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What is the optimal GnRH antagonist protocol for ovarian stimulation during ART treatment? A systematic review and network meta-analysis

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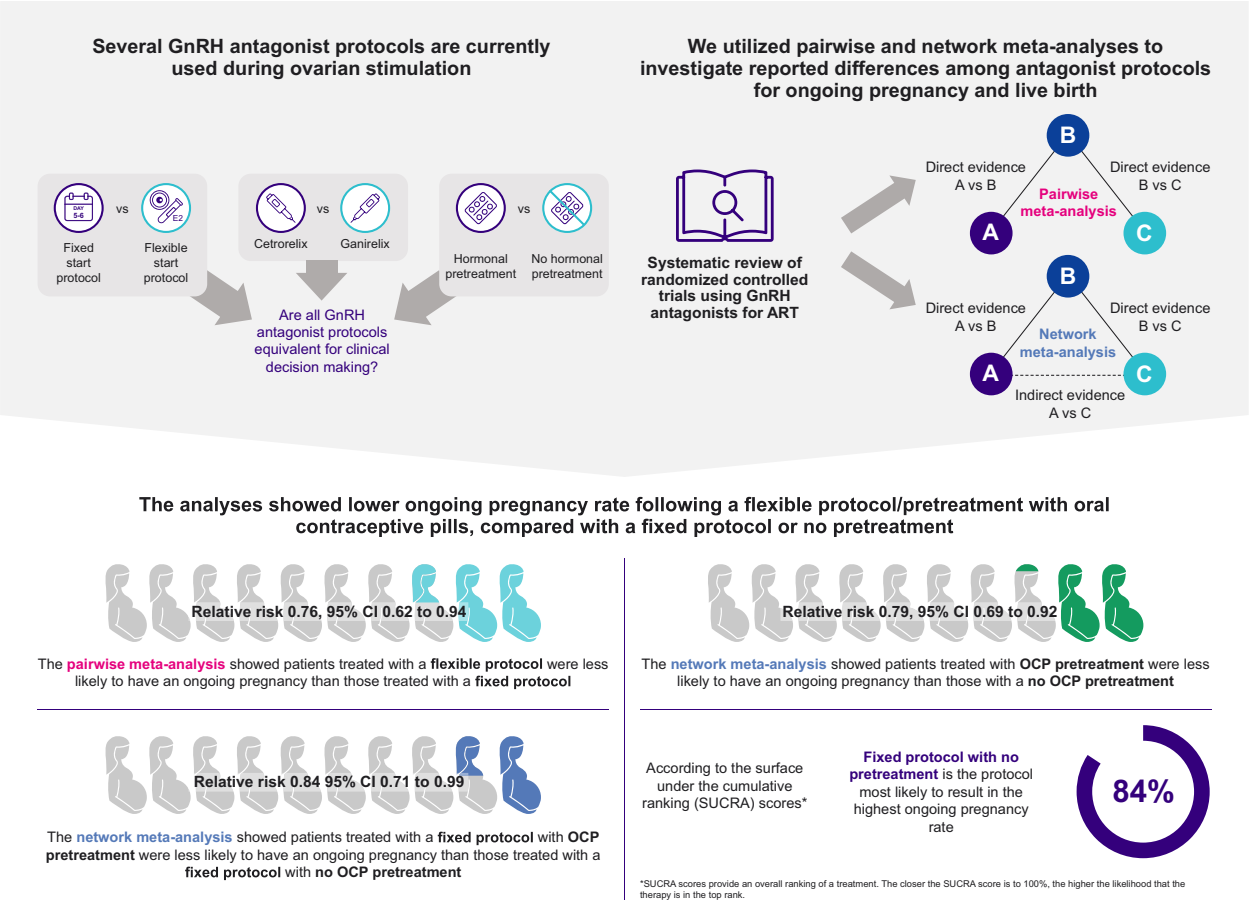
GRAPHICAL ABSTRACT



This meta-analysis showed lower ongoing pregnancy rates (OPRs) following controlled ovarian stimulation (COS) using a flexible GnRH antagonist protocol or pretreatment with an oral contraceptive pill (OCP) compared with COS using a fixed protocol or no OCP pretreatment, respectively.

BACKGROUND: Several GnRH antagonist protocols are currently used during COS in the context of ART treatments; however, questions remain regarding whether these protocols are comparable in terms of efficacy and safety.

OBJECTIVE AND RATIONALE: A systematic review followed by a pairwise and network meta-analyses were performed. The systematic review and pairwise meta-analysis of direct comparative data according to the PRISMA guidelines evaluated the effectiveness of different GnRH antagonist protocols (fixed Day 5/6 versus flexible, ganirelix versus cetrorelix, with or without hormonal pretreatment) on the probability of live birth and ongoing pregnancy after COS during ART treatment. A frequentist network meta-analysis combining direct and indirect comparisons (using the long GnRH agonist protocol as the comparator) was also performed to enhance the precision of the estimates.

SEARCH METHODS: The systematic literature search was performed using Embase (Ovid), MEDLINE (Ovid), Cochrane Central Register of Trials (CENTRAL), SCOPUS and Web of Science (WOS), from inception until 23 November 2021. The search terms comprised three different MeSH terms that should be present in the identified studies: GnRH antagonist; assisted reproduction treatment; randomized controlled trial (RCT). Only studies published in English were included.

OUTCOMES: The search strategy resulted in 6738 individual publications, of which 102 were included in the systematic review (corresponding to 75 unique studies) and 73 were included in the meta-analysis. Most studies were of low quality. One study compared a flexible protocol with a fixed Day 5 protocol and the remaining RCTs with a fixed Day 6 protocol. There was a lack of data regarding live birth when comparing the flexible and fixed GnRH antagonist protocols or cetrorelix and ganirelix. No significant difference in live birth rate was observed between the different pretreatment regimens versus no pretreatment or between the different pretreatment protocols. A flexible GnRH antagonist protocol resulted in a significantly lower OPR compared with a fixed Day 5/6 protocol (relative risk (RR) 0.76, 95%

CI 0.62 to 0.94, $I^2 = 0\%$; 6 RCTs; n = 907 participants; low certainty evidence). There were insufficient data for a comparison of cetrorelix and ganirelix for OPR. OCP pretreatment was associated with a lower OPR compared with no pretreatment intervention (RR 0.79, 95% CI 0.69 to 0.92; $I^2 = 0\%$; 5 RCTs, n = 1318 participants; low certainty evidence). Furthermore, in the network meta-analysis, a fixed protocol with OCP resulted in a significantly lower OPR than a fixed protocol with no pretreatment (RR 0.84, 95% CI 0.71 to 0.99; moderate quality evidence). The surface under the cumulative ranking (SUCRA) scores suggested that the fixed protocol with no pretreatment is the antagonist protocol most likely (84%) to result in the highest OPR. There was insufficient evidence of a difference between fixed/flexible or OCP pretreatment/no pretreatment interventions regarding other outcomes, such as ovarian hyperstimulation syndrome and miscarriage rates.

WIDER IMPLICATIONS: Available evidence, mostly of low quality and certainty, suggests that different antagonist protocols should not be considered as equivalent for clinical decision-making. More trials are required to assess the comparative effectiveness of ganirelix versus cetrorelix, the effect of different pretreatment interventions (e.g. progestins or oestradiol) or the effect of different criteria for initiation of the antagonist in the flexible protocol. Furthermore, more studies are required examining the optimal GnRH antagonist protocol in women with high or low response to ovarian stimulation.

Key words: assisted reproductive technologies / ART / cetrorelix / controlled ovarian stimulation / ganirelix / GnRH antagonist / live birth / pregnancy

Introduction

During controlled ovarian stimulation (COS) in the context of ART, GnRH analogues (agonists and antagonists) are used to suppress pituitary function and prevent premature luteinization by inhibiting the production of endogenous FSH and LH. The most recent Cochrane meta-analysis reports that live birth rates (LBRs) using GnRH antagonist protocols were no different to those obtained with long GnRH agonist protocols, whereas GnRH antagonist treatment was associated with a lower incidence of any grade of ovarian hyperstimulation syndrome (OHSS) than GnRH agonist protocols (Al-Inany *et al.*, 2016). Hence, based on the available evidence, GnRH antagonist protocols seem to be equally efficacious to long GnRH agonist protocols, whilst also being safer and more patient-friendly (Al-Inany *et al.*, 2016).

Several GnRH antagonist protocols are currently used during COS in the context of ART treatment, which is defined as pharmacological treatment in which women are stimulated to induce the development of multiple ovarian follicles to obtain multiple oocytes at follicular aspiration (Zegers-Hochschild *et al.*, 2009). These protocols are based on the use of the most commonly available GnRH antagonists (cetrorelix and ganirelix) using either a fixed or flexible protocol. In the fixed protocol, GnRH antagonists are usually started on Day 5 or 6 of COS during ART treatment (Al-Inany *et al.*, 2005), whereas in a flexible protocol, GnRH antagonists are started from the day when the largest ovarian follicle has reached a specific size, which may vary between 12 and 14 mm in most studies, and/or when serum oestradiol (E2) reaches a specific threshold (Al-Inany *et al.*, 2005). Both antagonist protocols may be initiated with or without hormonal pretreatment (e.g. with the oral contraceptive pill (OCP), E2 or progestins), which has been used by clinicians for different purposes, such as synchronization of follicular development, prevention of occurrence of early large follicles or spontaneous LH surge, reduction of cyst formation and scheduling of IVF cycles for the benefit of clinicians and patients (e.g. scheduling of oocyte retrieval) (Farquhar *et al.*, 2017). However, OCP pretreatment has previously been shown to be associated with decreased ongoing pregnancy rates (OPRs) compared with antagonist cycles without OCP pretreatment (Griesinger *et al.*, 2010). Furthermore, in the 2020 ESHRE Guidelines, pre-treatment with oestrogen or progestins before using the GnRH antagonist protocol was 'probably not recommended' for improving efficacy and safety, and pre-treatment with the OCP (12–28 days) was not recommended in an GnRH antagonist protocol because of reduced efficacy (Bosch *et al.*, 2020).

Despite the wide range of GnRH antagonist protocols currently used during COS in the context of ART treatment, after almost 20 years since their introduction in clinical practice it has not been properly evaluated whether all are equally efficacious and safe, and there are few studies that have directly compared outcomes from the different antagonist protocols. We have therefore performed a systematic review and pairwise meta-analysis of direct comparative data according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, with the primary aim of identifying the optimal antagonist protocol in terms of live birth and ongoing pregnancy. In addition, to enhance the precision of our estimates and utilize the large pool of randomized evidence available comparing antagonist protocols with the long GnRH agonist protocol, we performed a network meta-analysis, which combines both direct and indirect evidence from randomized controlled trials (RCTs).

Methods

Study design

A pairwise systematic review and meta-analysis was undertaken to identify the antagonist protocol for optimizing OPR and LBR. Owing to the relative paucity of studies that directly compare different antagonist protocols, a frequentist network meta-analysis was also performed to validate the findings of the direct comparisons and increase their precision in cases where the direct comparison data were inconclusive or underpowered. The frequentist network meta-analysis made use of a larger body of evidence by combining direct and indirect evidence from RCTs in which any GnRH antagonist protocol, with or without OCP pretreatment, was compared with the long GnRH agonist protocol for three pregnancy outcomes: LBR, OPR and clinical pregnancy rate (CPR). In the network meta-analysis, data from a fixed antagonist protocol with no pretreatment were used as the common comparator for all comparisons. The protocol for this systematic review and meta-analysis has been registered with the international prospective register of systematic reviews (PROSPERO;

CRD42019125037). The systematic review/meta-analysis was conducted according to the PRISMA guidelines (Moher et al., 2009) and the frequentist network meta-analysis was conducted according to the PRISMA network meta-analysis statement (Hutton et al., 2015).

Objectives

The primary objectives of the systematic review and pairwise and frequentist network meta-analyses were to compare the effectiveness of the GnRH antagonist protocols on the probability of live birth (using the definitions in the constituent studies) and ongoing pregnancy (defined as positive cardiac activity at 10–12 weeks of gestation) after COS during ART treatment. These outcomes were compared between women who had the fixed Day 5/6 GnRH protocol with those who had a flexible protocol, women who were treated with cetrorelix compared with women who were treated with ganirelix, and between GnRH antagonist protocols with or without any type of hormonal pretreatment, respectively.

The secondary objectives were to compare the effectiveness of the different GnRH antagonist protocols on the probability of clinical pregnancy (defined as ultrasound detection of at least one intrauterine gestation sac at 5–8 weeks of pregnancy), first trimester clinical miscarriage (defined as the failure of a clinical pregnancy to achieve ongoing pregnancy status), duration of stimulation (defined as the number of days from the beginning of COS during ART treatment (first gonadotrophin injection) until the day of triggering final oocyte maturation), total dose of FSH (IUs) required to complete COS, the number of oocytes retrieved and the occurrence of moderate or severe OHSS, as defined in the individual studies.

In addition, exploratory analyses were performed to investigate differences in reproductive outcomes caused by potential sources of heterogeneity among patient subpopulations, according to type of response (high, normal or low responders), the criteria used to initiate the flexible protocol (ultrasound, hormonal or both), the type of pretreatment (OCP, E2 or progestin) and the duration of the pretreatment-free interval.

Search strategy

The literature search was performed by two independent reviewers (A.S. and S.J.C.) using a predefined literature search strategy of selected databases (Supplementary Table SI). Searches were performed from inception up to 23 November 2021.

Selection of studies

RCTs published in English were included in the pairwise and network meta-analyses if they reported on at least one of the research questions of interest by comparing one or more of the following in women undergoing COS during ART treatment: flexible protocols versus fixed Day 5/6 GnRH antagonist protocol; ganirelix versus cetrorelix; any type of hormonal pretreatment (e.g. OCP, E2 or progestins) versus no pretreatment in the GnRH protocol or placebo; any two types of hormonal pretreatment in the GnRH antagonist protocol (e.g. OCP versus E2 pretreatment). Studies were included in the pairwise meta-analyses if they directly compared the aforementioned GnRH antagonist protocols. For the network meta-analysis, studies were also extracted if they reported on any of the eligible GnRH antagonist protocols compared with the long GnRH agonist protocol. In the event of RCTs comparing three or more interventions, only data from the interventions of interest were extracted.

Studies were excluded if they were pseudo-randomized or non-randomized studies, had asymmetrical co-interventions, did not report a clear description of the methodology, GnRH antagonists/agonists and gonadotrophins were not used (e.g. COS with clomiphene or letrozole) or they evaluated a Day 2/3/4 fixed antagonist or a short agonist protocol.

The selected studies were screened sequentially according to article title, abstract and full text, to determine eligibility by two independent reviewers (A.S. and S.J.C.). The reference lists of relevant publications or review articles were also manually searched for any additional relevant references.

Data extraction

Two independent reviewers (A.S. and S.J.C.) extracted the predefined primary and secondary outcome data from the included studies using standardized tables developed by the authors to ensure consistency. When multiple reports describing the same trial were published, the most recent or complete report was used. In the event of a disagreement about the eligibility of a study that could not be resolved through discussion, resolution via a third reviewer (C.A.V.) was sought. If further information was needed to decide on the inclusion of a study, the authors of the original study were contacted.

Statistical analysis

All data were extracted and analysed by using the intention-to-treat principle. Qualitative and quantitative syntheses of all direct (pairwise) comparative data were performed using the random effects model (restricted maximum likelihood method with Hartung–Knapp–Sidik–Jonkman correction (Hartung and Knapp, 2001; IntHout et al., 2014)). The effect size of choice was relative risk (RR) for dichotomous outcomes and weighted mean difference (WMD) for continuous data; uncertainty around these estimates was expressed using the 95% CIs. Statistical heterogeneity in each direct comparison was estimated with the I^2 statistic (Deeks et al., 2019) and assessed in several predefined subgroups: patient population (normal, high and unselected responders; polycystic ovary syndrome (PCOS)); criteria for the flexible protocol (ultrasound assessment only, hormone assessment or both); type of agonist (cetrorelix versus ganirelix); hormonal pretreatment versus no-pretreatment; duration of pretreatment-free interval. Publication bias was explored for the co-primary endpoints of this meta-analysis using the Harbord test (Harbord et al., 2006), as well as visually by constructing funnel plots and assessing for asymmetry (Sterne et al., 2011).

The frequentist network meta-analyses were performed using multivariate random effects meta-analysis models (White et al., 2012), with summary treatment effects presented as RR (95% CIs). Global inconsistency was assessed by using design-by-treatment interaction modelling (Higgins et al., 2012) and local inconsistency was assessed through the side-splitting approach (Dias et al., 2010). Surface under the cumulative ranking curve (SUCRA) scores were used to rank the treatments of interest (Salanti et al., 2011). The SUCRA score gives the overall ranking of a treatment as a value ranging from 0% to 100%, where the closer the SUCRA score is to 100%, the higher the likelihood that the

therapy is in the top rank (Mbuagbaw *et al.*, 2017). STATA software (version 16.1, StataCorp, College Station, TX, USA) was used to perform the statistical analysis, and the network meta-analysis was performed using 'network' command suite (White, 2015).

For the pairwise meta-analyses, sensitivity analyses for the co-primary endpoints as well as for CPR were performed for all comparisons, with the exclusion of randomized studies without a clear description of the randomization methodology in the publication. For the network meta-analyses, to guarantee the robustness of the findings, a series of sensitivity analyses were performed, including: flexible versus fixed agonist protocol (cetrorelix and no pretreatment, cetrorelix and OCP, ganirelix and no pretreatment, and ganirelix and OCP) and ganirelix versus cetrorelix (fixed protocol and no pretreatment, fixed protocol and OCP, flexible protocol and no pretreatment and flexible protocol and OCP).

Risk of bias of individual studies was assessed by using the Cochrane risk of bias tool for randomized trials (Higgins *et al.*, 2011). The overall certainty of the evidence obtained through direct comparisons was graded according to the Grading of Recommendations Assessment, Development and Evaluation (GRADE) Working Group guidelines (Guyatt *et al.*, 2008). The Confidence in Network Meta-analysis (CINeMA) application was used to assess the quality of evidence across the included studies in the network meta-analysis (Nikolakopoulou *et al.*, 2020).

Results

Selection of studies

Overall, 75 unique RCTs were included in the systematic review, and the data from 73 RCTs were included in the meta-analysis. Two studies were not included in the meta-analysis, because the number of women randomized in each intervention arm was not clearly defined (Batzofin *et al.*, 2002; Verpoest *et al.*, 2017). All studies were of low-to-moderate quality. The process involved in the screening and selection of eligible studies can be found in the PRISMA flow chart (Fig. 1). Of the 73 studies included in the meta-analysis, 7 compared GnRH fixed and flexible protocols, 14 compared different pretreatments with no pretreatments (2 of which contributed data both in the pairwise and the network meta-analysis), 2 compared ganirelix with cetrorelix, 1 compared NuvaRing® with OCP pretreatment (Supplementary

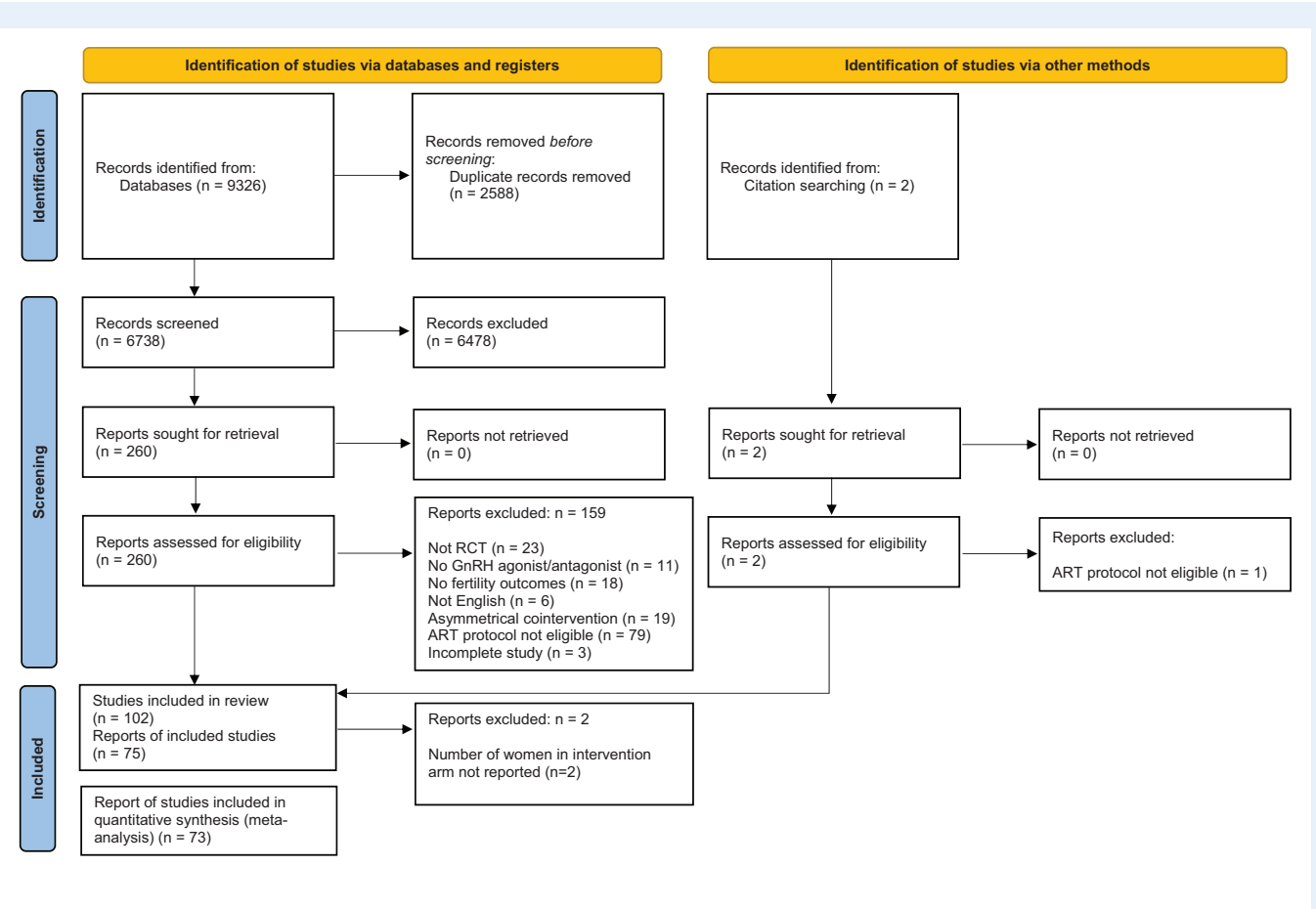


Figure 1. PRISMA diagram for a systematic review and network meta-analysis to determine the optimal GnRH antagonist protocol for controlled ovarian stimulation during ART treatment. OCP, oral contraceptive pill; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RCT, randomized controlled trial.

Table SII) and 49 compared a GnRH antagonist with an agonist (Supplementary Table SIII).

In the systematic review, 12 studies had a low overall risk of bias, 36 had moderate (e.g. some concerns) and 27 had a high risk of bias (Supplementary Table SIV). The risk of bias for outcome assessments was considered low owing to the use of pregnancy and live birth outcomes, which are considered to have a low risk of bias; however, the risk of performance bias was considered high, as participants were unlikely to be blinded, owing to the differences in ART protocols. Studies in which the method of randomization was unclear were graded as having a high overall risk. The occurrence of selective reporting was generally unclear, as most studies did not have a publicly available registered protocol. The GRADE analysis for the different outcomes in the pairwise meta-analysis generally suggested evidence of low certainty (Supplementary Table SV).

Primary outcomes (pairwise meta-analysis)

For the fixed versus flexible protocols, seven RCTs were identified for this outcome (Ludwig *et al.*, 2002; Escudero *et al.*, 2004; Mochtar, 2004; Kolibianakis *et al.*, 2011; Hossein Rashidi *et al.*, 2015; Depalo *et al.*, 2018; Luo *et al.*, 2021) (Supplementary Table SVI). Only one of these RCTs compared a flexible protocol with a fixed Day 5 protocol (Luo *et al.*, 2021), therefore only comparisons between flexible and fixed Day 6 protocols were included in the analysis. Furthermore, no RCTs reported on LBR. A significantly lower OPR was reported with flexible versus fixed protocols (RR 0.76, 95% CI 0.62 to 0.94, $I^2 = 0\%$; 6 RCTs; n = 907 participants; low certainty evidence) (Ludwig *et al.*, 2002; Mochtar, 2004; Kolibianakis *et al.*, 2011; Hossein Rashidi *et al.*, 2015; Depalo *et al.*, 2018; Luo *et al.*, 2021) (Fig. 2A). Publication bias could not be confidently detected using either the Harbord test (beta: 0.02, $P = 0.981$) or by inspecting the funnel plot. In the sensitivity analysis, after exclusion of studies with unclear methods of randomization, the effect size was similar (RR 0.73, 95% CI 0.55 to 0.97, $I^2 = 0\%$; 4 RCTs; n = 663 participants) (Mochtar, 2004; Kolibianakis *et al.*, 2011; Hossein Rashidi *et al.*, 2015; Depalo *et al.*, 2018).

Two RCTs were included for the comparison of cetrorelix versus ganirelix (Wilcox *et al.*, 2005; Kars *et al.*, 2007). Only one of the RCTs reported on LBR (RR 1.38, 95% CI 0.62 to 3.06) (Kars *et al.*, 2007); therefore, a meta-analysis could not be performed. Neither of the studies reported on OPR. Publication bias could not be assessed owing to the small number of studies.

Fourteen trials reporting on pretreatment versus no pretreatment were included (Fanchin *et al.*, 2003; Huirne *et al.*, 2006a; Kolibianakis *et al.*, 2006; Rombauts *et al.*, 2006; Cédrin-Durnerin *et al.*, 2007; Andersen *et al.*, 2011; Kim *et al.*, 2011; Vilela *et al.*, 2011; Blockeel *et al.*, 2012; Cédrin-Durnerin *et al.*, 2012; Hauzman *et al.*, 2013; Erdem *et al.*, 2015; Shahrokh Tehrani Nejad *et al.*, 2018; Fernandez-Prada *et al.*, 2022). OPR was lower with OCP pretreatment compared with no pretreatment (RR 0.79, 95% CI 0.65 to 0.97 $I^2 = 0\%$; 5 RCTs, n = 1318 participants; low certainty evidence) (Fig. 2B) (Huirne *et al.*, 2006a; Kolibianakis *et al.*, 2006; Rombauts *et al.*, 2006; Andersen *et al.*, 2011; Fernandez-Prada *et al.*, 2022). Publication bias could not be detected using either the Harbord test (beta: −0.41, $P = 0.691$) or by inspecting the funnel plot. The pool of RCTs included in the sensitivity analysis was identical, as all trials were considered to have adequate randomization. Two studies reported data for OPR for E2 versus no pretreatment (RR 0.96, 95% CI 0.32 to 2.93; $I^2 = 25.2\%$; 2 RCTs; n = 172 participants; very low certainty evidence) (Erdem *et al.*, 2015; Fernandez-Prada *et al.*, 2022). No data were available for progestin versus no pretreatment. There was no statistical difference in LBR following pretreatment with OCP versus no pretreatment (RR 0.93, 95% CI 0.56 to 1.54; $I^2 = 0\%$; 3 RCTs; n = 199 participants; low certainty evidence) (Cédrin-Durnerin *et al.*, 2007; Kim *et al.*, 2011), E2 versus no pretreatment (RR 0.88, 95% CI 0.66 to 1.16 $I^2 = 0\%$; 3 RCTs; n = 585 participants; moderate certainty evidence) (Cédrin-Durnerin *et al.*, 2007, 2012; Fernandez-Prada *et al.*, 2022) or progestin versus no pretreatment (RR 0.87, 95% CI 0.62 to 1.22; $I^2 = 0\%$; 2 RCTs; n = 416 participants; moderate certainty evidence) (Cédrin-Durnerin *et al.*, 2007, 2012).

For comparisons among different pretreatment regimens, there was no significant difference in live birth for E2 versus OCP (RR 0.91, 95% CI 0.60 to 1.39; $I^2 = 0\%$; 3 RCTs; n = 214 participants; low certainty evidence) (Cédrin-Durnerin *et al.*, 2007; Hauzman *et al.*, 2013; Fernandez-Prada *et al.*, 2022) or progestin versus E2 (RR 0.98, 95% CI 0.69 to 1.39; $I^2 = 0\%$; 2 RCTs; n = 418 participants; low certainty evidence) (Cédrin-Durnerin *et al.*, 2007; 2012). Only one study reported on progestin versus OCP (RR 1.52, 95% CI 0.41 to 5.60; $I^2 = 0\%$; 1 RCT; n = 44 participants; low certainty evidence) (Cédrin-Durnerin *et al.*, 2007). For OPR, there was no significant difference for E2 versus OCP (RR 0.92, 95% CI 0.61 to 1.38; $I^2 = 0\%$; 2 RCTs; n = 171 participants; low certainty evidence) (Hauzman *et al.*, 2013; Fernandez-Prada *et al.*, 2022). Only one study compared the NuvaRing with the OCP and no statistically significant difference in OPR was reported (RR 1.16, 95% CI 0.70 to 1.90, $I^2 = 0\%$; 1 RCT; n = 79 participants) (Karande *et al.*, 2007). Publication bias could not be detected for any of these comparisons, either by using the Harbord test or by inspecting the funnel plot. There were insufficient data to perform pairwise meta-analyses among other combinations of pretreatments.

Secondary outcomes (pairwise meta-analysis)

There was no significant difference in the number of oocytes retrieved with a flexible protocol compared with a fixed protocol (WMD 2.12 oocytes, 95% CI −0.58 to 4.83; 7 RCTs; n = 961 participants; $I^2 = 93.8\%$; very low certainty evidence) (Ludwig *et al.*, 2002; Escudero *et al.*, 2004; Mochtar, 2004; Kolibianakis *et al.*, 2011; Hossein Rashidi *et al.*, 2015; Depalo *et al.*, 2018; Luo *et al.*, 2021). No differences were reported for the other secondary outcomes (Table I).

There was no difference in CPR between cetrorelix and ganirelix (RR 0.99, 95% CI 0.20 to 4.93; 2 RCTs; n = 258 participants; $I^2 = 0\%$; very low certainty evidence) (Wilcox *et al.*, 2005; Kars *et al.*, 2007). No data were available for analysis of the other secondary outcomes (Table I).

Pretreatment with OCP was associated with a non-significant difference in the duration of stimulation compared with no pretreatment (WMD 0.66 days, 95% CI −0.48 to 1.81; 8 RCTs; n = 1223 participants; $I^2 = 95.9\%$; very low certainty evidence) (Huirne *et al.*, 2006a; Kolibianakis *et al.*, 2006; Rombauts *et al.*, 2006; Cédrin-Durnerin *et al.*, 2007; Kim *et al.*, 2011; Vilela *et al.*, 2011; Shahrokh Tehrani Nejad *et al.*, 2018; Fernandez-Prada *et al.*, 2022) and no significant difference

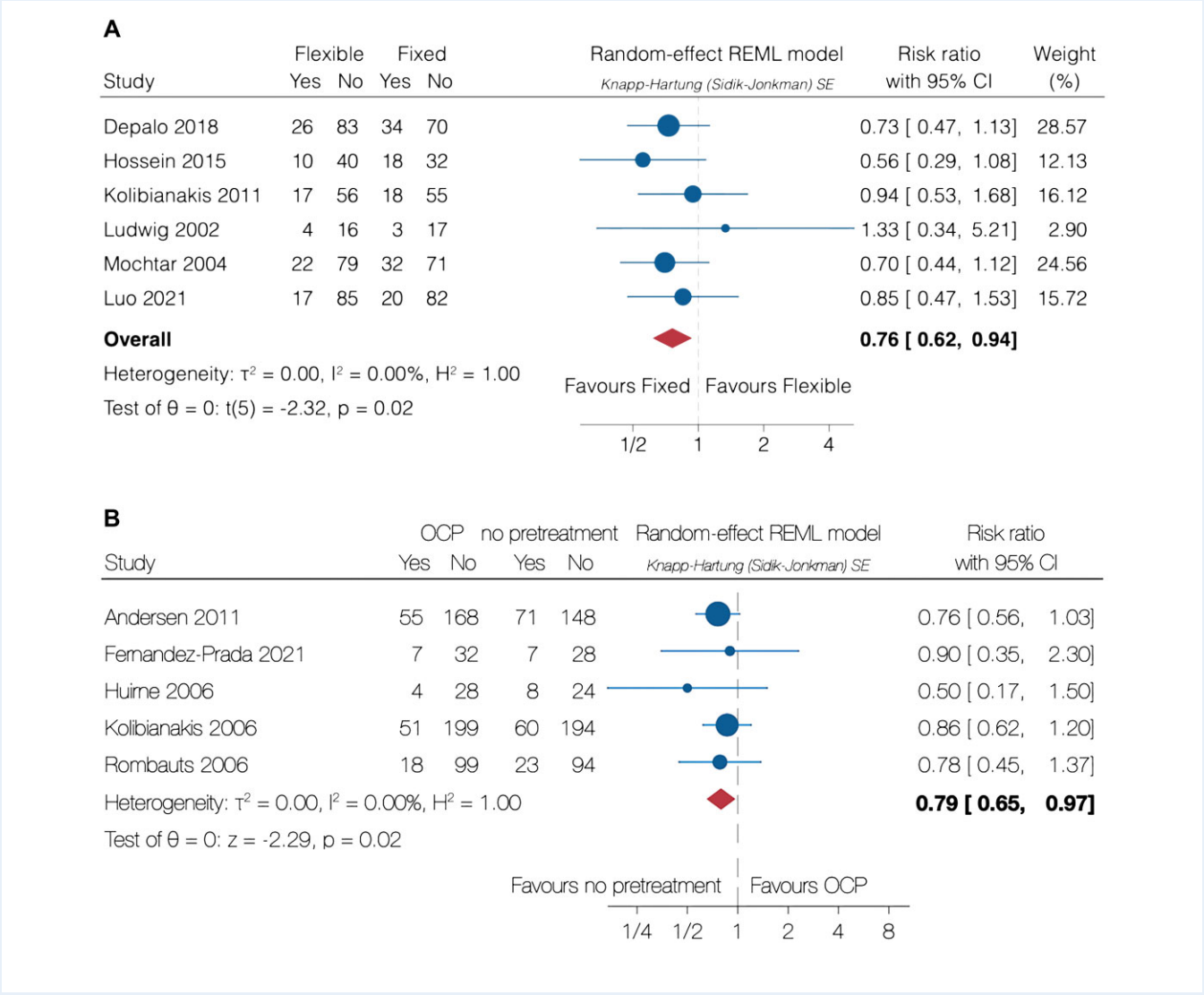


Figure 2. Forest plots of relative risk from the pairwise meta-analyses for achievement of ongoing pregnancy in women. (**A**) Forest plots of RR for achievement of pregnancy in women undergoing COS using a fixed or flexible GnRH antagonist protocol. (**B**) Forest plots of RR for achievement of pregnancy in women undergoing COS using OCP pretreatment versus no pretreatment GnRH antagonist protocol. Weights are from random effects model. OCP, oral contraceptive pill; REML, restricted maximum likelihood method; RR, relative risk; COS, controlled ovarian stimulation.

in the mean total dose of gonadotrophins compared with no pretreatment (WMD 289.43 IU, 95% CI −115.18 to 694.05; 7 RCTs; n = 1100 participants; $I^2 = 94.6\%$; very low certainty evidence) (Huirne *et al.*, 2006a; Kolibianakis *et al.*, 2006; Rombauts *et al.*, 2006; Cédrin-Durnerin *et al.*, 2007; Kim *et al.*, 2011; Vilela *et al.*, 2011; Fernandez-Prada *et al.*, 2022). Progestin pretreatment was not associated with a significantly higher mean total dose of gonadotrophins compared with no pretreatment (WMD 138.12 IU, 95% CI −287.06 to 563.30; 2 RCTs; n = 392 participants; $I^2 = 0\%$; low certainty evidence) (Cédrin-Durnerin *et al.*, 2007, 2012). However, E2 pretreatment compared with no pretreatment was associated with significantly higher mean total dose of gonadotrophins (WMD 192.73 IU, 95% CI 140.22 to 245.23; 5 RCTs; 711 participants; $I^2 = 4.6\%$; low certainty evidence) (Fanchin *et al.*, 2003; Cédrin-Durnerin *et al.*, 2007; Blockeel *et al.*, 2012; Cédrin-Durnerin *et al.*, 2012; Fernandez-Prada *et al.*, 2022). No significant difference in the number of oocytes retrieved was detected between E2 pretreatment compared with no pretreatment (WMD 0.39 oocytes, 95% CI −0.32 to 1.09; 6 RCTs; n = 851 participants; $I^2 = 0\%$; low certainty evidence) (Cédrin-Durnerin *et al.*, 2007; Blockeel *et al.*, 2012; Cédrin-Durnerin *et al.*, 2012; Erdem *et al.*, 2015; Shahrokh Tehrani Nejad *et al.*, 2018; Fernandez-Prada *et al.*, 2022). No difference was reported or there were insufficient data for pairwise meta-analyses of the remaining comparisons (Table II).

Exploratory analyses

Based on the available data for flexible versus fixed protocols, analyses were performed for the following subgroups: population (high/

Table I Summary of pairwise meta-analyses for the relative risk of secondary endpoints following controlled ovarian stimulation with different GnRH antagonist protocols.

Protocol	Outcome	Studies	Parameter estimate	Evidence quality
Flexible versus fixed	Clinical pregnancy	Ludwig, 2002; Escudero, 2004; Mochtar, 2004; Hossein Rashidi, 2015; Luo, 2021	RR 0.85, 95% CI 0.70 to 1.04	$I^2 = 0\%$; n = 657 participants; low-quality evidence (four used ultrasound only as criterion for antagonist; one used ultrasound and hormonal monitoring); low certainty assessment
	OHSS moderate-to-severe	NA	NA	No data available
	First trimester clinical miscarriage	Ludwig, 2002; Mochtar, 2004; Hossein Rashidi, 2015; Luo, 2021	RR 1.51, 95% CI 0.28 to 8.23	$I^2 = 0\%$; n = 548 participants; low-quality evidence (three used ultrasound only as criterion for antagonist; one used ultrasound and hormonal monitoring); very low certainty assessment
	Duration of stimulation (days)	Ludwig, 2002; Escudero, 2004; Mochtar, 2004; Kolibianakis, 2011; Hossein Rashidi, 2015; Depalo, 2018; Luo, 2021	WMD –0.25, 95% CI –0.71 to 0.21	$I^2 = 70.6\%$; n = 995 participants; low-quality evidence (four used ultrasound only as criterion for antagonist; three used ultrasound or hormonal monitoring); very low certainty assessment
	Total dose of FSH (IU)	Ludwig, 2002; Escudero, 2004; Mochtar, 2004; Kolibianakis, 2011; Luo, 2021	WMD 16.49, 95% CI –84.27 to 117.25	$I^2 = 0\%$; n = 693 participants; low-quality evidence (three used ultrasound only as criterion for antagonist; two used ultrasound or hormonal monitoring); low certainty assessment
	Number of oocytes retrieved	Ludwig, 2002; Escudero, 2004; Mochtar, 2004; Kolibianakis, 2011; Hossein Rashidi, 2015; Depalo, 2018; Luo, 2021	WMD 2.12, 95% CI −0.58 to 4.83	$I^2 = 93.8\%$; n = 961 participants; low-quality evidence (four used ultrasound only as criterion for antagonist; three used ultrasound or hormonal monitoring); very low certainty assessment
Ganirelix versus cetrorelix	Clinical pregnancy	Wilcox, 2005; Kars, 2007	RR 0.99, 95% CI 0.20 to 4.93	$I^2 = 0\%$; n = 258 participants; low-quality evidence; very low certainty assessment
	OHSS moderate-to-severe	NA	NA	No data available
	First trimester clinical miscarriage	NA	NA	No data available
	Duration of stimulation (days)	NA	NA	No data available
	Total dose of FSH (IU)	NA	NA	No data available
	Number of oocytes retrieved	NA	NA	No data available
OCP pretreatment versus no pretreatment	Clinical pregnancy	Huirne *et al.*, 2006a; Cédrin-Durnerin, 2007; Andersen, 2011; Kim, 2011; Shahrokh Tehrani Nejad, 2018; Fernandez-Prada, 2022	RR 0.75, 95% CI 0.56 to 1.01	$I^2 = 0\%$; n = 845 participants; low-quality evidence; moderate certainty assessment
	OHSS moderate-to-severe	Huirne *et al.*, 2006a; Rombauts, 2006; Andersen, 2011; Shahrokh Tehrani Nejad, 2018; Fernandez-Prada, 2022	RR 0.97, 95% CI 0.52 to 1.82	$I^2 = 0\%$; n = 954 participants; low-quality evidence; very low certainty assessment
	First trimester clinical miscarriage	Huirne *et al.*, 2006a; Andersen, 2011; Fernandez-Prada, 2022	RR 0.55, 95% CI 0.03 to 10.23	$I^2 = 26.4\%$; n = 580 participants; low-quality evidence; very low certainty assessment
	Duration of stimulation (days)	Huirne *et al.*, 2006a; Kolibianakis, 2006; Rombauts, 2006; Cédrin-Durnerin, 2007; Kim, 2011; Vilela, 2011; Shahrokh Tehrani Nejad, 2018; Fernandez-Prada, 2022	WMD 0.66, 95% CI 0.48 to 1.81	$I^2 = 95.9\%$; n = 1,223 participants; low-quality evidence; very low certainty assessment

(continued)

Table I Continued

Protocol	Outcome	Studies	Parameter estimate	Evidence quality
	Total dose of FSH (IU)	Huirne *et al.*, 2006a; Kolibianakis, 2006; Rombauts, 2006; Cédrin-Durnerin, 2007; Kim, 2011; Vilela, 2011; Fernandez-Prada, 2022	WMD 289.43, 95% CI −115.18 to 694.05	$I^2 = 94.6\%$; n = 1,100 participants; low-quality evidence; very low certainty assessment
	Oocytes retrieved	Huirne *et al.*, 2006a; Kolibianakis, 2006; Rombauts, 2006; Cédrin-Durnerin, 2007; Andersen, 2011; Kim, 2011; Vilela, 2011; Shahrokh Tehrani Nejad, 2018; Fernandez-Prada, 2022	WMD 0.29, 95% CI −1.07 to 1.65	$I^2 = 66.8\%$; n = 1,631 participants; low-quality evidence; low certainty assessment
E2 pretreatment versus no pretreatment	Clinical pregnancy	Fanchin, 2003; Cédrin-Durnerin, 2007, 2012; Blockeel, 2012; Erdem, 2015; Shahrokh Tehrani Nejad, 2018; Fernandez-Prada, 2022	RR 0.98, 95% CI 0.77 to 1.24	$I^2 = 0\%$; n = 1,006 participants; low-quality evidence; moderate certainty assessment
	OHSS moderate-to-severe	Blockeel, 2012; Shahrokh Tehrani Nejad, 2018; Fernandez-Prada, 2022	RR 1.01, 95% CI 0.86 to 1.20	$I^2 = 0\%$; n = 293 participants; low-quality evidence; very low certainty assessment
	First trimester clinical miscarriage	Erdem, 2015; Fernandez-Prada, 2022	RR 0.47, 95% CI 0.08 to 2.76	$I^2 = 0\%$; n = 172 participants; low-quality evidence; very low certainty assessment
	Duration of stimulation (days)	Fanchin, 2003; Cédrin-Durnerin, 2007, 2012; Blockeel, 2012; Shahrokh Tehrani Nejad, 2018; Fernandez-Prada, 2022	WMD 0.60, 95% CI 0.05 to 1.16	$I^2 = 89.5\%$; n = 844 participants; low-quality evidence; very low certainty assessment
	Total dose of FSH (IU)	Fanchin, 2003; Cédrin-Durnerin, 2007, 2012; Blockeel, 2012; Fernandez-Prada, 2022	WMD 192.73, 95% CI 140.22 to 245.23	$I^2 = 4.6\%$; n = 711 participants; low-quality evidence; low certainty assessment
	Oocytes retrieved	Cédrin-Durnerin, 2007, 2012; Blockeel, 2012; Erdem, 2015; Shahrokh Tehrani Nejad, 2018; Fernandez-Prada, 2022	WMD 0.39, 95% CI −0.32 to 1.09	$I^2 = 0\%$; n = 851 participants; low-quality evidence; low certainty assessment
Progestin pretreatment versus no pretreatment	Clinical pregnancy	Cédrin-Durnerin, 2007, 2012	RR 0.82, 95% CI 0.16 to 4.24	$I^2 = 0\%$; n = 416 participants; low-quality evidence; low certainty assessment
	OHSS moderate-to-severe	NA	NA	No data available
	First trimester clinical miscarriage	NA	NA	No data available
	Duration of stimulation (days)	Cédrin-Durnerin, 2007, 2012	WMD 0.24, 95% CI −1.02 to 1.49	$I^2 = 0\%$; n = 392 participants; low-quality evidence; low certainty assessment
	Total dose of FSH (IU)	Cédrin-Durnerin, 2007, 2012	WMD 138.12, 95% CI −287.06 to 563.30	$I^2 = 0\%$; n = 392 participants; low-quality evidence; low certainty assessment
	Oocytes retrieved	Cédrin-Durnerin, 2007, 2012	WMD 0.99, 95% CI −6.22 to 8.20	$I^2 = 0\%$; n = 392 participants; low-quality evidence; very low certainty assessment
E2 pretreatment versus OCP pretreatment	Clinical pregnancy	Cédrin-Durnerin, 2007; Hauzman, 2013; Shahrokh Tehrani Nejad, 2018; Fernandez-Prada, 2022	RR 1.01, 95% CI 0.63 to 1.61	$I^2 = 0\%$; n = 354 participants; moderate quality evidence; low certainty assessment
	OHSS moderate-to-severe	Shahrokh Tehrani Nejad, 2018; Fernandez-Prada, 2022	RR 1.10, 95% CI 0.32 to 3.74	$I^2 = 0\%$; n = 211 participants; low-quality evidence; very low certainty assessment

(continued)

Table I Continued

Protocol	Outcome	Studies	Parameter estimate	Evidence quality
	First trimester clinical miscarriage	Hauzman, 2013; Fernandez-Prada, 2022	RR 0.59, 95% CI 0.00 to 16178.76	$I^2 = 0\%$; n = 171 participants; low-quality evidence; low certainty assessment
	Duration of stimulation (days)	Cédrin-Durnerin, 2007; Hauzman, 2013; Shahrokh Tehrani Nejad, 2018; Fernandez-Prada, 2022	WMD −0.03, 95% CI −1.02 to 0.97	$I^2 = 72.7\%$; n = 318 participants; low-quality evidence; very low certainty assessment
	Total dose of FSH (IU)	Cédrin-Durnerin, 2007; Hauzman, 2013; Fernandez-Prada, 2022	WMD −133.61, 95% CI −802.07 to 534.84	$I^2 = 68.6\%$; n = 202 participants; low-quality evidence; low certainty assessment
	Oocytes retrieved	Cédrin-Durnerin, 2007; Hauzman, 2013; Shahrokh Tehrani Nejad, 2018; Fernandez-Prada, 2022	WMD 0.17, 95% CI −0.41 to 0.75	$I^2 = 0\%$; n = 318 participants; moderate quality evidence; low certainty assessment
Progestin pretreatment versus E2 pretreatment	Clinical pregnancy	Cédrin-Durnerin, 2007, 2012	RR 0.98, 95% CI 0.06 to 16.90	$I^2 = 16.2\%$; n = 418 participants; low-quality evidence; very low certainty assessment
	OHSS moderate-to-severe	NA	NA	No data available
	First trimester clinical miscarriage	NA	NA	No data available
	Duration of stimulation (days)	Cédrin-Durnerin, 2007, 2012	WMD −0.07, 95% CI −7.05 to 6.91	$I^2 = 92.1\%$; n = 403 participants; low-quality evidence; very low certainty assessment
	Total dose of FSH (IU)	Cédrin-Durnerin, 2007, 2012	WMD 92.68, 95% CI −2048.57 to 2233.92	$I^2 = 71.5\%$; n = 403 participants; low-quality evidence; very low certainty assessment
	Oocytes retrieved	Cédrin-Durnerin, 2007, 2012	WMD 0.05, 95% CI −1.99 to 2.10	$I^2 = 0\%$; n = 403 participants; low-quality evidence; low certainty assessment
Progestin pretreatment versus OCP pretreatment	Clinical pregnancy	NA	NA	Only one study
	OHSS moderate-to-severe	NA	NA	No data available
	First trimester clinical miscarriage	NA	NA	No data available
	Duration of stimulation (days)	NA	NA	Only one study
	Total dose of FSH (IU)	NA	NA	Only one study
	Oocytes retrieved	NA	NA	Only one study

E2, oestradiol; NA, not available; OCP, oral contraceptive pill; OHSS, ovarian hyperstimulation syndrome; RCT, randomized controlled trial; RR, relative risk; WMD, weighted mean difference.

Table II Summary of network meta-analyses for the relative risk of pregnancy outcomes following controlled ovarian stimulation with different GnRH antagonist protocols (compared with fixed-no pretreatment).

	Treatment	Studies	N participants	Relative risk, 95% CI (evidence quality)
Live birth 15 RCTs n = 3008	flexible-NP	Badrawi, 2005; Karabacak, 2006; Cédrin-Durnerin, 2007; Kim 2011; Xu, 2020; Fernandez-Prada, 2022	456	0.84, 0.57 to 1.25 (low-quality evidence)
	flexible-OCP	Barmat *et al.*, 2005; Cédrin-Durnerin, 2007; Kurzawa, 2008; Kim, 2011; Hauzman, 2013; Fernandez-Prada, 2022	227	0.86, 0.55 to 1.34 (low-quality evidence)
	flexible-E2	Cédrin-Durnerin, 2007; Ye *et al.*, 2009; Hauzman, 2013; Fernandez-Prada, 2022	213	0.81, 0.52 to 1.28 (low-quality evidence)
	fixed-P	Cédrin-Durnerin, 2012	135	0.87, 0.60 to 1.24 (low-quality evidence)
	fixed-E2	Cédrin-Durnerin, 2012	238	0.92, 0.69 to 1.24 (low-quality evidence)
	flexible-P	Cédrin-Durnerin, 2007	23	0.96, 0.35 to 2.61 (low-quality evidence)
	fixed-OCP	Garcia-Velasco *et al.*, 2011; Huirne *et al.*, 2006b	206	0.95, 0.62 to 1.47 (low-quality evidence)
Ongoing pregnancy 43 RCTs n = 8319	flexible-E2	Hauzman 2013; Erdem, 2015; Ye *et al.*, 2009; Fernandez-Prada, 2022	243	0.81, 0.62 to 1.06 (low-quality evidence)
	fixed-OCP	Cheung *et al.*, 2005; Huirne *et al.*, 2006a,b; Kolibianakis, 2006; Andersen, 2011; Garcia Velasco, 2011	744	0.84, 0.71 to 0.99 (moderate quality evidence)
	flexible-NP	Ludwig, 2002; Check *et al.*, 2004; Mochtar, 2004; Marci *et al.*, 2005; Rombauts, 2006; Baart *et al.*, 2007; Fornaro *et al.*, 2007; Pabuccu *et al.*, 2007; Tazegül *et al.*, 2008; Sbracia *et al.*, 2009; Tehraninejad *et al.*, 2009, 2011; Kolibianakis, 2011; Rabati and Zeidi, 2012; Sunkara *et al.*, 2014; Erdem, 2015; Hossein Rashidi, 2015; Depalo, 2018; Luo, 2021; Fernandez-Prada, 2022	1571	0.85, 0.73 to 1.00 (moderate quality evidence)
	flexible-OCP	Bahçeci *et al.*, 2005; Barmat *et al.*, 2005; Rombauts, 2006; Kurzawa, 2008; Lainas *et al.*, 2010; Tehraninejad *et al.*, 2010; Haydardedeoglu *et al.*, 2012; Hauzman, 2013; Fernandez-Prada, 2022	664	0.91, 0.76 to 1.08 (low-quality evidence)
Clinical pregnancy 61 RCTs n = 9623	flexible-P	Cédrin-Durnerin, 2007	23	0.68, 0.33 to 1.38 (low-quality evidence)
	fixed-OCP	Cheung *et al.*, 2005; Huirne *et al.*, 2006a,b; Andersen, 2011; Garcia Velasco, 2011; Prapas, 2013; Shin, 2018	687	0.76, 0.64 to 0.90 (moderate quality evidence)
	flexible-NP	Ludwig, 2002; Fanchin, 2003; Check *et al.*, 2004; Escudero, 2004; Loutradis *et al.*, 2004; Mochtar, 2004; Badrawi, 2005; Marci *et al.*, 2005; Xavier *et al.*, 2005; Ferrari *et al.*, 2006; Friedler *et al.*, 2006; Karabacak, 2006; Serafini *et al.*, 2006; Cédrin-Durnerin, 2007; Pabuccu *et al.*, 2007; Tazegül *et al.*, 2008; Sbracia *et al.*, 2009; Tehraninejad *et al.*, 2009, 2011; Al-Karaki *et al.*, 2011; Kim, 2011; Cota *et al.*, 2012; Rabati and Zeidi, 2012; Erdem, 2015; Hossein Rashidi, 2015; Shahrokh Tehrani Nejad, 2018; Xu, 2020; Luo, 2021; Fernandez-Prada, 2022	2111	0.82, 0.72 to 0.94 (moderate quality evidence)
	flexible-E2	Fanchin, 2003; Cédrin-Durnerin, 2007; Ye *et al.*, 2009; Hauzman, 2013; Erdem, 2015; Klement *et al.*, 2015; Shahrokh Tehrani Nejad, 2018; Fernandez-Prada, 2022	413	0.86, 0.70 to 1.05 (low-quality evidence)
	fixed-P	Cédrin-Durnerin, 2012	135	0.87, 0.63 to 1.19 (low-quality evidence)
	flexible-OCP	Bahçeci *et al.*, 2005; Barmat *et al.*, 2005; Cédrin-Durnerin, 2007; Moraloglu *et al.*, 2008; Hosseini *et al.*, 2010; Lainas *et al.*, 2010; Tehraninejad *et al.*, 2010; Kim, 2011; Hauzman, 2013; Mokhtar *et al.*, 2015; Trenkić *et al.*, 2016; Shahrokh Tehrani Nejad, 2018; Fernandez-Prada, 2022	688	0.90, 0.76 to 1.06 (low-quality evidence)
	fixed-E2	Blockeel, 2012; Cédrin-Durnerin, 2012	282	0.98, 0.78 to 1.23 (low-quality evidence)

Comparisons versus Fixed-NP: relative risk (RR) <1 favours the reference. E2, oestradiol; NP, no pretreatment; OCP, oral contraceptive pill; P, progestin; RCT, randomized controlled trial.

normal/low responders), antagonist (ganirelix versus cetrorelix) and criteria for initiating the antagonist protocol (ultrasound only, hormonal monitoring only or both). OPR was not significantly different for flexible protocols compared with fixed protocols when ultrasound only was used as the criterion for initiation of flexible protocols (RR 0.68, 95% CI 0.36 to 1.32; $I^2 = 0\%$; 3 RCTs; n = 344 participants; moderate quality evidence) (Ludwig *et al.*, 2002; Mochtar, 2004; Hossein Rashidi *et al.*, 2015) or when ultrasound and/or hormonal measurement was the criterion for initiation of the flexible protocol (RR 0.81, 95% CI 0.58 to 1.14; $I^2 = 0\%$; 3 RCTs; n = 563 participants; low-quality evidence) (Supplementary Table SVII) (Kolibianakis *et al.*, 2011; Depalo *et al.*, 2018; Luo *et al.*, 2021). No significant differences in effect sizes between the subgroups were observed ($P = 0.480$). No difference was reported or there were insufficient data for analyses for the other subpopulation subgroups (Supplementary Table SVII).

Subgroup analyses were not possible for cetrorelix versus ganirelix owing to insufficient data for all subpopulations.

Based on the available data, analyses for the following subgroups were performed for the comparison of pretreatment versus no pretreatment: population (normal/unselected), fixed versus flexible antagonist protocol, type of antagonist (iturelix, ganirelix or cetrorelix) and OCP-free interval (0 to 3 days versus 4 to 5 days). There was a significantly lower OPR with an OCP-free interval of 4–5 days compared with no pretreatment (RR 0.81, 95% CI 0.66 to 0.99; $I^2 = 0\%$; 3 RCTs; n = 1020 participants; low-quality evidence) (Huirne *et al.*, 2006a; Kolibianakis *et al.*, 2006; Andersen *et al.*, 2011). A statistically significant difference in OPR was not detected with an OCP-free interval 0–3 days when comparing OCP versus no OCP pretreatment (RR 0.71, 95% CI 0.07 to 7.18; $I^2 = 0\%$; 2 RCTs; n = 298 participants; low-quality evidence) (Huirne *et al.*, 2006a; Rombauts *et al.*, 2006). Importantly, no significant differences in effect sizes between the subgroups were observed ($P = 0.647$). A lower OPR was seen in studies that used ganirelix with OCP pretreatment compared with those that used ganirelix without OCP pretreatment (RR 0.80, 95% CI 0.67 to 0.96; $I^2 = 0\%$; 3 RCTs; n = 1,180 participants; low-quality evidence) (Kolibianakis *et al.*, 2006; Rombauts *et al.*, 2006; Andersen *et al.*, 2011). No data were available for the comparison of cetrorelix with and without pretreatment and only one study reported on studies using either cetrorelix or ganirelix (Fernandez-Prada *et al.*, 2022). There was no difference or there were insufficient data for analysis of the remaining outcomes in the subpopulations (Supplementary Table SVII).

Network meta-analysis

Nine interventions (protocol variations) were considered: agonist; flexible antagonist no pretreatment (flexible-NP); flexible antagonist OCP pretreatment (flexible-OCP); flexible antagonist E2 pretreatment (flexible-E2); flexible antagonist progestin (P) pretreatment (flexible-P); fixed antagonist no pretreatment (fixed-NP); fixed antagonist OCP pretreatment (fixed-OCP); fixed antagonist E2 pretreatment (fixed-E2); and fixed antagonist progestin pretreatment (fixed-P). None of the studies reported on all three outcomes of interest (LBR, OPR or CPR).

Overall, there was insufficient evidence of a difference between any protocol versus fixed-NP for LBR (15 RCTs; 3008 participants; all studies were of low quality) (Fig. 3A; Supplementary Fig. S1). The network meta-analysis for OPR included 43 RCTs (8319 participants). The use of a fixed-OCP protocol resulted in a significantly lower OPR compared with a fixed-NP protocol (RR 0.84, 95% CI 0.71 to 0.99; moderate quality evidence) (Fig. 3B; Supplementary Fig. S1). The use of flexible-NP and fixed-NP protocols was not associated with significantly different OPRs; however, the upper boundary of the CI was just at 1.00 (RR 0.85, 95% CI 0.73 to 1.00; moderate quality evidence) (Fig. 3B; Supplementary Fig. S1). The network meta-analysis for CPR included 61 RCTs (9623 participants). Overall, the use of a fixed-OCP intervention or flexible-NP intervention resulted in a significantly lower CPR compared with a fixed-NP protocol (RR 0.76, 95% CI 0.64 to 0.90, moderate quality evidence; RR 0.82, 95% CI 0.72 to 0.94; moderate quality evidence, respectively) (Fig. 3C; Supplementary Fig. S1). For all outcomes, the SUCRA scores suggested that the fixed-NP protocol was likely to be the optimal GnRH antagonist protocol (68% for LBR, 84% for OPR and 84% for CPR) (Fig. 4). There was no evidence of global inconsistency for LBR ($P = 0.6991$), OPR ($P = 0.6890$) or clinical pregnancy ($P = 0.4898$) in the studies included in the network meta-analysis. Local inconsistency was observed in the comparison of flexible-NP versus flexible-P for CPR ($P = 0.009$); however, there was no evidence of local inconsistency for LBR or OPR.

The sensitivity analysis showed reduced chances of LBR or OPR for flexible versus fixed protocols across all scenarios where data were available, although most findings were not statistically significant. An exception to this was in the scenario of ganirelix with no pretreatment, where the CPR was lower in a flexible protocol compared with the fixed protocol (RR 0.71, 95% CI 0.55 to 0.92; 11 RCTs; n = 1907 participants) (Supplementary Table SVIII). No other effect sizes were found to be statistically significantly different (Supplementary Table SIX).

Discussion

Overall, the results of the pairwise meta-analysis showed that there was a lower OPR following a flexible protocol compared with a fixed Day 5/6 protocol, and after OCP pretreatment compared with no pretreatment. The network meta-analysis showed that the use of the fixed-OCP protocol resulted in a significantly lower OPR compared with the fixed-NP protocol, and a flexible-NP protocol may result in a numerically lower OPR compared with the fixed-NP protocol; however, this was not statistically significant. By comparing all available protocols, the SUCRA score suggested that the fixed-NP protocol is most likely to result in the highest OPR. While we have tried to account for commonly encountered variations in the flexible protocols in this meta-analysis, there is extensive heterogeneity among the criteria used to initiate the flexible antagonist protocols, which may have had an effect on our results for the comparison of fixed versus flexible protocols for OPR. Furthermore, all studies included in this analysis were of low-to-moderate quality and therefore the results should be interpreted with caution.

Fixed versus flexible

There were no data available for live birth from studies directly comparing flexible and fixed protocols. A lower OPR was reported with the use of flexible protocols compared with fixed protocols. Five out of six studies used a fixed Day 6 protocol and only one study (Luo *et al.*, 2021) used a Day 5 fixed start; however, there was no difference between the subgroups in terms of the effect sizes. The finding of lower OPR with the flexible protocol with no pretreatment was also

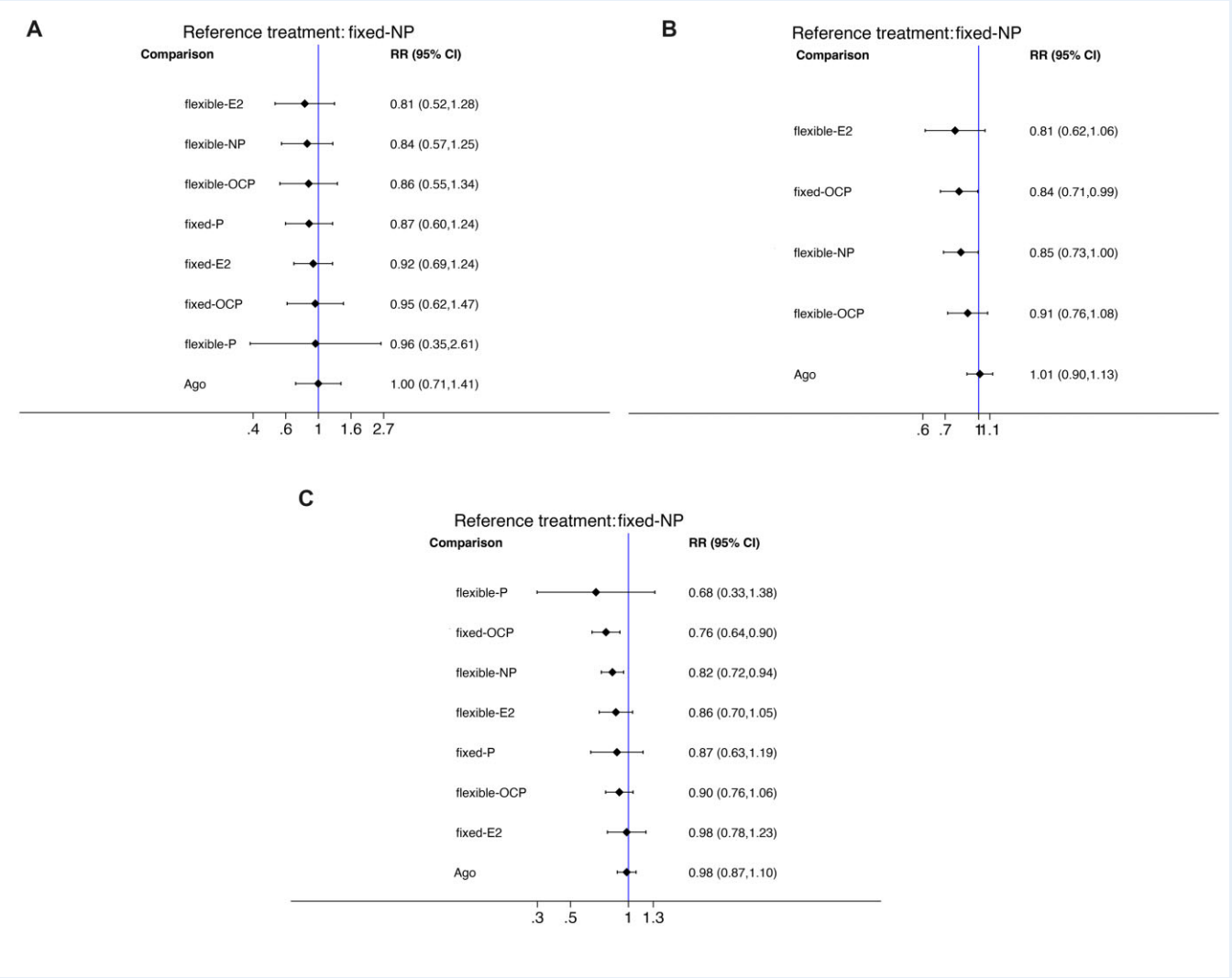


Figure 3. Forest plots of relative risk from the network meta-analyses for pregnancy outcomes in women. (**A**) Forest plots of RR for achievement of live birth in women when undergoing COS using different GnRH antagonist protocols. (**B**) Forest plots of RR for achievement of ongoing pregnancy in women when undergoing COS using different GnRH antagonist protocols. (**C**) Forest plots of RR for achievement of clinical pregnancy in women when undergoing COS using different GnRH antagonist protocols. Ago, agonist; E2, oestradiol; NP, no pretreatment; OCP, oral contraceptive pill; P, progestin; RR, relative risk; COS, controlled ovarian stimulation.

suggested by the network meta-analysis, however the 95% CI in the network meta-analysis included 1.00 (RR 0.85, 95% CI 0.73 to 1.00). It should be noted, however, that the included studies had a mixture of patient populations, antagonists and, importantly, heterogeneous criteria for initiating the antagonist (Supplementary Table SVI). In a fixed protocol, GnRH antagonist administration is usually started on Day 5 or 6 of COS during ART treatment, whereas more variable criteria are used in flexible protocols based on ultrasound only criteria (at least one follicle with a diameter of 14 or 15 mm) or on ultrasound and/or hormonal criteria (at least one dominant follicle with a diameter of >12, ≥14 or ≥15 mm, serum E2 per dominant follicle at a level of >150 or >200 pg/ml and/or serum LH level ≥10 IU/l) (Ludwig *et al.*, 2002; Escudero *et al.*, 2004; Mochtar, 2004; Kolibianakis *et al.*, 2011; Hossein Rashidi *et al.*, 2015; Depalo *et al.*, 2018; Luo *et al.*, 2021).

Differences in the criteria used may affect the reproductive outcomes and influence comparisons among not only the fixed and flexible protocols, but also among different flexible protocols (Lyttle Schumacher *et al.*, 2018). In view of recent data suggesting that a high serum E2 level could result in a decline in pregnancy rates, it is possible that initiating a GnRH antagonist too late in a fresh ART embryo transfer cycle may result in decreased receptivity of the endometrium or over-maturation of the oocytes, leading to decreased oocyte yield and lower pregnancy outcomes (Hernandez, 2000). Similarly, if a GnRH antagonist was initiated at a serum E2 level of <300 pg/ml, this early GnRH antagonist administration might directly impact the mitotic programming of cells involved in folliculogenesis, resulting in a decrease or an impaired increase in serum E2 levels during COS in the context of ART (Al-Inany *et al.*, 2016) or may interfere with endometrial development and implantation (Hernandez, 2000).

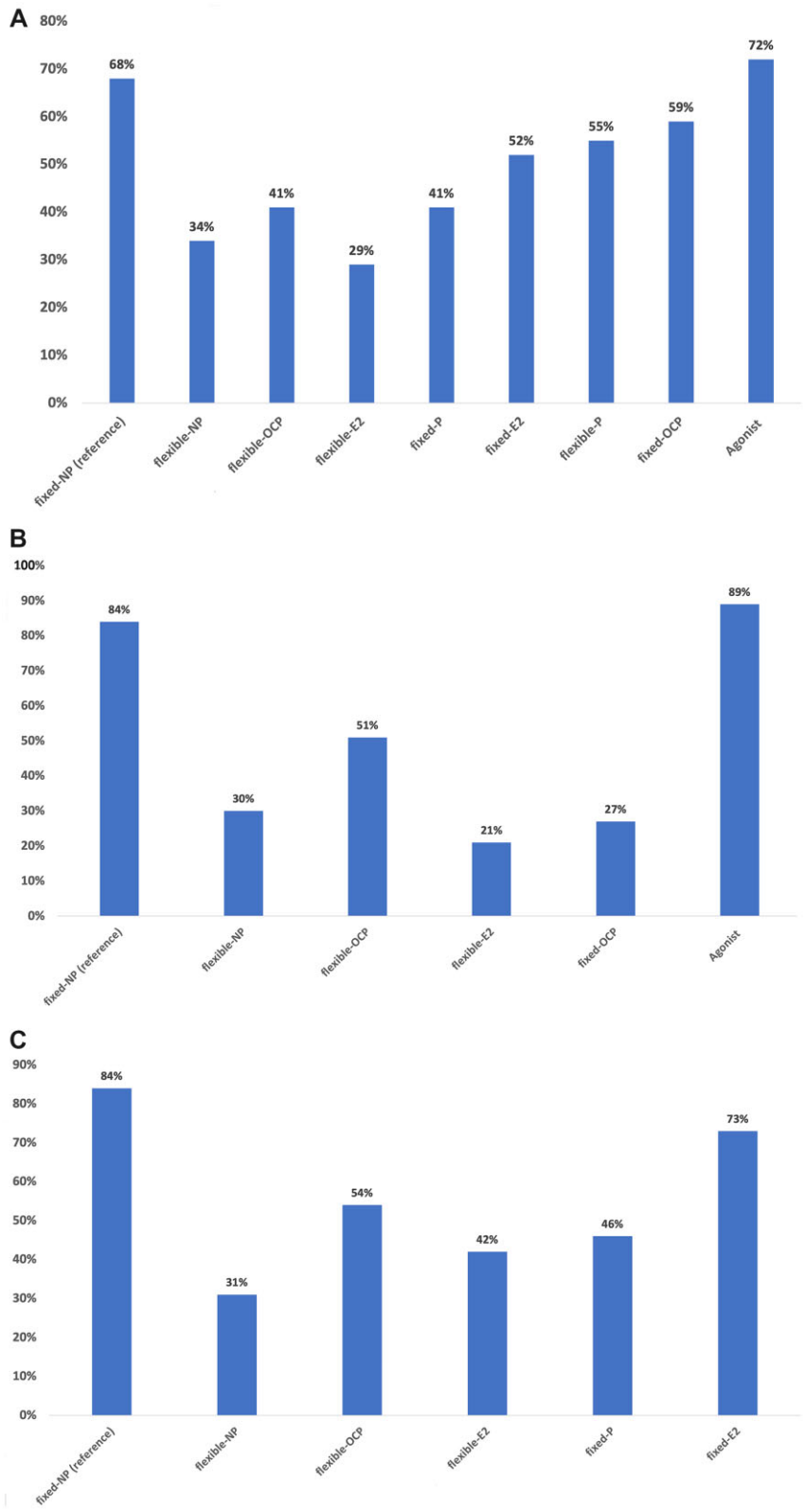


Figure 4. SUCRA scores for the network meta-analyses which are used to quantify GnRH antagonist treatment ranking. The higher the value of the SUCRA, the more superior the treatment type. (**A**) SUCRA scores for the achievement of live birth in women undergoing COS using different GnRH antagonist protocols. (**B**) SUCRA scores for the achievement of ongoing pregnancy in women undergoing COS using different GnRH antagonist protocols. (**C**) SUCRA scores for the achievement of clinical pregnancy in women undergoing COS using different GnRH antagonist protocols. Ant, antagonist; E2, oestradiol; NP, no pretreatment; OCP, oral contraceptive pill; P, progestin; SUCRA, surface under the cumulative ranking curve (on *Y*-axes); COS, controlled ovarian stimulation.

It should also be considered that there may be individual variations in patient response to COS. Notably, antagonist initiation in flexible protocols may occur before (although this is rare) or after the fixed Day 5/6, which means that patients may be at risk of a premature LH rise/surge if they have a fast ovarian response and the antagonist initiation is delayed for too long (Albano *et al.*, 2000; Borm and Mannaerts, 2000; Olivennes *et al.*, 2000; European and Middle East Orgalutran Study Group, 2001; Fluker *et al.*, 2001; Ludwig *et al.*, 2002; Mochtar, 2004) or the antagonist may be initiated too soon in patients with a slow response to gonadotrophin treatment (Tannus *et al.*, 2013; Ozturk Inal *et al.*, 2018). Future trials need to investigate the optimal timing and modality of monitoring ovarian response as well as the criteria for initiating the antagonist in the flexible protocol, since different criteria for the flexible protocol might lead to comparable results to the fixed protocol.

Cetrorelix versus ganirelix

The current systematic review and network meta-analysis did not provide evidence of a difference between cetrorelix and ganirelix in terms of LBR or OPR. More specifically, only one study reported LBR, suggesting no statistically significant difference, and no studies reported OPR. Furthermore, there was a lack of data regarding other secondary outcome measures. This lack of data precluded a formal network meta-analysis (since cetrorelix and ganirelix were considered equivalent). In theory, the different pharmacokinetic profiles (Duijkers *et al.*, 1998; Huirne and Lambalk, 2001; Tur-Kaspa and Ezcurra, 2009) of the two antagonists could lead to a clinical difference; however, until further evidence from properly designed RCTs is available, a clinical difference between cetrorelix and ganirelix cannot be supported based on the limited number of currently published RCTs.

Hormonal pretreatment

Several pretreatment regimens were assessed in the pairwise meta-analysis (E2, OCP and progestin) compared with no pretreatment or placebo. The direct pairwise comparison and the network meta-analysis failed to detect a significant difference in LBR between the different pretreatment regimens versus no pretreatment and between the different pretreatment protocols. However, there were a limited number of relevant RCTs that provided live birth data (two or fewer for all comparisons) and most studies were small in size. In the pairwise meta-analysis, a significantly lower OPR after pretreatment with the OCP compared with no pretreatment was observed, and the sensitivity analysis did not alter this result. These results are in agreement with those of Griesinger *et al.*, whose meta-analysis reported a 5% (95% CI −10% to −1%) lower OPR in women receiving OCP compared with those with no pretreatment (Griesinger *et al.*, 2010). Although the OCP is regularly used to schedule the start of IVF treatment, remnant adverse effects have been reported for the subsequent fresh embryo transfer cycle, which includes lower serum levels of LH and E2, and lower endometrial thickness in women with OCP-induced menses compared with those with spontaneous menses or progestin-induced menses (Wei *et al.*, 2017), as well as low serum LH levels that persisted from the beginning of COS for IVF until the day of hCG trigger (Kolibianakis *et al.*, 2006). An adverse effect of OCP treatment on LBR following freeze-all or frozen embryo transfers has also been shown, which has led to speculation that the remnant effect of using the OCP for scheduling endometrial preparation alone may be enough to negatively affect endometrial receptivity or the ensuing pregnancy rate (Wei *et al.*, 2017).

While it is thought that endogenous gonadotrophin secretion recovers within 5 days of OCP cessation (Cédrin-Durnerin *et al.*, 2007), the detrimental effects on conception may persist after discontinuation, although a review of the literature reported no difference in conception rates at 12 months after cessation from those seen for other contraceptive methods (Barnhart and Schreiber, 2009). Our own subgroup analyses of the pretreatment-free interval did not detect a statistically significant difference in the effect sizes between the two subgroups (0–3 days versus 4–5 days), although all these comparisons were based on a small number of RCTs and low-quality evidence. There were no data for treatment-free periods of >6 days.

Regarding pretreatment with E2 (Cédrin-Durnerin *et al.*, 2012) or progestin (Wei *et al.*, 2017), it has been supported that they do not confer a negative effect on pregnancy rates similar to that observed with OCP pretreatment. The present pairwise and network meta-analysis did not provide statistical evidence of a negative effect of E2 or progestin pretreatments on pregnancy rates. However, it did not also provide conclusive evidence of no difference, as the RRs for LBRs were <0.90 in both cases. It should be noted though that the number of available studies was limited. The network meta-analysis also failed to provide evidence of E2 or progestin pretreatments being preferable to OCP and this is reflected in the SUCRA scores where protocols including E2 and progestin pretreatment did not consistently have higher SUCRA scores compared to protocols with OCP pretreatment (Fig. 4).

Limitations

It should be acknowledged that there are some limitations in this analysis. As with any pairwise meta-analysis, the quality of the eventual output is determined by the quality of the primary studies. All studies included in this analysis were of low-to-moderate quality. For this reason, only RCTs evaluating the research question of interest were included. Furthermore, a sensitivity analysis with the exclusion of RCTs for which the randomization method was unclear was also performed. The presence of heterogeneity was quantified and explored based on predefined parameters using subgroup analyses although, in many cases, these analyses were underpowered owing to the lack of data. Another potential limitation is that this analysis was based on data from RCTs, which have strict inclusion/exclusion criteria and may not be representative of the patient populations typically undergoing ART treatment (Hershkop *et al.*, 2017). Therefore, real-world data are required to confirm the results of this analysis. In addition, our analysis only included fresh embryo transfer cycles, as only two small, recently published RCTs comparing different antagonist protocols reported data on cumulative pregnancy rates (Luo *et al.*, 2021; Fernandez-Prada *et al.*, 2022). Therefore, future studies reporting cumulative outcomes of frozen embryo transfer are needed, as certain outcomes can either be limited to fresh embryo transfers or persist through an effect on the embryo quality or a persistent effect on the endometrium. Furthermore, the number of studies used for each outcome analysis was relatively small, which could lead to biased estimation of heterogeneity (I^2) (von Hippel, 2015).

The use of indirect data for the assessment of specific interventions has been proposed in the last few years and, although it has been

shown to be a valuable and valid approach, it is also characterized by well-known limitations, meaning pairwise meta-analyses are always superior to analyses using indirect data. Most importantly, the feasibility of combining a network of studies performing different comparisons is based on the fundamental assumption of transitivity. This means that the studies that are indirectly pooled are essentially performed in similar populations using similar methodological and clinical characteristics. These limitations were considered during the interpretation of all results obtained during this research project; there was no evidence of global or local inconsistency both for live birth and OPRs.

Implications for clinical practice and future research

Almost 20 years after the introduction of GnRH antagonists in clinical practice, a number of protocol variations have been proposed and are being used worldwide. In view of the efficacy and safety data shown in this review, a fixed Day 5/6 GnRH antagonist protocol without any pretreatment appears to be the optimal protocol among women undergoing COS during ART treatment using GnRH antagonists. The fixed Day 5/6 GnRH antagonist protocol without any pretreatment appears to lead to better reproductive outcomes compared with a flexible antagonist protocol based on ultrasound monitoring only or using antagonist protocols with OCP pretreatment. It should, however, be noted that the studies included in this review included primarily normal responders or unselected populations and, therefore, there is not enough evidence to make recommendations specifically for poor responders or women with expected high response or PCOS.

High-quality research on the optimal criteria of a flexible protocol for antagonist initiation in low, normal and high responders is required. More trials are also required to assess the effectiveness of ganirelix versus cetrorelix, separately, as well as large trials that randomize women undergoing pretreatment with OCP, progestin or E2, and including women with high or low ovarian response. Pretreatment with progestin should be of particular interest for further research owing to the limited data currently available. Importantly, any such research needs to attempt to decipher how a potentially negative effect of these pretreatments is exerted.

Conclusion

The current systematic review and meta-analysis of direct and indirect randomized data suggests that it is likely that not all antagonist protocols are equivalent and that a fixed Day 5/6 antagonist protocol with no pretreatment might be associated with higher OPRs compared with other antagonist protocols for patients undergoing COS during ART treatment. However, as this analysis primarily included normal responders, there is insufficient evidence to make specific recommendations for poor responders or women with expected high response or PCOS. More trials are required to confirm these findings and also assess the comparative effectiveness of ganirelix versus cetrorelix, the effect of different pretreatment interventions or the effect of different criteria for initiation of the antagonist in the flexible protocol. Furthermore, more studies are required to examine the optimal GnRH antagonist protocol in women with high or low response to COS.

Supplementary data

Supplementary data are available at *Human Reproduction Update* online.

Data availability

The data underlying this article will be shared upon reasonable request to the Corresponding Author.

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Authors' roles

C.A.V., T.D.H. and S.L. conceived the idea of the study and worked out the study proposal. C.A.V. performed the literature search, drafted the protocol, interpreted the analysis and drafted the article. A.S. drafted the protocol, performed the study selection and data extraction, interpreted the analysis, drafted the report and critically revised the article for intellectual content. S.J.C. performed the study selection and data extraction, and critically revised the manuscript for intellectual content. S.L. and T.D.H. contributed to the study design, protocol revision, interpreted the results and critically revised the article. X.Y. performed a critical review of the statistical analysis methods and results and critically revised the article for intellectual content. All authors reviewed and approved the final version of the article.

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Conflict of interest

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EMD Serono Research & Development Institute, Inc., Billerica, MA, USA; a business of Merck KGaA, Darmstadt, Germany. S.L. is an employee of Merck Serono S.p.A., Rome, Italy, an affiliate of Merck KGaA, Darmstadt, Germany. T.D.H. is an employee of Merck KGaA, Darmstadt, Germany. S.J.C. and A.S. report no conflicts of interest.

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