

## Supplementary Material

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

*E. Gómez<sup>A,D</sup>, S. Carrocera<sup>A</sup>, S. Uzbekova<sup>B</sup>, D. Martín<sup>A</sup>, A. Murillo<sup>A</sup>, M. Alonso-Guervós<sup>C</sup>, F. Goyache<sup>A</sup> and M. Muñoz<sup>A</sup>*

<sup>A</sup>Centro de Biotecnología Animal, SERIDA, Camino de Rioseco 1225, 33394 Gijón, Spain.

<sup>B</sup>Institut National de la Recherche Agronomique , UMR8 Physiologie de la Reproduction et des Comportements, F-37380 Nouzilly, France.

<sup>C</sup>Unidad de Microscopía Fotónica y Proceso de Imágenes, Servicios Científico Técnicos, Universidad de Oviedo, Instituto Universitario de Oncología de Asturias (IUOPA), 33006 Oviedo, Spain.

<sup>D</sup>Corresponding author. Email: [egomez@serida.org](mailto:egomez@serida.org)

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

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

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

rev CCTTTGGTATAAGTGAGACCCG

protein 1 (p66Shc)

<b><i>SREBP1</i></b>	fw	ACCGCTCTTCCATCAATGAC	AB355703	190	Sterol regulatory element binding	1.87
	rev	TTCAGCGATTTGCTTTTGTG			transcription protein 1	
<b><i>TP53</i></b>	fw	AGCTGGTGTGGTAGGCAGT	NM_174201	180	Tumor protein p53	2.01
	rev	CCTCACCATCATCACACTGG				
<b><i>YWHAZ</i></b>	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)