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Immune factors in human embryo culture and their significance

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***Summary.** There is increasing evidence that human development before implantation is regulated by embryonically and maternally derived growth factors. The "regulators" of embryonic origin such as soluble human leukocyte antigen G, platelet-activating factor, Th1/Th2 cytokines, insulin-like growth factor, epidermal growth factor, transforming growth factor α, colony-stimulating factor, platelet-derived growth factor may be used as indicators of embryo viability and implantation potential. The data prove the influence of growth factors on the development and growth of preimplantation embryos. Though there is a lot of research in the field of biomarkers during folliculogenesis and maternal-fetal interface, only few of them deal with regulators derived from embryonic cells to the cultivation medium. The aim of our study was to summarize the research dealing with immune markers produced by embryos in vitro and to estimate their impact on the cell growth, viability and implantation potential.*

Introduction

A number of various exogenous and endogenous factors have been attributed to the regulation of embryo development and implantation. These embryonic "regulators" may be used as indicators of embryo viability and implantation potential. The data prove a cooperative interaction among preimplantation embryos and the role of growth factors on their development and growth. Human embryos produce transforming growth factor α and insulin-like growth factors, which are significantly involved in embryo ability to develop to the blastocyst stage (1). Platelet-activating factor (2), also produced by human embryos, is shown to be one of the markers of embryo viability. Menicuci et al. (3) reported an interaction between embryo implantation potential and soluble human leukocyte antigen G (sHLA-G) in early embryo cultures, and Fuzzi et al. (4) proved it later. It is clear that locally secreted cytokines of both the embryonic and the endometrium origin control the implantation process. Focusing on fetal-maternal dialogue during the implantation period promises to open a new era in assisted reproduction techniques that will be based on diagnostics of missing signaling molecules and impairments of uterine receptivity as well as on applications of embryo cultivating and transferring media.

Increasing interest is now being addressed to the soluble forms of biomarkers, because they might have prognostic properties in implantation and pregnancy process. Therefore, the aim of our study was to summarize research dealing with immunological markers produced by embryos to cultivation medium and their impact on the cell growth, viability, and implantation potential.

Soluble human leukocyte antigen G

sHLA-G is a nonclassical HLA class I molecule that can be expressed in membrane-bound or soluble form and is well known for its tolerogenic properties. Data show that HLA-G promotes the production of Th2 cytokines, down-regulates the production of Th1 cytokines (5), and protects cells against natural killer (NK) cell lysis (6). Taking into account the evidence, there is no doubt that sHLA-G of embryonic origin plays an important role in implantation process and is responsible for fetal allograft tolerance.

Investigations of sHLA-G as a possible marker of embryo developmental potential started in 1996, when Juriscova et al. (7) reported data on total mRNA and protein in human embryos. In 1999, Menicucci et al. (3) evaluated the presence of sHLA-G molecules in supernatant cultures of early human embryos obtained by in vitro fertilization (IVF). The similar study of 101 patients (4) showed that clinical pregnancy was obtained only if sHLA-G molecules were detected in culture supernatants of growing embryos. Until now, embryo selection is based only on morphological and cleavage criteria, though embryo viability is not strictly correlated with embryo quality (8, 9). sHLA-G may be used as a marker for

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embryo selection, because its production correlates with embryo viability and implantation potential, but not with percentage of fragmentation and size of blastomeres. Gardner and Sakkas (10) suggested that by selecting specific embryos for transfer based on their individual sHLA-G expression, pregnancy and implantation rates can be maximized while the number of embryos transferred can be reduced; therefore, the incidence of high-order multiple pregnancies could be minimized. In 2005, Noci et al. (11) published article where sHLA-G was proposed as a potential marker of embryo development, and enzyme-linked immunosorbent assay (ELISA) was introduced as a useful biochemical assay in addition to embryo morphology in embryo selection.

According to http://www.haveababy.com/, the detection of sHLA in vitro is a revolutionary new test that seems to be highly predictive of the ability of individual embryos to successfully produce a pregnancy following IVF. The one-year study carried out by Sher et al. (12) indicates that individually cultured embryos producing sufficient concentrations of a marker, sHLA-G, give previously infertile women a better than 60% pregnancy success rate, which is double the current success rates for IVF. In the study, the presence of high levels of sHLA-G had a positive predictive value greater than 70% in women under the age of 39 and greater than 50% in women aged 39 to 44 years.

Platelet-activating factor

Platelet-activating factor (PAF) is a lipid mediator endowed with a diversity of biological effects on various cells and tissues. PAF is a signaling phospholipid that has pleiotropic biological properties. Exogenous PAF binds to the cell surface receptors initiating the formation of inositol triphosphate and diacylglycerol (13), which affects intracellular levels of calcium. Calcium regulates the activity of signal transducing molecules and influences embryonic development.

The preimplantation embryos of a number of species – human, mouse, sheep, rabbit, pig – secrete PAF (14). The highest level of these molecules can be measured in the medium of expanded blastocysts. Evidence indicates that PAF has an important function in autocrine stimulation of embryonic metabolism, growth, and viability (15). Exogenous PAF stimulates embryo metabolism and cell division (16). Punjabi et al. (17) showed that PAF concentration was higher in media associated with clinical pregnancies when compared to preclinical pregnancies. The production of PAF by the preimplantation embryo was not related to follicle size or embryo morphology. However, differences in PAF concentrations in the culture media were related to the age of the embryo culture medium and the developmental stage of the embryo. Later Roudebush et al. (18) showed that patients who became pregnant had a higher PAF level in their culture mediums than patients who did not become pregnant. Embryo-derived platelet-activating factor may be an important mediator of early maternal recognition of pregnancy.

Th1/Th2 cytokines

Cytokines are low-molecular weight glycoproteins that exhibit pleiotropy and redundancy. Cytokines are responsible for intercellular interactions (19). Cytokines secreted by the embryos and cells within the uterus are important for implantation process, because can be responsible for causing miscarriages. The activity of these cytokines has been characterized as proinflammatory and anti-inflammatory. Prolonged exposure to Th1 cytokines is detrimental to pregnancy, while Th2 cytokines are necessary to stimulate the invasion of the blastocyst and formation of blood vessels during the implantation period.

Trophoblastic cells, as well as uterine epithelium and maternal immune cells, secrete cytokines, which promote immunotolerance. Some of these cytokines are transforming growth factor beta, progesterone-induced blocking factor, and regeneration and tolerance factor. The sources of proinflammatory cytokines, such as interleukins (IL), chemokines, tumor necrosis factor α (TNF-α), are macrophages and NK cells, which infiltrate the implantation sites destined to pregnancy loss. T cells produce anti-inflammatory cytokines – interferons (IFN) and IFN-like cytokines.

Human trophoblast in the fetal-maternal interface has been shown to produce IL-1 (20), IL-2 (21), IL-4 (20, 22, 23), TNF-α (20). Interleukin-10 was first recognized for its ability to inhibit activation and effector function of T cells, monocytes, and macrophages. IL-10 has a negative effect on macrophage activation and IFN-γ secretion. Interferon-γ, along with other Th2 cytokines, is related to antiviral, antiproliferative, and immunomodulatory activity. According to Wegman et al. (24), interleukin-10 is a potent immunomodulatory cytokine and promotes development of a humoral or T-helper 2 type immune response, which is associated with a successful outcome to pregnancy. Other authors showed that IL-10 is not associated with pregnancy, followed by embryo transfer after in vitro fertilization procedure, rate (19). According to Ozornec et al. (25), no effect of IL-10 and IFN-γ on fertilization process could be shown. Preimplantation human embryos express interleukin-1 (26), and this system may play an important role in embryo implantation (27). Zolti et al. (28) showed that IL-6 is released by cultured oocytes and early stage embryos. This fact suggests a potential role of IL-6 in embryogenesis.

IL-4, secreted by the maternal decidual tissue and by the developing embryo, may stimulate the production of IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1), macrophage colony-stimulating factor (M-CSF). Successful embryo implantation requires Th1/Th2 cytokine balance. The expression of these cytokines and growth factors is thought to be important in implantation and maintenance of early pregnancy (29).

Insulin-like growth factors

Insulin-like growth factors (IGF) I and II are the only members of the insulin-like family of growth factors. They have their own features different from proinsulin: are secreted by many cells and circulate in extracellular fluid, bound to IGF-binding proteins. IGF-I stimulates glucose uptake and is well known for its significance in the cell cycle (30). IGF-II is responsible for the growth rate during fetal development (31). In human embryos, transcripts for both insulin-like growth factors receptors – IGF-1R and IGF-2R – were proved by reverse transcription polymerase chain reaction (32). While searching for ligands, only IGF-II transcripts were detected in human embryo culture (32). IGF-II ligands are provided in human oocytes and persist through preimplantation development from fertilized oocyte to blastocyst. Hemmings et al. (1) proved that IGF-II is produced by human embryos. In cultivating medium, the concentration of IGF-II increases with the growth of embryo and is well detected at the time of morula-to-blastocyst transformation. Insulin-like growth factors are associated with effects on cell proliferation and differentiation in mouse embryos (33). Many authors investigated the effect of IGF-I, IGF-II, and their receptors employing mouse embryos carrying null mutations of the genes encoding these factors and their receptors (34–36). Studies showed that mentioned growth factors cannot be attributed to the major factors influencing preimplantation development, but perhaps they play some minor role.

Epidermal growth factor and transforming growth factor *α*

Epidermal growth factor (EGF) and transforming growth factor *α* (TGF-*α*) are the members of the epidermal growth factor family with similar amino acid sequence and ability to bind to the EGF receptor. In several species, EGF stimulates cell proliferation and differentiation (37), enhances mitogenesis, development, and implantation. Paria and Dey (38) showed that the development of singly cultured mouse embryos was markedly improved by addition of epidermal growth factor: blastocysts in the presence of EGF showed a higher incidence of zona hatching compared with those cultured singly in the absence of EGF. Earlier it was suggested that EGF might have a role in the differentiation of the trophectoderm cells (39). Transcripts for EGF were detected in human unfertilized oocytes and embryos between 8-cell and blastocyst stages on day 3 to 6 postinsemination (40). Due to the coculture of mouse embryos, the cell number and proportion developing to the blastocyst stage could be increased.

TGF, produced by embryo, may be bound to a transmembrane protein from which it is released by proteolytic cleavage. Hemmings et al. (1) showed that human embryos produce TGF-*α* to the cultivation medium and that activity of this protein can be documented on day 3 after fertilization. TGF-*α* may be involved in blastocyst growth, implantation, and early postimplantation development (41). Paria and Dey (38) found that TGF-*α* stimulated the development of 2-cell mouse embryos to blastocysts. Data show that TGF-*α* stimulates protein secretion into the blastocoels (42), affects formation of specific blastocyst proteins in mouse (43), and stimulates blastocyst growth in rabbits (44). Paria and Dey (38) suggested that TGF-*α* produced by the embryo participates in morula-to-blastocyst division, zona shedding, and blastocyst activation in an autocrine manner. Machida et al. (45) demonstrated the effect of growth factors on the mouse embryos. The results suggest that EGF and TGF-*α* play an important role in the implantation process by directly stimulating trophoblast development. There are multiple studies presenting clear evidence that specific growth factors of embryonic origin participate in preimplantation embryo development and blastocyst functions in an autocrine/paracrine way.

Platelet-derived growth factor

Platelet-derived growth factor B (PDGF-B) is a member of the platelet-derived growth factor family. The four members of this family are mitogenic factors for the cells of mesenchymal origin. Proteins can exist either as a homodimer (PDGF-BB) or as a heterodimer with the platelet-derived growth factor alpha polypeptide (PDGF-AB). PDGF is expressed in mouse preembryos (46) and in human placental cells (47). Lopata and Oliva (48) showed that PDGF receptor is expressed in human blastocysts as well. Transcripts for PDGF in human embryos were detected by reverse transcription-polymerase chain reaction (RT-PCR) (49). The authors proved the presence of PDGF-A at the oocyte, 8-cell, morula, and blastocyst stage, but not at 4-cell stage. According to Osterlund et al. (49), no RT-PCR products for PDGF B were detected at any developmental stage of human embryos. Although earlier Svalander et al. (50) reported PDGF presence in human blastocyst culture, no one later was able to prove these findings. Taking into account that PCR is considered

one of the most sensitive methods, it seems more likely that human embryos produce only PDGF-A. There is no doubt that PDGF is essential for formation of tissues and fetal development. Still little is known about the impact of PDGF A on preimplantation embryos. Taking into account that PDGF-A transcripts were found not in all embryos of the same developmental stage (49), there must be some correlation between embryo quality and developmental potential. Further investigation is required.

Colony-stimulating factor

Colony-stimulating factor 1 (CSF-1) is the growth factor for cells of the monocyte-macrophage lineage. It is known to support the proliferation, survival, and differentiation of macrophages and to induce macrophages to secrete cytokines and proteases. CSF-1 has been suggested to play a role in trophoblastic growth and embryonic development. Observation was made that during pregnancy CSF-1 is secreted by the uterine epithelium with the coordinate expression of CSF-1R in the trophoblasts. Macrophage colony-stimulating factor accelerates the formation of the blastocyst cavity and increases embryonic cell number (51). Gene transcripts for the CSF-1 were not detected in mouse preimplantation embryos (52). Human embryos cultured in GM-CSF are twice as likely to reach the blastocyst stage of de-

Table 1. Expression of ligands of preimplantation human embryo-derived immune factors

Factor	2–4-cell stage	6–8-cell stage	Blastocyst	Reference
sHLA-G	+	+		11
	+			12
PAF		+		18
CSF-1	+	+		28
	–	–	–	58
PDGF-A			+	49
PDGF-B			+	50
	–	–	–	49
IGF-I		+		59
	–	–	–	32
IGF-II			+	1
TGF-α			+	1
		+	+	40
	+	+		59
EGF		+	+	40
IL	+	+	+	28
	+	+	+	58
	+	+	+	60

Notes: +, ligand found; –, ligand not found. sHLA-G, soluble human leukocyte antigen G; PAF, platelet-activating factor; CSF-1, colony-stimulating factor 1; PDGF-A, platelet-derived growth factor A; PDGF-B, platelet-derived growth factor B; IGF- I, insulin-like growth factor I; IGF- II, insulin-like growth factor II; TGF-α, transforming growth factor α; EGF, epidermal growth factor; IL, interleukin.

Table 2. Effect of preimplantation human embryo-derived immune factors on cell development and implantation

Factor	Blastocyst formation	Cell proliferation and differentiation	Embryo implantation	Pregnancy rate
sHLA-G	n. d.	+ (63)	+ (12)	+ (61)
		+ (11)		+ (12)
				+ (11)
PAF	n. d.	n. d.	n. d.	+ (2)
				+ (18)
CSF	n. d.	n. d.	n. d.	n. d.
PDGF	n. d.	n. d.	n. d.	n. d.
IGF	+ (32)	+ (32)	n. d.	0 (63)
	+ (64)	+ (64)		
EGF	+ (65)	+ (37)	n. d.	+ (63)
IL	n. d.	n. d.	+ (27)	0 (19)

Notes: +, positive impact; 0, no impact; n.d., no data. sHLA-G, soluble human leukocyte antigen G; PAF, platelet-activating factor; CSF, colony-stimulating factor; PDGF, platelet-derived growth factor; IGF, insulin-like growth factor; EGF, epidermal growth factor; IL, interleukin. Numbers in brackets mean reference number.

velopment and have increased cell numbers both in the inner cell mass and trophectoderm (53) with reduced apoptosis (54). In both mouse and human embryos, the effects of GM-CSF are mediated through binding to the subunit of the GM-CSF receptor (54, 55). Experiments with mouse embryos (deficiency of CSF-1 leads to the reduced number of blastomeres) (56) strongly suggest that this factor may play important roles in cell development and growth.

Concluding remarks

There are multiple factors responsible for cell fertilization, differentiation, proliferation, and implantation processes. The ultimate goal in in vitro fertilization cycle is implantation. However, the success of assisted reproductive technologies (ART) (implantation, pregnancy, birth rate) seems to be far from desired. Data from 75 individual groups presented in "The World Collaborative Report on in Vitro Fertilization and Embryo Replacement: Current State of the Art in January 1984" (57) showed an overall clinical pregnancy rate of 14.2%. Until now, the probability of a successful pregnancy during an IVF cycle is approximately 18%, with a baby take-home rate of about 14%. These numbers are not very different from the numbers occurring during natural cycles and can vary throughout different IVF clinics around the world. Patients and specialists are extremely interested in the possibility to find new methods to increase fertilization, implantation, and pregnancy rate after ART procedures.

One of the main tasks leading to the success of IVF procedure is the selection of an embryo with the best chances to implant. Now embryo selection is based only on morphological and cleavage criteria, although embryo viability is not strictly correlated with embryo quality. There is no doubt that creating noninvasive techniques for the selection of the best embryo is of major significance. The detection of soluble markers in the surrounding environment may be the way to control embryo selection and implantation in its very early stage without damage to the patient and embryo.

There is increasing evidence that human development before implantation is regulated by embryonically and maternally derived growth factors. Studies on other mammalian species have shown that the preimplantation embryo expresses a number of immune factors (Table 1). Furthermore, a number of growth factors have been shown to affect embryo development to the blastocyst stage, number of blastomeres, and implantation (Table 2). They also influence metabolism and apoptosis of cells. Supplementation of culture medium with exogenous growth factors affects cell fate, development, and metabolism of embryos in vitro. Therefore, an interest in the molecular factors involved in each phase of implantation and early pregnancy is very understandable. Hopefully, research in this area will help to reveal the mechanism controlling reproduction and will enable us to achieve better results in infertility treatment.

Žmogaus embrionų kultūros imuniniai veiksniai ir jų svarba

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Raktažodžiai: imuniniai veiksniai, embrionas, apvaisinimas mėgintuvėlyje.

Santrauka. Pateikiama vis daugiau įrodymų, kad ikiimplantacinį žmogaus vystymąsi reguliuoja embrioninės ir motininės kilmės augimo veiksniai. Tokie „reguliatoriai", kaip tirpus žmogaus leukocitų antigenas G, trombocitų aktyvuojamasis faktorius, Th1/Th2 citokinai, epidermio augimo faktorius, transformuojamasis augimo faktorius α, kolonijas stimuliuojamasis faktorius, trombocitų augimo faktorius, gali būti svarbūs nuspėjant embriono gyvybingumo ir implantacijos potencialą. Duomenys patvirtina augimo faktorių įtaką ikiimplantacinio embriono vystymuisi. Daugelis tyrimų, susijusių su biologiniais žymenimis, apima folikulogenezės ir implantacijos etapus, o tyrimų, kurie tyrinėtų embrioninės kilmės veiksnius *in vitro*, yra vos keli. Šiame straipsnyje siekėme apibendrinti tyrimų duomenis apie embrionų į auginimo terpę išskiriamus imuninius veiksnius bei jų svarbą ląstelės vystymuisi, gyvybingumui ir implantacijos potencialui.

References

1. Hemmings R, Langlais J, Falcone T, Granger L, Miron P, Guyda H. Human embryos produce transforming growth factors alpha activity and insulin-like growth factors II. Fertil Steril 1992;58(1):101-4.
2. O'Neill C, Gidley-Baird AA, Pike IL, Saunders DM. Use of a bioassay for embryo-derived platelet-activating factor as a means of assessing quality and pregnancy potential of human embryos. Fertil Steril 1987;47(6):969-75.
3. Menicucci A, Noci I, Fuzzi B, Criscuoli L, Scarselli G, Barricordi O. Nonclassic sHLA class I in human oocyte culture medium. Hum Immunol 1991;60:1054-7.
4. Fuzzi B, Rizzo R, Criscuoli L, Noci I, Melchiorri L, Scarselli B, et al. HLA-G expression in early embryos is a fundamental prerequisite for the obtainment of pregnancy. Eur J Immunol 2002;32(2):311-5.
5. Kanai T, Fujii T, Kozuma S, Fujii T, Kozuma S, Yamashita T, et al. Soluble HLA-G influences the release of cytokines

from allogeneic peripheral blood mononuclear cells in culture. Mol Hum Reprod 2001;7(2):195-200.
6. Riteau B, Manier C, Khalil-daher I, Martonozzi S, Pla M, Dausset J, et al. HLA-G1 co-expression boosts the HLA class I-mediated NK lysis inhibition. Int Immunol 2001;13(2):193-201.
7. Juriscova A, Casper RF, MacLusky NJ, Mills GB, Librach CL. HLA-G expression during preimplantation human embryo development. Proc Natl Acad Sci U S A 1996;93:161-5.
8. Graham J, Han T, Porter R, Levy M, Stilman R, Tucker M J. Day 3 morphology is a poor predictor of blastocyst quality in extended culture. Fertil Steril 2000;74(3):495-7.
9. Balaban B, Urman B, Alatas B, Mercan C, Aksoy S, Isiklar A. Blastocyst-stage transfer of poor-quality cleavage-stage embryos results in higher implantation rates. Fertil Steril 2001;75(3):514-8.
10. Gardner DK, Sakkas D. Assessment of embryo viability: the ability to select a single embryo for transfer – a review. Placenta 2003;24:5-12.
11. Noci I, Fuzzi B, Rizzo R, Melchiorri L, Criscuoli L, Diabizzi S, et al. Embryonic soluble HLA-G as a marker of developmental potential in embryos. Hum Reprod 2005;20(1):138-46.
12. Sher G, Keskintepe L, Nouriani M, Roussev R, Batzofin J. Expression of sHLA-G in supernatants of individually cultured 46-h embryos: a potentially valuable indicator of "embryo competency" and IVF outcome. Reprod Biomed Online 2004;9:74-8.
13. Lapetina EG. Platelet-activating factor stimulates the phosphatidylinositol cycle. Appearance of phosphatidic acid is associated with the release of serotonin in horse platelets. J Biol Chem 1982;257(13):7314-7.
14. O'Neill C. A physiological role for PAF in the stimulation of mammalian embryonic development. Trends Pharmacol Sci 1991;12(3):82-4.
15. O'Neill C. PAF promotes the development of the preimplantation embryo. Adv Exp Med Biol 1996;416:255-61.
16. Roudebush WE, Duralia DR, Butler WJ. Effect of platelet-activating factor (PAF) on preimplantation mouse B6D2F1/J embryo formation. Am J Reprod Immunol 1996; 35(3): 272-6.
17. Punjabi U, Vereecken A, Delbeke L, Angle M, Gielis M, Gerris J, et al. Embryo-derived platelet activating factor, a marker of embryo quality and viability following ovarian stimulation for in vitro fertilization. J In Vitro Fert Embryo Transf 1990;7(6):321-6.
18. Roudebush WE, Wininger JD, Jones AE, Wright G, Toledo AA, Kort HI, et al. Embryonic platelet-activating factor: an indicator of embryo viability. Hum Reprod 2002; 17(5):1306-10.
19. Vujisic S, Zidovec S. Follicular immunology environment and the influence on in vitro fertilization outcome. Current Women's Health Reviews 2005;1:49-60.
20. Haynes MK, Jackson LG, Tuan RS. Cytokine production in first trimester chorionic villi: detection of mRNAs and protein products in situ. Cell Immunol 1993;151:300-8.
21. Boehm KD, Kelley MF, Ilan J. The interleukin 2 gene is expressed in the syncytiotrophoblast of the human placenta. Proc Natl Acad Sci USA 1989;86:656-60.
22. Jones CA, Williams KA, Finlay-Jones JJ. Interleukin 4 production by human amnion epithelial cells and regulation of its activity by glycosaminoglycan binding. Biol Reprod 1995;52;839-47.
23. De Moraes-Pinto MI, Vince GS, Flanagan BF. Localization of IL-4 and IL-4 receptors in the human term placenta, decidua and amniochorionic membranes. Immunology 1997;90:87-94.
24. Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? Immunol Today 1993;14:353-6.
25. Ozornek MH, Bielfeld P, Krussel JS, Maustafa M, Mikat-Drodzynski B, Koldovsky U, et al. Interferon gamma and interleukin 10 levels in preimplantation embryo culture media. Assisted Reprod Genet 1995;12:590-3.
26. Kruessel JS, Simon C, Rubio MC, Pape AR, Wen Y, Huang HY, et al. Expression of interleukin-1 system mRNA in single blastomeres from human preimplantation embryos. Hum Reprod 1998;13:2206-11.
27. Huang HY. Cytokine in embryo implantation. Chang Gung Med J 2006;29(1):25-34.
28. Zolti M, Ben-Rafael Z, Meirom R, Shemesh M, Bider D, Mashiach S, et al. Cytokine involvement in oocytes and early embryos. Fert Steril 1991;56:265-72.
29. Chard T. Cytokines in implantation. Hum Reprod Update 1995;1:385-96.
30. Lowe WL. Biological actions of the insulin-like growth factors. In: Insulin-like growth factors: molecular and cellular aspects. Le Roith D, editor. Boca Raton: CRC Press; 1991. p. 49-95.
31. Binoux M. The IGF system in metabolism regulation. Diabete Metab 1995;21:330-7.
32. Lighten AD, Hardy K, Winston RML, Moore GE. Expression of mRNR for the insulin-like growth factors and their receptors in human preimplantation embryos. Mol Reprod Dev 1997;47(2):134-9.
33. Harvey MB, Kaye PL. Insulin like growth factor-1 stimulates growth of mouse preimplantation embryos in vitro. Mol Reprod Dev 1992;31:195-9.
34. Liu JP, Baker J, Perkins AS. Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf 1r). Cell 1993;75(1):59-72.
35. Baker J, Liu JP, Robertson EJ, Efstratiadis A. Role of insulin-like growth factors in embryonic and postnatal growth. Cell 1993;75:73-82.
36. Wang ZQ, Fun MR, Barlow DP, Wagner EF. Regulation of embryonic growth lysosomal targeting by the imprinted Igf2/Mpr gene. Nature 1994;372:464-7.
37. Adamson ED. EGF receptor activities in mammalian development. Mol Reprod Dev 1990;27:16-22.
38. Paria BC, Dey SK. Preimplantation embryo development in vitro: cooperative interactions among embryos and role of growth factors. Proc Natl Acad Sci USA 1990;87(12):4756-60.
39. Wood SA, Kaye PL. Effects of epidermal growth factor on preimplantation mouse embryos. J Reprod Fertil 1989;85:575-82.
40. Chia CM, Winston RM, Handyside AH. EGF, TGF-α and EGFR expression in human preimplantation embryos. Development 1995;121:299-307.
41. Kane MPM, Coonan C. Peptide growth factors and preimplantation development. Hum Reprod Update 1997;3:137-57.
42. Dardik A, Schultz RM. Blastocoel expansion in the preimplantation mouse embryo; stimulatory effect of TGF-α and EGF. Development 1991;113:919-30.
43. Bababola GO, Schultz RM. Modulation of gene expression in the preimplantation mouse embryo by TGF-alpha and TGF-beta. Mol Reprod Dev 1995;41:133-9.
44. Hrabe de Angelis M, Kirchner C. Effects of TGF α and EGF on rabbit blastocysts in a protein-free culture medium. Fertilität 1993;9:5-8.
45. Machida T, Taga M, Minaguchi H. Effects of epidermal growth factor and transforming growth factor alpha on the mouse trophoblast outgrowth in vitro. Eur J Endocrin 1339;(6):741-6.
46. Rappolee DA, Brenner CA, Schultz R, Mark D, Werb Z. Developmental expression of PDGF, TGF-α and TGF-β genes in preimplantation mouse embryos. Science 1988;241:1823-5.
47. Goustin AS, Betscholtz C, Pfeifer-Ohlsson S, Persson H, Rydnert J, Bywater M, et al. Coexpression of the sis and myc proto-oncogenes in developing human placenta suggests autocrine control of trophoblast growth. Cell

1985;41(1):301-12.
48. Lopata A, Oliva K. Chorionic gonadotrophin secretion by human blastocysts. Hum Reprod 1993;8(6):932-8.
49. Osterlund C, Wramsby H, Pousette A. Temporal expression of platelet-derived growth factor (PDGF)-A and its receptor in human preimplantation embryos. Mol Hum Reprod 1996;2(7):507-12.
50. Svalander PC, Holmes PV, Olovsson M, Wikland M, Gemzell-Danielsson K, Bygdeman M. Platelet-derived growth factor is detected in human blastocyst culture medium but not in human follicular fluid – a preliminary report. Fertil Steril 1999;56(2):367-9.
51. Bhatnagar P, Papaioannou VE, Biggers JD. CSF-1 and mouse preimplantation development in vitro. Development 1995;121:1333-9.
52. Bartocci A, Pollard JW, Stanley ER. Regulation of colony stimulating factor 1 during pregnancy. J Exp Med 1989;164:956-61.
53. Sjoblom C, Wikland M, Robertson SA. Granulocyte-macrophage colony-stimulating factor promotes human blastocyst development in vitro. Hum Reprod 1999;14:3069-76.
54. Sjoblom C, Wikland M, Robertson SA. Granulocyte-macrophage colony-stimulating factor (GM-CSF) acts independently of the ß common subunit of the GM-CSF receptor to prevent inner cell mass apoptosis in human embryos. Biol Reprod 2002;67:1817-23.
55. Robertson SA, Sjoblom C, Jasper MJ, Norman RJ, Seamark RF. Granulocyte-macrophage colony-stimulating factor promotes glucose transport and blastomere viability in murine preimplantation embryos. Biol Reprod 2001;64:1206-15.
56. Pollard JW. Role of colony-stimulating factor-1 in reproduction and development. Mol Reprod Dev 1997;46:54-60.
57. Seppala M. The world collaborative report on in vitro fertilization and embryo replacement: current state of the art in January 1984. Ann N Y Acad Sci 1985;442:558-63.
58. Sharkey AM, Dellow K, Blayney M, Macnamee M, Charnock-Jones S, Smith SK. Stage-specific expression of cytokine and receptor messenger ribonucleic acids in human preimplantation embryos. Biol Reprod 1995;53(4):974-81.
59. Smotrich DB, Stillman RJ, Widra EA, Gindoff PR, Kaplan P, Graubert M, et al. Immunocytochemical localization of growth factors and their receptors in human pre-embryos and Fallopian tubes. Hum Reprod 1996;11:184-90.
60. De los Santos MJ, Mercader A, Frances A, Portoles E, Remohi J, Pellicer A, et al. Role of endometrial factors in regulating secretion of components of the immunoreactive human embryonic interleukin-1 system during embryonic development. Biol Reprod 1996;54(3):563-74.
61. Yie SM, Balakier H, Motamedi G, Librach CL. Secretion of human leukocyte antigen-G by human embryos is associated with a higher in vitro fertilization pregnancy rate. Fertil Steril 2005;83(1):30-6.
62. Spanos S, Becker DL, Winston RM, Hardy K. Anti-apoptotic action of insulin-like growth factor-I during human preimplantation embryo development. Biol Reprod 2000;63:1413-20.
63. Loutradis D, Koussidis GA, Bletsa R, Milingos S, Antsaklis A, Michalas S. EGF and IGF-I as predictors of ICSI outcome in human preimplantation embryo cultures. Clin Exp Obstet Gynecol 2005;32(3):166-8.

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