Single-Dose Versus Multiple-Dose GnRH Agonist for Luteal-Phase Support in Women Undergoing IVF/ICSI Cycles: A Network Meta-Analysis of Randomized Controlled Trials

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Background: Although gonadotropin-releasing hormone (GnRH) agonist has been introduced as a beneficial luteal phase support (LPS), the optimal strategy of GnRH agonist remains unclear. This network meta-analysis was therefore performed to determine the comparative efficacy and safety of multiple-dose versus single-dose GnRH agonist protocol for LPS in patients undergoing IVF/ICSI cycles.

Methods: We searched relevant studies in PubMed, Embase and the Cochrane Registry of Controlled Trials (CENTRAL) from their inception util to September 2021. Live birth, clinical pregnancy rate, multiple pregnancy rate, and clinical abortion rate was evaluated. Pairwise and network meta-analysis were conducted using RevMan and ADDIS based on random-effects model, respectively. Moreover, the prioritization of protocols based on ranking probabilities for different outcomes were performed.

Results: Sixteen RCTs met our eligibility criteria. Pairwise meta-analysis showed that multiple-dose protocol of GnRH agonist was effective for increasing live birth rate (OR 1.80, 95% CI 1.15 to 2.83, p=0.01) and clinical pregnancy rate (OR 1.89, 95% CI 1.01 to 3.56, p=0.05) as well as decreasing clinical abortion rate (OR 0.55, 95% CI 0.34 to 0.90, p=0.02). Meanwhile, single-dose protocol of GnRH agonist was effective for increasing clinical pregnancy rate (OR 1.45, 95% CI 1.11 to 1.89, p=0.007) and multiple pregnancy rate (OR 2.55, 95% CI 1.12 to 5.78, p=0.03). However, network meta-analysis only confirmed that multiple-dose protocol of GnRH agonist was the best efficacious strategy for live birth rate (OR 2.04, 95% CrI 1.19 to 3.93) and clinical pregnancy rate (OR 2.10, 95% CrI 1.26 to 3.54).

Conclusion: Based on the results of NMA, multiple-dose protocol may be the optimal strategy for patients undergoing IVF/ICSI cycles owing to its advantage in increasing live

birth and clinical pregnancy rate. Moreover, single-dose protocol may be the optimal strategy for improving multiple pregnancy rate. However, with the limitations, more RCTs are required to confirm our findings.

1 INTRODUCTION

In vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) has been extensively accepted for fertility aid among couples with infertility, and more than 1 million cycles were actually reported every year around the world (1). However, patients receiving IVF/ICSI cycles commonly suffered from luteal-phase deficiency (LPD) due to the use of controlled ovarian stimulation (COS) based on gonadotrophin-releasing hormone agonist (GnRH-a) or antagonist protocols (2–4). It's noted that LPD was linked to several adverse pregnancy outcomes, such as a relatively lower embryo implantation rate, clinical pregnancy rate and live birth rate (5). Therefore, it's vitally important to provide exogenous luteal-phase support (LPS) for paying compensation to the progesterone levels (6). Actually, a number of LPS protocols have been investigated, such as estradiol, progesterone, human chorionic gonadotropin (hCG), gonadotropin-releasing hormone (GnRH) agonists or combinations of these types (7, 8).

Among available exogenous LPS protocols, progesterone and hCG were widely used in clinical practice. However, compared with progesterone, hCG was linked to increased risk of ovarian hyperstimulation syndrome (5, 9). From the perspective of safety, progesterone should be preferentially selected. Nevertheless, the optimal administration route remains unclear (10, 11). Beyond that, some other modalities are currently under investigation, such as estrogen, ascorbic acid, and acupuncture (12). It's exciting that gonadotropin-releasing hormone (GnRH) agonist protocol recently introduced as a beneficial LPS (13, 14), and studies have indicated the positive role of the administration of a single-dose GnRH agonist protocol in IVF/ICSI cycles (2, 15–17).

However, in addition to single-dose administration, multiple-dose protocol of GnRH agonist as a LPS protocol has become more and more common (16). Up to now, the paucity of studies directly compared single-dose versus multiple-dose GnRH agonist protocol (18–20) although there were relatively numerous studies directly comparing single-dose (16) or multiple-dose (21–23) protocol with control protocols, respectively. As a result, optimal administration strategy of GnRH agonist remains debated. We therefore collected all available randomized controlled trials (RCTs) to conduct a network meta-analysis for the comparative efficacy and safety in single-dose versus multiple-dose GnRH agonist protocols among patients undergoing IVF/ICSI cycles. We also provided the hierarchies of the comparative live birth rate, clinical pregnancy rate, multiple pregnancy rate and clinical abortion rate on two protocols.

2 MATERIALS AND METHODS

We performed the present network meta-analysis according to the preferred reporting items for systematic reviews and meta-analysis (PRISMA) for network meta-analysis (PRISMA-NMA) (24, 25) and the Cochrane handbook for reviewer of systematic review (26). We did not register the formal protocol in public platform.

2.1 Search Strategy

We developed the search strategy according to a previous meta-analysis (16). We firstly identified five distinctive keywords as follows: gonadotropin-releasing hormone agonist, single-dose, multiple-dose, fertilization *in vitro*, and intracytoplasmic sperm injections. Then, we identified the medical subject heading (MeSH) based on MeSH database, and further determined possible expressions of all keywords. An electronic literature search was independently performed by two reviewers in PubMed, Embase (based on Ovid) and the Cochrane Registry of Controlled Trials (CENTRAL) (based on Ovid) from their inception until to 30 September 2021. Detailed search strategy were summarized in Table S1.

2.2 Eligibility Criteria

According to previous meta-analysis (16), we included RCTs which assessed the comparative efficacy and safety of single-dose versus multiple-dose GnRH agonist protocols as LPS on IVF/ICSI outcomes in this network meta-analysis. The following exclusion criteria were imposed: (a) studies with ineligible design, such as summary, discuss theory, letters, case reports, comments, meta-analysis, review, and other types of research literature; (b) duplicate publications and data were unavailable to odds ratios (OR); (c) patients with egg donation and frozen embryo transfer.

2.3 Study Selection

Results retrieved were firstly imported into EndNote software for the removal of duplicate records and initial screening. Manual forwards and backwards reference searching were done on all included studies to identify additional relevant studies. Titles, abstracts, and full texts were examined independently by two reviewers. Any conflicting was resolved through discussion until the consensus was achieved.

2.4 Definition of Outcomes

We evaluated four outcomes in this network meta-analysis, including live birth rate, clinical pregnancy rate, multiple pregnancy rate and clinical abortion rate. We used the ongoing pregnancy rate as the surrogate of the live birth rate when the data was not available because of the difference between both two

data can be ignored (27, 28). When studies reported on clinical pregnancy and ongoing pregnancy without miscarriage rates, the number of clinical abortion rate was seen as being equal to the difference between the number of clinical pregnancy rate and ongoing pregnancy rate.

2.5 Data Extraction
The following essential data were collected from each study, including the name of the first author, year of publication, sample size and country, details of ovarian stimulation protocol, details of LPS protocols, statistical findings and details of methodological quality. Two independent reviewers collected these data, and any conflicting between the two reviewers was settled by consensus principle.

2.6 Evidence Network
We displayed the current status of available evidence in terms of all outcomes through creating evidence network which was conducted using ADDIS software. In evidence network, solid line connecting two protocols indicated the presence of direct comparison and the numerical value marked in line indicated the number of eligible studies for each direct comparison.

2.7 Quality Assessment
Two independent reviewers used the Cochrane Risk of Bias tool (29) to assess the risk of bias from 6 aspects as follows: selection bias (random sequence generation and concealment of allocation), performance bias (blinding of investigators and participants), detection bias (blinding of outcome assessors), attrition bias (incomplete outcome data), reporting bias (selective reporting), and other sources of bias (e.g., inadequate sample size and funding bias). Each aspect was labeled as "low", "unclear", or "high" risk of bias according to the actual information. Consensus principle was utilized to address inconsistency between two reviewers.

2.8 Statistical Analysis
2.8.1 Pairwise Meta-Analysis
We firstly used RevMan version 5.4 (Review Manager, the Cochrane Collaboration, 2020) to conduct pairwise meta-analysis. Heterogeneity across studies was assessed using the Cochrane Q test (30) and I^2 value (31). All outcomes which were analyzed in this network meta-analysis were categorical variables, and thus we used the odds ratio (OR) with corresponding 95% confidence interval (CI) to express pooled estimates of all outcomes.

2.8.2 Network Meta-Analysis
We utilized the Aggregate Data Drug Information System (ADDIS) V.1.16.8 (Drugis, Groningen, NL) to conduct network meta-analysis (32). A Bayesian network meta-analysis was performed using a random effects model for integrating the direct and indirect evidence with the Markov Chain Monte Carlo (MCMC) method. The parameters for the network meta-analysis in the ADDIS were as follows: the number of chains, 4; tuning iterations, 20,000; simulation iterations, 50,000; thinning interval, 10; inference samples, 10,000; and variance scaling factor, 2.5 (33). The potential scale reduced factor (PSRF) was

used to evaluate convergence (34, 35). Good convergence was achieved when PSRF was close to or equaled to 1, indicating high reliability of the conclusions of the consistency model analysis. Model convergence will be assessed by visual inspection of the trace plots and after considering the Gelman-Rubin statistic. Results from network meta-analysis were expressed as OR, accompanied with 95% credible interval (CrI). Before conducted quantitative synthesis, we firstly used split node method to examine the possibility of inconsistency between direct and indirect effects (36, 37). A consistency model was used when the P value >0.05 in the node-splitting analysis; otherwise, the inconsistency model was used. Finally, the relevant rank plots based on probabilities of intervention for the different endpoints were shown by ADDIS (38).

2.8.3 Assessment of Publication Bias
We used funnel plot, which was created using RevMan software, to evaluate the robustness of pooled results when the accumulated numbers of eligible studies were more than 10 (39).

3 RESULTS
3.1 Identification of Studies
We identified 601 records from electronic databases. Total 420 studies were retained for initial screening based on the titles and abstracts after the removal of 188 duplicate records. Among them, 24 studies were retrieved for full-text evaluation after excluding 396 ineligible studies. Then, 9 unique studies (18, 19, 21, 40–45) were considered to meet selection criteria after excluding 8 ineligible studies due to conference abstract without sufficient data and ineligible topic. Additional 7 eligible studies (13, 22, 23, 46, 47) were retrieved from previous meta-analysis and reference lists, and then we included 16 eligible studies (13, 18, 19, 21–23, 40–49) in this network meta-analysis. Flow chart for the literature search and study selection was presented in **Figure 1**.

3.2 Characteristics of the Included Studies
Among 16 eligible studies, majority of studies were published in Turkey (18, 40–42) and Iran (21, 43–45), and remaining studies were published in Spain (49), Denmark (48), Japan (13), India (22), Belgium (46), Jordan (47), and China (19), respectively. Eight studies (40–45, 48, 49) compared single-dose versus controls, six studies (13, 21–23, 46, 47) compared multiple-dose versus controls, one study (19) compared single-dose versus multiple-dose, and one study (18) simultaneously single-dose, multiple-dose, and control regime. **Table 1** shown the basic characteristics of each study, and **Figure 2** displayed the evidence network of each outcome.

3.3 Quality Assessment of Included Studies
Figures S1 displayed the results of the Cochrane risk of bias assessment for 16 eligible studies. Only one study (21) was classified as high risk owing to inadequate random sequence

FIGURE 1 | Flow chart for the literature search and study selection.
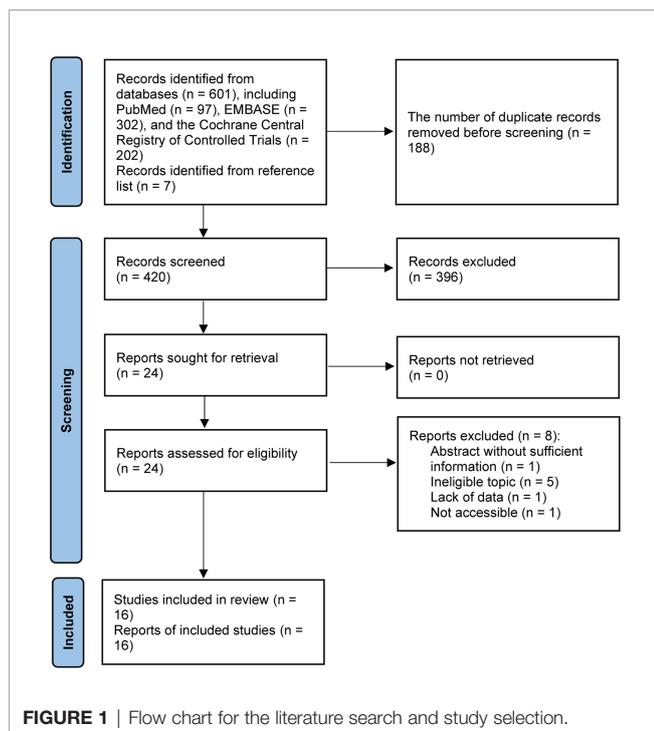
generation and allocation concealment. Two studies (19, 42) were classified as high risk because they did not use the correct blinding method. Four studies (18, 42, 45, 46) were classified as high risk owing to high patient attrition or inconsistencies in the amount of attrition between groups. Regarding other bias, two studies (40) were rated having a high risk of bias. Briefly, approximately 13.75% of the studies (18, 19, 21, 40, 42, 45, 46) were classified as high risk of overall bias.

3.4 Pairwise Meta-Analysis

We performed several pairwise meta-analyses to evaluate the comparative effects of two protocols with a combined effect size. According to the pooled results, multiple-dose GnRH agonist protocol was associated with increased live birth rate (6 RCTs; 27.42%% vs 19.45%; OR 1.80, 95% CI 1.15 to 2.83; I^2 = 53%; p = 0.01; low-quality evidence; **Figure S2**), higher clinical pregnancy rate (7 RCTs; 37.64%% vs 26.73%; OR 1.89, 95% CI 1.01 to 3.56; I^2 = 81%; p = 0.05; low-quality evidence; **Figure S3**) and lower clinical abortion rate (6 RCTs; 17.43%% vs 27.91%; OR 0.55, 95% CI 0.34 to 0.90; I^2 = 0%; p = 0.02; low-quality evidence; **Figure S5**) compared with control protocol. Single-dose GnRH agonist protocol significantly increased clinical pregnancy rate (9 RCTs; 39.98% vs 32.84%; OR 1.45, 95% CI 1.11 to 1.89; I^2 = 50.0%; p = 0.007; low-quality evidence; **Figure S3**) and multiple pregnancy rate (5 RCTs; 29.97% vs 17.91%; OR 2.55, 95% CI 1.12 to 5.78; I^2 = 67%; p = 0.03; low-quality evidence; **Figure S4**) compared with control protocol. However, multiple-dose GnRH agonist protocol reported higher live birth rate (2 RCTs; 42.74% vs 31.43%; OR 0.56, 95% CI 0.26 to 1.19; I^2 = 45%; p = 0.13; very low-quality evidence; **Figure S2**), clinical pregnancy rate (2 RCTs; 47.58% vs 35.0%; OR 0.51, 95% CI 0.20 to 1.31; I^2 = 65%; p = 0.16; very low-quality evidence; **Figure S3**) and multiple

pregnancy rate (2 RCTs; 30.51% vs 26.53%; OR 0.69, 95% CI 0.29 to 1.65; I^2 = 0%; p = 0.40; very low-quality evidence; **Figure S4**) as well as lower clinical abortion rate (2 RCTs; 10.17% vs 10.20%; OR 1.11, 95% CI 0.30 to 4.11; I^2 = 0%; p = 0.88; very low-quality evidence; **Figure S5**) compared with single-dose GnRH agonist protocol although no significant difference was detected.

3.5 Network Meta-Analysis

Network meta-analysis based on consistency model further confirmed the efficacious efficacy of multiple-dose GnRH agonist protocol in increasing live birth rate (OR 2.04, 95% CrI 1.19 to 3.93) and clinical pregnancy rate (OR 2.10, 95% CrI 1.26 to 3.54) compared with control protocol (protocol or no protocol). However, single-dose GnRH agonist protocol generated relatively lower point estimates in terms of live birth rate (OR 0.59, 95% CrI 0.27 to 1.11) and clinical pregnancy rate (OR 0.67, 95% CrI 0.36 to 1.24) and relatively higher estimates for multiple pregnancy rate (OR 1.48, 95% CrI 0.39 to 6.40) and clinical abortion rate (OR 1.39, 95% CrI 0.62 to 3.18) compared with multiple-dose GnRH agonist protocol although no statistical difference was detected. Results of network meta-analysis were shown in **Table 2**.

3.6 Consistency Examination

Node-splitting analysis was implemented to evaluate inconsistency by comparing the differences between direct and indirect evidence. For all available comparisons with at least one closed loop, split-node analysis did not suggest inconsistency between direct and indirect evidence, which were summarized in Table S2. As a result, we convinced that results calculated from consistency model were reliable and robust.

3.7 Ranking Probability

We generated a ranking probability matrix of each outcome using ADDIS software, and results revealed that multiple-dose GnRH agonist protocol was the most effective protocol for all outcomes compared with single-dose GnRH agonist protocol. Specific rank probability of each protocol for available outcomes was summarized in Table S3, and the rank probability diagram was shown in **Figure 3**. According to the results of rank probabilities, multiple-dose GnRH agonist protocol was the best efficacious option for live birth rate (95%), clinical pregnancy rate (91%) and clinical abortion rate (79%), however single-dose GnRH agonist protocol was the best efficacious option for multiple pregnancy rate (71%).

3.8 Publication Bias Examination

Owing to the accumulated number of included studies of individual comparison was not more than 10, we therefore did not draw funnel plot to inspect whether the presence of publication bias.

4 DISCUSSION

LPD has been identified as a common question in all IVF/ICSI cycles, and various LPS protocols have been routinely used to improve pregnancy outcomes (50). Among available LPS

TABLE 1 | Characteristics of the included studies (n=16).

Author	Country	Sample size		Condition	Ovarian stimulation protocol	LPS protocol		Control	Other protocol	Day after ER
		Randomization	Final Analysis			SD protocol	MD protocol			
Tesarik, et al., (49)	Spain	300 vs 300	286 vs 286	ICSI	Long GnRH agonist protocol and GnRH antagonist protocol	0.1mg triptorelin 6 days after ICSI	n.a.	placebo	4mg E2 valerate, 400mg vaginal micronized progesterone, 250μg human recombinant hCG	3
Ata, et al., (41)	Turkey	285 vs 285	285 vs 285	ICSI	Long GnRH agonist protocol and r-FSH	0.1mg triptorelin 6 days after ICSI	n.a.	placebo	90mg vaginal progesterone gel	3
Ata, et al., (40)	Turkey	n.r.	38 vs 52	ICSI	Long GnRH agonist protocol and r-FSH	0.1mg triptorelin 6 days after ICSI	n.a.	placebo	90mg vaginal progesterone gel	3
Isik, et al., (42)	Turkey	82 vs 82	74 vs 80	ICSI	GnRH antagonist protocol and r-FSH/hMG	0.5mg leuprolide acetate 6 days after ICSI	n.a.	no placebo	600mg intravaginal micronized progesterone and 1500 IU hCG	3
Razieh, et al., (43)	Iran	90 vs 90	90 vs 90	ICSI	Long GnRH agonist protocol and r-FSH	0.1mg triptorelin 5 or 6 days after ICSI		placebo	800mg vaginal micronized progesterone	2 or 3
Yildiz, et al., (18)	Turkey	100 vs 100 vs 100	100 vs 84 vs 95	ICSI	Long GnRH agonist protocol and r-FSH	1mg leuprolide acetate 6 days after ICSI	two sequential doses 1mg leuprolide acetate 3 and 6 days after ICSI	no placebo	600mg vaginal micronized progesterone, 4mg 17E2	3
Zafardoust, et al., (45)	Iran	50 vs 50	43 vs 40	ICSI	GnRH antagonist protocol and r-FSH/hMG	0.1mg decapeptil 6 days after ICSI	n.a.	no placebo	800mg vaginal progesterone	3
Benmachiche, et al., (48)	Denmark	165 vs 163	165 vs 163	IVF/ICSI	GnRH antagonist protocol and r-FSH	0.1mg triptorelin 6 days after ICSI	n.a.	no placebo	4mg E2, 600mg vaginal micronized progesterone, 1500IU hCG	2 or 3
Saharkhiz, et al., (44)	Iran	125 vs 125	122 vs 118	ICSI	GnRH antagonist protocol and r-FSH	0.1mg triptorelin 6 days after ICSI	n.a.	placebo	400mg vaginal progesterone	2 or 3
Eftekhar, et al., (21)	Iran	84 vs 84	84 vs 84	IVF/ICSI	GnRH antagonist protocol and r-FSH	n.a.	two sequential doses 1mg leuprolide acetate 3 and 6 days after ICSI	no placebo	progesterone	2 or 3
Fujii, et al., (13)	Japan	309 vs 280	309 vs 280	IVF/ICSI	Long GnRH agonist protocol and r-FSH	n.a.	continuous 600 μg/d IN buserelin twice daily for 14 days after oocyte retrieval	no placebo	10mg dydrogesterone	2 or 3
Inamdar, et al., (22)	India	213 vs 213	213 vs 213	IVF	r-hCG	n.a.	three 1 mg doses of lupride 6 days after oocyte retrieval	no placebo	400mg vaginal progesterone, 100mg natural micronized progesterone	2
Pirard, et al., (46)	Belgium	40 vs 20	35 vs 18	IVF/ICSI	hMG	n.a.	daily administration of 0.25mg buserelin the day before ovulation trigger	no placebo	hMG	3
Qublan, et al., (47)	Jordan	60 vs 60	60 vs 60	IVF	Long GnRH agonist protocol	n.a.	three 0.1mg triptorelin at 1, 3 and 6 day after oocyte retrieval	placebo	vaginal progesterone	3

(Continued)

TABLE 1 | Continued

Author	Country	Sample size		Condition	Ovarian stimulation protocol	LPS protocol		Control	Other protocol	Day after ER
		Randomization	Final Analysis			SD protocol	MD protocol			
Salehpour, et al., (23)	Iran	21 *vs* 23	21 *vs* 23	ICSI	GnRH agonist protocol, hCG	n.a.	daily dose of 0.2mg triptorelin for 10 weeks	no placebo	400mg vaginal progesterone	3
Qu, et al., (19)	China	40 *vs* 40	40 *vs* 40	IVF	r-FSH/hMG	0.1mg decapeptyl 6 days after ICSI	daily injection of 0.1mg decapeptyl for 14 days	n.a.	90mg vaginal progesterone gel, 20mg dydrogesterone tablets	3

LPS, luteal-phase support; SD, single-dose; MD, multiple-dose; ER, embryo transfer; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; GnRH, gonadotropin-releasing hormone; f-FSH, human follicle stimulating hormone; hMG, human menopausal gonadotropin; hCG, human chorionic gonadotropin; n.a., not available.

protocols, administration of GnRH agonist protocol introduced recently as a favorable LPS (14). Despite existing, several reports determined the efficacy of single-dose GnRH agonist as a LPS protocol, optimal strategy of GnRH agonist remains undetermined because multiple-dose GnRH agonist protocol commonly used in clinical practice. In this study, we firstly introduced the network meta-analysis to comprehensively investigate the comparative efficacy and safety of single-dose versus multiple-dose GnRH agonist protocols through combining direct and indirect evidence. Based on our results, multiple-dose GnRH agonist protocol was the best efficacious and safe option for increasing the live birth rate and clinical pregnancy rate compared with control protocols. Meanwhile, single-dose GnRH agonist protocol was the best efficacious option for increasing the multiple pregnancy rate although there was no statistical difference between single-dose and multiple-dose GnRH agonist protocols.

It's noted that GnRH agonist protocol was not routinely utilized as a LPS support in many IVF centers partially resulted from the poor elucidation of the underlying mechanism of GnRH agonist (9, 49). As a result, it's imperative to deeply optimize the administration strategy of GnRH agonist, such as determination of the optimal dose in the present network meta-analysis, so that the role of GnRH agonist protocol could be definitively clinically investigated during IVF/ICSI cycles. Additionally, safety may be the potential contributor to the application of GnRH agonist protocol (19).

Although previously published studies revealed that GnRH agonist protocol might be associated with the increased risk of congenital abnormality, pregnancy loss rates and ectopic pregnancy rates (51, 52), recently published studies further demonstrated the safety of GnRH agonist protocol as LPS protocol (19, 53–55).

Up to now, there were numerous traditional pairwise meta-analyses investigated the efficacy and safety of single-dose GnRH agonist protocol. Among two recent meta-analyses, Song et al. included 8 eligible studies in the final analysis and found that, compared with placebo or no LPS, administration of single-dose GnRH agonist in the luteal-phase significantly increased clinical pregnancy rate and multiple pregnancy rate (16), which was consistent with our findings from pairwise meta-analysis although we included additional one eligible study (44). Moreover, Ma et al. investigated the role of single-dose and multiple-dose GnRH agonist protocols based on subgroup analysis (2), and results suggested that single-dose protocol significantly increased clinical pregnancy rate, ongoing pregnancy rate and live birth rate but not increase multiple pregnancy rate and decrease abortion rate compared with control protocol. Meanwhile, no statistical difference was identified between multiple-dose and control protocol in terms of clinical pregnancy rate. Results from meta-analysis by Ma et al. was partially inconsistent with our findings in pairwise meta-analysis. It's noted that ongoing pregnancy and live birth were combined as an individual outcome rather than two independent outcomes in our meta-analysis. Moreover, meta-analysis by Ma et al. only included 3
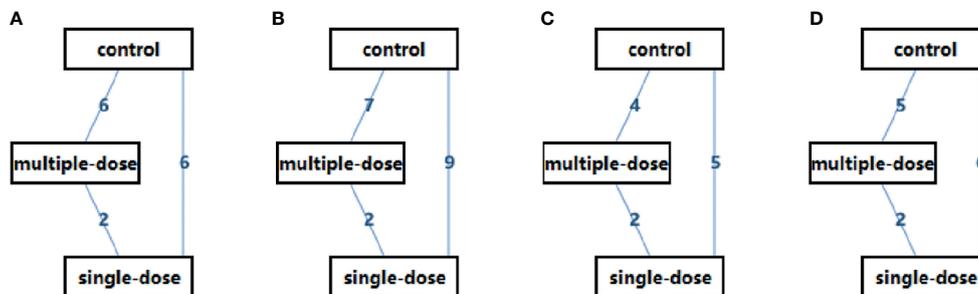

FIGURE 2 | Evidence Network of the eligible studies. **(A)** live birth rate, **(B)** clinical pregnancy rate, **(C)** multiple pregnancy rate, and **(D)** clinical abortion rate. D, single-dose; MD, multiple-dose.

TABLE 2 | Results of network meta-analysis.

Outcomes	Comparison, OR (95% CrI)		
	SD *vs* control	**MD *vs* control**	**SD *vs* MD**
Live birth rate	1.21 (0.69, 2.03)	**2.04 (1.19, 3.93)**	0.59 (0.27, 1.11)
Clinical pregnancy rate	1.40 (0.89, 2.19)	**2.10 (1.26, 3.54)**	0.67 (0.36, 1.24)
Multiple pregnancy rate	2.15 (0.68, 6.60)	1.45 (0.40, 4.51)	1.48 (0.39, 6.40)
Clinical abortion rate	0.94 (0.55, 1.71)	0.67 (0.36, 1.36)	1.39 (0.62, 3.18)

SD, single-dose; MD, multiple-dose; CrI, creditable interval. Bold numerical value indicates statistical significance.

eligible studies to calculate the pooled estimate of the multiple pregnancy rate, however 5 eligible studies were included for this outcome. Therefore, more sample size was accumulated to generate more reliable result in our pairwise meta-analysis. Additionally, Ma et al. also investigated the efficacy of multiple-dose protocol for clinical pregnancy rate based on 4 eligible studies and did not indicate statistical difference. However, our meta-analysis enrolled 7 eligible studies to calculate the pooled estimate and found that multiple-dose GnRH agonist protocol was linked to an increased clinical pregnancy rate. Compared with previous meta-analyses, our meta-analysis has a significant advantage because network meta-analysis technique was introduced to simultaneously combine direct and indirect evidence (56). Our network meta-analysis did not confirm the efficacy of single-dose GnRH agonist protocol for pregnancy outcomes but confirmed multiple-dose GnRH agonist protocol as the best efficacious and safe option due to its advantages in increasing the live birth rate and clinical pregnancy rate.

Our network meta-analysis has several methodological strengths as follow: (a) we applied a comprehensive search strategy to retrieve eligible studies and therefore decrease the risk of publication bias; (b) we firstly incorporated all available data from direct and indirect comparisons to investigate the comparative efficacy and safety of single-dose versus multiple-dose GnRH agonist protocols more precisely; and (c) we

calculated rank probabilities to distinguish the differences between single-dose and multiple-dose protocols.

Nevertheless, we recognized some limitations in our network meta-analysis. Firstly, inadequate number of eligible studies for the comparison between multiple-dose and control protocols as well as the comparison of single-dose and multiple-dose protocols was available, which may have adverse impact on our findings. Secondly, different ovarian stimulation protocols were used in eligible studies'; however, subgroup analysis was not performed to differentiate it due to inadequate eligible studies. Thirdly, despite the fact that GnRH agonist protocols were used in all eligible studies, different doses and drugs may introduce heterogeneity across studies. Therefore, our results should also be cautiously interpreted and further comparative study is required to determine the optimal dose and drug. Last but not least, three studies designed multiple-dose protocol, among them, two studies used two sequential doses at 3 and 6 days after ICSI, however another study sued daily injection protocol for 14 days.

5 CONCLUSION

Our network meta-analysis suggested that multiple-dose protocol of GnRH agonist has the significant advantage of higher live birth rate
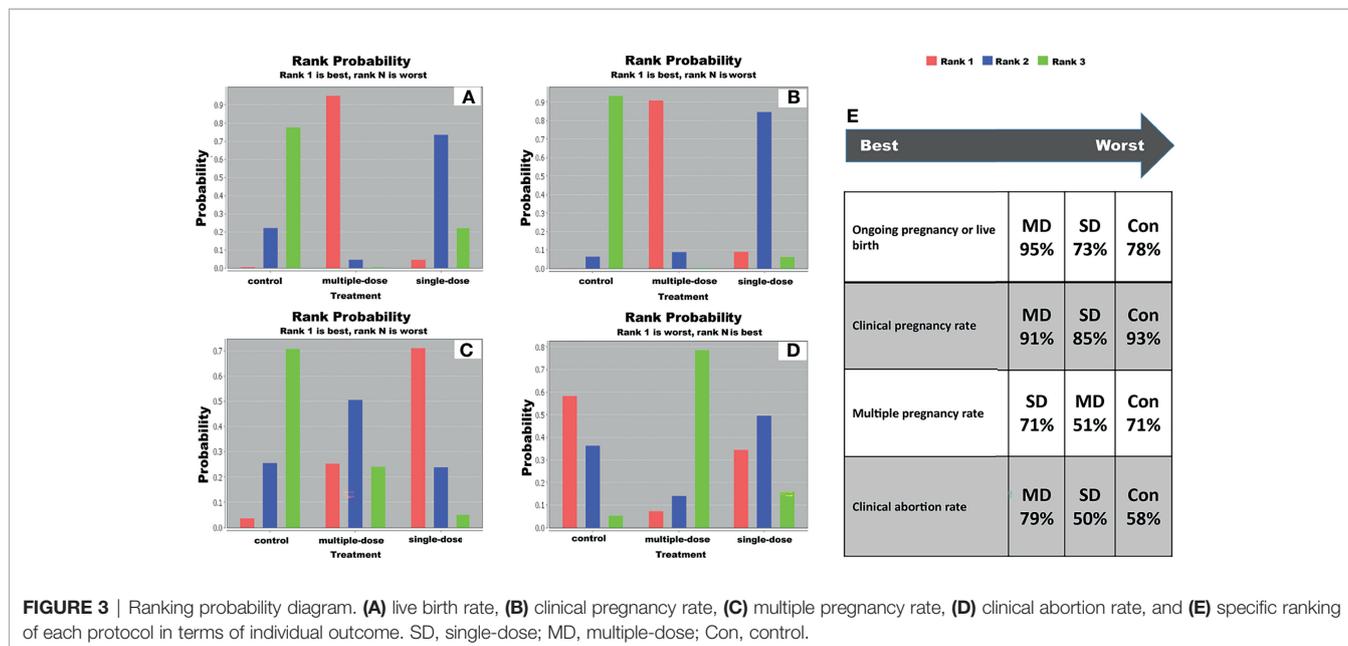

FIGURE 3 | Ranking probability diagram. **(A)** live birth rate, **(B)** clinical pregnancy rate, **(C)** multiple pregnancy rate, **(D)** clinical abortion rate, and **(E)** specific ranking of each protocol in terms of individual outcome. SD, single-dose; MD, multiple-dose; Con, control.

and clinical pregnancy rate than control protocol (placebo or no placebo), and multiple-dose protocol of GnRH agonist has relatively higher point estimates for effects and relatively lower point estimate for safety compared with single-dose protocol of GnRH agonist although no difference was detected. Therefore, multiple-dose protocol of GnRH agonist as the LPS protocol might be the most efficacious and safest option for increasing the live birth rate and clinical pregnancy rate among patients undergoing IVF/ICSI cycles. Moreover, single-dose protocol of GnRH agonist as the LPS protocol might be the most efficacious option for increasing the multiple pregnancy rate. However, as considering limitations of this network meta-analysis, our findings need additional and high-quality RCTs for further confirmation.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.802688/full#supplementary-material

Supplementary Figure 1 | Methodological quality assessment of 16 eligible studies. **(A)** risk of bias summary, and **(B)** risk of bias graph.

Supplementary Figure 2 | Meta-analysis of live birth rate. D, single-dose; MD, multiple-dose.

Supplementary Figure 3 | Meta-analysis of clinical pregnancy rate. D, single-dose; MD, multiple-dose.

Supplementary Figure 4 | Meta-analysis of multiple pregnancy rate. D, single-dose; MD, multiple-dose.

Supplementary Figure 5 | Meta-analysis of clinical abortion rate. D, single-dose; MD, multiple-dose.

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