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Supplementary Material

Insulin exposure during *in vitro* bovine oocyte maturation changes blastocyst gene expression and developmental potential

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Table S1.

Experimental design	
Definition of experimental and control groups	Control group INS0: Blastocysts obtained in regular maturation IVF media. Insulin groups, INS0.1 and INS10: Blastocysts obtained in maturation IVF media containing 0.1 ug/ml and 10 ug/ml of insulin, respectively.
Number within each group	n=4 (pool of 10)
Samples	
Description	For each experiment RNA extractions were performed with 4 independent experimental groups. Each RNA sample was obtained from an experimental group including 10 different Blastocysts. Real time RT-PCR was performed once for each examined gene, using 4 replicates for each cDNA.
Nucleic acid extraction	
Procedure/kit	RNA was extracted with AllPrepDNA/RNA micro kit (Qiagen). The DNA was eluted in 30 ul of water and the RNA was eluted in 15 ul of elution buffer and both were kept at -80° C
DNase treatment	No DNAse treatment was done as recommended in the kit.
Contamination	Absence of genomic DNA contamination in the RNA samples was tested with the Bioanalyzer (Agilent)
Quantification	Bioanalyzer (Agilent)
Integrity	RNA integrity number : 8.5-9.3
Reverse transcription	
Procedure/kit	qScript™ Flex cDNA Kit (Quanta Biosciences) with oligo-dT (10uM)
Amount of RNA	Equivalent of 5 blastocysts of total RNA

Reaction volume	20 µl
Temperature and time	65° C for 5 minutes 42° C for 1 hour 70° C for 15 minutes
Storage condition of cDNA	-20° C

RT-qPCR target information and oligonucleotide

Gene symbol	Gene Name	Genebank	Primer sequence (5'-3')		Annealing Temperature	Lenght	Efficiency
MYL6	myosin, light chain 6, alkali, smooth muscle and non-muscle	NM_175780.1	Fwr	TTCGGGTGTTTGACAAGGAAGGGA	57	182	2.03
			Rev	ATCCTCAGCCATTCAGCACCAT			
IGF2R	insulin-like growth factor 2 receptor	NM_174352.2	Fwr	AAGACCCTTGCTGCTTTAGG	57	250	1.88
			Rev	GGTGGAGTCTAACTGAGAGGATA			
INSIG1	insulin induced gene 1	NM_001077909.1	Fwr	CCCTATGGGATCTGCAATCTGTGA	57	200	1.80
			Rev	GGCTCAGATTGGTGTTCCTATAC			
APOA1	apolipoprotein A-I	NM_174242.3	Fwr	CCGTGTATGTGGAAGCAATCAAGG	57	100	2.04
			Rev	GTTGTCCAGGAGTTTCAGGTTGAG			
CYP11A1	cytochrome P450, family 11, subfamily A, polypeptide 1	NM_176644.2	Fwr	TAGCATCAAGGAGACGCTGAGA	57	469	1.86
			Rev	TAGCTGGATTGGTGGAAAGGG			
IGF2	insulin-like growth factor 2	NM_174087.3	Fwr	CACGCGCAGAACACCAAGTCAT	57	120	1.92
			Rev	TGGGATTGCGAGCGATAAAGGT			
SOX2	SRY (sex determining region Y)-box 2	NM_001105463.2	Fwr	CCCAAGAGAACCCTAAGATG	57	204	1.85
			Rev	GGCAGTGTGTACTTATCCTT			
GSC	goosecoid homeobox	NM_001192386.1	Fwr	CTCTTCCAGGAGACCAAGTA	57	61	1.80
			Rev	TCGTCAGTGAAGATGGTACG			
FASTK	Fas-activated serine/threonine kinase	NM_001035077.2	Fwr	CTCCAGTTCTTCAAAGGGTAG	57	652	2.07
			Rev	CCTCTGGAGCAGCAGTTTAT			
ADIPOR2	adiponectin receptor 2	NM_001040499.2	Fwr	CCAACCATGAAACGGAACTC	57	492	1.86
			Rev	GGATCTTCTCCAAACTGGATTA			
DHCR7	7-dehydrocholesterol reductase	NM_001014927.1	Fwr	CCCACAGGTATTCTTGACTTT	57	208	1.89
			Rev	CCTGCACTAACTCTGTTAGAC			
MVD	mevalonate (diphospho) decarboxylase	NM_001075424.1	Fwr	CCTGAGCACCTCTTTGATGG	57	221	2.17

			Rev	GGGAAAGGTGAGGCACTTAG			
KEAP1	kelch-like ECH-associated protein 1	NM_001101142.1	Fwr	GGTCACACATTCTTGGACAG	57	208	1.86
			Rev	AATACTCTGGATCGGACCTT			
ATP6AP2	ATPase, H+ transporting, lysosomal accessory protein 2	NM_001098022.1	Fwr	GGGCTACTGTTATGGTGATG	57	195	1.82
			Rev	CCCACCATGTACACTCTTTC			
PPAP2C	phosphatidic acid phosphatase type 2C	NM_001045890.1	Fwr	CTCCACTAACTCCACCTTCT	57	127	1.90
			Rev	CTCTCAGTCCCTTCCCTAAG			
ACAA1	acetyl-CoA acyltransferase 1	NM_001034319.2	Fwr	CTCTAGCCAGGTGAGTGATG	57	525	2.09
			Rev	GGTGTCTTGACTTGCTATCC			
DNMT3B	DNA (cytosine-5-)-methyltransferase 3 beta	NM_181813.2	Fwr	CCTGTGATAGCATCCAAGAAT	57	199	1.82
			Rev	GAAAGCCGAAGATCCTTTCT			
NDUFA10	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 10, 42kDa	NM_176655.2	Fwr	AGGTGGTCGAGGATATTGAG	57	198	1.90
			Rev	CTCTGTGAACTCCTGGAAGA			
MAP2K2	mitogen-activated protein kinase kinase 2	NM_001038071.2	Fwr	CGAGGTGGAAGAAGTGGATTT	57	221	1.97
			Rev	GAAGCAGGATCTGAGAAGACAG			
PSMD4	proteasome (prosome, macropain) 26S subunit, non-ATPase, 4	NM_001013598.2	Fwr	ATGCCCTGCTGAAGATGAC	57	199	1.92
			Rev	CAAAGTCACTGGCACCAAGA			
VIM	vimentin	NM_173969.3	Fwr	GTCCAAGTTTGCTGACCTCT	57	256	1.89
			Rev	GAGCCATCTCTTCCTTCATGTT			
FASD2	fatty acid desaturase 2	NM_001083444.1	Fwr	TCAGGAGACAGAGGGAAAGAG	57	217	1.87
			Rev	CTGGAGCTATCTACGGGTTAGT			
ACTB	actin, beta	NM_173979.3	Fwr	ATCGTCCACCGCAAATGCTTCT	59	101	1.82
			Rev	GCCATGCCAATCTCATCTCGTT			
PPIA	peptidylprolyl isomerase A (cyclophilin A)	NM_178320.2	Fwr	ACTTAAGCACCAGACCATTC	57	111	1.89
			Rev	TATGGAACCCAAAGAACTGTAG			
B2M	beta-2-microglobulin	NM_173893.3	Fwr	AGACACCCACCAGAAGATGG	54	234	1.91
			Rev	GGGGTTGTTCCAAAGTAACG			

RT-qPCR protocol	
Complete reaction conditions	LightCycler® 480 SYBR Green I Master (Roche)
Reaction volume amount of cDNA/primers/polymerase/buffer	Reaction volume: 20 µl Amount of cDNA: equivalent of 5 blastocysts Primer: 0.5 uM (final of each primer) Polymerase, nucleotides, $MgCl_2$ and buffer are included in the LightCycler® 480 SYBR Green I Master (Roche)
Complete thermo cycling parameters	Hold: 95° C for 10 minutes 50 cycles:

	95°C for 5 seconds Specific for each set of primers °C for 5seconds 72°C for 20 seconds
Real time RT-PCR instrument	Light Cycler 480 (Roche)
Data Analysis	
Statistical methods for results significance	Differences in expression between the INS0 group and the INS0.1 group and between INS0 group and the INS10 group were compared by unpaired t test (GraphPad Software ©, Prism 5) following log transformation of data. Differences in expression with p values <0.05 were considered as significant
Analysis of expression stability of endogenous reference genes	To analyze gene expression stability, Ct values of 3reference genes (*ACTB*, *PPIA* and *B2M*) were evaluated using Genorm software (Biogazelle). Under our experimental conditions, the 2 most stable reference genes were *ACTB* and *B2M* and the constant of their geometrical mean was use to normalized the genes.