Comparisons of GnRH antagonist protocol versus GnRH agonist long protocol in patients with normal ovarian reserve: A systematic review and meta-analysis

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Abstract

Objective
To evaluate the effectiveness and safety of gonadotropin-releasing hormone antagonist (GnRH-ant) protocol and gonadotropin-releasing hormone agonist (GnRH-a) long protocol in patients with normal ovarian reserve.

Methods
We searched the PubMed (1992–2016), Cochrane Library (1999–2016), Web of Science (1950–2016), Chinese Biomedical Database (CBM, 1979–2016), and China National Knowledge Infrastructure (CNKI, 1994–2016). Any randomized controlled trials (RCTs) that compared GnRH-ant protocol and GnRH-a long protocol in patients with normal ovarian reserve were included, and data were extracted independently by two reviewers. The meta-analysis was performed by Revman 5.3 software.

Results
Twenty-nine RCTs (6399 patients) were included in this meta-analysis. Stimulation days (mean difference (MD) [95% confidence interval (CI)] = -0.8 [-1.36, -0.23], P = 0.006), gonadotrophin (Gn) dosage (MD [95% CI] = -3.52 [-5.56, -1.48], P = 0.0007), estradiol (E2) level on the day of human chorionic gonadotrophin (HCG) administration (MD [95% CI] = -365.49 [-532.93, -198.05], P < 0.0001), the number of oocytes retrieved (MD [95% CI] = -1.41 [-1.84, -0.99], P < 0.00001), the embryos obtained (MD [95% CI] = -0.99 [-1.38, -0.59], P < 0.00001), incidence of ovarian hyperstimulation syndrome (OHSS) (OR [95% CI] = 0.69 [0.57, 0.83], P = 0.0001), the number of oocytes retrieved (MD [95% CI] = -1.41 [-1.84, -0.99], P < 0.00001), the embryos obtained (MD [95% CI] = -0.99 [-1.38, -0.59], P = 0.00001), incidence of ovarian hyperstimulation syndrome (OHSS) (OR [95% CI] = 0.69 [0.57, 0.83], P = 0.0001) were statistically significantly lower in GnRH-ant protocol than GnRH-a long protocol. However, the clinical pregnancy rate (OR [95% CI] = 0.90 [0.80, 1.01], P = 0.08), ongoing pregnancy rate (OR [95% CI] = 0.88 [0.77, 1.00], P = 0.05), live birth rate (OR [95% CI] = 0.05 [0.74, 1.09], P = 0.05), miscarriage rate (OR [95% CI] = 0.98 [0.69, 1.40], P = 0.93), and cycle cancellation rate (OR [95% CI] = 0.86 [0.52, 1.44], P = 0.57) showed no significant differences between the two groups.
Conclusion
GnRH-ant protocol substantially decreased the incidence of OHSS without influencing the pregnancy rate and live birth rate compared to GnRH-a long protocol among patients with normal ovarian reserve.

Introduction
Since gonadotropin-releasing hormone agonist (GnRH-a) was developed in the 1980s [1], it has played an important role in controlled ovarian hyperstimulation (COH) among patients who are undergoing assisted reproductive technology (ART). The advantage of GnRH agonist is to prevent premature luteinizing hormone (LH) surge, thereby increasing the number of retrieved oocytes and pregnancy rates and decreasing the number of cycle cancellations [2, 3]. These advantages, however, may lead to ovarian hyperstimulation syndrome (OHSS) or other side effects [4].

GnRH antagonist (GnRH-ant), which was discovered in the 1990s, can competitively block GnRH receptors and cause rapid suppression of Gn release [5]. This protocol has fewer complications and is more convenient for patients because of the shorter treatment time and fewer injections [6]. However, its effectiveness is still debated.

Multiple studies, including meta-analyses and randomized controlled trials (RCTs), of the GnRH-a protocol and GnRH-ant protocol on pregnancy rate and live birth rate have yielded controversial findings [6–8]. A 2006 Cochrane systematic review of 27 RCTs showed that GnRH-ant protocol has a significantly lower clinical pregnancy rate and live birth rates than those in GnRH-a long protocol, while the incidence of OHSS is significantly lower in GnRH-ant protocol [9]. However, a 2011 Cochrane systematic review of 45 RCTs found that there was no significant difference in the live birth rates between the GnRH-a and GnRH-ant groups [10]. A recent Cochrane systematic review of 73 RCTs in 2016 also concluded that these two protocols have equivalent live birth rates, and GnRH-ant protocol has a lower incidence of OHSS [11].

The finding that GnRH-ant protocol reduces the pregnancy rate may result from the fact that some centers only choose GnRH-ant protocol as their second treatment option in COH, or use it to treat the patients with an unfavorable prognosis, such as repeated implantation failures, older patients, and low responders [12]. This study’s purpose is to determine the effectiveness and safety of GnRH-a long protocol and GnRH-ant protocol among patients with normal ovarian reserve to unify the influencing factors.

Materials and methods
Search strategy
“GnRH agonist”, “GnRHa”, “GnRH antagonist”, “GnRH-ant”, “GnRHA”, “randomized controlled trial”, “RCT”, and “Normal ovarian reserve” were used as the keywords for the literature searches in the PubMed (1992–2016), Cochrane Library (1999–2016), Web of Science (1950–2016), Chinese Biomedical Database (CBM,1979–2016), and China National Knowledge Infrastructure (CNKI,1994–2016) databases. The retrieval time was from the first publication of the journal to the end of December 2016. References included in the studies were also searched.
Inclusion and exclusion criteria

Inclusion criteria were RCTs that compared the effectiveness and safety of GnRH-a long protocol and GnRH-ant protocol in patients with normal ovarian reserve. Exclusion criteria included failure to report appropriate randomized procedures, classification of participants as low or high ovarian response or endometriosis, and unclear or inappropriate outcomes. Editorials, letters to the editor, review articles, case reports and animal experimental studies were also excluded.

Data extraction and quality assessment

Studies were screened by two reviewers (R.W. and Y.W.) independently, and any disagreement was settled by consensus. First, the title and abstract of each study was read carefully to exclude the studies that clearly did not meet the inclusion criteria. Then, the full text of the remaining studies was read to determine which studies would be included in this study.

The quality assessment of RCTs was compiled using Cochrane’s risk of bias tool [13], which included sequence generation, allocation concealment blinding of participants, personnel and outcome assessors, incomplete outcome data, selective outcome reporting, and other sources of bias.

Outcome measures

The main efficacy outcome measures included the clinical pregnancy rate (defined as the presence of a gestational sac on ultrasound or gestational sac with fetal heart tones), the ongoing pregnancy rate (determined as pregnancies with over 12 weeks of gestation), and the live birth rate. The secondary efficacy outcome measures were ovarian stimulation outcomes, which included stimulation days, gonadotropin dosage, endometrial thickness and estradiol (E2) level on the day of human chorionic gonadotrophin (HCG) administration, the number of oocytes retrieved, and the embryos obtained. The safety outcome measures included the incidence of OHSS, the miscarriage rate, and the cycle cancellation rate.

Statistical analysis

All statistical analyses were performed using Revman 5.3 software. Dichotomous outcomes were expressed as odds ratios (ORs) with 95% confidence intervals (CIs). Continuous variables were expressed as weighted mean differences (MDs) with 95% CIs. Heterogeneity was evaluated using the Q-test and I²-index values, and reported for each outcome as a P-value and percentage, respectively. If heterogeneity was adopted (I² < 25% or > 50% with P > 0.1), meta-analysis used a fixed-effects model. Otherwise (I² ≥ 50% or I² > 25% with P ≤ 0.1), meta-analysis used a random-effects model. Sensitivity analysis was used to determine the stability of the results. Begg’s funnel plot and Begg’s test were used to assess publication bias by STATA software (version 12.0, Stata Corp).

Results

Eligible studies

The initial literature search yielded 1,304 studies. Screening of the titles and abstracts resulted in 68 published articles that could possibly compare the GnRH-a long protocol and GnRH-ant protocol in patients with normal ovarian reserve. There is no official definition of normal ovarian reserve. Therefore, the studies included in our article mainly depended on a consensus, which met any two of the following criteria: Age < 40 years, normal menstrual cycle, basal FSH ≤ 10 IU/L, basal E2 < 60 pg/ml, AFC > 5, previous IVF attempts < 3, no previous poor
response/OHSS history, no polycystic ovary syndrome (PCOS) / severe endometriosis. After reading the full papers, only 29 studies (6399 patients) finally met our inclusion criteria [8, 14–41]. Although the cited studies used different drugs and doses for COH, they all belong to either GnRH-ant protocol or GnRH-a protocol, so we included these studies in a general way. The literature screening process and the results are shown in Fig 1, and the basic characteristics of the included papers are shown in Table 1. The quality assessment results are shown in S1 Table.

Main outcome measures of effectiveness

The clinical pregnancy rate. A total of 25 studies [8, 14–23, 25–29, 32–39, 41](5,814 cases) were included in the clinical pregnancy rate meta-analysis. There was no heterogeneity (P = 0.91, I² = 0%) among the trials; therefore, the fixed-effects model was used for the meta-analysis. The results indicated that there was no statistically significant difference in the clinical pregnancy rate between the GnRH-ant group and the GnRH-a long-protocol group (OR [95% CI] = 0.90 [0.80, 1.01], P = 0.08, Fig 2).

The ongoing pregnancy rate. A total of 18 studies [8, 14–20, 23, 28, 30, 31, 34, 36–39, 41] (5,119 cases) were included in the ongoing pregnancy rate meta-analysis. There was no heterogeneity (P = 0.96, I² = 0%) among the trials; therefore, the fixed-effects model was used for the meta-analysis. The results indicated that there was no statistically significant difference in the ongoing pregnancy rate between the GnRH-ant group and the GnRH-a long-protocol group (OR [95% CI] = 0.88 [0.77, 1.00], P = 0.05, Fig 3).

The live birth rate. A total of 6 studies [8, 14, 31, 35, 37, 41](2,237 cases) were included in the live birth rate meta-analysis. There was no heterogeneity (P = 0.88, I² = 0%) among the trials; therefore, the fixed-effects model was used for the meta-analysis. The results indicated that there was no statistically significant difference in the live birth rate between the GnRH-ant group and the GnRH-a long-protocol group (OR [95% CI] = 0.95 [0.74, 1.09], P = 0.27, Fig 4).

Secondary outcome measures of effectiveness

The number of stimulation days. A total of 17 studies [8, 14, 16, 21–27, 31, 33–35, 39–41](3,171 cases) were included in the number of stimulation days meta-analysis. There was heterogeneity (P<0.00001, I² = 96%) among the trials; therefore, the random-effects model was used for the meta-analysis. The results indicated that the stimulation days were statistically significantly fewer in the GnRH-ant group than in the GnRH-a long-protocol group (MD [95% CI] = -0.8 [-1.36, -0.23], P = 0.006, Fig 5).

Gn dosage. A total of 18 studies [8, 14, 16, 21–27, 31–36, 40, 41](3,424 cases) were included in the Gn dosage meta-analysis. There was heterogeneity (P<0.00001, I² = 94%) among the trials; therefore, the random-effects model was used for the meta-analysis. The results indicated that the Gn dosage was statistically significantly less in the GnRH-ant group than in the GnRH-a long-protocol group (MD [95% CI] = -3.52 [-5.56, -1.48], P = 0.0007, Fig 6).

The endometrial thickness on the day of HCG administration. A total of 6 studies [23, 24, 27, 35, 36, 40](698 cases) were included in the meta-analysis of endometrial thickness on the day of HCG administration. There was no heterogeneity (P = 0.68, I² = 0%) among the trials; therefore, the fixed-effects model was used for the meta-analysis. The results indicated that there was no statistically significant difference in the endometrial thickness on the day of HCG administration between the GnRH-ant group and the GnRH-a long-protocol group (MD [95% CI] = -0.06 [-0.22, 0.11], P = 0.5, Fig 7).
The E2 level on the day of HCG administration. A total of 15 studies [8, 14, 16, 22–27, 32–34, 36, 39, 40](1,770 cases) were included in the E2 level on the day of HCG administration meta-analysis. There was heterogeneity ($P<0.0001, I^2 = 91\%$) among the trials; therefore, the random-effects model was used for the meta-analysis. The results indicated that the E2 level
Table 1. Characteristics of included studies.

| Study                          | Inclusion Criteria                                                                 | No. of patients | Protocol                              | Gn type & initial dosage (IU/d) |
|--------------------------------|-------------------------------------------------------------------------------------|-----------------|---------------------------------------|---------------------------------|
| Sauer et al. 2004              | Age <39 y, basal FSH <10 IU/L, regular menstrual cycle, previous IVF attempted ≤3   | 198             | Multiple dose (cetrorelix)            | Long, multiple dose (buserelin)  |
| European Orgalutran. 2000      | Age <39 y, BMI 18–29 kg/m², regular menstrual cycle                                | 486             | Multiple dose (ganirelix)            | Long, multiple dose (buserelin)  |
| Olivennes et al. 2000          | Age <39 y, basal FSH <10 IU/L, normal menstrual cycle, previous IVF attempts ≤3     | 126             | Single dose (cetrorelix)             | Long, single dose (triptorelin)  |
| European and Middle East        | Age <39 y, BMI 18–29 kg/m², regular menstrual cycle                                | 236             | Multiple dose (cetrorelix)            | Long, single dose (triptorelin)  |
| Fluker et al. 2001             | Age <39 y, basal FSH <10 IU/L, basal LH <10 IU/L, BMI 18–29 kg/m², regular menstrual cycle | 205             | Multiple dose (cetrorelix)            | Long, multiple dose (leuproline) |
| Sauer et al. 2004              | Age <39 y, BMI <35 kg/m², regular menstrual cycle, FSH level in normal range       | 25              | Single dose (cetrorelix)             | Long, multiple dose (leuproline) |
| Badrawi et al. 2005            | Age <39 y, basal FSH <10 IU/L, regular menstrual cycle                            | 50              | Multiple dose (cetrorelix)            | Long, multiple dose (buserelin)  |
| Barmat et al. 2005             | Age <39 y, BMI 19–32 kg/m², basal FSH <10 IU/L, E2<60 pg/ml, AFC >5, regular menstrual cycle, previous failed IVF cycle ≤1, no previous poor response/OHSS history | 40              | Multiple dose (cetrorelix)            | Long, multiple dose (leuproline) |
| Lee et al. 2005                | Age <39 y, BMI 18–29 kg/m², basal FSH <10 IU/L, regular menstrual cycle, no previous poor response history | 20              | Multiple dose (cetrorelix)            | Long, multiple dose (buserelin)  |
| Xavier et al. 2005             | Age <39 y, basal FSH <10 IU/L, previous IVF attempts ≤3                            | 66              | Multiple dose (cetrorelix)            | Long, multiple dose (buserelin)  |
| Ferrari et al. 2006            | Age <39 y, BMI 20–25 kg/m², basal FSH <10 IU/L, basal E2<45 IU/L, regular menstrual cycle | 30              | Multiple dose (cetrorelix)            | Long, multiple dose (leuproline) |
| Friedler et al. 2006           | Age <35 y, basal FSH <10 IU/L                                                    | 37              | Multiple dose (cetrorelix)            | Long, multiple dose (buserelin)  |
| Rombauts et al. 2006           | Age <39 y, BMI 18–29 kg/m², normal menstrual cycle, previous failed IVF cycle ≤3, no previous poor response history | 234             | Multiple dose (cetrorelix)            | Long, multiple dose (buserelin)  |
| Serafini et al. 2006           | Age ≤9 y, basal FSH<15 IU/L, basal E2<60 IU/L, previous IVF attempts <3, no previous poor response history | 217             | Multiple dose (cetrorelix)            | Long, multiple dose (buserelin)  |
| Baart et al. 2007              | Age <38, BMI 19–29 kg/m², regular menstrual cycle, no previous IVF attempt        | 67              | Multiple dose (orgalutran)            | Long, multiple dose (triptorelin) |
| Heijnen et al. 2007            | Age <38 y, BMI 18–28 kg/m², regular menstrual cycle, no previous IVF attempt       | 205             | Unclear                               | Long, unclear                    |
| Hsieh et al. 2008              | Age ≤39 y, body weight of 40–70 kg                                                | 86              | Multiple dose (cetrorelix)            | Long, multiple dose (leuproline) |
| Moraloglu et al. 2008          | Age ≤38 y, BMI <30 kg/m², basal FSH <10 IU/L, previous IVF attempts ≤3, no previous poor response history, no PCOS | 45              | Multiple dose (cetrorelix)            | Long, multiple dose (leuproline) |

(Continued)
Table 1. (Continued)

| Study                          | Inclusion Criteria                                                                 | No. of patients | Protocol | Gn type & initial dosage(IU/d) |
|-------------------------------|-------------------------------------------------------------------------------------|-----------------|----------|-------------------------------|
| Depalo et al. 2009            | Age \( \leq 42 \) y, basal FSH <10 IU/L, previous IVF attempts \( \leq 3 \), no PCOS/ severe endometriosis | 67,69           | Multiple dose (cetrorelix)    | Long, multiple dose (leuprolide) rFSH 225 |
| Ye et al. 2009                | Age \( \leq 35 \) y, BMI 18–25 kg/m\(^2\), previous IVF attempts \( < 3 \), no previous poor response history, normal-ovulatory cycles | 109, 111        | Multiple dose (cetrorelix)    | Long, multiple dose (leuprolide) rFSH 225 |
| Firouzabadi et al. 2010      | Age \( < 35 \) y, basal FSH <10 IU/L, no previous IVF attempt                       | 118, 107        | Multiple dose (cetrorelix)    | Long, multiple dose (buserelin) rFSH 150–225 |
| Papanikolaou et al. 2012      | Age \( < 39 \) y, basal FSH <12 IU/L, previous IVF attempts \( < 3 \)              | 96, 94          | Multiple dose (cetrorelix)    | Long, multiple dose (buserelin) rFSH 150–300 |
| Qiao et al. 2012              | Age \( \leq 35 \) y, BMI 18–29 kg/m\(^2\), normal menstrual cycle                 | 113, 120        | Multiple dose (ganirelix)     | Long, multiple dose (tripotrelin) rFSH unclear |
| Rabati et al. 2012            | basal FSH \( \leq 10 \) IU/L, first time of ART                                   | 69, 67          | Multiple dose (cetrorelix)    | Long, multiple dose (buserelin) rFSH 75 |
| Hershko et al. 2015           | Age \( < 37 \) y, previous failed IVF cycles \( \leq 3 \), without ovulatory factor(WHO I-III) | 31, 29          | Multiple dose (cetrorelix)    | Long, multiple dose (tripotrelin) rFSH/HMG |
| Toftager et al. 2016          | Age \( < 40 \) y, previous IVF attempts \( < 3 \)                               | 534, 516        | Multiple dose (ganirelix)     | Long, multiple dose (nafarelin) rFSH 150/225 |

https://doi.org/10.1371/journal.pone.0175985.t001

Fig 2. Forest plot comparing the clinical pregnancy rate per woman randomized between the GnRH-ant group and the GnRH-a long-protocol group.

https://doi.org/10.1371/journal.pone.0175985.g002
on the day of HCG administration was statistically significantly lower in the GnRH-ant group than in the GnRH-a long-protocol group (MD [95% CI] = -365.49 [-532.93, -198.05], P<0.0001, Fig 8).

The number of oocytes retrieved. A total of 22 studies [14–17, 20, 21, 23–27, 30–36, 38–41](4,919 cases) were included in the meta-analysis of the number of oocytes retrieved. There was heterogeneity (P = 0.01, I² = 45%) among the trials; therefore, the random-effects model was used for the meta-analysis. The results indicated that the retrieved oocytes were statistically significantly fewer in the GnRH-ant group than in the GnRH-a long-protocol group (MD [95% CI] = -1.41 [-1.84, -0.99], P<0.00001, Fig 9).

The number of embryos obtained. A total of 11 studies [16, 17, 22–24, 27, 30, 32, 33, 35, 38](1,588 cases) were included in the meta-analysis of the number of embryos obtained. There was no heterogeneity (P = 0.68, I² = 0%) among the trials; therefore, the fixed-effects model was
used for the meta-analysis. The results indicated that the obtained embryos were statistically significantly fewer in the GnRH-ant group than in the GnRH-a long-protocol group (MD [95% CI] = -0.99 [-1.38, -0.59], P < 0.00001, Fig 10).

Outcome measures of safety

The incidence of OHSS. A total of 21 studies [8, 14–19, 23–25, 28, 29, 31–33, 35–39, 41] (5,763 cases) were included in the incidence of OHSS meta-analysis. There was no

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**Fig 5.** Forest plot comparing the number of stimulation days per woman randomized between the GnRH-ant group and the GnRH-a long-protocol group.

https://doi.org/10.1371/journal.pone.0175985.g005

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**Fig 6.** Forest plot comparing the Gn dosage per woman randomized between the GnRH-ant group and the GnRH-a long-protocol group.

https://doi.org/10.1371/journal.pone.0175985.g006
heterogeneity ($P = 0.27, I^2 = 15\%$) among the trials; therefore, the fixed-effects model was used for the meta-analysis. The results indicated that the incidence of OHSS was statistically significantly lower in the GnRH-ant group than in the GnRH-a long-protocol group ($OR \ [95\% CI] = 0.69 \ [0.57, 0.83], P < 0.0001$, Fig 11).

The miscarriage rate. A total of 14 studies [8, 14–20, 23, 29–31, 33–36, 38, 41] (3,198 cases) were included in the miscarriage rate meta-analysis. There was no heterogeneity ($P = 0.94, I^2 = 0\%$) among the trials; therefore, the fixed-effects model was used for the meta-analysis. The results indicated that there was no statistically significant difference in the miscarriage rate between the GnRH-ant group and the GnRH-a long-protocol group ($OR \ [95\% CI] = 0.98 \ [0.69, 1.40], P = 0.93$, Fig 12).

The cycle cancellation rate. A total of 19 studies [8, 14–20, 23, 25, 29–31, 33–36, 38, 41] (5,209 cases) were included in the cycle cancellation rate meta-analysis. There was heterogeneity ($P = 0.004, I^2 = 53\%$) among the trials; therefore, the random-effects model was used for the meta-analysis. The results indicated that there was no statistically significant difference in the cycle cancellation rate between the GnRH-ant group and the GnRH-a long-protocol group ($OR \ [95\% CI] = 0.86 \ [0.52, 1.44], P = 0.57$, Fig 13).

Fig 7. Forest plot comparing the endometrial thickness on the day of HCG administration per woman randomized between the GnRH-ant group and the GnRH-a long-protocol group.

https://doi.org/10.1371/journal.pone.0175985.g007

Fig 8. Forest plot comparing the E2 level on the day of HCG administration per woman randomized between the GnRH-ant group and the GnRH-a long-protocol group.

https://doi.org/10.1371/journal.pone.0175985.g008
Sensitivity analysis and publication bias

The sensitivity analysis was performed by excluding the maximum weight studies [8, 17, 31, 36, 37, 41] in each outcome. The results showed that there was no influence on the pooled OR value; therefore, the outcomes are stable.

Begg’s funnel plots were symmetrical, and Begg’s tests had no significant publication bias (Begg’s test P > 0.05, S1–S12 Figs).

Discussion

Summary of main results

Ovarian reserve can be tested by biochemical measures, such as basal follicle stimulating hormone (bFSH), anti-Mullerian hormone (AMH), and inhibin B level, as well as imaging
measures, such as antral follicle count (AFC). The role of testing ovarian reserve is to help clinicians determine the appropriate protocol for individual patients, because ovarian reserve is the foundation of ovarian response to COH which may be vital for assisted reproductive technology (ART) outcome. Ovarian response can be divided into three types, low response, normal response, and high response. In 2011, the European Society of Human Reproduction and Embryology (ESHRE) standardized the definition of poor ovarian response [42], which usually occurs due to diminished ovarian reserve (DOR), and high response, which mainly refers to

![Fig 11. Forest plot comparing the incidence of OHSS per woman randomized between the GnRH-ant group and the GnRH-a long-protocol group.](https://doi.org/10.1371/journal.pone.0175985.g011)

![Fig 12. Forest plot comparing the miscarriage rate per woman randomized between the GnRH-ant group and the GnRH-a long-protocol group.](https://doi.org/10.1371/journal.pone.0175985.g012)
PCOS. However, there is no standard definition of normal ovarian reserve or normal response. Therefore, patients with normal ovarian reserve are included in our study to a certain extent based on a consensus.

Results of our meta-analysis mainly focused on the effectiveness and safety of GnRH antagonist protocol and GnRH agonist long protocol in COH. Regarding effectiveness, stimulation days, Gn dosage, E2 level on the day of HCG administration, and the number of oocytes retrieved, the embryos obtained were statistically significantly lower in GnRH-ant protocol than GnRH-a long protocol. The shorter stimulation period and lower Gn dosage in the GnRH-ant group may lead to the lower E2 level on the day of HCG administration, fewer retrieved oocytes, and fewer obtained embryos. However, regarding the main outcome measures of effectiveness, clinical pregnancy rate, ongoing pregnancy rate, and live birth rate had no significant differences between the two groups. The endometrial thickness on the day of HCG administration also showed no significant difference. This indicated that GnRH-ant protocol did not lead to a thicker endometrium on the day of HCG administration, and there was no difference in the reproductive outcome between the two groups. This means that even if the GnRH-ant protocol leads to fewer retrieved oocytes and fewer obtained embryos, this protocol does not reduce the chance of achieving a live birth. Moreover, GnRH-ant reduced the stimulation period and the Gn dosage, which is more convenient and cost-effective for patients.

The incidence of OHSS was statistically significantly lower in GnRH-ant protocol than in GnRH-a long protocol. This may because GnRH-ant protocol resulted in fewer retrieved oocytes, and a lower E2 level on the day of HCG administration, which had less possibility to induce OHSS than GnRH-a long protocol. No significant difference was found between the miscarriage rate, and the cycle cancellation rate between the two groups. Therefore, GnRH-ant protocol is safer because of the lower occurrence of OHSS without leading to miscarriage or cycle cancellation.
According to our results, GnRH-ant protocol can reduce the incidence of OHSS, faster and more cost-effectively, without affecting the reproductive outcome in normal ovarian reserve patients. Therefore, GnRH-ant protocol should be used as widely as GnRH-a protocol in these patients.

Comparison with other studies

The recent Cochrane systematic review of 73 RCTs in 2016 has concluded that GnRH-ant protocol and GnRH-a protocol have equivalent live birth rates, while GnRH-ant protocol has a lower incidence of OHSS [11]. Their results are similar to ours. However, the patients with all types of ovarian reserve were included in their study, which covered DOR and PCOS.

A meta-analysis focusing on the supposed normal ovarian responders, including 23 RCTs (n = 3961), was conducted in 2014 [43]. The results indicated that the incidence of OHSS was significantly lower, whereas the ongoing pregnancy and live birth rates were similar in the GnRH antagonist and the standard long GnRH agonist protocols, which is similar to the results of our meta-analysis.

Another systematic review and meta-analysis of 22 RCTs (n = 3176) comparing GnRH-ant and GnRH-a reported the live birth as their main outcome measure [7]. No significant difference was present in the probability of live birth between the two GnRH analogs [OR = 0.86; 95% CI = 0.72 to 1.02]. This result remains stable in subgroup analysis that ordered the studies by type of population studied, Gn type used for stimulation, type of agonist protocol used, type of agonist used, type of antagonist protocol used, type of antagonist used, presence of allocation concealment, presence of co-intervention, and the way the information on live birth was retrieved. However, their study also included all types of ovarian reserve.

Additionally, a meta-analysis of four RCTs (n = 874) in 2008 mainly measured ongoing pregnancy rate [44]. The ongoing pregnancy rate per randomized woman was not found to be significantly different between patients with and without oral contraceptive pill (OCP) pre-treatment (OR: 0.74, 95% CI: 0.53 to 1.03). Duration of Gn stimulation (WMD: 1.41 days, 95% CI: 1.13 to 1.68) and Gn consumption (WMD: 542 IU, 95% CI: 127 to 956) were significantly increased after OCP pretreatment. No significant differences were observed regarding the number of retrieved oocytes. However, the patients included in their study were also not classified by ovarian reserve.

Limitations of this study

First of all, this meta-analysis lacked a uniform definition of normal ovarian reserve, which made it difficult to define inclusion and exclusion criteria, and the studies included may be not strict enough. Second, although the random-effects model was used to minimize the heterogeneity, it cannot be abolished. The heterogeneity may relate to the size of studies that ranged from 40 to 1,050 patients, and to the time span between 2000 and 2016. Additionally, the quality of reported studies was uneven, which may also lead to heterogeneity. The methods of randomization, concealment, and blinding were unclear in some included studies, some of which also had incomplete and selective outcome data. Third, although comprehensive searches were undertaken to ensure that all eligible studies were included, there is still the possibility that some potentially eligible studies were left out. All of these factors may lead to bias.

Implications

The GnRH-ant protocol is a short, convenient, and cost-effective protocol in COH among patients with normal ovarian reserve, which can also reduce the incidence of OHSS without affecting the live birth rate when compared to GnRH-a long protocol. When referring to
patients with normal ovarian reserve, in future clinical practice, GnRH-ant protocol may also be a good choice for COH. Moreover, a uniform definition for ovarian reserve, especially for normal ovarian reserve, should be standardized as soon as possible. RCTs of GnRH-ant protocol and GnRH-a long protocol in patients with different ovarian reserve are still lacking. And the study design of future RCTs should be more rigorous according to the CONSORT statement [45].

Conclusion
In conclusion, our meta-analysis demonstrated that GnRH antagonist protocol substantially decreased the incidence of OHSS without influencing the pregnancy rate and live birth rate compared to GnRH agonist long protocol among patients with normal ovarian reserve. Therefore, for patients with normal ovarian reserve, GnRH-ant protocol should play a more important role in COH when clinicians are making individualizing and optimizing treatment decisions.

Supporting information
S1 Table. Quality assessment of studies.
(DOC)

S1 PRISMA Checklist. PRISMA 2009 checklist.
(DOC)

S1 Fig. Begg’s funnel plot and Egger’s test comparing the clinical pregnancy rate between the GnRH-ant group and the GnRH-a long-protocol group.
(TIF)

S2 Fig. Begg’s funnel plot and Egger’s test comparing the ongoing pregnancy rate between the GnRH-ant group and the GnRH-a long-protocol group.
(TIF)

S3 Fig. Begg’s funnel plot and Egger’s test comparing the live birth rate between the GnRH-ant group and the GnRH-a long-protocol group.
(TIF)

S4 Fig. Begg’s funnel plot and Egger’s test comparing the number of stimulation days between the GnRH-ant group and the GnRH-a long-protocol group.
(TIF)

S5 Fig. Begg’s funnel plot and Egger’s test comparing the Gn dosage between the GnRH-ant group and the GnRH-a long-protocol group.
(TIF)

S6 Fig. Begg’s funnel plot and Begg’s test comparing the endometrial thickness on the day of HCG administration between the GnRH-ant group and the GnRH-a long-protocol group.
(TIF)

S7 Fig. Begg’s funnel plot and Egger’s test comparing the E2 level on the day of HCG administration between the GnRH-ant group and the GnRH-a long-protocol group.
(TIF)
S8 Fig. Begg’s funnel plot and Egger’s test comparing the number of oocytes retrieved between the GnRH-ant group and the GnRH-a long-protocol group.
(TIF)

S9 Fig. Begg’s funnel plot and Egger’s test comparing the number of embryos obtained between the GnRH-ant group and the GnRH-a long-protocol group.
(TIF)

S10 Fig. Begg’s funnel plot and Egger’s test comparing the incidence of OHSS between the GnRH-ant group and the GnRH-a long-protocol group.
(TIF)

S11 Fig. Begg’s funnel plot and Egger’s test comparing the miscarriage rate between the GnRH-ant group and the GnRH-a long-protocol group.
(TIF)

S12 Fig. Begg’s funnel plot and Egger’s test comparing the cycle cancellation rate between the GnRH-ant group and the GnRH-a long-protocol group.
(TIF)

Acknowledgments
The authors thank Peking University Shenzhen Hospital and the Medical College of Shantou University.

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References

1. Fleming R, Adam AH, Barlow DH, Black WP, MacNaughton MC, Coutts JR: A new systematic treatment for infertile women with abnormal hormone profiles. *British journal of obstetrics and gynaecology* 1982, 89(1):80–83. PMID: 6800399

2. Porter RN, Smith W, Craft IL, Abdulwahid NA, Jacobs HS: Induction of ovulation for in-vitro fertilisation using buserelin and gonadotropins. *Lancet (London, England)* 1984, 2(8414):1284–1285.

3. Hughes EG, Fedorow DM, Daya S, Sagle MA, Van de Koppel P, Collins JA: The routine use of gonadotropin-releasing hormone agonists prior to in vitro fertilization and gamete intrafallopian transfer: a meta-analysis of randomized controlled trials. *Fertility and sterility* 1992, 58(5):888–896. PMID: 1426372

4. Forman RG, Frydman R, Egan D, Ross C, Barlow DH: Severe ovarian hyperstimulation syndrome using agonists of gonadotropin-releasing hormone for in vitro fertilization: a European series and a proposal for prevention. *Fertility and sterility* 1990, 53(3):502–509. PMID: 2106456

5. Coccia ME, Comparetto C, Bracco GL, Scarselli G: GnRH antagonists. *European journal of obstetrics, gynecology, and reproductive biology* 2004, 115 Suppl 1:S44–S56.

6. Bodri D, Sunkara SK, Coomarasamy A: Gonadotropin-releasing hormone agonists versus antagonists for controlled ovarian hyperstimulation in oocyte donors: a systematic review and meta-analysis. *Fertility and sterility* 2011, 95(1):164–169. https://doi.org/10.1016/j.fertnstert.2010.06.068 PMID: 20684954

7. Kolibianakis EM, Collins J, Tarlatzis BC, Devroye P, Diedrich K, Griesinger G: Among patients treated for IVF with gonadotrophins and GnRH analogues, is the probability of live birth dependent on the type of analogue used? A systematic review and meta-analysis. *Human reproduction update* 2006, 12(6):651–671. https://doi.org/10.1093/humupd/dml038 PMID: 16920869

8. Barmat LI, Chantilis SJ, Hurst BS, Dickey RP: A randomized prospective trial comparing gonadotropin-releasing hormone (GnRH) antagonist/recombinant follicle-stimulating hormone (rFSH) versus GnRH-agonist/rFSH in women pretreated with oral contraceptives before in vitro fertilization. *Fertility and sterility* 2005, 83(2):321–330. https://doi.org/10.1016/j.fertnstert.2004.06.076 PMID: 15705389

9. Al-Inany HG, Abou-Setta AM, Aboulghar M: Gonadotropin-releasing hormone antagonists for assisted conception. *The Cochrane database of systematic reviews* 2006(3):Cd001750. https://doi.org/10.1002/14651858.CD001750.pub2 PMID: 16855976

10. Al-Inany HG, Youssef MA, Aboulghar M, Broekmans F, Sterrenburg M, Smit J et al: Gonadotrophin-releasing hormone antagonists for assisted reproductive technology. *The Cochrane database of systematic reviews* 2011(5):Cd001750. https://doi.org/10.1002/14651858.CD001750.pub3 PMID: 21563131

11. Al-Inany HG, Youssef MA, Ayeleke RO, Brown J, Lam WS, Broekmans FJ: Gnadotrophin-releasing hormone antagonists for assisted reproductive technology. *The Cochrane database of systematic reviews* 2016, 4:Cd001750. https://doi.org/10.1002/14651858.CD001750.pub4 PMID: 27126581

12. Griesinger G, Felberbaum R, Diedrich K: GnRH antagonists in ovarian stimulation: a treatment regimen of clinicians’ second choice? Data from the German national IVF registry. *Human reproduction (Oxford, England)* 2005, 20(9):2373–2375.

13. Check JH, Slovis B: Choosing the right stimulation protocol for in vitro fertilization-embryo transfer in poor, normal, and hyper-responders. *Clinical and Experimental Obstetrics & Gynecology* 2011, 38(4):313–317.

14. Albano C, Felberbaum RE, Smit J, Riethmuller-Winzen H, Engel J, Diedrich K et al: Ovarian stimulation with HMG: results of a prospective randomized phase III European study comparing the luteinizng hormone-releasing hormone (LHRH)-antagonist cetrorelix and the LHRH-agonist buserelin. European Cetrorelix Study Group. *Human reproduction (Oxford, England)* 2000, 15(3):526–531.

15. Borm G, Mannaerts B: Treatment with the gonadotrophin-releasing hormone agonist ganirelix in women undergoing ovarian stimulation with recombinant follicle stimulating hormone is effective, safe and convenient: results of a controlled, randomized, multicentre trial. The European Orgalutra n Study Group. *Human reproduction (Oxford, England)* 2000, 15(7):1490–1498.

16. Olivennes F, Belaisch-Allart J, Empenaire JC, Dechaud H, Alvarez S, Moreau L et al: Prospective, randomized, controlled study of in vitro fertilization-embryo transfer with a single dose of a luteinizing hormone-releasing hormone (LH-RH) antagonist (cetrorelix) or a depot formula of an LH-RH agonist (tripotrem). *Fertility and sterility* 2000, 73(2):314–320. PMID: 10685535

17.Comparable clinical outcome using the GnRH antagonist ganirelix or a long protocol of the GnRH agonist tripotrem for the prevention of premature LH surges in women undergoing ovarian stimulation. *Human reproduction (Oxford, England)* 2001, 16(4):644–651.
18. Fluker M, Grifo J, Leader A, Levy M, Meldrum D, Muasher SJ et al: Efficacy and safety of ganirelix acetate versus leuprolide acetate in women undergoing controlled ovarian hyperstimulation. *Fertility and sterility* 2001, 75(1):38–45. PMID: 11163814

19. Hohmann FP, Macklon NS, Fauser BC: A randomized comparison of two ovarian stimulation protocols with gonadotropin-releasing hormone (GnRH) antagonist cotreatment for in vitro fertilization commencing recombinant follicle-stimulating hormone on cycle day 2 or 5 with the standard long GnRH agonist protocol. *The Journal of clinical endocrinology and metabolism* 2003, 88(1):166–173. https://doi.org/10.1210/jc.2002-020788 PMID: 12519847

20. Check ML, Check JH, Choiel JK, Davies E, Kiefer D: Effect of antagonists vs agonists on in vitro fertilization outcome. *Clinical and experimental obstetrics & gynecology* 2004, 31(4):257–259.

21. Loutradis D, Stefanidis K, Drakakis P, Milingos S, Antsaklis A, Michalas S: A modified gonadotropin-releasing hormone (GnRH) antagonist protocol failed to increase clinical pregnancy rates in comparison with the long GnRH protocol. *Fertility and sterility* 2004, 82(5):1446–1448. https://doi.org/10.1016/j. fertiandster.2004.04.051 PMID: 15533377

22. Sauer MV, Thornton MH 2nd, Schoolcraft W, Frishman GN: Comparative efficacy and safety of cetrorelix with or without mid-cycle recombinant LH and leuprolide acetate for inhibition of premature LH surges in assisted reproduction. *Reproductive biomedicine online* 2004, 9(5):487–493. PMID: 15588464

23. Badrawi A A-I H, Hussein M, Zaki S, Ramzy AM: Agonist versus antagonist in ICSI cycles: a randomized trial and cost effectiveness analysis. *Middle East Fertility Society Journal* 2005, 10(1):49–54.

24. Lee TH, Wu MY, Chen HF, Chen MJ, Ho HN, Yang YS: Ovarian response and follicular development.

25. Xavier P, Gamboa C, Calejo L, Silva J, Stevenson D, Nunes A et al: A randomised study of GnRH antagonist (cetrorelix) versus agonist (buserelin) for controlled ovarian stimulation: effect on safety and efficacy. *European journal of obstetrics, gynecology, and reproductive biology* 2005, 120(2):185–189.

26. Ferrari B, Pezzuto A, Barusi L, Coppola F: Follicular fluid vascular endothelial growth factor concentrations are increased during GnRH antagonist/FSH ovarian stimulation cycles. *European journal of obstetrics, gynecology, and reproductive biology* 2006, 124(1):70–76.

27. Friedler S, Gilboa S, Schachter M, Raziel A, Strassburger D, Ron El R: Luteal phase characteristics following GnRH antagonist or agonist treatment—a comparative study. *Reproductive biomedicine online* 2006, 12(1):27–32. PMID: 16459390

28. Rombauts L, Healy D, Norman RJ: A comparative randomized trial to assess the impact of oral contraceptive pretreatment on follicular growth and hormone profiles in GnRH antagonist-treated patients. *Human reproduction (Oxford, England)* 2006, 21(1):95–103.

29. Serafini P, Yadid I, Motta EL, Alegretti JR, Fioravanti J, Coslovsky M: Ovarian stimulation with daily late follicular phase administration of low-dose human chorionic gonadotropin for in vitro fertilization: a prospective, randomized trial. *Fertility and sterility* 2006, 86(4):830–838. https://doi.org/10.1016/j. fertster.2006.02.110 PMID: 16963040

30. Baart EB, Martini E, Eijkemans MJ, Van Opstal D, Beckers NG, Verhoeven A et al: Milder ovarian stimulation for in-vitro fertilization reduces aneuploidy in the human preimplantation embryo: a randomized controlled trial. *Human reproduction (Oxford, England)* 2007, 22(4):380–388.

31. Heijnjen EM EW, Eijkemans MJ, De Klerk C, Polinder S, Beckers NGM, Klinkert ER et al: A mild treatment strategy for in-vitro fertilisation: a randomised non-inferiority trial. *The Lancet* 2007, 369 (9563):743–749.

32. Hsieh YY, Chang CC, Tsai HD: Comparisons of different dosages of gonadotropin-releasing hormone (GnRH) antagonist, short-acting form and single, half-dose, long-acting form of GnRH agonist during controlled ovarian hyperstimulation and in vitro fertilization. *Taiwanese journal of obstetrics & gynecology* 2008, 47(1):66–74.

33. Moraloglu O, Kilic S, Karayalcin R, Yulkcu B, Tasdemir N, Isik A et al: Comparison of GnRH agonists and antagonists in normoresponder IVF/ICSI in Turkish female patients. *Advances in therapy* 2008, 25(3):266–273. https://doi.org/10.1007/s12325-008-0028-8 PMID: 18345521

34. Depalo R, Lorusso F, Palmisano M, Bassi E, Totaro I, Vacca M et al: Follicular growth and oocyte maturation in GnRH agonist and antagonist protocols for in vitro fertilisation and embryo transfer. In: *Gynecological endocrinology: the official journal of the International Society of Gynecological Endocrinology*. vol. 25; 2009: 328–334.

35. Ye H, Huang GN, Zeng PH, Pei L: IVF/ICSI outcomes between cycles with luteal estradiol (E2) pre-treatment before GnRH antagonist protocol and standard long GnRH agonist protocol: a prospective and randomized study. *Journal of assisted reproduction and genetics* 2009, 26(2–3):105–111. https://doi.org/10.1007/s10815-009-9300-3 PMID: 19225876
36. Firouzabadi RD, Ahmadi S, Oskouian H, Davar R: Comparing GnRH agonist long protocol and GnRH antagonist protocol in outcome the first cycle of ART. Archives of gynecology and obstetrics 2010, 281(1):81–85. https://doi.org/10.1007/s00404-009-1073-5 PMID: 19357861

37. Papanikolaou EG, Pados G, Grimbizis G, Bili E, Kyriazi L, Polyzos NP et al: GnRH-agonist versus GnRH-antagonist IVF cycles: is the reproductive outcome affected by the incidence of progesterone elevation on the day of HCG triggering? A randomized prospective study. In: Human reproduction (Oxford, England). vol. 27; 2012: 1822–1828.

38. Qiao J, Lu G, Zhang HW, Chen H, Ma C, Olofsson JI et al: A randomized controlled trial of the GnRH antagonist ganirelix in Chinese normal responders: high efficacy and pregnancy rates. In: Gynecological endocrinology: the official journal of the International Society of Gynecological Endocrinology. vol. 28; 2012: 800–804.

39. Rabati BK Z S: Investigation of pregnancy outcome and ovarian hyper stimulation syndrome prevention in agonist and antagonist gonadotropin-releasing hormone protocol. Journal of Research in Medical Sciences 2012, 17(11):1063–1066. PMID: 23635562

40. Hershko Klement A, Berkovitz A, Wiser A, Gonen O, Amichay K, Cohen I et al: GnRH-antagonist programming versus GnRH agonist protocol: a randomized trial. European journal of obstetrics, gynecology, and reproductive biology 2015, 185:170–173. https://doi.org/10.1016/j.ejogrb.2014.12.021 PMID: 25594526

41. Toftager MB JB TL KR JH TP LZ AN LP A: Risk of severe ovarian hyperstimulation syndrome in GnRH antagonist versus GnRH agonist protocol: RCT including 1050 first IVF/ICSI cycles. In: Human reproduction (Oxford, England). vol. 31; 2016: 1253–1264.

42. Ferrarretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L et al: ESHRE consensus on the definition of ‘poor response’ to ovarian stimulation for in vitro fertilization: the Bologna criteria. Human reproduction (Oxford, England) 2011, 26(7):1616–1624.

43. Xiao JS, Su CM, Zeng XT: Comparisons of GnRH antagonist versus GnRH agonist protocol in supposed normal ovarian responders undergoing IVF: a systematic review and meta-analysis. PloS one 2014, 9(9):e106854. https://doi.org/10.1371/journal.pone.0106854 PMID: 25216031

44. Griesinger G, Venetis CA, Marx T, Diedrich K, Tarlatzis BC, Kolibianakis EM: Oral contraceptive pill pre-treatment in ovarian stimulation with GnRH antagonists for IVF: a systematic review and meta-analysis. Fertility and sterility 2008, 90(4):1055–1063. https://doi.org/10.1016/j.fertnstert.2007.07.1354 PMID: 18054003

45. Schulz KF, Altman DG, Moher D: CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. Trials 2010, 11:32. https://doi.org/10.1186/1745-6215-11-32 PMID: 20334632