. Handyside. 1995. EGF, TGF-α and EGFR expression in human preimplantation embryos. Development, 121: 299-307.
11. Dardik, A. and R. M. Schultz. 1991. Blastocoel expansion in the preimplantation mouse embryo : stimulatory effect of TGF-α and EGF. Development, 113:919-930.
12. Doherty, A. M., G. L. Temeles and R. M. Schultz. 1994. Temporal pattern of IGF-I expression during mouse preimplantation embryogenesis. Mol. Reprod. Devel., 37: 21-26.
13. Donaldson, R. W., S. Nishibe and G. Capenter. 1989. The structure and physiology of epidermal growth factor and its receptor. In: advances in growth hormone and growth factor research. (Muller EE, Cocchi D, Locatelli V eds) Pythagora Press, Roma-Milano and Springer-Verlag, Berlin-Heidelberg, 165-180.
14. Erickson, R. P. 1995. Recent advances in developmental genetics: growth factors and morphogens. Mol. Reprod. Devel., 41: 109-125.
15. Hemmings, R., L. Granger, J. Langlais, P. Miron, T. Falcone and H. Guyda. 1992. Human embryos produce transforming growth factors a activity and insuline-like growth factor II. Fertil. Steril., 58:101-104.
16. Hill, D. J. 1989. Growth factors and their cellular actions. J. Reprod. Fert., 85:723-734.
17. Hofmann, G. E., M. R. Drews, J. R. B. T. Scott, D. Navot, D. Heller and L. Deligdisch. 1992. Epidermal growth factor and its receptor in human implantation trophoblast : immunohistochemical evidence for autocrine/paracrine function. J. Clin. Endocrinol. Metab., 74:981-988.
18. Imai, T., H. Kurachi, K. Adachi, H. Adachi, Y. Yoshimoto, H. Homma, C. Tadokora, S. Takeda, M. Yamaguchi, M. Sakata, Y. Sakoyama and A. Miyake. 1995. Changes in epidermal growth factor receptor and the levels of its ligands during menstrual cycle in human endometrium. Biol. Reprod., 52:928-938.
19. James, R. and R. A. Bradshaw. 1984. Polypeptide growth factors. Ann. Rev. Biochem., 53:259-292.
20. Kane, M. T., E. W. Carney and J. E. Ellington. 1992. The role of nutrients, peptide growth factors and co-culture cells in development of preimplantation embryos *in vitro*. Theriogenology, 38:297-313.
21. Lei, Z. M. and Ch. V. Rao. 1992. Expression of epidermal growth factor (EGF) receptor and its ligands, EGF and transforming growth factor-α, in human fallopian tubes. Endocrinology, 131:947-957.
22. Massague, J. 1990. Transforming growth factor-α. A model for membrane-anchored growth factors. J. Biol. Chem., 265:21393-21396.
23. Moessner, J. and W. C. Dodson. 1993. The role of growth factors and proteins in media for *in vitro* fertilization. Assi. Reprod. Reviews, 3(2):63-67.
24. Morishige, K., H. Kurachi, K. Amemiya, H. Adachi, K. Adachi, Y. Sakoyama, A. Miyake and O. Tanizawa. 1993. Menstrual stage-specific expression of epidermal growth factor and transforming growth factor-a in human oviduct epithelium and their role in early embryogenesis. Endocrinology, 133: 199-207.
25. Nielson, L. L., D. Benos and J. D. Biggers.

1986. Characterization of amiloride-sensitive Na^+/H^+ exchange in rabbit blastocysts. Biol. Reprod., 34(suppl.1): Abstract 132.

26. Paria, B. C. and S. K. Dey. 1990. Preimplantation embryo development *in vitro*: cooperative interactions among embryos and role of growth factors. Proc. Natl. Acad. Sci., USA, 87:4756-4760.
27. Paria, B. C., H. Tsukamura and S. K. Dey. 1991. Epidermal growth factor-specific protein tyrosine phosphorylation in preimplantation embryo development. Biol. Reprod., 45: 711-718.
28. Paria, B. C., S. K. Das, G. K. Andrews and S. K. Dey. 1993. Expression of the epidermal growth factor receptor gene is regulated in mouse blastocysts during delayed implantation. Proc. Natl. Acad. Sci., USA, 90: 55-59.
29. Paria, B. C., S. K. Das, R. A. Mead and S. K. Dey. 1994. Expression of epidermal growth factor receptor in the preimplantation uterus and blastocyst of the western spotted skunk. Biol. Reprod., 51:205-213.
30. Rappolee, D. A., C. A. Brenner, D. A. Schultz and Z. Werb. 1988. Developmental expression of PDGF, TGF-α, and TGF-β genes in preimplantation mouse embryos. Science, 241:1823-1825.
31. Re, R. N. 1989. The cellular biology of angiotensin: paracrine, autocrine and intracrine actions in cardiovascular tissues. J. Mol. Cell Cardiol., 21:63-69.
32. Roblero, L. and L. Izquierdo. 1976. Effect of progesterone on the cleavage rate of mouse embryos *in vitro*. J. Reprod. Fert., 46:475-476.
33. Rosenblum, I. Y., M. Farber, K. Ramos and M. L. Pritchard. 1989. Hormonal signaling mechanism : the role of protein phosphorylation in early development. In: growth factors in mammalian development. (Rosenblum IY, Heyner S eds) CRC Press, New York, 33-46.
34. Schultz, R. M., D. M. Worrad, W. Davis and P. A. De Sousa. 1995. Regulation of gene expression in the preimplantation mouse embryo. Theriogenology, 44:1115-1131.
35. Sporn, M. B. and G. Todaro. 1980. Autocrine secretion and malignant transformation of cells. N. Engl. J. Med., 303:878-880.
36. Takahashi, Y. and N. L. First. 1992. *In vitro* development of bovine one-cell embryos : influence of glucose, lactate, pyruvate, amino acid and vitamins. Theriogenology, 37:963-978.
37. Tamada, H., S. Das, G. K. Andrews and S. K. Dey. 1991. Cell-type-specific expression of transforming growth factor-a in the mouse uterus during the peri-implantation period. Biol. Reprod., 45:365-372.
38. Tamada, H., Y. Kai and J. Mori. 1994. Epidermal growth factor-induced implantation and decidualization in the rat. Prostaglandins, 47:467-475.
39. Telford, N. A., A. J. Watson and G. A. Schultz. 1990. Transition from maternal to embryonic control in early mammalian development: a comparison of several species. Mol. Reprod. Dev., 26:90-100.
40. Viuff, D., P. Hyttel, T. Greve, T. Eriksen and S. Alexandersen. 1995. Transcription and locaization of growth factor mRNA in the bovine oviduct. Mol. Reprod. Devel., 42:28-34.
41. Wales, R. G. 1992, Growth factors in the early embryonic development : summary and perspective. Reprod. Fertil. Dev., 4:355-359.
42. Werb, Z. 1990. Expression of EGF and TGF-α genes in early mammalian development. Mol. Reprod. Dev., 27:10-15.
43. Wiley, L. M. and M. F. Obasaju. 1989. Gen-

etics of early mouse development. In: growth factors in mammalian development. (Rosenblum IY, Heyner S eds) CRC Press, New York, 1-18.

44. Wiley, L. M., J. X. Wu, I. Harari and E. D. Adamson. 1992. Epidermal growth factor receptor mRNA and protein increase after the four-cell preimplantation stage in murine development. Dev. Biol. 149:247-260.
45. Wood, S. A. and P. L. Kaye. 1989. Effects of epidermal growth factor on preimplantation mouse embryos. J. Reprod. Fert., 85: 575-582.
46. Yang, B. K., X. Yang and R. H. Foote. 1993. Effect of growth factors on morula and blastocyst development of *in vitro* matured and *in vitro* fertilized bovine oocytes. Theriogenology, 40:521-530.

(접수일자 : 1997. 2. 17 / 채택일자 : 1997. 3. 25)