Lactate Can Modulate the Expression of Lactate Dehydrogenase and Aquaporin Genes in Mouse Preimplanation Embryos

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ABSTRACT : It is suggested that carbohydrate metabolites may involve in the development of morula to blastocyst but many of the mechanisms are not unmasked. Two-cell stage embryos were collected and examined the effects of lactate on the development of blastocyst *in vitro*. The expression profiles of lactate dehydrognase (*Ldh*) genes and aquaporin (*Aqp*) genes were analyzed with RT-PCR. The successful development from morula to blastocyst was dependent on lactate concentrations. The expression profiles of *Ldh* genes were changed by the lactate concentration. *Ldha* was expressed in morula stage at 10 mM lactate, and in blastocyst stage at lactate free condition. *Ldhb* was expressed in morula stage at 10 mM and 20 mM lactate, and in blastocyst stage at 10 mM lactate. *Aqp* genes were also showed different expression patterns by the lactate concentrations. *Aqp3* was expressed in hatching embryo at 120 hr post hCG administration (hph) which was cultured in BWW medium and lactate free condition. *Aqp7* was expressed in hatching embryos at 120 hph which was cultured at 10 mM lactate condition. Also *Aqp8* was expressed in hatching embryo at BWW and 20 mM lactate condition. *Aqp9* was expressed in morula at BWW and 10 mM lactate condition, and in blastocyst at BWW. Based on these results, it is suggested that concentration of lactate in the medium and the level of lactate synthesis in embryo is critical factor for blastocoels formation. In addition it is suggested that LDH may involve the AQPs expression in embryos.

Key words : Early stage embryo, Carbohydrate metabolism, Lactate dehydrogenase, Aquaporin

INTRODUCTION

Blastocoel formation is dramatic event in development of preimplantation stage embryo and is cue for implantation. It is accomplished by expressing a set of genes which facilitate the transport and retention of the blastocoel fluid as it accumulates in the nascent blastocyst cavity (Watson et al., 2001). During cavitation, the trophoblast cells secrete fluid into blastocoel. Na^+ and Cl^- in the medium imported through the trophectoderm into the blastocoels. These secreted fluids, Na^+, and Cl^- generate an osmotic gradient (Manejwala et al., 1989). Na^+/H^+ exchanger (NHE) facilitate the transtrophectodermal Na^+ flux. The specific inhibitor of NHE-3 blocks the blastocyst formation in a dose dependent manner (Kawagishi et al., 2004). In addition polarized mural trophectodermal Na^+/K^+-ATPase establishes and maintains an ionic gradient across the trophectoderm. The concentration gradient formed by those transporter promotes the osmotic accumulation of water across the epithelium and result in formation of blastocoels (Watson et al., 2004).

On the other hand, carbohydrate, energy substrate is important to support early embryonic development. The pattern of energy metabolism of the zygote is dependent on developmental stages and pyruvate is the main energy substrate which can be used directly by the oocyte and zygote (Biggers et al., 1967). Lactate supports development from the 2-cell stage and glucose supports after compaction (Leese, 1995). Generation of high-energy molecules (ATP and NADH) as cellular energy sources as part of aerobic

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respiration and anaerobic respiration. For aerobic respiration or anaerobic respiration pyruvate is synthesized in cytoplasm. And, as a necessary, pyruvate is converted to lactate by lactate dehydrogenase (LDH) that concomitant conversion of NAD^+ to NADH in the cytosol (Lane et al., 2005). Recently, in cancer cells, it is known that LDH activity is increased in aerobic condition (Hayashi et al., 2007). It is also known that the production of CO_2 and lactate increase in mourla and blastocyst through glucose metabolism in mouse (Khurana et al., 1989). Base on them, we can suspect that lactate may involve in cavitation of morula.

Transport of lactate across the plasma membrane of all cells is mediated by proton-linked monocarboxylate transporters (MCTs). The rate-limiting step for net lactate flux appears to be the return of the free carrier across the membrane which is required to complete the translocation cycle, and this is reflected in rates of monocarboxylate exchange being substantially faster than those of net transport. That is important in the critical regulation of pH and mono-carboxylate transport, and implies a role for glucose in the control of MCT1 expression (Jansen et al., 2006). In the brain, lactate induces swelling of C6 glial cells via Na^+/H^+ exchange (Jakubovicz & Klip, 1989). The formation of edema and water homeostasis in the brain is affected by aquaporin water channels (Iacovetta et al., 2012). Cell swelling of human astrocytoma cell is achieved via Roh a, Na^+/H^+ exchanger, and AQPs . It is also known that lactate increases AQP expression in the brain (Mirishiam et al., 2008).

Put together with our laboratory study, we could suspect that lactate may a factor for development of blastocoel. To evaluate the possible role of lactate in blastocyst formation, the expression profiles of *Ldh* genes and *Aqp* genes were examined in the various culture condition.

MATERIALS AND METHODS

1. Animals

Six- to 8 week-old female CD-1 mice were used in this study. All experimental animal studies followed to the Guide for the Care and Use of Laboratory. Animals were maintained under standard conditions at Sungshin Women's University. Animals were fed a standard rodent diet and water *ad libitum* from weaning at 21 days of age. The condition is maintained by 14 / 10 hr light and dark cycle.

2. Superovulation Induction and 2-Cell Stage Embryo Collection

Embryos were obtained from females that were super-ovulated with 5 IU of pregnant mares serum gonadotrophin (PMSG, Sigma, Missouri, USA) followed 48 hr later by 5 IU of human chronic gonadotrophin (hCG, Sigma, Missouri, USA). Immediately after the hCG injection, females were placed with males of the same strain. The next morning of finding a vaginal plug was defined day 1 of pregnancy. Preimplantation mouse embryos were collected at 48 hr post hCG injection (hph). The embryos were flushed from oviducts by BWW medium containing 0.4% bovine serum albumin (BSA, Sigma, Missouri, USA).

3. Embryos Culture

The collected healthy 2-cell embryos were cultured in the 10 μl microdrops of BWW medium containing different concentrations of lactate. Embryos were cultured in groups according to the different lactate concentrations (Table 1): group 1, BWW (25 mM lactate, control); group 2, 20 mM lactate modified BWW; group 3, 15 mM lactate modified BWW; group 4, 10 mM lactate modified BWW; group 5, 5 mM lactate modified BWW; group 6, 0mM modified BWW. The embryos were cultured at 37℃ with 5% CO_2 in air until 144 hph. The embryo development was evaluated every 12 or 24 hr under the inverted microscope (Olympus, IX70). The number and percentage of embryos reaching the 4-cell embryo, 8-cell embryo, morula (Mor), blastocyst (Bla), hatching (Hat) blastocyst were recorded at 48, 72, 84, 96, 108, and 120 hph (the total number of embryos are 623).

Table 1. Composition of media (Unit: mM)

Componant	BWW	Modified BWW; Lactate
NaCl	94.59	94.59
KCl	4.78	4.78
Ca-lactate	1.71	-
KH_2PO_4	1.19	1.19
$MgSO_4$	1.19	1.19
$NaHCO_3$	25.07	25.07
Na-pyruvate	0.25	0.25
Na-lactate	21.58	-
Glucose	5.56	5.56
$CaCl_2$	-	1.71
	Na-lactate	NaCl
20 mM	20	1.58
15 mM	15	6.58
10 mM	10	11.58
5 mM	5	16.58
0 mM	-	21.58

All medium containing antibiotics and phenol red and 0.4% bovine serum albumin (BSA).

Table 2. Sequences of primers

Gene		Primer sequence (5'-3')	Amplified length (bp)
AQP1	S	tgg ttt gag cat cgc tac tct g	347
	AS	tag tca atc gcc agc agg tgt	
AQP3	S	tga cct tcg caa tgt gct tc	379
	AS	tga aga ggc gag gtc caa agt	
AQP6	S	ttt acg ggg taa ctc cag gag gta	343
	AS	tag atc agc gaa gcc agg aca	
AQP7	S	gca gct atc tcg gtg tca act tg	340
	AS	acg aat gcc tca tcc agg aa	
AQP8	S	gat gcc gtg tgt tct ggt atg a	338
	AS	ctc tgg act cac cac ttt agc caa	
AQP9	S	tct gag ttc ctg ggc acc ttt	398
	AS	cct ggc acg gat aca aat ggt t	
LDH A	S	act gtg taa ctg cga act cca agc t	452
	AS	ctg ctt gtg aac ctc ctt cca	
LDH B	S	gct caa cct ggt gca gag aaa	344
	AS	ctg tcc cca ttt ctg gat tca g	
LDH C	S	cac ggc agt ctt ttc ctt agc act	399
	AS	gag tcc cca tgt tct cca aga a	
β-Actin	S	cag ggt gtg atg gtg gga at	287
	AS	Tgt ggt acg acc aga ggc ata ca	

4. Embryos Sampling

The embryos were sampled to examine the expression of *Ldh* genes and *Aqp* genes. Sampling for morula stage embryos did at the 84 hph, the embryos were quickly frozen using liquid nitrogen and stored at −80℃ until used. The blastocyst stage embryos were sampled by same manner at 108 hph. Hatching embryos were sampled at 120 hph as mentioned above. To analysis the express patterns of *Aqa* genes and *Ldh* genes, the early stage embryos and oocytes were collected by time schedule. These were containing unfertile egg (UF, 16 hph in no mating female mouse), pronucleus (PN, 16 hph), 2-cell, 4-cell, 8-cell, morula, blastocyst, and hatching embryos.

5. Total RNA Extract and RT-PCR

Total RNAs were extracted using SideStep™ Lysis & Stabilization Buffer (Strategene, cat#. 400901-21, CA, USA) according to the manufacturer's instruction. First strand cDNA was synthesized using First-Strand synthesis system (Stratagene, Cat #.200420, CA, USA). Briefly, the mixtures were incubated at 65℃ for 5 minutes and place tube at room temperature for 10 minutes for the primers to anneal to the RNA. And incubated at 42℃ for 60 minutes and incubated at 70℃ for 15 minutes to terminate cDNA synthesis.

Transcripts of target genes were amplified using PCR method (Table 2) with the *Ldh* and *Aqp* genes specific primers (Bionics, Table 2). PCR products were analyzed on 1% agarose gel and were stained with ethidiumbromide.

6. Statistics

The *t*-test was used to evaluate the difference between controls and experiment groups. Results were presented as MEAN ± SD. A *p*-value less than 0.05 were considered to be a significant difference.

RESULTS

1. Lactate Concentration in Preimplantation Stage Embryo

The amount of lactate in culture media could not disturb the embryonic development until morula stage (Fig. 1A). However, the development rate of morula to blastocysts was significantly reduced (respectively 26%, 25%) ($P < 0.05$) at 96 hph in 10, 5 and 0 mM groups compared with BWW control (Fig 1A). As expected the embryos could adapt to the condition of nutrients. At 72 hr after incubation (120 hph) most of embryos developed to the blastocyst stage (Fig. 1B).

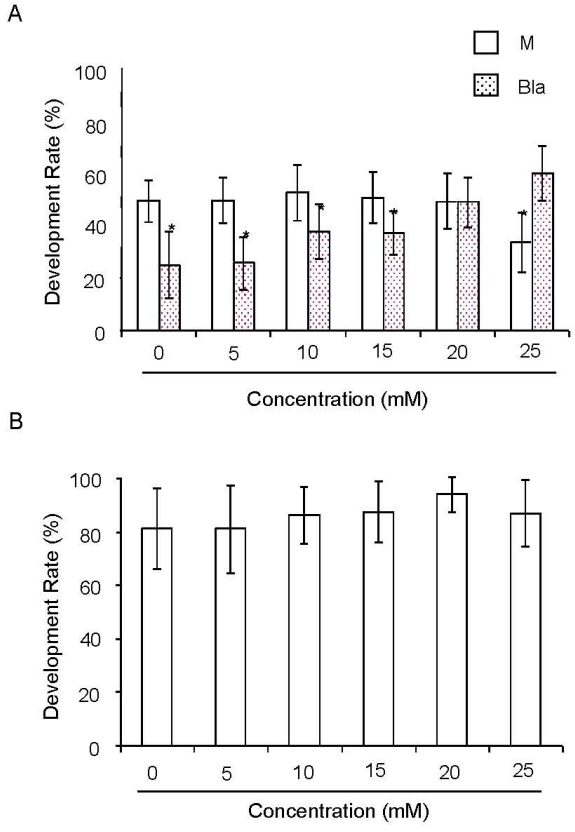

Fig. 1. Effects of lactate on the development of mouse embryos *in vitro*. A: Percentage of morula and blastocyst at 48 hr (96 hr post hCG) after incubation in conditioned media. B: Percentage of blastocyst at 72 hr (120 hr post hCG) after incubation in conditioned media. Mor; morula, Bla; blastocyst. * Significance: $P > 0.05$ Control vs. experimental group.

2. Profiles of *Ldh* Genes Expression during Early Embryonic Stage

Ldha, *Ldhb*, and *Ldhc* specific mRNAs were detected in embryos of various stages and lactate concentrations. In the BWW control condition, *Ldha* mRNA was detected morula and blastocyst stage embryos in BWW control.

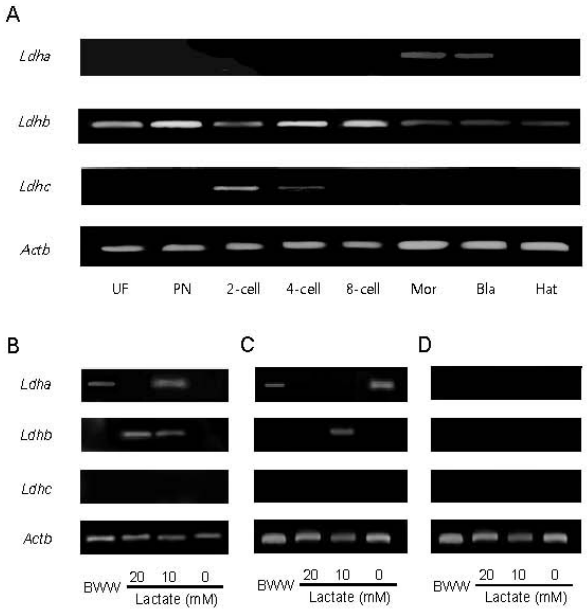

Fig. 2. Expression profiles of *Ldh* genes in periimplantation embryo. A: Expression profiles of *Ldha*, *Ldhb*, and *Ldhc* in unfertile eggs (UF), pronucleus stage embryos (PN), 2 cell, 4 cell, 8 cell, morula (Mor), blastocyst (Bla), hatching embryo (Hat). These embryos are cultured in BWW media. B: Expression profiles of *Ldha*, *Ldhb*, and *Ldhc* in morula stage in cultured different of lactate concentration. These embryos were cultured in 20 mM lactate modified BWW, 10 mM lactate modified BWW, and lactate free modified BWW. C: Expression profiles of *Ldha*, *Ldhb*, and *Ldhc* in blastocyst and over stage in cultured different of lactate concentration. These embryos were cultured in 20 mM lactate-modified BWW, 10 mM lactate-modified BWW, and lactate-free modified BWW. D: Expression profiles of *Ldha*, *Ldhb*, and *Ldhc* in hatching embryos in timely 120 hr post hCG injection. These embryos were cultured in 20 mM lactate-modified BWW, 10 mM lactate-modified BWW, lactate -free modified BWW.

Ldhb mRNA was detected in all examined stage (UF, PN, 2-cell, 4-cell, 8-cell, Mor, Bla, and Hat embryos). *Ldhc* mRNA detected only 2-cell and 4-cell embryos in BWW control (Fig. 2A). Interestingly, *Ldh* genes expression profiles were changed by lactate concentration. The *Ldha* mRNA was detected in morula stage embryo in 10 mM lactate group, and in blastocyst stage embryo in 0 mM lactate group (Fig. 2B, C). The *Ldhb* mRNA was detected in morula stage embryo at 20 mM and 10 mM lactate group, and in blastocyst stage in 10 mM lactate group (Fig 2B, C, D). *Ldhc* mRNA expression was not changed by lactate administration.

3. Expression Profile of *Aqp* Genes in Preimplantation Embryo

Aqp1, *3*, *6*, *7*, *8*, and *9* specific mRNAs were monitored in various embryo stage and concentration of lactate. In the embryos which were cultured in BWW medium, *Aqp3* mRNA was expressed in 4-cell and morula, and *Aqp8* mRNA was expressed in hatching stage embryo. And *Aqp9* mRNA was detected 4-cell, 8-cell and blastocyst (Fig. 3A). *Aqp1*, *6*, and *7* mRNAs were not detected in any stage embryos (Fig. 3A).

4. *Aqp* Genes Expression in Different Lactate Concentration

To examine, the lactate can effect on *Aqp* mRNAs expression in embryo, late 2-cell stage embryo were cultured in various conditional media and collected as mentioned at Materials and Methods. *Aqp1* mRNA expression was not modified by lactate concentration in the media in morula, blastocyst, and hatching stage embryos. *Aqp3* mRNA expression was induced only in hatching stage embryos by 0 mM lactate group (Fig. 3B, C, D). *Aqp7* mRNA expression was modified only in hatching stage embryos by 10 mM lactate group (Fig. 3B, C, D). *Aqp8* mRNA expression was modified in hatching stage embryos by 20 mM lactate group (Fig. 3B, C, D). *Aqp9* mRNA expression was modified only in morula of 10 mM lactate group (Fig. 3B, C, D).

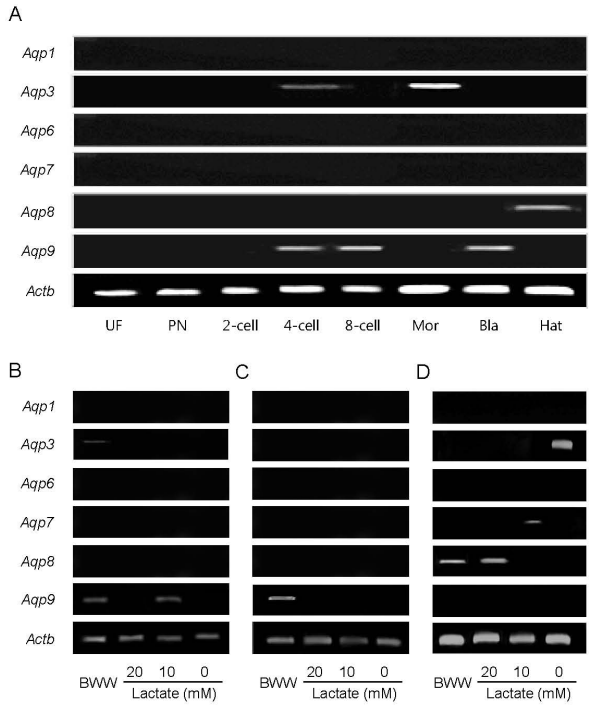

Fig. 3. Expression profiles of *Aqp* genes in preimplantation stages embryos and the modulation of those genes expression by the concentration of lactate. A: Expression profiles of *Aqp1*, *3*, *6*, *7*, *8*, and *9* expression in unfertile egg (UF), pronucleus (PN), 2 cell, 4 cell, 8 cell, morula, blastocyst, hatching stage embryo were analyzed with RT-PCR method. These embryos were cultured in BWW media. B: *Aqp1*, *3*, *6*, *7*, *8*, and *9* expression profiles in morula stage embryo. These embryos were cultured in 20 mM lactate-modified BWW, 10 mM lactate-modified BWW, and lactate-free modified BWW. C: *Aqp1*, *3*, *6*, *7*, *8*, and *9* expression patterns by lactate in blastocyst. These embryos were cultured in 20 mM lactate-modified BWW, 10 mM lactate-modified BWW, and lactate-free modified BWW. D: C: *Aqp1*, *3*, *6*, *7*, *8*, and *9* expression patterns by lactate in hatching embryo in timely 120 hr post hCG injection. These embryos were cultured in 20 mM lactate-modified BWW, 10 mM lactate-modified BWW, and lactate-free modified BWW.

DISCUSSION

Carbohydrate metabolism in early embryos shows substrate, specificity and metabolic adaptation (Leese, 1995; Cheon, 2008). Low level of lactate in medium did not inhibit the

development of 2-cell to morula stage. However, blastocoel formation rate was much low in the media containing less lactate at 96 hph. Blastocoel formation rate was dependent on lactate concentration in media. Besides in lactate free condition, blastocoel formation rate was dramatically reduced. Based on them it is suggested that lactate is a factor to control the embryonic development to blastocyst.

Metabolic adaptation of early stage embryos have been well known, even though the quality of embryos is different by the nutrient condition. Interestingly, as expected, those embryos formed blastocoel and reached to the blastocyt stage. Therefore it is suggested that the metabolic pathway to synthesize lactate is a critical factor for development of morula to blastocyst.

The metabolic pathway, LDH is a key enzyme to produce lactate. In mammals, LDH is constructed by assembly in homo- and heterodimer of *Ldha*, *Ldhb* and *Ldhc*. Differential expression of these genes determines the LDH isozyme composition of tissues. The A subunits predominate in skeletal muscle and the B subunits are abundantly produced in brain and heart. In oocytes, the LDH2 isozyme is the most abundant form. The C subunits are the primary LDH of spermatozoa (Coonrod et al, 2006). It means that the changes of the expression profiles of Ldh genes by the lactate concentration may make different functional dimers in the early stage embryos.

The embryonic stage specific isoform was examined in this study. In our result, *Ldh* genes mRNA were detected in oocyte and preimplantation embryos. *Ldhc* was expressed only 2-cell and 4-cell embryos. *Ldha* was expressed in morula stage and blastocyst stage. And *Ldhb* mRNA was expressed all stage in preimplantation embryo and oocyte. This result suggests that LDHA may involve in blastoceal formation and LDHB involve in preimplantation embryo development.

The embryo cultured in conditioned media showed different expression profiles of *Ldh* genes. *Ldha* mRNA was detected in morula stage at 10 mM lactate media, and in blastocyst stage at lactate free media. *Ldhb* mRNA expressed in morula stage at 20 mM and 10 mM lactate media. Also this gene expressed in blastocyst stage at 10mM lactate media. *Ldhc* mRNA did not expression at morula and blastocyst stage in cultured associated of lactate. These results showed that the expression of *Ldh* genes can be modulated by the concentration of lactate.

Recently, many studies have indicated that the water channel protein AQP is involved in the homeostasis of water level in the brain and reduction of brain edema. Here, these have demonstrated that lactate increases the AQP4 expression level on the membrane of cultured rat astrocytes, and it may be a new regulatory mechanism of AQP4 in the brain (Morishima et al., 2008). As for any substrate transported by aquaporins, the driving force for lactate through AQP9 is provided by its concentration gradient. The concentration gradient would be particularly pronounced in ischemia and hypoxia, which are characterized by an increased lactate formation and a reduction in cytoplasmic pH (Moghaddam et al., 2005). It has been suggested that AQP is involved in water transport during blstocoel formation (Barcroft et al., 2003).

Aqp1, *3*, *6*, *7*, *8*, and *9* gene expression was examined in oocytes and preimplantation embryos. As mentioned by Barcroft et al. (2003), *Aqp3*, *8*, and *9* mRNAs could detect in this study. *Aqp3* mRNA was expressed 4-cell and morula. *Aqp8* mRNA was expressed only hatching embryo. And *Aqp9* mRNA was detected 4-ell, 8-cell and blastocyst stage embryo. But *Aqp1*, *6*, *7* specific mRNAs were not detected at any stage. However, the stage which were detected a *Aqp* gene was not exactly same with the results of other reports (Brcroft et al., 2003; Edashige et al., 2000; Offenderg et al., 2000; Offender & Thomsen, 2005). Although, so far the results are controversy, these results show that oocyte does not express *Aqp*s and the development of blastocyst is effected by AQPs expression. In our experiment of associate with lactate culture, *Aqp* genes expression pattern have some different with BWW control embryo. *Aqp9* mRNA was expressed in morula in BWW control and 10mM lactate group. *Aqp3* mRNA was

detected in hatching embryos at lactate free condition. *Aqp7* mRNA was expressed in hatching embryo at 10mM lactate condition. *Aqp8* mRNA was detected at hatching embryo at 20mM lactate condition. Based on these results, *Aqp3* and *Aqp9* express during early stage embryo and these expression profiles can be modulated by the lactate concentration in media.

In summary, lactate involves in the development of pre-implantation stage embryos to blastocyst stage. In addition the expression profiles of *Ldh* and *Aqp* genes were modified by the lactate concentration in media. Based on them, it is suggested that lactate is a modulating factor of the expression of *Ldh* and *Aqp* genes in early stage embryos and a regulating molecule for early stage embryo development to the blastocyst stage.

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REFERENCES

Barcroft LC, Offenberg H, Thomsen P, Watson AJ (2003) Aquaporin proteins in murine trophectoderm mediate transepithelial water movements during cavitation. Dev Biol 256:342-354.

Biggers JD, Whittingham DG, Donahue RP (1967) The pattern of energy metabolism in the mouse oocyte and zygote. Proc Natl Acad Sci U S A 58:560-567.

Cheon YP (2008) Carbohydrate metabolism in preimplantation stage embryos and the role of metabolites. Dev Reprd 12:19-30.

Coonrod S, Vitare A, Duan C, Gould SB, Herr J, Goldburg E (2006) Testis-specific lactate dehydrogenase (LD H-C4; Ldh3) in murine oocytes and preimplantation embryos. J Androl 27:502-509.

Edashige K, Sakamoto M, Kasai M (2000) Expression of mRNAs of the aquaporin family in mouse oocytes and embryos. Cryobiology 40:171-175.

Hayashi Y, Edwards NA, Proescholdt MA, Oldfield EH, Merrill MJ (2007) Regulation and function of aquaporin-1 in glioma cells. Neoplasia 9:777-787.

Iacovetta C, Rudloff E, Kirby R (2012) The role of aquaporin 4 in the barin. Vet Clin Pathol 41:32-44.

Jakubovicz DE, Klip A (1989) Lactic acid-induced swelling in C6 glial cells via Na^+/H^+ exchange. Brain Res 485:215-224.

Jansen S, Esmaeilpour T, Pantaleon M, Kaye PL (2006) Glucose affects monocarboxylate cotransporter (MCT) 1 expression during mouse preimplantation development. Reproduction 131:469-479.

Kawagishi R, Tahara M, Sawada K, Morishige K, Sakata M, Tasaka K, Murata Y (2004) Na^+/H^+ exchanger-3 is involved in mouse blastocyst formation. J Experimental Zoology 301:767-775.

Khurana NK, Wales RG (1989) Effects of oxygen concentration on the metabolism of [U-14C] glycose by mouse morulae and early blastocysts *in vitro*. Reprod Fertil Dev 1:99-106.

Lane M, Gardner DK (2005) Mitochondrial malate-aspartate shuttle regulates mouse embryo nutrient consumption. J Biol Chem 280:18361-18367.

Leese HJ (1995) Metabolic control during preimplantation mammalian development. Hum Reprod Update 1:63-72.

Manejwala FM, Cragoe EJ, Schultz RM (1989) Blastocoel expansion in the preimplantation mouse embryo: Role of extracellular sodium and chloride and possible apical routes of their entry. Dev Biol 133:210-220.

Moghaddam MA, Lindland H, Zelenin S, Roberg BA, Gundersen BB, Petersen P, Rinvik E, Torgner IA, Ottersen OP (2005) Brain mitochondria contain aquaporin water channels: evidence for the expression of a short AQP9 isoform in the inner mitochondrial membrane. FASEB J 19:1459-1467.

Morishima T, Aoyama M, Iida Y, Yamamoto N, Hirate H, Arima H, Fujita Y, Sasano H, Tsuda T, Katsuya H, Asai K, Sobue K (2008) Lactic acid increases aquaporin

4 expression on the cell membrane of cultured rat astrocytes. Neurosci Research 61:18-26.

Offenberg H, Barcroft LC, Caveney A, Viuff D, Thomsen PD, Watson AJ (2000) mRNAs encoding aquaporins are present during murine preimplantation development. Mol Reprod Dev 57:323-330.

Offenberg H, Thomsen PD (2005) Functional challenge affects aquaporin mRNA abundance in mouse blastocysts. Mol Reprod Dev 71:422-430.

Saito M, Tanaka H, Sasaki M, Kurose H, Nakahata N (2010) Involvement of aquaporin in thromboxane A2 receptor-mediated, G12/13/RhoA/NHE-sensitive cell swelling in 1321N1 human astrocytoma cells. Cell Siganl 22: 41-46.

Watson AJ, Barcroft LC (2001) Regulation of blastocyst formation. Front Biosci 6:708-730.

Watson AJ, Natale DR, Barcroft LC (2004) Morecular regulation of blastocyst formation. Animal Repred Sci 82-83:583-592.

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