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Carbohydrate Metabolism in Preimplantation Stage Embryos and the Role of Metabolites

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착상전 초기 배아에서 탄수화물 대사와 그 대사물의 역할

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ABSTRACT : Proper development of fertilized oocyte to blastocyst is a key step in mammalian development to implantation. During development of preimplantation embryos, the mammalian embryo needs supply the energy substrate for keep viability. Usually mammalian oocyte get substrate especially energy substrate from oviduct and uterus, because it does not store much substrate into cytoplasm during oogenesis. Carbohydrates are known as a main energy substrate for preimplantation stage embryos. Glucose, lactate and pyruvate are essential component in preimplantation embryo culture media and there are stage specific preferences to them. Glucose transporter and H^+-monocarboxylate cotransporter are a main mediator for carbohydrate transport and those expression levels are primarily under the control of intrinsic or extrinsic factors like insulin and glucose. Other organic substances, amino acids, lipids and nucleotides are used as energy substance and cellular regulation factor. Though since 1960s, successful development of fertilized embryo to blastocyst has been accomplished with chemically defined medium for example BWW and give rise to normal offspring in mammals, the role of metabolites and the regulation of intermediary metabolism are still poorly understood. Glucose may permit expression of metabolic enzymes and transporters in compacting morula, capable of generating the energy required for blastocyst formation. In addition, it has been suggested that the cytokines can modulate the metabolic rate of carbohydrate in embryos and regulate the preimplantation embryonic development through control the metabolic rate. Recently we showed that lactate can be used as a mediator for preimplantation embryonic development. Those observations indicate that metabolites of carbohydrate are required by the early embryo, not only as an energy source, but also as a key substrate for other regulatory and biosynthetic pathways. In addition metabolites of carbohydrate may involve in cellular activity during development of preimplantation embryos. It is suggested that through these regulation and with other regulation mechanisms, embryo and uterus can prepare the embryo implantation and further development, properly.

Key words : Carbohydrate metabolism, Preimplantation embryo, Metabolite, Regulator.

요 약 : 수정란이 포배로 분화하는 것은 착상을 통하여 개체 발생이 성립되는 포유동물의 발생에 있어서 핵심적인 현상이다. 초기 배아 발생 시기동안 배아는 생존을 위한 에너지원을 공급받아야 한다. 포유동물의 난자는 보통의 경우 난자 형성 동안 많은 양의 에너지원을 세포질에 비축하지 않기 때문에 발생 동안 수란관과 자궁으로부터 물질대사와 관련돼 여러 물질, 특히 에너지원을 획득해야 한다. 탄수화물은 착상전 배아의 주 에너지원으로 알려져 있다. 포도당, 젖산염, 피르브산염은 착상 전 배아 배양액에서 없어서는 않될 성분으로, 초기 배아는 그 발생 단계에 따라 이들 물질에 대한 선호도를 각기 다르게 갖고 있다. 포도당수송체(glucose transporter)와 수소이온-단당류 동향수송체(H^+-monocarboxylate cotransporter)는 탄수화물을 수송하는 주된 매개자로 이들의 발현 수준은 일차적으로 내인성 또는 인슐린이나 포도당과 같은 외인성 요인에 동시적으로 조절을 받는다. 비록 1960년대 이후 화학적으로 규명된 BWW와 같은 배양액을 이용하여 수정란이 성공적으로 포배로 발생되고 이식 후 정상적인 새끼가 태어났어도, 발생조절에 있어서 이들 탄수화물 물질대사 산물의 역할은 잘 알려져 있지 않다. 포도당은 밀착이 진행되는 상실배에서 물질대사 관련 효소와 수송체의 발현을 조절하고, 포배강 형성에 필요로 하는 에너지를 생산하는데 관련된 것으로 인식되고 있다.

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다른 한편으로 cytokine은 배아에서 탄수화물의 대사율, 그리고 물질대사율 조절을 통하여 배아 발생을 조절할 수 있는 것으로 제안되어 왔다. 또한, 근래 들어 본인 등은 젖산염이 착상 전 배아의 발생을 조절할 수 있는 물질임을 밝히고 있다. 이러한 결과들은 탄수화물의 물질대사물이 초기 배아 시기에 에너지원으로서 뿐만이 아니라 생합성 경로 및 다른 조절경로에 참여하고 있음을 의미한다. 따라서 초기 배아 발생 동안 탄수화물 대사와 대사물질은 에너지원으로서 뿐만이 아니라 수정란이 착상할 수 있는 능력을 갖춘 포배로 발생하는 것을 조절하는 조절물질로 그 중요성이 있다.

The metabolisms of preimplantation embryos are up to the intrinsic control and extrinsic control. The intrinsic metabolic control mainly works during early stage embryos and the best characterized intrinsic metabolic control mechanism is the switch from pyruvate to glucose as major energy substrate during preimplantation development. On the other hand, extrinsic control occurs during differentiation of the blastocyst (Leese, 1995).

Preimplantation embryos exhibit metabolic adaptation, whereby their metabolism responds to changes in the external environment. The very early stage embryos can develop to the blastocyst stage embryo in a variety of culture media, which are containing carbohydrate sources and other substrates, though there is little difference in developmental scores. Main ideas for the metabolic products of preimplantation embryos are focused on the ATP synthesis and structural molecules for cell division. However, in mammals the preimplantation embryonic development is maintained by intrinsic and extrinsic factors and the carbohydrate metabolites may involve in cellular regulation. Therefore it is asked to extend the role of carbohydrate metabolism in developmental biology.

FACTORS INVOLVED IN METABOLIC SUPPORT DURING PREIMPLANTATION USING CARBOHYDRATE

During oogenesis, oocytes have to store nutrients for ontogenesis and to accumulate the maternal transcripts for regulator and structural proteins of early embryonic development. In oviparous and ovoviviporus animals, the oocyte should be accumulating the enough huge amounts of nutrients to support proper ontogeny. However in viviparous animals including mammals the oocyte usually dose not needs to accumulate a huge amount of nutrients because the placenta develops during ontogeny and support the embryo.

Organic substances including glucose and its metabolites play a fundamental role during oogenesis and embryogenesis. Mammalian embryos can use the stored carbon source or imported nutrients from reproductive tracts (Leese, 1995). The ovulated oocyte of mammals usually get nutrients from the reproductive tracts (Gardner et al., 1996; Gray et al., 2004; Harris et al., 2005; Hugentobler et al., 2008; Stewart et al., 2000). For example, early mouse embryo can store glucose as glycogen (Houghton et al., 1996) and can use for the synthesis of amino acids.

After ovulation the oocyte is exposed to the oviductal fluid and after fertilization it travels the oviduct and finally enters into the uterus. As expected the preimplantation embryos have to develop for implantation to blastocyst, and that are finely tuned by environmental factors and maternal or zygotic transcripts. One of the environmental factors is the kinds and amount of nutrients in cytoplasm or extracellular fluid of preimplantation embryo.

According to Harris and colleagues, the fertilized oocytes meet a various nutrient environment during development. The levels of carbohydrates and amino acids show difference between reproductive tracts in estrous stage of mouse. Glucose concentrations in follicular, oviduct (with/ without cumulus cells) and uterine fluids are 0.46, 1.09/ 1.65 and 0.61 mmol/L, respectively. Pyruvate concentrations are 0.38, 0.37/0.17 and 0.25 mmol/L, respectively, and lactate concentrations are 17.34, 10.92/11.68 and 9.41 mmol/L, respectively. Taurine, glycine, alanine, glutamine and glutamate are the major amino acids detected (Harris et al.,

2005). Oviductal concentration of pyruvate, glucose and lactate in near the cumulus mass are 0.37, 3.40, and 4.79 mM, respectively. In the absence of cumulus pyruvate is 0.14 mM, while the glucose is 5.19 mM. The concentration of glutamine is 0.20 mM (Gardner and Leese, 1990). It is also similar in human and the early human embryo is exposed to high lactate levels *in vivo* (Gardner et al., 1996).

In pregnant mice uterus, the levels of lactate and glucose are low on day 1 after mating. From day 2 onwards approximately glucose is 1 mM, lactate shows peak at 4 mM on day 2 of pseudopregnancy. In general, the concentration of pyruvate is 10% of the lactate value (Wales and Edirisinghe, 1989).

From *in vitro* studies, it is revealed that metabolic activity of preimplantation embryos is depending on the kinds of media which contain a various carbohydrate or other organic substances (Sagirkaya et al., 2006). Medium with a high potassium concentration inhibit cleavage and blastocyst formation. It is partially alleviated by the removal of phosphate, and by adding of amino acids, vitamins, insulin, epidermal growth factor and transferring (AVIET). Glucose uptake by cultured blastocysts is not affected by the ionic or metabolite composition of the medium, but is significantly reduced by the inclusion of AVIET. Lactate production is also significantly reduced in the presence of AVIET (Gardner and Sakkas, 1993). Usually we can observe such a response in preimplantation embryo during culture (Gandhi et al., 2001 Leppens-Luisier and Sakkas, 1997).

Energy metabolic activities of preimplantation embryos are depending on their stage and the nutrient environment. During bovine early development in *in vivo* condition , the oxidation range of pyruvate and lactate keeps 1~3 pmol until the morula state, after then it is increased 2- to 4-fold and the amount of incorporated carbon from pyruvate and lactate is very limited at this time. In the case of glucose, it can not be used in immature oocyte, but gradually increase of the utilize amount through cleavage with first marked increase occurring between the 12- and 16-cell stage. The incorporation is increased more than 15-fold from the immature oocyte to the blastocyst stage (Khurana and Niemann, 2000). However the utility of substances are changed by the components of nutrient.

Mitochondrial activity is co-regulated with cellular activity and the structure or number are different from cell type to cell type. During early embryogenesis the mitochondrial structures change dramatically according to their energy metabolic activities (Hillman and Tasca, 1969). In oocytes, mitochondria are spherical with few transverse cristae, whereas between the zygote and two-cell stage the mitochondria assume a dumb-bell shape and contain concentrically located cristae. From the four-cell to the morula stage, mitochondria become more elongated, containing transverse cristae with a proportion being vacuolated. At the blastocyst stage, two distinct forms of mitochondria are present; in the ICM they are spherical, whereas in the TE they are long and slender (Houghton, 2006; Stern et al., 1971).

The number of mitochondria in a cell could be counted with the copy number of mitochondrial DNA. Recently, Smith and his colleages account the copy number of mitochondrial DNA in rat. The copy number of mitochondrial DNA is very stable and almost same during preimplantation development (Kameyama et al., 2007 Sathananthan and Trounson, 2000). Based on them, the ATP synthesis in aerobic condition is dependingon the mitochondrial activity.

In blastocyst, trophectoderm and inner cell mass exposed to the different environment and biological function. There is difference in mitochondrial number between the two cell lineages of the blastocyst. It may be ascribed to the greater oxygen consumption observed in the TE compared with the ICM, because prominent cristae and an increase in mitochondrial number are associated with a high respiratory rate (Houghton, 2006).

As mentioned, metabolism of preimplantation stage embryo is regulated by multifactors and finely tuned through

communication between embryo and maternal factors in mammals. It means that metabolites in environment and the acquisition ways are essential factor in proper development of very early embryos.

TRANSPORT INTO CYTOPLASM

Transport of metabolic substances in early stage embryosare mediated by various transport system including diffusion, facilitated diffusion, and active transport. Glucose uptake is facilitated by a number of transporters. Import of glucose is mediated through sodium-coupled glucose transporter (sodium-glucose co-transporter, SGLT) and by the sodium-independent facilitative glucose transporters (GLUTs) (Heilig et al., 2003; Jensen et al., 2006; Wood and Trayhurn, 2003).

The facilitated hexose transporter, GLUT, family has 13 members and designated as GLUT1-12 and HMIT (or SLC2A1-12 and HMIT) (Uldry and Thorens, 2004). Expression patterns of GLUTs correlated with the glucose dependence during preimplantation. The GLUT2 and GLUT3 mRNA expression increase at eight-cell stage. The abundance of GLUT4 and GLUT6 mRNAs is increase at the eight-cell stage relative to the pronuclear or two-cell stages in monkey (Moley et al., 1998; Zheng et al., 2007). The expression of GLUT1 mRNA detect from 2-cell stage and regulate the glucose transport (Moley et al., 1998; Zheng et al., 2007).

In GLUT1 deficient mice, glucose uptake rate suppress 95% and the embryo shows delayed or impaired development (Heilig et al., 2003). Reduce of glucose uptake is cause of apoptosis of blastomere (Chi et al., 2003; Heilig et al., 2003). Similar results also observed in antisense-GLUT1 treated preimplantation embryos (Chi et al., 2001).

The SGLT (SLCA5) is constituted with 220 or more members in animal and bacterial cells. There are 11 human genes expressed in tissues ranging from epithelia to the central nervous system. Six are tightly coupled plasma membrane Na^+/substrate cotransporters for solutes such as glucose, myo-inositol and iodide; one is a Na^+/Cl^-/choline cotransporter; one is an anion transporter; and another is a glucose-activated ion channel. In preimplantation stage embryo, only the sodium-dependent-glucose transporter (SGLT-1) is studies. SGLT-1 is detectable from oocyt to blastocyst in bovine (Augustin et al., 2001).

The quantity of glucose transporter is strictly regulated in somatic cell, oocyte and embryos by endocrine regulators including insulin and insulin-like growth factor-1 (Fladeby et al., 2003; Jensen et al., 2006; Navarrete et al., 2004a; Russo et al., 2004). Insulin stimulates glucose uptake by regulating the transporter activities at both the transcriptional and post-translational levels (Benomar et al., 2006; Riley et al., 2005, 2006; Zheng et al., 2007). Result from the mechanisms for homeostasis, certain-glucose transporters reduced in the plasma membrane in the condition of hyperglycemia (Moley et al., 1998b; Keim et al., 2001). Therefore embryos can keep the viability and properly develop to blastocyst.

Lactate or pyruvate transport through the plasma membrane does not need energy input other than the that provided by the concentration gradients of lactate and protons, although the latter, in the form of a pH gradient, can drive the accumulation or exclusion of the lactate anion (Poole and Halestrap, 1993; Juel, 1997).

H^+-monocarboxylate cotransporter (MCTs) have a central role in mammalian metabolism and are critical for metabolic communication between cells (Poole and Halestrap, 1993). MCT proteins facilitate the coupled diffusion of a proton and a monocarboxylate ion (e.g. pyruvate, lactate, or acetoacetate). Nine MCT-related sequences have so far been identified in mammals, each having a different tissue distribution. MCT1 expression increase in response to increased work and suggested to have a role in lactic acid oxidation. MCT1 is ubiquitously expressed and prominent in heart and red muscle. MCT4 is highly expressed in lactate efflux predominate cells such as white muscle and other cells with a high glycolytic rate including tumor cells and white blood cells. MCT2 has a ten-fold higher

affinity for substrates than MCT1 and MCT4 and is found in cells where rapid uptake, including the proximal kidney tubules, neurons and sperm tails (Halestrap and Price, 1999).

ROLE AND REGULATION OF CARBOHYDRATE METABOLISM

As expected the major metabolic sources in preimplantation embryo are pyruvate, lactate, amino acid and glucose (Biggers, 1987). The appropriate metabolism of preimplantation is essential for successful implantation. Therefore it is critical to regulate the metabolic rate during preparation for implantation in preimplantation embryos.

Primarily the substrate requirement is depend on the intrinsic potency including the pools of metabolic enzymes and the auxotrophy of cell. Oocye or embryo shows substrates requirement which is strictly regulated during development. From fertilization, the embryo exhibits substrate preference for pyruvate, and lactate supports development from the two-cell stage. Glucose uptake is limited initially but increases after compaction and it are support the development (Lane et al., 2001; Leese, 1995; Leese, 2002; Summers and Biggers, 2003).

According to Wiebold and Anderson, 75% of the energy available at the 2-cell stage would be consumed in the synthesis of protein. At morula, blastocyst, and late blastocyst stages, protein synthesis accounts for 64%, 49%, and 24%, respectively (Merz et al., 1981; Wiebold and Anderson, 1985). The second major energy-consuming process is ion pumping which is associated with blastocoel cavity formation mediated by Na^+/K^+ ATPase (Biggers et al., 1988).

Recently using high through-put methodology we identified many genes which are specifically expressed in the uteri of various physiological conditions. Among the identified genes, a few genes are suspected as a paracrine factor for regulation of preimplantation embryonic development (Cheon et al., 2002). It is known that some of the crine factors may serve to regulate glucose, glycogen and lipid metabolism (Gardner and Sakkas, 1993). Growth hormone receptor is detectable from day 3 after fertilization in embryo (Sinclair et al., 2003). Insulin is acting as a growth factor and regulates GLUT-1 and -4 expressions (Navarrete Santos et al., 2004). Insulin-like growth factor-1 and -2 improve the embryonic development and it is related with increase of metabolism (Oropeza et al., 2004; Pantaleon et al., 2003). Fibroblast growth factor-4 expression associated with glucose concentration and involved in trophoblast differentiation (Leunda-Casi et al., 2001). Granulocyte-macrophage colony-stimulating factor (GM-CSF) facilitates the glucose transportand improve the survivability of preimplantation stage embryo in murine (Robertson et al., 2001). Tumor necrosis factor alpha (TNF- alpha) synthesis has the potential to influence the preimplantation development of rats through glucose metabolism (Pampfer et al., 1994).

Physical condition also can involve in metabolism of preimplantation embryos. Oxygen works as a modulator in glucose metabolism during preimplantation developmental period. The oxygen concentration used in the incubation atmosphere during embryo culture influences embryo development rates and embryo quality. Many of oxygen-regulated genes have important roles in embryonic development and metabolism. Expression of glucose transporter (GLUT)-1, GLUT-3, and vascular endothelial growth factor (VEGF) in blastocysts is increased by 2- to 4-fold in embryos cultured under 2% oxygen, when compared to embryos cultured under 20 or 7% oxygen, and when compared to embryos developed *in vivo* (Harvey et al., 2004; Kind et al., 2005). Oxygen stress caused by its concentration modulates the pentose-phosphate pathway (PPP) activity (Kimura et al., 2004).

Interestingly preimplantation stage embryos response to the stress such as glucose starvation or hypoxia, and express several genes including glucose-regulated protein (GRP) −8 (HSPA5) and −9 (HSP90B1), and hypoxia induced factor 1A (HIF1A). HIF1A is a transcription factor

capable of mediating the response to hypoxia by regulating the transcription of genes involved in glycolysis and angiogenesis (Zheng et al., 2007).

During early preimplantation stages, energy is largely generated by oxidative phosphorylation, whereas from the blastocyst stage onwards, there is a combination of mitochondrial respiration and aerobic glycolysis. In the mouse preimplantation embryo, pyruvate is the predominant energy substrate utilized prior to the blastocyst stage (Leese and Barton, 1984; Gardner and Leese, 1986). On the other hand, glucose consumption is low at early stages of preimplantation development but increases at the morula stage to become the predominant substrate utilized (Martin and Leese, 1995). Besides, the amount of oxygen consumption in embryos of early preimplantation stages is quiescent compared with the blastocyst stage (Houghton et al., 1996; Sturmey and Leese, 2003 Thompson et al., 1996).

Pyruvate is a cytosolic oxidant but a mitochondrial reductant, while lactate is a strong cytosolic reductant via the activity of lactate dehydrogenase (LDHA). Unexpectedly, lactate-derived pyruvate appears to be diverted from mitochondrial oxidation (Dumollard et al., 2007). Such a pattern of substrate utilization supports sufficient ATP production and biosynthetic pathways.

DL-lactate uptake kinetics in mouse embryo depends on the concentration of lactate and the number of transporter. Zygote uptake shows a K_m almost four-fold higher than blastocyst. Increase of affinity with development is apparent in morula. V_{max} does not change during preimplantation development. Furthermore, it does not affect of presence of glucose in medium (Jansen et al., 2006). Lactate transport via MCT involves the initial binding of a proton followed by the lactate anion, which are then translocated across the membrane and subsequently released (Halestrap and Price, 1999).

Pyruvate uptake and metabolism by mouse embryos is significantly affected by increasing the lactate concentration in the culture medium. In contrast, glucose uptake is not affected by lactate in the culture medium. At the zygote stage, the percentage of pyruvate taken up and oxidized is significantly reduced in the presence of 20 mM lactate, while at the blastocyst stage, 20 mM lactate increased the percentage of pyruvate oxidized. Lactate oxidation is determined to be 3-fold higher (when lactate is present at 20 mM) at the blastocyst stage compared to the zygote. Analysis of the kinetics of LDHA determined that while the V_{max} of LDHA is higher at the zygote stage, the K_m of LDHA is identical for both stages of development, confirming that the LDHA isozyme is the same. Furthermore, the activity of LDHA isolated from both stages is reduced by 40% in the presence of 20 mM lactate. The observed differences in lactate metabolism between the zygote and blastocyst must therefore be attributed to in situ regulation of LDHA.

Switching of predominant substrate is focused on the energy supply, but in the field of cellular activity glucose involved from very early stage. Glucose supports oocyte maturation or pronucleus formation via the pentose phosphate pathway (PPP) (Comizzoli et al., 2003; Herrick et al., 2006a). Besides, exposure to glucose prior to eight cell stage require absolutely for blastocyst formation to occur (Chatot et al., 1994; Martin and Leese 1995). Because this exposure facilitates expression of the glucose transporter protein (Pantaleon et al. 2001a) and the adaptive response associated with increased pyruvate utilization (Martin and Leese 1995). The incorporation of glucose increased steadily 15-fold from the 1-cell to the blastocyst stage (Khurana and Niemann, 2000). Based on them, it is suggested that glucose may act as a signal during early cleavage that induces metabolic differentiation of the developing embryo. Although glucose is the predominant substrate utilized, it makes only a modest (17%) contribution to ATP production in quantitative terms, while the oxidation of pyruvate accounts for 40% of the ATP produced (Houghton and Leese, 2004). Therefore, glucose may permit expression of metabolic enzymes and transporters in compacting morula, capable of generating the

energy required for blastocyst formation (Jansen et al., 2006).

To use glucose as a substrate, embryo has to metabolic pathway constructed with glycolysis enzymes. The glycolysis enzymes, phosphofructokinase muscle type (PFKM), aldolase (ALDOA1), phosphoglycerate kinase (PGK1), enolase (ENO1) and pyruvate kinase muscle type and liver type (PKM2 and PKLR, respectively) are abundantly expressed in oocytes and embryos. ALDOA1, PGK1, and ENO1 are predominantly expressed in embryos from the eight-cell stage. The PKM2 mRNA decrease in abundance during oocyte maturation and appears variably increased in abundance thereafter (Zheong et al., 2007).

The metabolism of glucose, pyruvate and lactate differed significantly between the ICM and trophectoderm. At the blastocyst stage, bulk of glucose is metabolized through aerobic glycolysis. Isolated trophectoderm has a higher pyruvate and lower glucose consumption, and higher lactate-production than does ICM. The consumption or production of amino acids by ICM and trophectoderm also differed, with the trophectoderm displaying a higher turnover than that of ICM (Gopichandran and Leese, 2003). The TE consumes significantly more oxygen, produces more ATP and contains a greater number of mitochondria than the ICM. Amino acid turnover is significantly greater in the TE compared with the ICM. The TE produces approximately 80% of the ATP generated and is responsible for 90% of amino acid turnover compared with the ICM.

It is suggested that much of the remainder of the oxygen taken up at the blastocyst stage may be accounted for by reactive oxygen species, as 30～40% is consumed by non-oxidative mechanisms (Trimarchi et al., 2000). About 40～60% of the ATP produced is used by the sodium pump (Na^+/K^+ ATPase) located on basolateral surfaces of the TE (Houghton, 2006; Houghton et al., 2003), with the remainder thought to be composed of the synthesis of macromolecules: DNA, RNA, protein, and lipid. Na^+-K^+ ATPase activity is minimal at the very early embryonic stage, and being first detected at the morula stage (Vorbrodt et al., 1977; Watson and Kidder, 1988). However, as the blastocyst stage is reached, there is a large increase in protein synthesis and Na^+ pumping, coincident with the formation of the blastocoel cavity (Epstein, 1975; Biggers et al., 1991).

44～81% of the glucose taken up at the blasocyst stage is converted to lactate and remained the level after implantation (Gardner and Leese, 1990; Houghton et al., 1996; Khurana and Niemann, 2000). Glycolysis produces two molecules of lactate for every glucose molecule consumed. Lactate must be transported out of the cells if they are to keep high rate of glycolysis (Denton and Halestrap, 1979; Juel, 1997; Poole and Halestrap, 1993). When embryos are cultured in 5.55mM glucose, 11.5～12.5 mM lactate and 0.25～0.33 mM pyruvate, the concentrations of glucose, L-lactate and pyruvte in blastocoel fluid of mouse and rat are 2.30 and 2.75 mM, 14.6 and 19.6 mM, and 0.13 and 0.50 mM, respectively. When cultured in the presence of 1.0 mM glucose and 1.0 mM L-lactate, concentrations in the blastocoel fluid are 0.50 and 0.59 mM glucose and 2.22 and 3.70 mM L-lactate, respectively (Brison et al., 1993).

Interestingly, glucose can induce MCT1 expression in preimplantation stage embryos and it is support the viability. MCT1 is important in the critical regulation of pH and monocarboxylate transportduring preimplantation development (Harding et al., 1999; Herubel et al., 2002; Jansen et al., 2006). MCT1 may be involved in lactate transport in blastocyst and result in maintaining intracellular pH and helping blastocoel formation.

On the other hand, osmotically induced water transport pathways have been demonstrated to be associated with the glucose transporter (Fischbarget et al., 1990; Zhang et al., 1991). cAMP-activated cystic fibrosis transmembrane conductance regulator (CFTR) (Schreiber et al., 1999), urea transporter UT3 (Yang et al., 1998), and multiple Na-solute cotransporters (Loo et al., 1999). D-glucose and fructose can induce the AQP1 expression like dexamethasone, plate-

let-derived growth factor, and hypoxia. Induction of AQP1 expression is upregulated by aerobic glycolysis conditions and higher lactate concentration (Rajendran et al., 2004). Upregulation of AQP1, LDH, and cathepsin B contribute to acidification of the extracellular milieu and invasive potential (Gatenby et al., 2006; Hayashi et al., 2007). Based on them, it is expected that lactate accumulate in blastocoel through the MCTs.

CONCLUDING REMARKS

In adult cells, primary energy substrate is carbohydrate. Secondarily carbohydrates used for a part of building blocks of nucleotide or lipid. During oogenesis and embryo development, carbohydrate can be used both an energy substance and an intermediates of other organic substances. Besides, glucose involve in regulation the protein function and stability, and also for the formation of extracellular matrix molecules. On the other hand, proteins are used primarily cellular regulator or structural molecule and secondarily energy substance. Lipids also used as cellular regulator and energy substrate. So far, as a cellular regulator the carbohydrate is not clearly explained but new knowledge continuously exposed in these days. From recent experiments, it is suspected that glucose is required by the early embryo, not only as an energy source, but also as a key substrate for other regulatory and biosynthetic pathways as well. In addition it is accumulated that the metabolites involved in cellular physiological regulation through gene expression or cellular signaling.

Ontogeny of animals is up to the successful development of preimplantation stage embryo. From *in vitro* experiment, we can learn how the carbohydrate important in embryonic development. Priority or preference in carbohydrate substance by the embryonic stage is very clear and critical in preimplantation embryonic development. It may the results of its role as both energy substance and cellular regulator. In addition with intrinsic regulation, extrinsic regulation originated from oviduct or uterus is suspected as critically regulator in the developmental speed and embryonic gene expression. Even though carbohydrate metabolism in preimplantation embryo is matched with intrinsic or extrinsic regulation mechanism, most of them are not evaluated so far. Therefore it is needed to studies about the cooperative regulations between carbohydrate metabolism and cellular regulation mechanism to improve the embryonic developmental control and to expend our knowledge. With other regulation mechanisms involved with preimplantation development and implantation, we can understand how carbohydrate collaborates with other organic substances and with other cellular regulation factors including growth factors and membrane transporters.

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