Assisted Reproduction Interrupt the Placental Lipid Metabolism Resulted to Genomic Imprinting Aberration linked to Slow Fetal Growth

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Figure S1 Methylation status of CpG sites of *Gnas, Grb10.*

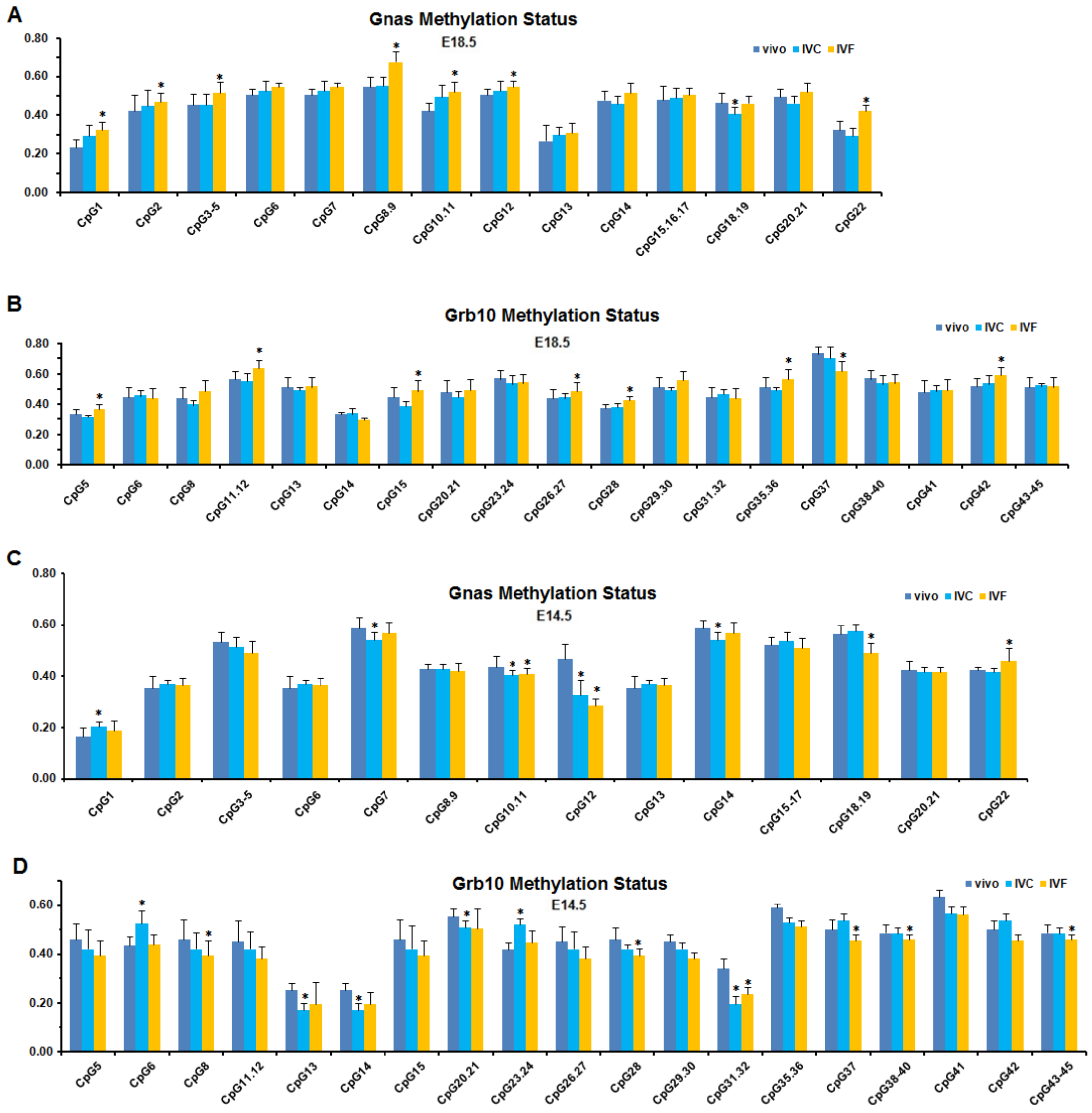

Table S1 The quantities of 15 lipid classes.

E18.5 placentae	Average content (uM/g)			Ratio of changes	
	in vivo	IVC	IVF	IVC/vivo	IVF/vivo
Acylglycerols					
TAG (triacylglycerides)	4.481	3.563	9.256	0.795	2.066
DAG (diacylglycerol)	0.278	0.255	0.351	0.917	1.263
Cholesterols					
CE (cholesteryl esters)	4.080	3.372	10.419	0.826	2.554
Free Cho (free cholesterol)	29.695	22.617	48.092	0.762	1.620

Sphingolipids					
Cer (ceramide)	0.377	0.337	0.803	0.893	2.128
GluCer (Glucosylceramide)	0.218	0.262	0.359	1.203	1.649
SM (sphingomyelin)	7.000	6.342	12.594	0.906	1.799
GM3 (anglioside mannoside 3)	0.062	0.118	0.168	1.887	2.688
Phospholipids					
PC (phosphatidylcholine)	26.105	22.297	36.031	0.854	1.380
PE (phosphatidylethanolamine)	4.983	4.780	7.108	0.959	1.427
PG (Phosphatidylglycerol)	0.187	0.176	0.393	0.942	2.104
PI (phosphatidylinositol)	4.735	4.530	5.831	0.957	1.231
PS (phosphatidylserine)	4.739	3.897	7.103	0.822	1.499
LBPA (Lysobisphosphatidic acid)	0.058	0.037	0.079	0.641	1.372
LPC (lysophosphatidylcholine)	0.526	0.476	0.698	0.904	1.326

E14.5 placentae	**Average content (uM/g)**			**Ratio of changes**	
	in vivo	**IVC**	**IVF**	**IVC/vivo**	**IVF/vivo**
Acylglycerols					
TAG (triacylglycerides)	14.162	16.976	5.901	1.199	0.417
DAG (diacylglycerol)	0.598	0.546	0.249	0.913	0.416
Cholesterols					
CE (cholesteryl esters)	9.214	9.180	4.882	0.996	0.530
Free Cho (free cholesterol)	48.677	51.873	25.674	1.066	0.527
Sphingolipids					
Cer (ceramide)	0.732	0.742	0.356	1.013	0.487
GluCer (Glucosylceramide)	0.770	0.829	0.503	1.077	0.654
SM (sphingomyelin)	10.633	9.895	5.514	0.931	0.519
GM3 (anglioside mannoside 3)	0.260	0.243	0.099	0.935	0.381
Phospholipids					
PC (phosphatidylcholine)	48.050	40.162	21.763	0.836	0.453
PE (phosphatidylethanolamine)	11.414	9.994	5.698	0.876	0.499
PG (Phosphatidylglycerol)	0.344	0.400	0.213	1.163	0.619
PI (phosphatidylinositol)	8.313	7.598	4.659	0.914	0.560
PS (phosphatidylserine)	7.453	5.837	4.054	0.783	0.544
LBPA (Lysobisphosphatidic acid)	0.116	0.078	0.040	0.668	0.348
LPC (lysophosphatidylcholine)	0.791	1.572	0.556	1.988	0.703

Table S2 qPCR primer sequences for the genes related to lipid uptake, transport and metabolism

Genes	**Forward primers**	**Reverse primers**	**Gene ID**
ABCG1	CTTTCCTACTCTGTACCCGAGG	CGGGGCATTCCATTGATAAGG	11307
Acaca	CCGATTCATAATTGGGTCTGTGT	CCATCCTGTAAGCCAGAGATCC	NM_133360
Acly	CAGCCAAGGCAATTTCAGAGC	CTCGACGTTTGATTAACTGGTCT	NM_134037
Apoe	CTGACAGGATGCCTAGCCG	CGCAGGTAATCCCAGAAGC	11816
Lrp8	TCCTGCCGAGAAGTTAAGCTG	AAGAACGCAAGTCCCATCCC	16975
Actb	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT	11461
Agrp	ATGCTGACTGCAATGTTGCTG	CAGACTTAGACCTGGGAACTCT	11604
Apoc1	TCCTGTCCTGATTGTGGTCGT	CCAAAGTGTTCCCAAACTCCTT	11812
CD31	ACGCTGGTGCTCTATGCAAG	TCAGTTGCTGCCCATTCATCA	18613
Cpt1a	GGGTCGAAAGCCCATGTTGTA	CAGTGCTGTCATGCGTTGGA	NM_013495
Cpt1b	GCACACCAGGCAGTAGCTTT	CAGGAGTTGATTCCAGACAGGTA	NM_009948
Ctps1	GCAGTGTGGGCACAATACTTA	GCGCTCCTTGTTAATGACGTA	51797
Cat	AGCGACCAGATGAAGCAGTG	TCCGCTCTCTGTCAAAGTGTG	12359
EL	CTTCCAGTGCACAGACTCCA	GGGTGTCCCCACTGTTATTG	NM_010720.3
Fasn	GGAGGTGGTGATAGCCGGTAT	TGGGTAATCCATAGAGCCCAG	NM_007988
Slc27a1	CGCTTTCTGCGTATCGTCTG	GATGCACGGGATCGTGTCT	26457
Slc27a4	ACTGTTCTCCAAGCTAGTGCT	GATGAAGACCCGGATGAAACG	26569
Slc2a1	CAGTTCGGCTATAACACTGGTG	GCCCCCGACAGAGAAGATG	20525
Gpx1	AGTCCACCGTGTATGCCTTCT	GAGACGCGACATTCTCAATGA	14775
Gpihbp1	AGCAGGGACAGAGCACCTCT	AGACGAGCGTGATGCAGAAG	NM_026730.1
Hmgcs2	TCGAGGGCATAGATACCACCAAC	CCGCGCTTCAGTTCAGTGTC	NM_008256
Hnf4a	CACGCGGAGGTCAAGCTAC	CCCAGAGATGGGAGAGGTGAT	15378
HSL	GCTGGGCTGTCAAGCACTGT	GTAACTGGGTAGGCTGCCAT	NM_010719.5
LDLR	TGACTCAGACGAACAAGGCTG	ATCTAGGCAATCTCGGTCTCC	16835

LIPIN1	CATGCTTCGGAAAGTCCTTCA	GGTTATTCTTTGGCGTCAACCT	14245
LIPIN3	CAAACCTCGTGGTGAAAATCAAC	CCACAGTGCTCTCAGGTAAGT	64899
LPL	GGGAGTTTGGCTCCAGAGTTT	TGTGTCTTCAGGGGTCCTTAG	16956
Sorl1	AGCAGGAGGGAGTCGAGAC	GTTCCTAGCCGGAGATCGC	20660
LRP 1	ACAAGGACTGTACCGATGGC	GGGTACTCACACTCAGGGGA	NM_008512.2
LRP2	GTTCGGGTTGATGTTCTGGAC	TGCATCGTTTATGATGAGTCTCC	14725
Ldlr	GGATGTCGACTGTGTTGACG	GCACACTGGAATTCATCAGG	NM_010700.2
Lepr	GTCTTCGGGGATGTGAATGTC	ACCTAAGGGTGGATCGGGTTT	16847
Lrp1	ACTATGGATGCCCCTAAAACTTG	GCAATCTCTTTCACCGTCACA	16971
PPARd	CAAGTGGGGTCAGTCATGGAA	GCTGGAAGGAAGCGTGTGTT	19015
PPARa	AGAGCCCCATCTGTCCTCTC	ACTGGTAGTCTGCAAAACCAAA	19013
PPARg	TCGCTGATGCACTGCCTATG	GAGAGGTCCACAGAGCTGATT	19016
Plin2	GACCTTGTGTCCTCCGCTTAT	CAACCGCAATTTGTGGCTC	11520
Prdx2	GGTAACGCGCAAATCGGAAAG	TCCAGTGGGTAGAAAAAGAGGT	21672
Pla2g10	GTGCAGGTGTGACGAGGAG	CACTTGGGAGAGTCCTTCTCA	26565
Pla2g15	GGGTAACCAGTTGGAAGCAAA	TTGTCAATCCAGCAGTCAATGAT	192654
RBP	GGAGAACTTCGAACAAGGCTC	CTGCACACACTTCCCAGTTG	NM_001159487.1
Rbp1	CTGAGCAATGAGAATTTCGAGGA	GCGGTCGTCTATGCCTGTC	19659
Sepp1	AGCTCTGCTTGTTACAAAGCC	CAGGTCTTCCAATCTGGATGC	20363
Sc4mol	AAACAAAAGTGTTGGCGTGTTC	AAGCATTCTTAAAGGGCTCCTG	66234
VLDLR	GGCAGCAGGCAATGCAATG	GGGCTCGTCACTCCAGTCT	22359
UGT1A1	TGACCACGCGCAGCAGAAAAGAAT	TGACCACGCGCAGCAGAAAAGAAT	394431
UGT1A2	TGCCCCTTCGAGGAATCTCAGG	CTCCCGCACAACATCCCTCATG	[1]
UGT1A6	CTGGCTGATGGTGGCTGACTG	ACTGAGGCCCAAAGCACTAGGAA	[1]
Pgf	TCTGCTGGGAACAACTCAACA	GTGAGACACCTCATCAGGGTAT	18654
Bcl2	ATGCCTTTGTGGAACTATATGGC	GGTATGCACCCAGAGTGATGC	12043
Bax	TGAAGACAGGGGCCTTTTTG	AATTCGCCGGAGACACTCG	12028
GSR	GACACCTCTTCCTTCGACTACC	CCCAGCTTGTGACTCTCCAC	14782

Gsx1	CTTCCCTCCCTTCGGATCG	GTCCACAGAGATGCAGTGAAA	14842
SOD1	AACCAGTTGTGTTGTCAGGAC	CCACCATGTTTCTTAGAGTGAGG	20655
casp3	TGGTGATGAAGGGGTCATTTATG	TTCGGCTTTCCAGTCAGACTC	12367
Casp8	TGCTTGGACTACATCCCACAC	TGCAGTCTAGGAAGTTGACCA	12370
casp9	GACGCTCTGCTGAGTCGAG	GGTCTAGGGGTTTAACAGCCTC	12371
Cytb	AACATACGAAAAACACACCCATT	AGTGTATGGCTAAGAAAAGACCTG	[2]
COXII	ACGAAATCAACAACCCCGTA	CTAGGGAGGGGACTGCTCAT	[2]
Gapdh	CATGGCCTTCCGTGTTCCTA	TGTCATCATACTTGGCAGGTTT	[2]
HPRT	CAGGCCAGACTTTGTTGGAT	TTGCGCTCATCTTAGGCTTT	[2]

1 Wassef L, and Quadro L. (2011) Uptake of Dietary Retinoids at the Maternal-Fetal Barrier J Biol Chem 286: 32198-32207.

2 Collier AC, Milam KA, Rougée LR, Sugawara A, Yamauchi Y, et al. (2012) Upregulation of Ugt1a genes in placentas and fetal livers. Placenta 33: 77-80.

Table S3 qPCR primer sequences for the genes related to lipid metabolism regulation

Genes	**Forward primers**	**Reverse primers**	**Gene ID**
CD36	ATGGGCTGTGATCGGAACTG	GTCTTCCCAATAAGCATGTCTCC	12491
Dgkk	CCTGGCTCTACATCAGTAACTGC	AATTTGAGGAGTGACCCAACAG	331374
Dkk1	CTCATCAATTCCAACGCGATCA	GCCCTCATAGAGAACTCCCG	13380
Hmox1	AAGCCGAGAATGCTGAGTTCA	GCCGTGTAGATATGGTACAAGGA	15368
Pthlh	CATCAGCTACTGCATGACAAGG	GGTGGTTTTTGGTGTTGGGAG	19227
Mtor	ACCGGCACACATTTGAAGAAG	CTCGTTGAGGATCAGCAAGG	56717
Pgf	TCTGCTGGGAACAACTCAACA	GTGAGACACCTCATCAGGGTAT	18654
P53	GTAGGAAGGCGCGTGGTAG	CAGTTACAGGAACCCCGAG	[1]
SRBI	TCCCTCATCAAGCAGCAGGT	TTCCACATCCCGAAGGACA	NM_016741.1
Stat3	AGCTGGACACACGCTACCT	AGGAATCGGCTATATTGCTGGT	20848
Stat1	CGGAGTCGGAGGCCCTAAT	ACAGCAGGTGCTTCTTAATGAG	20846
TNF	GACGTGGAACTGGCAGAAGAG	TTGGTGGTTTGTGAGTGTGAG	21926

1 Collier AC, Milam KA, Rougée LR, Sugawara A, Yamauchi Y, et al. (2012) Upregulation

of Ugt1a genes in placentas and fetal livers. Placenta 33: 77-80.

Table S4 qPCR primer sequences for the imprinted genes.

Genes	**Forward primers**	**Reverse primers**	**GeneID**
GNAS	CAGAGCCTCCATTGGGGTC	GCTTCTCGCTCAACTGGGG	14683
Peg3	TCATGCACACTAGGGAGAACC	GGCAGCACTCCTACTGAAGG	18616
Dlk1	AGTGCGAAACCTGGGTGTC	GCCTCCTTGTTGAAAGTGGTCA	13386
Cobl	CTTAGGGGGAAGCTATGGACT	ACACATCCCTGTCATAACACCT	12808
Commd1	CTGCACAGCCAACTCTATCCG	GCCTCTAACTGGTTGAAATCCA	17846
Copg2	GAGGAGTCTGGTAGTGGTTCC	TGTTCACCCTGGTTGAGTAAGT	54160
Ddc	TAGCTGACTATCTGGATGGCAT	GTCCTCGTATGTTTCTGGCTC	13195
Esx1	TTGGAGGGAGCAGACTACCAG	CCAAAGTCGGAGTAGAAAGTTGT	13984
Gab1	GAAGTTGAAGCGTTATGCGTG	AGAAAATCCGGTCGATGGTGT	14388
Grb10	TGCACCACTTCTTGAGGATG	ACCAGTGAGCTCCGGAAATG	14783
Igf2p0	GCATCCCCGGTCCTCTTTAT	GCTCTGGCTGGACGAGAAGT	16002
Impact	GTGAAGAAATCGAAGCAATGGC	GGTACTCACTTGGCAACATCA	16210
Klf14	CTCCGTGTGCCTCAACTAGC	CAGGCGCATCCAGGATAGC	619665
Meg3	TCCTCACCTCCAATTTCCCCT	GAGCGAGAGCCGTTCGATG	17263
Nap1l5	GCCGAGGACGAGGTAATGG	CATTTCACGGAATTGGGCAAG	58243
Peg1	GTGGTGGGTCCAAGTAGGG	AAGCACAACTATCTCAGGGCT	17294
Peg10	GTGGCATCGCAGAGGAAT	GTGAGAGGGGCTTCACTCC	170676
Pon2	GCTCTGAGTTTGCTGGGCAT	GGCAGTTTGGAAGGTCTACAGAT	330260
Rtl1	CCATGAGTCGAGCTTTGGTTC	TCCGGGTTTGAATCATAGCTTG	353326
Sfmbt2	GGCCAATTCTTACCCTCTGAC	TCTCAATAGGCACTGGGGATAAT	353282
Slc22a2	CCAGTGCATGAGGTATGAGGT	CTGAAACAGGTCCAGCATCCA	20518
Slc22a3	CAGCCCGACTACTATTGGTGT	TGAGCTGGTATTAGTGGCTTCC	20519
Usp29	TCCGCAGCACAAACAGGAG	CTCACCACTAACCACTACGCC	57775
Zdbf2	ACTCTGATGGAACGCTTTTTGC	ACCACCACCACTTCAGGTGA	73884

Zim1	GGAGAACTACGAGAACCTGATCT	TGTCTTAGAATTGTCTGGCTTCC	NM_011769.4
Zrsr1	CAGACCGCGATTCCTGAGAAA	CTCTGCAAGTTCTTCGTTAGCA	22183

Table S5 Sequenom MassArray primer for *Gnas, Grb10*

GNAS (NC_000068.7 from: 174120497 to 17412110)

Target region：351-546 length: 196 bp

5’ primer: aggaagagagTGGATATGGTTATTTTGATAGGGG

3’ primer: cagtaatacgactcactatagggagaaggctCTAAACACAAACCCCACACCTAC

Grb10 (GenBank: AL663087.14)

Target region: 65461-66250 length: 790 bp

5’ primer: aggaagagag GGGTTTTGGAGTTTAGAGGAG

3’ primer: cagtaatacgactcactatagggagaaggctAAAATTAACCCCCTTACTAAAATAAC

Table S6 qPCR primer sequences for *DNMTs* and *Tets*.

Genes	**Forward primers**	**Reverse primers**
DNMT1	AAGAATGGTGTTGTCTACCGAC	CATCCAGGTTGCTCCCCTTG
DNMT3A	GAGGGAACTGAGACCCCAC	CTGGAAGGTGAGTCTTGGCA
DNMT3B	AGCGGGTATGAGGAGTGCAT	GGGAGCATCCTTCGTGTCTG
DNMT3L	GCTCTAAGACCCTTGAAACCTTG	GTCGGTTCACTTTGACTTCGTA
Tet1	ACACAGTGGTGCTAATGCAG	AGCATGAACGGGAGAATCGG
Tet2	AGAGAAGACAATCGAGAAGTCGG	CCTTCCGTACTCCCAAACTCAT
Tet3	TGCGATTGTGTCGAACAAATAGT	TCCATACCGATCCTCCATGAG