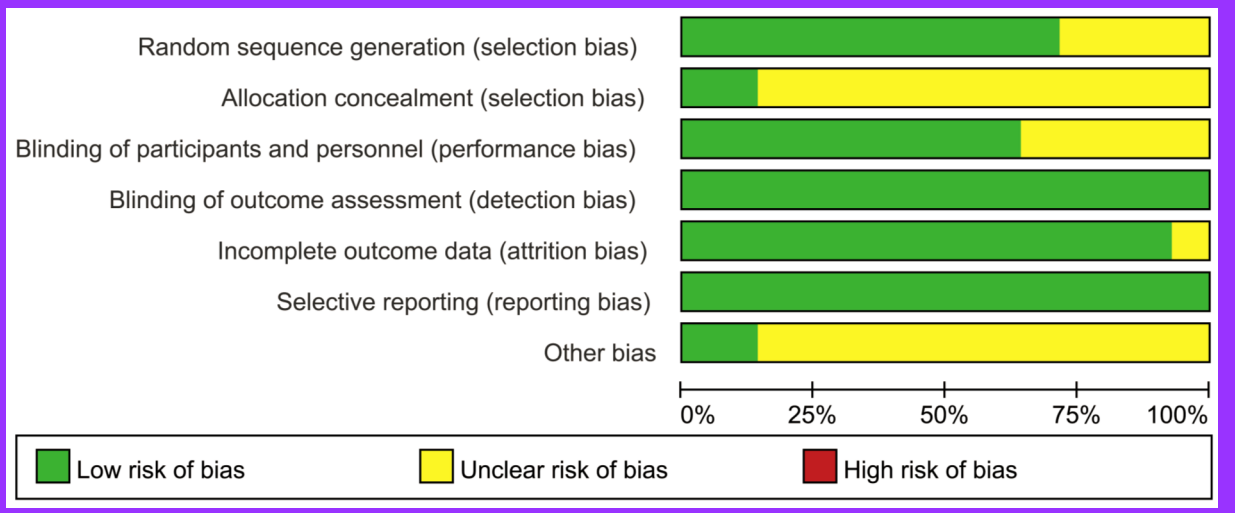

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Meta-analysis

Lymphocyte active immunotherapy for unexplained recurrent spontaneous abortion

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Abstract

This study is to evaluate the curative effect of lymphocyte active immune treatment for unexplained recurrent spontaneous abortion. Fourteen randomized controlled trials of 994 subjects were included. Blocking antibody positive rate of patients who experienced a new pregnancy showed significant differences between immunotherapy and placebo. Moreover, live birth rate among paternal or third-party donor lymphocytes and placebo groups had significant difference [RR= 1.63, 95% CI (1.38-1.92), $p<0.05$]. Trial sequential analysis showed that the Z-curve crosses the trial sequential monitoring boundaries, confirming that the clinical benefit of active immunotherapy was significant. Subgroup analysis suggests that immunotherapy with lymphocytes might be more effective for primary recurrent spontaneous abortion than for secondary recurrent miscarriage. Taken together, women with primary recurrent spontaneous abortion who are negative for blocking antibody prior to treatment seemed most likely to obtain a potential beneficial effect of lymphocyte active immunotherapy, but not secondary recurrent spontaneous abortion.

Introduction

Recurrent spontaneous abortion, generally refers to pregnancy loss not less than twice or birth weight less than 500 g before 20th gestational week with the same partner, which the incidence in women of childbearing age is approximately 1-5% (Kuon et al., 2012). With the increase of the number of abortions, the abortion rate of recurrent spontaneous abortion patients will also increase, the risk of spontaneous abortion can be as high as above 50% after twice abortions, which should cause enough attention and clinical intervention to prevent the recurrence of abortion (Alijotas-Reig and Garrido-Gimenez, 2013). For now, known causes of recurrent spontaneous abortion including: Chromosomal abnormalities, genital tract anatomic abnormalities, endocrine disorders, autoimmunological factors, infectious diseases, former state of thrombosis, etc. In addition, there are at least half of the patients with unknown etiology, also known as unexplained recurrent spontaneous abortion, which is associated with the homogeneous immunity (Rai and Regan, 2006; Cohen and Bischof, 2007), may be due to the rise of human leukocyte antigen (HLA) compatibility, changes in the pattern of the functions of immune cells and Th1/Th2 cytokines balance disorders, lack of blocking antibodies or protective antibodies (Kheshtchin et al., 2010). Hypothesis of blocking antibody has attracted many attentions of the scholars, who believe that the mother produces blocking antibody against the harmful immunological reaction of male-specific minor histocompatibility antigens (MiHA) on the fetus or trophoblast, if the blocking antibody is insufficient, it eventually leads to miscarriage.

In 1981, Taylor made four unexplained recurrent

spontaneous abortion patients with negative anti-paternal lymphocyte antibody (APLA) vaccinated with their husbands' lymphoid cells subcutaneously as active immunotherapy, 3 of them achieved successful pregnancy and childbirth. This method is widely used, known as lymphocytes active immunity, currently used as injecting the paternal or the third-party donor lymphocyte intravenously or subcutaneously, and usually conducted before and after three months of pregnancy. The mechanisms of lymphocyte active immunotherapy are still not entirely clear, there exist different opinions on the effect. The system evaluation on immune treatment against recurrent miscarriage was updated (Wong et al., 2014), which involved in literatures from 1985 to 2004 in 11 countries, focused on patients with 3 or more consecutive miscarriages prior to 20 weeks' gestation with 1 sexual partner or (and) no more than 1 previous live birth, controlled measures are placebo or no treatment. According to the WHO, the clinical guidelines and relevant literature about the diagnostic criteria of primary and secondary recurrent miscarriage (Regan and Rai, 2000), also considering the existing clinical practice for a variety of treatment of recurrent abortion patients, the inclusion and exclusion criteria of this research will be different from Wong et al. (2014). Such as the research objects refer to patients who have 2 or more consecutive miscarriages prior to 20 weeks' gestation, controlled measures are placebo or no treatment, literature published before November 2016 in either English or Chinese, were retrieved and reviewed, to determine the curative effect of lymphocytes active immunotherapy for unexplained recurrent spontaneous abortion patients, including the Chinese patients.

Materials and Methods

Selection criteria

Types of participants

Studies with objects conforming to the diagnostic criteria of primary and secondary recurrent spontaneous abortion, according to the WHO and clinical guidelines. Primary recurrent spontaneous abortion refers to the extent of which patients have history of 2 or more consecutive miscarriages prior to 20 weeks' gestation with 1 sexual partner and no more than 1 previous live birth. Secondary recurrent spontaneous abortion refers to the extent in which the patient has the history of 2 or more consecutive miscarriages prior to 20 weeks' gestation with 1 sexual partner and at least more than 1 previous live birth. Excluding the patient in any age group with uterine or parental chromosome abnormality, genetic abnormality in miscarriage specimen, family heredity, birth canal abnormality, maternal endocrine abnormality, acquired or hereditary thrombophilia, or caused by environmental and other known factors.

Types of interventions

The experimental group patients immunized with paternal or third-party leukocytes whereas the control group patients received placebo or no treatment.

Types of outcome measures

The primary outcome measure was the live birth rate (number of live births/number of women receiving immunotherapy or placebo). The secondary outcome measure was the successful pregnancy rate (number of successful pregnancy/number of women receiving immunotherapy or placebo). The successful pregnancy refers to the pregnancy success more than 3 months, B-scan images did not show any abnormality.

Types of studies

The studies were published randomized controlled trials either in English or Chinese. All the studies involving "random", "randomized-control" or "randomization" were included.

Exclusion criteria

Researches without clear criterion of diagnosis, inclusion and exclusion for the participants; Experimental groups adopted treatments other than the immunization with leukocytes (paternal or health irrelevant third-party donor) in the peripheral blood; Reported data incorrect or incomplete, cannot provide outcome studies; Repeated published research.

Electronic searches

Searches of PubMed (1966 to November 2017), EMBASE (1980 to November 2017), Ovid Medline (1980 to November 2017), Chinese National Knowledge Infrastructure (CNKI, 1980 to November 2017), China Biology Medicine disc (CBM, 1978 to November 2017) and Wan fang database (1998 to November 2017).

English keywords

Abortion, Habitual, Habitual Abortion, Recurrent abortion, Miscarriage, Recurrent, Lymphocyte Activation, Immunotherapy, Lymphocyte, Active Immunotherapy, Immunity, Active, Allocation, Random, Randomization Controlled Clinical Trials, Randomized, Clinical Trials, Randomized, Trials, Randomized Clinical.

Search strategies

In PubMed, using the following terms: (((Randomization) OR Allocation, Random) OR Clinical Trials, Randomized))) AND (((Active Immunotherapy) OR Lymphocyte) OR Lymphocyte Activation [Mesh]))) OR Immunotherapy, Active [Mesh])) AND (Abortion, Recurrent) OR (("Abortion, Habitual" [Mesh]) OR Habitual Abortion) OR Miscarriage, Recurrent)).

Selection of studies

Trials identified through the searching activities described above were each assigned to a review topic (or topics). Data extracted from the review were entered into the Thomson Research Software (EndNote X4), and checked for accuracy. When the information regarding any of the above was unclear, the original report would be provided for further details. "Included", "pending", "excluded (reason)" were indicated into the "notes" column, "pending" reports would be retraced from the references.

Quality assessment

The quality of included studies was assessed according to the Cochrane handbook for Systematic Reviews of Interventions 5.1.0 recommended by the Cochrane Collaboration. Evaluation items mainly included seven aspects: Random sequence generation; allocation concealment; blinding of participants and provider; blinding of outcome assessment; incomplete outcome data; selective reporting; other bias. Each document in accordance with the above seven items respectively was judged as "low-risk", "unclear-risk" and "high-risk".

Data extraction

A form designed for data extraction, including the lead authors, years of publication, definitions of objects, types of abortion, types of treatment, comparability of baseline, interventions and sample sizes of experimental group and control group.

The process of literature selection, quality assessment and data extraction were carried out by two authors. Discussion or a third person consultation would be necessary in case disagreement.

Statistical analysis

The statistical analysis was carried out within the STATA 13.1 (StataCorp LP, USA). Relative risk (RR) and its 95% CI presented the result of meta-analysis for binary classification data.

Heterogeneity among all the studies measured by chi-square test, and the estimates of I^2. If the heterogeneity was not significant among included studies ($p>0.1$, $I^2 \leq 50\%$), fixed-effects model would be used. If there was statistical heterogeneity among the included studies ($p \leq 0.1$, $I^2>50\%$), subgroup analyses based on interventions would be proposed to detect the sources of heterogeneity, combined random-effects model would be better if it was unable to identify the sources.

Funnel plots were used for reporting the bias if there was more than 10 studies included. Funnel plot asymmetry assessed visually suggests that there might be publication bias.

Trial sequential analysis was performed 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 (DARIS) in accordance with the diversity in the intervention effect estimates among the included trials. DARIS was estimated using two-sided α=5%, β=20%, the control event proportions calculated from the placebo group, and the relative risk reduction (RRR) of 20% in outcomes. While meta-analysis aims to detect the efficacy of an intervention as early as possible, trial sequential analysis with monitoring boundaries were used to decide whether trials should be terminated early to prevent wastage of medical and research resources.

Results

Literature retrieval result

Fourteen randomized clinical trials were included after duplicate checking, early screening, full text intensive reading among 234 article. A summary for included trials overall is set out in Figure 1.

Quality assessment result

In total, 14 studies were included. Seven studies (Mowbray et al., 1985; Cauchi et al., 1991; Gatenby et al., 1993; Illeni et al., 1994; Ober et al., 1999; Kumar Pandey and Halder, 2003; Pandey and Agrawal, 2004) had used computer-generated random sequence. The rest were just reported as randomized trials; 1 trial (Illeni et al., 1994) allocated patients by phone, 1 trial (Ober et al., 1999) prepared opaque, sequentially numbered, sealed envelope, other trials did not provide sufficient information for allocation concealment. Eight studies (Mowbray et al., 1985; Cauchi et al., 1991; Clark and Daya, 1991; Gatenby et al., 1993; Christiansen et al., 1994; Ober et al., 1999; Kumar Pandey and Halder, 2003; Pandey and Agrawal, 2004) ensured double-blind, that the rest trials did not mention; 1 study recorded incomplete information, the rest did not have incomplete report nor selective report. Eight trials (Ho et al., 1991; Christiansen et al., 1994; Illeni et al., 1994; Ober et al., 1999; Kumar Pandey and Halder, 2003; Pandey and Agrawal, 2004; Liang et al., 2007; Cui et al., 2011) did not mention the basis of sample size estimation. Seven trials (Mowbray et al., 1985; Clark and Daya, 1991; Illeni et al., 1994; Mowbray James and Underwood Jennifer, 1994; Carp et al., 1997; Liang et al., 2007; Cui et al., 2011) did not have comparable baselines (Table I, Figure 2).

Characteristics of included literature

There were 994 patients (experimental group 475, placebo group 519). Three trials enrolled only women

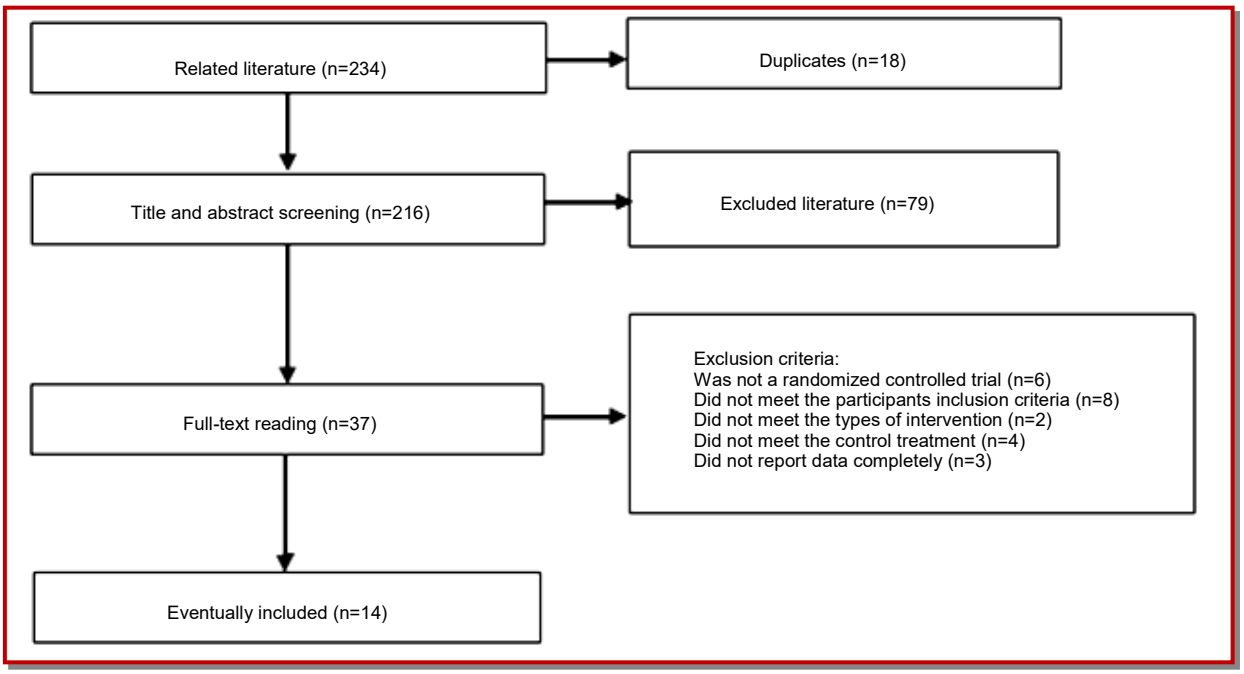

Figure 1: Flow diagram for literature screening and selection

with primary recurrent spontaneous abortion (Clark and Daya, 1991; Gatenby et al., 1993; Illeni et al., 1994), and 11 trials enrolled women with primary and secondary recurrent miscarriage (Mowbray et al., 1985; Cauchi et al., 1991; Ho et al., 1991; Christiansen et al., 1994; Mowbray and Underwood, 1994; Carp et al., 1997; Ober et al., 1999; Pandey and Halder, 2003; Pandey and Agrawal, 2004; Liang et al., 2007; Cui et al., 2011). The experimental intervention was paternal or third-party lymphocytes active immunotherapy in all the trials. Dosage of infusions varied between the trials. Five trials used normal saline as placebo (Clark and Daya, 1991; Gatenby et al., 1993; Ober et al., 1999; Pandey and Halder, 2003; Pandey and Agrawal, 2004), nine trials used maternal leukocytes as placebo (Mowbray et al., 1985; Cauchi et al., 1991; Ho et al., 1991; Christiansen et al., 1994; Illeni et al., 1994; Mowbray and Underwood, 1994; Carp et al., 1997; Pandey and Halder, 2003; Pandey and Agrawal, 2004), and four trials had no treatment in the control group (Pandey and Halder, 2003; Pandey and Agrawal, 2004; Liang et al., 2007). Characteristics of each included study are shown in Table II.

Blocking antibody level trend analysis in unexplained recurrent spontaneous abortion patients who experienced a new pregnancy after immunotherapy

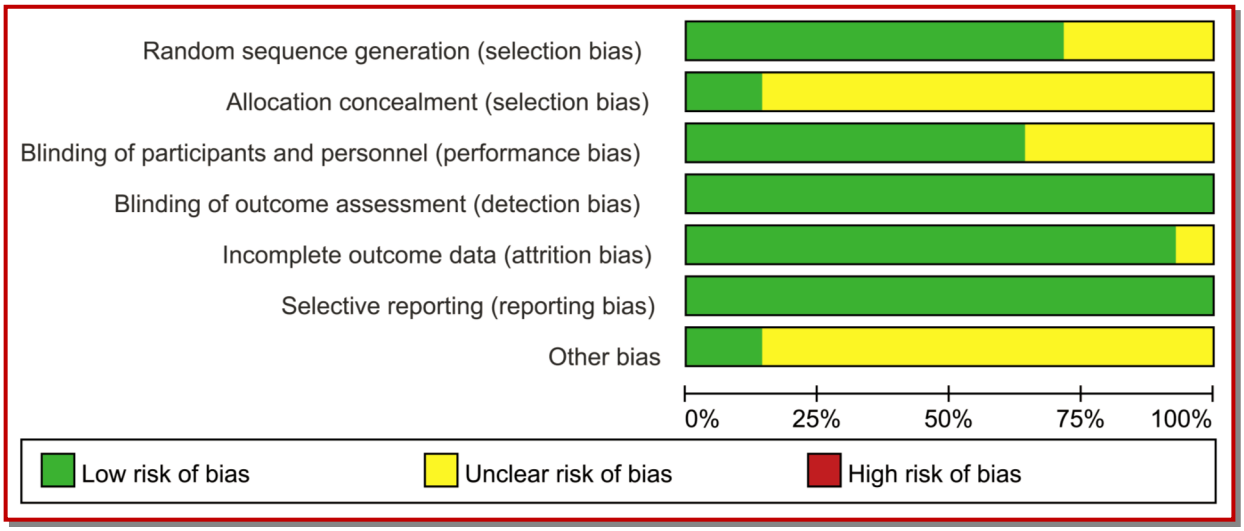

Figure 2: Quality assessment summary for all included studies

Table I

Quality assessment of included studies

Included studies	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other bias
Mowbray et al., 1985	Low risk (computer-generated list of numbers)	Unclear risk	Low risk (blinding of participants and obstetrician)	Low risk	Unclear risk	Low risk	Unclear risk (Insufficient information for baseline comparison)
Ho et al., 1991	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk	Low risk	Unclear risk (Insufficient information for sample size estimation)
Cauchi et al., 1991	Low risk (computer-generated list of numbers)	Unclear risk	Low risk (blinding of participants and obstetrician)	Low risk	Low risk	Low risk	Low risk
Clark et al., 1991	Low risk	Unclear risk	Low risk (blinding of participants and obstetrician)	Low risk	Low risk	Low risk	Unclear risk (Insufficient information for baseline comparison)
Gatenby et al., 1993	Low risk (computer-generated list of numbers)	Unclear risk	Low risk (blinding of participants, obstetrician and personnel)	Low risk	Low risk	Low risk	Low risk
Christiansen et al., 1994	Unclear risk	Unclear risk	Low risk (blinding of participants and obstetrician)	Low risk	Low risk	Low risk	Unclear risk (Insufficient information for sample size estimation)
Illeni et al., 1994	Low risk (computer-generated list of numbers)	Low risk (allocation by phone)	Unclear risk	Low risk	Low risk	Low risk	Unclear risk (Insufficient information for baseline comparison and sample size estimation)
Mowbray et al., 1994	Low risk	Unclear risk	Low risk	Low risk	Low risk	Low risk	Unclear risk (Insufficient information for baseline comparison)
Carp et al., 1997	Low risk	Unclear risk	Unclear risk	Low risk	Low risk	Low risk	Unclear risk (Insufficient information for baseline comparison)
Ober et al., 1999	Low risk (computer-generated list of numbers)	Low risk (using sealed envelopes)	Low risk (blinding of participants and obstetrician)	Low risk	Low risk	Low risk	Unclear risk (Insufficient information for sample size estimation)
Pandey et al., 2003	Low risk (computer-generated list of numbers)	Unclear risk	Low risk (blinding of participants, obstetrician and personnel)	Low risk	Low risk	Low risk	Unclear risk (Insufficient information for sample size estimation)
Pandey et al., 2004	Low risk (computer-generated list of numbers)	Unclear risk	Low risk (blinding of participants, obstetrician and personnel)	Low risk	Low risk	Low risk	Unclear risk (Insufficient information for sample size estimation)
Liang et al., 2007	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk	Low risk	Unclear risk (Insufficient information for baseline comparison and sample size estimation)
Cui et al., 2009	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk	Low risk	Unclear risk (Insufficient information for baseline comparison and sample size estimation)

Table II

Characteristics of included studies

Included studies	Participants	Interventions	Patient category	Experimental group				Control group			
				Interventions	Live birth	Successful pregnancy	Sample size	Interventions	Live birth	Successful pregnancy	Sample size
Mowbray et al., 1985	≥3 spontaneous abortion with the same partner and no more than 1 live birth	400 mL citrated Blood, lymphocytes intravenous 3 mL, intradermal 1 mL and subcutaneous 1 mL	Primary/ secondary RSA	Paternal leukocytes	17/22	--	22	Maternal leukocytes	10/27	--	27
Ho et al., 1991	≥3 consecutive spontaneous abortion with the same husband	200–400×10^7 lymphocytes, injected intradermally 2 mL	Primary/ secondary RSA	Paternal or third-party leukocytes	PL 21/39 TPL 5/11	PL 31/39 TPL 8/11	PL 39 TPL 11	Maternal leukocytes	23/49	32/49	49
Cauchi et al., 1991	≥3 consecutive consecutive first trimester miscarriages with the same partner	100–150×10^7-10^8 lymphocytes, intravenous 1 mL, intradermal 0.5 mL, subcutaneous 0.5 mL	Primary RSA	Paternal leukocytes	--	13/21	21	Normal saline	--	19/25	25
Clark et al., 1991	≥3 spontaneous abortion with the same partner and no live birth	40×10^6 lymphocytes, injected intradermally	Primary RSA	Paternal leukocytes		7/11	11	Normal saline		2/7	7
Gatenby et al., 1993	≥3 consecutive spontaneous abortion prior to 20 weeks' gestation with 1 sexual partner and no more than 1 previous live birth.	400×10^6 peripheral blood mononuclear leukocytes, injected intravenous 3 mL, intradermal 1 mL and subcutaneous 1 mL	Primary/ secondary RSA	Paternal leukocytes	13/19	--	19	Maternal leukocytes	9/19	--	19
Christiansen et al., 1994	≥ 3 spontaneous abortion and no more than 1 live birth	150–460×10^7 lymphocytes intravenously	Primary/ secondary RSA	third-party leukocytes	29/43		43	Maternal leukocytes	10/23		23
Illeni et al., 1994	≥ 3 spontaneous abortion and no live births	400 ml blood , 200×10^7 lymphocytes injected intravenously, intradermally	Primary RSA	Paternal leukocytes	10/22	16/22	22	Maternal leukocytes	11/22	14/22	22
Mowbray et al., 1994	≥2 abortion with the same partner and no more than 1 live birth	400 mL citrated Blood, lymphocytes intravenous 3 mL, intradermal 1 mL and subcutaneous 1 mL	Primary/ secondary RSA	Paternal leukocytes	25/35		35	Maternal leukocytes	14/30		30

Table II

Characteristics of included studies (Cont.)

Included studies	Participants	Interventions	Patient category	Experimental group				Control group			
				Interventions	Live birth	Successful pregnancy	Sample size	Interventions	Live birth	Successful pregnancy	Sample size
Carp et al., 1997	≥3 consecutive spontaneous abortion prior to 20 weeks' gestation with 1 sexual partner	80–100×10^6 lymphocytes, 2/3 intravenous, 1/6 intradermal, 1/6 subcutaneous	Primary/ secondary RSA	Paternal leukocytes	5/11		11	Maternal leukocytes	11/31		31
Ober et al., 1999	≥3 abortion with the same partner and no more than 1 live birth	200×10^7 lymphocytes, injected intravenously, intramuscularly and subcutaneously	Primary/ secondary RSA	Paternal leukocytes		31/86	86	Normal saline		41/85	85
Pandey et al., 2003	≥3 consecutive spontaneous abortion prior to 20 weeks' gestation with 1 sexual partner	5×10^6 lymphocytes, injected intravenously, intramuscularly and subcutaneously	Primary/ secondary	Paternal leukocytes	12/14		14	Maternal leukocytes or Normal saline or no treatment	5/18		18
Pandey et al., 2004	≥ 3 consecutive spontaneous abortion prior to 20 weeks' gestation with 1 sexual partner	5×10^6 lymphocytes, injected intravenously, intramuscularly and subcutaneously	Primary/ secondary RSA	Paternal or third-party leukocytes	TPL 6/31 PL 21/32	TPL 19/31 PL 25/32	TPL 31 PL 32	Maternal leukocytes (AL) Normal saline No treatment	AL 4/28 NS 2/19 NT 4/14	AL 12/28 NS 8/19 NT 9/14	AL 28 NS 19 NT 14
Liang et al., 2007	≥2 miscarriages, maternal blocking antibodies was negative	Lymphocytes injected intradermally	Primary/ secondary RSA	Paternal leukocytes	26/50	42/50	50	No treatment	14/50	26/50	50
Cui et al., 2009	≥2 miscarriages, maternal blocking antibodies was negative	2–4×10^7 lymphocytes, injected intradermally 2 mL	Primary/ secondary RSA	Paternal leukocytes	24/50	41/50	50	No treatment	13/50	24/50	50

Table III

Blocking antibody level changes before and after the lymphocyte treatment

Included studies	Blocking antibody (+) before treatment (%)		Blocking antibody (+) after treatment (%)	
	Experimental group	Control group	Experimental group	Control group
Mowbray et al., 1985	0	0	76% (37/49)[a]	0
Ho et al., 1991	11% (5/46) 5% (2/37)[b] 33% (3/9)[c]	9% (4/44)	26% (12/46) 22% (8/37)[ab] 44% (4/9)[c]	7% (3/44)
Christiansen et al., 1994	0	0	33% (13/40)[a]	0
Ober et al., 1999	0	0	26% (22/86)[a]	0
Pandey et al., 2003	0	0	93% (13/14)[a]	0
Pandey et al., 2004	0	0	78% (25/32)[a]	0
Liang et al., 2007	0	0	72% (36/50)[a]	0
Cui et al., 2009	0	0	68% (34/50)[a]	0

[a]Treatment effect on blocking antibody level change is significant ($p<0.05$); [b]Primary RSA; [c]Secondary RSA

Prior blocking antibody negative patients were included in seven studies (Mowbray et al., 1985; Gatenby et al., 1993; Christiansen et al., 1994; Kumar Pandey and Halder, 2003; Pandey and Agrawal, 2004; Liang et al., 2007; Cui et al., 2011) in addition to Ho NH's. The results of Gatenby et al., 1993; Cauchi et al., 1991; Clark and Daya, 1991; Illeni et al., 1994; Mowbray and Underwood, 1994; Carp et al., 1997 did not describe the change of blocking antibody levels after treatment. Mowbray et al., 1985; Ho et al., 1991; Christiansen et al., 1994; Ober et al., 1999; Kumar Pandey and Halder, 2003; Pandey and Agrawal, 2004; Liang et al., 2007; Cui et al., 2011 showed that blocking antibody positive rate was significantly increased in intervention group after treatment. Details were reported in Table III.

Pregnancy outcome analysis of lymphocyte active immunotherapy against unexplained recurrent spontaneous abortion

Live birth rate comparison between paternal or third-party lymphocyte and placebo

There were 379 and 380 unexplained recurrent spontaneous abortion patients who in experimental group (paternal or third-party lymphocytes active immunotherapy) and placebo group, 214 and 130 live births respectively. Meta-analysis showed no statistical heterogeneity between studies ($p=0.191$, $I^2=26.6\%$), fixed effect model could be used for merging, which showed that the difference of live-birth rate between the two groups was statistically significant (RR=1.63, 95% CI (1.38 1.92), $p<0.05$) as shown in Figure 3.

Trial sequential analysis for live birth rate between paternal or third-party lymphocyte and placebo

Trial sequential analysis showed that the required information size was again 1,590 patients and the obtained information was 759 patients. The number of participants did not reached the required information size. While the Z-value calculated at the fourth significance test is 'extreme enough'; the Z-curve crosses the trial sequential monitoring boundaries, thereby confirming that the clinical benefit of active immunotherapy is significant.

Successful pregnancy rate comparison between paternal or third-party lymphocyte and placebo

There were 353 and 349 unexplained recurrent spontaneous abortion patients who in experimental group (paternal or third-party lymphocytes active immunotherapy) and placebo group, 233 and 187 patients had successful pregnancy respectively. Meta-analysis showed no statistical heterogeneity between studies ($p=0.003$, $I^2=67.0\%$), random effect model could be used for merging, which showed that the difference of successful pregnancy rate between two groups was not statistically significant (RR=1.23, 95% CI (0.98, 1.54), $p=0.068$) as shown in Figure 4.

Subgroup analysis between primary and secondary recurrent spontaneous abortion

There were 115 and 117 primary recurrent spontaneous abortion patients in experimental group (paternal or third-party lymphocytes active immunotherapy) and placebo, 67 and 44 patients live births respectively. 32 and 35 secondary recurrent spontaneous abortion patients in experimental group (paternal or third-party lymphocytes active immunotherapy) and placebo, 20 and 21 patients live births respectively. The pooled effect of immunotherapy was significant in improving the live birth rate in women with primary recurrent spontaneous abortion (RR= 1.51, 95% CI (1.13, 2.01), $p<0.05$), while active immunotherapy was not significant in improving the live birth rate in women with secondary recurrent spontaneous abortion (RR= 0.99, 95% CI (0.68, 1.44), $p= 0.95$) as shown in Figure 5, Figure 6 and Figure 7.

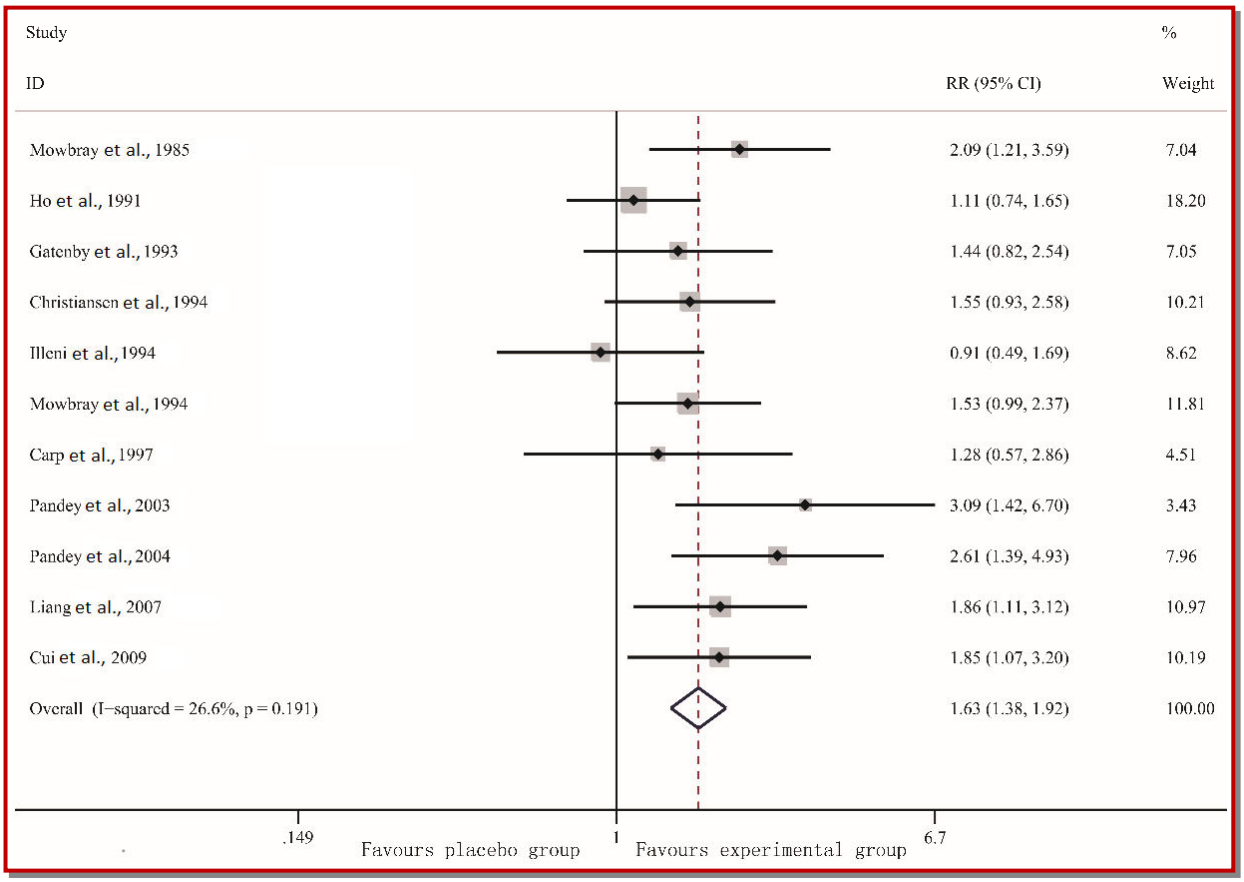

Figure 3: Live birth rate comparison between paternal or third-party lymphocyte and placebo

Publication bias

A funnel plot was used for reporting bias, which appeared asymmetric (Figure 8), suggesting that there may be publication bias.

Discussion

Immune rejection is considered to be the most important mechanism of recurrent spontaneous abortion. Recently, whereby blocking antibody combines with fetal antigens and sustain pregnancy continuation, has been one of the most important hypothesis of pregnancy immune tolerance. Stimulation on maternal with paternal or third-party lymphocytes to produce similarly antibody for pregnancy immune tolerance has been commonly used in practice against recurrent spontaneous abortion, which effect has been in doubt for a long time. Lymphocytes source is very important for fetal immune system, which is inherited from the couple. Theoretically, blocking antibody stimulated by paternal lymphocyte is more targeted to protect the fetus. Blocking antibody refers to specific IgG antibodies in pregnant women serum resisting partner's lymphocytes, which can provide inhibition of mixed lymphocyte reaction (MLR). BA block trophocyte antigen, prevent helper T cell recognition to fetal antigens, stop the mother's immune system's attack against the embryo, which is considered to be an important mechanism of pregnancy immune tolerance. However, the mechanism is still unclear, thus analysis was conducted on this issue.

In our review, 13 included studies adopted paternal lymphocytes, 3 studies used third-party lymphocytes. The blocking antibody hypothesis may be proved that the unexplained recurrent spontaneous abortion patients had significant higher blocking antibody positive rate after active immunotherapy, while blocking antibody positive rate had no significant difference after treatment in placebo group. Such a ubiquitous appearance of blocking antibody after immunotherapy may indicate the generation of an appropriate immune reaction in patients, which may be considered to contribute to a successful continuation of the subsequent pregnancy. Overall, treatment with active immunotherapy improved live birth rate in women with unexplained recurrent spontaneous abortion when compared with placebo. Trial sequential analysis on the outcome 'live birth' showed that the cumulative Z-curve crossed the monitoring boundaries for benefits, harms, or futility. Accordingly, we can interpret that the clinical benefit of active immunotherapy is significant.

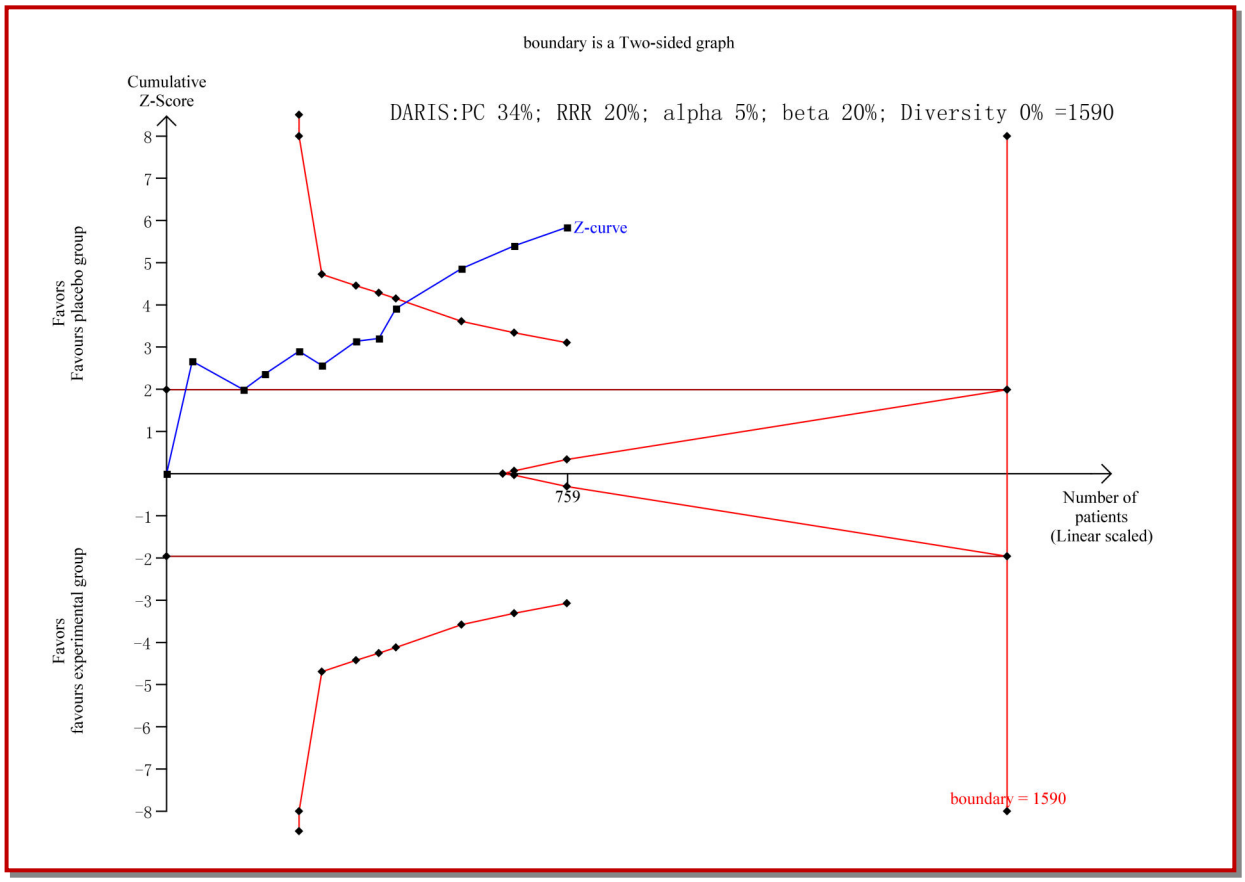

Figure 4: Trial sequential analysis for live birth rate between paternal or third-party lymphocyte and placebo

While the difference of successful pregnancy rate between immunotherapy group and placebo group was not statistically significant in unexplained recurrent spontaneous abortion patients, suggesting that active immunotherapy may be beneficial for specific patient subset. Thus, recurrent spontaneous abortion was divided into primary or secondary recurrent spontaneous abortion for further meta-analysis in this review. From the 14 included studies, 5 studies enrolled primary recurrent spontaneous abortion patients (Mowbray et al., 1985; Ho et al., 1991; Christiansen et al., 1994; Carp et al., 1997; Illeni et al., 1994), and 4 studies recruited secondary recurrent spontaneous abortion patients (Mowbray et al., 1985; Ho et al., 1991; Christiansen et al., 1994; Carp et al., 1997). Our review suggests that women with primary recurrent spontaneous abortion seemed more likely to obtain a potential beneficial effect of active immunotherapy compare to secondary recurrent spontaneous abortion, which is consistent with the conclusion made by Christiansen et al. (1994) Judgments of blocking antibody theory and clinical value of immunotherapy proposed the following ponders: Women with secondary recurrent spontaneous abortion (i.e., women in whom recurrent abortions were preceded by at least one pregnancy that resulted in a live birth or stillbirth beyond 20 weeks' gestation), autoimmunity, and evidence of antipaternal antibody. The presence of pretreatment antipaternal antibody appeared to reduce the effect of lymphocyte immunization therapy, which can be approved in study Ho et al. (1991) that blocking antibody Level had significant change after active immunotherapy in primary recurrent spontaneous abortion, while provided no significant change in secondary RSA (Table III). The second possibility is that reproductive age is generally agreed as one of reasons which affect the pregnancy outcome, as the growth of the reproductive age, the overall abortion rate also rose and live birth rate fell.

Fertility decline with age has a variety of factors, including sexual intercourse frequency decreases, the number of ovulation, oocyte chromosome abnormality and relevant pelvic diseases are also on the increase. In addition to the conception of reducing embryo grow success rate also fell, probably because the increase of the genetic abnormality associated with age, embryo quality and endometrial let down by sex. Generally speaking, primary recurrent spontaneous abortion younger than secondary recurrent spontaneous abortion patients, which explain treatment effect is better among primary recurrent spontaneous abortion with lymphocyte immunization therapy.

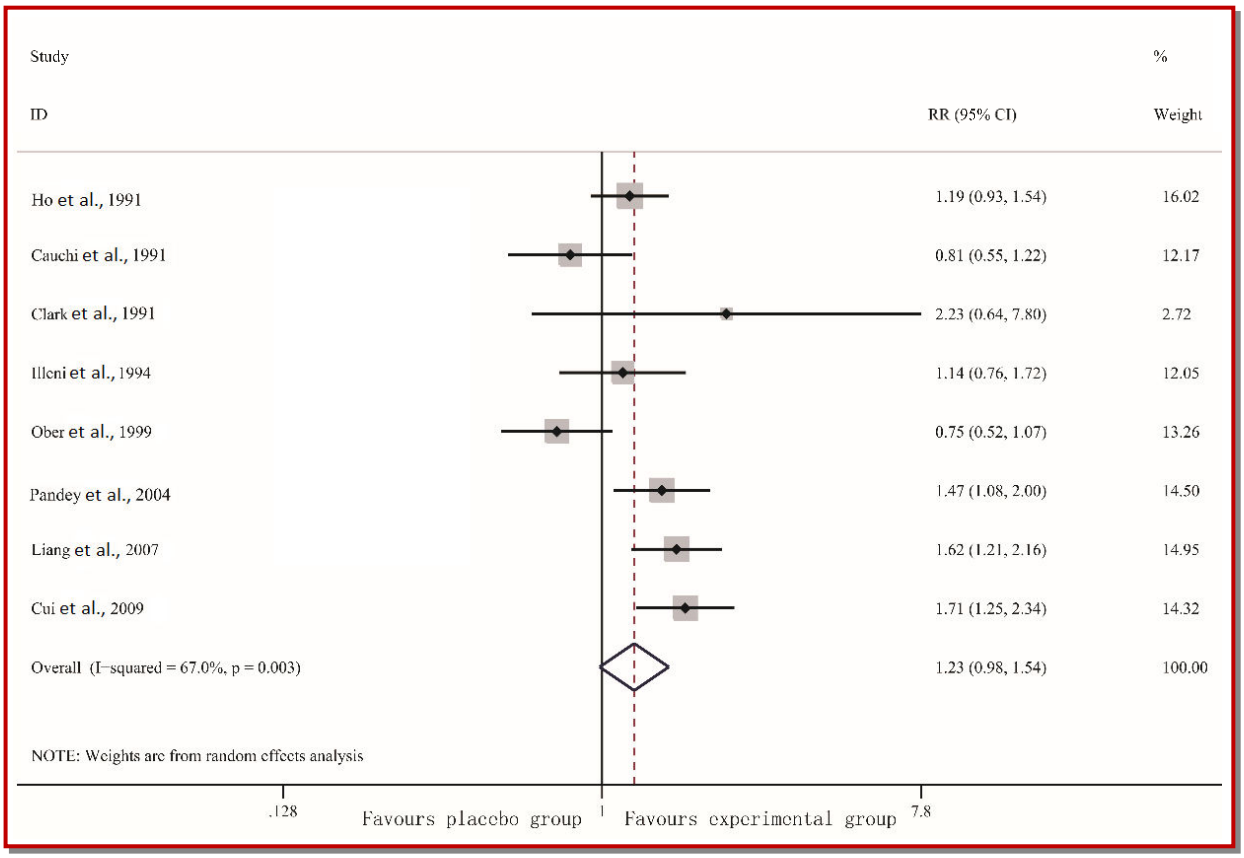

Figure 5: Successful pregnancy rate comparison between paternal or third-party lymphocyte and placebo

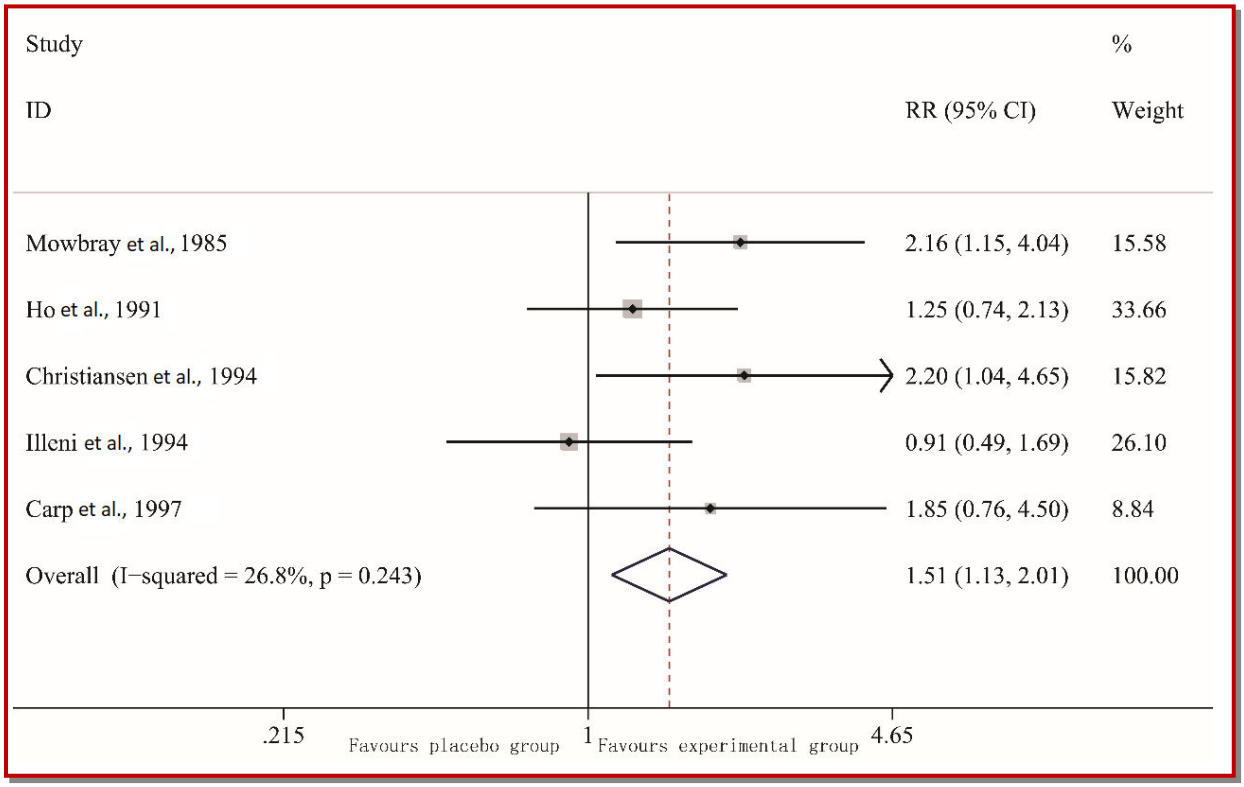

Figure 6: Forest plot comparing primary RSA in immunotherapy and placebo groups

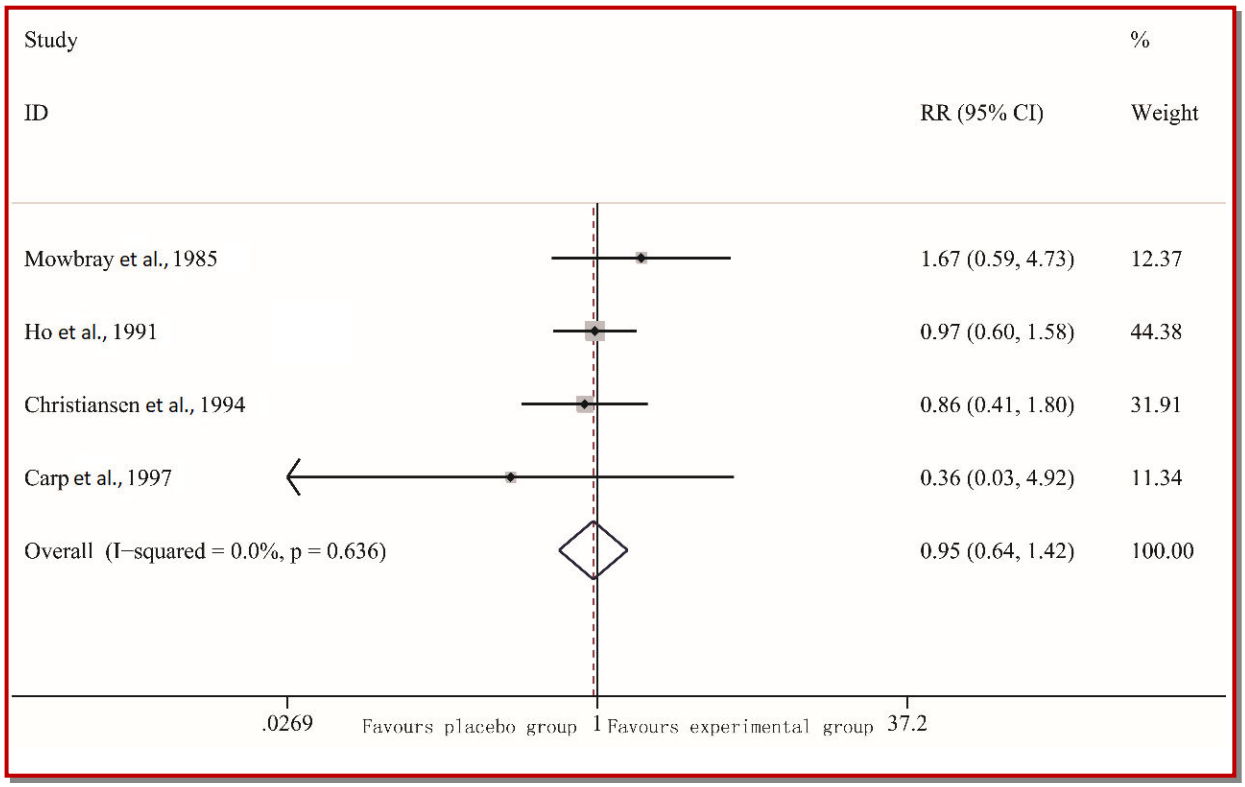

Figure 7: Forest plot comparing secondary RSA in immunotherapy and placebo groups

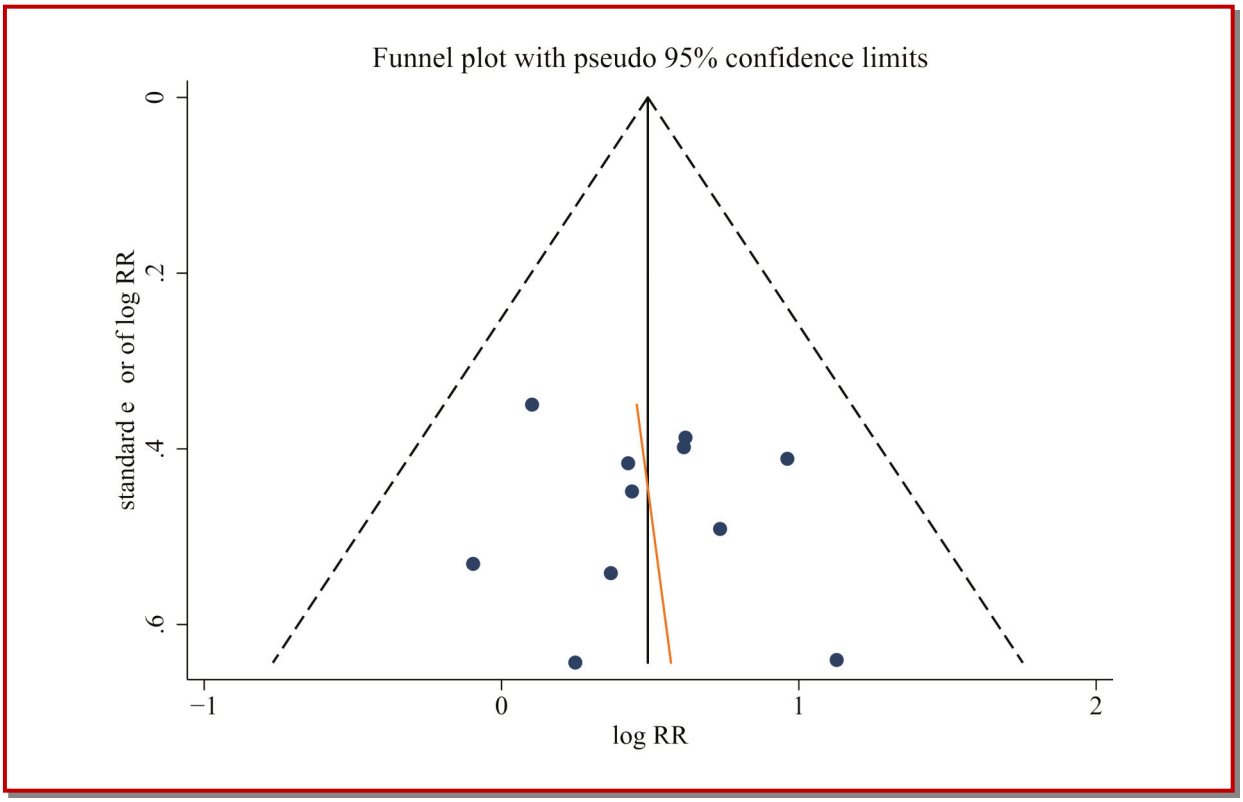

Figure 8: The funnel plot for live birth rate comparisons in active immunotherapy trials

Recently, Egerup et al. and Christiansen et al. (Christiansen, 2014; Egerup et al., 2015) suggested that intravenous immunoglobulin may be effective in secondary recurrent spontaneous abortion patients, but not primary recurrent miscarriage patients. Passive immunization through intravenous immunoglobulin to suppress and neutralize autoantibodies, which is the main mechanism to prevent abortion. Accordingly, patients undergoing immunization therapy or placebo-treatment should be monitored for changes of some of the immunological parameters that have drawn attention during recent years. More large placebo-controlled studies of, in particular, active immuno-therapy exclusively should be done among patients with primary recurrent miscarriage without high titers of auto- or allo-antibodies. Furthermore, more placebo-controlled studies of passive immunization should be done among patients with secondary recurrent miscarriage.

Funnel plot appeared asymmetry, suggesting that there was reporting bias, as included studies are published literature, which tend to prefer "was statistically significant" results rather than "no statistical signifi-cance" or invalid research results, leading to exaggerate the effect of the experimental group. If gray literature or unpublished data is included, the evidence of effect assessment for these therapies will be more persuasive. This review and the included researches showed that there was significance improve in blocking antibody positive rate after active immunotherapy, but there is no evidence to support that blocking antibody positive conversion after active immunotherapy can improve the pregnancy outcome, and the number of blocking antibody positive women get normal pregnancy compare to blocking antibody negative women.

Conclusion

Based on our results, treatment with lymphocyte active immunization compared with placebo seems to improve the live birth rate. Subgroup analysis suggests that women with primary recurrent spontaneous abortion who are negative for blocking antibody prior to treatment seemed most likely to obtain a potential beneficial effect of lymphocyte active immunization.

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Conflict of Interest

All authors stated that there is no conflicts of interest relevant to this article.

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