Association of microRNA gene polymorphisms with recurrent spontaneous abortion: An updated meta-analysis

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Abstract. Numerous studies have reported single nucleotide polymorphisms (SNPs) in microRNAs (miRNAs) associated with unexplained recurrent spontaneous abortion (URSA). The present study aimed to conduct an updated meta-analysis to confirm a pooled effect size of the association between miRNA SNPs and URSA. The relevant literature was searched on PubMed, EMBASE, Web of Science and Cochrane Library before July 2022 to identify case-control studies. The pooled odds ratio and confidence intervals at 95% of the eligible studies were extracted and evaluated under five genetic models. A total of 18 studies involving 3,850 cases and 4,312 controls were included. miR499a rs3746444 A>G, miR-149 rs2292832 T>C, miR-125a rs41275794 G>A and miR-10a rs3809783 A>T may enhance the risk of recurrent spontaneous abortion (RSA) under various genetic models. Although no separate association was found between the miR-125a rs12976445 C>T and miR-27a rs895819 A>G polymorphisms and RSA, statistical significance was found in certain ethnic groups only. The current analysis suggests a high significance of an up-to-date meta-analysis for screening out and preventing URSA among high-risk women by testing miRNA SNPs and RSA susceptibility.

Introduction

Recurrent spontaneous abortion (RSA) or recurrent pregnancy loss (RPL) are common and significant pregnancy issue occurring in ~1-3% of couples trying to conceive. It is defined as at least two consecutive spontaneous abortions before the 20th week of pregnancy (1) Although several etiologic factors, such as uterine abnormalities, infectious or immune factors, endocrine and metabolic disorders, genetic abnormalities, acquired and inherited thrombophilia and chemical factors, are considered risk factors for RSA (2), the etiology of 40-55% of pregnant women suffering RSA remains to be elucidated (3), namely unexplained recurrent spontaneous abortion (URSA). Therefore, it is urgent to identify risk factors for the prevention and treatment of RSA. An increasing number of studies have focused on genetic factors, especially single nucleotide polymorphisms (SNPs) (4).

MicroRNAs (miRNAs) are a class of noncoding RNAs that regulate gene expression at the post-tanscriptional level by suppressing the translation of protein-coding genes by targeting mRNA 3'UTR and are involved in a wide range of life processes, including proliferation, development, differentiation, immune response and hormone secretion (5). miRNAs are estimated to regulate ~60% of human mRNA (6). According to studies (7-9), abnormal miRNA expression is implicated in the pathogenesis of RSA. Sequence variants in miRNA genes may contribute to their dysregulation. The presence of an SNP or mutation in an miRNA gene may alter the binding affinity of the miRNA to its mRNA targets, the transcription of miRNA primary transcripts and the process of the pre-miRNA into its mature, epigenetic regulation of miRNA genes (10-12). SNPs in the miRNA gene region may affect the properties and function of miRNAs, consequently contributing to RSA susceptibility by altering miRNA expression or maturation (13).

A number of studies have been conducted to investigate the association between miRNA SNPs and RSA risk, including well-known SNPs in pre-miRNA sequences such as miR-146a C/G (rs2910164), miR-196a2 T/C (rs11614913), miR-499 A/G (rs3746444) and other SNPs (14-30), but the results are not conclusive and consistent. Srivastava *et al* (31) first reported a meta-analysis of miRNA SNPs and RSA. The results showed that miR-196a-2 rs11614913, miR-499 rs3746444 and miR-149 rs2292832 could reduce the risk of RSA under certain genetic models. The present study performed a meta-analysis of 18 case-control studies to assess the association between miRNA SNPs and RSA susceptibility and improve understanding of the association between these polymorphisms and RSA risk.

Materials and methods

The present systematic review and meta-analysis design was prospectively based on the Preferred Reporting

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Items for Systematic Reviews and Meta-Analyses (PRISMA) (http://www.prisma-statement.org/PRISMA-Statement/PISMAStatement.aspx). The study has been registered on PROSPERO (https://www.crd.york.ac.uk/prospero/) (ID, CRD42021230598).

Literature search strategy. The authors Xueqin Wang and Yan Xing systematically searched the online databases, including PubMed (MEDLINE, https://pubmed.ncbi.nlm.nih.gov/), EMBASE (https://www.embase.com/), Web of Science (http://www.webofscience.com/) and Cochrane Library (https://www.cochranelibrary.com/), without language limitations up till July 2022. The following keywords were used: 'miRNA' AND ('recurrent pregnancy loss or RPL' OR 'recurrent spontaneous abortion or RSA' OR 'recurrent miscarriage') AND ('polymorphism' OR 'single nucleotide polymorphism'). In addition, the reference lists from the identified articles were searched manually.

Inclusion and exclusion criteria. The present meta-analysis comprised case-control studies that met the following criteria: i) The study assessed the association between microRNA gene polymorphisms and the risk of recurrent spontaneous abortion, ii) RSA was defined as at least two consecutive spontaneous abortions before the 20th week of pregnancy, iii) in all evaluated studies, a patient group (women with RSA) compared with a control group (healthy women), iv) the distribution of genotypes or alleles in both cases and controls was extracted for calculating the odds ratios (ORs) and 95% confidence intervals (CIs), v) for repeated studies, only the studies with more complete data and longer study periods were included, vi) the selected SNPs with two or more published studies were included in the current study. Studies were excluded if i) they were letters, editorials, abstracts, reviews, case reports and studies performed on animals, ii) they did not quantify the information to calculate OR and 95% CI, iii) they were copies of previous publications, or iv) they did not meet the criteria for RSA.

Data extraction. The data from eligible studies were extracted independently by two of the authors (Xueqin Wang and Yan Xing) based on the following inclusion and exclusion criteria: First author name, year of publication, study country, ethnicity, diagnostic criteria for RSA, numbers of cases and controls, genotyping technology and polymorphisms studied. Differences were resolved by a third author (Jing Gao).

Quality assessment of included studies. Study quality assessment was independently performed by two authors (Xueqin Wang and Yan Xing) according to the Newcastle-Ottawa Scale (NOS) (32). The NOS determined the research quality based on three parameters: Study object selection, group comparability and exposure factor measurement. The NOS employs a star grading system that ranges from zero stars (worst) to nine stars (best). In brief, each study received a maximum of nine points: Four for selection, two for comparability and three for outcomes. Studies with a score of ≥6 points were considered high quality.

Statistical analysis. In the present study, ORs and 95% CIs were used to assess the association between microRNA gene polymorphisms and RSA risk. The pooled ORs and 95% CIs were calculated and their significance was determined by P-values to clarify the potential relationships. P<0.05 was considered to indicate a statistically significant difference. The present study analyzed five genetic patterns of each microRNA (allele pattern, homozygous model, heterozygous model, recessive model and dominant model) (33). Heterogeneity was measured using the chi-square test-based Q-test and I^2 statistics. If significant heterogeneity existed (significant heterogeneity, P<0.10 and I^2>50%), the random-effects model was used and if not (no heterogeneity, P>0.10 and I^2<50%), the fixed effect model was used. The present study conducted a sensitivity analysis to evaluate the effect of each study on the combined OR by sequentially excluding individual studies to investigate the potential sources of heterogeneity and verify the reliability of the meta-analysis. As the number of included studies in each SNP was <10, publication bias evaluation was not performed.

Results

Characteristics of eligible studies. The PRISMA flow chart of the literature search and selection process is detailed in Fig. 1. A total of 41 articles were collected from the databases through a literature search using different combinations of key terms. After removing the duplicate literature and meta-analysis, 37 studies were evaluated for eligibility. A total of 13 studies were excluded (eight were not about miRNA polymorphisms, three were about recurrent implantation failure, one was a missing genotype in miRNA SNPs and one was about spontaneously aborted fetuses). Therefore, 24 studies were considered eligible for the current meta-analysis (13-30,34-39). A total of five studies were excluded because the selected SNPs in these studies were reported in only one study (34-38). Finally, the quality of 19 studies (13-30) was assessed using the NOS and all studies scored ≥6 stars or more, indicating high quality.

Table I summarized study characteristics of the 19 included studies. There were a total of 3,850 cases and 4,312 controls involving 10 SNPs of microRNAs: miR-196a-2 rs11614913 (seven studies), miR-449 rs3746444 (five studies), miR-146 rs2910164 (four studies), miR-125a rs12976445 (four studies), miR-149 rs2292832 (four studies), miR-27a rs895819 (four studies), miR-423 rs6505162 (two studies), miR-125a rs41275794 (three studies), miR-10a rs3809783 (two studies) and miR-323b rs56103835 (two studies). The distributions of microRNA gene polymorphism alleles and genotypes are shown in Table II.

Quantitative synthesis. The present meta-analysis included 10 SNPs discovered in miRNA gene loci. Table III summarizes the ORs with corresponding 95% CIs for the association between those SNPs and the risk for RSA base on different genetic models. After all included studies were pooled into the meta-analysis of each selected SNP, it was discovered that miR-149 rs2292832, miR-499a rs3746444, miR-125a rs12976445, miR-10a rs3809783, miR-125a rs41275794 and miR-323b rs56103835 SNPs were significantly associated with RSA risk (Table III). Forest plots were constructed from the findings of all included studies to show the relationship

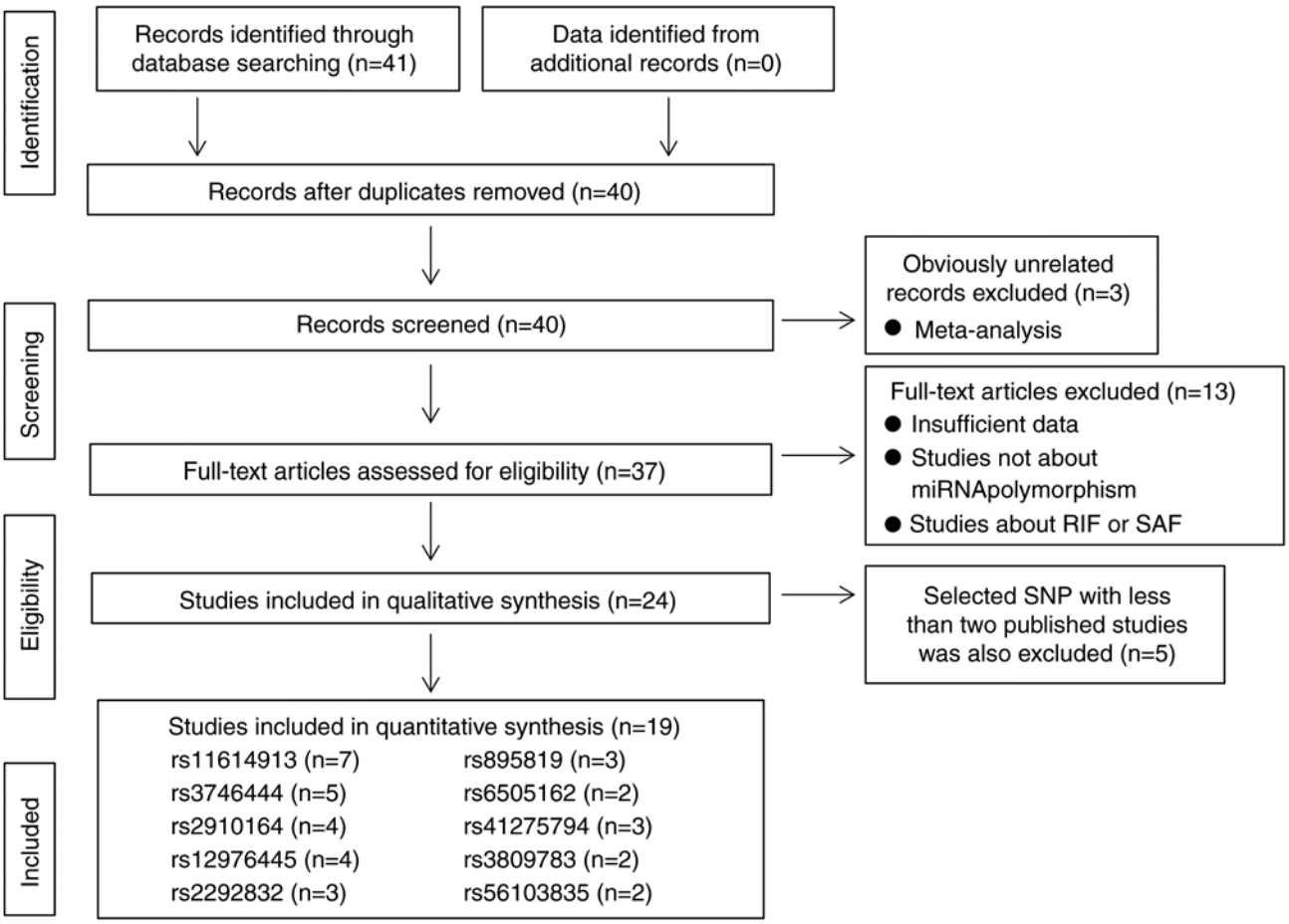

Figure 1. Flowchart illustrating the search strategy used to identify association studies of miRNA SNPs and RSA risk. miRNA, microRNA; SNPs, single nucleotide polymorphisms; RSA, recurrent spontaneous abortion; RIF, repeated implantation failure; SAF, spontaneously aborted fetuses.

between miRNA SNPs and RSA risk under a homogeneous model (Fig. 2). Statistical heterogeneity was found in nine SNPs. A total of seven SNPs underwent subgroup analysis to detect the source of heterogeneity, while miR-423 rs6505162 and miR-125a rs41275794 were not subjected to subgroup analysis because the number of included studies was too small (n=2).

miR-196a2 rs11614913. The present study examined seven relevant papers to determine the possible association between miR196a2 rs11614913 and RSA risk. When all the eligible studies were pooled into the analysis under various models, no significant risk associations were observed, indicating they were not genetic-related risk factors for RSA risk (Table III; Fig. 2A). Additionally, substantial heterogeneity was observed. The meta-analysis did not show any correlation when subgroup analyses were performed between ethnic backgrounds.

miR-499a rs3746444. A total of five studies related to rs3746444 were included in the meta-analysis. The allele contrast and heterogeneity model showed protective ORs with significant P-values (G vs. A: OR=0.66; 95% CI=0.51-0.86; $P_{heterogeneity}$=0.03, P=0.002; AG vs. AA: OR=0.73; 95% CI=0.59-0.90; $P_{heterogeneity}$=0.15; P=0.003) (Fig. 2B; Table III). There was significantly increased association between miR499a rs3746444 A>G and RSA risk susceptibility in the recessive, dominant and homogeneous model (GG vs. GA + AA: OR=1.99; 95% CI=1.41-2.80; $P_{heterogeneity}$=0.59; P<0.0001; GG + GA vs. AA: OR=1.54; 95% CI=1.12-2.12; $P_{heterogeneity}$=0.06; P=0.007; GG vs. AA: OR=2.26; 95% CI=1.53-3.36; $P_{heterogeneity}$=0.38; P<0.00001) (Table III). The findings of subgroup analysis results demonstrated that this SNP contributed to RSA susceptibility in Asian (Korean and Indian) populations under all models (Table IV).

miR-146 rs2910164. The present analysis included four studies on the miR-146a SNP. There was no significant association in any genetic model between the miR-146a rs2910164 C>G polymorphism and RSA risk (Fig. 2C; Table III). The meta-analysis did not find any correlation when subgroup analyses among ethnic backgrounds were performed (Table IV).

miR-125a rs12976445. There were four articles related to miR-125a rs12976445 C>T and URSA. Under a homogeneous model, the TT allele had a protective OR (TT vs. CC: OR=0.51; 95% CI: 0.31-0.84; P=0.008). There was no heterogeneity in the recessive model (TT vs. TC + CC: I^2=0.00%; $P_{heterogeneity}$=0.65) or homogeneous model (TT vs. CC: I^2=0.00%; $P_{heterogeneity}$=0.48). Significant heterogeneity was found in allele contrast (T vs. C: I^2=0.85%; P=0.001), dominant model (TT + TC vs. CC: I^2=0.87%; P<0.001) and

Table I. Characteristics of 19 included studies in this meta-analysis.

First author, year	No	Country	Ethnicity	Diagnostic criteria for RSA	Case	Control	Genotype	Polymorphisms studied	Quality score	(Refs.)
Alipour M, 2019	1	Iran	Caucasian	two or more	120	90	PCR-RFLP	miR-146a rs2910164 (G/C) miR-149 rs2292832 (T/C) miR-196a rs11614913 (C/T) miR-499 rs3746444 (A/G)	8	14
Amin-Beidokhti M, 2017	2	Iran	Caucasian	two or more	200	200	PCR-RFLP	miR-196a-2 rs11614913 (C/T) miR-499 rs3746444 (T/C)	8	15
Babakhanzadeh E, 2021	3	Iran	Caucasian	two or more	214	147	PCR-RFLP	miR-146a rs2910164 (G/C) miR-196 rs11614913 T/C	7	16
Fazli M, 2018	4	Iran	Caucasian	three or more	100	100	PCR-RFLP	miR-196a rs11614913 (C/T) miR-499 rs3746444 (A/G)	6	17
Hu Y, 2011	5	China	Asian	two or more	214	431	PCR-Sequencing	miR-125a rs41275794 (G/A) miR-125a rs12976445 (C/T)	8	13
Hu Y, 2014	6	China	Asian	two or more	426	370	PCR-Sequencing	miR-125a rs41275794 (G/A) miR-125a rs12976445 (C/T)	8	18
Jeon YJ, 2012	7	Republic of Korea	Asian	two or more	234	330	PCR-RFLP	miR-146 rs2910164 (G/C) miR-149 rs2292832 (T/C) miR-196a-2 rs11614913 (C/T) miR-499 rs3746444 (A/G)	7	19
Lee JY, 2020	8	Republic of Korea	Asian	two or more	361	272	PCR-RFLP	miR-25 rs1527423 (T>C) miR-32 rs7041716 (C>A) miR-125a rs12976445 (C>T) miR-222 rs34678647 (G>T)	8	20
Li Y, 2016	9	China	Asian	two or more	200	200	TaqMan miRNA RT-PCR, sequencing	miR-10a rs3809783 (A>T)	7	21
Manzoor U, 2022	10	India	Asian	two or more	150	180	PCR-RFLP	miR-125a rs12976445 (C/T) miR-125a rs10404453 (A/G)	9	22
Parveen F, 2014	11	India	Asian	three or more	200	300	PCR-RFLP	miR-146a rs2910164 (G/C) miR-149 rs2292832 (T/C) miR-196a rs11614913 (C/T) miR-499 rs3746444 (A/G)	8	23
Rah H, 2017	12	Republic of Korea	Asian	two or more	225	387	PCR-RFLP	miR-27a rs895819 (A/G) miR-423 rs6505162 (C/A)	7	24

Table I. Continued.

First author, year	No	Country	Ethnicity	Diagnostic criteria for RSA	Case	Control	Genotype	Polymorphisms studied	Quality score	(Refs.)
								miR-449b rs10061133 (A/G) miR-605 rs2043556 (A/G)		
Shaker M, 2020	13	Egypt	Caucasian	two or more	99	100	PCR-RFLP	leptin rs7799039 (G/A) miR-27a rs895819 (A/G)	7	25
Vahedi SN, 2021	14	Iran	Caucasian	two or more	116	89	PCR-SNaPshot	miR-125a rs41275794 (G>A) miR-10a rs3809783 (A>T) miR-323b rs56103835 (T>A)	6	26
Wang CY, 2016	15	China	Asian	two or more	138	142	PCR-Sequencing	miRNA-27a rs895819 A/G	8	27
Wang XQ, 2018	16	China	Asian	two or more	206	182	PCR-Sequencing	miR-323b rs56103835 (T>A)	8	28
Wang XQ, 2019	17	China	Asian	two or more	316	309	PCR-Sequencing	miR-423 rs6505162 (C/A) miR-423 rs8067576 (A/T)	8	29
Wang XQ, 2020	18	China	Asian	two or more	300	313	PCR-Sequencing	miR-196 rs11614913 T/C	8	30
Stavros S, 2022	19	Greece	Caucasian	two or more	199	200	PCR-RFLP	miR-149 rs2292832 (T/C) miRNA-27a rs895819 (A/G)	8	39

Diagnostic criteria for RSA, the number of consecutive spontaneous abortions; RSA, recurrent spontaneous abortions; RFLP, restriction fragment length polymorphism; miRNA, microRNA.

Table II. Alleles and genotypes distributions of microRNAs gene polymorphisms.

			Alleles (n, %)				Genotypes (n, %)							
			RSA		Control		RSA			Control				
miR-196a-2 rs11614913														
First author, year	RSA	Control	C	T	C	T	CC	CT	TT	CC	CT	TT	HWE	(Refs.)
Alipour M, 2019	120	90	34 (14.15)	206 (85.85)	20 (11.11)	160 (88.89)	3 (2.5)	28 (23.3)	89 (74.2)	1 (1.1)	18 (20.0)	71 (78.9)	0.91	14
Amin-Beidokhti M, 2017	200	200	236 (59.0)	164 (41.0)	268 (67.0)	132 (33.0)	68 (34.0)	100 (50.0)	32 (16.0)	84 (42.0)	100 (50.0)	16 (8.0)	0.06	15
Babakhanzadeh, 2021	214	147	307 (71.7)	121 (28.3)	194 (66.0)	100 (34.0)	104 (49.0)	99 (46.0)	11 (5.0)	62 (43.0)	70 (47.0)	15 (10.0)	0.73	16
Fazli M, 2018	100	100	119 (59.5)	81 (40.5)	120 (60.0)	80 (40.0)	33 (33.0)	53 (53.0)	14 (14.0)	29 (29.0)	62 (62.0)	9 (9.0)	0.004	17
Jeon YJ, 2012	330	234	323 (48.9)	337 (51.1)	211 (45.1)	257 (54.9)	82 (24.8)	159 (48.2)	89 (27.0)	41 (17.5)	129 (55.1)	64 (27.4)	0.08	19
Parveen F, 2014	200	300	175 (43.7)	225 (56.3)	234 (39.0)	366 (61.0)	40 (20.0)	95 (47.5)	65 (32.5)	38 (12.6)	158 (52.6)	104 (34.6)	0.06	23
Wang XQ, 2020	300	313	248 (41.3)	352 (58.7)	307 (49.0)	319 (51.0)	54 (18.0)	140 (46.7)	106 (35.3)	76 (24.3)	155 (49.5)	82 (26.2)	0.87	30
miR-499 rs3746444														
			A	G	A	G	AA	AG	GG	AA	AG	GG		
Alipour M, 2019	120	90	86 (35.84)	154 (64.16)	83 (46.1)	97 (53.9)	15 (12.5)	57 (47.5)	48 (40.0)	16 (17.8)	51 (56.7)	23 (25.5)	0.18	14
Amin-Beidokhti M, 2017	200	200	286 (71.5)	114 (28.5)	284 (71)	116 (29)	100 (50)	86 (43)	14 (7)	96 (48)	92 (46)	12 (6)	0.10	15
Fazli M, 2018	100	100	96 (48.0)	104 (52.0)	126 (63.0)	74 (37.0)	29 (29.0)	38 (38.0)	33 (33.0)	45 (45.0)	36 (36.0)	19 (19.0)	0.02	17
Jeon YJ, 2012	330	234	529 (80.2)	131 (19.8)	404 (86.3)	64 (13.7)	211 (63.9)	107 (32.4)	12 (3.6)	173 (73.9)	58 (24.8)	3 (1.3)	0.45	19
Parveen F, 2014	200	300	318 (79.5)	82 (20.5)	531 (88.5)	69 (11.5)	130 (65)	58 (29)	12 (6)	237 (79)	57 (19)	6 (3)	0.25	23
miR-146 rs2910164														
			C	G	C	G	CC	CG	GG	CC	CG	GG		
Alipour M, 2019	120	90	197 (82.09)	43 (17.91)	132 (73.35)	48 (26.65)	81 (67.5)	35 (29.2)	4 (3.3)	45 (50.0)	42 (46.7)	3 (3.3)	0.07	14
Babakhanzadeh, 2021	214	147	291 (68.0)	137 (32.0)	214 (73.0)	80 (27.0)	92 (43.0)	105 (49.0)	17 (8.0)	78 (53.0)	59 (40.0)	10 (7.0)	0.80	16
Jeon YJ, 2012	330	234	390 (59.1)	270 (40.9)	283 (60.5)	185 (39.5)	116 (35.2)	158 (47.9)	56 (17.0)	79 (33.8)	125 (53.4)	30 (12.8)	0.07	19
Parveen F, 2014	200	300	233 (58.3)	167 (41.7)	372 (62.0)	228 (38.0)	63 (31.5)	107 (53.5)	30 (15.0)	108 (36.0)	156 (52.0)	36 (12.0)	0.07	23

Table II. Continued.

			Alleles (n, %)				Genotypes (n, %)							
			RSA		Control		RSA			Control				
miR-125a rs12976445														
			C	T	C	T	CC	CT	TT	CC	CT	TT		
Hu, Y 2011	217	431	322 (75.2)	106 (24.8)	707 (82.0)	155 (18.0)	111 (51.9)	100 (46.7)	3 (1.4)	285 (66.1)	137 (31.8)	9 (2.1)	0.11	13
Hu Y, 2014	370	631	526 (71.1)	214 (28.9)	1011 (80.1)	251 (19.1)	158 (42.7)	210 (56.8)	2 (0.5)	392 (62.1)	227 (36.0)	12 (1.9)	0.001	18
Lee JY, 2020	361	272	617 (85.6)	105 (14.5)	469 (86.2)	75 (13.8)	263 (72.9)	91 (25.2)	7 (1.9)	203 (74.6)	63 (23.2)	6 (2.2)	0.67	20
Manzoor U, 2022	150	180	128 (42.7)	172 (57.3)	125 (34.7)	235 (65.3)	29 (19.3)	70 (46.7)	51 (34.0)	19 (10.6)	87 (48.3)	74 (41.1)	0.37	22
miR-149 rs2292832														
			T	C	T	C	TT	TC	CC	TT	TC	CC		
Alipour M, 2019	120	90	188 (78.3)	52 (21.7)	157 (87.2)	23 (12.8)	70 (58.3)	48 (40.0)	2 (1.7)	68 (75.6)	21 (23.3)	1 (1.1)	0.66	14
Jeon YJ, 2012	330	234	477 (72.3)	183 (27.7)	352 (75.2)	116 (24.8)	173 (52.4)	131 (39.7)	26 (7.9)	132 (56.4)	88 (37.6)	14 (6.0)	0.90	19
Parveen F, 2014	200	300	318 (79.5)	82 (20.5)	498 (83.0)	102 (17.0)	128 (64.0)	62 (31.0)	10 (5.0)	207 (69.0)	84 (28.0)	9 (3.0)	0.89	23
Stavros S, 2022	199	200	272 (68.3)	126 (31.7)	278 (69.5)	122 (30.5)	102 (51.3)	68 (34.2)	29 (14.6)	110 (55.0)	58 (29.0)	32 (16.0)	<0.001	39
miR-27a rs895819														
			A	G	A	G	AA	AG	GG	AA	AG	GG		
Rah HC, 2017	387	225	502 (64.9)	272 (35.1)	268 (59.6)	182 (40.4)	166 (42.9)	170 (43.9)	51 (13.2)	74 (32.9)	120 (53.3)	31 (13.8)	0.11	24
Shaker M, 2019	99	100	101 (51.0)	97 (49.0)	142 (71.0)	58 (29.0)	34 (34.3)	33 (33.3)	32 (32.4)	56 (56.0)	30 (30.0)	14 (14.0)	0.007	25
Wang CY, 2016	138	142	172 (62.3)	104 (373.)	207 (72.9)	77 (27.1)	56 (40.7)	60 (43.4)	22 (15.9)	78 (54.9)	51 (35.9)	13 (9.2)	0.28	27
Stavros S, 2022	199	200	206 (51.8)	192 (42.2)	268 (67.0)	132 (33.0)	58 (29.1)	90 (45.2)	51 (25.7)	87 (43.5)	94 (47.0)	19 (9.5)	0.37	39
miR-423 rs6505162														
			C	A	C	A	CC	CA	AA	CC	CA	AA		
Rah HC, 2017	387	225	594 (76.7)	180 (23.3)	363 (80.7)	87 (19.3)	232 (59.9)	130 (33.6)	25 (6.5)	149 (66.2)	65 (28.9)	11 (4.9)	0.27	24
Wang XQ, 2019	316	309	552 (87.3)	80 (12.7)	503 (81.4)	115 (18.6)	240 (75.9)	72 (22.8)	4 (1.3)	206 (66.7)	91 (29.4)	12 (3.9)	0.63	29

Table II. Continued.

			Alleles (n, %)				Genotypes (n, %)							
			RSA		Control		RSA			Control				
miR-125a rs41275794														
			G	A	G	A	GG	GA	AA	GG	GA	AA		
Hu Y, 2011	217	431	333 (77.8)	95 (22.2)	734 (85.2)	128 (14.8)	122 (57.0)	89 (41.6)	3 (1.4)	310 (71.9)	114 (26.5)	7 (1.6)	0.34	13
Hu Y, 2014	370	631	501 (67.7)	239 (32.3)	1072 (84.9)	190 (15.1)	141 (38.1)	219 (59.2)	10 (2.7)	450 (71.3)	172 (27.3)	9 (1.4)	0.10	18
Vahedi SN, 2021	116	89	151 (65.1)	81 (34.9)	139 (78.1)	39 (21.9)	46 (39.7)	59 (50.9)	11 (9.4)	54 (46.7)	31 (34.8)	4 (4.5)	0.87	26
miR-10a rs3809783														
			A	T	A	T	AA	AT	TT	AA	AT	TT		
Li Y, 2016	200	200	303 (75.8)	97 (24.2)	354 (88.5)	46 (11.5)	103 (51.5)	97 (48.5)	0 (0.0)	154 (77.0)	46 (23.0)	0 (0.0)	0.07	21
Vahedi SN, 2021	116	89	162 (69.8)	70 (30.2)	142 (79.8)	36 (20.2)	53 (45.7)	56 (48.3)	7 (6.0)	56 (62.9)	30 (33.7)	3 (3.4)	0.67	26
miR-323b rs56103835														
			T	C	T	C	TT	TC	CC	TT	TC	CC		
Vahedi SN, 2021	116	89	150 (64.7)	82 (35.3)	117 (65.7)	61 (34.3)	45 (38.8)	60 (51.7)	11 (9.5)	31 (34.8)	55 (61.8)	3 (3.4)	0.0005	26
Wang XQ, 2018	206	182	252 (61.2)	160 (38.8)	252 (69.6)	112 (30.4)	26 (12.6)	108 (52.4)	72 (35.0)	18 (9.4)	76 (42.0)	88 (48.6)	0.79	28

RSA, recurrent spontaneous abortions; HWE, Hardy-Weinberg equilibrium; miRNA, microRNA.

Table III. Overall result of meta-analysis of eligible SNPs.

Model	Studies (n)	Test of association		Test of heterogeneity		
		OR (95% CI)	P-value	Model	P-value	I^2 (%)
miR-196a-2 rs11614913						
Allele contrast (C vs. T)	7	0.99 (0.80, 1.22)	0.93	Random	0.003	70
Recessive model (CC vs. CT + TT)		0.91 (0.80, 1.03)	0.12	Random	<0.00001	91
Dominant model (CC + CT vs. TT)		1.20 (0.89, 1.62)	0.22	Random	0.05	54
CC vs. TT		0.98 (0.58, 1.66)	0.95	Random	0.0006	75
CT vs. TT		1.18 (0.98, 1.43)	0.08	Fixed	0.13	39
miR-499 rs3746444						
Allele contrast (G vs. A)	5	0.66 (0.51, 0.86)	**0.002**	Random	0.03	64
Recessive model (GG vs. GA + AA)		1.99 (1.41, 2.80)	**<0.0001**	Fixed	0.59	0
Dominant model (GG + GA vs. AA)		1.54 (1.12, 2.12)	**0.007**	Random	0.06	56
GG vs. AA		2.26 (1.53, 3.36)	**<0.00001**	Fixed	0.38	6
AG vs. AA		0.73 (0.59, 0.90)	**0.003**	Random	0.15	41
miR-146 rs2910164						
Allele contrast (G vs. C)	4	0.58 (0.22, 1.53)	0.27	Random	<0.00001	97.00
Recessive model (GG vs. GC + CC)		1.30 (0.95, 1.79)	0.10	Fixed	0.97	0.00
Dominant model (GG + GC vs. CC)		0.99 (0.66, 1.48)	0.95	Random	0.01	73.00
GG vs. CC		1.30 (0.95, 1.79)	0.10	Fixed	0.97	0.00
CG vs. CC		0.77 (0.56, 1.08)	0.13	Fixed	0.83	0.00
miR-125a rs12976445						
Allele contrast (T vs. C)	4	1.18 (0.82, 1.69)	0.38	Random	0.001	85
Recessive model (TT vs. TC + CC)		0.68 (0.47, 1.00)	0.05	Fixed	0.65	0.00
Dominant model (TT + TC vs. CC)		1.28 (0.76, 2.13)	0.35	Random	<0.001	87
TT vs. CC		0.51 (0.31, 0.84)	**0.008**	Fixed	0.48	0.00
TC vs. CC		1.35 (0.81, 2.24)	0.25	Random	<0.0001	87
miR-149 rs2292832						
Allele contrast (C vs. T)	4	1.21 (1.03-1.42)	**0.02**	Fixed	0.31	17
Recessive model (CC vs. TC + TT)		1.15 (0.79-1.68)	0.46	Fixed	0.62	0
Dominant model (CC + TC vs. TT)		1.28 (1.05-1.56)	**0.01**	Fixed	0.30	18
CC vs. TT		1.25 (0.85-1.85)	0.26	Fixed	0.67	0
TC vs. TT		1.71 (1.12-2.62)	**0.01**	Random	0.01	72
miR-27a rs895819						
Allele contrast (G vs. A)	4	1.53 (0.92-2.55)	0.10	Random	<0.00001	91
Recessive model (GG vs. AG + AA)		2.44 (0.96-6.23)	0.06	Random	<0.00001	90
Dominant model (GG + AG vs. AA)		1.28 (0.73-2.26)	0.39	Random	0.0001	85
GG vs. AA		2.19 (0.90-5.31)	0.08	Random	<0.0001	86
AG vs. AA		1.24 (0.73-2.12)	0.43	Random	0.002	80
miR-423 rs6505162						
Allele contrast (A vs. C)	2	0.90 (0.46, 1.77)	0.75	Random	0.001	90
Recessive model (AA vs. CA + CC)		0.72 (0.17, 3.11)	0.66	Random	0.03	79
Dominant model (AA + CA vs. CC)		0.91 (0.45, 1.86)	0.80	Random	0.004	88
AA vs. CC		0.69 (0.14, 3.39)	0.64	Random	0.02	82
AC vs. CC		0.93 (0.50, 1.74)	0.83	Random	0.01	83
miR-125a rs41275794						
Allele contrast (A vs. G)	3	2.07 (1.47, 2.92)	**<0.0001**	Random	0.02	75
Recessive model (AA vs. GA + GG)		1.68 (0.90, 3.13)	0.10	Fixed	0.54	0
Dominant model (AA + GA vs. GG)		2.68 (1.59, 4.52)	**0.0002**	Random	0.003	83
AA vs. GG		2.61 (1.39, 4.90)	**0.003**	Fixed	0.35	5
AG vs. GG		2.68 (1.59, 4.51)	**0.0002**	Random	0.004	82

Table III. Continued.

Model	Studies (n)	Test of association		Test of heterogeneity		
		OR (95% CI)	P-value	Model	P-value	I^2 (%)
miR-10a rs3809783						
Allele contrast (T vs. A)	2	2.12 (1.58, 2.85)	**<0.00001**	Fixed	0.23	31
Recessive model (TT vs. AT + AA)		1.84 (0.46, 7.33)	0.39	Fixed	Not estimable	Not estimable
Dominant model (TT+AT vs. AA)		2.68 (1.90, 3.77)	**<0.00001**	Fixed	0.22	34
TT vs. AA		0.41 (0.10, 1.65)	0.21	Fixed	Not estimable	Not estimable
AT vs. AA		2.67 (1.89, 3.77)	**<0.00001**	Fixed	0.20	38
miR-323b rs56103835						
Allele contrast (C vs. T)	2	1.28 (1.01, 1.63)	**0.04**	Fixed	0.23	30
Recessive model (CC vs. TC + TT)		1.16 (0.23, 5.82)	0.85	Random	0.02	82
Dominant model (CC + TC vs. TT)		0.80 (0.53, 1.23)	0.32	Fixed	0.81	0
CC vs. TT		1.06 (0.25, 4.53)	0.94	Random	0.05	73
CT vs. TT		0.84 (0.54, 1.31)	0.45	Fixed	0.55	0

Bold numbers indicate P<0.05. SNPs, single nucleotide polymorphisms; n, number of cohorts; OR, odd ratio; CI, confidence interval.

heterogeneity model (TT vs. TC + CC: I^2=0.87%; P<0.0001) (Fig. 2D, Table III). Subgroup analysis revealed that ethnic Chinese had an elevated risk in allelic contrast, dominant model and heterozygous model (Table IV).

miR-149 rs2292832. A total of four articles associated with miR-149 rs2292832 T>C and URSA were included. The results showed risk ORs for C allele, CC+TC and TC in allele contrast, dominant model and heterogeneity model respectively (C vs. T: OR=1.21; 95% CI=1.03-1.42; P=0.02; CC + TC vs. TT: OR=1.28; 95% CI=1.05-1.56; P=0.01; TC vs. TT: OR=1.24; 95% CI=0.73-2.12; P=0.43; Fig. 2E, Table III). Except for the heterogeneity model (TC vs. TT: I^2=0.72%; $P_{heterogeneity}$=0.002), no heterogeneity was observed in any of the models. Subgroup analysis showed no significant association among different ethnic backgrounds under any models (Table IV).

miR-27a rs895819. A total of four eligible studies were included in the analysis. There was no significant connection between the miR-27a rs895819 A>G polymorphism and RSA risk in any genetic model (Fig. 2F; Table III). All of the models showed significant heterogeneity. However, subgroup analysis revealed an increased risk under allelic contrast, recessive model homozygote model and heterozygous model in the Caucasian population (G vs. A: OR=2.35; 95% CI=1.56-3.56; P<0.001; GG vs. AG + AA: OR=2.93; 95% CI=1.45-5.94; P=0.003; GG vs. AA: OR=3.63; 95% CI=1.70-7.77; P=0.009; AG vs. AA: OR=1.54; 95% CI=1.07-2.22; P=0.02; Table IV).

miR-423 rs6505162. The analysis included three eligible studies. No significant association was found between miR-423 rs6505162 C>A polymorphism and RSA risk in any genetic model (Fig. 2G, Table III). Significant heterogeneity was found in all models.

miR-125a rs41275794. For overall studies, there was a significant association of rs41275794 and RSA susceptibility in allele contrast (A vs. G: OR=2.07; 95% CI=1.47-2.92; P<0.0001), dominant model (AA + GA vs. GG: OR=2.68; 95% CI=1.59-4.52; P=0.0002), homogeneous model (AA vs. GG: OR=2.61; 95% CI=1.39-4.90; P=0.003) and heterogeneity model (GA vs. GG: OR=2.68; 95% CI=1.59-4.51; P=0.0002; Fig. 2H; Table III). Significant heterogeneity was found in the allele contrast, dominant model and heterogeneity model. Considering heterogeneity in the above gene model, a subgroup analysis by ethnicity was performed. The results showed significant and increased risk in the Chinese population under the allelic contrast, recessive, dominant, homozygote and heterozygous model. Significantly, there was increased risk for non-Chinese under the allelic contrast, dominant model and heterozygous model (Table IV).

miR-10a rs3809783. A significant association with increased risk was observed in the allele contrast (T vs. A: OR=2.12; 95% CI=1.58-2.85; P<0.00001), dominant model (TT+AT vs. AA: OR=2.68; 95% CI=1.90-3.77; P<0.00001) and heterogeneity model (AT vs. AA: OR=2.67; 95% CI=1.89-3.77; P<0.00001; Fig. 2I, Table III) when two studies were pooled into meta-analysis. No heterogeneity was found in the meta-analysis process except that the P-value and I^2 in test of heterogeneity was not estimable.

miR-323b rs56103835. A significant association with increased risk was observed in the allele contrast (C vs. T: OR=1.28; 95% CI=1.01-1.63; P=0.04) with no heterogeneity (I^2=30%; $P_{heterogeneity}$ = 0.23) as shown in Fig. 2J and Table III, when two studies were pooled into meta-analysis.

Sensitivity analysis. Sensitivity analysis was used to examine the impact of each study on the overall OR by excluding one

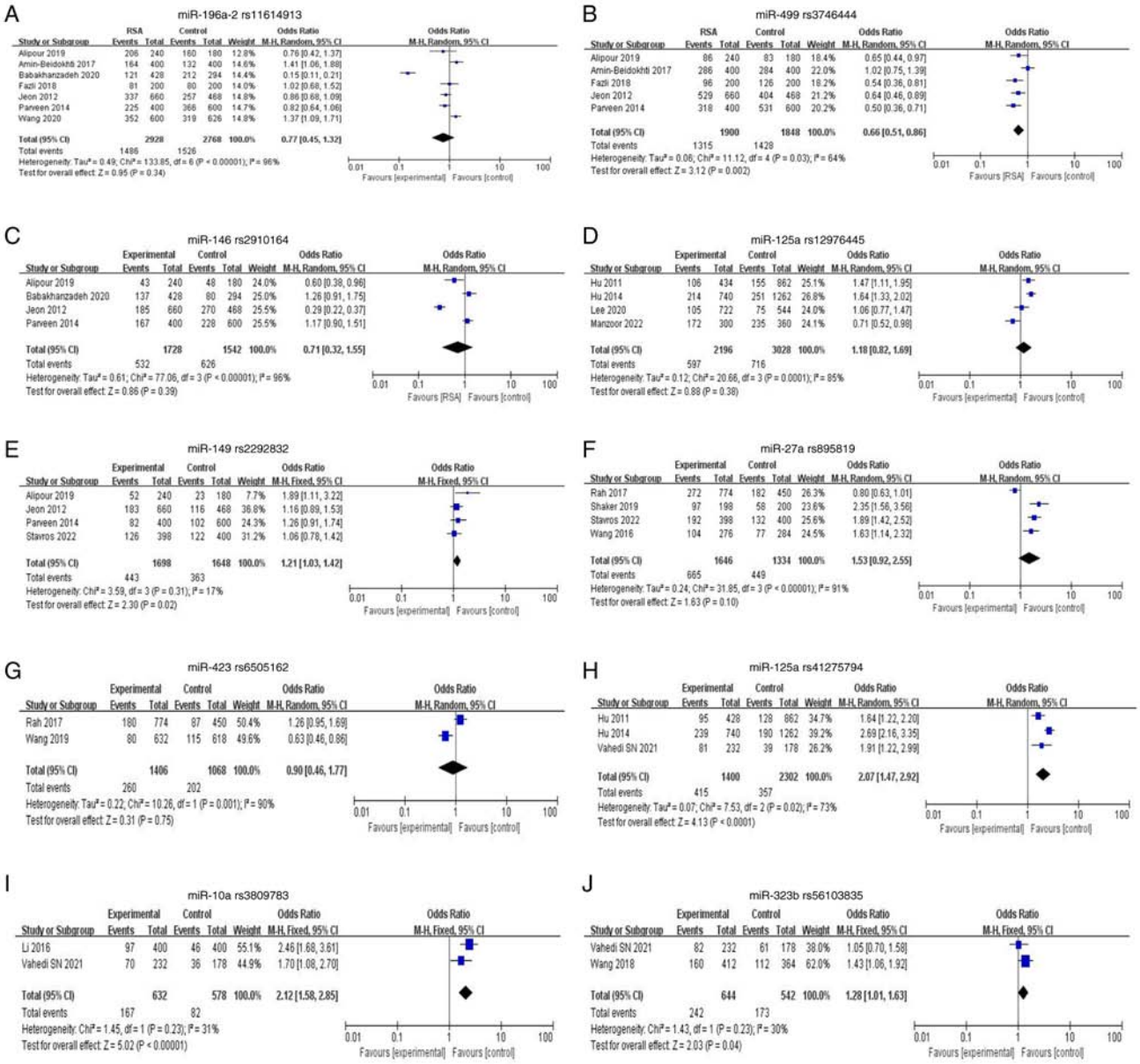

Figure 2. Forest plots of RSA risk association with miRNA SNPs under the homogeneous model. (A) miR196a2 rs11614913. (B) miR499a rs3746444 T>C. (C) miR-146a rs2910164 C>G. (D) pri-miRNA-125a rs12976445 C>T. (E) miR-149 rs2292832 T>C. (F) miRNA-27a rs895819 A>G. (G) miR423 rs6505162 C >A. (H) pri-miRNA-125a rs41275794 G>A. (I) miR-10a rs3809783 A>T. (J) miR-323b rs56103835 T>C. RSA, recurrent spontaneous abortion; miRNA, microRNA; SNPs, single nucleotide polymorphisms.

study at a time. The sensitivity analysis results suggested that overall effects were not influenced by any specific study, ensuring the credibility and reliability of the results of the present study (data not shown).

Discussion

RSA is a common pregnancy complication affecting 1-3% of couples trying to conceive. Studies have shown that miRNAs may play an important role in URSA and SNPs located both in the pre-miRNAs or within miRNA-binding sites are likely to influence the expression and function of the miRNA target and thus may contribute to susceptibility to URSA (28-30). The most common and widely studied SNPs in miRNAs are miR-146a rs2910164, miR-196a2 rs11614913 and miR499a rs3746444. Several studies have been conducted to investigate the relationship between these SNPs and the risks of RSA (14-30). However, the results are contradictory and inconclusive. Srivastava *et al* (31) performed the first meta-analysis on miRNA SNPs in RSA, suggesting that rs11614913, rs3746444 and rs2292832 biomarkers may decrease the risk of RSA under different genetic models. However, the most recent study of the above meta-analysis was published in June 2021 (31). The present study conducted an independent meta-analysis on all available studies to assess the RSA risk with miRNA SNPs as well as subgroup analyses by ethnicity with larger sample size to improve understanding of the association between these polymorphisms and RSA risk. This meta-analysis reviewed the case-control literature on the association between miRNA polymorphisms and RSA risk and

Table IV. Summary of overall results and subgroup for the association between the microRNAs genes polymorphisms and RSA.

			Sample size		Allelic contrast		Recessive model		Dominant model		Homozygote model		Heterozygous model	
Gene	Subgroup	n	Case	Control	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
miR196a2	Ethnicity													
rs11614913	Caucasian	4	634	537	0.98 (0.71,1.37)	0.92	1.00 (0.93,1.08)	0.97	1.08 (0.54,2.16)	0.83	1.03 (0.39,2.67)	0.96	1.11 (0.59,2.09)	0.74
	Asian	3	734	943	0.99 (0.71,1.37)	0.96	0.82 (0.67,1.00)	0.05	1.12 (0.80,1.56)	0.50	0.92 (0.45,1.87)	0.81	1.20 (0.96,1.50)	0.11
miR499a	Ethnicity													
rs3746444	Caucasian	3	420	390	0.73 (0.49,1.08)	0.11	1.78 (1.21,2.61)	**0.003**	1.37 (0.81,0.33)	0.24	1.93 (1.22,3.05)	**0.005**	0.92 (0.67, 1.27)	0.63
	Asian	2	530	534	0.57 (0.45,0.73)	**<0.00001**	3.03 (1.38, 6.68)	**0.006**	1.78 (1.35,2.33)	**<0.0001**	3.49 (1.57,7.72)	**0.002**	0.61 (0.46,0.80)	**0.0005**
miR-146	Ethnicity													
rs2910164	Caucasian	2	334	237	0.89 (0.43,1.83)	0.74	1.14 (0.56,2.33)	0.72	0.86 (0.28,2.63)	0.80	1.14 (0.56,2.33)	0.72	0.93 (0.44,1.96)	0.85
	Asian	2	530	534	0.58 (0.15,2.30)	0.44	1.35 (0.95,1.91)	0.10	1.06 (0.82,1.37)	0.65	1.35 (0.95,1.91)	0.10	0.74 (0.51,1.07)	0.11
miR-125a	Ethnicity													
rs12976445	Caucasian	2	587	1062	1.58 (1.33,1.87)	**<0.00001**	0.43 (0.16,1.15)	0.09	2.02 (1.64,2.50)	**<0.00001**	0.51 (0.19,1.37)	0.18	2.13 (1.73,2.62)	**<0.00001**
	Asian	2	511	452	0.87 (0.59,1.29)	0.49	0.76 (0.50,1.15)	0.19	0.77 (0.35,1.67)	0.50	0.51 (0.28,0.91)	0.02	0.81 (0.39,1.67)	0.56
Diagnostic criteria for RSA														
miR-149	Ethnicity													
rs2292832	Caucasian	2	319	290	1.35 (0.77-2.37)	0.30	0.92 (0.54-1.56)	0.76	1.54 (0.82-2.86)	0.18	1.02 (0.58-1.77)	0.96	1.61 (0.93-2.78)	0.09
	Asian	2	434	630	1.20 (0.98-1.48)	0.08	1.46 (0.84-2.51)	0.18	1.21 (0.94-1.55)	0.14	1.54 (0.88-2.68)	0.13	1.79 (0.81-3.98)	0.15
miR-27a	Ethnicity													
rs895819	Caucasian	2	525	367	1.12 (0.56,2.26)	0.74	1.26 (0.65,2.44)	0.49	1.06 (0.40,2.85)	0.90	1.26 (0.40, 4.01)	0.69	1.00 (0.39,2.54)	1.00
	Asian	2	298	300	2.03 (1.60-2.32)	**<0.00001**	4.63 (2.00-10.7)	**0.0003**	1.57 (0.71-3.50)	0.27	3.86 (2.39-6.26)	**<0.00001**	1.54 (1.07-2.22)	**0.02**
miR-125a	Ethnicity													
rs41275794	Caucasian	2	587	1062	2.12 (1.30,3.45)	**0.003**	2.12 (1.30,3.45)	0.30	2.81 (1.37,5.80)	**0.005**	2.36 (1.13,4.96)	**0.02**	2.86 (1.42,5.78)	**0.003**
	Asian	1	116	89	1.91 (1.22,2.99)	**0.004**	2.23 (0.68,7.24)	0.18	2.35 (1.33,4.13)	**0.003**	3.23 (0.96,10.83)	0.06	2.23 (1.24,4.02)	**0.007**

Bold numbers indicate P<0.05. n, number of cohorts; OR, odd ratio; CI, confidence interval; RSA, recurrent spontaneous abortions.

conducted an independent meta-analysis of eligible studies. It included 18 studies involving 3,850 cases and 4,312 controls involving 20 SNPs. miR499a rs3746444, miR-149 rs2292832, miR-125a rs41275794 and miR-10a rs3809783 may enhance the risk of RSA under different genetic models. Although there was no association between the miR-125a rs12976445 and miRNA-27a rs895819 polymorphisms and RSA, they were found to be statistically significant in certain ethnic groups of populations.

miR-196a and RSA. Preliminary data suggested a significant association of miR-196a with RSA. However, the results of the present study showed no significant association. These results were consistent with the study by Alipour *et al* (14), Babakhanzadeh *et al* (16) and Fazli *et al* (17). The results of the present study contradicted the findings of the meta-analysis conducted by Srivastava *et al* (31), which suggested that miR-196a2 T>C polymorphism may be responsible for recurrent spontaneous abortion. Significantly, some errors existed when genotypic frequencies were abstracted by Srivastava *et al* (31). For example, the CC and TT genotypic frequencies in the case and control groups from studies of Amin-Beidokhti *et al* (15) and Wang *et al* (30) were reversed. This could explain the differences in the current results.

miR-499a rs3746444 and RSA. Human SRY-box containing gene 6 (SOX6) can recruit c-terminal binding protein 2 (CtBP2) to repress transcription of fibroblast growth factor-3 (FGF-3), which is involved in cell proliferation and differentiation during developing embryonic tissues and SOX6 was identified as a direct target of miR-499 (40,41). It is hypothesized that miR-499 expression deregulation and dysfunctions caused by gene mutations can affect female reproduction and fertility. Studies conducted by Alipour *et al* (14), Fazli *et al* (17) and Parveen *et al* (23) found a significant association of miR-499a with patients with RSA, which is consistent with the conclusion of the present study. Other trials yielded inconsistent results with no significant correlation with RSA (15,19).

miR-146 rs2910164 and RSA. Alipour *et al* (14) suggested a positive association between miR-146a C>G polymorphism and RSA. This result is inconsistent with previous studies (16,19,23) and the present study. Studies have shown that miR-146C>G polymorphism enhances the expression of mature miR146a which suppresses breast cancer metastasis (42,43). It has also been reported that miR-146a significantly alters mRNA levels of Fas by targeting its 3'-UTR of this gene (44). Women with idiopathic infertility and recurrent pregnancy loss have lower expression of FAS, which induces apoptosis in oocytes during folliculogenesis (45).

miR-125a rs12976445 and RSA. Except for the homogeneous model, no significant association was observed in the present study in any genetic model. No significant association was observed in studies by Srivastava *et al* (31) in any genetic model; in their study, the genotype frequencies from pri-miR-125a rs12976445 were reversed between case and control group studies by Hu *et al* in 2014 (18). This can somewhat explain the inconsistency with the results of the present study.

miR-149 rs2292832 and RSA. The present study observed statistical evidence for a significant association of SNP rs2292832 within the miR-149 gene with RSA under three genetic models, which indicated that the C allele and CC genotype are risk factors for RSA. This result is inconsistent with previous studies (14,31). The target genes of miR-149 are Akt1 and E2F1, which are involved in promoting cell growth and cell cycle progression (46).

miR-27a rs895819 and RSA. miR-27a rs895819 is significantly associated with increased frequency of RSA risk and repeated implantation failure (33). However, the findings of the present study did not show any association, consistent with the results of Rah *et al* (24) and Srivastava *et al* (31). The subgroup study showed no association in the Asian group but a significant association in the Caucasian group.

miR-423 rs6505162 and RSA. A study by Wang *et al* (29) found that SNP rs6505162C>A in coding region of miR-423 was associated with an increased risk of human URSA in 316 RSA cases and 309 controls, while Rah *et al* (24) and Srivastava *et al* (31) observed no significant correlation with RSA, which is consistent with results of the present study. Studies by Srivastava *et al* (31), which included the same two studies, reached the same conclusion.

miR-125a rs41275794 and RSA. Hu *et al* (18) identified that two functional SNP sites in pri-miR-125a affected the expression of LIFR and ERBB2 and thus increased the RSA risk. Vahedi *et al* (26) also reported that the number of alleles in pre-miR-125a was significantly different and the dominant inheritance model was proposed. Except for the recessive model, the present study showed that miR-125a rs41275794 significantly increases the risk of RSA in all models. Subgroup analysis also indicated that miR-125a rs41275794 may increase susceptibility to RSA. Srivastava *et al* (31) found no significant connection in any genetic model other than the homogeneous model. In that study, the genotype frequencies from pri-miR-125a rs41275794 were reversed between case and control group studies by Hu *et al* (18) in 2014. This can explain the inconsistency with the results of the present study.

miR-10a rs3809783 and RSA. Studies by Li *et al* (21) and Vahedi *et al* (26) discovered that miR-10a rs3809783 A>T is conducive to a genetic predisposition to RSA, which is consistent with the current findings. miR-10a rs3809783 A>T disrupts the production of mature miR-10a and reinforces the expression of Bim (21).

miR-323b rs56103835 and RSA. Studies by Wang *et al* (28) discovered that miR-323b rs56103835 T>C was associated with an increased risk of human URSA, while Vahedi *et al* (26) found no significant association with RSA. No significant association was observed in any genetic model except the allele contrast in the present study.

The present meta-analysis has the advantages of including more literature, studying more gene sites and conducting more in-depth subgroup analysis than the previous meta-analysis (31). However, in addition to the significant heterogeneity, a limitation of the present meta-analysis was that the number

of eligible studies included in the total is insufficient to obtain a precise assessment between SNPs in miRNA and RSA.

In conclusion, the current meta-analysis suggested a strong association between miR499a rs3746444 A>G, miR-149 rs2292832 T>C, miR-125a rs41275794 G>A and miR-10a rs3809783 A>T and RSA risk. Thus, these SNPs might be recommended as a predictor for susceptibility to RSA. However, the results of the present meta-analysis should be interpreted carefully because of the heterogeneity among study designs. To obtain a more scientific result, more relevant case-control studies with multiple sample sources must be conducted and included in the meta-analysis.

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Availability of data and materials

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XW and JG conceived the study. XW and YX searched the databases and extracted the data. XW, YW, CZ and ZD analyzed and interpreted the data. XW wrote the draft of the paper. JG and ZD reviewed the manuscript. XW and JG confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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