Altered dietary ratio of folic acid and vitamin B12 during pregnancy influences the expression of imprinted H19/IGF2 locus in C57BL/6 mice

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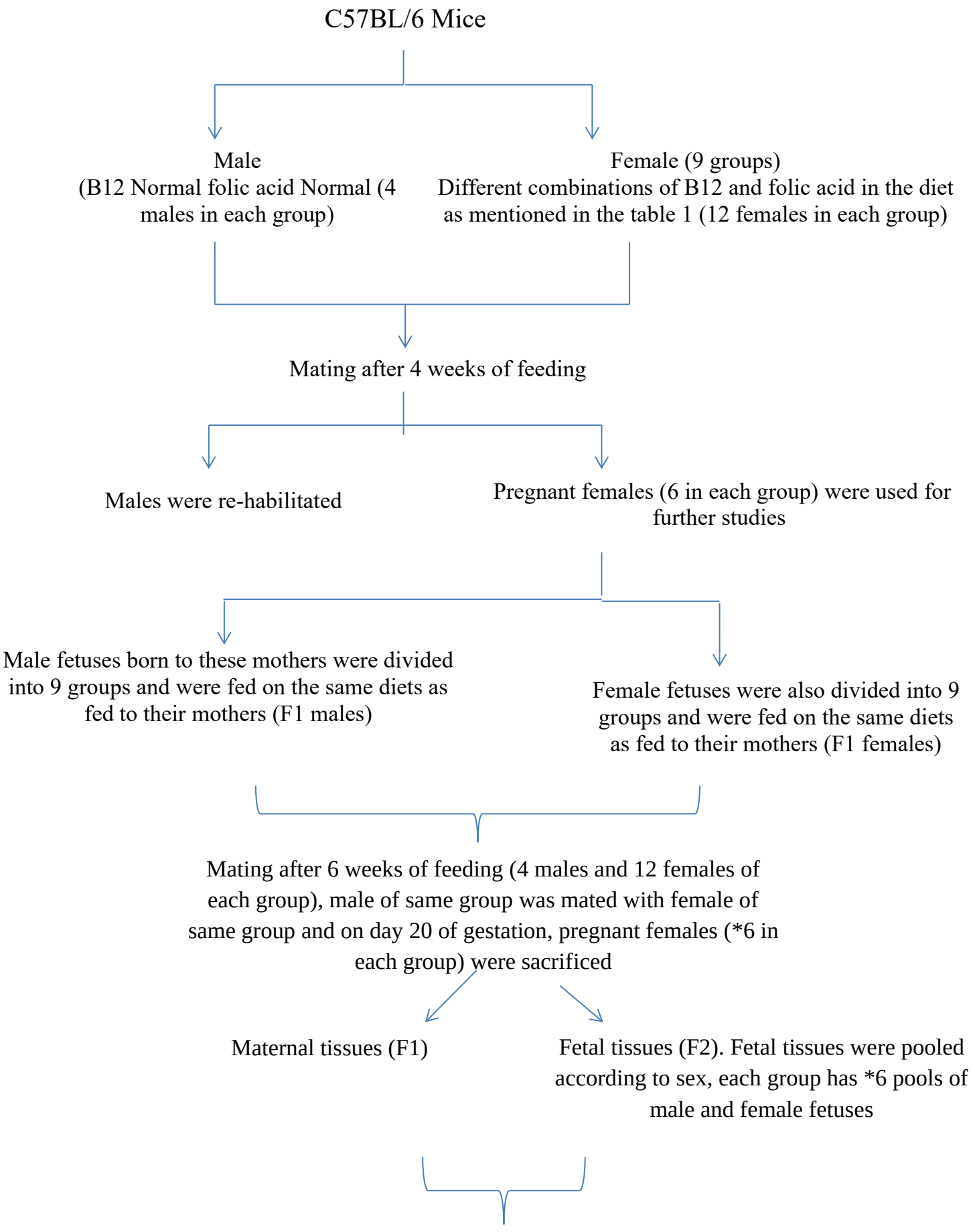


Supplementary figure 1: Work flow of the study

H19

(A).

H19 Placenta								
B12	BD		BN			BO		
Folate	FD	FN	FD	FN	FO	FD	FN	FO
mRNA	NC	NC	↑	C	NC	↑	NC	NC
DNA methylation	↑	↓	↑	C	↑	NC		
H3K9me3	↓	↓	↑	C		↑		
H3K27me3	NC	NC	NC	C		↑		
H3K4me2	NC	↑	↑	C		↑		

C= control
NC= No change
↑ = up-regulated
↓ = down-regulated
= up/downregulation of expression associated with either DNA methylation/histone modification.

(B)

H19 Fetal Liver																
B12	BD (Male)		BD (Female)		BN (Male)			BN (Female)			BO (Male)			BO (Female)		
Folate	FD	FN	FD	FN	FD	FN	FO	FD	FN	FO	FD	FN	FO	FD	FN	FO
mRNA	↑	NC	NC	NC	↑	C	NC	↑	C	NC	NC	NC	NC	NC	NC	NC
DNA methylation	↓	↓	↓	↓	↓	C	↓	↑	C	↓	↓			↑		
H3K9me3	↓		NC		NC	C		↓	C		↑			↓		
H3K27me3	↓		↑		↑	C		↑	C		↑			NC		
H3K4me2	NC		↑		NC	C		↑			NC			↓		

(C)

H19 Fetal Kidney																
B12	BD (Male)		BD (Female)		BN (Male)			BN (Female)			BO (Male)			BO (Female)		
Folate	FD	FN	FD	FN	FD	FN	FO	FD	FN	FO	FD	FN	FO	FD	FN	FO
mRNA	NC		NC		↑	C	NC	↑	C	NC	NC	NC	NC	NC	NC	NC
DNAmethylation	↓		↓		NC	C	↓	↑	C	↓	↓			↓		
H3K9me3	NC		↓		NC	C		↓	C		↑			↑		
H3K27me3	NC		↓		NC	C		NC	C		↑			NC		
H3K4me2	NC		↓		NC	C		↓	C		↑			↓		

(D)

H19 Fetal Brain																
B12	BD (Male)		BD (Female)		BN (Male)			BN (Female)			BO (Male)			BO (Female)		
Folate	FD	FN	FD	FN	FD	FN	FO	FD	FN	FO	FD	FN	FO	FD	FN	FO
mRNA	NC	NC	↑	NC	↑	C	NC	↑	C	↑	NC	NC	NC	↑	NC	NC
DNA methylation	↓	↓	NC	↓	↓	C	↓	↑	C	↓	↓			↑		
H3K9me3	↓		↓		↓	C		↑	C		↑			↑		
H3K27me3	↓		↑		↑	C		↓	C		NC			↓		
H3K4me2	↓		NC		↓	C		NC	C		↓			↑		

Supplementary figure 2: Summary of changes observed in expression, DNA methylation and histone modifications of **H19** with different dietary combinations of folic acid and B12.

IGF2

(A)

IGF2 Placenta								
B12	BD		BN			BO		
Folate	FD	FN	FD	FN	FO	FD	FN	FO
mRNA	NC	↓	NC	C	↑	NC	NC	NC
DNA methylation	NC	NC	NC	C	↑	NC		
H3K9me3	↑	↑	NC	C		↑		
H3K27me3	↑	↑	NC	C		↑		
H3K4me2	↓	↓	↓	C		↓		

C= control
NC= No change
↑ = up-regulated
↓ = down-regulated
▭ = up/downregulation of expression associated with either DNA methylation/histone modification.

(B)

IGF2 Fetal Liver																
B12	BD (Male)		BD (Female)		BN (Male)			BN (Female)			BO (Male)			BO (Female)		
Folate	FD	FN	FD	FN	FD	FN	FO	FD	FN	FO	FD	FN	FO	FD	FN	FO
mRNA	NC	NC	↑	NC	NC	C	NC	NC	C	NC	↑	↑	NC	NC	↑	NC
DNA methylation	↓	↓	↓	↓	NC	C	↓	NC	C	↓	↓			↑		
H3K9me3	NC		NC		↑	C		↑	C		↑			NC		
H3K27me3	↑		NC		NC	C		↑	C		↑			↑		
H3K4me2	↑		↓		NC	C		NC	C		↑			↓		

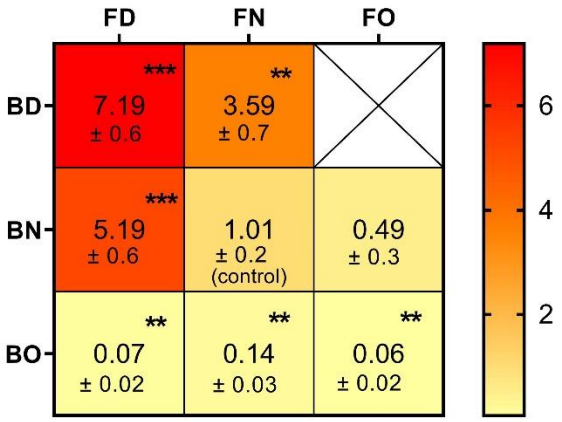
(C)

IGF2 Fetal Kidney																
B12	BD (Male)		BD (Female)		BN (Male)			BN (Female)			BO (Male)			BO (Female)		
Folate	FD	FN	FD	FN	FD	FN	FO	FD	FN	FO	FD	FN	FO	FD	FN	FO
mRNA	↓		↓		↓	C	↑	↓	C	NC	NC	↑	↑	NC	↑	NC
DNA methylation	↓		↓		NC	C	↑	NC	C	NC	NC			NC		
H3K9me3	NC		NC		↑	C		↑	C		↑			NC		
H3K27me3	NC		NC		NC	C		NC	C		↑			NC		
H3K4me2	NC		NC		NC	C		↑	C		↑			NC		

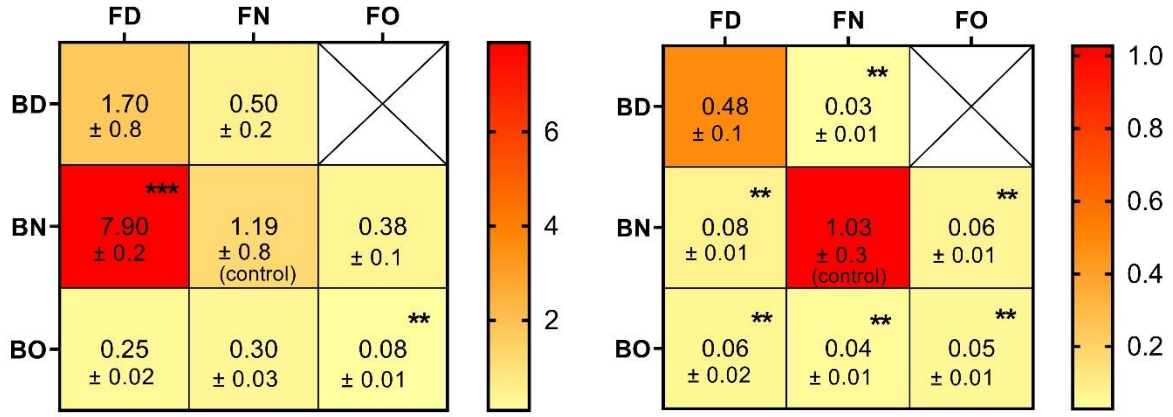
(D)

IGF2 Fetal Brain																
B12	BD (Male)		BD (Female)		BN (Male)			BN (Female)			BO (Male)			BO (Female)		
Folate	FD	FN	FD	FN	FD	FN	FO	FD	FN	FO	FD	FN	FO	FD	FN	FO
mRNA	↓	NC	↓	NC	NC	C	↑	NC	C	NC	↓	NC	NC	↓	NC	NC
DNA methylation	↓	↓	↓	↓	NC	C	NC	↑	C	↓	NC			↑		
H3K9me3	↑		↓		↑	C		NC	C		↑			↓		
H3K27me3	↑		↑		NC	C		NC	C		↑			NC		
H3K4me2	NC		NC		↑	C		NC	C		↑			NC		

Supplementary figure 3: Summary of changes observed in expression, DNA methylation and histone modifications of **IGF2** with different dietary combinations of folic acid and B12.

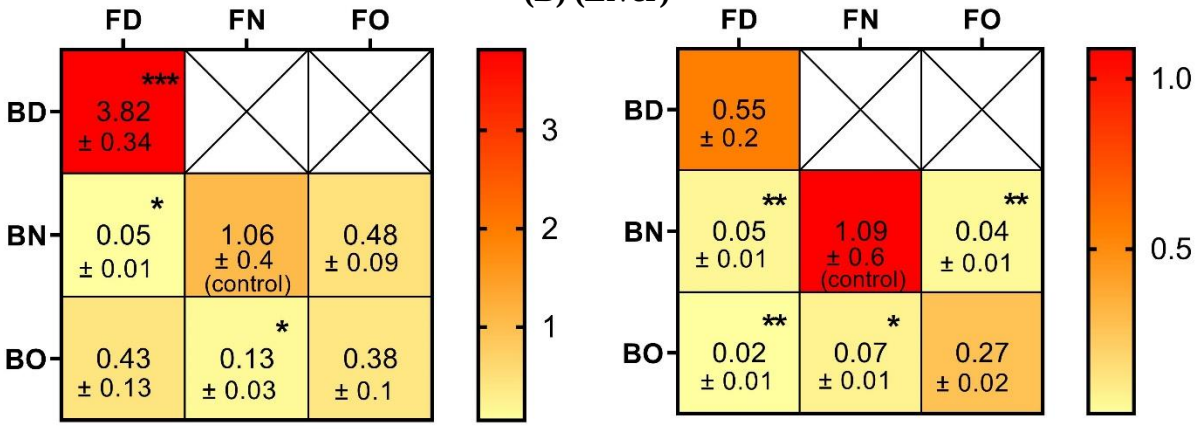


(A)



(Male) (Female)

(B) (Liver)



(Male) (Female)

(C) (Kidney)

Supplementary figure 4 : Quantification of miRNA 675 in placenta and fetal tissues (A) Mother Placenta (B) Fetal Liver (C) Fetal Kidney. *p < 0.05, **p < 0.01, ***p < 0.001 vs BNFN. The data is presented as mean± SD.B12 normal folic acid normal (BNFN), B12 normal folic acid over-supplemented (BNFO), B12 normal folic acid deficient (BNFD), B12 deficient folic acid normal (BDFN), B12 deficient folic acid over-supplemented (BDFO), B12 deficient folic acid deficient (BDFD), B12 over-supplemented folic acid normal (BOFN), B12 over-supplemented folic acid over-supplemented (BOFO), B12 over-supplemented folic acid deficient (BOFD).

miR-675

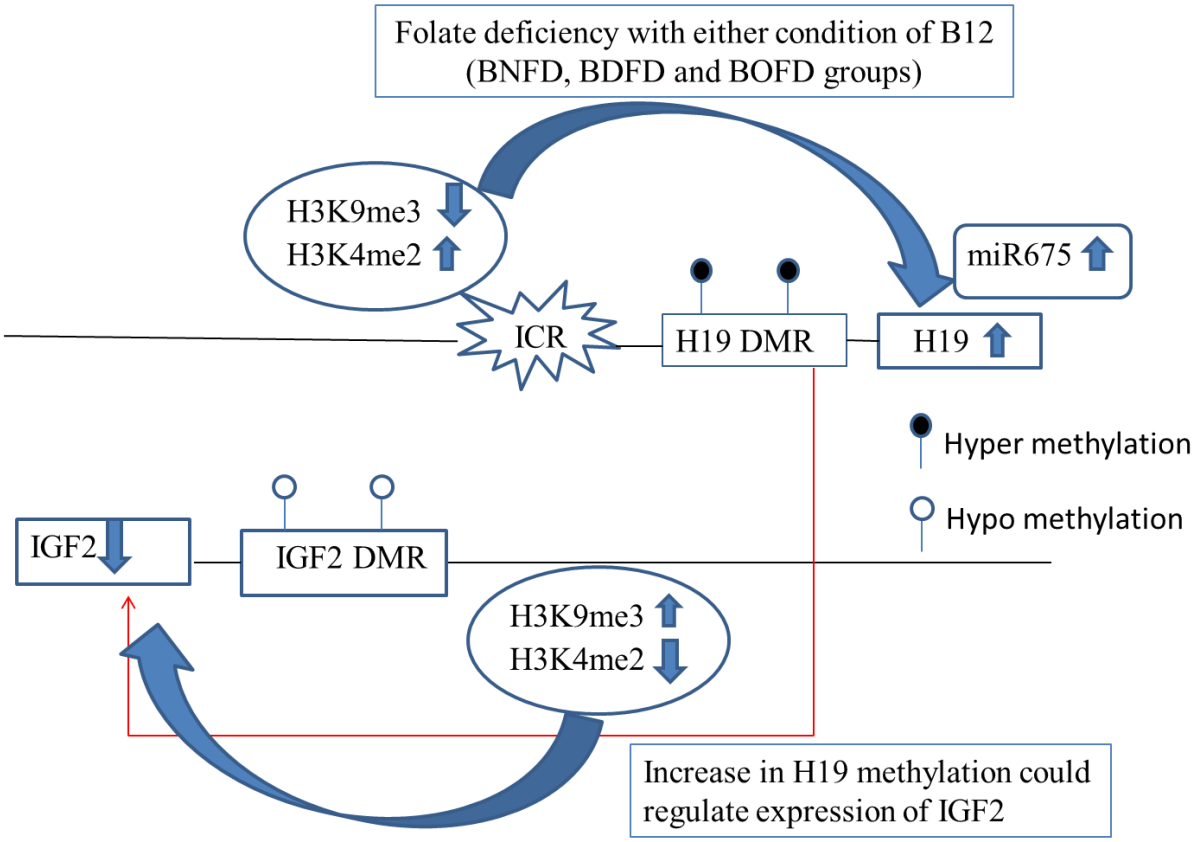
C= control
NC= No change
↑ = up-regulated
↓ = down-regulated

miR675 Placenta								
B12	BD		BN			BO		
Folate	FD	FN	FD	FN	FO	FD	FN	FO
mRNA	↑	↑	↑	C	NC	↓	↓	↓

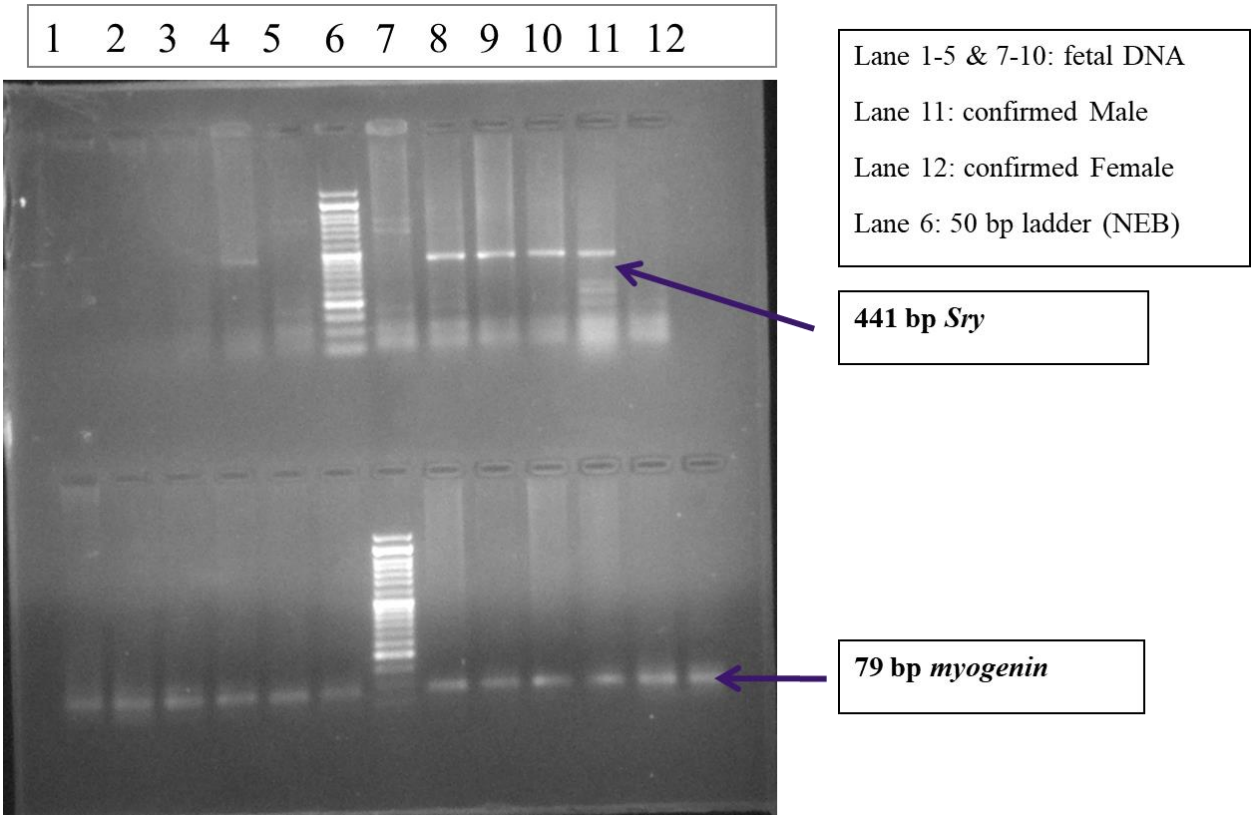
miR675 Fetal Liver																
B12	BD (Male)		BD (Female)		BN (Male)			BN (Female)			BO (Male)			BO (Female)		
Folate	FD	FN	FD	FN	FD	FN	FO	FD	FN	FO	FD	FN	FO	FD	FN	FO
mRNA	NC	NC	NC	↓	↑	C	NC	↓	C	↓	NC	NC	↓	↓	↓	↓

miR675 Fetal Kidney																
B12	BD (Male)		BD (Female)		BN (Male)			BN (Female)			BO (Male)			BO (Female)		
Folate	FD	FN	FD	FN	FD	FN	FO	FD	FN	FO	FD	FN	FO	FD	FN	FO
mRNA	↑		NC		↓	C	NC	↓	C	↓	NC	↓	NC	↓	↓	NC

Supplementary figure 5: Summary of changes observed in the expression of **miR-675** with different dietary combinations of folic acid and B12.



Supplementary figure 6: Overall diagrammatic representation of results showing that the dietary conditions of folate deficiency (BNFD, BDFD and BOFD) leads to increase in expression of maternally expressed gene (H19), which is regulated by increase in activating histone mark (H3K4me2) and decrease in repressive histone mark (H3K9me3) at its ICR. IGF2, a paternally expressed gene was found to be downregulated under dietary deficiency which was regulated by an increase in suppressive mark (H3K9me3) and decrease in activating mark (H3K4me2). Further, increased H19 DNA methylation at DMR may be responsible for decrease in the expression of IGF2.



Supplementary Figure 7: Fetal sex determination by using SRY gene. PCR reaction generated a male-specific SRY band of 441 bp against a control MYOGENIN band of 79 bp which was run on 1.5% agarose gel

Supplementary Table 1: Primer sequences for SRY and **MYOGENIN**

Gene	**Primer sequence**	**Length**
SRY	**F:** 5'-TCATGAGACTGCCAACCACAG- 3' **R:** 5'-CATGACCACCACCACCACCAA-3'	**441bp**
MYOGENIN	**F:** 5'-CGTGGGCATGTAAGGTGTGTA-3' **R:** 5'-CCTGCGCTTCTCCCTCAGT-3'	**79bp**

Supplementary Table 2: mRNA primer sequence

Gene	Primers sequence	Product
GAPDH	F: AGCTTGTCATCAACGGGAAG R: TTTGATGTTAGTGGGGTCTCG	61 bp
H19	F: TGTGCCTGCCAGTCACTGA R: CCATCACACCGGACCATGT	79bp
IGF2	F: CAACTCAGTCCCTCTAACATGCAT R: GTTTGCGAGCGTTAACAGGTT	79bp

Supplementary Table 3: miRNA primer sequence

MiRNA	**Sequence**
miRNA-675	F:GGACTGGTGCGGAAAGGGCCCACAGTGGACTTGGTACACTGTATG
Universal reverse primer	GAATCGAGCACCAGTTACGC

Supplementary Table 4: MS-HRM primer sequence

Gene	Primer sequence
H19	F:CGAACGTGCGTTGGAACGATAT R:CGATACTTCGAACCCTCTAACC
IGF2 DMR2	F: CGTGTTAATCGTCGTAGTCGTGGT R: TCGCTACCTACCGAAAATACGACC

Supplementary Table 5: ChIP primer sequence

H19 (ICR)	F:TCCCCCGGACATGAAAATAG R:TTGCCTACAGTTCCCGAATCA
IGF2	F:CAGAACATGAAAACTGGAAATAGAACTT R:AGTATAATCATGTGCTTTTTAGGGTAGGT

Supplementary table 6: Interaction between folic acid and B12 for H19 imprinted gene (mRNA, DNA methylation and histone modifications) in maternal placenta

H19 Placenta											
Dependent variable:		mRNA (fold change)		DNA methylation (% Methylation)		H3K9me3 (fold enrichment)		H3K27me3 (fold enrichment)		H3K4me2 (fold enrichment)	
B12	Folate	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BN	FN	1.15	0.62	42.18	1.20	12.65	1.65	3.88	1.15	0.32	0.24
	FD	15.89	2.24	62.73	2.33	18.01	0.14	4.72	0.07	18.12	0.70
	FO	1.92	0.47	77.10	0.92						
BD	FN	0.78	0.07	10.41	0.40	2.76	0.75	5.94	0.75	5.64	0.65
	FD	3.95	1.08	80.91	2.93	2.49	0.30	2.32	1.40	1.17	0.06
BO	FN	0.84	0.25								
	FD	6.77	3.61	45.19	3.27	24.28	0.36	37.29	1.11	24.42	1.04
	FO	0.42	0.38								

Supplementary table 7: Interaction between folic acid and B12 for H19 imprinted gene (mRNA, DNA methylation and histone modifications) in fetal brain (Male)

H19 Fetal Brain (Male)											
Dependent variable:		mRNA (fold change)		DNA methylation (% Methylation)		H3K9me3 (fold enrichment)		H3K27me3 (fold enrichment)		H3K4me2 (fold enrichment)	
B12	Folate	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BN	FN	1.02	0.25	90.81	2.38	20.89	0.77	3.55	0.89	8.31	0.70
	FD	46.24	8.02	23.70	0.45	1.85	0.70	45.38	1.41	2.10	0.37
	FO	6.03	2.25	41.84	2.32						
BD	FN	7.04	2.17	79.76	1.04						
	FD	3.55	0.88	69.44	1.81	0.41	0.09	0.42	0.23	0.71	0.14
BO	FN	2.86	1.74								
	FD	8.44	2.23	84.26	3.54	24.66	1.41	2.52	0.61	0.46	0.17
	FO	1.91	0.45								

Supplementary table 8: Interaction between folic acid and B12 for H19 imprinted gene (mRNA, DNA methylation and histone modifications) in fetal brain (female)

H19 Fetal Brain (female)											
Dependent variable:		mRNA (fold change)		DNA methylation (% Methylation)		H3K9me3 (fold enrichment)		H3K27me3 (fold enrichment)		H3K4me2 (fold enrichment)	
B12	Folate	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BN	FN	1.13	0.13	59.26	2.00	4.82	0.95	5.32	0.42	0.74	0.08
	FD	25.58	1.81	84.30	2.28	7.88	0.63	1.94	0.25	0.81	0.14
	FO	16.52	3.49	36.44	1.42						
BD	FN	4.18	1.32	49.52	4.17						
	FD	40.55	19.25	56.27	.26	2.52	0.47	22.38	2.12	1.17	0.19
BO	FN	1.98	1.16								
	FD	18.86	4.97	83.91	3.47	11.40	0.98	2.52	0.61	28.28	0.70
	FO	5.79	2.50								

Supplementary table 9: Interaction between folic acid and B12 for H19 imprinted gene (mRNA, DNA methylation and histone modifications) in fetal kidney (Male)

H19 Fetal Kidney (Male)											
Dependent variable:		mRNA (fold change)		DNA methylation (% Methylation)		H3K9me3 (fold enrichment)		H3K27me3 (fold enrichment)		H3K4me2 (fold enrichment)	
B12	Folate	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BN	FN	1.04	0.30	82.37	1.26	1.63	0.73	1.03	0.14	2.63	0.77
	FD	7.94	2.39	81.10	2.76	0.42	0.11	0.63	0.28	0.76	0.13
	FO	0.57	0.09	39.86	1.97						
BD	FN										
	FD	2.28	0.63	57.51	.96	2.33	0.36	0.54	0.24	0.26	0.17
BO	FN	0.18	0.05								
	FD	2.02	0.86	55.91	0.008	55.43	2.82	25.13	0.70	8.28	0.70
	FO	0.54	0.29								

Supplementary table 10: Interaction between folic acid and B12 for H19 imprinted gene (mRNA, DNA methylation and histone modifications) in fetal kidney (female)

H19 Fetal Kidney (female)											
Dependent variable:		mRNA (fold change)		DNA methylation (% Methylation)		H3K9me3 (fold enrichment)		H3K27me3 (fold enrichment)		H3K4me2 (fold enrichment)	
B12	Folate	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BN	FN	1.45	0.03	83.23	1.89	11.21	0.37	2.23	0.53	20.66	1.41
	FD	3.92	1.17	93.00	0.64	2.92	0.81	0.78	0.21	5.26	0.56
	FO	1.42	0.12	43.50	1.651						
BD	FN										
	FD	3.00	1.71	65.03	3.35	0.60	0.20	0.42	0.28	1.86	0.10
BO	FN	1.13	0.07								
	FD	1.45	0.45	75.73	3.03	14.15	0.70	1.95	0.46	3.58	0.34
	FO	2.69	0.58								

Supplementary table 11: Interaction between folic acid and B12 for H19 imprinted gene (mRNA, DNA methylation and histone modifications) in fetal liver (Male)

H19 Fetal Liver (Male)											
Dependent variable:		mRNA (fold change)		DNA methylation (% Methylation)		H3K9me3 (fold enrichment)		H3K27me3 (fold enrichment)		H3K4me2 (fold enrichment)	
B12	Folate	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BN	FN	1.15	0.11	82.36	1.05	3.21	0.89	1.81	0.81	0.95	0.05
	FD	11.25	2.51	39.30	0.81	1.98	0.11	6.69	0.70	1.45	0.28
	FO	1.51	1.03	32.28	3.67						
BD	FN	2.35	1.29	37.21	2.53						
	FD	3.59	2.22	45.93	1.51	0.25	0.07	0.26	0.07	0.47	0.29
BO	FN	0.67	0.18								
	FD	2.47	0.78	73.84	0.09	88.55	3.18	93.98	2.03	0.71	0.30
	FO	0.34	0.37								

Supplementary table 12: Interaction between folic acid and B12 for H19 imprinted gene (mRNA, DNA methylation and histone modifications) in fetal liver (female)

H19 Fetal Liver (female)											
Dependent variable:		mRNA (fold change)		DNA methylation (% Methylation)		H3K9me3 (fold enrichment)		H3K27me3 (fold enrichment)		H3K4me2 (fold enrichment)	
B12	Folate	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BN	FN	1.24	0.22	63.64	2.36	6.95	1.03	1.66	0.76	2.61	0.32
	FD	4.40	2.18	89.18	2.60	3.23	0.40	19.48	1.60	11.41	0.99
	FO	1.59	0.21	31.88	1.26						
BD	FN	1.65	0.55	16.10	2.03						
	FD	1.93	0.72	54.62	2.80	5.38	0.82	5.29	0.73	27.63	1.63
BO	FN	1.35	0.08								
	FD	2.07	0.89	90.06	5.88	0.31	0.14	1.62	0.43	0.11	0.13
	FO	2.64	1.20								

Supplementary table 13: Interaction between folic acid and B12 for IGF2 imprinted gene (mRNA, DNA methylation and histone modifications) in maternal placenta

IGF2 Placenta											
Dependent variable:		mRNA (fold change)		DNA methylation (% Methylation)		H3K9me3 (fold enrichment)		H3K27me3 (fold enrichment)		H3K4me2 (fold enrichment)	
B12	Folate	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BN	FN	1.00	0.10	42.37	3.09	1.08	0.30	0.88	0.17	9.38	0.20
	FD	0.97	0.22	40.39	2.87	0.43	0.10	0.85	0.13	2.09	0.13
	FO	5.81	0.46	55.71	4.32						
BD	FN	0.04	0.01	41.83	1.70	3.75	0.34	3.26	0.21	1.80	0.21
	FD	0.84	0.06	37.80	2.53	5.51	0.42	2.79	0.13	0.14	0.06
BO	FN	1.15	0.60								
	FD	1.19	0.35	40.57	3.84	4.18	0.45	8.42	0.12	4.01	0.64
	FO	0.86	0.37								

Supplementary table 14: Interaction between folic acid and B12 for IGF2 imprinted gene (mRNA, DNA methylation and histone modifications) in fetal brain (Male)

IGF2 Fetal Brain (Male)											
Dependent variable:		mRNA (fold change)		DNA methylation (% Methylation)		H3K9me3 (fold enrichment)		H3K27me3 (fold enrichment)		H3K4me2 (fold enrichment)	
B12	Folate	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BN	FN	1.09	0.34	67.26	2.64	1.72	0.58	0.60	0.15	0.67	0.21
	FD	0.95	0.41	63.42	8.87	23.68	1.60	2.39	0.24	7.93	0.85
	FO	2.08	0.96	72.05	2.51						
BD	FN	0.67	0.35	55.42	1.12						
	FD	0.59	0.19	22.23	1.88	5.65	0.97	21.64	1.85	0.78	0.27
BO	FN	1.25	0.26								
	FD	0.55	0.23	70.97	4.16	26.92	2.42	9.91	0.42	5.43	1.27
	FO	1.15	0.26								

Supplementary table 15: Interaction between folic acid and B12 for IGF2 imprinted gene (mRNA, DNA methylation and histone modifications) in fetal brain (female)

IGF2 Fetal Brain (female)											
Dependent variable:		mRNA (fold change)		DNA methylation (% Methylation)		H3K9me3 (fold enrichment)		H3K27me3 (fold enrichment)		H3K4me2 (fold enrichment)	
B12	Folate	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BN	FN	1.12	0.56	56.70	2.09	8.41	1.57	1.94	0.80	4.08	0.61
	FD	0.89	0.17	66.72	1.46	10.18	0.79	1.55	0.73	2.17	1.08
	FO	1.91	0.49	11.10	0.07						
BD	FN	0.93	0.35	41.88	0.44						
	FD	0.66	0.14	36.90	3.11	1.92	1.47	20.27	1.51	6.29	0.95
BO	FN	1.27	0.18								
	FD	0.73	0.32	74.14	4.29	5.12	0.36	0.46	0.38	1.83	0.23
	FO	1.18	0.33								

Supplementary table 16: Interaction between folic acid and B12 for IGF2 imprinted gene (mRNA, DNA methylation and histone modifications) in fetal kidney (Male)

IGF2 Fetal Kidney (Male)											
Dependent variable:		mRNA (fold change)		DNA methylation (% Methylation)		H3K9me3 (fold enrichment)		H3K27me3 (fold enrichment)		H3K4me2 (fold enrichment)	
B12	Folate	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BN	FN	0.82	0.26	71.24	5.07	4.97	0.28	1.41	0.30	0.53	0.10
	FD	0.17	0.01	77.21	4.60	10.07	0.77	2.36	0.24	1.03	0.08
	FO	2.98	0.47	78.18	1.40						
BD	FN										
	FD	0.27	0.03	42.77	3.49	3.50	0.18	1.15	0.10	0.96	0.12
BO	FN	4.00	0.93								
	FD	0.42	0.09	76.98	4.86	46.51	3.41	73.44	2.54	9.21	1.54
	FO	2.77	0.49								

Supplementary table 17: Interaction between folic acid and B12 for IGF2 imprinted gene (mRNA, DNA methylation and histone modifications) in fetal kidney (female)

IGF2 Fetal Kidney (female)											
Dependent variable:		mRNA (fold change)		DNA methylation (% Methylation)		H3K9me3 (fold enrichment)		H3K27me3 (fold enrichment)		H3K4me2 (fold enrichment)	
B12	Folate	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BN	FN	1.64	0.07	71.81	3.73	0.69	0.16	1.31	0.20	1.47	0.43
	FD	0.38	0.10	73.60	1.92	1.95	0.35	2.16	0.35	2.78	0.70
	FO	1.37	0.34	76.70	1.67						
BD	FN										
	FD	0.24	0.04	43.41	1.42	0.68	0.30	0.20	0.08	0.63	0.12
BO	FN	3.10	0.86								
	FD	0.78	.004	75.96	2.50	0.74	0.14	1.09	0.28	0.60	0.24
	FO	1.13	0.35								

Supplementary table 18: Interaction between folic acid and B12 for IGF2 imprinted gene (mRNA, DNA methylation and histone modifications) in fetal liver (Male)

IGF2 Fetal Liver (Male)											
Dependent variable:		mRNA (fold change)		DNA methylation (% Methylation)		H3K9me3 (fold enrichment)		H3K27me3 (fold enrichment)		H3K4me2 (fold enrichment)	
B12	Folate	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BN	FN	0.99	0.38	74.96	0.69	1.54	0.58	1.04	0.24	0.19	0.02
	FD	0.67	0.26	67.31	3.50	4.84	0.38	1.06	0.10	0.60	0.05
	FO	1.41	0.21	12.03	0.90						
BD	FN	0.60	0.37	44.03	1.41						
	FD	0.71	0.25	27.45	0.99	2.95	0.12	2.67	0.93	4.68	0.34
BO	FN	5.17	0.01								
	FD	3.25	0.78	65.35	4.49	10.36	0.18	4.86	0.38	4.51	1.19
	FO	1.40	0.21								

Supplementary table 19: Interaction between folic acid and B12 for IGF2 imprinted gene (mRNA, DNA methylation and histone modifications) in fetal liver (female)

IGF2 Fetal Liver (female)											
Dependent variable:		mRNA (fold change)		DNA methylation (% Methylation)		H3K9me3 (fold enrichment)		H3K27me3 (fold enrichment)		H3K4me2 (fold enrichment)	
B12	Folate	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BN	FN	1.11	0.15	58.32	10.42	0.85	0.12	0.97	0.29	2.9	0.20
	FD	1.61	0.79	67.06	2.76	7.45	0.92	8.96	2.31	4.31	1.20
	FO	1.22	0.07	20.27	1.06						
BD	FN	0.50	0.16	46.84	4.15						
	FD	2.33	0.80	42.86	4.48	1.39	0.30	0.68	0.06	0.31	0.09
BO	FN	2.93	0.50								
	FD	1.20	0.33	77.98	3.87	2.24	0.13	14.71	0.69	0.54	0.09
	FO	1.35	0.39								

Supplementary table 20: Interaction between folic acid and B12 for miR-675 (mRNA) in maternal and fetal tissues

MiRNA-675 (mRNA) (fold change)											
Dependent variable:		Placenta		Fetal Kidney (Male)		Fetal Kidney (Female)		Fetal liver (Male)		Fetal liver (Female)	
B12	Folate	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BN	FN	1.01	0.20	1.05	0.48	0.66	0.02	1.19	1.04	0.79	0.001
	FD	5.18	0.63	0.05	0.04	0.04	0.01	7.89	0.21	0.07	0.006
	FO	0.48	0.39	0.48	0.09	0.03	0.008	0.38	0.34	0.06	0.008
BD	FN	3.59	0.76					0.49	0.25	0.02	0.01
	FD	7.18	0.63	3.81	0.33	0.55	0.25	1.70	1.88	0.47	0.29
BO	FN	0.14	0.03	0.12	0.03	0.07	0.01	0.30	0.02	0.04	0.03
	FD	0.06	0.02	0.42	0.13	0.01	0.0007	0.24	0.20	0.06	0.04
	FO	0.05	0.01	0.38	0.16	0.26	0.002	0.08	0.01	0.05	0.01

Supplementary table 21: Pearson correlation and multiple regression analysis for H19 gene in maternal and fetal tissues

	Tissue		Pearson correlation analysis: r				Regression analysis with mRNA as dependent variable: r^2(SEM)#
			H3K9me3	H3K27me3	H3K4me2	mRNA vs DNA methylation	
F1 generation mother	Placenta	mRNA	0.542 *	0.098	0.642**	0.438	0.895***(2.173)
		DNA methylation	0.088	-0.136	0.014		
F2 generation fetus	Liver (male)	mRNA	-0.283	-0.231	0.651**	-0.740**	0.767**(2.426)
		DNA methylation	0.446	0.404	-0.304		
	Liver (female)	mRNA	-0.081	0.896***	0.220	0.421	0.856***(0.713)
		DNA methylation	0.155	0.527*	0.057		
	Kidney (male)	mRNA	-0.276	-0.259	-0.347	0.334	0.639*(2.108)
		DNA methylation	-0.627**	-0.600*	-0.451		
	Kidney (female)	mRNA	-0.828***	-0.738**	-0.480	0.081	0.806**(0.816)
		DNA methylation	0.101	0.227	0.374		
	Brain (male)	mRNA	-0.482	0.941***	-0.243	-0.907***	0.907**(0.799)
		DNA methylation	0.738**	-0.932***	0.352		
	Brain (female)	mRNA	0.140	0.629**	0.107	0.325	0.780**(0.702)
		DNA methylation	0.910***	-0.369	0.562**		

R = Pearson coefficient, r^2 = regression determination coefficient, SEM = standard error of measurement. #Equation for Prediction for each gene was "mRNA expression = β1 H3K9me3 + β2 H3K27me3 +β3 H3K4me2 + β4 DNA methylation + α ", where β1, β2, β3, β4 are regression coefficients and α is intercept of test. *p < 0.05 and **p < 0.01, **p< 0.001

Supplementary table 22: Pearson correlation and multiple regression analysis for IGF2 gene in maternal and fetal tissues

	Tissue		Pearson correlation analysis: r				Regression analysis with mRNA as dependent variable: r^2(SEM)#
			H3K9me3	H3K27me3	H3K4me2	mRNA vs DNA methylation	
F1 generation mother	Placenta	mRNA	-0.135	0.168	0.333	-0.184	0.285 (0.434)
		DNA methylation	-0.305	-0.065	0.305		
F2 generation fetus	Liver (male)	mRNA	0.821***	0.787**	0.428	0.315	0.931***(0.321)
		DNA methylation	0.173	-0.229	-0.702**		
	Liver (female)	mRNA	0.581**	0.131	0.034	-0.033	0.739***(0.522)
		DNA methylation	0.450*	0.863***	0.157		
	Kidney (male)	mRNA	-0.061	-0.011	-0.057	0.215	0.804**(0.148)
		DNA methylation	0.464	0.376	0.374		
	Kidney (female)	mRNA	-0.380	0.092	0.061	0.485	0.885***(0.218)
		DNA methylation	0.372	0.783**	0.435		
	Brain (male)	mRNA	-0.425	-0.715**	-0.250	0.470	0.817***(0.176)
		DNA methylation	0.453	-0.840***	0.472		
	Brain (female)	mRNA	0.358	-0.476*	-0.144	0.207	0.561*(0.264)
		DNA methylation	0.615**	-0.635**	-0.405		

R = Pearson coefficient, r^2 = regression determination coefficient, SEM = standard error of measurement. #Equation for Prediction for each gene was "mRNA expression = β1 H3K9me3 + β2 H3K27me3 +β3 H3K4me2 + β4 DNA methylation + α ", where β1, β2, β3, β4 are regression coefficients and α is intercept of test. *p < 0.05 and **p < 0.01, **p< 0.001

Supplementary table 23: Pearson correlation for miRNA-675 with H19 in maternal and fetal tissues

Comparison of expression of H19 with miRNA-675		
	Tissue	Pearson correlation analysis: r (H19 mRNA vs miRNA-675)
F1 generation mother	Placenta	0.43*
F2 generation fetus	Liver (Male)	0.86***
	Liver (Female)	-0.30
	Kidney (Male)	-0.07
	Kidney (Female)	-0.02

R = Pearson coefficient *p < 0.05 and **p < 0.01, ***p< 0.001.