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PERICONCEPTION, PREGNANCY AND CHILD OUTCOMES

REVIEW

The effect of intravenous immunoglobulin passive immunotherapy on unexplained recurrent spontaneous abortion: a meta-analysis

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Abstract The aim of this study was to investigate the effect of passive immunotherapy using intravenous immunoglobulin (IVIG) on unexplained recurrent spontaneous abortion (RSA). Live birth rates were analysed and binary data were calculated using risk ratio and 95% confidence interval. Meta-analysis of 11 studies showed that the difference in the live birth rate between the IVIG treatment and placebo groups was on the margin of significance (RR = 1.25, 95% CI 1.00 to 1.56, $P = 0.05$). Both cumulative and trial sequential meta-analyses indicated potential beneficial effect of IVIG but the evidence was inconclusive. Subgroup analysis showed that the live birth rate in primary (RR = 0.88, 95% CI 0.71 to 1.07) and secondary (RR = 1.26, 95% CI 0.99 to 1.61) RSA patients was not significantly different between the IVIG and placebo groups. Live birth rate was significantly different when IVIG was administered before conception (RR = 1.67, 95% CI 1.30 to 2.14, $P < 0.0001$) but not after implantation (RR = 1.10, 95% CI 0.93 to 1.29). Evidence is insufficient to support the beneficial effects of IVIG on an unexplained RSA. Further high quality studies are needed to elucidate the effectiveness of IVIG.

KEYWORDS: IVIG, meta-analysis, primary RSA, randomized controlled trial, recurrent spontaneous abortion, secondary RSA

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Introduction

Recurrent spontaneous abortion (RSA) is defined as two or more spontaneous abortions (with the same partner) before 20th gestational week of pregnancy or loss of pregnancy when fetal weight is less than 500 g. It is further categorized into primary RSA and secondary RSA. Primary RSA refers to a series of spontaneous abortions without a previous live birth; and secondary RSA refers to a series of spontaneous abortions ensuing a live birth or stillbirth. Between 1 and 5% of all women are affected by RSA during childbearing years (Kuon et al., 2012). The risk of subsequent spontaneous abortion in women who have experienced RSA also increases with the number of previous spontaneous abortions. This risk can reach as high as 50% after two pregnancy losses. Clinically, it is crucial to prevent RSA by certain interventions when possible (Xiao and Zhao, 2014). Various factors contribute to the occurrence of RSA, including chromosome abnormalities, genital anatomic abnormality, endocrine disturbances, autoimmune disturbance, infectious disease and pro-thrombophilic status. Some patients, however, experience unexplained RSA, which may be related to alloimmunity (Cohen and Bischof, 2007), including elevated compatibility in human leukocyte antigen (HLA), phenotypic changes in immune cell subsets, Th1/Th2 cytokine imbalance, and deficiencies in generation of blocking (protective) antibodies (Lin, 2003).

Immunological disturbances were reported to play an important role in RSA. High levels of natural killer cell subsets, autoantibodies and inflammatory cytokines were found in the peripheral blood of patients who had experienced RSA. Activated leukocyte and certain specific natural killer cells that were beyond normal level were also found in the decidua of these patients (Hill et al., 1995; Kruse et al., 2004; Quack et al., 2001; Xu et al., 1990). Further evidence indicated that secondary RSA showed worse immune rejection than that of primary RSA, particularly in immunological disturbances of white blood cell heterogeneity. Additionally, gene expressions of *HLA-DR3* and *HLA-G* were higher in secondary RSA compared with primary RSA and control groups (Christiansen et al., 2012). A previous study also showed that the risk of RSA was higher in those with a firstborn boy compared with a firstborn girl, owing to the reaction of male-specific (H-Y) antigens to the immune system (Nielsen et al., 2010).

In the late 1980s, passive immunization with intravenous immunoglobulin (IVIG) was used to treat RSA, and was reported to have beneficial effects. The underlying mechanism might be associated with neutralization of autoantibodies in the circulatory system, inhibition of natural killer cell, attenuation of complement-mediated cytotoxicity and release of regulatory T lymphocytes (Schwab and Nimmerjahn, 2013). Today, unexplained RSA is commonly treated off-label with IVIG in a clinical setting (Hutton et al., 2007). Immunoglobulin is extracted from normal blood donors, and therefore might pose some potential risks to receivers including allergies and infectious diseases, such as HIV, hepatitis and prions. Although, IVIG is generally well tolerated, common adverse reactions (headaches, myalgia, fevers, chills, dizziness, nausea, and vomiting) occur in less than 5% of the patients. In addition, IVIG therapy does not increase the risk of premature birth (Schwab and Nimmerjahn, 2013).

The effectiveness of IVIG treatment on unexplained RSA has been controversial. Coulam et al. (1995) and Christiansen et al. (1995) both suggested that passive immunization with IVIG improved or showed trend of improving the live birth rate in women who had experienced RSA women relative to treatment with placebo. Randomized controlled trials also demonstrated otherwise (Christiansen et al., 2002; Group, 1994; Jablonowska et al., 1999; Perino et al., 1997; Stephenson et al., 1998, 2010). Recently, diverging results suggest that, although the effect of IVIG treatment observed in patients with primary RSA is not beneficial, women with secondary RSA have a higher live birth rate (Christiansen, 2014; Christiansen and Nielsen, 2005; Christiansen et al., 1995, 2002; Hutton et al., 2007; Stephenson et al., 1998). These observations, however, need to be confirmed, as each study was limited by the relatively low number of participants.

Therefore, our present study aimed to review the currently available randomized controlled trials to determine the effectiveness of IVIG in improving the chance of live birth in unexplained RSA patients, which included studies on the Chinese population, and to further identify the efficacy of IVIG in different subgroups.

Materials and methods

Inclusion criteria and participants

Primary RSA is defined as two or more spontaneous abortions occuring before the 20th gestational week of pregnancy without a history of live birth. Secondary RSA is defined as three or more spontaneous abortions occuring before the 20th gestational week of pregnancy subsequent to a live birth or stillbirth. Women at any age who have the following conditions were excluded: chromosomal abnormality in either couple, chromosomal abnormality in abortion specimen, abnormality in family genetic histories, maternal reproductive tract abnormalities, uterine malformations, maternal endocrine abnormalities, acquired or inherited thrombophilia, environmental factors and other unexplained recurrent abortions.

Intervention

The treatment group received IVIG before pregnancy or during the first trimester of pregnancy. The control group received a placebo.

Studies outcomes

The primary outcomes are the live birth rates, the number of achieved pregnancies and the number of live births.

Types of studies

Studies were selected from both Chinese and English languages. Publications with randomized, randomized-controlled,

or randomized-allocated trials were included irrespective of the blinding methods.

Exclusion criteria

The exclusion criteria were as follows: studies that did not report clear diagnosis and criteria of inclusion and exclusion on participants' selection; studies that were involved in other treatments beyond IVIG; studies that reported inaccurate or incomplete data and were unable to provide outcomes; and studies that were duplicated.

Literature search

Electronic databases

The relevant published literatures searches were conducted in the following databases: *PubMed* (1966–2016), *EMBASE* (1980–2016), *ScienceDirect* (1980–2016), *MEDLINE* (1980–2016), *OVID* (1980–2016), Chinese journals full-text database (*CNKI*) (1980–2016), VIP Chinese scientific and technical journals database (*VIP*), 1989–2016), and articles electronic journals database (1998–2016). The last update was May 2016.

Keywords searches

'Abortion', 'habitual', 'habitual hbortion', 'recurrent abortion', 'miscarriage', 'recurrent', 'abortion', 'immunoglobulins', 'intravenous', 'IVIG', 'passive immunity', 'allocation', 'random', 'randomization', 'controlled clinical trials', 'randomized', 'clinical trials', 'trials', 'randomized clinical'.

PubMed search strategy

(((((((randomization) OR allocation, random) OR trials, randomized clinical) OR clinical trials, randomized) OR controlled clinical trials, randomized)) AND (((((('immunoglobulins, intravenous'[Mesh]) OR IVIG) OR passive immunity) OR 'immunoglobulins'[Mesh]) OR immunoglobulin)) AND ((('abortion, habitual'[Mesh]) OR habitual abortion) OR (((miscarriage, recurrent) OR recurrent abortion) OR abortion, recurrent)).

Selection of studies

Articles were imported into EndNote X4 to record important information and the most complete and best bibliographies were saved. Articles were evaluated on the basis of volume, issue, completeness in abstracts and if the articles were accessible. Articles were screened by reading the full-text. Eligible articles were labelled 'to be included' in the notes column of EndNote X4 whereas others were labelled as 'to be determined' or 'to be excluded (with reasons specified)'. Pending literatures were further evaluated by checking references.

Quality assessment of included studies

The quality of each included study was evaluated on the basis of the randomized controlled trail risk of bias assessment recommended by Cochrane Centre (Cochrane handbook version 5.1.0). Evaluation of the entries included the following seven aspects: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting and other bias. Each entry was then classified as low risk, unclear risk, or high risk.

Data extraction

The data extraction form was self-designed. The following information was recorded: first author, publication year, inclusion criteria of participants, types of spontaneous abortion, therapeutic strategy, baseline compatibility, intervention in treatment and placebo groups, sample size of intervention and outcomes in both treatment and placebo groups. Two reviewers worked independently on literature screening, quality assessment and data extraction. Disagreement was discussed for consensus or determined by the assistance of a third reviewer.

Primary outcome measurement

Live birth rate (number of live births/number of achieved pregnancies).

Statistical analysis

Binary data outcomes were calculated using risk ratio and 95% confidence interval through Cochrane RevMan 5.2 software provided by Cochrane Centre.

The chi-squared method was used to assess heterogeneity, and the degree of heterogeneity was assessed with I^2 statistic. If no significant difference in heterogeneity was detected between groups ($P > 0.1$, $I^2 \leq 50\%$), fixed-effect model was used for analysis. If a significant difference in heterogeneity was detected between groups ($P \leq 0.1$, $I^2 > 50\%$), subgroups of interventional strategy was used to analyse the source of clinical heterogeneity. Random-effect model was used for combined analysis when sources of heterogeneity were uncertain.

Cumulative meta-analysis was performed using STATA 13.1 (StataCorp LP, USA). Outcomes were accrued according to the year of publication. The cumulative effect size and trends were analysed to evaluate the effectiveness of IVIG, to assess the effect of an individual study on the overall effect size, and to determine whether additional studies are required.

Trial sequential analysis (TSA) was carried outo to minimize the risk of false positive errors (type I errors) produced by random errors due to sparse data and repetitive testing in meta-analysis. The required information size refers to the required number of participants to produce statistically significant result in meta-analysis. We estimated a diversity-adjusted required information size in accordance with the diversity in the intervention effect estimates among the included trials. Diversity-adjusted required information size was estimated using two-sided $\alpha v = 5\%$, $\beta = 20\%$, the control event proportions calculated from the placebo group, and the relative risk reduction of 20% in outcomes. Although meta-analysis aims to detect the efficacy of an intervention as early

as possible, TSA with monitoring boundaries are used to decide whether trials should be terminated early to prevent wastage of medical and research resources.

Sensitivity analysis was carried out by removing one study at a time to determine if the effect size were dependent on a certain single study.

If more than 10 studies were included, a funnel plot was used to assess for publication bias. An asymmetry indicated existence of publication bias. Harbord's modified and Egger's linear regression tests were further used to assess funnel plot asymmetry. $P < 0.05$ was considered to be significant.

Results

Study selection

A preliminary search yielded 175 relevant citations, with 23 citations eliminated because they included duplicated studies. A total of 119 citations did not meet the screening criteria and were excluded. The remaining 33 articles were retrieved for detailed evaluation and, of these, 22 articles were excluded for the following reasons: not a randomized controlled study ($n = 4$), did not fulfill inclusion criteria ($n = 7$), did not meet intervention strategy ($n = 2$), control group did not meet intervention strategy ($n = 5$) and insufficient data report ($n = 4$). Only 11 articles met the inclusion criteria and were included in this meta-analysis (**Figure 1**) (Christiansen, 2014; Christiansen et al., 1995, 2002; Coulam et al., 1995; Group, 1994; Jablonowska et al., 1999; Lin and Li, 2015; Liu and Chen, 2010; Perino et al., 1997; Stephenson et al., 1998).

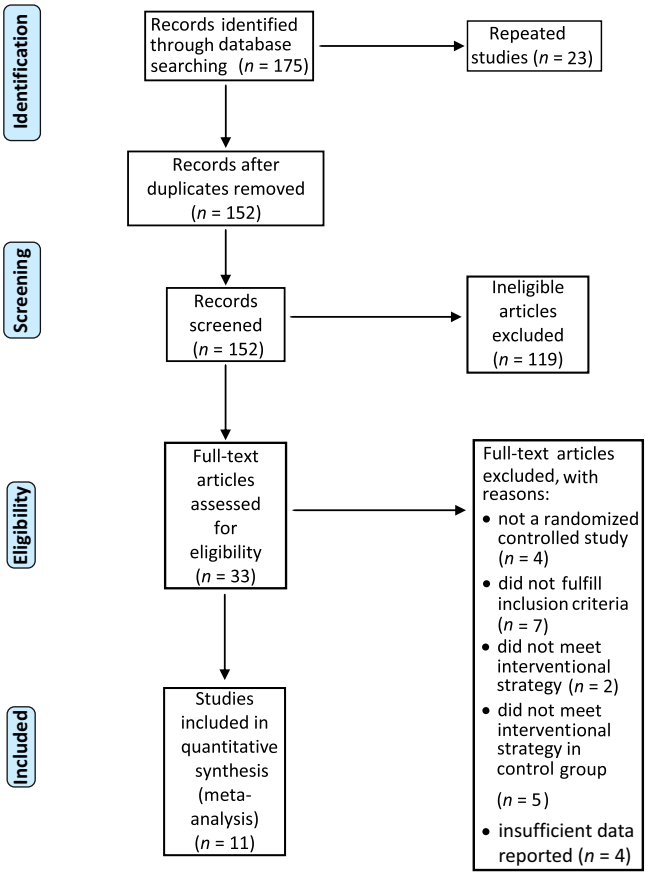

Figure 1 Article selection.

Quality assessment

The methodology of each included trial was evaluated using the risk of bias assessment tool. Overall, the 11 citations included trials that were considered as low-risk studies. Six studies were classified as low risk whereas the other five had uncertain risk of biases in the following aspects: random sequence generation, allocation concealment, blinding of participants and intervention providers, and other source of bias. The quality assessment for each study is shown in **Table 1**, **Figure 2** and **Figure 3**.

Characteristics of included studies

The characteristics of the 11 included studies are presented in **Table 2**. These studies were reported between 1994 and 2015. All of the studies recruited women who had experienced at least two or more spontaneous abortions. A total of 582 patients achieved pregnancy; 297 patients were in the IVIG treatment group for RSA and 285 patients were in the placebo group. A total of 202 live births occurred in the IVIG group and 151 in placebo groups. In the primary RSA subgroup, 57 out of 93 women treated with IVIG and 63 out of 90 women treated with placebo achieved a live birth. Meanwhile in the secondary RSA subgroup, of the 112 women treated with IVIG, 69 achieved a live birth and of the 108 treated with placebo, 53 achieved a live birth. All of the studies were randomized and double-blinded. The age of participants, dosage and timing of IVIG administration, however, as well as the placebo used varied among studies.

Data analysis of IVIG passive immunotherapy on unexplained RSA

Comparison of live birth rate between IVIG-treated and placebo groups

As shown in **Figure 4**, there was strong heterogeneity between the studies ($P = 0.003$, $I^2 = 62\%$). Therefore, a random-effects model was used to produce an overall summary. Our random-effects analyses showed that the difference in the live birth rates between IVIG treatment and placebo groups was on the margin of significance (RR = 1.25, 95% CI 1.00 to 1.56; $P = 0.05$) (**Figure 4**). Our cumulative meta-analysis showed that, after the inclusion of studies, the point estimates for relative risk and the confidence interval became stable, with a trend favouring the treatment of IVIG (**Figure 5**). We noted a marginal significance (RR = 1.25, 95% CI 1.00 to 1.56; $P = 0.05$) after the inclusion of Lin and Li (2015) study. The clinical trial conducted by Lin et al. was comprised of a small sample size and therefore had little effect on the overall effect size. This significant observation after the inclusion of Lin et al. study is therefore not sufficiently convincing. Further trials are required to validate this observation. Our TSA showed that

Table 1 Quality assessment of each included study.

Author	*Random sequence generation*	*Allocation concealment*	*Blinding of participants and personnel*	*Blinding of outcome assessment*	*Incomplete outcome data*	*Selective reporting*	*Other bias*
German RSA/IVIG (Group, 1994)	Low risk (random digital packet by computer)	Unclear risk (not described)	Low risk (blinding of participants and obstetricians)	Low risk	Low risk	Low risk	Unclear risk (insufficient information on baseline compatibility; no evidence on sample size estimation)
Coulam et al. (1995)	Low risk (random digital packet by computer)	Unclear risk (not described)	Low risk (blinding of participants and obstetricians)	Low risk	Low risk	Low risk	Low risk
Christiansen et al. (1995)	Low risk (random digital packet by computer)	Low risk (digital encoding)	Low risk (blinding of participants and obstetricians)	Low risk	Low risk	Low risk	Low risk
Perino et al. (1997)	Low risk (random digital packet by computer)	Low risk (digital encoding)	Low risk (double blind)	Low risk	Low risk	Low risk	Unclear risk (no evidence on sample) size estimation)
Stephenson et al. (1998)	Low risk	Low risk (digital encoding)	Low risk (blinding of participants, obstetricians, and personnel)	Low risk	Low risk	Low risk	Low risk
Jablonowska et al. (1999)	Low risk (centralized distribution)	Low risk (opaque sealed envelope method)	Low risk (blinding of participants and Obstetricians)	Low risk	Low risk	Low risk	Low risk
Christiansen et al. (2002)	Low risk (random digital packet by computer)	Low risk (digital encoding)	Low risk (blinding of participants and obstetricians)	Low risk	Low risk	Low risk	Low risk
Stephenson et al. (2010)	Low risk (random digital packet by computer)	Low risk	Low risk (blinding of participants, Obstetricians, and personnel)	Low risk	Low risk	Low risk	Low risk
Christiansen (2014)	Low risk (random digital packet by computer)	Low risk (digital encoding)	Low risk (blinding of participants and obstetricians)	Low risk	Low risk	Low risk	Low risk
Liu and Chen (2010)	Unclear risk (randomization not described)	Unclear risk (not described)	Unclear risk (not described)	Low risk	Low risk	Low risk	Unclear risk (insufficient information on baseline compatibility, no evidence on sample size estimation)
Lin and Li (2015)	Unclear risk (randomization not described)	Unclear risk (not described)	Unclear risk (not described)	Low risk	Low risk	Low risk	Unclear risk (no evidence on sample size estimation)

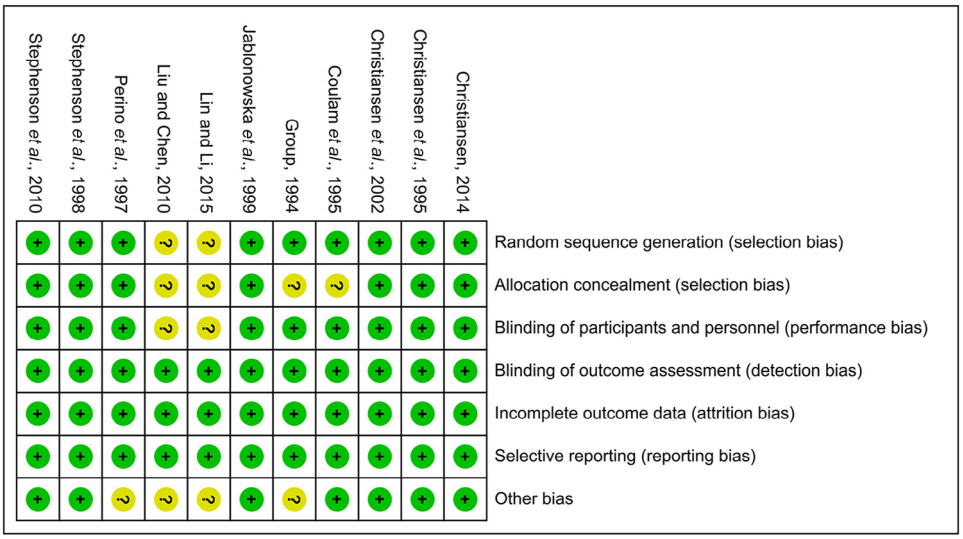

Figure 2 Risk of bias assessment summary for each included study. + = low risk of bias; ? = unclear risk of bias.

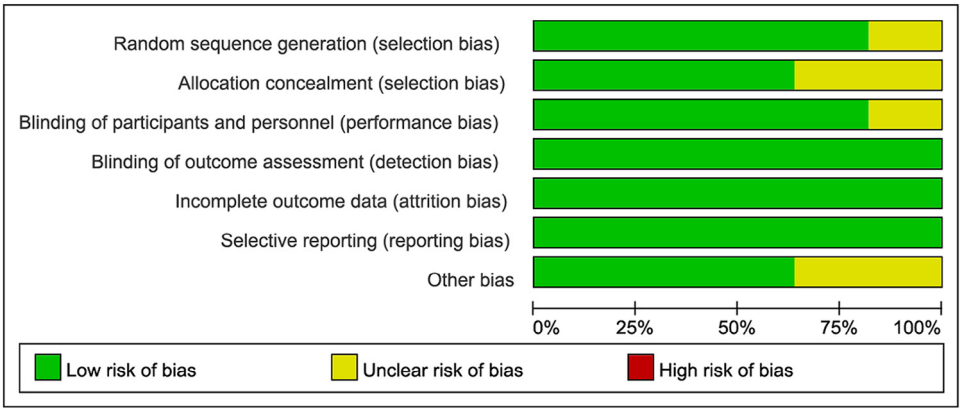

Figure 3 Risk of bias assessment for all included studies.

the cumulative Z-curve was marginally at the conventional boundary for benefit but had not crossed the trial sequential monitoring boundary for benefit. The number of participants had not reached the required information size, suggesting that the conventional meta-analysis might yield false positive conclusion (**Figure 6**). More trials are required to conclude the effectiveness of IVIG.

Analysis of RSA subgroups

RSA can be divided into primary and secondary RSA. Five studies were identified that enrolled patients with primary RSA (Christiansen et al., 2002; Group, 1994; Jablonowska et al., 1999; Perino et al., 1997; Stephenson et al., 1998). Among the 93 RSA patients in the IVIG treatment group who became pregnant, 57 patients successfully delivered live birth, whereas the placebo group showed that 63 out of 90 RSA patients successfully delivered live birth. Meta-analysis showed little heterogeneity between studies (I^2 = 0%). Fixed-effect meta-analysis showed no significant difference in the live birth rate between the IVIG treatment and placebo groups (RR = 0.88, 95% CI 0.71 to 1.07). On the other hand, six studies involved secondary RSA (Christiansen, 2014; Christiansen et al., 1995, 2002; Stephenson et al., 1998, 2010). A total of 112 RSA patients in the IVIG group were pregnant and 69 of them successfully delivered live birth. In the placebo group, a total of 108 pregnancies occurred, in which 53 patients successfully delivered live birth. Meta-analysis showed little heterogeneity between placebo and treatment groups (I^2 = 0%) and fixed-effect analyses showed marginal insignificant difference in the live birth rates between the two groups (RR = 1.26, 95% CI 0.99 to 1.61) (**Figure 7**). Further cumulative and TSA meta-analyses were carried out on the secondary RSA subgroup. Cumulative meta-analysis showed that after inclusion of studies, the point estimates for RR and the CI became stable, and no significant difference was observed in the live birth rate between IVIG treatment and placebo groups (**Figure 8**). The TSA analysis showed that the cumulative Z-curve was marginally at the conventional boundary for benefit and had not reached the trial sequential monitoring boundary for benefit (**Figure 9**). The number of participants had not reached the required information size. Our data suggest that the clinical benefit of IVIG is not significant. More

Table 2 Details of trials and pregnancy outcomes comparing intravenous immunoglobulin treatment and placebo for women who have experienced recurrent spontaneous abotion.

Included studies	*Participants*	*Pregnancy confirmation*	*Therapeutic strategy*
German RSA/IVIG 1994 (Group, 1994)	Women aged <40 years who had three or more spontaneous abortions at <16 weeks of gestation and no live children.	Positive beta-HCG test (≥75 IU/l)	30 g of IVIG or 5% albumin (control) initiated at <8 weeks of gestation and 20 g given every 3 weeks until 25 weeks of gestation
Coulam et al. (1995)	Women aged 18–45 years who had two or more consecutive RSA and no other cause found for spontaneous abortion	The presence of embryonic cardiac activity by ultrasonography	500 mg/kg IVIG versus 0.5% albumin given during follicular phase and every 28 days until pregnancy was achieved and then continued until 28–32 weeks of gestation.
Christiansen et al. (1995)	Three or more spontaneous abortions and no more than one live birth or one fetal death after 14 weeks of gestation, and no other cause found for spontaneous abortion.	The presence of embryonic cardiac activity by ultrasonography	IVIG or human albumin given at weeks 5–8, then every other week. IVIG was adjusted according to weight: 60–80 kg: 35 g in week 5–6; 25 g in week 7–26; 30 g in week 28, 30, 32 and 34; >80 kg: 5 g more at each infusion
Perino et al. (1997)	Women aged <42 years who had three or more consecutive RSA with the same partner in the first trimester and had no live birth	An appropriate cranio-caudal length and the presence of embryonic cardiac activity by ultrasonography	IVIG 25 g/day for two consecutive days and 25 g administered 3 weeks later with ultrasound confirmation of viable pregnancy or saline solution with 5% human albumin (control)
Stephenson et al. (1998)	Two or more documented consecutive RSA under 20 weeks of gestation and no other cause found for spontaneous abortion.	Levels of serum beta-HCG and the presence of embryonic cardiac activity by endovaginal ultrasonography.	IVIG 500 mg/kg (or normal saline). Infusions were given initially at a rate of 60 ml/h and gradually increased to 180 ml/h. If conception did not occur within six menstrual cycles, participation ended. With documentation of pregnancy, the participant received the same infusion every 4 weeks until 18 weeks of gestation.
Jablonowska et al. (1999)	Three or more consecutive RSA under 20 weeks of gestation and no other cause found for spontaneous abortion (primary RSA or secondary were included).	The presence of embryonic cardiac activity by ultrasonography.	Women received IVIG (20 g Gammonativ 400 ml) or placebo (saline 400 ml) every 3 weeks on five occasions if a viable pregnancy was confirmed by ultrasound before each treatment. By error, one patient with a successful outcome of pregnancy in the IVIG group received only four infusions.
Christiansen et al. (2002)	Four or more spontaneous abortions at <26 weeks of gestation and no other cause found for spontaneous abortion.	Levels of serum beta-HCG and the presence of embryonic cardiac activity by endovaginal ultrasonography	Weekly IVIG or human albumin given at week 5 until week 10. Infusions were subsequently carried out every second week until the 26th week of gestation. Until the 20th gestational week, a total of 0.8 g of study drug per kg body weight was administered. From gestational week 20 to 26, 1.0 g of study drug per kg body weight was given.

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Table 2 (*continued*)

Included studies	*Participants*	*Pregnancy confirmation*	*Therapeutic strategy*
Stephenson et al. (2010)	Women aged 18–45 years who had experiencedthree or more idiopathic secondary spontaneous abortions with their present partner and in whom the most recent conception took place less than 1 year ago	Levels of serum berta-HCG and the presence of embryonic cardiac activity by ultrasonography	IVIG 500 mg/kg (or normal saline). Participants were administered 14–21 days from the projected next menstrual period. The infusion rate was 60 ml/h for the first hour, then increased to a maximum of 180 ml/h. If conception did not occur within six menstrual cycles, participation ended. With documentation of pregnancy, the participant received the same infusion every 4 weeks until 18–20 weeks of gestation, with adjustment for weight based on her prior visit.
Christiansen (2014)	Women aged 18–45 years who had four or more confirmed spontaneous abortions before gestational week 14 and at least one birth occurring after gestational week 28; ≥3 of the spotnaneous abortions should be consecutive after the last birth and be fathered by the present partner.	Levels of serum beta-HCG and the presence of embryonic cardiac activity by ultrasonography	A total of eight IVIG or human albumin was given until gestational weeks 14–15. Infusions were given over 3–4 h on an outpatient basis. The second infusion was given 3–6 days after the first and subsequently three infusions were given at intervals of 6–8 days and three infusions at intervals of 12–16 days. The normal active drug was immunoglobulin human CSL Behring 120 mg/ml (number 41, Privigen CSL Behring 100 mg/ml). At each infusion, participants weighing <75 kg before pregnancy were given 24 g (25 g), and for those weighing ≥75 kg, 36 g (35 g). In the placebo group, 200 ml or 300 ml of human albumin CSL Behring 5% was given at each infusion according to weight respectively.
Liu and Chen (2010)	Three or or more RSA	The presence of embryonic cardiac activity by ultrasonography	Administered IVIG gamma type before pregnancy with 20 g every 3 weeks on four occasions. Then participants were infused with same treatments twice after pregnancy. Prevention of spontaneous abortion was given once a visible pregnancy was confirmed.
Lin and Li (2015)	Three or more RSA with the same partner	The presence of embryonic cardiac activity by ultrasonography	Once pregnant, participants received 5 g of gamma albumin (2.5 g/vial by Yuhualan pharmaceutical) per day for 7 days or 10 g/day for 3 days. The therapeutic strategy was given every 3 weeks until 12 weeks of gestation.

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Table 2 (*continued*)

Included studies	*Treatment group*			*Control group*			*Study design*
	Intervention	*Number of live births*	*Number of achieved pregnancies*	*Intervention*	*Number of live births*	*Number of achieved pregnancies*	
German RSA/IVIG 1994 (Group, 1994)	IVIG	Primary RSA 20	33	5% albumin	Primary RSA 21	31	Multi-centre; randomized; double blind; centralized distribution
Coulam et al. (1995)	IVIG	Total RSA 18	29	0.5% albumin	Total RSA 11	32	Multi-centre; Randomized; double blind
Christiansen et al. (1995)	IVIG	Secondary RSA 9 Recurrent late spontaneous abortions	14 3	Human albumin	Secondary RSA 2 Recurrent late spontaneous abortions 3	10 7	Multi-centre; randomized; double blind
Perino et al. (1997)	IVIG	Primary RSA 16	22	5% human albumin	Primary RSA 20	24	Multi-centre;randomized, double blind, centralized distribution
Stephenson et al. (1998)	IVIG	Primary RSA 5 Secondary RSA 7	10 10	Normal saline	Primary RSA 4 Secondary RSA 6	10 11	Randomized; double blind; centralized distribution
Jablonowska et al. (1999)	IVIG	Primary RSA 9 Secondary RSA 8	11 11	Normal saline	Primary RSA 8 Secondary RSA 7	9 10	Multi-centre; randomized; double blind
Christiansen et al. (2002)	IVIG	Primary RSA 7 Secondary RSA 6	17 12	Human albumin	Primary RSA 10 Secondary RSA 3	16 13	Randomized; double blind
Stephenson et al. (2010)	IVIG	Secondary RSA 16	23	Normal saline	Secondary RSA 15	24	Multi-centre, randomized; double blind
Christiansen (2014)	IVIG	Secondary RSA 23	42	Human albumin	Secondary RSA 20	40	Multi-centre; randomized; double blind
Liu and Chen (2010)	IVIG	Total RSA 34	35	HCG	Total RSA 11	29	–
Lin and Li (2015)	IVIG	Total RSA 24	25	Regular spontaneous abortion prevention	Total RSA 10	19	–

Total RSA: a series of two or more spontaneous abortions before 20th gestational week of pregnancy with the same partner, including primary and secondary RSA.
Primary RSA: a series of two or more spontaneous abortions before 20th gestational week of pregnancy without a history of live birth.
Secondary RSA: aseries of three or more spontaneous abortions before 20th gestational week of pregnancy subsequent to a live birth or stillbirth.
Recurrent late spontaneous abortions: ≥1 second trimester sponaneous abortion.
IVIG, intravenous immunoglobulin; RSA, recurrent spontaneous abortion.

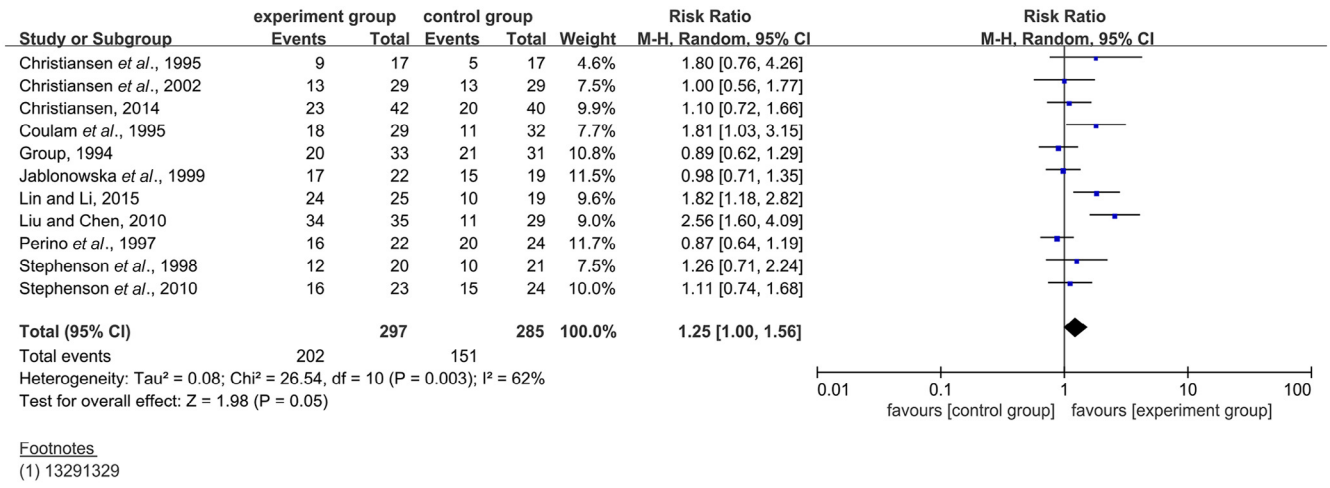

Figure 4 Live birth rate between intravenous immunoglobulin treatment and placebo groups in patients who have experienced recurrent spontaneous abortion.

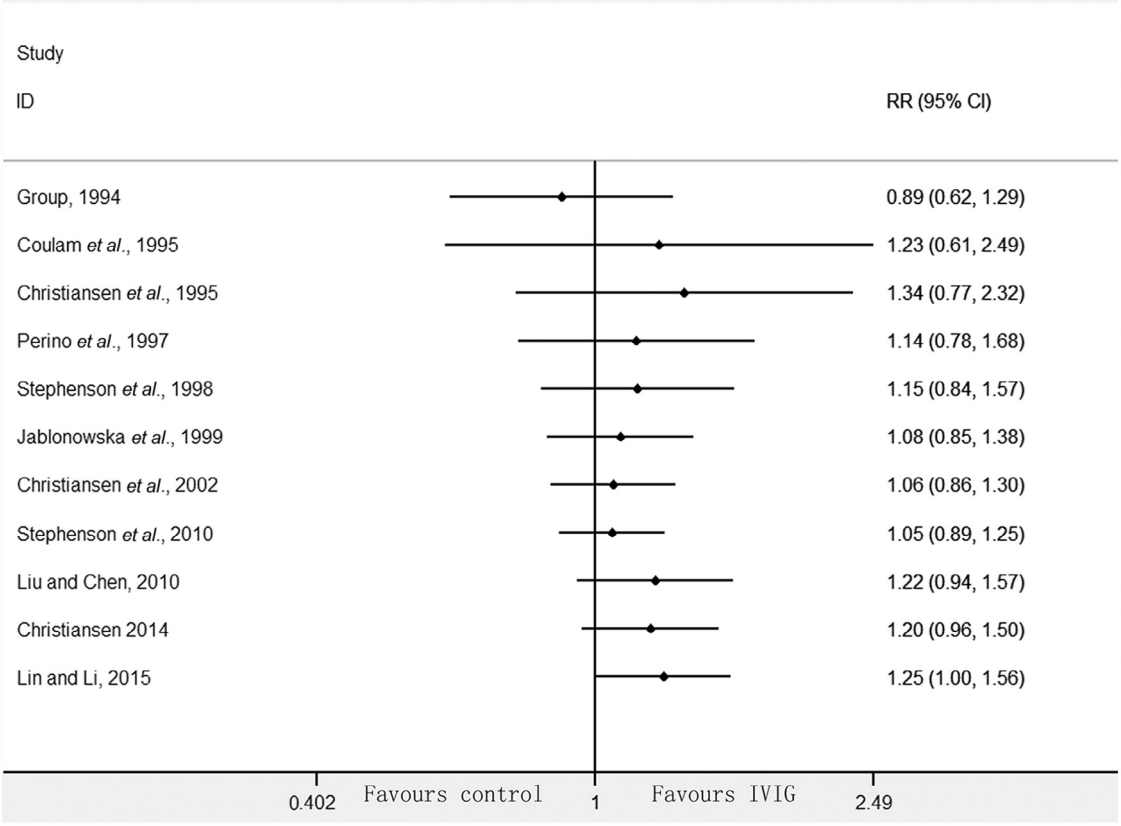

Figure 5 Cumulative meta-analysis of randomized trials comparing intravenous immunoglobulin with placebo in patientw who have experienced unexplained recurrent spontaneous abortion. IVIG, intravenous immumoglobulin; RR, relative risk.

studies are required to validate the efficacy of IVIG in secondary RSA patients.

Analysis of IVIG administration timing

The effect of IVIG administered before conception or after implantation on the live birth rate was also analysed. Four studies were identified (Coulam et al., 1995; Liu and Chen, 2010; Stephenson et al., 1998, 2010), with a total of 213 women who had experienced RSA who started treatment with either IVIG or placebo before conception and seven studies (Christiansen, 2014; Christiansen et al., 1995, 2002; Group, 1994; Jablonowska et al., 1999; Lin and Li, 2015; Perino et al., 1997) with 369 RSA patients treated with IVIG or placebo after confirmation of pregnancy. Meta-analysis for administration of IVIG before conception showed a significant difference in the live birth rate between IVIG treatment and placebo groups

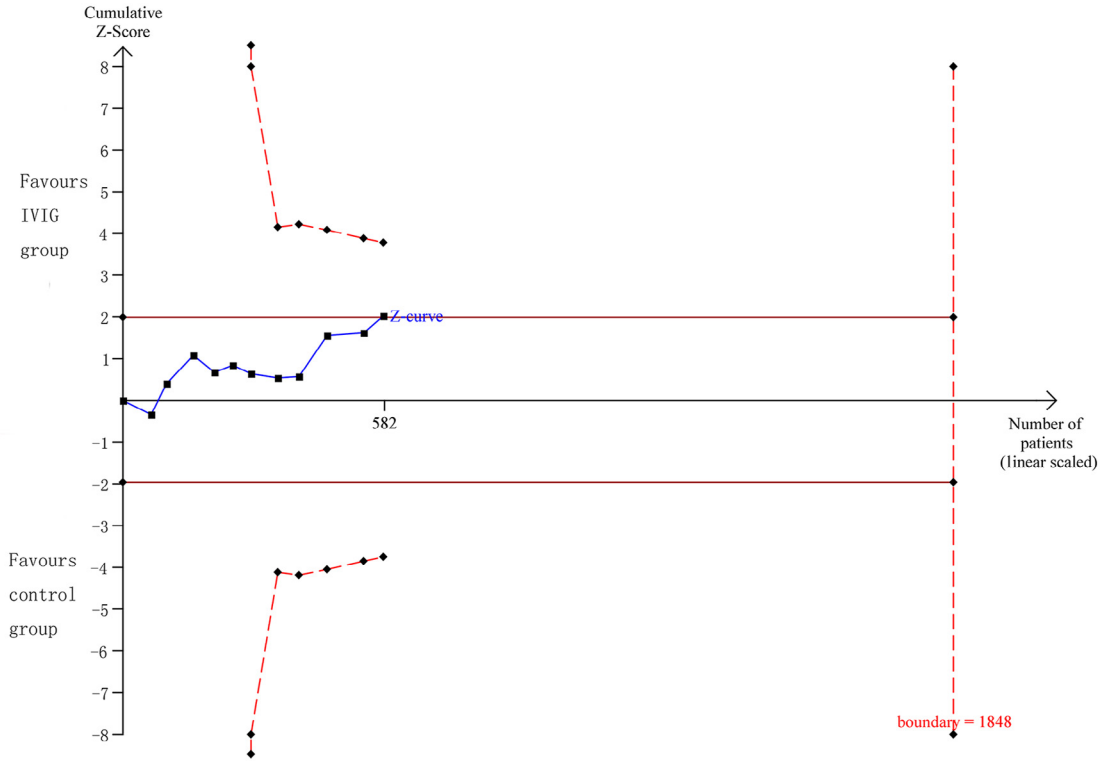

Figure 6 Trial sequential analysis of all included studies on the effect of intravenous immunoglobulin on live birth rate in unexplained recurrent spontaneous abortion patients. A diversity-adjusted required information size of 1848 patients was calculated on the basis of a live birth rate of 52.98% in the control group, relative risk reduction of 20%, $\alpha = 5\%$ (two-sided), and $\beta = 20\%$. The cumulative Z-curve did not cross the trial sequential monitoring boundaries for benefits, harms, or futility, and the required information size was not reached.

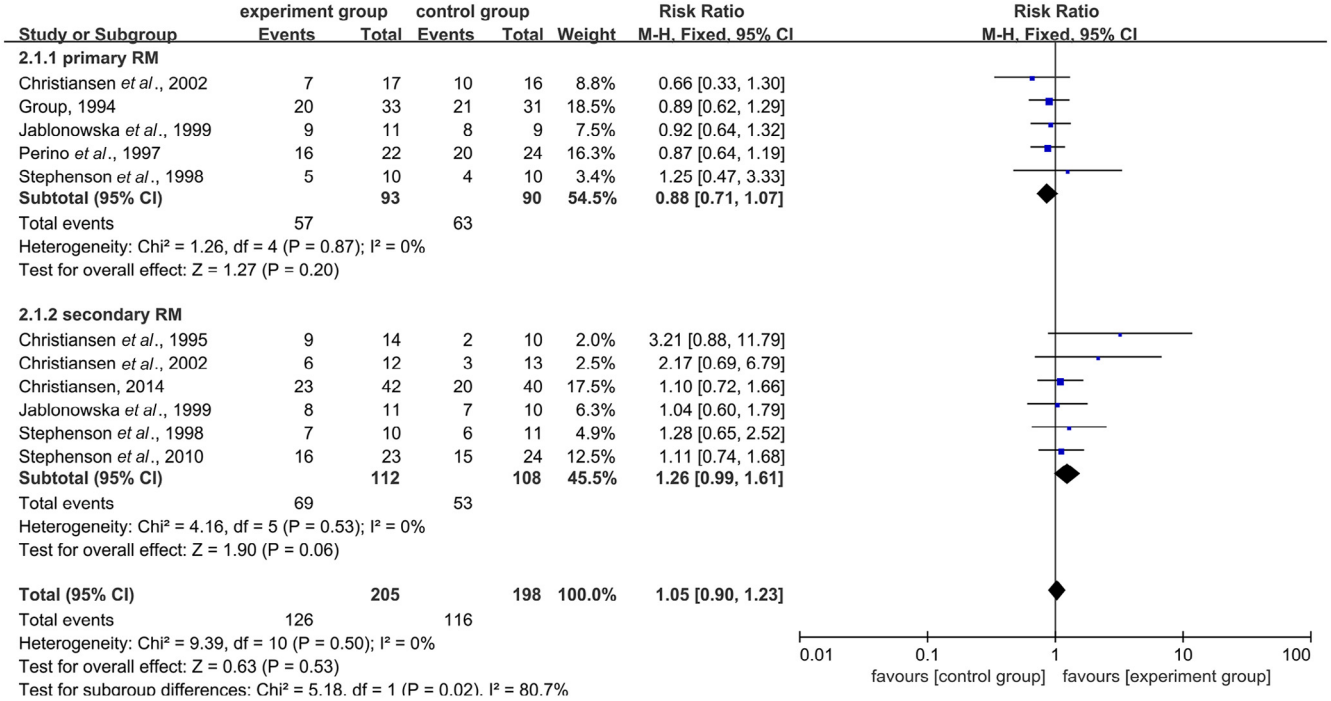

Figure 7 Comparison of primary and secondary recurrent spontaneous abortion in intravenous immunoglobulin treated and placebo groups.

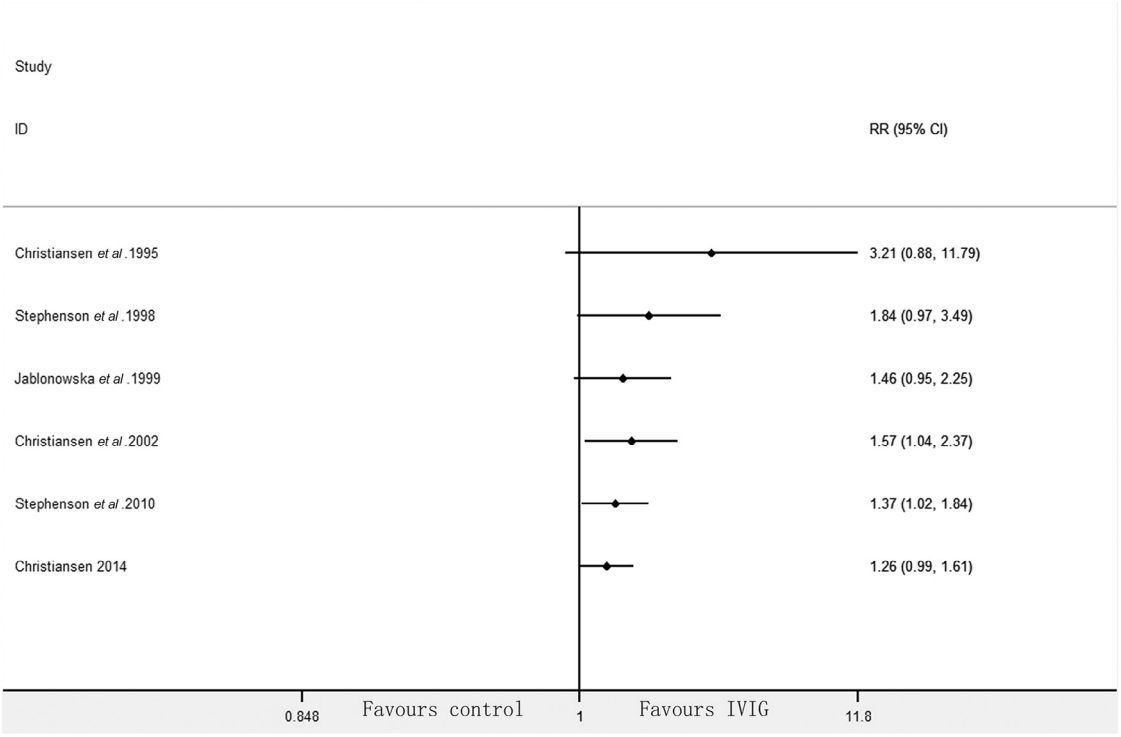

Figure 8 Cumulative meta-analysis of randomized trials comparing intravenous immunoglobulin with placebo in secondary recurrent spontaneous abortion patients.

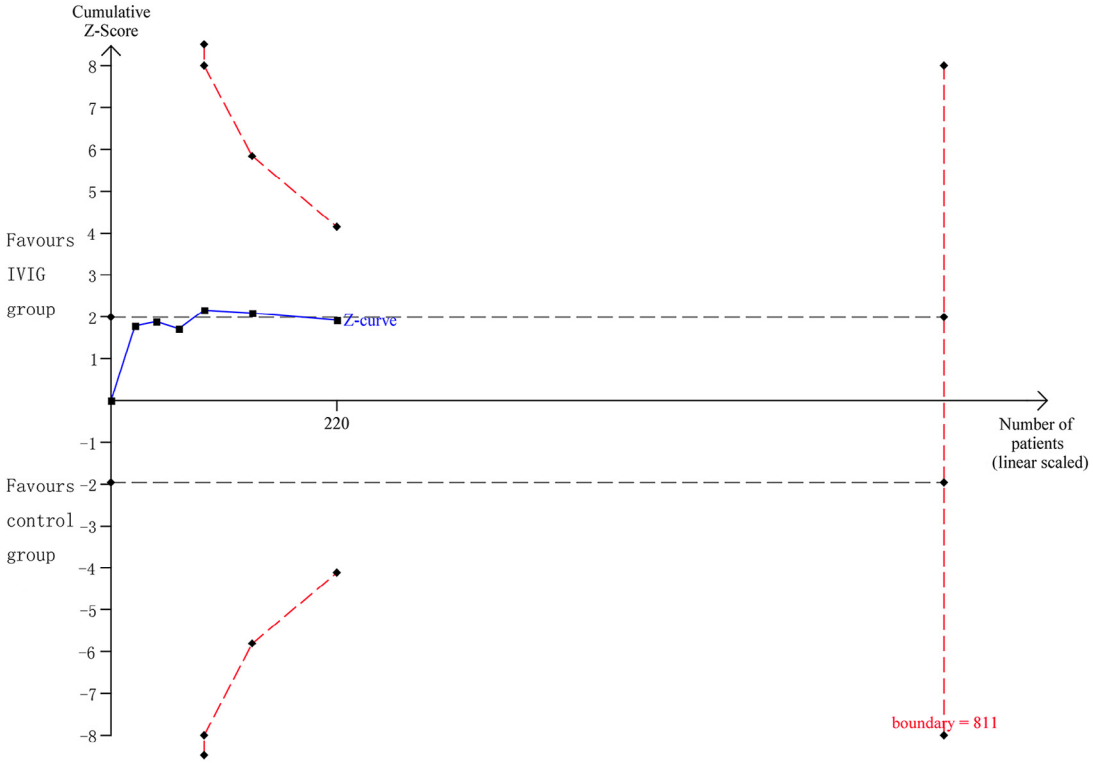

Figure 9 Trial sequential analysis of the effect of intravenous immunoglobulin on live birth rate in secondary recurrent spontaneous abortion patients. A diversity-adjusted required information size of 811 patients was calculated on the basis of a live birth rate of 58.59% in the control group, relative risk reduction of 20%, $\alpha = 5\%$ (two-sided), and $\beta = 20\%$. The cumulative Z-curve did not cross the trial sequential monitoring boundaries for benefits, harms, or futility, and the required information size was not reached. IVIG, intravenous immunoglobulin.

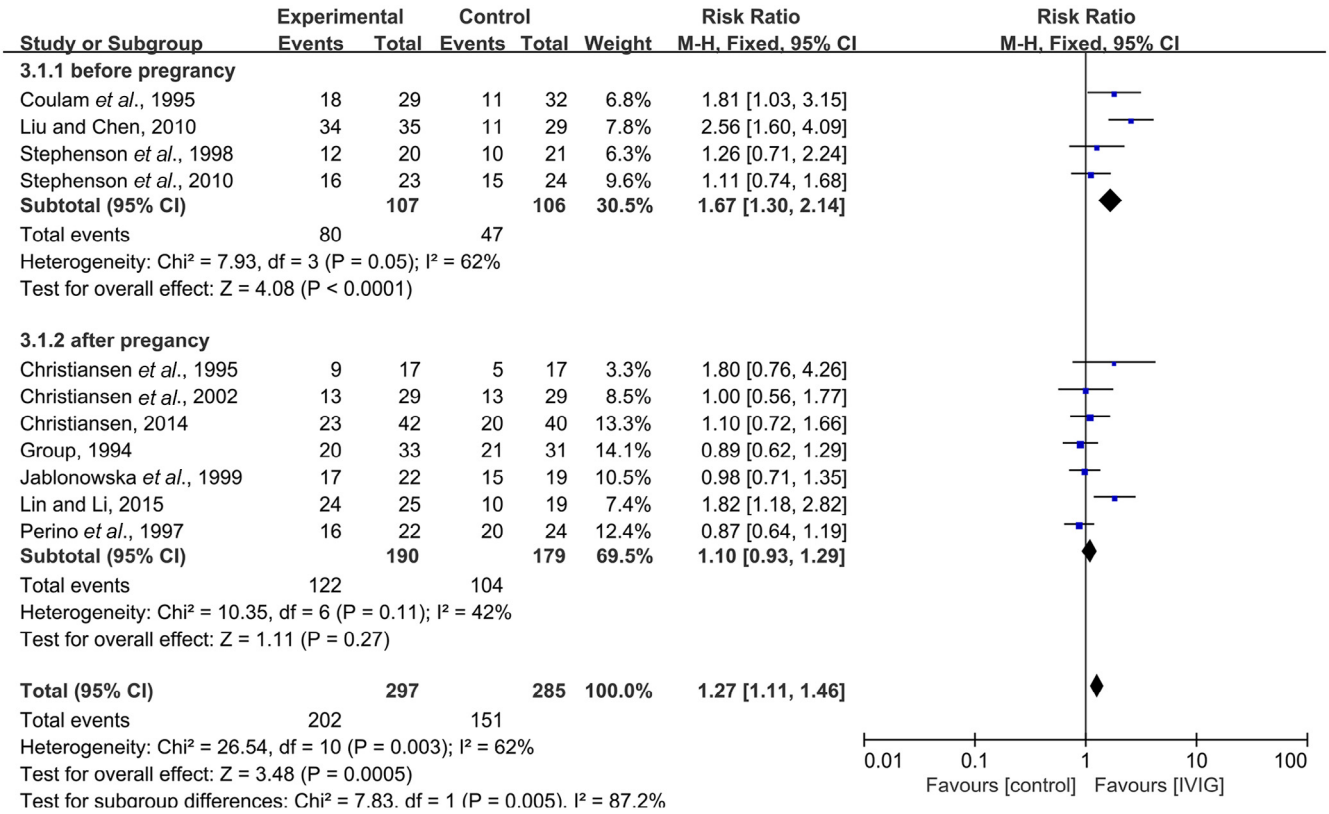

Figure 10 Comparison of live birth rate between pre-conception and post-implantation intravenous immunoglobulin administration. CI, confidence interval.

(RR = 1.67, 95% CI 1.30 to 2.14; $P < 0.0001$). Meta-analysis for administration of IVIG after embryo implantation showed no significant difference in the live birth rate between IVIG treatment and placebo groups (RR = 1.10, 95% CI 0.93 to 1.29 (**Figure 10**). Our data suggest that women with RSA may benefit from IVIG administered before conception.

Sensitivity analysis and publication bias

A sensitivity analysis was conducted to evaluate the robustness of our findings. Each study was removed one at a time and the effect size was recalculated. Our leave-one-out sensitivity analysis showed similar, consistent results, indicating that no individual study significantly affected the overall effect size (**Figure 11**).

A funnel plot was used to check for the existence of publication bias. Our funnel plot showed asymmetry and indicated potential publication bias (**Figure 12**). Accordingly, we carried out Harbord's modified and Egger's linear regression tests, in which no evidence of publication bias were found.

Discussion

Cliically, IVIG has been widely used to treat unexplained RSA, although its efficacy has not yet been clinically proven (Hutton et al., 2007). The high cost for this immunotherapy, its limited supply as well as its potential side-effects call for a guideline for appropriate applications (Hutton et al., 2007). A total of 175 relevant published literatures were searched between 1966 and 2016 in *PubMed*, *EMBASE*, *CNKI*, *VIP*, articles electronic journals database, *ScienceDirect*, *MEDLINE*, and *OVID*. Eleven randomized-controlled studies were included in the meta-analysis after screening (Christiansen, 2014; Christiansen et al., 1995, 2002; Coulam et al., 1995; Group, 1994; Jablonowska et al., 1999; Lin and Li, 2015; Liu and Chen, 2010; Perino et al., 1997; Stephenson et al., 1998, 2010). The 11 included studies were high-quality trials with low risk of biases. Of the 582 women who achieved pregnancy, 353 had live births. Our analysis showed that IVIG might be of beneficial value to treat unexplained RSA. Cumulative and TSA indicated the need for more clinical trials to validate the effectiveness of IVIG as its therapeutic value is still inconclusive.

As Hutton et al. (2007) demonstrated that IVIG was more effective in secondary RSA than in primary RSA, RSA was divided into primary or secondary RSA for further meta-analysis in this review. From the 11 included studies, five studies enrolled primary RSA patients (Christiansen et al., 2002; Group, 1994; Jablonowska et al., 1999; Perino et al., 1997; Stephenson et al., 1998), and six studies recruited secondary RSA patients (Christiansen, 2014; Christiansen et al., 1995, 2002; Jablonowska et al., 1999; Stephenson et al., 1998, 2010). Following the review by Hutton et al. (2007), our present meta-analysis included additional two high-quality, randomized controlled studies published in 2010 and 2014, composed of large sample sizes (Christiansen, 2014; Stephenson et al., 2010). Our results, however, did not support the clinical benefit of IVIG on secondary RSA patients, which contradicted the conclusion made by Hutton et al. (2007).

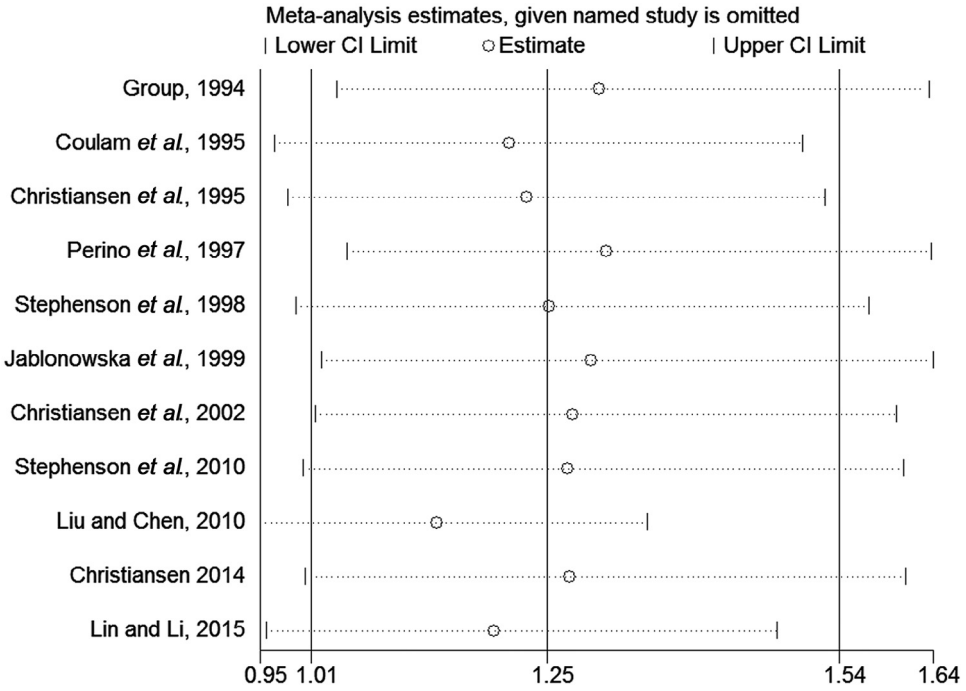

Figure 11 Sensitivity analysis for all included studies.

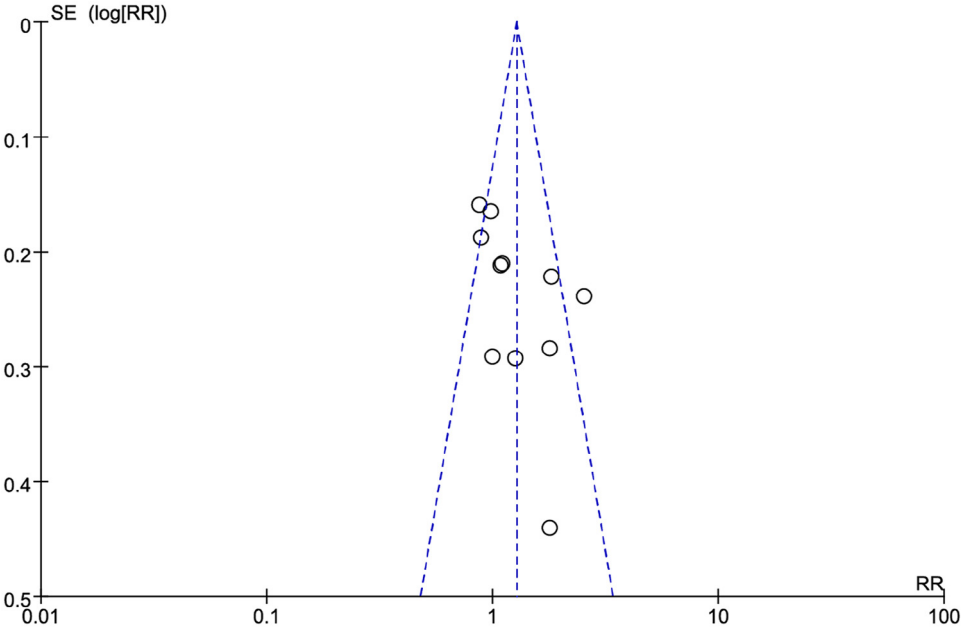

Figure 12 Assessment of publication biases. RR, relative risk; SE, standard error.

Cumulative meta-analysis and TSA analyses indicate that present studies are not conclusive and further trials are warranted.

The present meta-analysis observed heterogeneity among included studies. Leave-one-out sensitivity analysis showed no individual study which significantly affected the overall effect size. The heterogeneity may be associated with the inconsistency of the study design, especially in terms of the selection of participants and control group, interventional strategy, and therapeutic regimen. Age and number of previous spontaneous abortions varied among patient populations. As age increased, fertility declined and the chance for spontaneous abortion also increased (Daya et al., 1999). Daya et al. (1999) demonstrated that the success rate for pregnancy (≥20 weeks of gestation) declined with age and the number of previous spontaneous abortions (Daya et al., 1999). Moreover, the immunological characteristics of an individual could also vary among patients. For instance, high levels

of peripheral natural killer cells have been correlated with poor pregnancy outcomes (Seshadri and Sunkara, 2014). Multiple reports have indicated that the treatment of IVIG suppresses the peripheral levels and activities of natural killer cells (Kwak et al., 1996; Morikawa et al., 2001; Rigal et al., 1994; Roussev et al., 2007; Ruiz et al., 1996; Szereday et al., 1999; Yamada et al., 2015), improving reproductive outcomes in RSA patients (Cohen and Machupalli, 2015; Moraru et al., 2012; Perricone et al., 2006; Ramos-Medina et al., 2014; Shimada et al., 2009; van den Heuvel et al., 2007). Therefore, patients with higher peripheral nautral killer cell levels may benefit from IVIG treatment compared with those with lower levels. These variables were not considered during the recruitment of participants, which might thus affect the outcomes of the studies.

Variation in the intervention method, for example, the timing of IVIG administration, either during pre-conception or post-implantation, might also affect the outcome. Sapir et al. (2005) reported that IVIG administration before conception increased chance of live delivery in primary RSA women, whereas in secondary RSA women, IVIG given after embryo implantation had a higher success rate. Hutton et al. (2007), however, showed that IVIG infusion given to RSA women before ovulation was not as effective compared with those who were given administration after confirmation of pregnancy (Hutton et al., 2007). These diverging reports affecting interventional strategy necessitate an analysis for determining the optimal administration timing. Interestingly, our meta-analysis showed that IVIG treatment given before pregnancy would increase the rate of live birth in RSA patients.

To date, the optimal dose for IVIG administration in RSA women remains unknown (Daya et al., 1999). In most of the published trials, the IVIG doses administered were relatively low compared with autoimmune diseases that could hinder a possible treatment effect (Christiansen et al., 2002). No dose-response study has ever been conducted to identify the optimal dose. The placebo given in each trial also varied and might have potential influence on the outcome. Reports suggested that albumin, which was used as placebo in trials, had been associated with ovarian hormone regulation and therefore might have led to beneficial effects in the patients (Asch et al., 1993).

Over time, we have gained a deeper understanding of RSA and its complications. Recent studies have revealed multiple new contributors in RSA, including genetic polymorphisms, circulating microparticles, glycoproteins, as well as novel immunologic risk factors (Alijotas-Reig and Garrido-Gimenez, 2013). These factors were not known beforehand, and therefore trials as early as 1994 did not consider these factors when recruiting RSA participants. Certain sub-populations with unexplained RSA but not all may benefit from IVIG populations. Limited knowledge of RSA, therefore, hampers the effort to design a well-controlled clinical trial to validate the effectiveness of IVIG.

In addition, double-blinded, randomized trials are difficult to execute. The unwillingness of participants to be randomized to the placebo groups (Silver, 2015), and the invasive nature of the treatment itself, are some of the reasons for the low number of participants in a trial. The high cost of treatment involved also prevents the initiation of clinical studies and limits the recruitment of participants and therefore, hinders the determination of an optimal dose and duration of IVIG. Apart from that, differences in treatment protocol among studies may also generate variations in outcomes. For instance, the conventional treatment protocols include: administration of IVIG at 400–500 mg/kg per day for 1–3 days with an interval of 3–4 weeks, and given until week 26–32; administration of IVIG at low dose (200 mg/kg per day) once in 4 weeks until week 26–32; continuous administration of IVIG at a single high dose (20–30 g/day) after pregnancy for 5 days; administration of IVIG (400–500 mg/kg per day) in combination with heparin (5000 IU subcutaneous, twice a day) and aspirin (75 mg/day, oral) continuously for 1–3 days with an interval of 3–4 months. Therefore, these variations call for standardization of protocol for optimal IVIG administration.

From our 11 high-quality, low-risk, randomized controlled trials, we conclude that, although IVIG treatment does not benefit primary RSA patients, the overall effect of IVIG in RSA patients, or in women with secondary RSA, is still inconclusive. Further high-quality studies with adequate number of participants and rigorous study designs are warranted. Future trials should include efforts to address the following issues which may influence the outcome: trials should be double-blinded and randomized to reduce bias; participants should be controlled in age, history of spontaneous abortion, endocrine status, genetic polymorphisms, and immunological characteristics; to identify the best timing for IVIG administration (pre-conception versus post-implantation); and to ascertain the most effective and optimal dosage for treatment.

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