



Effects of IGF-I and EGF Supplemented to PZM3 Culture Medium on the Development of Porcine Embryos *In vitro*

J. Y. Kim, M. C. Park, S. B. Kim, H. D. Park¹, J. H. Lee² and J. M. Kim^{2,*}
Infertility Medical Center of CHA General Hospital, Daegu, 705-809, Korea

ABSTRACT : This study investigated the effects of IGF-I and EGF on the development of blastocysts or hatched blastocysts during the *in vitro* culture of embryos from immature porcine oocytes. After the *in vitro* maturation and fertilization of cumulus-oocyte complexes (COCs) and their culture *in vitro* in PZM3 medium, we examined the embryo development rate for 168 h. When different concentrations of IGF-I (0, 1, 10, 20 ng/ml) were supplemented to fertilized porcine embryos *in vitro*, there were no significant differences in cleavage rate, blastocyst development rate or blastocyst hatching rate among the treated groups. On the other hand, when different concentrations of EGF (0, 1, 10, 20 ng/ml) were supplemented to the *in vitro* culture medium, blastocyst development rate was highest in the group in which EGF was not supplemented and, specifically, it was higher than in the 20 ng/ml treatment group ($p < 0.05$). When 10 ng/ml IGF-I and 1 ng/ml EGF were supplemented separately or simultaneously, there were no significant differences among the treated groups in blastocyst hatching rate and the number of cells in each condition. This study demonstrated that the addition of IGF-I and EGF into PZM3 medium did not enhance development of the blastocyst stage and total cell number in blastocysts. (**Key Words :** Embryo Development, Porcine Embryo, Growth Factor, PZM3)

INTRODUCTION

The *in vitro* production of porcine embryos has been an important basis for the study of the generation process of mammalian embryos; furthermore, it is widely adopted for cutting-edge biotechnologies such as transgenic animal cells, development of micromanipulation (Lee et al., 2007), and stem cell studies, etc.

In general, the *in vitro* production of mammalian embryos is generally classified into maturation, fertilization and culture of embryos. Also, the system of *in vitro* embryo production technologies has been founded on the production of a number of offspring through the transplantation of *in vitro*-produced blastocysts (Kikuchi et al., 2002). However, in the case of the porcine species in particular, as compared with other animal species, nuclear maturation and cytoplasmic maturation are not coordinated, so it is difficult to obtain good quality embryos due to

unstable *in vitro* maturation (Wang et al., 1997), unstable male pronucleus production (Motlik et al., 1984), polyspermy during *in vitro* fertilization (Abeydeera and Day, 1997), and developmental delays or stoppage phenomena while in the four-cell stage during *in vitro* culture (Camous et al., 1984; Heyman et al., 1987; Uhm et al., 2009). In order to overcome these problems, numerous studies have been conducted regarding the relevant factors for *in vitro* culture using immature porcine oocytes, specifically on topics such as the optimization of culture solution components (Kim et al., 1993), the presence of co-culture with somatic cells (Xu et al., 1992), static/perfusion culture systems (Lim et al., 1996; Kim et al., 2007), supplementation of growth factor (May, 1988; Chang et al., 2002; Makarevich et al., 2006) and supplementation of antioxidant (Ali et al., 2003; Lee et al., 2004).

In general, in the culture of porcine embryos Whitten's medium, Beltsville embryo culture medium3 (BECM-3), North Carolina State University23 (NCSU-23) and Porcine zygote Medium 3 (PZM3) are used. Among these, PZM3 is most commonly used and has recently resulted in better outcomes than any other medium (Yoshioka et al., 2002). The culture medium for embryos is composed of simple minerals, energy sources, amino acids, pH buffers, trace elements and antibiotics. Recently, there have been

* Corresponding Author: Jae-Myeoung, Kim. Tel: +82-53-656-4200, Fax: +82-53-656-0198, E-mail: dangi2359@hanmail.net

¹ Department of Biotechnology, Daegu University, Gyeongsangbuk-do, 712-714, Korea.

² College of Medicine, CHA University, Gyeonggi-do, 487-801, Korea.

Received January 19, 2009; Accepted April 22, 2009

continuous studies on the effects on embryo development of growth factor, antioxidants and chelators supplemented to basic culture medium.

It was reported that the growth factor, one of the most important materials that are supplemented to an *in vitro* culture medium, controls proliferation, differentiation and shape of mammalian embryonic cells *in vivo* (Hill et al., 1992). The growth factor also has the ability to promote the development of endometrium and embryo by controlling the functions of the ovary and uterus during the gestational period (Adashi et al., 1985; Echterkamp et al., 1994). In addition, it promotes embryo development in mice (Demeestere et al., 2004), rabbits (Herrler et al., 1998), cattle (Narula et al., 1996; Moreira et al., 2002), and humans (Lighten et al., 1997), as well as increasing the overall cell numbers of blastocysts produced. Among the growth factors, insulin-like growth factor I (IGF-I) maintains the pregnancy with the interaction between endometrium and embryo (Simmen et al., 1993), and it plays an important role in inhibiting the cell death of various cell types such as hematopoietic cells (Minshall et al., 1996), fibroblasts (Kulik et al., 1997) and oocytes (Morita et al., 1999).

In contrast, regarding the cytoplasmic maturation of immature mouse, cow and human oocytes (Harper and Brackett, 1993; Park and Lin, 1993), EGF is effective for nuclear membrane disruption (Down et al., 1989; Das et al., 1991) and expansion of cumulus cells (Larson et al., 1990). Furthermore, it was reported that in mice, during the *in vitro* culture of embryos, EGF promoted the blastocoele formation (Wood and Kaye, 1989), and it also increased the blastocyst development rate of bovine and porcine embryos (Harper and Brackett, 1993; Wei et al., 2001). Although there have been comparisons between the effects of IGF-I and EGF on embryo development as well as hatching of porcine embryos, there have been no reports as yet regarding the effects of PZM3.

Therefore, in this study, the effects of IGF-I and EGF supplemented to PZM3 culture medium on the development of *in vitro* porcine embryos were examined in order to improve the understanding of culture conditions for the basal molecular metabolic processes involved in the *in vitro* production of porcine embryos.

MATERIALS AND METHODS

Chemicals and media

Unless otherwise stated, all chemicals used in this study were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Solutions are expressed as percent dilutions (v/v) and all media used for IVM, IVF, and *in vitro* culture (IVC) were pre-warmed to 39°C in a 5% CO₂ incubator with maximum humidity for 4 h before use.

Oocyte collection and *in vitro* maturation

Ovaries were collected from prepubertal gilts at a local slaughterhouse and transported to the laboratory in saline supplemented with 25 µg/ml gentamicin at 25-30°C within 2-3 h. Cumulus oocyte complexes (COCs) were obtained by aspiration from follicles 2 to 6 mm in diameter using an 18-gauge needle connected to a 10-ml disposable syringe. Only COCs with compact cumulus cell layers and evenly granulated ooplasm were selected. The COCs were washed three times in HEPES-Tyrode-albumin-lactate-pyruvate medium (TALP medium) supplemented with 25 mM HEPES and 3 mg/ml BSA. Groups of 50 COCs were placed into 500 µl of BSA-free NCSU-23 solution with 0.57 mM cysteine, 10% porcine follicular fluid (pFF), 2.5 mM β-mercaptoethanol, 10 ng/ml epidermal growth factor (EGF), 10 IU/ml human chorionic gonadotropin (hCG) and 10 IU/ml pregnant mare serum gonadotropin (PMSG) in each well of a 4-well multidish (Nunc, Roskilde, Denmark). After 22 h for maturation, oocytes were washed twice in the same maturation medium without PMSG and hCG and cultured in this medium for 22 h at 39°C in an atmosphere of 5% CO₂ and maximum humidity.

Sperm preparation and *in vitro* fertilization

Diluted porcine semen was produced by Dabby A.I. Center and stored at 17°C for 5 days. The semen was layered on top of a discontinuous Percoll density gradient (2 ml of 45% Percoll over 2 ml of 90% Percoll) in a 15-ml centrifuge tube. The sample was centrifuged for 20 min at 500×g at room temperature. The spermatozoa collected in the bottom fraction were washed three times: twice in D-PBS containing 1 mg/ml BSA, 100 µg/ml penicillin and 75 µg/ml streptomycin at 500×g for 5 minutes, and once in mTBM. The spermatozoa were diluted with mTBM to give a final concentration of 3×10⁶ spermatozoa/ml.

After the IVM period, oocytes were briefly treated with 0.1% hyaluronidase in Dulbecco's phosphate-buffered saline (D-PBS, Gibco, USA) supplemented with 1 mg/ml BSA to remove cumulus cells, and were washed 2-3 times with modified Tris-buffered medium (mTBM) containing 1 mg/ml BSA and 2.5 mM caffeine sodium benzoate. After washing, groups of 25-30 oocytes were placed in 48-µl droplets of mTBM in 60-mm petri dishes that had been covered with warm mineral oil. Two microliters of spermatozoa suspension was added to each fertilization drop, resulting in a final concentration of 2.5×10⁵ spermatozoa/ml. Oocytes and spermatozoa were co-incubated for 6 h at 39°C and 5% CO₂ with maximum humidity.

In vitro culture

The presumptive zygotes (day 0) were washed three

times with TL-HEPES solution, the impurities including sperm removed, and washed 2-3 times with PZM3 solution to which IGF-I and/or EGF (0, 1, 10 and 20 ng/ml) were supplemented. Then, the oocytes were inserted into 50 μ l of PZM3 solution and *in vitro* development conducted by culturing at 39°C and 5% CO₂ in the culture medium. After 48 h of *in vitro* culture, the fertilization rate was examined. Then, 168 h after *in vitro* culture began, the embryo development of blastocysts and hatched blastocysts was examined.

Blastocyst differential staining

The zona pellucida of the blastocysts was removed with a 0.5% protease solution and washed 4-5 times in TL-HEPES solution with 0.1% PVA (TL-PVA). Zona-free blastocysts were incubated in a 1:5 dilution of rabbit anti-bovine whole serum in TL-PVA medium for 1 h. After being washed five more times in TL-PVA medium, blastocysts were re-incubated in a 1:10 dilution of a guinea pig complement in TL-PVA medium supplemented with 4 μ g/ml propidium iodide (PI) and 4 μ g/ml bisbenzimidazole for 1 h. The presumptive stained blastocysts were mounted on a slide and the cells were counted under a fluorescence microscope (Olympus, Tokyo, Japan). The bisbenzimidazole-stained inner cell mass (ICM) nuclei fluoresced blue, and the trophectoderm (TE) nuclei, which were stained with both bisbenzimidazole and PI, fluoresced red or pink.

Statistical analysis

Data on embryo development were analyzed by the χ^2 -test. All data for cell number were arcsine-transformed and analyzed by the General Linear Models Procedure with the Statistical Analysis System (SAS; Cary, USA). Treatment means were compared with Duncan's multiple range test. *p*-values less than 0.05 were considered statistically

significant.

RESULTS

Embryo development during IGF-I supplementation

The *in vitro* fertilization rates were 66.9%, 75.8%, 68.8% and 76.7% for the treatment groups, and there were no significant differences among the groups. The blastocyst development rates were 12.1%, 8.9%, 14.6% and 12.7% for the groups, and there were no significant differences among the groups. In addition, the blastocyst hatching rates were 5.3%, 7.1%, 21.7% and 5.3%, and again, there were no significant differences among the groups.

Embryo development during the EGF supplementation

The *in vitro* fertilization rates were 74.6%, 80.7%, 69.3% and 64.9% for the treatment groups, and the highest rate was that of the 1-ng/ml treatment group, which was significantly higher than the 10-ng/ml and 20-ng/ml treatment groups (*p*<0.05). In contrast, the blastocyst development rates were 16.7%, 13.2%, 8.8% and 7.2% for the treatment groups, and the highest rate was found in the non-supplemented group, where it was significantly higher than the 20-ng/ml treatment group (*p*<0.05). Also, the blastocyst hatching rates were 15.8%, 20.0%, 0.0% and 12.5%, and there were no significant differences among the groups.

Embryo development during the simultaneous supplementation of IGF-I and EGF

The *in vitro* fertilization rates were 67.6%, 69.2%, 76.9% and 63.5% for the treatment groups, and there were no significant differences among the groups. The blastocyst development rates were 13.5%, 10.9%, 12.2% and 13.5% for the treatment groups, and showed similar trends among

Table 1. Effects of different concentrations of IGF-I supplement on the development of porcine *in vitro*-fertilized embryos

Concentration of IGF (ng/ml)	No. of oocytes examined	No. (%) of embryos developed to		
		≥Two-cell	Blastocysts	Hatched blastocysts
0	157	105 (66.9)	19 (12.1)	1 (5.3)
1	157	119 (75.8)	14 (8.9)	1 (7.1)
10	157	108 (68.8)	23 (14.6)	5 (21.7)
20	150	115 (76.7)	19 (12.7)	1 (5.3)

Table 2. Effects of different concentrations of EGF supplement on development of porcine *in vitro*-fertilized embryos

Concentration of EGF (ng/ml)	No. of oocytes examined	No. (%) of embryos developed to		
		≥Two-cell	Blastocysts	Hatched blastocysts
0	114	85 (74.6) ^{ab}	19 (16.7) ^b	3 (15.8)
1	114	92 (80.7) ^b	15 (13.2) ^{ab}	3 (20.0)
10	114	79 (69.3) ^a	10 (8.8) ^{ab}	0 (0.0)
20	111	72 (64.9) ^a	8 (7.2) ^a	1 (12.5)

^{a, b} Within the same column, values with different superscripts differ significantly (*p*<0.05).

Table 3. Effects of simultaneous IGF-I and EGF supplements on development of porcine *in vitro*-fertilized embryos

Supplement types	No. of oocytes examined	No. (%) of embryos developed to		
		≥Two-cell	Blastocysts	Hatched blastocysts
Control	148	100 (67.6)	20 (13.5)	3 (15.0)
IGF (10 ng/ml)	156	108 (69.2)	17 (10.9)	3 (17.6)
EGF (1 ng/ml)	156	120 (76.9)	19 (12.2)	2 (10.5)
IGF (10 ng/ml)+EGF (1 ng/ml)	156	99 (63.5)	21 (13.5)	2 (9.5)

the groups, while the blastocyst hatching rates were 15.0%, 17.6%, 10.5% and 9.5%, and there were no significant differences among the groups.

Number of cells

Figure 1 shows the number of cells of blastocysts produced under each condition. The total cell numbers were $20.5 \pm 4.2 \sim 22.4 \pm 4.9$ for the treatment groups, and there were no significant differences among the groups. The ICM cell numbers ranged from $3.6 \pm 2.1 \sim 7.4 \pm 3.6$, and there were no significant differences among the groups. Also, the TE cell numbers ranged from $13.5 \pm 2.4 \sim 17.1 \pm 4.5$, and there were no significant differences among the groups.

DISCUSSION

In the various types of cells in the reproductive organs, diverse growth factors such as the IGF family, colony stimulating factor-I, transforming growth factor- β , leukemia inhibitor factor, granulocyte-macrophage colony-stimulating factor, EGF, and platelet-activating factor (Schell et al., 1994; Lonergan et al., 1996; Davies et al., 2004) are produced. In particular, the growth factor that is secreted from ovaries affects both nuclear and cytoplasmic maturation in the maturation process of oocytes (Motlik and Fulka, 1986), and the above growth factors play important

roles in the development of blastocysts. Considering that the receptors for these growth factors appear on the cytoplasm surface, it was proved that as the embryo develops, these growth factors are necessary for the formation of blastocysts (Collier et al., 1990; Osterlund and Fried, 2000; Lee et al., 2005).

It was reported that among the above growth factors, IGF-I, which is a single chain polypeptide of 7.6 kDa that is structurally similar to proinsulin, is synthesized in the liver and fibroblast and found in follicular fluid (Spier and Echtenkamp, 1995), uterine tubes (Schmidt et al., 1994), uterus (Geisert et al., 1991) and ovary, so as to affect the maturation of ova as well as embryo development (Watson et al., 1999), controlling the growth as well as the division of cells. Therefore, IGF-I supplementation to *in vitro* maturation and *in vitro* culture media of mouse (Doherty et al., 1994), rabbit (Herrler et al., 1998) and human (Spanos and Becker, 2000) oocytes enhanced embryo development by decreasing necrocytosis and increasing cell division (Devreker and Hardy, 1997). In previous studies, the amount of IGF-I supplementation during *in vitro* culture was 50-100 ng/ml in the case of cows (Byrne et al., 2002; Sirisathien et al., 2003) and 30-100 ng/ml (Desai et al., 2000; Fabian et al., 2004) for mice. The cell number of blastocysts which developed under each concentration was increased (mouse: Kurzawa et al., 2002; cow: Sirisathien et al., 2003; human: Spanos et al., 2000). Specifically, in porcines, the blastocyst formation rate as well as the cell number of blastocysts was increased (Lighten et al., 1997). However, in this study, there were no significant effects with regard to embryo development due to IGF-I supplementation of the *in vitro* culture medium of porcine embryos.

Among the growth factors in the reproductive organs, EGF, which is composed of 53 amino acids, plays a role as the cell division accelerator and also promotes growth of the epithelial cell, mesoderm cell, connective tissue, nerve cell and granule cell (Shiraga et al., 1997). In addition, it also exists on uterine tube cells, uterus cells and the oocyte cell surface (Kane et al., 1992) and it has been reported to be relevant to nuclear membrane disruption (Das et al., 1991), expansion of cumulus cells of immature bovine oocytes (Larson et al., 1990) and the cytoplasmic maturation of mouse, cow and human oocytes (Harper and Brackett, 1993; Park and Lin, 1993).

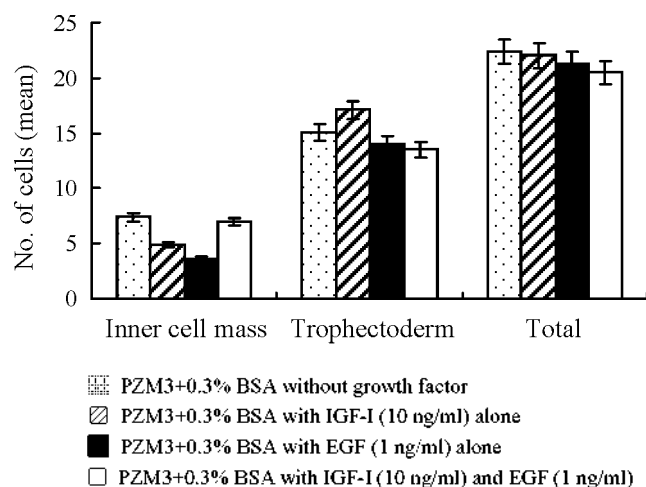


Figure 1. Effects of IGF-I and EGF supplements on cell numbers of porcine blastocysts developed from *in vitro*-produced embryos.

The effects of EGF on the *in vitro* culture of oocytes (Wood and Kaye, 1989; Werb, 1990) have also been reviewed. In the case of mice, EGF promoted the formation of blastocoes (Wood and Kaye, 1989; Paria et al., 1994) and increased blastocyst development rate of bovine embryos (Coskun et al., 1991; Harper and Brackett, 1993), as well as the cell number of blastocysts (Lee and Fukui, 1995) so as to increase embryo development rate (Wei et al., 2001). However, Abeydeera et al. (1998) reported that the supplementation of EGF to *in vitro* culture medium did not result in significant differences in blastocyst formation rate or cell numbers.

In this study, the supplementation of EGF to *in vitro* culture medium was not significantly effective and was in agreement with results of Abeydeera et al. (1998). When IGF-I and EGF were simultaneously supplemented, it was effective for the *in vitro* development of bovine (Rieger et al., 1998) and porcine (Yoon et al., 2003) embryos, but in this study the simultaneous supplementation of IGF-I and EGF to the *in vitro* culture medium did not affect blastocyst development rate or blastocyst hatching rate.

In terms of *in vitro* production of embryos, the differences in growth factors depending on animal species cannot be compared directly due to different culture conditions. However, it is considered that the effects of the growth factors on embryos were cancelled out by unknown factors due to the supplementation of protein sources such as BSA to the *in vitro* culture medium, PZM3.

REFERENCES

- Abeydeera, L. R. and B. N. Day. 1997. Fertilization and subsequent development *in vitro* of pig oocytes inseminated in a modified Tris-buffered medium with frozen-thawed ejaculated spermatozoa. *Biol. Reprod.* 57:729-734.
- Abeydeera, L. R., W. H. Wang, T. C. Cantley, A. Rieke, R. S. Prather and B. N. Day. 1998. Presence of epidermal growth factor during *in vitro* maturation of pig oocytes and embryo culture can modulate blastocyst development after *in vitro* fertilization. *Mol. Reprod. Dev.* 51:395-401.
- Adashi, E. Y., C. E. Resnick, A. J. D' Ercole, M. E. Svoboda and J. J. Van Wyk. 1985. Insulin-like growth factors as intraovarian regulators of granulosa cell growth and function. *Endocr. Rev.* 3:400-420.
- Ali, A. A., J. F. Bilodeau and M. A. Sirard. 2003. Antioxidant requirements for bovine oocytes varies during *in vitro* maturation, fertilization and development. *Theriogenology* 59:939-949.
- Byun, T. H. and S. H. Lee. 1992. Morphological and cellular criteria ovaries, follicles and oocytes for *in vitro* maturation in the pig. *Korean J. Emb. Tran.* 7:97-110.
- Camous, S., Y. Heyman, W. Menezes and Y. Menezes. 1984. Cleavage beyond the block stage and survival after transfer of early bovine embryos cultured with trophoblastic vesicles. *J. Reprod. Fertil.* 72:479-485.
- Chang, H. S., W. T. Cheng, H. K. Wu and K. B. Choo. 2000. Identification of genes expressed in the epithelium of porcine oviduct containing early embryos at various stages of development. *Mol. Reprod. Dev.* 56:331-335.
- Chollier, M., C. O'Neill, A. J. Ammit and D. M. Saunders. 1990. Measurement of human embryo-derived platelet-activating factor (PAF) using a quantitative bioassay of platelet aggregation. *Hum. Reprod.* 5:323-328.
- Coskun, S., A. Sanbuissho, Y. C. Lin and Y. Rikihisa. 1991. Fertilizability and subsequent developmental ability of bovine oocytes matured in medium containing epidermal growth factor (EGF). *Theriogenology* 36:485-494.
- Das, K., L. E. Stout, H. C. Hensleigh, G. E. Tagatz, W. R. Phipps and B. S. Leung. 1991. Direct positive effect of epidermal growth factor on the cytoplasmic maturation of mouse and human oocytes. *Fertil. Steril.* 55:1000-1004.
- Davies, S., M. C. Richardson, F. W. Anthony, D. Mukhtar and I. T. Cameron. 2004. Progesterone inhibits insulin-like growth factor binding protein-1 (IGFBP-1) production by explants of the Fallopian tube. *Mol. Hum. Reprod.* 10:935-939.
- Demeestere, I., C. Gervy, J. Centner, F. Devreker, Y. Englert and A. Delbaere. 2004. Effect of insulin-like growth factor-I during preantral follicular culture on steroidogenesis, *in vitro* oocyte maturation and embryo development in mice. *Biol. Reprod.* 70:1664-1669.
- Desai, N., J. Lawson and J. Goldfarb. 2000. Assessment of growth factor effects on post-thaw development of cryopreserved mouse morulae to the blastocyst stage. *Hum. Reprod.* 15:410-418.
- Devreker, F. and K. Hardy. 1997. Effects of glutamine and taurine on preimplantation development and cleavage of mouse embryos *in vitro*. *Biol. Reprod.* 57:921-928.
- Doherty, A. S., G. L. Temeles and R. M. Schultz. 1994. Temporal pattern of IGF-I expression during mouse preimplantation embryo genesis. *Mol. Reprod. Dev.* 37:21-26.
- Down, S. M. 1989. Specificity of epidermal growth factor action on maturation of the murine oocytes and cumulus oophorus *in vitro*. *Biol. Reprod.* 41:371-379.
- Echtemkamp, S. E., L. J. Spicer, J. Klindt and R. K. Vernon. 1994. Administration of porcine somatotropin by a sustained-release implant: Effects on follicular growth, concentrations of steroids and insulin-like growth factor I, and insulin-like growth factor binding. *J. Anim. Sci.* 72:2431 (Abstr.).
- Fabian, D., G. Il'kova, P. Rehak, S. Czikkova, V. Baran and J. Koppel. 2004. Inhibitory effect of IGF-I on induced apoptosis in mouse preimplantation embryos cultured *in vitro*. *Theriogenology* 61:745-755.
- Geisert, R. D., C. Y. Lee, F. A. Simmen, M. T. Zary, A. E. Fliss and F. W. Bazer. 1991. Expression of messenger RNAs encoding insulin-like growth factor-I, II, and insulin-like growth factor binding protein-2 in bovine endometrium during the estrous cycle and early pregnancy. *Biol. Reprod.* 45:975-983.
- Harper, K. M. and B. G. Brackett. 1993. Bovine blastocysts development after *in vitro* maturation in a defined medium with epidermal growth factor and low concentrations of gonadotropins. *Biol. Reprod.* 48:409-416.
- Herrler, A., C. A. Krusche and H. M. Beier. 1998. Insulin and Insulin-like growth factor-I promote rabbit blastocyst development and prevent apoptosis. *Biol. Reprod.* 59:1302-1310.

- Heyman, Y., Y. Menezes, P. Chesne, S. Camous and V. Garnier. 1987. *In vitro* cleavage of bovine and ovine early embryos: Improved development using co-culture with trophoblastic vesicles. *Theriogenology* 27:59-68.
- Hill, D. J. and J. Hogg. 1989. Growth factors and the regulation of pre- and postnatal growth. *Baillieres Clin. Endocrinol. Metab.* 3:579-625.
- Hill, D. J. 1992. Peptide growth factor interaction in embryonic and fetal growth. *Horm. Res.* 38:199-202.
- 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.
- Kikuchi, K., A. Onishi, N. Kashiwazaki, M. Iwamoto, J. Noguchi, H. Kaneko, T. Akita and T. Nagai. 2002. Successful piglet production after transfer of blastocysts produced by a modified *in vitro* system. *Biol. Reprod.* 66:1033-1041.
- Kim, J. H., K. Niwa, J. M. Lim and K. Okuda. 1993. Effects of phosphate, energy substrates, and amino acids on development of *in vitro*-matured, *in vitro*-fertilized bovine oocytes in a chemically defined, protein-free culture medium. *Biol. Reprod.* 48:1320-1325.
- Kim, J. Y., S. B. Kim, M. C. Park, H. Park, Y. S. Park, H. D. Park, J. H. Lee and J. M. Kim. 2007. Addition of macromolecules to PZM-3 culture medium on the development and hatching of *in vitro* porcine embryos. *Asian-Aust. J. Anim. Sci.* 20:1820-1826.
- Kulik, G., A. Klippe and M. J. Weber. 1997. Antiapoptotic signaling by the insulin-like growth factor I receptor, phosphatidylinositol 3-kinase and Akt. *Mol. Cell. Biol.* 17:1595-1606.
- Kurzawa, R., W. Glabowski, T. Baczowski and P. Brelik. 2002. Evaluation of mouse preimplantation embryos exposed to oxidative stress cultured with insulin-like growth factor-I and II epidermal growth factor, insulin, transferrin and selenium. *Reprod. Biol.* 2:143-162.
- Larson, S. F. and J. E. Parks. 1990. *In vitro* maturation and fertilization of bovine oocytes in defined medium. *Biol. Reprod.* 92:156 (Abstr.).
- Lee, E. S. and Y. Fukui. 1995. Effects of various growth factors in a defined culture medium on *in vitro* development of bovine embryos mature and fertilized *in vitro*. *Theriogenology* 44:71-83.
- Lee, G. S., H. S. Kim, S. H. Hyun, H. Y. Jeon, D. H. Nam, Y. W. Jeong, S. Kim, J. H. Kim, S. K. Kang, B. C. Lee and W. S. Hwang. 2005. Effect of epidermal growth factor in preimplantation development of porcine cloned embryos. *Mol. Reprod. Dev.* 71:45-51.
- Lee, H. K., C. K. Lee, B. K. Yang, G. J. Jeon, K. D. Choi, S. S. Lee, H. S. Kong and H. Y. Jang. 2004. Expression of the antioxidant enzyme and apoptosis genes in *in vitro* maturation / *in vitro* fertilization porcine embryos. *Asian-Aust. J. Anim. Sci.* 17:33-38.
- Lee, Y. S., S. A. Ock, S. K. Cho, B. G. Jeon, T. Y. Kang, S. Balasubramanian, S. Y. Choe and G. J. Rho. 2007. Effect of donor cell type and passage on preimplantation development and apoptosis in porcine cloned embryos. *Asian-Aust. J. Anim. Sci.* 20:711-717.
- Lighten, A. D., K. Hardy, R. M. Winston and G. E. Moore. 1997. Expression of mRNA for the insulin-like growth factors and their receptors in human preimplantation embryos. *Mol. Reprod. Dev.* 47:134-139.
- Lim, J. M., B. C. Reggio, R. A. Godke and W. Hansel. 1996. Culture of bovine embryos in a dynamic culture system: Effect of bovine oviduct epithelial cells on the development of 8-cell embryos to the blastocyst stage. *Annual conf.* 199.
- Loneragan, P., C. Carolan, A. Van Langendonck, I. Donnay, H. Khati and P. Mermillod. 1996. Role of epidermal growth factor in bovine oocyte maturation and preimplantation embryo development *in vitro*. *Biol. Reprod.* 54:1420-1429.
- Makarevich, A. V., P. Chrenek and P. Flak. 2006. The influence of microinjection of foreign gene into the pronucleus of fertilized egg on the preimplantation development, cell number and diameter of rabbit embryos. *Asian-Aust. J. Anim. Sci.* 19:171-175.
- May, J. V., J. P. Frost and D. W. Schomberg. 1988. Differential effects of epidermal growth factor, somatomedin-C/insulin-like growth factor I and transforming growth factor-beta on porcine granulosa cell DNA synthesis and cell proliferation. *Endocrinology* 123:168-179.
- Minshall, C., S. Arkins, G. G. Freund and K. W. Kelly. 1996. Requirement for phosphatidylinositol 3'-kinase to protect hemopoietic progenitors against apoptosis depends upon the extracellular survival factor. *J. Immunol.* 156:939-947.
- Moreira, F., F. F. Paula-Lopes, P. J. Hansen, L. Badinga and W. W. Thatcher. 2002. Effects of growth hormone and insulin-like growth factor-I on development of *in vitro* derived bovine embryos. *Theriogenology* 57:895-907.
- Morita, Y., T. F. Manganaro, X. J. Tao, S. Martimbeau, P. K. Donaho and J. L. Tilly. 1999. Requirement for phosphatidylinositol 3-kinase in cytokine-mediated germ cell survival during fetal oogenesis in the mouse. *Endocrinology* 140:941-949.
- Motlik, J. and J. Fulka. 1986. Factors affecting meiotic competence in pig oocytes. *Theriogenology* 25:87-96.
- Motlik, J., N. Grozet and Fulka. 1984. Meiotic competence *in vitro* of pig oocytes isolated from early antral follicles. *J. Reprod. Fertil.* 72:323-328.
- Narula, A., M. Tanej and S. M. Totey. 1996. Morphological development, cell number, and allocation of cells to trophoblast and inner cell mass of *in vitro* fertilized and parthenogenetically developed buffalo embryos: the effect of IGF-I. *Mol. Reprod. Dev.* 44:343-351.
- Osterlund, C. and G. Fried. 2000. TGFbeta receptor types I and II and the substrate proteins Smad 2 and 3 are present in human oocytes. *Mol. Hum. Reprod.* 6:498-503.
- 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.
- Park, Y. S. and Y. C. Lin. 1993. Effect of epidermal growth factor and defined simple media on *in vitro* bovine oocyte maturation and early embryonic development. *Theriogenology* 39:475-484.
- Schell, D. L., P. A. Mavrogianis, A. T. Fazleabas and H. G. Verhage. 1994. Epidermal growth factor, transforming growth factor-alpha, and epidermal growth factor receptor localization in the baboon (*Papioanubis*) oviduct during steroid treatment and the menstrual cycle. *J. Soc. Gynecol. Investig.* 1:269-276.

- Schmidt, A., R. Einspanier, W. Amselgruber, F. Sinowatz and D. Schams. 1994. Expression of insulin-like growth factor I (IGF-I) in the bovine oviduct during the oestrous cycle. *Exp. Clin. Endocrinol.* 102:364-369.
- Shiraga, M., S. Takahashi, T. Miyahke, S. Takeuchi and H. Fukamachi. 1997. Insulin-like growth factor-I stimulates proliferation of mouse uterine epithelial cells in primary culture. *Proc. Soc. Exp. Biol. Med.* 215:412-417.
- Simmen, R. C. M., Y. Ko and F. A. Simmen. 1993. Insulin-like growth factors and blastocyst development. *Theriogenology* 39:163-175.
- Sirisathien, S., H. J. Hernandez-Foneseca and B. G. Brackett. 2003. Influences of epidermal growth factor and insulin-like growth factor-I on bovine blastocyst development *in vitro*. *Anim. Reprod. Sci.* 77:21-32.
- Spanos, S., D. L. Becker, R. M. Winston and K. Hardy. 2000. Anti-apoptotic action of insulin-like growth factor-I during human preimplantation embryo development. *Biol. Reprod.* 63:1413-1420.
- Spier, L. J. and S. E. Echternkamp. 1995. The ovarian insulin-like growth factor system with an emphasis on domestic animals. *Domest. Anim. Endocrinol.* 12:223-245.
- Uhm, S. J., M. K. Gupta, H. J. Chung, J. H. Kim, C. K. Park and H. T. Lee. 2009. Relationship between developmental ability and cell number of day 2 porcine embryo produced by parthenogenesis or somatic cell nuclear transfer. *Asian-Aust. J. Anim. Sci.* 22:483-491.
- Wang, W. H., L. R. Abedeera, T. C. Cantley and B. N. Day. 1997. Effect of oocyte maturation media on development of pig embryos or produced by *in vitro* fertilization. *J. Reprod. Fertil.* 111:101-108.
- Watson, A. J., M. E. Westhusin and Q. A. Winger. 1999. IGF paracrine and autocrine interactions between conceptus and oviduct. *J. Reprod. Fertil. Suppl.* 54:303-315.
- Wei, Z., K. W. Park, B. N. Day and R. S. Prather. 2001. Effect of epidermal growth factor on preimplantation development and its receptor expression in porcine embryos. *Mol. Reprod. Dev.* 60:457-462.
- Werb, Z. 1990. Expression of EGF and TGF- α genes in early mammalian development. *Mol. Reprod. Dev.* 27:10-15.
- Wood, S. A. and P. L. Kaye. 1989. Effects of epidermal growth factor on preimplantation mouse embryo. *J. Reprod. Fertil.* 85:575-582.
- Xu, X. J., Q. Z. Yang, R. C. Qian and Z. Nakahara. 1992. Development of *in vitro* fertilized bovine ova in two co-culture systems. *Anim. Reprod.* 1:372-374.
- Yoon, S. Y., S. Y. Lee, H. T. Cheong, B. K. Yang, C. I. Kim and C. K. Park. 2003. Effects of growth and hexoses on *in vitro* development in porcine embryos. *Korean J. Anim. Reprod.* 27: 249-258.
- Yoshika, K., C. Suzuki, A. Tanaka, I. M. Anas and S. Iwamura. 2002. Birth of piglets derived from porcine zygotes cultured in a chemically defined medium. *Biol. Reprod.* 66:112-119.