Title

# First-line ovulation induction for polycystic ovary syndrome: an individual participant data meta-analysis

Running title

Ovulation induction for PCOS

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## Abstract

**Background**: Polycystic ovary syndrome (PCOS) is the most frequent cause of anovulatory infertility. In women with PCOS, effective ovulation induction serves as an important first-line treatment for anovulatory infertility. Individual participant data (IPD) meta-analysis is considered as the gold standard for evidence synthesis which provides accurate assessments of outcomes from primary randomised controlled trials (RCTs) and allows additional analyses for time-to-event outcomes. It also facilitates treatment-covariate interaction analyses and therefore offers an opportunity for personalised medicine.

**Objective and rationale**: We aimed to evaluate the effectiveness of different ovulation induction agents, in particular letrozole alone and clomiphene citrate (CC) plus metformin, as compared to CC alone, as the first-line choice for ovulation induction in women with PCOS and infertility, and to explore interactions between treatment- and participant-level baseline characteristics.

**Search methods**: We searched electronic databases including MEDLINE, EMBASE and Cochrane Central Register of Controlled Trials up to 20th December 2018. We included RCTs comparing the following interventions with each other or placebo/ no treatment in women with PCOS and infertility: CC, metformin, CC plus metformin, letrozole, gonadotrophin and tamoxifen. We excluded studies on treatment-resistant women. The primary outcome was live birth. We contacted the investigators of eligible RCTs to share the IPD and performed IPD meta-analyses. We assessed the risk of bias by using the Cochrane risk of bias tool for RCTs.

**Outcomes:** IPD of 20 RCTs including 3962 women with PCOS were obtained. Six RCTs compared letrozole and CC in 1284 women. Compared with CC, letrozole improved live birth rates (3 RCTs, 1043 women, risk ratio [RR] 1.43, 95% confidence interval [CI] 1.17-1.75, moderate-certainty evidence) and clinical pregnancy rates (6 RCTs, 1284 women, RR 1.45, 95% CI 1.23-1.70, moderate-certainty evidence), and reduced time-to-pregnancy (6 RCTs,

1235 women, hazard ratio [HR] 1.72, 95%CI 1.38-2.15, moderate-certainty evidence). Meta-analyses of effect modifications showed a positive interaction between baseline serum total testosterone levels and treatment effects on live birth (interaction RR 1.29, 95%CI 1.01-1.65). Eight RCTs compared CC plus metformin to CC alone in 1039 women. Compared with CC alone, CC plus metformin might improve clinical pregnancy rates (8 RCTs, 1039 women, RR 1.18, 95% CI 1.00-1.39, low-certainty evidence) and might reduce time-to-pregnancy (7 RCTs, 898 women, HR 1.25, 95%CI 1.00-1.57, low-certainty evidence), but there was insufficient evidence of a difference on live birth rates (5 RCTs, 907 women, RR 1.08, 95% CI 0.87-1.35, low-certainty evidence). Meta-analyses of effect modifications showed a positive interaction between baseline insulin levels and treatment effects on live birth in the comparison between CC plus metformin and CC (interaction RR 1.03, 95% CI 1.01-1.06).

**Wider implications**: In women with PCOS, letrozole improves live birth and clinical pregnancy rates and reduces time-to-pregnancy compared to CC and therefore can be recommended as the preferred first-line treatment for women with PCOS and infertility. CC plus metformin may increase clinical pregnancy and may reduce time-to-pregnancy compared to CC alone, while there is insufficient evidence of a difference on live birth. Treatments effects of letrozole are influenced by baseline serum levels of total testosterone, while those of CC plus metformin are affected by baseline serum levels of insulin. These interactions between treatments and biomarkers on hyperandrogenaemia and insulin resistance provide further insights into a personalised approach for the management of anovulatory infertility related to PCOS.

**Key words:**

polycystic ovary syndrome, infertility, anovulation, ovulation induction, letrozole, clomiphene, metformin, individual participant data, meta-analysis.

## Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder of reproductive age women, and the prevalence among different geographic regions ranges from 5% to 21%, depending on the criteria used (Lizneva, et al., 2016). PCOS is a heterogeneous syndrome comprising of at least two of the following clinical characteristics according to the Rotterdam diagnostic criteria: oligo-/ anovulation, clinical and/or biochemical hyperandrogenism, or polycystic ovaries morphology based on ultrasound assessment (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004).

Anovulatory infertility is usually one of the key features that women with PCOS are confronted with. Simple and effective infertility treatments as the first-line choice are therefore important. Our previous network meta-analysis compared available first-line treatment options for women with PCOS with infertility and found that letrozole and combined clomiphene citrate (CC)-metformin were superior to other ovulation induction medications in terms of clinical pregnancy and that letrozole resulted in more live births than other interventions, including CC (Wang, et al., 2017). These findings are in agreement with the evidence summarised in the International evidence based guideline for the assessment and management of PCOS (Teede, et al., 2018).

As women with PCOS represent a heterogeneous population according to the diagnostic criteria, it is important to identify which individuals benefit most from a particular treatment so that clinicians can provide personalised care (Wang and Mol, 2017). However, primary RCTs are usually underpowered to detect subgroup effects (Riley, et al., 2010). Subgroup analyses in meta-analyses of aggregate data are at risk of ecological bias due to the ignorance of within-study interactions, or are even impossible to perform due to heterogeneous reporting of subgroup data in the primary trials (Riley, et al., 2010).

Moreover, time-to-pregnancy is also an important patient-centred outcome, but it has never been reported in previous meta-analyses on PCOS. This is likely due to the unavailability of the data in the publication as well as the methodological challenges on data extraction and synthesis. In addition, the primary trials are not always of high quality in terms of analyses and reports (Eshre Capri Workshop Group, 2018), which can directly affect the data extraction, analysis and risk of bias assessment process in subsequent meta-analyses.

These deficiencies in aggregate data meta-analyses can potentially be overcome by using individual participant data (IPD). IPD meta-analysis has been described as the gold standard in evidence synthesis, by engaging investigators of the primary trials to provide the raw data of the primary trials (Broeze, et al., 2010). Such strategy facilitates derivation of the information beyond the primary publication, standardisation of inclusion criteria, outcomes and analyses across trials, and investigations of subgroup effects and time-to-event outcomes. (Broeze, et al., 2010, Riley, et al., 2010).

We therefore performed an IPD meta-analysis to evaluate the effectiveness of different ovulation induction agents, in particular letrozole alone and CC plus metformin, as compared to CC alone, as the first-line choice for ovulation induction in women with PCOS and infertility, and to explore interactions between treatment- and participant-level baseline characteristics.

## Methods

### Registration and literature search

This IPD meta-analysis was conducted based on a registered protocol (PROSPERO CRD42017059251) and reported according to the Preferred Reporting Items for Systematic

Review and Meta-Analyses of individual participant data (PRISMA-IPD) statement (Stewart, et al., 2015).

We updated the searches in MEDLINE, EMBASE and Cochrane Central Register of Controlled Trials in September 2017, based on our previous search strategies for a network meta-analysis on treatment strategies for World Health Organization (WHO) II anovulation (Wang, et al., 2017). In brief, the search terms included both index terms as well as free words on PCOS, anovulation and ovulation induction. After completing data requesting process, we further updated the search on 20th December 2018 to identify the latest studies. We also searched the WHO International Clinical Trials Registry Platform (WHO ICTRP) and U.S. National Institutes of Health (clinicaltrials.gov) and ISRCTN registry to identify ongoing trials. In addition, we reviewed the references lists of relevant papers and corresponded with trialists in PCOS to identify potential eligible trials that we might have missed.

**Eligibility criteria**

We included RCTs comparing the following interventions with each other or placebo/no treatment: clomiphene citrate (CC), metformin, CC and metformin combined, letrozole, gonadotrophins and tamoxifen in women with WHO II anovulation, including PCOS. We excluded trials reporting on treatment-resistant women, trials comparing different doses of the same intervention and quasi-RCTs. We did not apply language restrictions. For crossover trials, we only included the data in the first phase.

The primary outcome was live birth. The secondary outcomes were clinical pregnancy, ovulation, miscarriage, multiple pregnancy and time to pregnancy.

**Study selection and data collection**

Two members of the review team (from RW, WL and EMB) independently assessed the titles and abstracts to exclude irrelevant studies and subsequently reviewed the full-text articles to evaluate their eligibility. Disagreements were resolved by discussion with a third author (BWM, MvW or RJN).

We contacted investigators of eligible RCTs to share the de-identified IPD and established the International Ovulation Induction IPDMA Collaboration. We sent at least two more reminders when we did not receive responses.

We obtained de-identified IPD including baseline characteristics including age, body mass index (BMI), ethnicity, type of infertility (primary/secondary), treatment history (treatment-naïve or not), fasting glucose, fasting insulin, total testosterone, sex hormone binding globulin (SHBG), ovarian volume and the Ferriman-Gallwey score for hirsutism. We also obtained data on allocated treatments, number of ovulation induction cycles, ovulation and fertility outcomes including live birth, clinical pregnancy, miscarriage and multiple pregnancy.

We checked data for consistency by comparing the analyses from obtained IPD with the original publications. We discussed any inconsistencies or obvious errors with investigators of primary RCTs and solved discrepancies by consensus.

**Risk of bias assessment**

Two members of the review team independently evaluated the risk of bias in each included RCT, using the domain-based evaluation tool described in the Cochrane Handbook for Systematic Reviews of Interventions (Higgins and Green, 2011). We assessed the following domains as low risk of bias, unclear or high risk of bias: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessors, incomplete outcome data, selective reporting (reporting bias) and other sources of bias. When

the risk of bias for a domain was unclear, investigators of these RCTs were asked to provide additional information to resolve the uncertainty.

We assessed the overall certainty of the evidence across RCTs by using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach, including the risk of bias, consistency of effect, imprecision, indirectness and publication bias.

**Data synthesis**

We conducted all analyses based on an intention-to-treat principle using woman randomised per allocated group as the unit of all analyses. We performed two-stage random-effects IPD meta-analyses for letrozole versus CC alone and CC with metformin versus CC alone. For dichotomous outcomes, we calculated risk ratios (RRs) and 95% confidence intervals (CIs) and presented statistical heterogeneity by using $I^2$ statistic (Higgins and Green, 2011). For time-to-event outcomes, we used the number of treatment cycles as an approximate estimate for time and visualised the summary time-to-event in simple non-stratified Kaplan-Meier curves. We also estimated hazard ratios (HR) in Cox proportional hazards regression models for discrete time and pooled HRs and 95% CI, by using the generic inverse variance method (Fisher, 2015). Subgroup effects were estimated for the primary outcome by treatment-covariate interaction terms within trials and subsequent meta-analyses of interactions, as interactions using within-trials information alone without considering between-trials interactions are recommended as the standard practice to avoid ecological bias (Fisher, et al., 2017). We explored the treatment-covariate interactions of the following pre-specified baseline covariates: age, BMI, ethnicity, primary/secondary infertility, treatment history, hirsutism score, insulin resistance (serum glucose and insulin level), hyperandrogenaemia status (testosterone, SHBG, free androgen index) and ovarian volume. We also added the analysis of homeostatic model assessment for insulin resistance (HOMA-IR) as requested during the peer review process. For dichotomous

263 covariates with statistically significant interaction, we further performed stratified analyses to
264 illustrate the treatment effects in different strata of the subgroups. Continuous variables were
265 analysed as such without categorisation. For continuous covariates with statistically significant
266 interaction, we further presented a weighted mean curve and pointwise confidence interval
267 based on treatment-covariate interactions estimated in relevant studies. Due to the potential
268 type I error, the results of subgroup analyses were all considered exploratory.
269 To evaluate the IPD availability bias, we performed a network meta-analysis of RCTs with IPD
270 in a random-effects multivariate meta-analysis model (Riley, et al., 2017, White, 2015) on live
271 birth and clinical pregnancy, and then compared the results with a network meta-analysis of all
272 eligible RCTs. If these results were consistent, we considered the included RCTs with IPD
273 representative of all the eligible RCTs.
274 We performed a sensitivity analysis on studies with low risk of bias in allocation concealment
275 as planned. As the majority of eligible studies focused only on treatment-naïve women with
276 PCOS, these studies did not contribute to within-study interaction for treatment history and
277 were not included in the treatment-covariate analysis. We performed a post-hoc sensitivity
278 analysis by including only treatment-naïve women to demonstrate the robustness of the results.
279 We conducted all the analyses in Stata software version 15.1 (Stata Corp, College Station, TX,
280 USA).
281
282 **Results**
283
284 **Characteristics of included studies**
285 The final updated search yielded 709 non-duplicated studies (Figure 1). After screening the
286 titles and abstracts, 636 irrelevant studies were excluded. Finally, a total of 62 studies (61
287 publications, 9356 women) fulfilled the inclusion criteria and were included. These studies

were published in English (n=58), French (n=1) (Boudhraa, et al., 2010), Italian (n=1) (Santonocito, et al., 2009), Turkish (n=1) (Aygen, et al., 2007) and Persian (n=1) (Lorzadeh, et al., 2011).

IPD was not sought from eight studies (575 women), due to insufficient contact information (n=6; 359 women) (Beigi, 2006, Boudhraa, et al., 2010, Cudmore and Tupper, 1966, El-Biely and Habba, 2001, Garcia, et al., 1985, Johnson, et al., 1966) or because the studies were identified after our data requesting timeline (n=2; 216 women) (Fatima, et al., 2018, Topçu, et al., 2017). For the remaining 54 studies (8781 women), the primary investigators were contacted to share IPD of the primary studies. IPD from 34 studies (4819 women) were not available, due to no response (n=23; 3258 women) (Abuelghar, et al., 2013, Atay, et al., 2006, Ayaz, et al., 2013, Banerjee Ray, et al., 2012, Basirat, et al., 2012, Boostanfar, et al., 2001, Chen, et al., 2016, Dasari and Pranahita, 2009, Dehbashi, et al., 2009, Hossein-Rashidi, et al., 2016, Jahan, 2015, Karimzadeh, et al., 2007, Karimzadeh and Javedani, 2010, Lopez, et al., 2004, Lorzadeh, et al., 2011, Maged, et al., 2015, Robinson, et al., 2003, Roy, et al., 2012, Selim and Borg, 2012, Seyedoshohadaei, et al., 2012, Sharief and Nafee, 2015, Sheikh-El-Arab Elsedeek and Elmaghraby, 2011, Zeinalzadeh, et al., 2010), data loss (n=10; 1411 women) (Aygen, et al., 2007, Badawy, et al., 2009, Badawy and Gibreal, 2011, Fleming, et al., 2002, Keikha and Shahraki Mojahed, 2011, Khorram, et al., 2006, Mobusher, 2014, Santonocito, et al., 2009, Tang, et al., 2006, Zain, et al., 2009) or legal reasons (n=1; 150 women) (Moussa, et al., 2016). The details of these studies are listed in Supplementary Table 1.

IPD were available for at least one outcome from 20 studies (3962 women), including three from the US (Legro, et al., 2007, Legro, et al., 2014, Williams, et al., 2009), three from Italy (Leanza, et al., 2014, Palomba, et al., 2005, Vegetti, et al., 1999), three from Turkey (Bayar, et al., 2006, Nazik and Kumtepe, 2012, Sahin, et al., 2004), two from the UK (Amer, et al., 2017, Lord, et al., 2006), two from China (Liu, et al., 2017, Wu, et al., 2017), two from India (Kar,

2012, Kar and Sanchita, 2015), two studies (in one publication) from New Zealand (Johnson, et al., 2010), one from The Netherlands (Moll, et al., 2006), one from Finland (Morin-Papunen, et al., 2012) and one from multiple countries (The Netherlands, UK, Malta, Belgium, Argentina and Colombia) (Homburg, et al., 2012). These RCTs were published in English between 1999 and 2017, with 11 (55%) published after 2010.

Participants in all 20 RCTs were women with PCOS. In one RCT, participants were diagnosed with PCOS by fulfilling at least three of the following: PCO morphology, oligo/amenorrhoea, hirsutism, hyperandrogenaemia and elevated serum LH/FSH ratio (Sahin, et al., 2004); while in the remaining 19 RCTs, the participants were women with PCOS based on the Rotterdam criteria (Bayar, et al., 2006, Kar, 2012, Leanza, et al., 2014, Liu, et al., 2017, Nazik and Kumtepe, 2012) or different phenotypes, including Phenotype B (ovulatory dysfunction + androgen excess) (Amer, et al., 2017, Homburg, et al., 2012, Johnson, et al., 2010, Kar and Sanchita, 2015, Legro, et al., 2007, Legro, et al., 2014, Lord, et al., 2006, Morin-Papunen, et al., 2012, Palomba, et al., 2005, Williams, et al., 2009, Wu, et al., 2017) or Phenotype D (ovulatory dysfunction + PCO) (Moll, et al., 2006, Vegetti, et al., 1999).

For RCTs involving two stages of different interventions, including cross-over studies, we only included the data in the first stage. We included the IPD comparing letrozole versus CC before crossing over (Amer, et al., 2017) and included the IPD comparing metformin versus placebo within the first three months before starting other ovulation induction agents (Morin-Papunen, et al., 2012). In one RCT (Nazik and Kumtepe, 2012), switching between intervention and the control after the first cycle was allowed during the trial and the analysis in the primary publication was on a per-cycle basis; and therefore we only included the IPD of the first cycle.

In summary, four RCTs compared three interventions (CC plus metformin or CC alone versus metformin (Johnson, et al., 2010, Kar and Sanchita, 2015, Legro, et al., 2007) or CC with metformin or letrozole versus CC (Liu, et al., 2017)) and the remaining 16 compared two

interventions. The most common comparisons were CC with metformin versus CC alone (8 RCTs) (Johnson, et al., 2010, Kar and Sanchita, 2015, Leanza, et al., 2014, Legro, et al., 2007, Liu, et al., 2017, Moll, et al., 2006, Sahin, et al., 2004, Williams, et al., 2009) and letrozole versus CC alone (6 RCTs) (Amer, et al., 2017, Bayar, et al., 2006, Kar, 2012, Legro, et al., 2014, Liu, et al., 2017, Nazik and Kumtepe, 2012).

**Quality of evidence of individual studies**

The details of risks of bias assessments within individual studies are presented in Figure 2. All RCTs (n=20) reported adequate methods of random sequence generation. Sixteen RCTs (80%) reported adequate methods of allocation concealment while the other four used an open allocation schedule without concealment (Kar, 2012, Kar and Sanchita, 2015, Liu, et al., 2017, Nazik and Kumtepe, 2012). Fourteen RCTs (70%) blinded the participants and personnel during the trial while six RCTs applied an open label design (Homburg, et al., 2012, Kar, 2012, Kar and Sanchita, 2015, Liu, et al., 2017, Nazik and Kumtepe, 2012, Vegetti, et al., 1999). Given that all outcomes of interest were objective outcomes, it is unlikely that the non-blinded design will affect the outcome measurement and therefore detection bias was rated at low risk for all the included studies. One RCT (5%) had high risk of attrition bias, with 22% overall missing outcome data and 31% missing outcome data in the metformin group (Kar and Sanchita, 2015). One RCT (5%) was at another risk of bias due to allowing imbalanced co-intervention (CC) in both groups.

**Meta-analyses of letrozole versus CC**

***Live birth***

IPD were available in six RCTs comparing letrozole and CC, including 1284 women with PCOS. The forest plot of IPD Meta-analysis on live birth is presented in Figure 3a. Compared

with CC, letrozole increased live birth rates (3 RCTs, 1043 women, RR 1.43, 95% CI 1.17-1.75, $I^2$=0, moderate certainty of evidence). Sensitivity analysis on studies with low risk of bias at allocation concealment and on treatment-naïve women were consistent with the main findings (2 RCTs, 909 women, RR 1.42, 95% CI 1.14-1.76, $I^2$=0; 3 RCTs, 627 women, RR 1.41, 95%CI 1.11-1.79, $I^2$=0) (Supplementary Table 2).

***Secondary outcomes***

Compared with CC alone, letrozole improved clinical pregnancy (6 RCTs, 1284 women, RR 1.45, 95%CI 1.23-1.70, $I^2$=0, moderate certainty of evidence, Figure 3b) and ovulation rates (5 RCTs, 1210 women, RR 1.13, 95%CI 1.07-1.20, $I^2$=0, moderate certainty of evidence, Table 2). There was insufficient evidence of a difference between letrozole and CC alone in terms of multiple pregnancy or miscarriage (Table 2).

The summary Kaplan-Meier curve for time to pregnancy is presented in Figure 4a. Subsequent pooled analysis of HRs showed that compared to CC, letrozole improved time-to-pregnancy (6 RCTs, 1235 women, HR 1.72, 95%CI 1.38-2.15, $I^2$=0, moderate certainty of evidence).

***Treatment-covariate interactions***

A meta-analyses of effect modifications showed a positive interaction between baseline serum total testosterone levels and treatment effects on live birth in the comparison between letrozole and CC (interaction RR 1.29, 95%CI 1.01-1.65, 3 RCTs, 1039 women, Figure 5a). This suggests that women with a higher baseline serum total testosterone level have a larger treatment effect of letrozole versus CC on live birth, compared to women with a lower baseline serum total testosterone level. Such an interaction was consistent across studies ($I^2$=0). To directly illustrate the association between baseline serum total testosterone level and relative treatment effects, this interaction is also presented in a weighted mean curve with 95% CI (Figure 5b). Meta-analyses did not find any other treatment-covariate interactions (Table 3).

**Meta-analyses of CC plus metformin versus CC**

***Live birth***

IPD were available in eight RCTs comparing CC with metformin and CC alone, including 1039 women with PCOS. The forest plot of IPD Meta-analysis on live birth is presented in Figure 3c. Compared with CC alone, there was insufficient evidence of a difference between CC with metformin and CC alone on live birth (5 RCTs, 907 women, RR 1.08, 95%CI 0.87-1.35, $I^2$=5.6%, low certainty of evidence). Sensitivity analyses on studies with low risk of bias at allocation concealment and on treatment-naïve women showed very small treatment effects with wide CIs (3 RCTs, 714 women, RR 1.02, 95%CI 0.76-1.37, $I^2$=33.2%; 5 RCTs, 662 women, RR 1.06, 95%CI 0.83-1.34, $I^2$=3.9%) (Supplementary Table 2).

***Secondary outcomes***

Compared with CC alone, CC with metformin might improve clinical pregnancy (8 RCTs, 1039 women, RR 1.18, 95% CI 1.00-1.39, $I^2$=6.9%, low certainty of evidence, Figure 3b). There was insufficient evidence of a difference between CC with metformin and CC alone on ovulation, multiple pregnancy or miscarriage (Table 2).

The summary Kaplan-Meier curve is presented in Figure 4b. Pooled analysis of HRs showed that compared to CC alone, CC with metformin might improve time-to-pregnancy (7 RCTs, 898 women, HR 1.25, 95%CI 1.00-1.57, $I^2$=0, low certainty of evidence).

***Treatment-covariate interactions***

Meta-analyses of effect modifications showed a positive interaction between baseline insulin levels and treatment effects on live birth in the comparison between CC with metformin and CC alone (interaction RR 1.03, 95%CI 1.01-1.06, 4 RCTs, 741 women, Figure 5c). Such an interaction was consistent across studies ($I^2$=0). This suggests that women with a higher baseline serum insulin level have larger treatment effects of CC with metformin versus CC alone on live birth, compared to women with a lower baseline serum insulin level. Such an

interaction was also presented in a weighted mean curve with 95%CI (Figure 5d). Additional meta-analysis of interactions for HOMA-IR was performed as requested during the peer review process and it also showed a positive interaction between baseline HOMA-IR and treatment effects on live birth in the comparison between CC with metformin and CC alone (interaction RR 1.14, 95%CI 1.03-1.25, 4 RCTs, 736 women, $I^2$=0, Table 3). Meta-analyses did not find any other treatment-covariate interactions (Table 3).

**IPD availability bias**

With regards to IPD availability bias, network meta-analyses of 20 RCTs with IPD showed similar results to network meta-analyses of all eligible RCTs on both live birth and clinical pregnancy (Supplementary Table 3). Therefore, the participants in RCTs with IPD were representative of all the eligible participants with PCOS. The transitivity assumption of network meta-analyses was considered valid as the interventions of interest and placebo/no treatment were jointly randomisable.

## Discussion

**Summary of evidence**

This IPD meta-analysis showed that in women with PCOS, letrozole increased live birth rates compared to CC alone and the overall certainty of evidence was moderate. Such treatment benefits of letrozole compared to CC alone were more predominant in women with higher baseline serum levels of total testosterone. There was insufficient evidence of a difference between CC plus metformin and CC alone in live birth rates and the overall certainty of evidence was low, mainly due to risk of bias and imprecision. The potential benefit of CC in combination with metformin compared to CC alone were more pronounced in women with

higher baseline serum insulin or HOMA-IR levels. We did not find other treatment-covariate interactions on live birth for other prespecified covariates including age, BMI, ethnicity, primary/secondary infertility, treatment history, Ferriman–Gallwey score for hirsutism, SHBG, free androgen index, fasting glucose levels or ovarian volume.

**Strengths and limitations**

Establishing the International Ovulation Induction IPDMA Collaboration facilitated a platform for key trialists in PCOS to collaborate and share the IPD of the primary trials. It provided us the opportunity to collect unpublished information of the primary trials including the details of randomisation and allocation concealment, treatment history, subgroup data and time-to-pregnancy. Such information allowed us to assess the quality of included trials precisely, to investigate treatment-covariate interactions and to take account of the time in the analyses. The findings of this IPD meta-analysis provide the best available up-to-date evidence.

Moreover, we applied a comprehensive search strategy without language restrictions and updated the search after completing data requesting in case we missed the most recent RCTs. Of the newly identified RCTs, one compared CC plus metformin vs CC in 128 women but did not report live birth (Fatima, et al., 2018), while the other one compared tamoxifen vs CC in 88 women (Topçu, et al., 2017). Although we did not seek IPD from two RCTs identified after the data requesting deadline, adding IPD of these two studies is unlikely to change the main findings.

In addition, the investigation of subgroup effects includes within-study interaction only according to current statistical practice for IPD meta-analyses (Fisher, et al., 2017) and therefore are free from ecological bias. For continuous covariates, without categorisation of the data, the statistical power was not compromised. Further illustration of interactions in weighted mean curve makes the interactions easier to interpret.

Nevertheless, this IPD meta-analysis has a few limitations. First, we were not able to access the IPD of all eligible studies. IPD were available for 32% (20/62) of the included trials, comprising 42% (3962/9356) of the eligible women with PCOS and the proportions of IPD availability was higher for studies reporting live birth (44% trials including 65% eligible women, Supplementary Table 3). This seems to be partly due to the long history of research on ovulation induction, with the first trial published in 1966. We were however able to access IPD of the highest-quality trials published within the last 15 years and we did not detect evidence of availability bias. Second, most of the planned subgroup analyses were based on two to three of the included studies and therefore may still be underpowered due to the unavailability of data on relevant covariates and/or live birth. Some primary trials only included a relatively homogeneous ethnicity group and therefore IPD in such trials could not contribute to the analysis of treatment-ethnicity interaction as no within-trial interaction was available. Third, as treatment-resistant women were excluded from this IPD meta-analysis, the findings can be applied in clinical practice on the choice of first-line treatment only. Last, we planned a one-stage IPD meta-analysis in the protocol but decided to use a two-stage approach before the final analysis. A two-stage approach allows graphical presentations for both overall treatment effects and treatment-covariate interactions, which is important for clinical interpretation, while it is not obvious how best to present graphically the results of a one-stage model (Fisher, et al., 2017). In addition, the two-stage approach automatically avoids ecological bias by accounting for within-trial interactions only (Fisher, et al., 2017). Given the relatively large number of participants, low heterogeneity and overall good to moderate quality of included studies, we would expect both approaches to give very similar results.

**Interpretations and clinical implications**

The overall effects of letrozole and CC plus metformin vs CC on live birth and clinical pregnancy in this IPD meta-analysis were in agreement with existing systematic reviews (Franik, et al., 2018, Morley, et al., 2017, Wang, et al., 2017) as well as the most recent the international evidence-based guideline recommendations (Teede, et al., 2018). Based on the findings of this IPD meta-analysis, letrozole can be recommended as the first-line ovulation induction medication in women with PCOS and infertility, provided off-label use is allowed and women are fully informed. Compared to CC alone, CC plus metformin may increase clinical pregnancy rates but the evidence on live birth was insufficient. Sensitivity analysis showed that the treatment effects on live birth seemed very small. The discrepancies between clinical pregnancy and live birth were likely due to the bias arising from low quality of studies which did not report live birth. Further evidence is needed to address this question.

Subgroup analyses showed that women with higher baseline serum levels of total testosterone may benefit more from letrozole compared to CC and women with higher baseline serum levels of insulin may benefit more from CC plus metformin compared to CC alone. Such positive interactions were consistent across trials and supported from a biological perspective. Letrozole has been introduced as an ovulation induction agent since 2001 and it inhibits aromatase, therefore increasing gonadotropin secretion by release of the hypothalamic/pituitary axis from estrogenic negative feedback and resulting in stimulation of ovarian follicle development (Mitwally and Casper, 2001). According to the recent "two triangles hypothesis" for folliculogenesis in PCOS, pre-antral follicle growth is excessive due to intrinsic androgen excess that renders granulosa cells hypersensitive to FSH, with consequently excessive AMH expression (Dewailly, et al., 2016) Therefore, hyperandrogenaemia may improve the response to letrozole by enhancing the sensitivity of FSH receptors. However, such an interaction was not observed in other biomarkers of hyperandrogenaemia or hirsutism. This is likely due to the fact that the severity of hirsutism does not correlate well with the magnitude of androgen

excess, as hirsutism is an expression of hyperandrogenism on hair follicles mediated through different pathways from those affecting the ovaries and follicles (Escobar-Morreale, et al., 2012). Metformin is an insulin sensitising agent that decreases gluconeogenesis and lipogenesis and enhances peripheral glucose uptake and therefore increases insulin sensitivity (Naderpoor, et al., 2015). The addition of metformin may further improve insulin resistance in women with higher fasting insulin or HOMA-IR levels and therefore improve pregnancy outcomes. We acknowledge that insulin levels are affected by many factors, ranging from physical activity and pre-test duration of fasting to sample handling and assay variability (Cassar, et al., 2016). Therefore the international evidence-based guideline does not recommend clinical measurement of insulin resistance at present due to the lack of accuracy (Teede, et al., 2018). In addition, SHBG has been proposed as a measure of insulin resistance (Cassar, et al., 2016), but the findings in our IPD meta-analysis did not support treatment-by-SHBG interactions. Our work provides preliminary evidence that there may be a role for assessing insulin resistance in PCOS and infertility and supports the need to assess insulin resistance in infertility studies.

We did not find ethnicity differences on treatment effects. This could be partly due to self-reported ethnicity without objective or DNA validation in all trials. We also did not find other treatment-covariate interactions on live birth for other prespecified covariates including age, BMI, primary/secondary infertility, treatment history, Ferriman–Gallwey score for hirsutism, SHBG, free androgen index, fasting glucose levels or ovarian volume. Although analyses of subgroup effects were prespecified in the protocol, these results should still be considered exploratory due to multiplicity.

Time is an important measurement for infertility outcomes, especially in the assessment of the effectiveness of multi-cycle treatments. However, time-to-event outcomes have seldomly been reported in meta-analyses of infertility trials as fertility outcomes are usually considered as dichotomous outcomes and Kaplan-Meier curves are rarely presented. Our IPD meta-analysis

used number of cycles as a measure of time and evaluated time-to-pregnancy by estimating HRs and presenting summary Kaplan-Meier curves. Time-to-event analysis takes time and censored participants into account and provides more accurate estimates of treatment effect. Our analyses on time-to-pregnancy were inconsistent with those of clinical pregnancy.

**Research implications**

IPD meta-analyses are useful to inform the design, conduct, analysis, and interpretation of trials (Tierney, et al., 2015). Given the consistent treatment benefits of letrozole across different fertility outcomes, future trials investigating new interventions for PCOS should choose letrozole as the reference arm. New trials are encouraged to incorporate treatment selection markers in their design to guide treatment decision (Janes, et al., 2011), and the impact of these, including age, BMI and other biomarkers, need to be confirmed in future trials. More specifically, biomarkers for hyperandrogenaemia and insulin resistance could be applied in trials that evaluate metformin. Due to the limited accuracy for measuring existing insulin resistance biomarkers, optimal methods to assess insulin resistance in future trials should also be considered.

Developing and implementing a core outcome set for infertility (Duffy, et al., 2018) and PCOS should be recommended to ensure outcomes are reported and collected consistently across future trials on infertility and PCOS to reduce research waste .

**Conclusions**

Our IPD meta-analysis shows that in women with PCOS, letrozole improves live birth and clinical pregnancy rates and reduces time-to-pregnancy compared to CC alone. CC plus metformin may improve clinical pregnancy rates and may reduce time-to-pregnancy compared to CC alone, but there is insufficient evidence of a difference on live birth.

562 Treatments effects of letrozole are influenced by baseline serum levels of total testosterone
563 while those of CC plus metformin are affected by baseline serum levels of insulin. These
564 interactions between treatments and biomarkers on hyperandrogenaemia and insulin resistance
565 provide further insights into a personalised approach towards the clinical management of
566 anovulatory infertility related to PCOS and therefore should be confirmed in future studies.
567

## Authors' roles

RW, RSL, SB, RJN, MvW and BWM conceptualised and designed the study. RW, WL, EMB, RJN, MvW and BWM collected the data. RSL, HZ, XW, JG, LMP, RH, TEK, EM, SK, WH, NPJ, SAA, WV, SP, AF, UO, HN, CDW, GF, JL and YS provided and interpreted data from the included trials. RW, WL, EMB, MvW and BWM cleaned and analysed the data. RW drafted the first manuscript. All authors interpreted the pooled data, critically revised the manuscript for important intellectual content, and approved the final version.

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**Conflict of interest**

RSL reports consultancy fees from Abbvie, Bayer, Fractyl and Ogeda and research sponsorship from Ferring. NPJ has received conference expenses from Bayer Pharma, Merck-Serono and Merck, Sharp and Dohme (MSD), research funding from AbbVie and Myovant Sciences, and is a consultant to Vifor Pharma, Guerbet and Myovant Sciences. WV has received conference expenses from Ferring and Merck-Serono, and his department has received research funding from Ferring and Merck-Serono. SB is Editor in Chief of Human Reproduction Open and receives an honorarium and support for travel to conferences from Oxford University Press for his role. RJN has received grant funding from Ferring and conference support from Merck. BWM is supported by a NHMRC Practitioner Fellowship (GNT1082548) and reports consultancy for ObsEva, Merck and Guerbet. The other authors have no conflict of interest to declare.

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Figure legends

Figure 1. PRISMA-IPD flow diagram

Figure 2. Risk of bias assessments of individual RCTs

Figure 3. Meta-analyses of letrozole versus CC and CC plus metformin versus CC on live birth and clinical pregnancy

Figure 4. Summary Kaplan-Meier curves for time-to-event outcomes

Figure 4a-4b illustrate the non-stratified summary Kaplan-Meier curves for time-to-pregnancy in the comparisons of letrozole versus CC and CC plus metformin versus CC, respectively.

Participants with pregnancy before the first treatment cycles were not included in the 'Numbers at risk' table below and data were not stratified by trial in this Kaplan-Meier curve. The figures were intended to visualise time-to-event outcomes, but not to show statistical significance.

Figure 5. Forest plots and weighted mean curves for treatment-covariate interactions

5a. Forest plot of interactions between baseline serum total testosterone (TT) level and effect of letrozole versus CC on live birth.

5b. Weighted mean curve with pointwise 95% CI of interactions between baseline serum total testosterone level and relative effect of letrozole versus CC on live birth. 5c. Forest plot of interactions between baseline serum insulin level and effect of CC plus metformin versus CC on live birth.

5d. Weighted mean curve with pointwise 95% CIs of interactions between baseline serum insulin level and effect of CC plus metformin versus CC on live birth.

5a,c. Circles are used to depict the interaction effects within individual trials as well as the overall interaction effect. The sizes of the circles are in proportion to the inverse of the variance of the estimates.

899 5b,d. Blue line represents for the weighted mean effect of covariate on log risk ratios in the
900 comparison between letrozole and CC. Red lines represent for pointwise 95% CI of
901 interactions.
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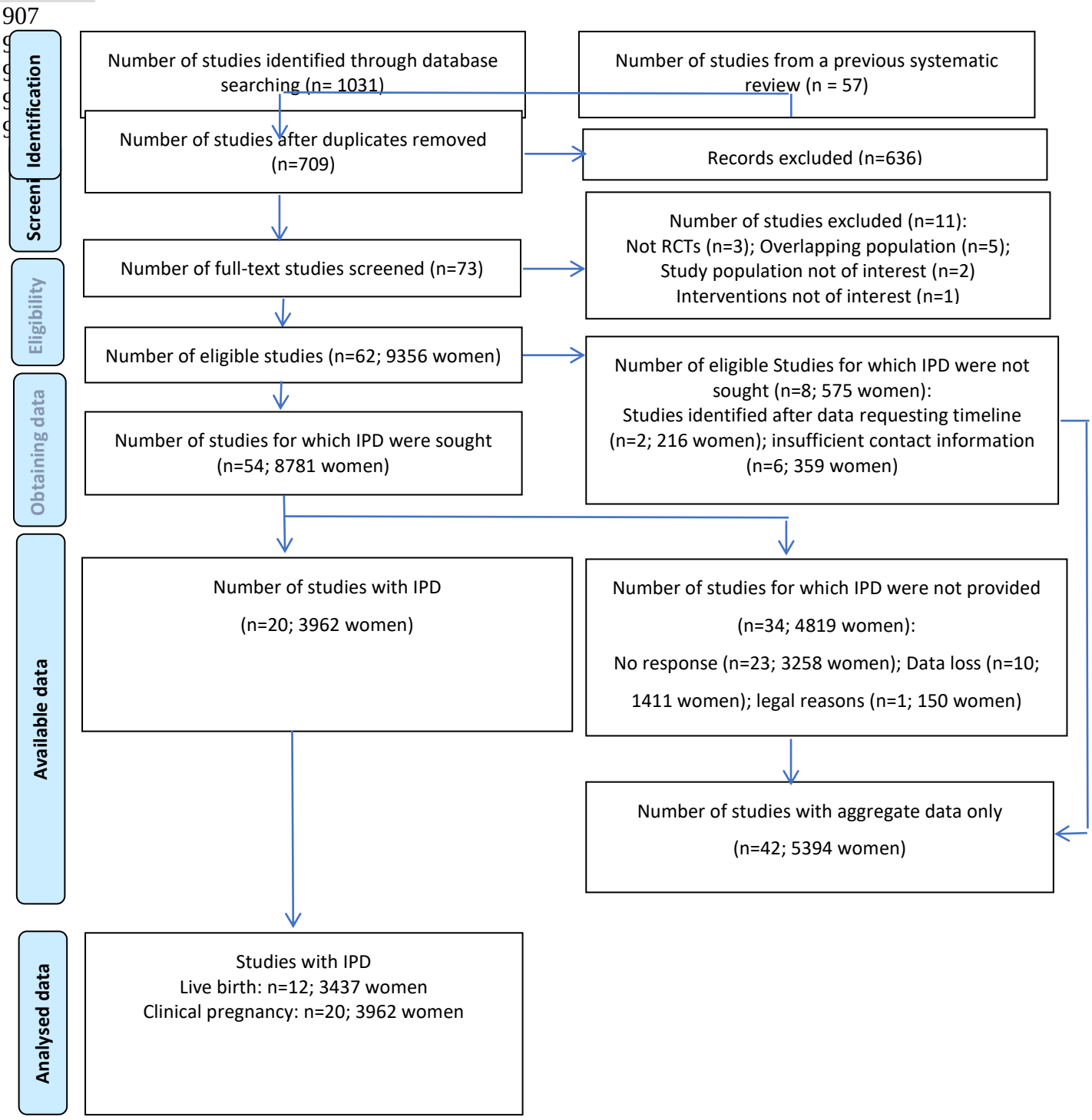
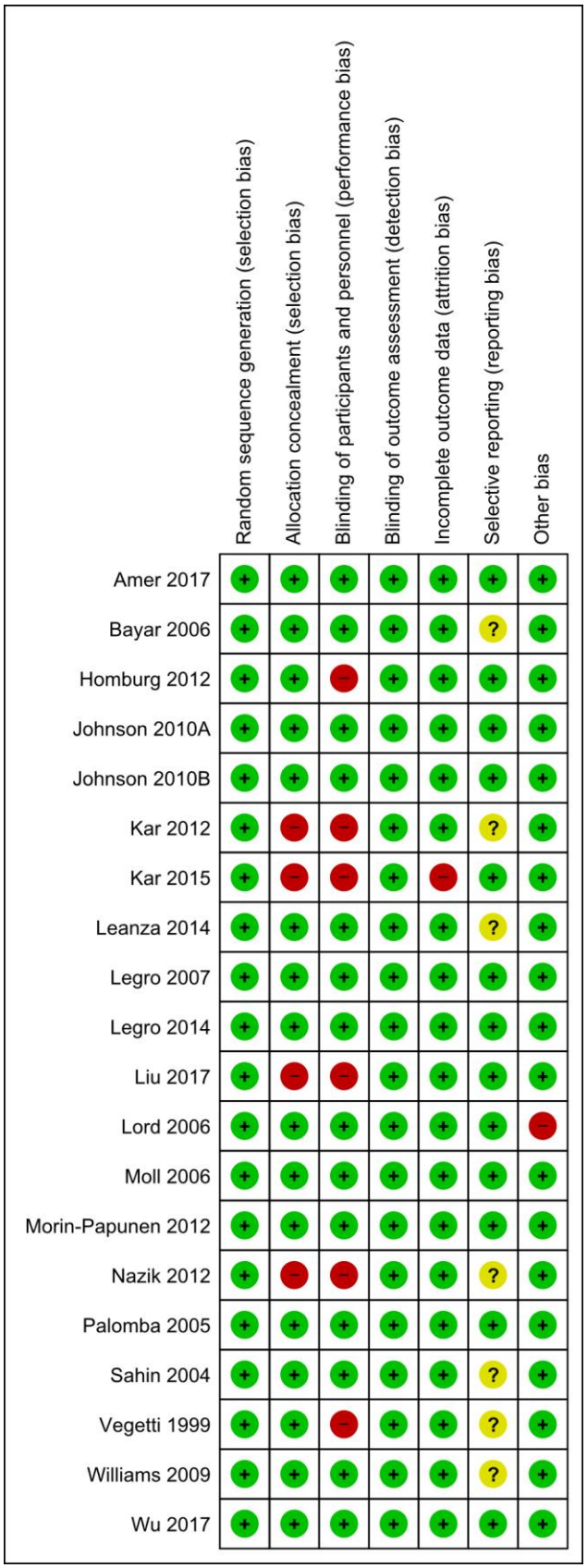
903




The PRISMA IPD flow diagram

Fig 2


Random sequence generation (selection bias)
Allocation concealment (selection bias)
Blinding of participants and personnel (performance bias)
Blinding of outcome assessment (detection bias)
Incomplete outcome data (attrition bias)
Selective reporting (reporting bias)
Other bias
Amer 2017
Bayar 2006
Homburg 2012
Johnson 2010A
Johnson 2010B
Kar 2012
Kar 2015
Leanza 2014
Legro 2007
Legro 2014
Liu 2017
Lord 2006
Moll 2006
Morin-Papunen 2012
Nazik 2012
Palomba 2005
Sahin 2004
Vegetti 1999
Williams 2009
Wu 2017

917 Fig 3

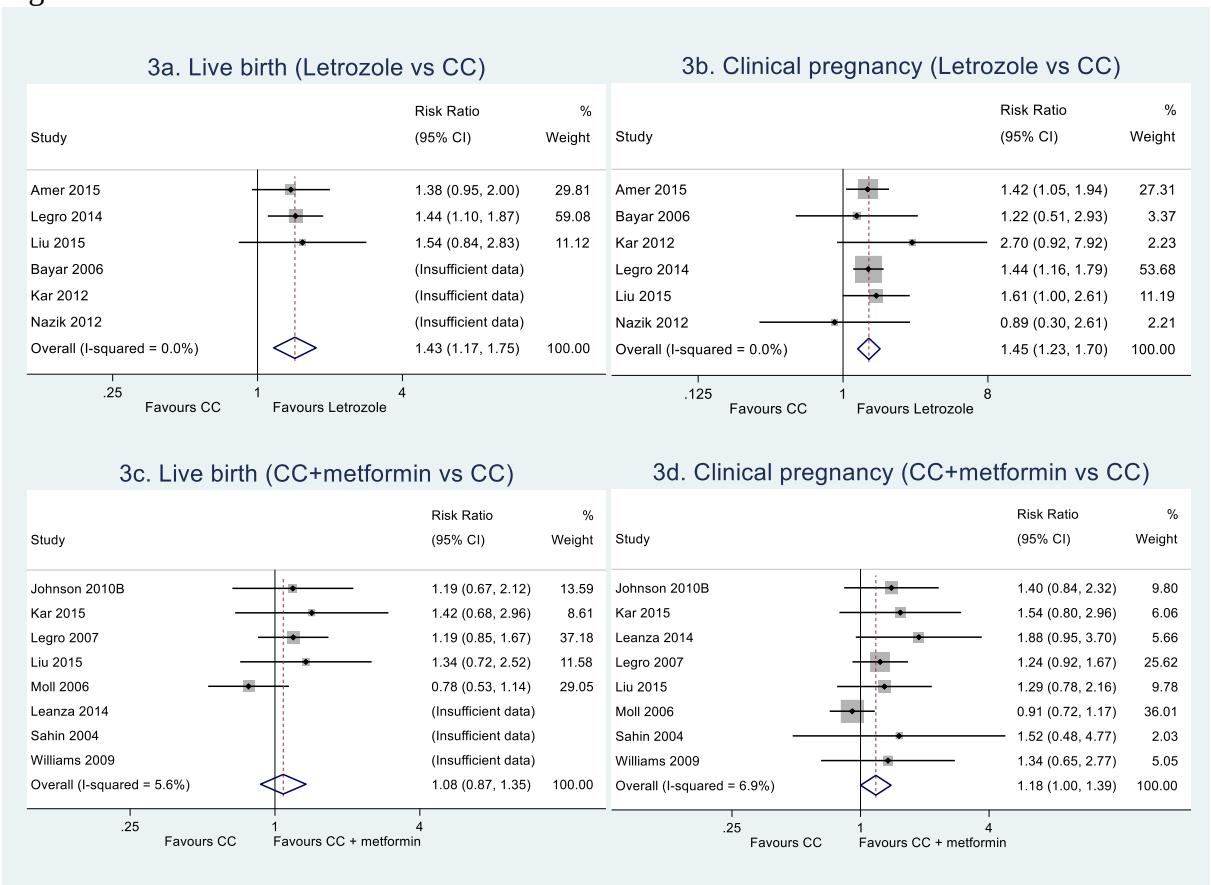



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Figure 4

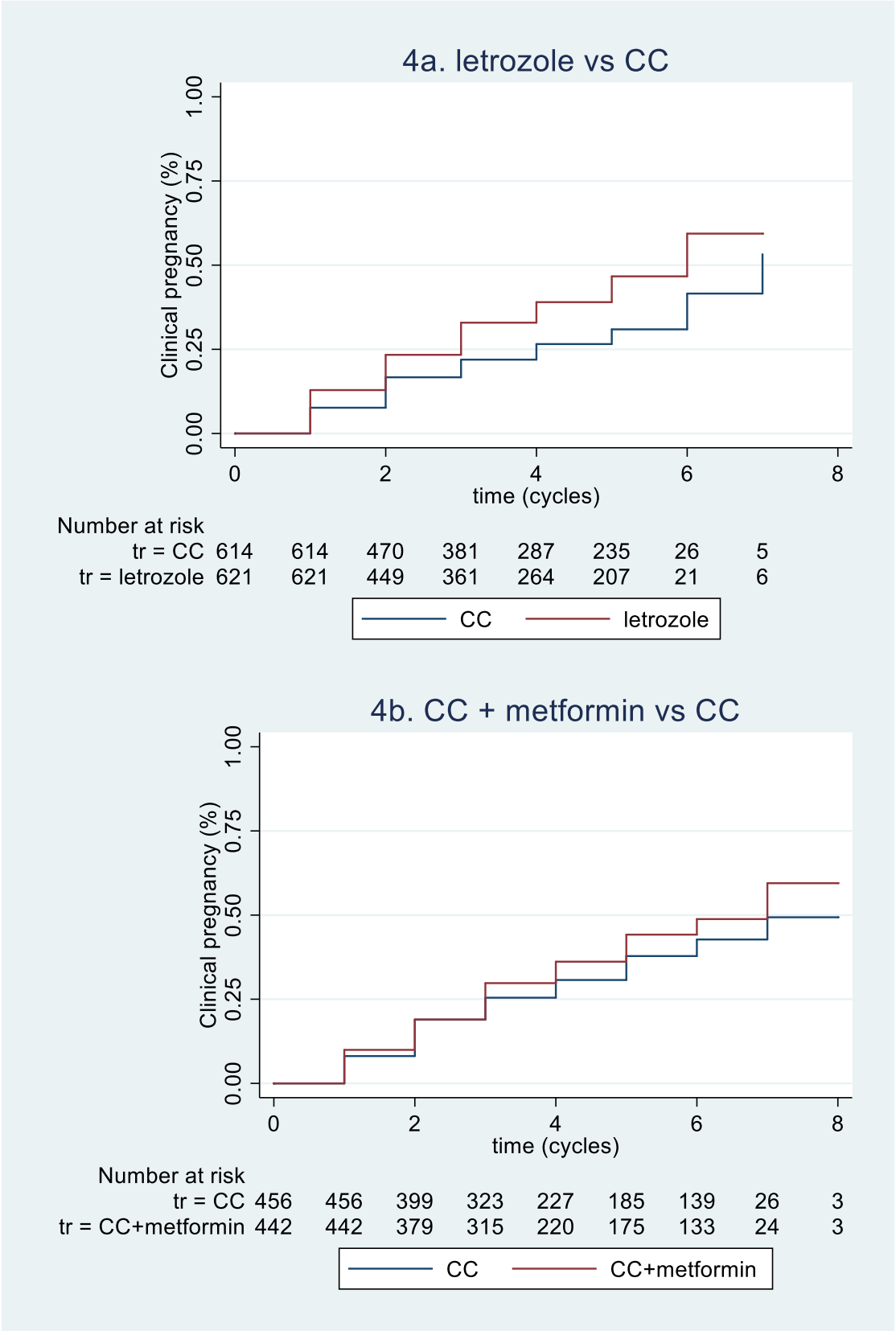
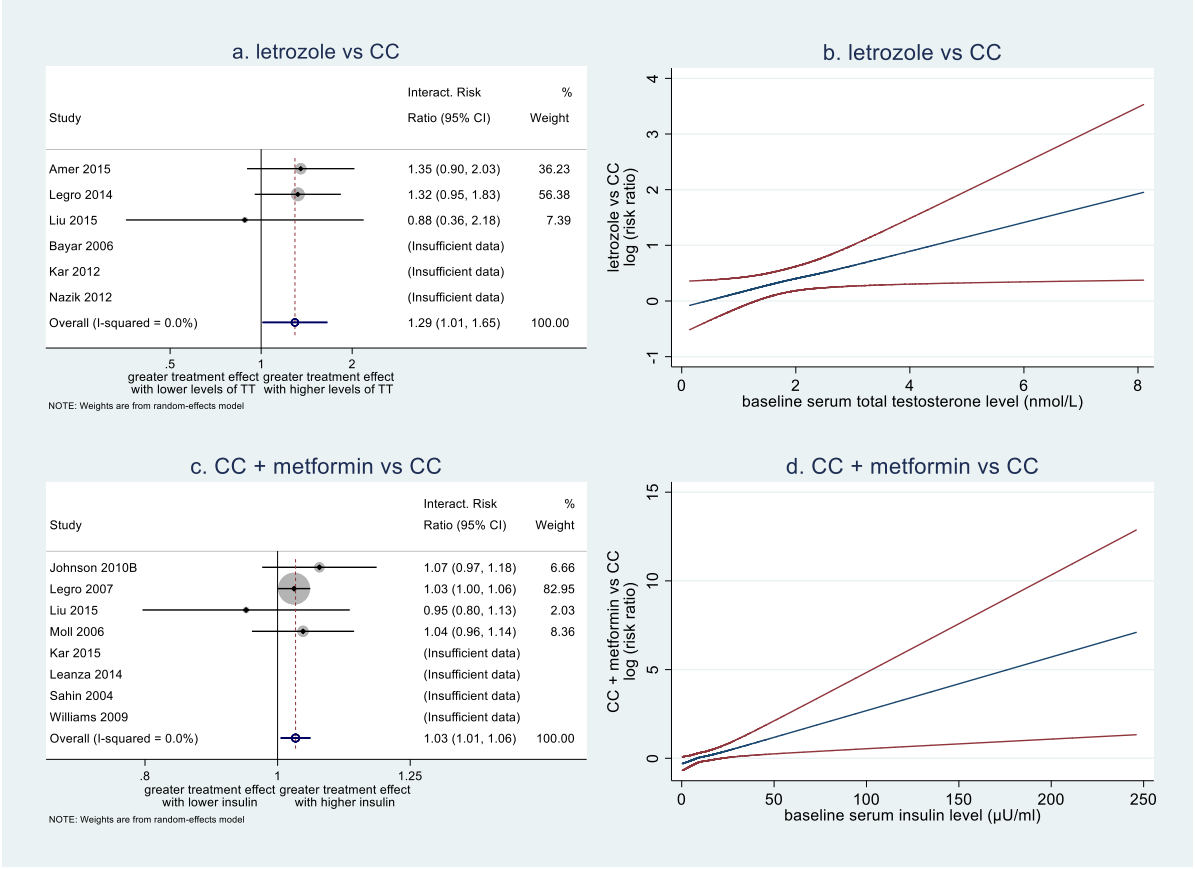

4a. letrozole vs CC
Clinical pregnancy (%)
0.00
0.25
0.50
0.75
1.00
0
2
4
6
8
time (cycles)
Number at risk
tr = CC 614 614 470 381 287 235 26 5
tr = letrozole 621 621 449 361 264 207 21 6
CC
letrozole
4b. CC + metformin vs CC
Clinical pregnancy (%)
0.00
0.25
0.50
0.75
1.00
0
2
4
6
8
time (cycles)
Number at risk
tr = CC 456 456 399 323 227 185 139 26 3
tr = CC+metformin 442 442 379 315 220 175 133 24 3
CC
CC+metformin

Figure 5


a. letrozole vs CC
Study
Interact. Risk Ratio (95% CI)
% Weight
Amer 2015 1.35 (0.90, 2.03) 36.23
Legro 2014 1.32 (0.95, 1.83) 56.38
Liu 2015 0.88 (0.36, 2.18) 7.39
Bayar 2006 (Insufficient data)
Kar 2012 (Insufficient data)
Nazik 2012 (Insufficient data)
Overall (I-squared = 0.0%) 1.29 (1.01, 1.65) 100.00
.5
1
2
greater treatment effect with lower levels of TT
greater treatment effect with higher levels of TT
NOTE: Weights are from random-effects model
b. letrozole vs CC
letrozole vs CC log (risk ratio)
-1
0
1
2
3
4
0
2
4
6
8
baseline serum total testosterone level (nmol/L)
c. CC + metformin vs CC
Study
Interact. Risk Ratio (95% CI)
% Weight
Johnson 2010B 1.07 (0.97, 1.18) 6.66
Legro 2007 1.03 (1.00, 1.06) 82.95
Liu 2015 0.95 (0.80, 1.13) 2.03
Moll 2006 1.04 (0.96, 1.14) 8.36
Kar 2015 (Insufficient data)
Leanza 2014 (Insufficient data)
Sahin 2004 (Insufficient data)
Williams 2009 (Insufficient data)
Overall (I-squared = 0.0%) 1.03 (1.01, 1.06) 100.00
.8
1
1.25
greater treatment effect with lower insulin
greater treatment effect with higher insulin
NOTE: Weights are from random-effects model
d. CC + metformin vs CC
CC + metformin vs CC log (risk ratio)
0
5
10
15
0
50
100
150
200
250
baseline serum insulin level (µU/ml)