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Supplementary Material

Differential effects of high and low glucose concentrations during lipolysis-like conditions on bovine *in vitro* oocyte quality, metabolism and subsequent embryo development

J. De Bie^{A,D}, *W. F. A. Marei*^{A,B}, *V. Maillo*^C, *L. Jordaens*^A, *A. Gutierrez-Adan*^C, *P. E. J. Bols*^A and *J. L. M. R. Leroy*^A

^AGamete Research Centre, Laboratory for Veterinary Physiology and Biochemistry, Department of Veterinary Sciences, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium.

^BDepartment of Theriogenology, Faculty of Veterinary Medicine, Cairo University, Giza square, 12211 Giza, Egypt.

^CDepartamento de Reproduccion Animal, Instituto nacional de Investigacion y Tecnologia Agraria y Alimentaria (INIA), Crta. De la Coruña, km 7,5 28040 Madrid, Spain.

^DCorresponding author. Email: jessie.debie@uantwerpen.be

Table S1. Details of primers used for RT- qPCR

GENE SYMBOL	GENE NAME	ACCESSION NO.	FORWARD PRIMER (5'-3')	REVERSE PRIMER (5'-3')	PRODUCT LENGTH
<i>ACACA</i>	Acetyl-CoA carboxylase alpha	FN185963.1	AAGCAATGGATGAACCTTCTTC	GATGCCCAAGTCAGAGAGC	196
<i>ACTB</i>	Actin, beta	AF191490.1	GAGAAGCTCTGCTACGTCG	CCAGACAGCACCGTGTTGG	264
<i>CAT</i>	Catalase	NM_001035386.2	GAATATGGCTCCCGCATCCAGG	TTCACAAGCGGACGGCAACGTG	169
<i>CPT1B</i>	Carnitine palmitoyltransferase 1B	NM_001034349.2	CTGCCCGCCTGGGAAATGCTGT	CAGTCTCTCCTCCCCGGGCTGG	332
<i>G6PD</i>	Glucose-6-phosphate dehydrogenase	NM_001244135.1	CGCTGGGACGGGGTGCCCTTCATC	CGCCAGGCCTCCCGCAGTTCATCA	347
<i>GPX1</i>	Glutathione Peroxidase 1	NM_174076.3	GCAACCAGTTTGGGCATCA	CTCGCACTTTTCGAAGAGCATA	116
<i>H2AFZ</i>	H2A histone family, member Z	NM_174809	AGGACGACTAGCCATGGACGTGTG	CCACCACCAGCAATTGTAGCCTTG	209
<i>IGF2R</i>	Insulin-like growth factor 2 receptor	NM_174352.2	GCTGCGGTGTGCCAAGTGAAAAAG	AGCCCCTCTGCCGTTGTTACCT	201
<i>LDHA</i>	Lactate dehydrogenase A	NM_174099.2	TTCTTAAGGAAGAACATGTC	TTCACGTTACGCTGGACCAA	310
<i>NFE2L2</i>	Nuclear factor, erythroid 2-like 2	NM_001011678.2	CAGGACATTGAGCAAGTTTGG	GTGGAAAGGATGCTGTTGAAG	234
<i>PDHA1</i>	Pyruvate dehydrogenase (lipoamide) alpha 1	NM_001101046.2	TGGAAAGAGCAGCAGCCAGCAC	GCGAGATTGCTGTTCCACCATCC	295
<i>PKFM</i>	Phosphofructokinase, muscle	NM_001075268.1	AAGAACGTGCTGGGCCACATG	CTTCAGCCACCACTGCTCCTTG	268
<i>SCL2A1</i>	Solute carrier family 2 (facilitated glucose transporter) member 1 (former <i>GLUT1</i>)	NM_174602.2	CTGATCCTGGGTCGCTTCAT	ACGTACATGGGCACAAAACCA	68
<i>SOD2</i>	Superoxide Dismutase 2, Mitochondrial (former <i>MnSOD</i>)	S67818.1	GCTTACAGATTGCTGCTTGT	AAGGTAATAAGCATGCTCCC	101
<i>TFAM</i>	Transcription factor A, mitochondrial	NM_001034016.2	CAAATGATGGAAGTTGGACG	AGCTTCCGGTATTGAGACC	150

<i>UCP2</i>	Uncoupling protein 2 (mitochondrial, proton carrier)	NM_001033611.2	CGCTCGCAATGCCATTGTCAAC	CAGGATCCCAAGCGGAGAAAG	302
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Table S2. Glucose consumption and lactate production by individual embryos split by D7 blastocyst stage in each treatment

Data are presented as means \pm s.e.m. (7 repeats). Number of embryos evaluated are indicated between brackets. ^{a,b}Data with different superscripts in the same column within 1 stage are significantly different ($P < 0.05$).

D7 stage (all embryos)	Treatment	Glucose consumption (pmol/embryo/h)	Lactate production (pmol/embryo/h)	Lactate/2glucose ratio
Young	CONT	21.32 \pm 6.84 (7)	11.54 \pm 3.77 (12) ^{ab}	0.37 \pm 0.13 (7)
	HI NEFA	25.39 \pm 4.92 (12)	9.15 \pm 3.57 (12) ^a	0.17 \pm 0.06 (12)
	HI NEFA + HI GLUC	29.86 \pm 6.41 (12)	18.82 \pm 3.82 (14) ^b	0.42 \pm 0.19 (12)
	HI NEFA + LO GLUC	27.38 \pm 8.23 (10)	14.15 \pm 3.37 (10) ^{ab}	0.35 \pm 0.20 (10)
Normal	CONT	23.33 \pm 2.20 (22)	16.71 \pm 3.79 (23) ^a	0.41 \pm 0.10 (22) [§]
	HI NEFA	20.62 \pm 1.95 (17)	11.39 \pm 2.82 (18) ^{ab}	0.30 \pm 0.10 (17)
	HI NEFA + HI GLUC	30.75 \pm 5.25 (16)	8.86 \pm 2.34 (21) ^b	0.13 \pm 0.06 (16) [§]
	HI NEFA + LO GLUC	22.32 \pm 6.95 (10)	10.73 \pm 3.07 (14) ^{ab}	0.39 \pm 0.13 (10)
Expanded	CONT	29.77 \pm 2.56 (35)	24.75 \pm 3.29 (39)	0.44 \pm 0.08 (35)
	HI NEFA	32.74 \pm 2.83 (30)	28.79 \pm 3.32 (31)	0.48 \pm 0.07 (30)
	HI NEFA + HI GLUC	31.51 \pm 2.82 (15)	27.83 \pm 4.04 (17)	0.56 \pm 0.15 (15)
	HI NEFA + LO GLUC	23.57 \pm 3.42 (11)	27.56 \pm 3.36 (15)	0.61 \pm 0.13 (11)
Hatched	CONT	40.10 \pm 5.06 (9) ¹	36.20 \pm 8.15 (9) ¹	0.62 \pm 0.21 (9) ¹
	HI NEFA	33.01 \pm 14.16 (3) ¹	40.90 \pm 22.25 (3) ¹	0.64 \pm 0.48 (3) ¹
	HI NEFA + HI GLUC	42.56 \pm 21.19 (3) ¹	12.36 \pm 7.24 (2) ¹	0.34 \pm 0.24 (2) ¹
	HI NEFA + LO GLUC	44.92 (1) ¹	32.04 (1) ¹	0.36 (1) ¹

¹ Numbers of embryos in these groups are too low to perform statistical analysis.

[§] Data marked with dollar sign in the same column within 1 stage tend to be different from each other ($P < 0.1$).

Table S3. Glucose consumption and lactate production by individual growing embryos (developing to a more advanced stage from Day 7 to Day 8) per D7 stage in each treatment

Data are presented as means \pm s.e.m. (7 repeats). Number of embryos evaluated are indicated between brackets. ^{a,b}Data with different superscripts in the same column within 1 stage are significantly different ($P < 0.05$).

D7 stage (growing embryos)	Treatment	Glucose consumption (pmol/embryo/h)	Lactate production (pmol/embryo/h)	Lactate/2glucose ratio
Young	CONT	21.32 \pm 6.84 (7)	12.39 \pm 4.02 (11) ^{ab}	0.37 \pm 0.13 (7)
	HI NEFA	25.39 \pm 4.92 (12)	9.15 \pm 3.57 (12) ^a	0.17 \pm 0.06 (12)
	HI NEFA + HI GLUC	30.72 \pm 6.96 (11)	18.92 \pm 3.66 (12) ^b	0.46 \pm 0.20 (11)
	HI NEFA + LO GLUC	29.69 \pm 8.84 (9)	14.96 \pm 3.66 (9) ^{ab}	0.33 \pm 0.22 (9)
Normal	CONT	24.94 \pm 2.31 (19) ²	20.24 \pm 4.14 (19) ²	0.47 \pm 0.11 (19)
	HI NEFA	22.05 \pm 1.91 (15) ²	10.27 \pm 3.11 (15) ²	0.24 \pm 0.09 (15)
	HI NEFA + HI GLUC	30.67 \pm 4.34 (13) ²	10.85 \pm 2.55 (17) ²	0.17 \pm 0.07 (13)
	HI NEFA + LO GLUC	29.51 \pm 10.82 (6) ²	12.18 \pm 4.11 (10) ²	0.33 \pm 0.09 (6)
Expanded	CONT	30.25 \pm 2.60 (28) ¹	24.05 \pm 3.79 (31) ^a	0.41 \pm 0.08 (28) ¹
	HI NEFA	36.28 \pm 3.21 (21) ¹	32.97 \pm 3.79 (21) ^b	0.51 \pm 0.08 (21) ¹
	HI NEFA + HI GLUC	31.95 \pm 3.24 (10) ¹	27.52 \pm 4.88 (12) ^{ab}	0.48 \pm 0.15 (10) ¹
	HI NEFA + LO GLUC	10.36 (1) ¹	34.06 \pm 6.56 (3) ^{ab}	1.05 (1) ¹

¹ Numbers of embryos in these groups are too low to perform statistical analysis.

² Statistical analysis cannot be performed due to a significant interaction Treatment*Repeat (the effect of the treatment was dependent on the experimental repeat).