Administration effects of single-dose GnRH agonist for luteal support in females undertaking IVF/ICSI cycles: A meta-analysis of randomized controlled trials

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Abstract. The aim of the present meta-analysis was to evaluate the effects of the addition of single-dose gonadotropin-releasing hormone agonist (GnRHa) for luteal support on pregnancy outcomes in females partaking in *in vitro* fertilization or intracytoplasmic sperm injection cycles. In total, the studies were hand-searched from six electronic databases to compare the pregnancy outcomes between single-dose GnRHa administered as luteal phase support (GnRHa group) and regular luteal support (control group). In the GnRHa group, single-dose GnRH agonist were administered at 5/6 days after IVF/ICSI procedures. In the control group, single-dose GnRH agonist was not added during luteal phase support. Only randomized controlled trials were included. Sensitivity analysis was performed using Revman 5.3 software; the high heterogeneity identified in the present analysis was primarily caused by one study included. Following exclusion of this particular study, the meta-analysis results indicated significantly higher rates of ongoing pregnancy or live birth per transfer (P=0.002), clinical pregnancy per transfer (CPR; P=0.001) and multiple pregnancy per pregnancy (P=0.020) in the GnRHa group compared with those in the control group. Meta-analysis of a subgroup of trials with long-acting GnRH-a ovarian treatment protocols indicated that the rate of ongoing pregnancy or live birth (P=0.080), CPR (P=0.090) and multiple pregnancy per pregnancy (P=0.140) were not significantly different between the two groups. However, the results from trials that had used a multi-dose GnRH antagonist ovarian treatment protocol indicated a significantly higher ongoing pregnancy or live birth rate per transfer (P=0.010), CPR per transfer (P<0.0001) and multiple pregnancy rate per pregnancy (P=0.003) compared with those in the control group. The present results suggested that administration of single-dose GnRH agonist in the luteal phase may be an ideal choice for patients undergoing IVF/ICSI therapy.

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Introduction

In the natural reproductive cycle, the luteal phase is the result of intermittent stimulation of the corpus luteum by pituitary luteinizing hormone (LH), which is different from assisted reproductive technology (ART) cycles. Luteal-phase deficiency is common during the follicular stimulation phase of the menstrual cycle (1) and leads to a decreased embryo implantation rate, a lower pregnancy rate and an increased miscarriage rate (2). Controlled ovarian stimulation is usually involved in *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI)/embryo transfer (ET) technology, causing the development of multiple follicles and the formation of multiple embryos to increase the clinical pregnancy rate (CPR) and live birth rate. The development of multiple follicles leads to abnormally high levels of estrogen and multiple corpora lutea are formed after aspiration, which maintain the abnormally high production of steroids (3,4). In a natural pregnancy, LH is continuously produced after the ovulation surge until human chorionic gonadotropin (hCG) is secreted by the proliferating trophoblast. In addition, the high steroid levels exert a negative feedback on the pituitary gland, thus prematurely inhibiting the production of LH during ART cycles (4). By contrast, aspiration of granulosa cells may interfere with the production of progesterone (P) (5), leading to a reduction in the luteal phase to cause a condition known as premature luteolysis (3).

To overcome this issue, pharmacological support, including combinations of estradiol, P and hCG, have been frequently applied to directly or indirectly increase the low levels of P (6). Recently, gonadotropin-releasing hormone (GnRH) agonist has been used for luteal-phase support (LPS) (7,8). A possible explanation for the effect of GnRH agonist is that it extends

LH production, thus preventing the occurrence of premature luteolysis (3). However, it was observed that a single dose GnRH agonist at the time of implantation improved the pregnancy rate in recipients after artificial endometrial preparation, inducing the downregulation of GnRH followed by a decrease in the levels of estrogen and P (8). In addition, GnRH agonist may directly influence the quality of the early embryo for the recipients without corpus luteum (4,8), although a direct effect on the endometrium cannot be excluded. A previous *in vitro* study provided similar results, since GnRH agonist receptor was indicated to be broadly expressed in the human morula and at the blastocyst stage (9). The development of porcine and murine pre-implantation embryos is enhanced when incubated with GnRH agonist and diminished when incubated with GnRH antagonist (10,11).

In total, four previous systematic reviews concluded that there may be benefits from the addition of single-dose GnRH agonist to improve luteal support (3,12-14), and it was suggested that this treatment is relatively safe and effective (15). However, it is required to perform a comprehensive and unbiased systematic review analyzing intensive studies and recent results (16). The aim of the present meta-analysis was to identify, analyze and summarize evidence from randomized controlled trials (RCTs) and examine the effects of single-dose GnRH agonist for luteal support in females undergoing IVF/ICSI cycles.

Materials and methods

Eligibility criteria. All published and ongoing RCTs assessing the administration effect of single-dose GnRH agonist during the luteal phase on IVF/ICSI outcomes were included in the present meta-analysis. Trials including egg donation and frozen embryo transfer cycles were excluded. Studies with multiple-dose GnRH agonist treatments in the luteal phase were excluded due to the lack of safety assessment and the large difference in GnRH agonist application protocols.

Grouping. In total, four groups were considered: i) Single-dose GnRH agonist administered as LPS at 5/6 days after IVF/ICSI procedures (GnRHa group); ii) regular support, where progesterone/estradiol/hCG was used for LPS (control group); iii) GnRH agonist was used to suppress premature LH surge (GnRH-a group); and iv) GnRH antagonist was used to suppress premature LH surge (GnRH-A group). In the GnRH-a group, a single GnRH agonist (Decapeptyl, 1.25-3.75 mg) was administered on day 24-26 of the cycle. After 2 weeks, Gn (Gonadotropin) are used to stimulate follicular development. In GnRH-A group, GnRH antagonist (Ganirelix acetate) was given 0.25 mg/d from day 5 or 6 of Gn administration until hCG day.

Outcome measures. The outcome measures used for the present meta-analysis were as follows: i) Ongoing pregnancy or live birth rate per transfer; ii) CPR per transfer; iii) multiple pregnancy rate; and iv) clinical abortion rate. Data in which the live birth rate was recorded was generally preferred. However, when live birth was not reported, data from an ongoing pregnancy (intrauterine live fetus with a gestational age ≥12 weeks) were used as a measure for live birth, since the difference between live birth and ongoing pregnancy rates are limited and <1% of pregnancies result in stillbirth (17,18). When studies reported on clinical pregnancy and ongoing pregnancy without miscarriage rates, the number of clinical abortions was considered as being equal to the difference between the number of clinical pregnancies and ongoing pregnancies.

Search strategy. Published studies were searched in the following electronic databases: i) China National Knowledge Infrastructure (CNKI), ii) Wanfang database, iii) Chinese Biomedicine Literature Database (CBM), iv) Pubmed, v) EMBASE and vi) Cochrane Controlled Trials Register. The entries analyzed were published prior to June 1st, 2018. There was no language restriction. The following terms were used, adjusting for each database as necessary: 'Fertilization *in vitro*' OR '*in vitro* fertilization' OR *in vitro* fertilizations' OR 'test-tube fertilization' OR 'fertilization, test-tube' OR 'fertilizations, test-tube' OR 'test tube fertilization' OR 'test-tube fertilizations' OR 'fertilizations *in vitro*' OR 'test-tube babies' OR 'babies, test-tube' OR 'baby, test-tube' OR 'test tube babies' OR 'intracytoplasmic sperm injection' OR 'IVF' OR 'ICSI' AND 'luteal support' OR 'luteal phase support' AND 'gonadotropin-releasing hormone agonist' OR 'GnRH-a' OR 'GnRHa' AND 'randomized controlled trial' OR 'randomized'.

Study selection and search results. The two researchers (MLS and CLL) independently screened the studies as follows: i) Duplicated articles were removed using NoteExpress software (version 3.2; Aegean Software Corp.); ii) two researchers read the titles and abstracts and manually removed the articles that did not meet the inclusion criteria of the present study; ii) the articles were further screened by reading the full text and excluded or included according to the inclusion and exclusion criteria of the present study. When inconsistent results were obtained, the issues were resolved by discussing with relevant experts in the field. Missing data were obtained from the authors of the respective studies whenever possible. In total, 468 records were retrieved in the initial electronic search: i) 92 records were from CNKI; ii) 70 records were from WANFANG; iii) 18 records were from CBM; iv) 65 records were from Pubmed; v) 112 records were from Embase; and vi) 111 records were from Cochrane. A total of 33 additional records were retrieved through manual search of potentially eligible studies and relevant reviews. A total of 501 records were assessed for eligibility. After removal of duplicate articles, 376 documents were retained and after reading the titles and abstracts, 318 records were removed. In addition, 38 articles that did not meet the inclusion criteria were excluded. Finally, among the 20 potentially relevant studies were obtained and a total of 8 studies fulfilled the inclusion criteria (19-26). A flowchart depicting the selection process is provided in Fig. 1.

Data extraction. In three studies (20,22,24), the long GnRH-a ovarian treatment protocol starting in the mid-luteal phase of the preceding cycle was used, in four studies (21,23,25,26), the GnRH-A multi low-dose ovarian treatment protocol was applied and in one study (19), both ovarian treatment protocol types were used. The trials published by Tesarik *et al* (8) used both the GnRH-a and the GnRH-A treatment protocol. In all studies, IVF or ICSI were performed. The characteristics of the eight studies identified, including the eligibility criteria, are provided in Table I.

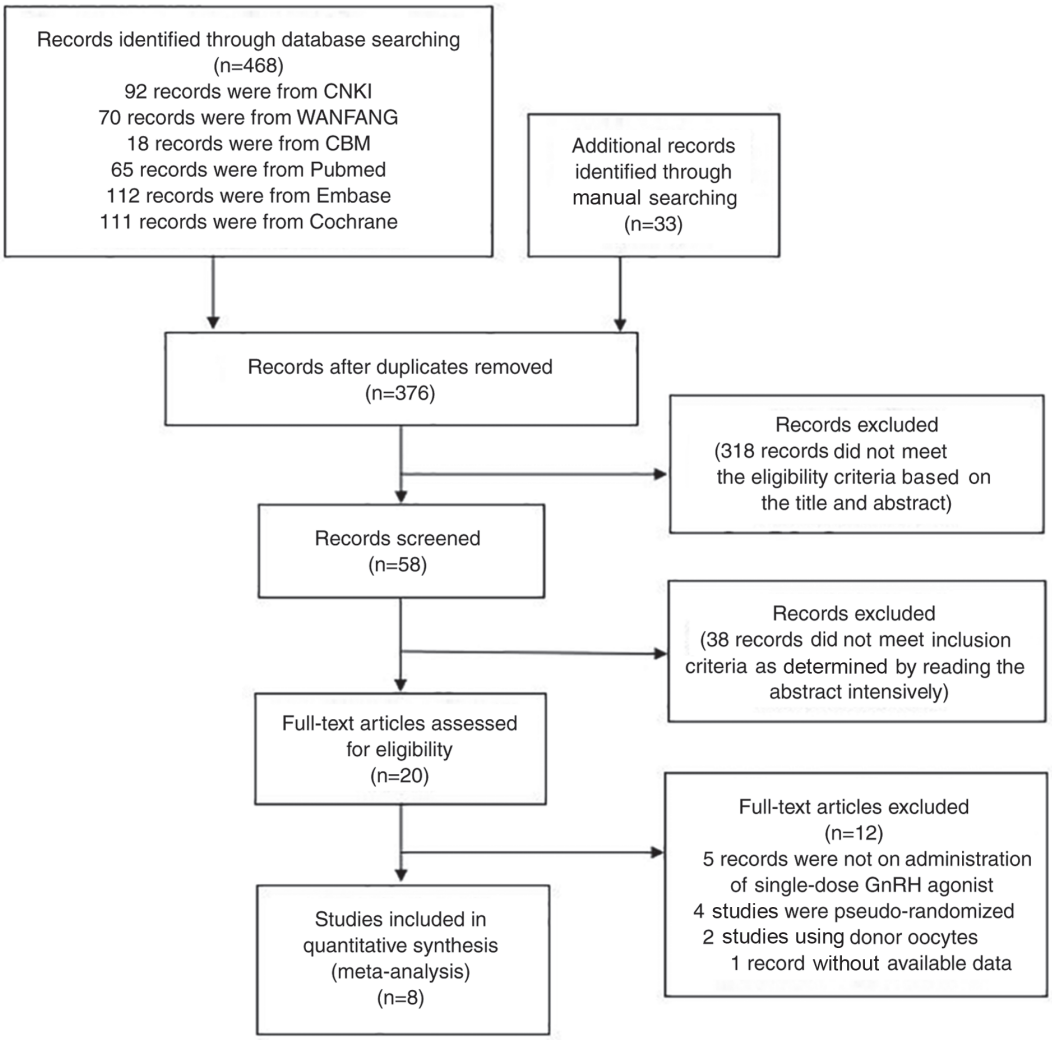

Figure 1. Flow diagram depicting the process of study selection. Published studies were searched in various electronic databases. All published and ongoing randomized controlled trials assessing the effect of single-dose GnRH agonist administration on *in vitro* fertilization/intracytoplasmic sperm injection outcomes were included. GnRH, gonadotropin-releasing hormone; CNKI, China National Knowledge Infrastructure; CBM, Chinese Biomedicine Literature Database.

Risk of bias within trials. The two researchers (MLS and CLL) evaluated the methodological quality of the studies included according to the RCT bias risk assessment tool recommended by the Cochrane systematic review guidelines (16). The evaluation included the following: i) Selection bias (random sequence generation and allocation concealment); ii) performance bias (blinding of participants and personnel); iii) detection bias (blinding of outcome assessment); iv) attrition bias (incomplete outcome data); v) reporting bias (selective reporting); and vi) other bias. In total, nine trials from eight studies were rated as 'low risk', 'unknown risk' or 'high risk'. In the case of disagreement, the two researchers discussed or resolved the issue by discussion with a third researcher (RH).

Statistical analysis. The results for each of the studies eligible for the present meta-analysis were expressed as the risk ratio (RR) and the precision of estimates were evaluated using the 95% CI. The results of the previous studies were combined for the present meta-analysis using the DerSimonian and Laird method and a random-effects model was applied. The heterogeneity analysis was performed using the I^2 test. When $P \leq 0.05$ and $I^2 \geq 50\%$, the studies were considered heterogeneous; $0 \leq I^2 \leq 25\%$ indicated low heterogeneity, $25\% < I^2 \leq 50\%$ indicated moderate heterogeneity and $50\% < I^2 \leq 75\%$ indicated high heterogeneity. All results were combined for the meta-analysis using Revman 5.3 software (Cochrane Collaboration). In addition, sensitivity analysis and subgroup analysis were performed for the GnRH-a and GnRH-A groups according to a pituitary downregulation protocol, where the levels of FSH and LH are reduced. Sensitivity analysis was performed using a method that removed one document at a time to assess the impact of a single study on the results of the present meta-analysis. A re-analysis was performed after excluding the study identified as the source of heterogeneity.

Results

Ongoing pregnancy or live birth rate per transfer. In total, seven records from six studies were included to determine the effect of the treatments on ongoing pregnancy or live birth

Table I. Major characteristics of randomized controlled trials on the administration GnRH agonist in the luteal phase.

Author (year)	Funding source	Randomization method/ allocation concealment	Protocol stimulation	Luteal phase study group GnRH-a	Control group	Others medicines (all patients)	Fertilization	Day of embryo transfer	Embryos transfered (n)	(Refs.)
Tesarik (2006)	Not reported	Embryo transfer day Computer-generated list Opaque envelopes Blinding	GnRH-a long protocol/GnRH-ant multiple doses + r-FSH/HMG	GnRH-a long protocol (n=150) GnRH-A protocol (n=150) Single injection Dose: 0.1 mg triptorelin Day 6 after ICSI	GnRH-a long protocol (n=150) GnRH-A protocol Placebo (n=150)	E_2 valerate (4 mg) + vaginal micronized progesterone (400 mg) + r-HCG (single dose)	ICSI	3	1-3	(19)
Ata (2008)	Not reported	Embryo transfer day Computer-generated list Opaque envelopes Blinding	GnRH-a long protocol + r-FSH	Single injection (n=285) Dose: 0.1 mg triptorelin Day 6 after ICSI	Placebo (n=285)	Vaginal progesterone gel 90 mg	ICSI	3	1-3	(20)
Isik (2009)	Not reported	Embryo transfer day Computer-generated list Blinding	GnRH-A multiple doses + r-FSH/HMG	Single injection (n=74) Dose: 0.5 mg leuprolide Day 6 after ICSI	No placebo (n=80)	Vaginal micronized progesterone (600 mg) + HCG (single dose)	ICSI	3	1-5	(21)
Razieh (2009)	Same institution	Drawing piece of paper from a bag	GnRH-a long protocol + r-FSH	Single injection (n=90) Dose: 0.1 mg triptorelin Day 5/6 after ICSI	Placebo (n=90)	Vaginal micronized progesterone (800 mg)	ICSI	2 or 3	2 or 3	(22)
Ata and Urman (2010)	Not reported	Embryo transfer day Computer-generated list Opaque envelopes Blinding	GnRH-A multiple doses + r-FSH	Single injection (n=38) Dose: 0.1 mg triptorelin Day 6 after ICSI	Placebo (n=52)	Vaginal progesterone gel 90 mg	ICSI	3	1-3	(23)
Yildiz (2014)	Not reported	Computer-generated list	GnRH-a long protocol + r-FSH	Single injection (n=100) Dose: 1 mg leuprolide Day 6 after ICSI	No placebo (n=100)	17βE2 (4 mg) + Vaginal micronized progesterone (600 mg)	ICSI	3	1-3	(24)
ZS[a] (2015)	Not reported	OCP administration day Computer-generated list	GnRH-ant multiple doses + r-FSH/HMG	Single injection (n=43) Dose: 0.1 mg triptorelin Day 6 after ICSI	No placebo (n=40)	Vaginal progesterone (800 mg)	ICSI	3	1-3	(25)
BA[b] (2017)	Same institution	Embryo transfer day Computer-generated list Blinding	GnRH-ant multiple doses + r-FSH	Single injection (n=165) Dose: 0.1 mg triptorelin Day 6 after OPU	No placebo (n=163)	E2 (4 mg) + Vaginal micronized progesterone (600 mg) + r-HCG (single dose)	IVF/ ICSI	2 or 3	1-3	(26)

IVF, *in vitro* fertilization; ICSI, intracytoplasmic sperm injection; GnRH, gonadotropin-releasing hormone; r-FSH, r-FSH, recombinant follicle-stimulating hormone; HMG, human menopausal gonadotropins; HCG, human chorionic gonadotropin; GnRH, gonadotropin-releasing hormone; GnRH-a, long GnRH agonist ovarian stimulation protocol; GnRH-A, GnRH antagonist multi low-dose ovarian stimulation protocol; GnRHa, single-dose GnRH agonist for luteal support. OCP, oral contraceptive pill; OPU, oocyte pick up. [a]Zafadourst S; [b]Benmachiche A.

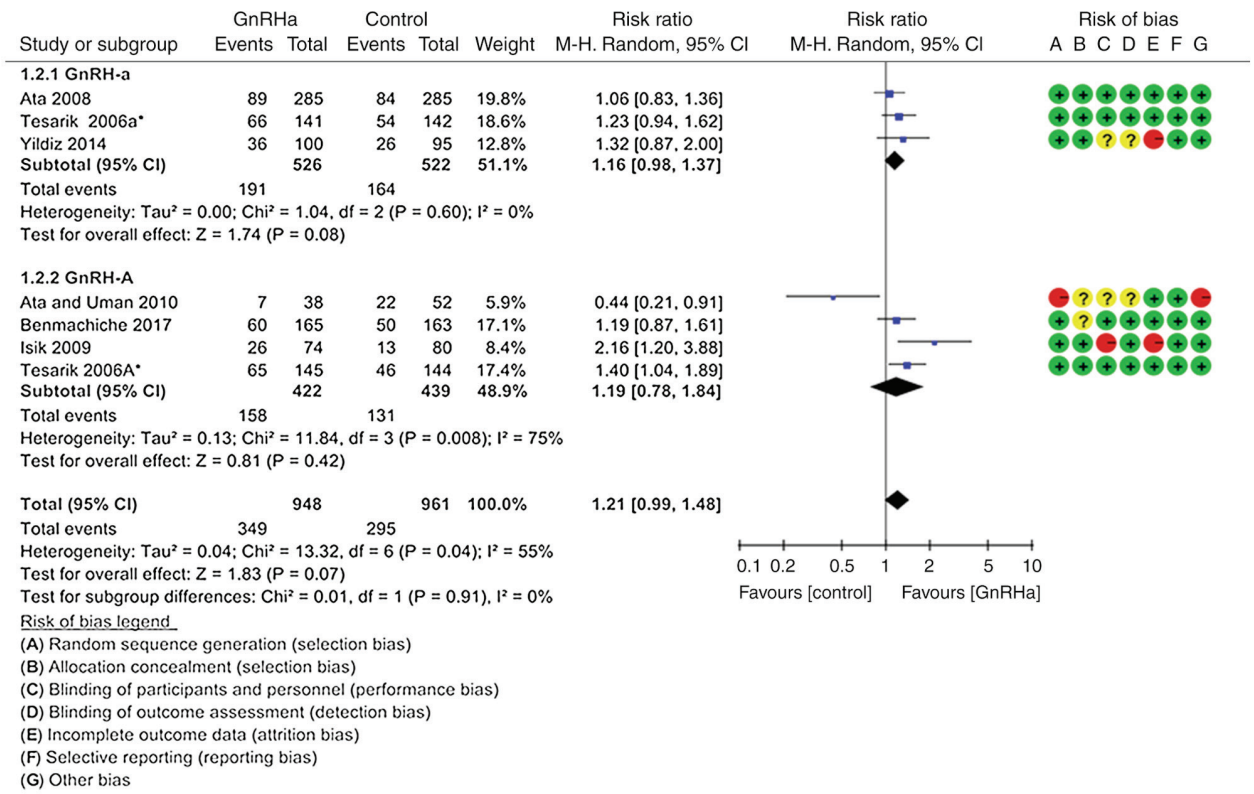

Figure 2. Forest plot for the meta-analysis on the influence of GnRHa on the ongoing pregnancy or live birth rate. In total, seven records from six studies were included. The pooled ongoing pregnancy or live birth rates per transfer were not significantly different between the GnRHa group (36.81%; 349/948) and the control group (30.70%; 295/961). Subgroup analysis according to GnRH agonists or antagonists used for luteinizing hormone suppression did not change the direction or the magnitude of the effect observed. GnRH, gonadotropin-releasing hormone; GnRH-a, long GnRH agonist ovarian treatment protocol; GnRH-A, GnRH antagonist ovarian treatment protocol; GnRHa, single-dose GnRH agonist for luteal support; Control, regular luteal support; MH, Mantel-Haentzel; df, degrees of freedom.

rate per transfer (Fig. 2) (19-21,23,24,26). The pooled ongoing pregnancy or live birth rate per transfer was not significantly different between the GnRHa group (36.81%, 349/948) and the control group (30.70%, 295/961; P=0.070; RR=1.21; 95% CI=0.99-1.48). Subgroup analysis according to the type of GnRH analogue used for LH suppression did not change the direction or the magnitude of the effect observed; there were no significant differences between the GnRH-a (P=0.080; RR=1.16, 95% CI=0.98-1.37) (19,20,24) and GnRH-A groups (P=0.420; RR=1.19, 95% CI=0.78-1.84; Fig. 2) (19,21,23,26).

However, there was high heterogeneity in the studies using a GnRH-A ovarian stimulation protocol (P=0.008; I^2=75%). Therefore, sensitivity analysis under exclusion of the study by Ata and Urman (23), the source of heterogeneity, was performed (Fig. 3). The results suggested that the ongoing pregnancy or live birth rate per transfer was significantly higher in the GnRHa group (37.5%; 342/910) compared with that in the control group (30.03%; 273/909; P=0.002; RR=1.25, 95% CI=1.09-1.44) (19-21,24,26). This trend was more pronounced in the GnRH-A ovarian stimulation protocol (P=0.010; RR=1.41, 95% CI=1.08-1.84) (19,21,26). However, there were no significant differences in the GnRH-a ovarian treatment protocol compared with control (P=0.080; RR=1.16, 95% CI=0.98-1.37; Fig. 3) (19,20,24).

CPR per transfer. In total, nine records from eight studies were included to calculate the CPR (Fig. 4) (19-26). The pooled CPR per transfer was significantly higher in the GnRHa group (41.58%; 437/1,051) compared with that in the control group (33.64%; 367/1,091; P=0.010; RR=1.28; 95% CI=1.06-1.55). There was significant heterogeneity in this comparison (P=0.020; I^2=57%). However, in the subgroup of trials where the long GnRH-a ovarian stimulation protocol was used, the pooled CPR per transfer did not differ significantly between the GnRHa group (41.72%; 257/616) and the control group (35.62%; 218/612; P=0.090; RR=1.24, 95% CI=0.97-1.58), and the comparison did not exhibit significant heterogeneity (P=0.070; I^2=57%) (19,20,22,24). On the other hand, in the subgroup of trials where the GnRH-A ovarian stimulation protocol was used, the CPR per transfer was significantly superior in the GnRHa group (41.38%; 180/435) than in the control group (31.11%; 149/479; P=0.040; RR=1.32, 95% CI=0.96-1.81; Fig. 4) (19,21,23,25,26).

As above, a sensitivity analysis excluding the study by Ata and Urman (23) was performed (Fig. 5), and the pooled CPR per transfer was significantly higher in the GnRHa group (41.95%; 425/1,013) than that in the control group (32.92%; 342/1,039; P=0.001; RR=1.34, 95% CI=1.12-1.59) (19-22,24-26). This trend was more evident with the GnRH-A ovarian stimulation protocol (P<0.0001; RR=1.45, 95% CI=1.20-1.74) (19,21,25,26). However, there were no significant differences in the GnRH-a ovarian stimulation protocol compared with control (P=0.090; RR=1.24, 95% CI=0.97-1.58; Fig. 5) (19,20,22,24).

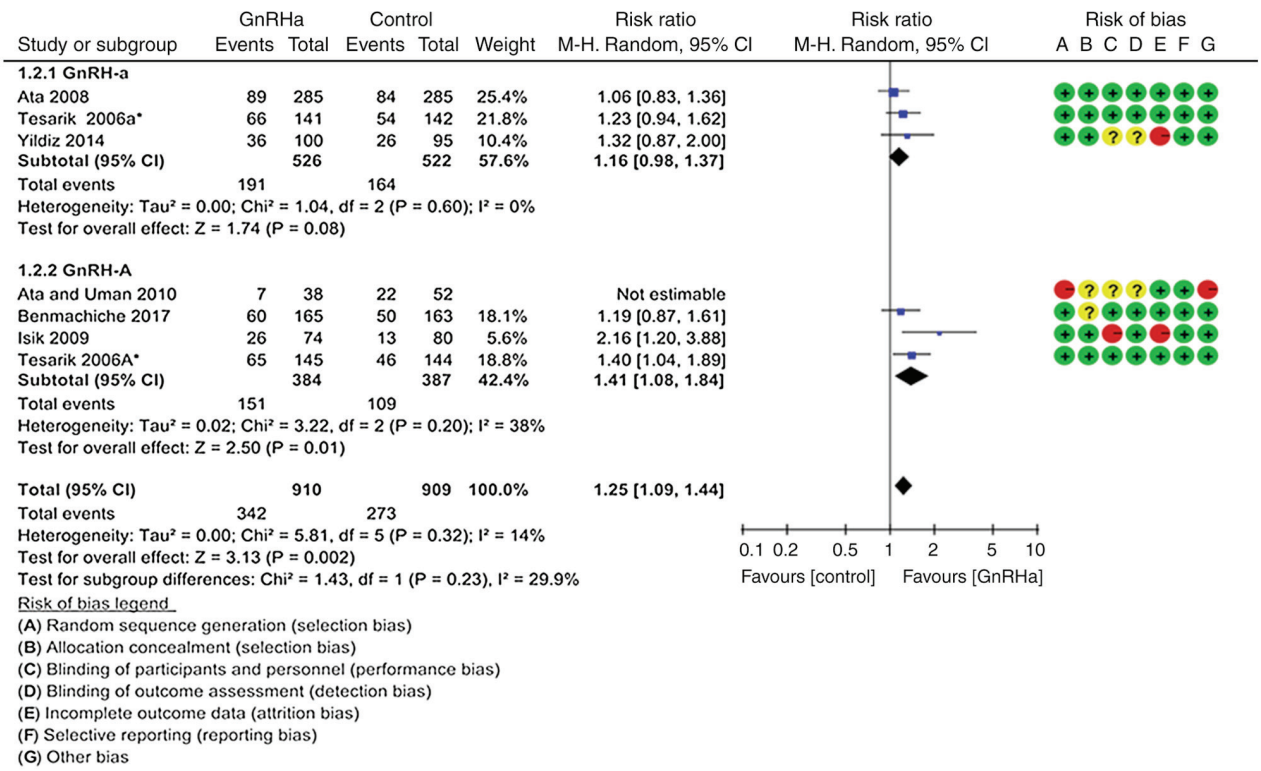

Figure 3. Forest plot for the sensitivity analysis for ongoing pregnancy or live birth rate. Sensitivity analysis excluding the study by Ata and Urman was performed, and the results indicated that the pooled ongoing pregnancy or live birth rate per transfer in the GnRHa group was significantly higher than that in the control group. This trend was more significant following GnRH-A ovarian stimulation protocol. GnRH, gonadotropin-releasing hormone; GnRH-a, long GnRH agonist ovarian stimulation protocol; GnRH-A, GnRH antagonist multi low-dose ovarian stimulation protocol; GnRHa, single-dose GnRH agonist for luteal support; Control, regular luteal support; MH, Mantel-Haentzel; df, degrees of freedom.

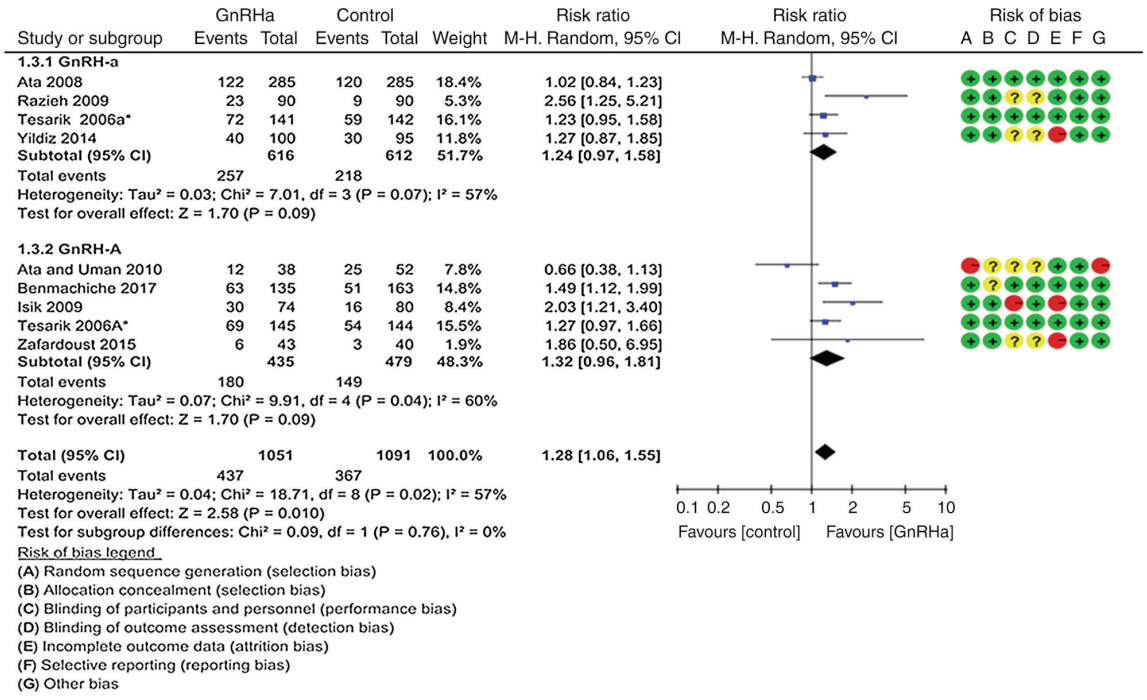

Figure 4. Forest plot for the CPR. In total, nine records from eight studies were included. The pooled CPR per transfer rate was significantly higher in the GnRHa group than in the control group. There was heterogeneity in this comparison. However, in the subgroup of trials using the long GnRH-a ovarian stimulation protocol, the pooled CPR per transfer did not differ significantly between the GnRHa group and the control group (35.62%; 218/612). Furthermore, this comparison exhibited a certain heterogeneity (P=0.070; I^2=57%) (19,20,22,24). By contrast, in the subgroup of trials using the GnRH-A ovarian stimulation protocol, the CPR per transfer was significantly different in the GnRHa group compared with that in the control group. CPR, clinical pregnancy rate; GnRH, gonadotropin-releasing hormone; GnRH-a, long GnRH agonist ovarian stimulation protocol; GnRH-A, GnRH antagonist multi low-dose ovarian stimulation protocol; GnRHa, single-dose GnRH agonist for luteal support; Control: Regular luteal support; MH, Mantel-Haentzel; df, degrees of freedom.

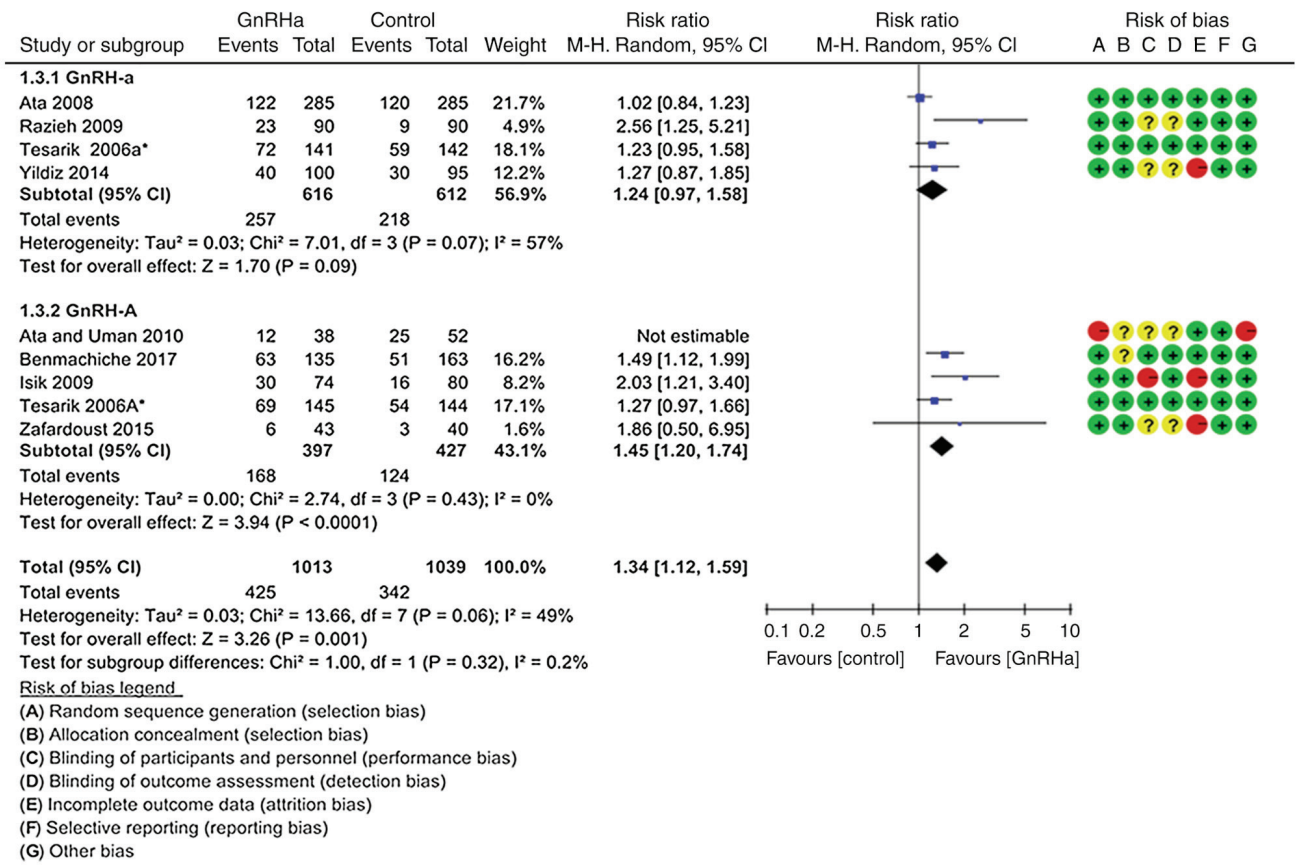

Figure 5. Forest plot for the sensitivity analysis for the CPR. Sensitivity analysis excluding the study by Ata and Urman was performed, and the results suggested that the pooled CPR per transfer was significantly higher in the GnRHa group compared with that in the control group. This trend was more significant following the GnRH-A ovarian stimulation protocol. However, there were no significant differences in the GnRH-a ovarian stimulation protocol compared with the control group. CPR, clinical pregnancy rate; GnRH, gonadotropin-releasing hormone; GnRH-a, long GnRH agonist ovarian stimulation protocol; GnRH-A, GnRH antagonist multi low-dose ovarian stimulation protocol; GnRHa, single-dose GnRH agonist for luteal support; Control, regular luteal support; MH, Mantel-Haentzel; df, degrees of freedom.

Multiple pregnancy rate per pregnancy. As indicated in Fig. 6, the rate of multiple pregnancy per pregnancy was significantly higher in the GnRHa group (32.82%; 106/323) compared with that in the control group (19.17%; 51/266; P=0.020; RR=2.37, 95% CI=1.17-4.81). In total, four studies were included (19-21,24). In the subgroup of trials where the GnRH-A ovarian stimulation protocol was used, the multiple pregnancy rate per pregnancy was significantly higher in the GnRH-A group (33.68%; 32/95) than that in the control group (8.06%; 5/62; P=0.003; RR=3.70; 95% CI=1.57-8.69) (19,21). However, the difference between the GnRH-a ovarian stimulation protocol and the control group was not significant (P=0.140; RR=1.88; 95% CI=0.82-4.34; Fig. 6) (19,20,24).

Clinical abortion rate. As indicated in Fig. 7, the clinical abortion rate was not significantly different between the GnRHa group (14.46%; 59/408) and the control group (16.90%; 60/355; P=0.790; RR=0.94; 95% CI=0.61-1.45). These results were obtained from six studies (19-21,23,24,26). There were also no significant differences in the subgroup analyses.

According to the sensitivity analysis excluding the study by Ata and Urman (23), the clinical abortion rates were not significantly different between the GnRHa group and the control group (P=0.310; RR=0.84, 95% CI= 0.61-1.17; I^2=0%; Fig. 8). In addition, the subgroup analyses did not provide any significant differences.

Risk of bias of the individual studies are presented in the forest plots shown in Figs. 2-8. There was 'high risk' and high heterogeneity in the study by Ata and Urman (23). The high risk of this study mainly comes from selection bias (random sequence generation and allocation concealment) and other bias.

Discussion

In 1993, Wilshire *et al* (27) reported that the use of GnRH agonist during early pregnancy did not have any adverse effect on pregnancy outcomes. Over the past years, the question regarding whether GnRH agonist may be used during LPS to improve pregnancy outcomes has attracted increasing attention. Zafardoust *et al* (25) indicated that subcutaneous injection of 0.1 mg triptorelin on the 6th day after oocyte collection increased the rate of embryo implantation and pregnancy rate in ICSI patients who were downregulated following GnRH antagonist regimen. A previous study identified increased levels of LH, as well as increased embryo implantation and CPRs, following intranasal inhalation of buserelin as LPS treatment compared with those following vaginal administration

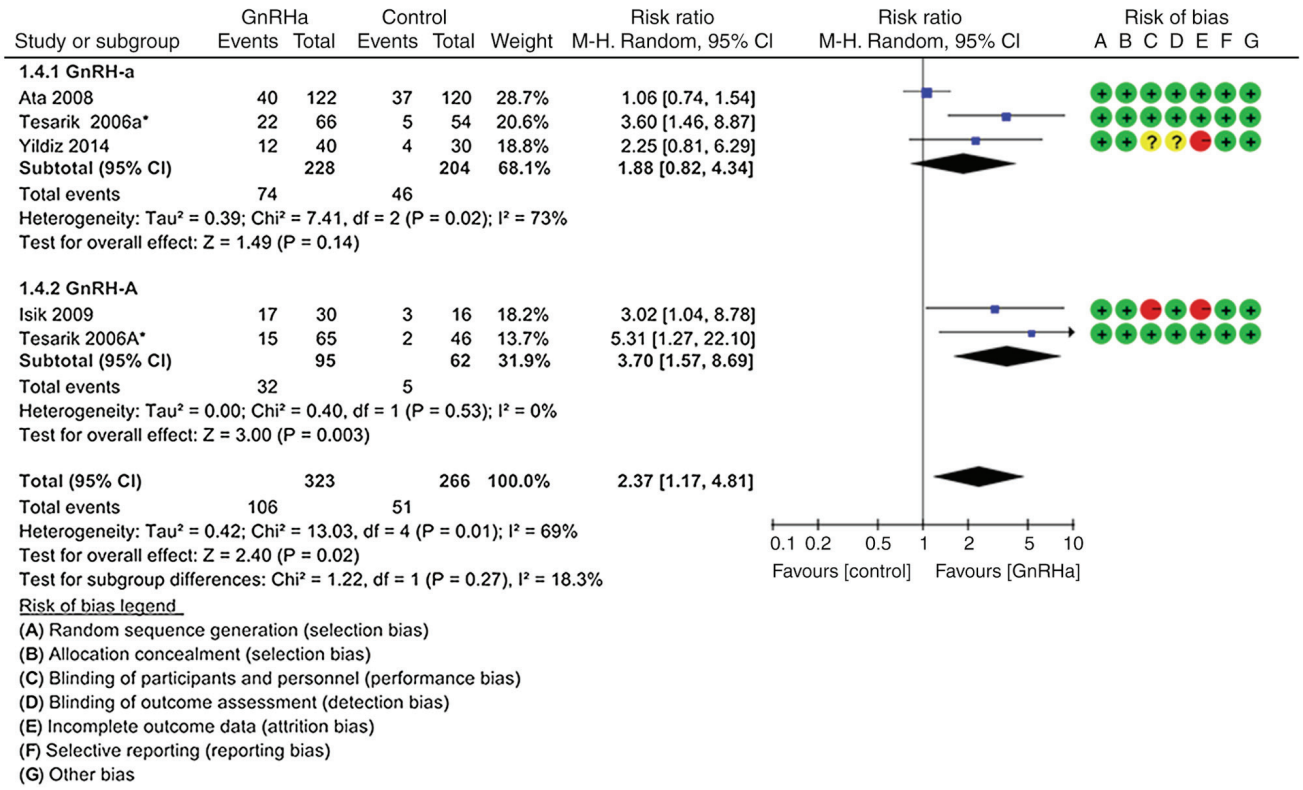

Figure 6. Forest plot for the multiple pregnancy rate. The probability of multiple pregnancy per pregnancy was significantly higher in the GnRHa group compared with that in the control group. In total, four studies were included. In the subgroup of trials using the GnRH-A ovarian stimulation protocol, the multiple pregnancy rate was higher in the GnRHa group than in the control group. However, there were no significant differences in the GnRH-a ovarian stimulation protocol. GnRH, gonadotropin-releasing hormone; GnRH-a, long GnRH agonist ovarian stimulation protocol; GnRH-A, GnRH antagonist multi low-dose ovarian stimulation protocol; GnRHa, single-dose GnRH agonist for luteal support; Control, regular luteal support; MH, Mantel-Haentzel; df, degrees of freedom.

of progesterone, but the differences were not statistically significant (28). However, Aboulghar *et al* (29) indicated that continuous injection of GnRH agonist at 0.1 mg/day from the luteal phase to hCG test day (14 days after embryo transfer) was not sufficient to increase the rate of ongoing pregnancy and CPR, and exhibited a negative effect; therefore, the effect of GnRH treatment in LPS remains controversial.

The studies included were single-center RCTs with a small sample size, and the present study included nine records from eight studies with a total of 2,142 embryo transfer cycles. The present results suggested that addition of GnRH agonist during LPS significantly increased the ongoing pregnancy or live birth rate per transfer, CPR per transfer and multiple pregnancy rate per pregnancy. The present meta-analysis suggested that the majority of the present results exhibited high heterogeneity. Therefore, a sensitivity analysis was performed using Revman 5.3 software, and it was indicated that the high heterogeneity was primarily caused by the study by Ata and Urman (23). Sensitivity analysis was performed following the exclusion of this study, resulting in significantly higher rates of ongoing pregnancy or live birth per transfer (P=0.002), clinical pregnancy per transfer (P=0.001) and multiple pregnancy per pregnancy (P=0.020) in the GnRHa group compared with those in the control group. In addition, meta-analysis was performed for subgroups of trials that had used a long GnRH-a ovarian stimulation protocol, indicating that ongoing pregnancy or live birth rate per transfer (P=0.080), CPR per transfer (P=0.090) and multiple pregnancy rate per pregnancy (P=0.140) were not significantly different between the two groups. Furthermore, the results from trials that had used GnRH-A multi-dose ovarian stimulation protocols indicated significantly higher ongoing pregnancy or live birth rate per transfer (P=0.010), CPR per transfer (P<0.0001) and multiple pregnancy rate per pregnancy (P=0.003) compared with those in the control group. Therefore, the present results suggested that the addition of single-dose GnRH agonist during LPS was clinically beneficial for pregnancy outcomes.

At present, the mechanisms underlying the use of GnRH agonist to improve pregnancy outcomes remain to be fully elucidated. Previous studies have suggested various possible mechanisms of action of GnRH agonist. GnRH agonist may act on the pituitary gland or ovary during the luteal phase (30-32); GnRH agonist may stimulate the ovaries to produce estrogen and P by stimulating the pituitary to produce LH, and GnRH agonist may produce estrogen and P by acting directly on the corpus luteum (20). Furthermore, GnRH agonist may directly act on the embryo and placenta during implantation (8); GnRH agonist promotes the expression of GnRH agonist receptor in placental cytotrophoblasts and syncytiotrophoblasts and increases serum hCG levels by upregulating GnRH agonist receptor levels and stimulating placental production of hCG, thus improving the implantation ability of the embryo (33,34). In addition, GnRH agonist promoted the growth of mouse embryos when it was added in the culture medium; however,

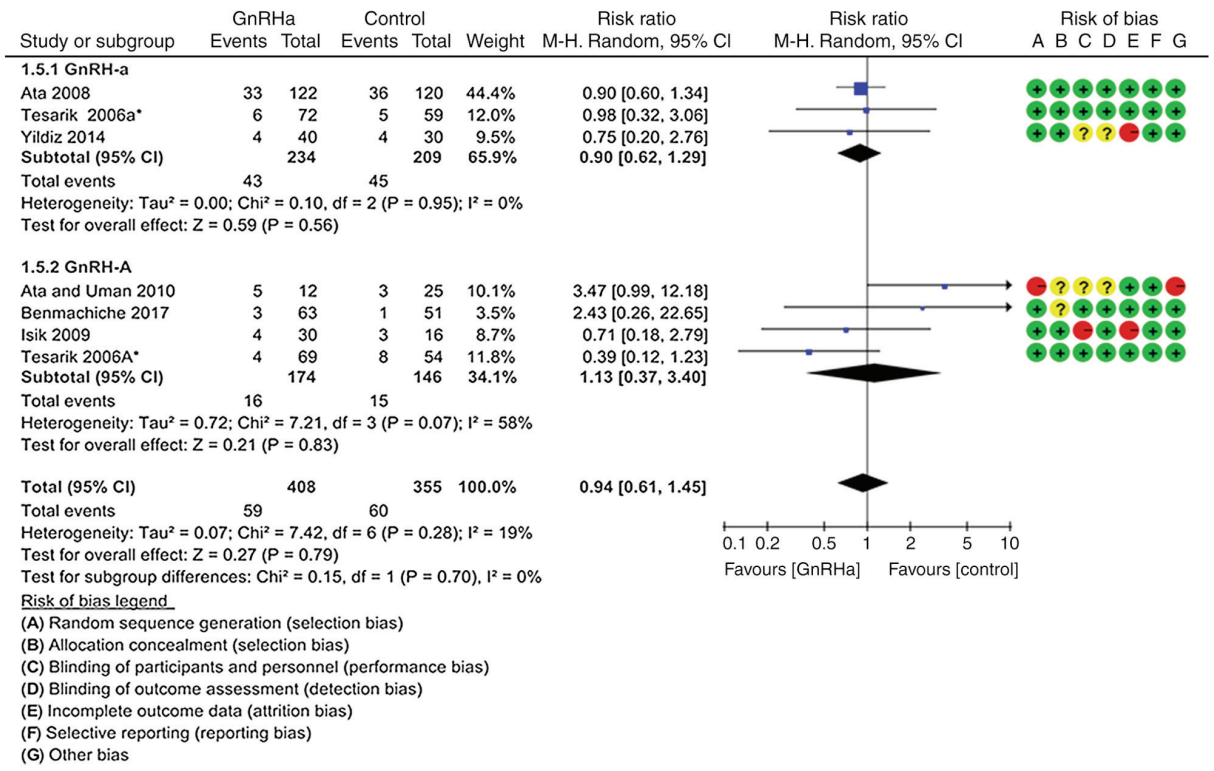

Figure 7. Forest plot for the clinical abortion rate. The clinical abortion rate was not significantly different between the GnRHa group and the control group. The results were derived from six studies. GnRH, gonadotropin-releasing hormone; GnRH-a, long GnRH agonist ovarian stimulation protocol; GnRH-A, GnRH antagonist multi low-dose ovarian stimulation protocol; GnRHa, single-dose GnRH agonist for luteal support; Control, regular luteal support; MH, Mantel-Haentzel; df, degrees of freedom.

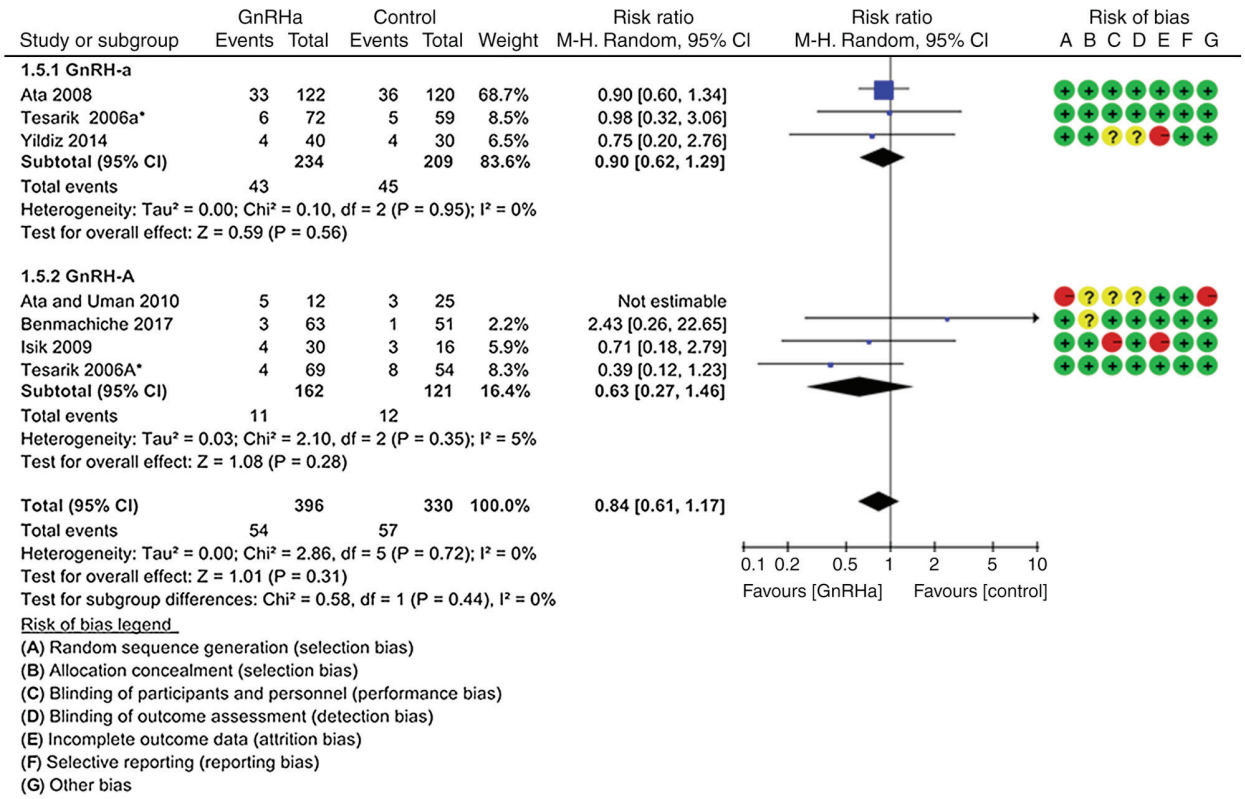

Figure 8. Forest plot for the sensitivity analysis for the clinical abortion rate. Sensitivity analysis excluding the study by Ata and Urman comprised five studies, where no significance was observed. GnRH, gonadotropin-releasing hormone; GnRH-a, long GnRH agonist ovarian stimulation protocol; GnRH-A, GnRH antagonist multi low-dose ovarian stimulation protocol; GnRHa, single-dose GnRH agonist for luteal support; Control, regular luteal support; MH, Mantel-Haentzel; df, degrees of freedom.

GnRH antagonist had a detrimental effect on mouse embryos (11). Therefore, it is possible that pre-implantation mouse embryos may express the GnRH agonist receptor. Furthermore, GnRH agonist may directly act via the GnRH agonist receptor on the surface of the endometrium (8,9).

The major limitation of the present meta-analysis is that there may be publication bias in the studies analyzed, and this may be due to a number of reasons; the present analysis only included studies including full texts, and abstract-only papers were excluded due to the lack of complete statistical data. In addition, three of articles included in the meta-analysis (21,24,25) exhibited a high risk of bias. In addition, the evidence is limited to recommend the use of GnRHa in the luteal phase support. Therefore, multi-center randomized controlled studies following a unified standardized scheme are required.

In conclusion, the present meta-analysis study suggested that administration of single-dose GnRH agonist for LPS in females partaking in IVF/ICSI was able to increase the ongoing pregnancy or live birth rate per transfer, CPR per transfer and multiple pregnancy rate per pregnancy. However, GnRH agonist did not affect the clinical abortion rate. In addition, since the multiple pregnancy risk increased significantly, GnRH agonist administration may be a better option for LPS with single embryo transfer. Therefore, GnRH agonist treatment may be an ideal choice for LPS in patients undergoing IVF/ICSI. However, further RCTs or multi-center randomized controlled studies are required prior to clinical application of GnRH agonist.

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Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

Authors' contributions

MLS conceived the study and was a major contributor in writing the manuscript. CLL participated in the design and coordination of the study and helped to draft the manuscript. RH performed the statistical analysis. FMW and ZHH contributed to the collection, analysis of data and edited the manuscript. All authors have 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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