

Supplementary Material

Protein in culture and endogenous lipid interact with embryonic stages *in vitro* to alter calf birthweight after embryo vitrification and warming

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Table S1. Oligonucleotides used for real-time PCR gene expression analysis

Gene	primer	Sequence 5'-3'	Accession	Amplicon (bp)	Gene product	Primers' efficiency (%)
<i>ABHD6</i>	fw	ACCCCGAAGGAGATGAGTGA	NM_001075196	276	Abhydrolase domain containing 6	1.93
	rev	CTGGGAGTTGGCGATTGACT				
<i>ACACA</i>	fw	TGCTTCCCATTTGCCATC	NM_174224	188	Acetyl coenzyme A carboxylase	1.90
	rev	CTGCCATCCTCACGACCT				
<i>ACTB</i>	fw	GCTGTCCCTGTATGCCTCTGG	NM_173979	349	Actin, beta	2.01
	rev	GAACCGCTCATTGCCGATGG				
<i>BAX</i>	fw	AGAGGATGATCGCAGCTGTGGA	NM_173894	300	Bcl-2-associated X protein	1.99
	rev	CAAAGATGGTCACTGTCTGCCATGT				
<i>CPT1A</i>	fw	TCCTGGTGGGCTACCAATTA	FJ415874	181	Carnitine palmitoyltransferase 1A	1.95
	rev	TGCGTCTGTAAAGCAGGATG				
<i>CPT2</i>	fw	TGTGCCTTCCTTCCTGTCTTGG	NM_001045889	111	Carnitine palmitoyltransferase 2	1.98
	rev	CGATGGGGTCTGGGTAAACGA				
<i>DGATI</i>	fw	CGCCTTCTTCCACGAGTACC	NM_174693	159	Diacylglycerol O-acyltransferase 1	1.96
	rev	CCGATGATGAGTGACAGCCA				
<i>FABP3</i>	fw	ATCGTGACGCTGGATGGCGG	NM_174313	210	Fatty acid binding protein 3	2.04
	rev	GCCGAGTCCAGGAGTAGCCCA				
<i>FABP5</i>	fw	TGGCGCATTGGTTCAACATCAGG	NM_174315	193	Fatty acid binding protein 5	2.03
	rev	TGAACTGAGCTTGTCATCCTCGC				
<i>FASN</i>	fw	CACTCCATCCTCGCTCTCC	AY343889	181	Fatty acid synthetase	2.03
	rev	GCCTGTCATCATCTGTCACC				
<i>GLUT1</i>	fw	CTGATCCTGGGTCGCTTCAT	NM_174602	68		2.04

	rev	ACGTACATGGGCACAAAACCA			Solute carrier family 2 (facilitated glucose transporter), member 1 (SLC2A1)	
GPXI	fw	GCAACCAGTTTGGGCATCA	NM_174076	116	Glutathione peroxidase 1	2.06
	rev	CTCGCACTTTTCGAAGAGCATA				
GPX4	fw	CGATACGCCGAGTGTGGTTTAC	NM_174770	261	Glutathione peroxidase 4	1.96
	rev	ACAGCCGTTCTTGTC AATGAGG				
LIPE	fw	GAGTTTGAGCGGATCATTCA	NM_001080220	102	Hormone-sensitive lipase	1.98
	rev	TGAGGCCATGTTTGCTAGAG				
PLIN2	fw	ACAACACACCCCTCAACTGG	NM_173980	211	Adipophilin (perilipin 2)	1.97
	rev	CTGCCTGCCTACTTCAGACC				
PPLA2	fw	ATGGTGCCCTACACTCTGCC	NM_001046005	152	Patatin-like phospholipase domain containing 2	1.88
	rev	AGCTTCCTCTTGCGCGTAT				
PTSG2	fw	AGGTGTATGTATGAGTGTAGGA	NM_174445	483	Prostaglandin G/H synthase 2	2.05
	rev	GTGCTGGGCAAAGAATGCAA				
RBP4	fw	TTCGACAAGGCTCGCTTCGCC	NM_001040475	427	Retinol binding protein 4	2.02
	rev	CCTGCCTCTGCCGCACGATT				
RPL19	fw	AATCGCCAATGCCAACTC	NM_001040516	156	Ribosomal protein L19	1.98
	rev	CCCTTTCGCTTACCTATAACC				
RPS9	fw	GGAGACCCTTCGAGAAGTCC	BC148016	180	Ribosomal protein S9	2.05
	rev	GGGCATTACCTTCGAACAGA				
SDHA	fw	GCAGAACCTGATGCTTTGTG	NM_174178	185	Succinate dehydrogenase complex, subunit A, \pm	1.95
	rev	CGTAGGAGAGCGTGTGCTT				
SHC1	fw	GCAGTTGGAACCGGTAGCTT	NM_00116406	119	Src homology 2 domain containing) transforming	1.85

rev CCTTTGGTATAAGTGAGACCCG

protein 1 (p66Shc)

<i>SREBP1</i>	fw	ACCGCTCTTCCATCAATGAC	AB355703	190	Sterol regulatory element binding	1.87
	rev	TTCAGCGATTTGCTTTTGTG			transcription protein 1	
<i>TP53</i>	fw	AGCTGGTGTGGTAGGCAGT	NM_174201	180	Tumor protein p53	2.01
	rev	CCTCACCATCATCACACTGG				
<i>YWHAZ</i>	fw	GCATCCCACAGACTATTTCC	NM_174814	120	Tyrosine 3-monooxygenase / tryptophan 5-monooxygenase	2.05
	rev	GCAAAGACAATGACAGACCA			activation protein, zeta	

Table S2. Progesterone increases from Day-0 up to Day-7 in recipients transferred with Day-7 expanded blastocysts in terms of their Day-6 origin from the morula or the early blastocyst stage and their pregnancy status on Day-40

Day-6 stage	Pregnant	N	P4 [Day-7] – [Day-0]
Early blastocyst	(+)	13	15.3±3.5
Early blastocyst	(-)	14	12.5±3.0
Morula	(+)	27	17.1±2.1
Morula	(-)	27	18.8±2.7

Differences were not significant (P>0.10)

Table S3. Progesterone increases from Day-0 up to Day-7 in recipients transferred with Day-7 expanded blastocysts in terms of their culture with or without protein from Day-6 onwards and their pregnancy status on Day-40

Day-6 culture	Pregnant	N	P4 [Day-7] – [Day-0]
Protein	(+)	22	18.4±3.0
Protein	(-)	26	14.7±2.6
No protein	(+)	18	14.7±2.7
No protein	(-)	15	16.5±2.9

Differences were not significant (P>0.10)