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Effect of TGF-β1 and IGF-I on Bovine *In Vitro* Maturation and Embryo Culture

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TGF-β1와 IGF-I이 소 난포란의 체외성숙 및 체외수정란의 배양에 미치는 영향

서 태 광

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요 약

본 연구는 혈청첨가 또는 무첨가에 따른 소 난포란의 체외성숙에 있어서 참가된 TGF-β1과 IGF-I이 그후의 수정 및 발생에 미치는 영향과, 이들 growth factor의 농도에 따른 8세포기 소 체외수정란의 발달에 미치는 영향을 조사하고자 실시하였다. 도축장에서 얻어진 난소로 부터 채취된 난포란을 20% FBS가 첨가 또는 첨가되지 않은 TCM-199에 TGF-β1, IGF-I 또는 TGF-β1 + IGF-I을 각각 10ng/ml 첨가하여 38.5℃에서 24시간 배양하여 체외성숙을 유기하였다. 성숙된 난자를 1×10^6/ml 정자농도로 수정후 24시간에 glucose가 첨가되지 않은 CZB 배양액으로 옮겨 48시간 배양한 다음, TCM-199 + 20% FBS에서 96시간 추가배양하였다.

본 연구에서 혈청이 첨가된 난포란의 체외성숙배양액에 첨가된 growth factor 들은 수정후의 배분할 및 배발생에 영향을 미치지 않았다. 혈청이 첨가되지 않은 경우에서는 TGF-β1의 첨가는 배분할 및 배발생율을 향상시켰다($P<0.05$). 한편 TCM-199 + 20% FBS에 5, 10ng/ml의 TGF-β1 및 5, 10, 50, 100ng/ml의 IGF-I을 각각 첨가후 8세포기 체외수정란을 배양한 결과, 10ng/ml TGF-β1의 첨가는 배반포기로의 발생율을 향상시켰다($P<0.05$). 결론적으로, 혈청이 포함되지 않은 소 난포란의 체외성숙 배양액, 또는 수정란의 체외배양액에 10 ng/ml TGF-1 의 첨가는 배반포기로의 발생율을 향상시킨다.

Key words: *in vitro* fertilization, maturation, bovine, TGF-β1, IGF-I.

I. INTRODUCTION

In vitro development of bovine embryos is known to be developmentally slower with fewer embryos reaching the blastocyst stage compared to *in vivo* development. A wide range of studies have been aimed at improving *in vitro* maturation (IVM) systems and/or culture systems (IVC). The improvement of IVM and IVC systems include defining essential factors necessary for full developmental capacity. Growth factors are considered to be essential components in IVM and IVC and have been shown to act in both a paracrine and/or autocrine fashion(Heyner et al., 1993; Kane et al., 1991; Nilson-Hamilton, 1989). The addition of several growth factors including epidermal growth factor, transforming growth factor-α, transforming growth factor-β and/or insul-

in-like growth factor-I in IVM or IVC system for embryo development has been reported in murine and bovine embryos(Coskun et al., 1991; Flood et al., 1993; Herrler et al., 1992; Keefer, 1992; Larson et al., 1990; Lorenzo et al., 1993; Paria and Dey, 1990; Park and Lin, 1993; Yang et al., 1993).

Transforming growth factor-β(TGF-β)and insulin-like growth factor(IGF-I) are know to be produced by follicular cells and are involved in granulosa cell growth *in vitro*(Adashi et al., 1985; Schams et al., 1988; Skinner et al., 1987). This may account for the beneficial effect of coculture with granulosa cells in IVM or IVC system (Berg and Brem, 1990; Critser et al., 1986; Fukui and Ono, 1989; Goto et al., 1992; Mochizuki et al., 1991; Suh et al., 1993) for the improvement of embryo development. Moreover, mRNA transcripts for TGF-β and receptors for IGF-I in bovine preimplantation embryos were found suggesting a possible role for these growth factors during embryo development (Rappolee et al., 1988, 1990; Watson et al., 1991, 1992). Nevertheless, any direct beneficial effects of these factors is still unclear as a function of the culture conditions(Herrler et al., 1992; Keefer, 1992). The purpose of this experiment was to evaluate the effect of TGF-β1 and IGF-I in bovine oocyte maturation, in the presence and absence of serum, on fertilization and subsequent embryo development to blastocyst. Further, to evaluate various concentration of these growth factors for the ability to promote development of 8-cell bovine embryos to blastocyst stage during *in vitro* culture.

Ⅱ. MATERIALS AND METHODS

Transforming growth factor-β1 (TGF-β1, Calbiochem. Cat. # 619350) was dissolved in 4mM HCl and stored at −80℃. The tubes and handling pipettes for TGF-β1 were siliconized with dimethyldichlorosilane (Sigma). Insulin-like growth factor-I (IGF-I, Boehringer Mannheim Biochem. Cat. # 1048058) was dissolved in 10mM HCl and stored at −20℃.

Ovaries were obtained form cows and heifers at a local slaughterhouse and were transported in sterile saline(0.9% NaCl) at room temperature to the laboratory within 3~4 h of slaughter. Ovaries were washed once with 70% ethanol and twice with sterile saline. Cumulus-oocyte complexes (COCs) were recovered in TL-HEPES (Bavister, 1989) medium by dissection and subsequent rupture of follicles of 2~6mm in diameter using a scalpel and the COCs were washed three times with TL-HEPES. Only cumulus-oocyte complexes with evenly granulated cytoplasm and 3~5 complete layers of compact cumulus cells were selected and used for this experiment. The medium used for *in vitro* maturation was TCM-199 (Earles salt with L-glutamine and 25mM HEPES. Sigma Cat # M2520) supplemented with or without 20% fetal bovine serum (FBS, Hyclone Lab. Inc., Cat # A-1115) with 0.05μg/ml FSH (NIH-oFSH), 1μg/ml LH (NIH-bLH) and 1μg/ml estradiol-17β(Sigma). Ten cumulus-oocyte complexes were cultured in each 1 ml drop of medium in Nunclon multidish (Nunc. Cat # 176740) covered with sterile-filtered paraffin oil at 38.5℃ in 5% CO_2 in air with maximum humidity for 24 h.

Frozen Wagyu semen (IVF-tested fertile bulls) was thawed at 37℃ for 1 min, overlaid on top of 45% and 90% Percoll (Sigma) bilayer and centrifuged at 300×g for 30 min at room temperature. The sperm pellet was suspended in 10ml of Sp-TL (Parrish et al., 1985) medium (pH 7.4), centrifuged at 300×g for 10min and resuspended in Sp-TL medium to a final concentration of 25×10^6 cells/ml. Matured oocytes were washed three times in TL-HEPES medium, transferred

to IVF-TL(Bavister and Yanagimachi, 1977) medium (pH 7.4) containing 5 units/ml heparin (Elkins-Sinn Inc. NJ) and 6mg/ml fatty acid free BSA (Sigma. Cat. # A-8806), and fertilized at a final concentration of 1×10^6 cells/ml (40 μl of Sp-TL).

After 24 hours of sperm-egg incubation, the embryos were washed three times in TL-Hepes medium, transferred to CZB medium without glucose (Chatot et al., 1989) for 48 h. Cumulus cells were removed by vortexing for 30 sec and the resulting embryos were washed 2× in TL-HEPES. Cleavage retes were determined at this time for Exiperiment 1. The embryos were cultured in 1 ml drop of TCM-199/20% FBS covered with sterile-filtered paraffin oil. Culture medium was replaced every 48 h and the development to the blastocyst stage was observed at 70× magnification during 7 days of incubation.

Experiment 1 was designed to evaluate the effect of TGF-β1 and IGF-I in the presence or absence of serum during *in vitro* maturation on subsequent embryonic development. The concentrations of TGF-β1 and IGF-I in media were 10 ng/ml each. Experiment 2 was designed to evaluate the effect of various concentrations of TGF-β1 and IGF-I in the presence of serum on 8-cell enbryo development to blastocyst. Eight-cell embryos obtained following 3 days of culture after sperm-egg incubation were cultured in the medium for 4 days. Growth factors were added to the medium in the beginning of culture in TCM-199 and the concentrations of TGF-β1 were 5, 10 ng/ml and those of IGF-I were 5, 10, 50, 100 ng/ml. The data were analyzed by Analysis of Variance using general linear model(GLM) procedure of SAS(SAS Inst. Inc., 1985).

Ⅲ. RESULTS

No significant differences between control and treated groups in cleavage rete and development to blastocyst stage were observed in the presence of serum (Table 1). In the absence of serum during bovine oocyte maturation, significantly more oocytes cultured in the medium supplemented with TGF- 1 cleaved and developed to the blastocyst stage when compared to control ($p<0.05$). The supplementation of IGF-I or TGF- 1 combined with IGF-I in serum-free media exhibited a trend toward greater develop-

Table 1. Effect of growth factors in *in vitro* maturation medium on subsequent bovine embryo development *in vitro*

Treatment	No. oocytes	% cleaved (No.)	% Morula (No.)	% Blastocyst (No.)
With serum				
Control	39	79.5 (31)	58.9 (23)	28.2 (11)
IGF-I	39	66.7 (26)	56.4 (22)	33.3 (13)
TGF-β1	39	82.1 (32)	66.7 (26)	33.3 (13)
IGF-I+TGF-β1	39	89.7 (35)	69.2 (27)	46.2 (18)
Serum free				
Control	30	53.3 (16)^{b}	20.0 (6)	3.3 (1)^{b}
IGF-I	36	52.8 (19)^{b}	22.2 (8)	16.7 (6)^{ab}
TGF-β1	42	81.0 (34)^{a}	40.5 (17)	23.8 (10)^{a}
IGF-I+TGF-β1	39	69.2 (27)^{ab}	41.0 (16)	17.9 (7)^{ab}

$^{a, b}$ Different superscripts within the same columns are significantly different ($p<0.05$)

Table 2. Effect of growth factors on *in vitro* development of eight-cell bovine embryos produced by IVM/IVF

Treatment	(ng /ml)	No. embryos	% Morula (No.)	% Blastocyst (No.)
Control		31	74.2 (23)	25.8 (8)[a]
TGF-β1	5	28	82.1 (23)	42.9 (12)[ab]
	10	28	67.9 (19)	50.0 (14)[b]
IGF-I	5	37	56.8 (21)	29.7 (11)[ab]
	10	28	71.4 (20)	32.1 (9)[ab]
	50	27	81.5 (22)	37.0 (10)[ab]
	100	26	61.5 (16)	30.8 (8)[ab]

[a, b] Different superscripts within the same column are significantly different ($p<0.05$)

ment to blastocyst.

The supplementation of 10 ng /ml of TGF- 1 to the culture medium improved the development of 8-cell stage embryos to the blastocyst stage compared with that of the control group ($p<0.05$). There were no significant differences found between various concentrations of IGF-I and the control (Table 2). Although no differences were observed between the treatments of TGF-β, a concentration of 10 ng /ml of TGF-β1 was more beneficial in improving 8-cell development to blastocyst in serum-containing medium when compared to the control.

Ⅳ. DISCUSSION

This study showed that in the presence of serum, the supplementation of growth factors to the oocyte maturation medium had no effect on cleavage and subsequent embryo development to the blastocyst stage. However, Lorenzo et al. (1993) reported improved fertilization rate by the supplementation of IGF-I to a bovine oocyte maturation medium consisting of TCM-199 containing 10 % estrous cow serum (ECS). They used a higher concentration of IGF-I (100 ng /ml) in conjunction with EGF (50 ng /ml) when compared to this study. In a similar experiment, Herrler et al. (1992) added IGF-I (50 ng /ml) to their bovine oocyte maturation medium of TCM-199 + 20% ECS, and found no beneficial effect on cleavage or development to the blastocyst stage (20%) compared to control (16%). The positive results with 10% ECS + 100 ng /ml IGF-I and the absence of an effect in 20 % ECS+50 ng /ml IGF-I, suggests that growth factors addition is dependent on original serum concentration in the medium. Serum is known to contain many growth factors(Maurer, 1986). The addition of growth factors to media containing serum may be ineffective due to the already high levels present in serum. The results of this study showed that the addition of growth factors in the 20% serum-containing TCM-199 had no effect.

Recently, many researchers have focused on the expression of growth factor genes and receptors in preimplantation embryos(Rappolee et al., 1988, 1990; Watson et al., 1991, 1992). Transcripts for TGF- mRNAs appeared immediately after fertilization in murine embryos (Rappolee et al., 1988) and have been detected at all stages of early development in bovine embryos (Watson et al., 1992). The mRNAs encoding the receptors for IGF-I (Watson et al., 1991), and receptors for IGF-I (Watson et al., 1992) have also been detected throughout bovine preimplantation development. These findings support the

result of Yang et al. (1993), which showed the beneficial effect of TGF-β on bovine embryo development and those of Larson et al. (1990), which showed the development promoting effect of TGF-β beyond the bovine 8-cell block in defined medium. The development of murine embryos in culture was improved by the addition of TGF-β and EGF, with IGF-I having no effect on embryo development (Paria and Dey, 1990) which is consistent with the present study involving bovine embryos. However, the result of Keefer (1992) showed no improvement in blastocyst development with the addition of TGF-β in defined medium and that of Herrler et al. (1992) showed no beneficial effect of IGF-I in culture medium leaving a question on the role of growth factors in embryo culture. Although the involvement of growth factors in early embryo development has been studied in several species, additional research is needed in many aspects including the cooperative action with the component(s) in medium to define the action of growth factors *in vitro*.

V. SUMMARY

This experiment was designed to evaluate the effect of transforming growth factor-β (TGF-β) and insulin-like growth factor-I (IGF-I) in bovine oocyte maturation in the presence or absence of serum on subsequent fertilization and embryo development. In addition, various concentrations of these growth factors were evaluated for the ability to promote development of eight-cell stage embryos to the blastocyst stage. Cumulus-oocyte complexes were recovered from 2 to 6 mm follicles obtained from slaughterhouse ovaries and cultured at 38.5℃ for 24 hours in TCM-199 (HEPES Modification) with or without 20 % fetal bovine serum (FBS) to which the following growth factors were added : TGFβ, IGF-I or TGF-β + IGF-I, all at 10 ng/ml each. The matured oocytes were fertilized in IVF-TL medium with frozen-thawed semen at a concentration of 1×10^6 cells/ml of fertilization medium following Percoll separation. After 24 hours of sperm-egg incubation, the embryos were transferred to CZB medium without glucose for 48 hours and then cultured in TCM-199 with 20 % fetal bovine serum (FBS) for 96 hours. The addition of growth factors to IVM medium in the presence of serum had no effect on cleavage and subsequent embryo devlopment to blastocyst. In the absence of serum, TGF- improved cleavage and development to blastocyst compared to control's($p<0.05$) and no synergistic effect of IGF-I + TGF-β was observed. In the second experiment, eight-cell embryos obtained by *in vitro* maturation (IVM) in TCM-199 + 20 % FBS without growth facrors and *in vitro* fertilization (IVF) were cultured in the *in vitro* culture (IVC) medium supplemented with 5, 10 ng/ml TGF-β or 5, 10, 50, 100 ng/ml IGF-I. Cleavage rate and development to the blastocyst stage was observed during seven days of incubation. The supplementation of 10 ng/ml TGF-β to IVC medium for eight-cell embryos improved development to blastocyst ($p<0.05$) compared to control. In conclusion, these data indicate that the supplementation of growth factors to IVM medium in the presence of serum does not influence cleavage and subsequent embryo development. However, significantly more oocytes matured in serum-free TCM-199 and eight-cell embryos cultured in IVC medium developed to blastocyst with supplementation of 10 ng/ml TGF-β.

VI. REFERENCES

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