발생과 생식 제 4권 제 1호, 29~35 (2000)
Dev. Reprod. Vol. 4, No. 1, 29~35 (2000)

Involvement of Phosphatidylinositol 3-Kinase in the Insulin Signaling in Preimplantation Mouse Embryos

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생쥐 착상전 배아의 인슐린 신호전달 과정에 Phosphatidylinositol 3-Kinase의 관련성

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ABSTRACT: A phosphatidylinositol 3-kinase (PI3K) is a upstream component of insulin signaling by which protein synthesis can be stimulated in many systems. To elucidate involvement of PI3K and its downstream mammalian target of rapamycin (mTOR) in the insulin signaling in preimplantation mouse embryos, 8-cell embryos were cultured to blastocysts in the presence or absence of insulin and / or inhibitor drugs. The number of blastomeres per blastocyst, protein synthesis, and protein phosphorylation were examined. There was significant difference in embryonic development to blastocyst stage and hatching was potentiated by the insulin supplementation. The increase in the mean cell numbers per blastocyst was apparent in the insulin culture. Wortmannin, a PI3K inhibitor and rapamycin, an inhibitor of mTOR abolished the stimulatory effect of insulin on morphological development, mitosis and protein synthesis. In autoradiography, phosphoproteins pp22 and pp30 which undergo phosphorylation in response to insulin were identified. Taken together, it can be suggested that PI3K and mTOR engaged in insulin signaling in the mouse embryo 8-cell onward and mediate embryotropic effect of insulin.

Key words: Insulin, PI3 Kinase, mTOR, Embryos, Mouse.

요 약: A phosphatidylinositol 3-kinase (PI3K)는 인슐린 신호전달의 상위구성 요소로 다양한 세포에서 단백질합성을 촉진한다. PI3K와 하위의 mammalian target of rapamycin (mTOR)가 착상전 생쥐 배아의 인슐린 신호전달에 관여하고 있는지의 여부를 조사하고자 하였다. 생쥐의 8-세포기 배아를 인슐린 또는 PI3K 및 mTOR의 억제제를 포함한 조건에서 배양하면서 발생율, 할구수, 단백질합성 및 인산화를 조사하였다. 인슐린의 첨가는 포배형성과 부화 등 형태발생을 촉진하며 포배내 평균 할구수, 8-세포기 배아의 단백질 합성과 인산화를 유의하게 증가시켰다. PI3K의 억제제인 wortmannin과 mTOR를 억제하는 rapamycin은 인슐린에 의한 발생율, 포배내 할구수, 단백질합성의 증가 효과를 상쇄하였다. 오토라디오그라피에서 두종의 인산화단백질인 pp22와 pp30의 인산화가 인슐린 처리에 의해 증가함을 확인하였다. 이상의 결과에서 생쥐 8-세포기 배아의 발생을 촉진하는 인슐린 신호의 전달에 PI3K와 mTOR가 관여함을 알 수 있다.

INTRODUCTION

Insulin is a highly conserved peptide with diverse biological functions. The expressions of insulin receptors and insulin like growth factor-I (IGF-I) receptors was found in 8-cell mouse embryo onward (Heyner et al., 1989b; Rappolee et al., 1990; Schultz et al., 1990). Insulin and IGF family have been known to promote cell proliferation (Paria & Dey, 1990; Gardner & Kaye, 1991; Harvey & Kaye, 1992), inhibit apoptosis (Herrler et al., 1998; Moley et al., 1998), stimulate synthesis of nucleic acids and protein (Heyner et al., 1989a; Harvey & Kaye, 1988; Rao et al., 1990), glucose uptake (Pantaleon & Kaye, 1996), and morphological development (Gardner & Kaye, 1991) of preimplantation embryos. On the other hand, the increased cell

This work was supported by Korea Research Foundation Grant (1998-015-D00241)

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death and expression of clusterin, a gene associated with the apoptosis were reported in the blastocysts exposed to maternal diabetes (Pampfer et al., 1997). However, the molecular evidences supporting the embryotropic effect of insulin was poorly understood in mammals.

When a stimulatory agonist molecule binds at the exterior of the cell membrane, a second messenger transduces the signal to the interior of the cell. Second messengers can be derived from phospholipids in the membrane by the action of the phospholipase C or phosphoinositide-3-OH kinase (PI3K) (Toker & Cantley, 1997). It has been suggested that the PI3K is involved in the insulin signaling pathway, one branch leading through protein kinase C (PKC) zeta to general protein synthesis and one, through protein kinase B (Akt) and the mammalian target of rapamycin (mTOR), to growth-regulated protein synthesis and cell cycle progression (Mendez et al., 1997; Yenush & White, 1997). PI3K is composed of two subunits (Carpenter & Cantley, 1996), a regulatory 85 kDa subunit, which contains the SH2 domains, and a 110 kDa subunit displaying the catalytic site that confers sensitivity to wortmannin, a potent inhibitor of PI3K (Arcaro & Wymann, 1993). Although the role as second messengers of the 3-phosphorylated inositol lipids is still not fully understood, it is clear that PI3K is found in virtually all cell types (Carpenter & Cantley, 1996; Shpetner et al., 1996). At the molecular level, PI3K has been reported to affect the activities of p70S6 kinase (Karnitz et al., 1995), a number of PKC isoforms (Toker and Cantley, 1997) and mitogen activated protein kinase (MAPK) (Cross et al., 1994). To uncover the insulin signaling in preimplantation mouse embryo, the development, protein synthesis, and protein phosphorylation were examined in the presence or absence of PI3K inhibitor or mTOR inhibitor in combination with insulin.

MATERIALS AND METHODS

1. Embryo retrieval and culture

Six weeks-old mice (ICR strain) were injected with 5 IU of pregnant mare serum gonadotropin (PMSG) and followed by injection of 5 IU of human chorionic gonadotropin (hCG) 48 h later. Hormone injected mice were mated with 3 months-old males. Eight-cell embryos were collected by flushing the oviduct and uterus with M2 medium (0.4% BSA) 68 h after hCG injection, placed in HTF medium (0.4% BSA, insulin-free) under mineral oil, and cultured at 37°C, 5 % CO_2 in air and 100% humidity. Embryos were cultured in the presence or absence of insulin (Sigma, 400 ng/ml) and wortmannin (Sigma, 100 nM) or rapamycin (Sigma, 1 uM) in the HTF medium (Quinn et al., 1985) for 60 h. Development of embryos to blastocysts was recorded 12 h interval, and mean cell numbers per blastocyst were evaluated at the end of culture. More than 56 embryos were subjected to culture and chi square independence test was done for statistical analysis.

2. Nuclear staining of blastocysts

Zona pellucidae was removed by brief incubation in acid Tyrode solution (Hogan et al., 1994). After rinse in M2 / BSA, zona-free blastocysts were fixed in 1% glutaraldehyde in phosphate buffered saline (PBS), washed in PBS, and stained with bisbenzamide (Sigma, 10 μg/ml in PBS) for 10 min. After several washing in PBS, embryos were mounted in glycerol and observation was done under epifluorescence microscope (BX50, Olympus, WU filter block). More than 30 embryos per group were evaluated for their cell numbers. Mean cell numbers per embryo were statistically analyzed by Student's *t*-test.

3. Peptide labeling with radioisotopes

To label the newly synthesized peptides, 8-cell embryos developed from 2-cell were preincubated for 15 min in the presence of [^{35}S] Met (1 mCi/ml, specific activity > 1000Ci/mmol) in HTF. After brief washing in HTF, insulin and inhibitors were introduced to culture, and embryos were incubated for 2 h. At the end of labeling, 20 embryos were washed with M2 and directly lysed in the sample buffer (Laemmli, 1970). For the analysis of protein phosphorylation, embryos were placed in phosphate-free HTF for 15 min before labeling. Labeling was conducted in the presence of [^{32}P] orthophosphate (1 mCi/ml, specific activity > 1000Ci/mmol) and 1 mM sodium orthophosphate for 2 h in the presence or absence of insulin and inhibitors. After labeling, embryos were washed with M2 and directly lysed in the sample buffer (Laemmli, 1970). For fluorography, labeled samples were boiled for 5 min at 100°C and separated by SDS-PAGE in 10% acrylamide gel. After electrophoresis, gel was stained with silver nitrate (Merril et al., 1981) and followed by fluorography according to Bonner

and Lasky (1974). For the autoradiography of labeled samples, SDS-PAGE in 8% acrylamide gel and direct exposure to X-ray film were conducted. Densitometric analysis of image on the X-ray film was done using the Bioprofil (France) software.

RESULTS

1. Effects of insulin on the development of 8-cell embryos

Effects of insulin on the development of 8-cell embryos were summarized in Table 1. At the end of culture, there was a significant difference in the embryonic development beyond the blastocysts between insulin culture and control one ($p<0.05$). In addition, in the presence of insulin, hatching was faster than control one and percent of degenerated embryos decreased. Developmental retardation and degeneration of embryos were apparent in the presence of wortmannin (100 nM) or rapamycin (1 uM) regardless of insulin. Rapamycin was the most potent in degeneration of embryos regardless of insulin.

Table 1. The development of embryos in the presence or absence of insulin or inhibitor drugs

Post hCG	Treatment	Total Embryos	No. of embryos (% development)				
			M	EB	LB	H	D
80hr	C	56	52 (92.6)	2 (1.8)	2 (1.8)		
	I*	58	50 (86.2)	5 (8.6)	3 (5.2)		
	W	57	56 (98.2)	1 (1.8)			
	I+W	57	55 (96.5)	2 (3.5)			
	R	56	54 (96.4)	2 (3.6)			
	I+R	56	54 (96.4)	2 (3.6)			
92hr	C	56	22 (39.3)	4 (7.1)	31 (55.4)		
	I	58	16 (27.6)	5 (8.6)	37 (63.8)		
	W	57	23 (40.4)	5 (8.8)	29 (50.9)		
	I+W	57	27 (47.4)	4 (7.0)	24 (42.1)		2 (3.5)
	R*	56	28 (50.0)	6 (10.7)	21 (37.5)		1 (1.8)
	I+R*	56	28 (50.0)	6 (10.7)	21 (37.5)		1 (1.8)
104hr	C	56	5 (8.9)	3 (5.4)	41 (73.2)	4 (7.1)	3 (5.3)
	I*	58	4 (6.9)	1 (1.7)	50 (86.2)	2 (3.4)	1 (1.7)
	W*	57	10 (17.5)	4 (7.0)	36 (63.2)	3 (5.3)	4 (7.0)
	I+W*	57	13 (22.8)	1 (17.5)	32 (56.1)	2 (3.5)	10 (17.5)
	R*	56	7 (12.5)	4 (7.1)	32 (57.1)		13 (23.2)
	I+R*	56	8 (14.3)		32 (57.1)	2 (3.6)	14 (25.0)
116hr	C	56		1 (1.8)	37 (66.1)	11 (19.6)	7 (12.5)
	I	58	2 (3.4)		38 (65.5)	14 (24.1)	4 (6.9)
	W*	57		2 (3.5)	33 (57.9)	9 (15.8)	13 (22.8)
	I+W	57	1 (1.8)		31 (54.4)	10 (17.5)	15 (26.3)
	R	56		2 (3.6)	26 (46.4)	8 (14.3)	20 (35.7)
	I+R	56	1 (1.8)	2 (3.6)	22 (39.3)	10 (17.9)	21 (37.5)
128hr	C	24			14 (58.3)	4 (16.7)	6 (25.0)
	I*	24			16 (66.7)	6 (25.0)	2 (8.3)
	W	24			19 (79.2)		5 (20.8)
	I+W	24		1 (4.2)	14 (58.3)		9 (37.5)
	R*	24			14 (58.3)	1 (4.2)	9 (37.5)
	I+R	24			12 (50.0)	3 (12.5)	9 (37.5)

The compacted 8-cell mouse embryos collected at 68 h post hCG and cultured for 60 h. M, morula; EB, early blastocyst; LB, late blastocyst; H, hatching; D, degeneration; C, control; I, insulin; W, wortmannin; I+W, insulin plus wortmannin; R, rapamycin; I+R, insulin plus rapamycin. Values were obtained from three independent experiments. Significant difference between experimental groups was analyzed by chi-square test. *, $p<0.05$.

2. Effects of insulin on the cell numbers of mouse blastocysts

The effects of insulin and inhibitor drugs on the mean cell numbers per blastocyst were summarized in Table 2. Insulin treatment significantly increased the mean cell numbers of the blastocyst compared to control culture (102.8±21.2 vs. 90.4±17.9, respectively) ($p<0.05$). In the presence of wortmannin (100 nM) or rapamycin (1 uM), the mean cell numbers of the blastocysts were significantly decreased (78.8±27.4 and 83.8±19.7, respectively) ($p<0.05$). Inclusion of wortmannin or rapamycin in the presence of insulin significantly ($p<0.05$) decreased the mean cell numbers of the blastocysts (82.4±25.8 and 88.0±9.8, respectively) compared to insulin-treated embryos (102.8±21.2) ($p<0.05$).

3. Protein synthesis and phosphorylation of embryos

Eight-cell embryos showed increase in total protein incorporation in response to insulin treatment (Fig. 1A). Insulin increased general protein synthesis 1.2 fold. However, in the presence of wortmannin or rapamycin, the potentiation of protein synthesis by insulin was not observed (Fig. 1B). Insulin (Fig. 2A). Wortmanin slightly decreased the total protein phosphorylation when compared to insulin treatment (Fig. 2B). There was a specific increase in the phosphoproteins of embryos treated with insulin. For example, phosphorylation of a 30 kDa

Table 2. The number of the blastomeres per blastocyst in the presence or absence of insulin or inhibitor durgs

Treatment	No. of Embryos	No. of Blastomere per Blastocyst
C	40	90.40±17.9
I	38	102.8 ±21.2*
W	44	78.80±27.4**
I+W	30	82.40±25.8**
R	35	83.80±19.7**
I+R	46	88.00±9.80**

The compacted 8-cell mouse embryos collected at 68 h post hCG cultured for 60 h and followed by nuclear staining. C, control; I, insulin; W, wortmannin; I+W, insulin plus wortmannin; R, rapamycin; I+R, insulin plus rapamycin. Values are represented by mean±SE and obtained from three independent experiments. Significant difference between experimental groups was analyzed by Student's *t*-test. * and ** , $p<0.05$; *(vs. C); **(vs. I).

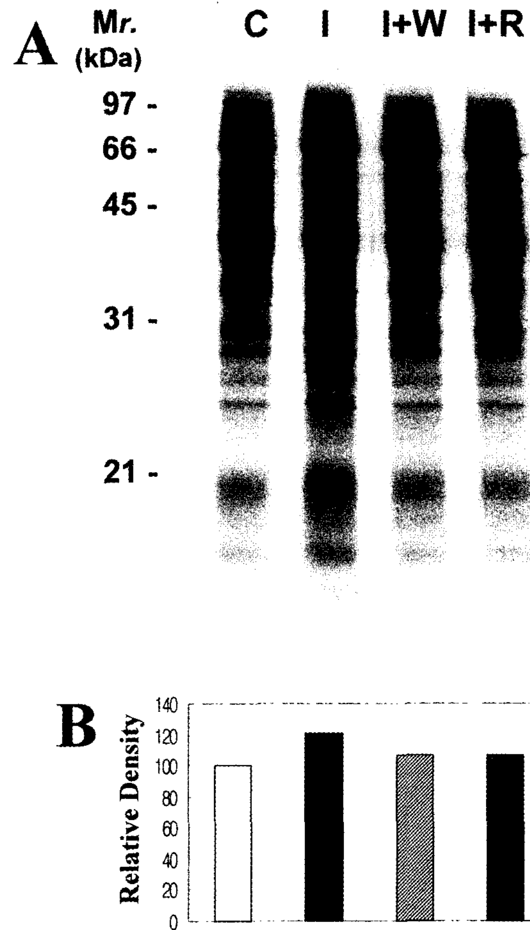

Fig. 1. Protein synthesis of 8-cell embryos in response to insulin. (A) Fluorography of 8-cell embryos after the culture in the presence or abesence of insulin and/or wortmannin and rapamycin for 2 h. C, control culture (no supplementation of insulin); I, insulin (400 ng/ml) supplementation in culture; I+W, insulin+wortmannin (100 nM); I+R, insulin + rapamycin (1 uM). (B) Densitometric analysis of fluorography. Relative density of each lane was analyzed by Bioprofil system.

protein (pp30) increased 1.7 fold, and phosphorylation of a 22 kDa protein (pp22) increased 1.8 fold after insulin treatment (Fig. 2B).

DISCUSSION

Results from this study showed that insulin treatment significantly potentiated the development of 8-cell embryos to blastocysts and increased the mean cell numbers per embryo. It was reported that embryonic expression of insulin receptor which mediates biological effect of insulin initiated at 8-cell stage in mouse embryos (Heyner et al., 1989b; Rappolee et al.,

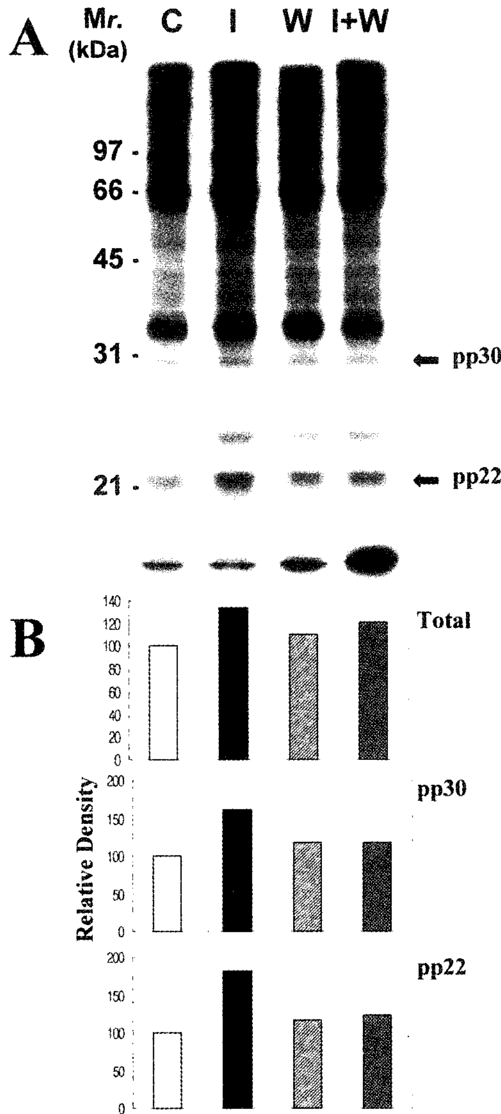

Fig. 2. Protein phosphorylation of 8-cell embryos in response to insulin. (A) Autoradiography of 8-cell embryos after the culture in the presence or abesence of insulin and/or wortmannin and rapamycin for 2h. C, control culture (no supplementation of insulin); I, insulin(400 ng/ml) supplementation in culture; W, wortmannin(100 nM); I+W, insulin+wortmannin. (B) Densitometric analysis of autoradiography. Relative density of each lane was analyzed by Bioprofil system. Total, protein phosphorylation in total; pp30, phosphoprotein of *Mr*. 30 kDa protein; pp22, phosphoprotein of *Mr*. 22 kDa protein.

1990). Therefore, it can be suggested that the embryotropic effect of insulin was temporally coincident with the onset of embryonic expression of cognate receptor at 8-cell stage. It has been known that short term effect of insulin was metabolic (Harvey & Kaye, 1988; Gardner & Kaye, 1991) and mitogenic in long term (Harvey & Kaye, 1992) in mouse embryos. However, even the absence of insulin supplementation, most of embryos developed to blastocysts. It suggested that the development of embryo was not impaired in the absence of insulin supplementation. Because of the embryonic expression of receptor for some growth factors (Paria & Dey, 1990), it might be resulted from paracrine or autocrine action of growth factors in our experimental condition. The increment in cell number of insulin-treated embryos was apparent, suggesting the mitogenic effect of insulin in preimplantation mouse embryos. It has been known that the morphological development of the early embryos depends on the embryonic clock rather than cell number per embryo (Satoh, 1982; Valdimarsson & Kidder, 1995). Taken together, it can be suggested that culture condition which guarantees paracrine and autocrine actions of embryo-derived factor is sufficient for morphological development to blastocyst.

On the fluorography, there was 1.2 fold increase in total amount of protein synthesis in insulin culture, coinciding with Shi et al. (1994). However, no detectable increase in specific peptides in response to insulin supplementation was found in short term. The total protein phosphorylation in embryo increased 1.3 fold by insulin. Intersetingly, the phosphorylations of Mr. 30 and 22 kDa proteins were significantly enhanced by insulin. It suggested that these proteins might be mediator or target of insulin signaling in 8-cell embryos. Taken together, it can be suggested that the embryotropic effect of insulin was related to the changes in protein synthesis and phosphorylation.

Under the insulin supplementation, wortmannin attenuated the development and the increase in cell mass of embryos in response to insulin. It suggested that PI3K involved in insulin signaling leading to mitotic activity in the mouse embryos. When the rapamycin, an inhibitor of mTOR that is a downstream target of insulin receptor signaling in animal cells (Yenush et al., 1997) was added to embryo in the presence of insulin, embryonic development was significantly impaired and the mean cell numbers of blastocyst were also greatly reduced.

Inclusion of wortmannin or rapamycin in the presence of insulin abolished effect of insulin on the protein synthesis and phosphorylation. It suggested that PI3K and mTOR were closely related to insulin signaling leading to potentiation of embryonic development.

In summary, it suggested that insulin supplementation is not essential for embryonic development to blastocyst. However, insulin potentiated the mitotic activity, morphological develo-

pment over the blastocyst, and protein synthesis and phosphorylation of 8-cell embryos, suggesting the possible benefit of insulin supplementation of *in vitro* culture of preimplamtation embryos. Inhibition of embryotropic effect of insulin by wortmannin or rapamycin suggested that potentiation of embryonic development by insulin might be mediated by upstream PI3K and downstream mTOR and that insulin signaling was highly conserved in animal development.

REFERENCES

Arcaro A, Wymann MP (1993) Wortmannin is a potent phosphatidylinositol 3-kinase inhibitor: the role of phosphatidylinositol 3,4,5-trisphosphate in neutrophil responses. Biochem J 296: 297-301.

Bonner WM & Lasky RA (1974) A film detection method for tritium-labeled proteins and nucleic acids in polyacrylamide gels. Eur J Biochem 46: 83-88.

Carpenter CL, Cantley LC (1996) Phosphoinositide kinases. Curr Opin Cell Biol 8: 153-158.

Gardner HG, Kaye PL (1991) Insulin increases cell numbers and morphological development in mouse preimplantation embryos *in vitro*. Reprod Fertil Dev 3: 79-91.

Cross DA, Alessi DR, Vandenheede JR, McDowell HE, Hundal HS, Cohen P (1994) The inhibition of glycogen synthase kinase-3 by insulin or insulin-like growth factor 1 in the rat skeletal mjuscle cell ine L6 is blocked by wortmannin, but nor by rapamycin: evidence that wortmannin blocks activation of the mitogen-activated protein kinase pathway in L6 cells between Ras and Raf. Biochem J 303: 21-26.

Harvey MB, Kaye PL (1988) Insulin stimulates protein synthesis in compacted mouse embryos. Endocrinology 122: 1182-1184.

Harvey MB, Kaye PL (1992) IGF-I stimulates growth of mouse preimplantation embryos *in vitro*. Mol Reprod Dev 31: 195-199.

Herrler A, Krusche CA, Beier HM (1998) Insulin and insulin-like growth factor-I promote rabbit blastocyst development and prevent apoptosis. Biol Reprod 59: 1302-1310.

Heyner S, Rao LV, Jarett L, Smith RM (1989a) Preimplantation mouse embryos internalize maternal insulin via receptor mediated endocytosis: Pattern of uptake and functional correlations. Dev Biol 134: 48-58.

Heyner S, Smith RM, Schultz GA (1989b) Temporally regulated expression of insulin and insulin-like growth factors and their receptors in early mammalian development. Bioessays 11: 171-176.

Hogan B, Beddington R, Costantini F, Lacy E (1994) Manipulating the Mouse Embryo: A laboratory manual. Cold Spring Harbor Press, New York, pp 191.

Karnitz LM, Burns LA, Sutor SL, Blenis J, Abraham RT (1995) Interleukin-2 triggers a novel phosphatidylinositol 3-kinase-dependent MEK activation pathway. Mol Cell Biol 15: 3049-3057.

Laemmli UK (1970) Cleavage of structural proteins during the assembly on the head of bacteriophage T4. Nature 227: 680-685.

Mendez R, Kollmorgen G, White MF, Rhoads RE (1997) Requirement of protein kinase C zeta for stimulation of protein synthesis by insulin. Mol Cell Biol 17: 5184-5192.

Merril CR, Goldman D, Sedman SA, Ebert MH (1981) Ultrasensitive stain for proteins in polyacrylamide gels shows regional variation in cerebrospinal proteins. Science 211: 1437-1438.

Moley KH, Chi MM, Knudson CM, Korsmeyer SJ, Mueckler MM (1998) Hyperglycemia induces apoptosis in pre-implantation embryos through cell death effector pathways. Nat Med 4: 1421-1424.

Pampfer S, Vanderheyden I, McCracken JE, Vesela J, De Hertogh R (1997) Increased cell death in rat blastocysts exposed to maternal diabetes in utero and to high glucose or tumor necrosis factor-alpha *in vitro*. Development 124: 4827-4836.

Pantaleon M, Kaye PL (1996) IGF-I and insulin regulate glucose transport in mouse blastocysts via IGF-I receptor. Mol Reprod Dev 44: 71-76.

Paria BC, Dey SK (1990) Preimplantation embryo development *in vitro*: Cooperative interactions among embryos and role of growth factors. Proc Natl Acad Sci USA 87: 4756-4760.

Quinn P, Kerin JF, Warnes GM (1985) Improved pregnancy rate in human *in vitro* fertilization with the use of a medium based on the composition of human tubal fluid. Fertil Steril 44: 493-498.

Rao LV, Wikarczuk ML, Heyner S (1990) Functional roles of insulin and insulinlike growth factors in preimplantation mouse embryo development. *in vitro* Cell Dev Biol 26: 1043-8.

Rappolee DA, Sturm KS, Schultz GA, Perdesen RA, Werb Z (1990) The expression of growth factor ligand and receptors in preimplantation embryos. In: Heyner S and Wiley L (eds), Early Embryo Development and Paracrine Relationships, UCLA Symposia on Molecular and Cellular Biology, New Series 117. Alan R Liss, New York, pp 11-25.

Satoh N (1982) Timing mechanisms in early embryonic development. Differentiation 22: 156-163.

Schultz GA, Dean W, Hahnel A, Telford N, Rappolee DA, Werb Z, Perdesen R (1990) Changes in RNA and protein synthesis during the development of the preimplantation mouse embryo. In: Heyner S and Wiley L (eds), Early Embryo Development and Paracrine Relationships, UCLA Symposia on Molecular and Cellular Biology, New Series 117. Alan R Liss, New York, pp 27-46.

Shi CZ, Collins HW, Buettger CW, Garside WT, Matschinsky FM, Heyner S (1994) Insulin family growth factors have specific effects on protein synthesis in preimplantation mouse embryos. Mol Reprod Dev 37: 398-406.

Shpetner H, Joly M, Hartley D, Corvera S (1996) Potential sites of PI-3 kinase function in the endocytic pathway revealed by the PI-3 kinase inhibitor, wortmannin. J Cell Biol 132: 595-605.

Toker A, Cantley LC (1997) Signalling through the lipid products of phosphoinositide-3-OH kinase. Nature 387: 673-676.

Valdimarsson G, Kidder GM (1995) Temporal control of gap junction assembly in preimplantation mouse embryos. J Cell Sci 108: 1715-1722.

Yenush L, White MF (1997) The IRS-signaling system during insulin and cytokine action. Bioessays 19: 491-500.