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Supplementary Material

Lipid profile of bovine blastocysts exposed to insulin during *in vitro* oocyte maturation

Denise Laskowski[A,E,F], *Göran Andersson*[B,E], *Patrice Humblot*[A,E], *Marc-André Sirard*[C], *Ylva Sjunnesson*[A,E], *Christina R. Ferreira*[D], *Valentina Pirro*[D] *and Renée Båge*[A,E]

[A]Department of Clinical Sciences, PO Box 7054, SE-750 07 Uppsala, Sweden.

[B]Animal Breeding and Genetics, Swedish University of Agricultural Sciences, PO Box 7023, SE-750 07 Uppsala, Sweden.

[C]Departement des Sciences Animales, Centre de Recherche en Biologie de la Reproduction, Pavillon Des Services, Local 2732, University Laval, Québec G1V 0A6, Canada.

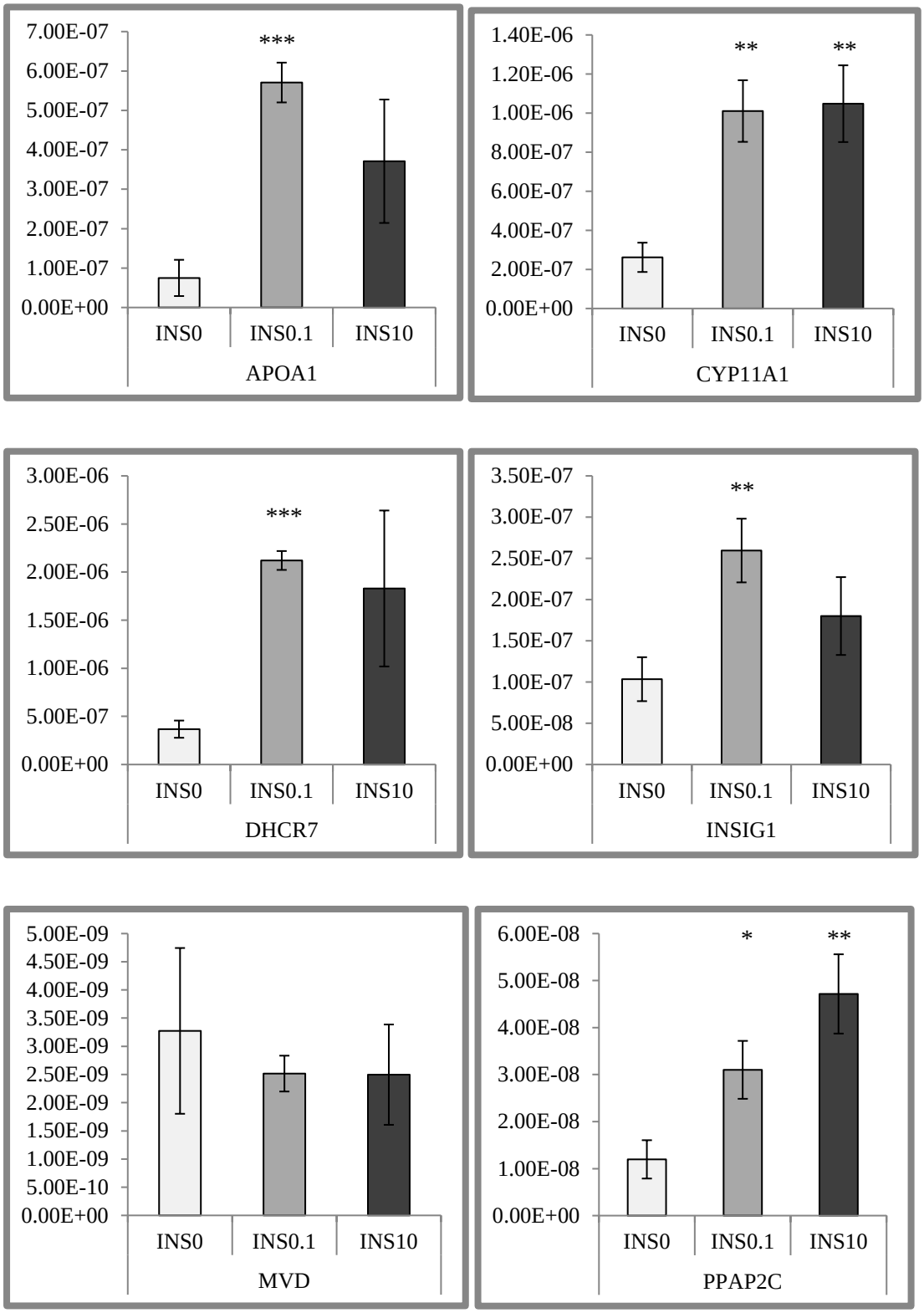
[D]Department of Chemistry, Purdue University, 560 Oval Drive, West Lafayette, IN 47907-2084, USA.

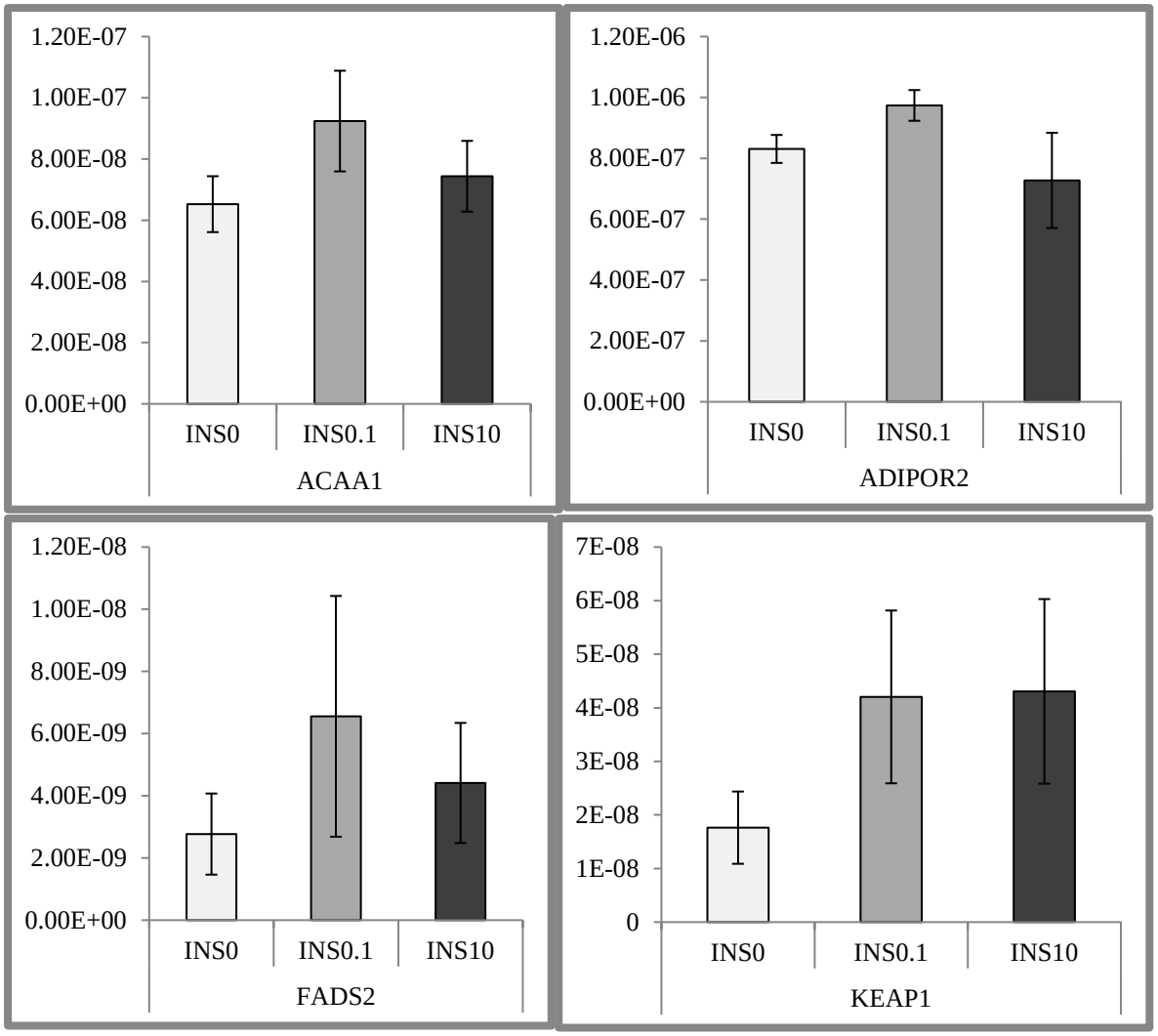
[E]Centre for Reproductive Biology in Uppsala (CRU), Box 7054, 75007 Uppsala, Sweden.

[F]Corresponding author. Email: denise.laskowski@slu.se

Fig. S1. Validation of microarray data. The validation of 10 candidate genes showed the same pattern as the microarray data with the exception of MVD.

Validation of genes linked to cholesterol metabolism (APOA1, CYP11A1, DHCR7, INSIG1, MVD, PPAP2C).





Microarray fold change differences

Gene	INS0	INS0.1	INS10
ACAA1	1	1,679	1,504
ADIPOR2	1	1,584	1,775
APOA1	1	1,569	1,785
CYP11A1	1	1,338	2,325
DHCR7	1	1,933	2,325
FADS2	1	1,872	1,678
INSIG1	1	1,286	1,509
KEAP1	1	1,698	1,959
MVD	1	1,557	1,935
PPAP2C	1	1,473	1,897

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 µg/ml and 10 µg/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 groups.
Nucleic acid extraction	
Procedure/kit	RNA was extracted with AllPrepDNA/RNA micro kit (Qiagen). The DNA was eluted in 30 µl of water and the RNA was eluted in 15 µl 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

Amplicon specificity was verified on an agarose gel and by sequencing. Efficiency of the reaction was analysed with standard curves analysis. The RT-qPCR analyses were done by using the second derivative method included in the Lightcycler 480 software.

Gene Symbol	Gene Name	Genebank	Primer sequence (5'-3')	Annealing temperature	Efficiency

INSIG1	insulin induced gene 1	NM_001077909.1	Fwr	CCCTATGGGATCTGCAATCTGTGA	57	1.80
			Rev	GGCTCAGATTGGTGTTCCTATAC		
APOA1	apolipoprotein A-I	NM_174242.3	Fwr	CCGTGTATGTGGAAGCAATCAAGG	57	2.04
			Rev	GTTGTCCAGGAGTTTCAGGTTGAG		
CYP11A1	cytochrome P450, family 11, subfamily A, polypeptide 1	NM_176644.2	Fwr	TAGCATCAAGGAGACGCTGAGA	57	1.86
			Rev	TAGCTGGATTGGTGGAAAGGG		
ADIPOR2	adiponectin receptor 2	NM_001040499.2	Fwr	CCAACCATGAAACGGAACTC	57	1.86
			Rev	GGATCTTCTCCAAACTGGATTA		
DHCR7	7-dehydrocholesterol reductase	NM_001014927.1	Fwr	CCCACAGGTATTCTTGACTTT	57	1.89
			Rev	CCTGCACTAACTCTGTTAGAC		
MVD	mevalonate (diphospho) decarboxylase	NM_001075424.1	Fwr	CCTGAGCACCTCTTTGATGG	57	2.17
			Rev	GGGAAAGGTGAGGCACTTAG		
KEAP1	kelch-like ECH-associated protein 1	NM_001101142.1	Fwr	GGTCACACATTCTTGGACAG	57	1.86
			Rev	AATACTCTGGATCGGACCTT		
PPAP2C	phosphatidic acid phosphatase type 2C	NM_001045890.1	Fwr	CTCCACTAACTCCACCTTCT	57	1.90
			Rev	CTCTCAGTCCCTTCCCTAAG		
ACAA1	acetyl-CoA acyltransferase 1	NM_001034319.2	Fwr	CTCTAGCCAGGTGAGTGATG	57	2.09
			Rev	GGTGTCTTGACTTGCTATCC		
FASD2	fatty acid desaturase 2	NM_001083444.1	Fwr	TCAGGAGACAGAGGGAAAGAG	57	1.87
			Rev	CTGGAGCTATCTACGGGTTAGT		

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 0.125 blastocysts Primer: 0.5 mM (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 3 reference 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.

(*d*) qPCR information and protocol

Table S1. Values of m/z. predicted ion molecular formula, ion description, and tentative attribution of lipids detected in positive ion modes by DESI-MS (Gonzalez-Serrano *et al.* 2013; Pirro *et al.* 2014)

Abbreviations: DAG. diacylglycerol. TAG. triacylglycerol. Attribution of lipids is based on Lipid Maps (www.lipidmaps.org) and Metlin (http://metlin.scripps.edu) searches of the predicted molecular formulae and possible adducts.

[a] (C:U) represents the number of carbon atoms (C) and the number of unsaturations (U) of the fatty acyl chains.

Ion *m/z*	Ion molecular formula	Ion description	Tentative attribution
686.2	$C_{30}H_{50}O_3NAg_2$	$[M+Ag_2NO_3]^+$	Squalene
725.4	$C_{39}H_{70}O_5Ag$		DAG (36:2)[a]
729.4	$C_{43}H_{74}O_2Ag$		16:1 Cholesteryl ester
755.4	$C_{45}H_{76}O_2Ag$		18:2 Cholesteryl ester
757.5	$C_{45}H_{78}O_2Ag$		18:1 Cholesteryl ester
797.4	$C_{43}H_{78}O_6Ag$		TAG(40:2)
799.5	$C_{43}H_{80}O_6Ag$		TAG(40:1)
823.5	$C_{45}H_{80}O_6Ag$		TAG(42:3)
825.5	$C_{45}H_{82}O_6Ag$		TAG(42:2)
827.5	$C_{45}H_{84}O_6Ag$		TAG(42:1)
851.5	$C_{47}H_{84}O_6Ag$		TAG(44:3)
853.5	$C_{47}H_{86}O_6Ag$		TAG(44:2)
881.5	$C_{49}H_{90}O_6Ag$		TAG(46:2)
883.5	$C_{49}H_{92}O_6Ag$		TAG(46:1)
911.6	$C_{51}H_{96}O_6Ag$	$[M+Ag]^+$	TAG (48:1)
935.6	$C_{53}H_{96}O_6Ag$		TAG (50:3)
937.6	$C_{53}H_{98}O_6Ag$		TAG (50:2)
939.6	$C_{53}H_{100}O_6Ag$		TAG (50:1)
963.6	$C_{55}H_{100}O_6Ag$		TAG (52:3)
965.6	$C_{55}H_{102}O_6Ag$		TAG (52:2)
967.6	$C_{55}H_{104}O_6Ag$		TAG (52:1)
989.6	$C_{57}H_{102}O_6Ag$		TAG (54:4)
991.6	$C_{57}H_{104}O_6Ag$		TAG (54:3)
993.6	$C_{57}H_{106}O_6Ag$		TAG (54:2)
995.6	$C_{57}H_{108}O_6Ag$		TAG (54:1)
1015	$C_{59}H_{104}O_6Ag$		TAG (56:3)
1017	$C_{59}H_{106}O_6Ag$		TAG (56:2)
1019	$C_{59}H_{108}O_6Ag$		TAG(56:1)
1106.2	$C_{53}H_{96}O_9NAg_2$		TAG (50:3)
1108.2	$C_{53}H_{98}O_9NAg_2$		TAG (50:2)
1132.2	$C_{55}H_{100}O_9Nag_2$		TAG (52:3)
1134.2	$C_{55}H_{102}O_9Nag_2$	$[M+Ag_2NO_3]^+$	TAG (52:2)
1140.4	$C_{59}H_{90}O_7NAg_2$		Ubiquinone (Coenzyme Q10)
1160.2	$C_{57}H_{104}O_9NAg_2$		TAG (54:3)
1162.2	$C_{57}H_{106}O_9NAg_2$		TAG (54:2)