Figure S1 Standard curves of miR-16 using synthetic miRNAs. miRNA, microRNA.
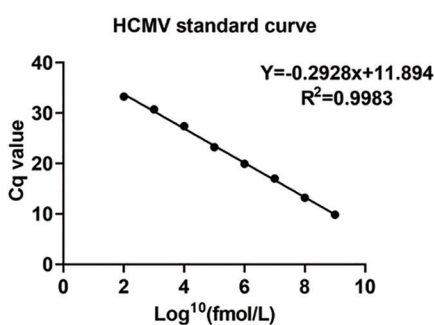

Figure S2 Standard curve of recombinant plasmid that contained the HCMV target sequence. HCMV, human cytomegalovirus.
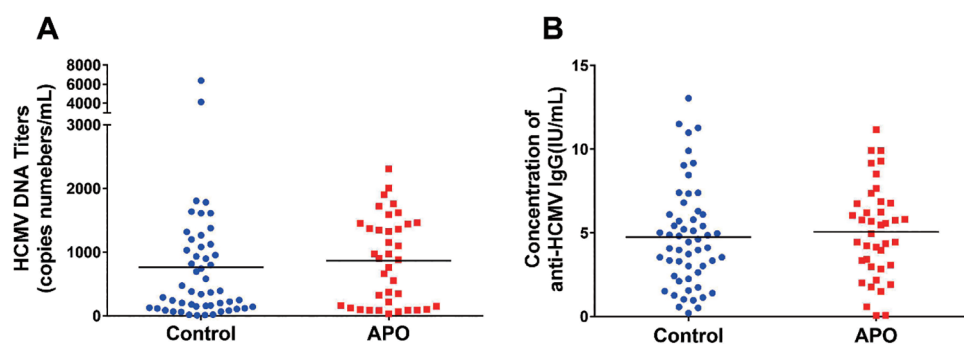

Figure S3 Detection of HCMV and its relationship with HCMV-encoded miRNAs. (A) The HCMV DNA titers were calculated in PBLs from pregnant women with APOs and compared with those from normal controls. (B) Comparison of the concentrations of anti-HCMV IgG in the APO group (n=40) versus the control group (n=55). HCMV, human cytomegalovirus; miRNA, microRNA; PBL, peripheral blood leukocyte; APO, adverse pregnancy outcome; IgG, immunoglobulin G.

Table S1 Gestational age of pregnant women with adverse pregnancy and normal controls in the present study

Training set			Validation set			Placenta tissues set		
Case number	Gestational age	Type of pregnancy	Case number	Gestational age	Type of pregnancy	Case number	Gestational age	Type of pregnancy
N1	20	Normal	N29	29	Normal	N1	20	Normal
N2	36	Normal	N30	18	Normal	N2	36	Normal
N3	6	Normal	N31	37	Normal	N3	6	Normal
N4	40	Normal	N32	13	Normal	N4	40	Normal
N5	19	Normal	N33	39	Normal	N5	19	Normal
N6	38	Normal	N34	39	Normal	N7	9	Normal
N7	9	Normal	N35	9	Normal	N11	28	Normal
N8	39	Normal	N36	39	Normal	N12	18	Normal
N9	38	Normal	N37	38	Normal	N23	5	Normal
N10	37	Normal	N38	40	Normal	N24	11	Normal
N11	28	Normal	N39	16	Normal	N25	8	Normal
N12	18	Normal	N40	38	Normal	N26	13	Normal
N13	37	Normal	N41	38	Normal	N27	7	Normal
N14	40	Normal	N42	40	Normal	N28	9	Normal
N15	38	Normal	N43	19	Normal	N29	29	Normal
N16	38	Normal	N44	33	Normal	N30	18	Normal
N17	37	Normal	N45	40	Normal	N26	13	Normal
N18	39	Normal	N46	17	Normal	N45	40	Normal
N19	39	Normal	N47	16	Normal	N48	40	Normal
N20	39	Normal	N48	40	Normal	N49	27	Normal
N21	39	Normal	N49	27	Normal	C4	27	FSA
N22	38	Normal	N50	36	Normal	C8	25	FSA
N23	5	Normal	N51	13	Normal	C11	17	FSA
N24	11	Normal	N52	16	Normal	C13	23	FSA
N25	8	Normal	N53	17	Normal	C21	38	FSA
N26	13	Normal	N54	16	Normal	C22	15	FSA
N27	7	Normal	N55	5	Normal	C23	20	FSA
N28	9	Normal	C21	38	FSA	C24	39	FSA
C1	30	FSA	C22	15	FSA	C26	23	FSA
C2	24	FSA	C23	20	FSA	C28	27	FSA
C3	33	FSA	C24	39	FSA	C15	10	SA
C4	27	FSA	C25	20	FSA	C17	9	SA
C5	19	FSA	C26	23	FSA	C18	27	SA
C6	22	FSA	C27	14	FSA	C19	8	SA
C7	24	FSA	C28	27	FSA	C33	6	SA
C8	25	FSA	C29	22	FSA	C34	12	SA
C9	26	FSA	C30	29	FSA	C35	10	SA
C10	25	FSA	C31	20	FSA	C36	9	SA
C11	17	FSA	C32	26	FSA	C39	12	SA
C12	17	FSA	C33	6	SA	C40	12	SA
C13	23	FSA	C34	12	SA			
C14	18	SA	C35	10	SA			
C15	10	SA	C36	9	SA			
C16	11	SA	C37	9	SA			
C17	9	SA	C38	26	SA			
C18	27	SA	C39	12	SA			
C19	8	SA	C40	12	SA			
C20	7	SA						

FSA, fetal structural anomaly; SA, spontaneous abortion.

Table S2 TaqMan advanced miRNA assays (Applied Biosystems)

Assay name	Mature miRNA sequence	Assay ID
hcmv-miR-UL22A-5p	UAACUAGCCUUCCCGUGAGA	007677
hcmv-miR-UL22A-3p	UCACCAGAAUGCUAGUUUGUAG	006040
hcmv-miR-UL36-5p	UCGUUGAAGACACCUGGAAAGA	197212-mat
hcmv-miR-UL36-3p	UUUCCAGGUGUUUUCAACGUGC	006481
hcmv-miR-UL112-5p	CCUCCGGAUCACAUGGUUACUCA	469687
hcmv-miR-UL112-3p	AAGUGACGGUGAGAUCCAGGCU	006621
hcmv-miR-UL148D	UCGUCCUCCCCUUCUUCACCG	197215-mat
hcmv-miR-US33-5p	GAUUGUGCCCGGACCGUGGGCG	197227-mat
hcmv-miR-US33-3p	UCACGGUCCGAGCACAUCCAA	467895-mat
hcmv-miR-US5-1	UGACAAGCCUGACGAGAGCGU	004641-mat
hcmv-miR-US25-1-5p	AACCGCUCAGUGGCUCGGACC	197211-mat
hcmv-miR-US25-1-3p	UCCGAACGCUAGGUCGGUUCU	467895-mat
hcmv-miR-US25-2-5p	AGCGGUCUGUUCAGGUGGAUGA	197201-mat
hcmv-miR-US25-2-3p	AUCCACUUGGAGAGCUCCCGCGGU	468261-mat
hcmv-miR-US4-5p	UGGACGUGCAGGGGGAUGUCUG	469977-mat
hcmv-miR-US4-3p	UGACAGCCCGCUACACCUCU	469699-mat
hcmv-miR-US5-2-5p	CUUUCGCCACACCUAUCCUGAAAG	469274-mat
hcmv-miR-US5-2-3p	UAUGAUAGGUGUGACGAUGUCU	469255-mat
hcmv-miR-US29-5p	UGGAUGUGCUCGGACCGUGACG	CS1RUMR
hcmv-miR-US29-3p	CCCACGGUCCGGGCACAAUCA	468621-mat
hcmv-miR-US22-5p	UGUUUCAGCGUGUGUCCGCGGG	468736-mat
hcmv-miR-US22-3p	UCGCCGGCCGCGCUGUAACCAGG	468548-mat
peu-MIR2911	GGCCGGGGGACGGGCUGGGA	242025-mat
hsa-miR-16	UAGCAGCACGUAAAUAUUGGCG	00391

miRNA, microRNA.

Table S3 The concentration of HCMV-encoded miRNAs in pregnant women with APOs and normal controls in the validation set

HCMV encoded miRNAs	APOs (n=20)	Controls (n=27)	Fold change	P value[a]
hcmv-miR-UL148D	3.91±0.25	3.08±0.27	1.27	0.035
hcmv-miR-US25-1-5p	0.11±0.01	0.08±0.01	1.38	0.006
hcmv-miR-US5-1	3.18±0.43	1.90±0.14	1.67	0.003
hcmv-miR-US33-3p	0.06±0.01	0.06±0.01	1.05	0.875

Data are presented as the mean ± SEM. [a], Student t-test. HCMV, human cytomegalovirus; miRNA, microRNA; APO, adverse pregnancy outcome.

Table S4 The concentration of HCMV-encoded miRNAs in pregnant women with APOs and normal controls

HCMV encoded miRNAs	APOs (n=40)	Controls (n=55)	Fold change	P value[a]
hcmv-miR-UL148D	5.01±0.49	3.32±0.20	1.51	<0.001
hcmv-miR-US25-1-5p	0.11±0.01	0.08±0.01	1.58	<0.001
hcmv-miR-US5-1	3.44±0.35	2.11±0.16	1.63	<0.001
hcmv-miR-US33-3p	0.09±0.01	0.06±0.01	1.43	0.041

Data are presented as the mean ± SEM. [a], Student t-test. HCMV, human cytomegalovirus; miRNA, microRNA; APO, adverse pregnancy outcome.

Table S5 The concentration of HCMV-encoded miRNAs in two types of pregnant women with APOs and normal controls

HCMV encoded miRNAs	FSA (n=25)	SA (n=15)	Controls (n=55)	Fold change			P value[a]		
				FSA *vs.* controls	SA *vs.* controls	FSA *vs.* SA	FSA *vs.* controls	SA *vs.* controls	FSA *vs.* SA
hcmv-miR-UL148d	5.41±0.53	4.35±0.96	3.32±0.20	1.63	1.31	1.24	<0.001	0.105	0.298
hcmv-miR-US25-1-5p	0.16±0.02	0.10±0.01	0.09±0.01	1.83	1.16	1.57	<0.001	0.356	0.018
hcmv-miR-US5-1	3.42±0.38	3.46±0.70	2.11±0.16	1.62	1.64	0.99	<0.001	0.005	0.954

Data are presented as the mean ± SEM. [a], Student t-test. HCMV, human cytomegalovirus; miRNA, microRNA; APO, adverse pregnancy outcome; FSA, fetal structural anomaly; SA, spontaneous abortion.

Table S6 The respective AUCs of three candidate miRNAs in ROC curve analyses for control and APO cohorts

Test result variable(s)	APOs vs. controls				
	Area	Std. error	Asymptotic sig.	Asymptotic 95% CI	
				Lower bound	Upper bound
hcmv-miR-UL148D	0.689	0.055	0.002	0.581	0.797
hcmv-miR-US25-1-5p	0.735	0.051	<0.0001	0.635	0.836
hcmv-miR-US5-1	0.688	0.058	0.002	0.574	0.801
The panel[a]	0.685	0.058	0.002	0.573	0.798

[a], combination of three miRNAs. AUC, area under the ROC curve; miRNA, microRNA; ROC, receiver-operating characteristic; APO, adverse pregnancy outcome.

Table S7 Univariate logistic regression analyses of plasma HCMV miRNAs for pregnant women with APOs

Variables	B	Std. error	OR	95% CI for OR		P value
				Lower	Upper	
hcmv-miR-US25-1-5p	10.185	4.662	26,500.657	2.848	246,567,602.202	0.029
hcmv-miR-US5-1	0.341	0.162	1.406	1.024	1.931	0.035

HCMV, human cytomegalovirus; miRNA, microRNA; APO, adverse pregnancy outcome; OR, odds ratio.

Table S8 Clinical characteristics associated with the change of plasma hcmv-miR-25-1-5p in linear regression model

Variables	Unstandardized coefficient	P value
Age	4.973E–04	0.711
Gestational age	–3.438E–04	0.585
HCMV DNA titers in PBLs	1.423E–05	0.072
Anti-HCMV IgG concentrations	–2.168E–04	0.931

HCMV, human cytomegalovirus; PBLs, peripheral blood leukocyte; IgG, immunoglobulin G.