Suplementary figures

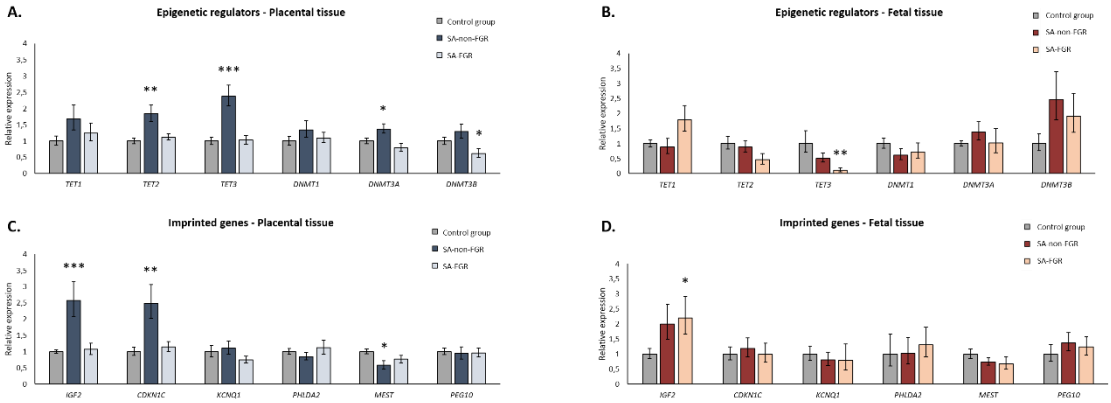

Figure S1: Relative expression of epigenetic regulators (*TET1*, *TET2*, *TET3*, *DNMT1*, *DNMT3A* and *DNMT3B*) and imprinted genes (*IGF2*, *CDKN1C*, *KCNQ1*, *PHLDA2*, *MEST* and *PEG10*) in placental (A-C) and fetal tissues (B-D). Bars represent $2^{\Delta\Delta Ct}$ ± SEM; * represents p<0.05, ** represents p<0.01, *** represents p<0.001.

Supplementary tables

Table S1: Global 5-hmC levels in placental and fetal tissues.

Cases	5-hmC (%) in placentas	5-hmC (%) of placental group	p value	5-hmC (%) in fetal tissue	5-hmC (%) of fetal tissue group	p value
Control 1	0,351608	0,32	-	0,208574	0,27	-
Control 2	0,303792			0,383759		
Control 3	0,326876			0,154163		
Control 4	0,325227			0,20981		
Control 5	0,333059			0,119538		
Control 6	0,353669			0,142209		
Control 7	0,387057			0,729596		
Control 8	0,159934			0,191261		
Case 1	0,201979	0,28	0,064	0,056884	0,17	0,268
Case 2	0,225062			0,309151		
Case 3	0,154988			0,223001		
Case 4	0,119538			0,04864		
Case 5	0,418796			0,328936		
Case 6	0,27535			0,07296		
Case 7	0,234542			0,100989		
Case 8	0,859027			0,263809		
Case 9	0,305853			0,115416		
Case 10	0,211871			0,110882		
Case 11	0,239901			0,100989		
Case 12	0,179308			0,20981		
Case 13	0,187139			0,276587		

Table S2: Data on maternal ages and gestational ages of SA cases.

Sample characteristics	Controls (n=16)	Idiopathic SA (n=19)	p-value
Maternal age at diagnosis (years)	31,94 (1,29)	32,11 (1,28)	0,927
Gestational age (weeks)	18,25 (0,95)	18,32 (0,79)	0,957
Data are expressed as MEAN (SD) T-test was used to obtain the p-value SA: Spontaneous Abortion; FGR: Fetal Growth Restriction			

Table S3: Samples characteristics corresponding to pregnancy losses and patient clinical history.

Sample type	Fetal growth restriction	karyotype	Maternal age at diagnosis (years)	Gestational age (weeks)	Clinical history
Control	-	46,XY	39	19	3G 1P 1A
Control	-	46,XX	25	18	6G 2P 3A
Control	-	46,XX	32	22	1G
Control	-	46,XX	36	15	2G 1A
Control	-	46,XX	30	19	2G 1P
Control	-	46,XY	27	14	2G 1P
Control	-	46,XY	24	23	1G
Control	-	46,XX	26	14	2G 1P
Control	-	46,XY	37	21	1G
Control	-	46,XY	38	21	1G
Control	-	46,XY	28	15	2G 1P
Control	-	46,XY	35	16	7G 5P 1A
Control	-	46,XY	31	16	6G 2P 2A 1FD (at 32 weeks)
Control	-	46,XX	30	23	2G 1A
Control	-	46,XY	33	24	1G
Control	-	46/XY	40	12	3G 2A
ISA	Yes	46,XY	30	23	1G
ISA	Yes	46,XY	32	20	2G 1P
ISA	Yes	46,XY	27	17	1G
ISA	Yes	46,XY	33	23	4G 3A
ISA	Yes	46,XY	29	20	3G 2P
ISA	Yes	46,XY	41	18	2G 1P
ISA	Yes	46,XY	31	18	1G
ISA	Yes	46,XX	37	19	2G 1P
ISA	No	46,XY	29	12	No information
ISA	No	46,XY	38	19	3G 2P
ISA	No	46,XY	31	24	2G 1A
ISA	No	46,XY	18	18	1G
ISA	No	46,XX	35	14	5G 4P
ISA	No	46,XX	24	23	2G 1P
ISA	No	46,XX	38	20	1G
ISA	No	46,XX	35	15	7G 6P
ISA	No	46,XX	38	15	11G 10P
ISA	No	46,XY	35	14	3G 2P
ISA	No	46,XY	29	16	1G

FGR- Fetal growth restriction; G –gravida, P- Para; A- abortion, FD –Fetal death

Table S4: Primers for qRT-PCR and amplification of bisulfite-modified DNA.

Gene	Technique	Sequence	Product	Reference
TET1	qRT-PCR	*Fw*: 5' TGGAAAGAAGAGGGCTGCGATGA 3'	164	Designed in primer-BLAST
		Rv: 5' GCACGGTCTCAGTGTTACTCCCTAA 3'		
TET2	qRT-PCR	*Fw*: 5' AAGGCTGAGGGACGAGAACGA 3'	115	(Klug, M. *et al*., 2013)[46]
		Rv: 5' TGAGCCCATCTCCTGCTTCCA 3'		
TET3	qRT-PCR	*Fw*: 5' CCCACAAGGACCAGCATAAC 3'	129	Designed in primer-BLAST
		Rv: 5' CCATCTTGTACAGGGGGAGA 3'		
DNMT1	qRT-PCR	*Fw*: 5' TGGACGACCCTGACCTCAAAT 3'	168	Joana Marques, C. *et al.*, (2011)[47]
		Rv: 5' TGCTTACAGTACACACTGAAGCAG 3'		
DNMT3A	qRT-PCR	*Fw*: 5' TATTGATGAGCGCACAAGAGAGC 3'	111	Joana Marques, C. *et al.*, (2011)[47]
		Rv: 5' TGGTACATGGCTTTTCGATAGGA 3'		
DNMT3B	qRT-PCR	*Fw*: 5' GGCAAGTTCTCCGAGGTCTCTG 3'	113	Joana Marques, C. *et al.*, (2011)[47]
		Rv: 5' TGGTACATGGCTTTTCGATAGGA 3'		
IGF2	qRT-PCR	*Fw*: 5' ATGGGGAAGTCGATGCTGGT 3'	154	Designed in primer-BLAST
		Rv: 5' CGGGCCTGCTGAAGTAGAA 3'		
CDKN1C	qRT-PCR	*Fw*: 5' CGGCGATCAAGAAGCTGTCC 3'	186	Designed in primer-BLAST
		Rv: 5' TGGGCTCTAAATTGGCTCACC 3'		
KCNQ1	qRT-PCR	*Fw*: 5' AGCAGAAGCAGAGGCAGAAGC 3'	160	Designed in primer-BLAST
		Rv: 5' CTGGGTGACAGCAGAGTGTGG 3'		
PHLDA2	qRT-PCR	*Fw*: 5' GCGACAGCCTCTTCCAGCTAT 3'	178	Designed in primer-BLAST
		Rv: 5' TCGGTGGTGACGATGGTGAAGT 3'		
MEST	qRT-PCR	*Fw*: 5' AGCTCTTGCCTCTGTAACTATCCC 3'	104	Designed in primer-BLAST
		Rv: 5' GCGGCAGCGTTTTCCTGTA 3'		
PEG10	qRT-PCR	*Fw*: 5' CTGAGGAGAACAGCGGAGAAGG 3'	170	Designed in primer-BLAST
		Rv: 5' CGCTTATTTCACGCGAGGAC 3'		
ACTB	qRT-PCR	*Fw*: 5' CGAGCACAGAGCCTCGCCTTT 3'	195	Designed in primer-BLAST
		Rv: 5' CACCATCACGCCCTGGTGCCT 3'		
RPLP0	qRT-PCR	*Fw*: 5' CCTCGTGGAAGTGACATCGTCT 3'	134	Designed in primer-BLAST
		Rv: 5' CGGATAATCATCCAATAGTTGGATG 3'		
TBP	qRT-PCR	*Fw*: 5' TGCACAGGAGCCAAGAGTGAAGA 3'	174	Designed in primer-BLAST
		Rv: 5' TTGGTGGGTGAGCACAAGGC 3'		
MEST	Bisulfite	*Fw*: 5' TYGTTGTTGGTTAGTTTTGTAYGGTT 3'	289	Marques, C. J. *et al., (2008)*[48]
		Rv: 5' AAAAATAACACCCCCTCCTCAAAT 3'		
KvDMR1	Bisulfite	*Fw*: 5' TGTTTTTGTAGTTTATATGGAAGGGTTAA 3'	265	Khoueiry, R. *et al., (2012)*[49]
		Rv: 5' CTCACCCCTAAAAACTTAAAACCTC 3'		
H19 DMR	Bisulfite	*Fw*: 5' AGGTGTTTTAGTTTTATGGATGATGG 3'	322	Marques, C. J. *et al., (2008)*[48]
		Rv: 5' TCCTATAAATATCCTATTCCCAAATAACC 3'		
P3 *IGF2*	Bisulfite	*Rv*: 5' GGTTATGTAGGTAGGATTTGAGTT 3'	364	(Li, Y. *et al*., (2009)[50]
		Rv: 5' CCCCACCAAAAAACTACACTAC 3'		

Table S5: Gene expression value and p-value for epigenetics regulators genes, obtained by comparing idiopathic spontaneous abortion (ISA) and control groups from placental and fetal tissues. Blue color represents the significantly altered expression. PL-Placenta; FT-Fetal tissue.

	TET1		***TET2***		***TET3***		***DNMT1***		***DNMT3A***		***DNMT3B***	
	PL	FT	PL	FT	PL	FT	PL	FT	PL	FT	PL	FT
p-value	0,111	0,581	0,015	0,140	0,013	0,008	0,271	0,062	0,372	0,239	0,891	0,127
Ratio ISA	1,486	1,177	1,498	0,681	1,606	0,260	1,236	0,661	1,092	1,213	0,947	2,220
Ratio control group = 1												

Table S6: Gene expression value and p-value for imprinted genes, obtained by comparing idiopathic spontaneous abortion (ISA) and control groups from placental and fetal tissues. Blue color represents the significantly altered expression. PL-Placenta; FT-Fetal tissue.

	IGF2		***CDKN1C***		***KCNQ1***		***PHLDA2***		***MEST***		***PEG10***	
	PL	FT	PL	FT	PL	FT	PL	FT	PL	FT	PL	FT
p-value	0,017	0,013	0,014	0,9703	0,987	0,4654	0,612	0,711	0,022	0,128	0,987	0,341
Ratio ISA	1,773	2,066	1,798	1,098	0,937	0,801	0,952	1,139	0,650	0,711	0,949	1,315
Ratio control group = 1												