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Pregnancy Outcomes Following Letrozole Use in Frozen-thawed Embryo Transfer Cycles: A Systematic Review and Meta-analysis

Schwangerschafts-Outcome nach der Anwendung von Letrozol bei Transferzyklen mit konservierten/aufgetauten Embryos: eine systematische Übersicht und Metaanalyse

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ABSTRACT

While widely used for ovulation induction in assisted reproductive technology, the clinical efficacy of letrozole for endometrial preparation prior to frozen-thawed embryo transfer (FET) cycles remains yet to be elucidated. We performed a meta-analysis to compare pregnancy outcomes after letrozole use with those of other endometrial preparation protocols in patients undergoing FET. PubMed, Scopus, Embase and the Cochrane Library were searched for eligible studies. Clinical pregnancy rate (CPR), live birth rate (LBR) and birth defect rate (BDR) were analysed using odds ratio (OR) and 95% confidence interval (CI). A total of 10 studies representing 75 968 FET cycles were included. Comparable CPR and LBR were observed when comparing letrozole administration with natural cycle (OR 1.24, 95% CI: 0.69–2.24; OR 1.18, 95% CI: 0.60–2.32), artificial cycle (OR 1.46, 95% CI: 0.87–2.44; OR 1.39, 95% CI: 0.77–2.52), and artificial cycle with gonadotropin-releasing hormone agonist suppression (OR 1.11, 95% CI: 0.78–1.59; OR 1.18, 95% CI: 0.82–1.68). Pooled results of the limited studies comparing letrozole with human menopausal gonadotropin demonstrated a similar CPR between groups (OR 1.46, 95% CI: 0.29–7.21, two studies), but the letrozole group had a statistically lower LBR (OR 0.67, 95% CI: 0.52–0.86, one study). No increased BDR was observed in the letrozole group compared to natural cycles or artificial cycles (OR 0.98, 95% CI: 0.60–1.61; OR 1.39, 95% CI; 0.84–2.28). This pooled analysis supports the use of letrozole as an efficacious and safe alternative to mainstream regimens for endometrial preparation in FET cycles.

ZUSAMMENFASSUNG

Obwohl Letrozol oft in der assistierten Reproduktionstechnik zur Einleitung der Ovulation eingesetzt wird, ist die klinische Wirksamkeit von Letrozol bei der Vorbereitung des Endometriums vor Transferzyklen von konservierten/aufgetauten Embryos (FET) noch unklar. Wir führten eine Metaanalyse durch, um das Schwangerschafts-Outcome nach dem Einsatz von Letrozol mit denen anderer Protokolle zur Vorbereitung des

* Dongjia Chen and Xiaoting Shen contributed equally to this work, and both should be considered as first authors.

Endometriums bei Patientinnen, bei denen ein FET vorgenommen wurde, zu vergleichen. Die Datenbanken von PubMed, Scopus, Embase und the Cochrane Library wurden nach passenden Studien durchsucht. Die klinische Schwangerschaftsraten (SR), Lebendgeburtenraten (LGR) und Geburtsfehlerraten wurden mithilfe von Odds Ratio (OR) und 95%-Konfidenzintervallen (KI) analysiert. Insgesamt wurden 10 Studien, die 75 968 FET-Zyklen darstellten, in die Analyse aufgenommen. Die SR und die LGR für Letrozol waren statistisch vergleichbar mit den Werten für natürliche Zyklen (OR 1,24; 95%-KI 0,69–2,24 bzw. OR 1,18, 95%-KI 0,60–2,32), künstliche Zyklen (OR 1,46; 95%-KI 0,87–2,44 bzw. OR 1,39; 95%-KI 0,77–2,52), sowie künstliche Zyklen mit Gonadotropin-Releasing-Hormon-Agonisten-Suppression (OR 1,11; 95%-KI 0,78–1,59 bzw. OR 1,18; 95%-KI 0,82–1,68). Die gepoolten Ergebnisse der begrenzten Anzahl an Studien, die Letrozol mit humanem Menopausengonadotropin verglichen, zeigten eine ähnliche SR für beide Gruppen (OR 1,46; 95%-KI 0,29–7,21, 2 Studien), wobei die Letrozol-Gruppe eine statistisch niedrigere LGR aufwies (OR 0,67; 95%-KI 0,52–0,86, 1 Studie). Die Geburtsfehlerrate in der Letrozol-Gruppe war nicht erhöht verglichen mit den Geburtsfehlerraten für natürliche Zyklen bzw. künstliche Zyklen (OR 0,98; 95%-KI 0,60–1,61 bzw. OR 1,39; 95%-KI 0,84–2,28). Diese gepoolte Analyse weist darauf hin, dass Letrozol eine wirksame und sichere Alternative zu den Standardprotokollen bei der Vorbereitung des Endometriums in FET-Zyklen darstellen könnte.

Introduction

Frozen embryo transfer (FET) is an essential component of assisted reproductive technology (ART), and has the merit of retaining superfluous embryos, increasing cumulative pregnancy rates and avoiding ovarian hyperstimulation syndrome (OHSS) [1, 2]. Choosing an appropriate endometrial preparation protocol is one of the critical factors for successful FET [3]. Natural cycle (NC), artificial cycle (AC), and ovarian induction cycle are all widely-used endometrial preparation protocols in FET. However, elucidating which is the best option remains to be determined [4].

Letrozole is a potent, reversible, and highly-selective aromatase inhibitor, which can disrupt the conversion of androstenedione to oestradiol, and stimulate pituitary gonadotropin secretion in a negative-feedback manner, thus facilitating follicular development [5]. Although it is primarily used in chemotherapy for postmenopausal breast cancer patients [6], since 2001, letrozole has been extensively administered to induce ovulation prior to intrauterine insemination (IUI), in vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI) [7–9], and its efficacy and safety has been demonstrated in clinical trials [10]. Furthermore, letrozole has recently replaced traditional clomiphene citrate (CC) as the first-line treatment for infertile patients with polycystic ovary syndrome (PCOS), contributing to improved pregnancy rates, higher live birth rates and shorter time-to-pregnancy rates [11, 12].

Numerous researchers have investigated the characteristics of letrozole for induction of ovulation. Letrozole has been proved to promote the endometrial thickness required for embryo implantation and does not impair endometrial receptivity [13, 14]. Moreover, unlike CC, it has no anti-oestrogenic effects on the endometrium and cervical mucus [15]. Based on previous findings, letrozole could theoretically be considered an effective option for endometrial preparation in FET. However, its efficacy and safety in preparing the endometrium have not been previously systematically evaluated. We therefore performed this meta-analysis to compare pregnancy outcomes following letrozole use to outcomes after other endometrial preparation protocols in patients undergoing FET.

Methods

The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines for reporting systematic reviews [16] were strictly followed in this review. This study was registered with PROSPERO, number CRD42020164027.

Definition of protocols

In NC protocols, transvaginal ultrasound is carried out to assess follicular and endometrial development. Clinical physicians monitor spontaneous ovulation based on luteinizing hormone (LH) levels in urine and serum oestradiol and LH levels, or trigger ovulation by injecting human chorionic gonadotropin (HCG). When the time of ovulation is determined, progesterone is added and the timing of embryo transfer is scheduled.

In AC protocols, 4–6 mg oestrogen is administered daily to promote endometrial growth from day 2 to day 5 of the menstrual cycle (with or without prior pituitary suppression by gonadotropin-releasing hormone agonist [GnRH-a]). Transvaginal ultrasound is performed every 3–5 days to monitor the thickness of the endometrium and adjust oestrogen dosages. When endometrial thickness is more than 8 mm, progesterone is added and the timing of embryo transfer is scheduled.

In letrozole cycles, patients receive letrozole 2.5–5 mg/day from day 2 or 3 of the menstrual cycle for 3 to 5 days. The remaining steps are the same as in NC. Letrozole cycles without additional human menopausal gonadotropin (HMG) administration were defined as letrozole alone cycles, while letrozole cycles with HMG supplement were referred to as letrozole plus HMG cycles.

In HMG cycles, patients receive a daily intramuscular injection of HMG at a dose of 37.5–75 IU from day 3 (traditional HMG regimen) or day 10 (modified HMG regimen) of the menstrual cycle. Transvaginal ultrasonography is performed to detect follicular development and endometrial development, and the dose of HMG is increased when necessary. The remaining steps are the same as in NC.

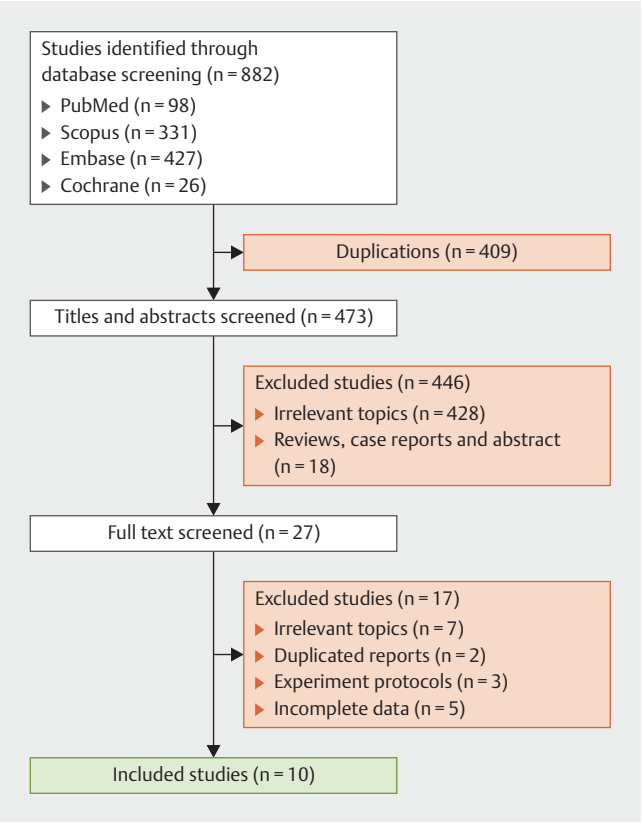
In CC cycles, patients receive oral CC 50 mg/day from day 2 to 6. The remaining steps are the same as in NC.

Search strategy

We identified all English-language medical papers published in PubMed, Scopus, Embase and the Cochrane Library from the inception of each database up to 7th August 2019. We used a combination of MeSH terms, search terms, and their combinations to generate two subsets of citations, one of studies on letrozole (letrozole, aromatase inhibitor) and the other of studies of frozen-thawed embryo transfers (frozen or cryopreserved or vitrified embryo transfer, frozen or cryopreserved or vitrified embryo replacement, FET, FER, cryopreservation). These two subsets were combined using "AND" to generate a subset of citations relevant to our topic. The search was performed on titles and abstracts of studies. Subsequently, the reference lists of the retrieved studies were searched further manually. If the same study population was repeatedly reported, the latest or more complete study was included in our review.

Selection criteria

Two of the authors (Chen and Fu) independently assessed the titles and abstracts of all included studies. Each study was read in its entirety prior to final inclusion. Disagreements during assessment were solved after consensus discussions with a senior author (Shen). Inclusion criteria for the analysis were randomised controlled trials (RCTs), cohort or case-control studies comparing letrozole with the following endometrial preparation protocols: NC, AC, AC + GnRH-a, HMG, and CC. Abstracts, reviews, case reports, experimental animal studies, letters to the editor, editorials, book chapters and articles in languages other than English were excluded.



▶ **Fig. 1** Flowchart of study selection.

Data extraction and outcomes of interest

Two of the authors (Chen and Ding) independently extracted and summarised the data from the included studies. Discrepancies were resolved after consensus discussions with a third review author (Zhong). If any data was missing, e-mails were sent to the corresponding authors.

The primary outcomes of concern were clinical pregnancy rate (CPR) per FET cycle and live birth rate (LBR) per FET cycle. Secondary outcomes were ongoing pregnancy rate (OPR) per FET cycle, miscarriage rate (MR) per clinical pregnancy, birth defect rate (BDR) per birth child and peak endometrial thickness (EMT) (in millimetres on ultrasound scan) before transfer.

Quality assessment

The level of evidence of each included study was evaluated using the criteria provided by the Centre for Evidence-Based Medicine in Oxford, UK [17]. Additionally, the Cochrane risk of bias tool was applied to assess the quality of RCTs, while the modified Newcastle-Ottawa scale (NOS) was applied to assess the quality of nonrandomised studies (including case-control studies and cohort studies) [18, 19]. RCTs and nonrandomised studies with 6 or more stars in NOS were regarded as high-quality studies.

Statistical analysis

Statistical analysis was performed using the Review Manager 5.0 software (Cochrane Collaboration, Oxford, UK). Binary variables were analysed with odds ratio (OR) and 95% confidence intervals (CI), while continuous variables were analysed with weighted mean difference (WMD) and 95% CI. Heterogeneity between studies in each comparison was quantified by I^2 statistics and indicated at a level of $I^2 > 40\%$. The random-effects model was employed for all analyses to minimise the effects of heterogeneity. If > 3 studies were included in one comparison, sensitivity analyses which excluded one study at a time were conducted using Review Manager 5.0 to seek the potential source of heterogeneity, and the differences of pooled outcomes were simultaneously compared before and after exclusion. Outcomes were considered as unstable when the exclusions reversed pooled outcomes. If the required data were available, subgroup analyses were performed for letrozole with and without HMG supplementation and for women with or without a diagnosis of PCOS. In addition, funnel plots were applied to assess publication bias.

Results

Characteristics of included studies

A total of 10 studies representing 122418 FET cycles were included in the final analysis. The flow diagram for the study selection is shown in ▶ **Fig. 1**.

The following 5 types of endometrial preparation regimens described in the included studies were used for comparisons: letrozole (letrozole alone or letrozole plus HMG), NC, AC, AC with GnRH-a suppression (AC + GnRH-a) and HMG. No study compar-

▶ **Table 1** Characteristics of included studies.

Author (year)	Country	Design	Study population	Cycles (n)	Outcomes	Quality scores[a]
Aleyasin (2017) [20]	Iran	RCT	Ovulatory and anovulatory	Letrozole[b] (50), AC + GnRH-a (50)	CPR/LBR/EMT	RCT
Chaudhuri (2013) [21]	India	P	Ovulatory and anovulatory	Letrozole (115), NC (55), AC + GnRH-a (100)	CPR/LBR	5
Guan (2016) [22]	China	R	Ovulatory and anovulatory	Letrozole (132), NC (427), AC (794), AC + GnRH-a (129)	CPR/BDR/OPR/MR/EMT	6
Guo (2016) [23]	China	R	Advanced endometriosis	Letrozole[c] (142), NC (233), AC (167)	CPR/LBR/BDR/OPR/MR/EMT	7
Hu (2014) [24]	China	R	PCOS	Letrozole[d] (40), AC (76), HMG (32)	CPR/OPR/EMT	7
Huang (2018) [25]	China	R	Ovulatory	Letrozole (340), NC (1838), AC (1666), HMG (1226)	CPR/LBR/MR/EMT	6
Li (2014) [29]	China	CCS	ovulatory in NC, anovulatory in AC/letrozole	Letrozole (359), NC (517), AC (354)	CPR/LBR/MR/EMT	5
Sibai (2016) [26]	Egypt	R	Not stated	Letrozole (94), AC (96)	CPR/OPR/MR/EMT	7
Tatsumi (2017) [27]	Japan	R	Not stated	Letrozole (2409), NC (41470), AC (66843)	CPR/LBR/BDR	6
Zhang (2019) [28]	China	R	PCOS	Letrozole[d] (1571), AC (1093)	CPR/LBR/OPR/MR	8

P: prospective cohort; R: retrospective cohort; CCS: case-control study; RCT: randomised controlled trail; NC: natural cycles; AC: artificial cycles; AC + GnRH-a: artificial cycles with gonadotropin-releasing hormone agonist; HMG: human menopausal gonadotropin; PCOS: polycystic ovary syndrome; CPR: clinical pregnancy rate; LBR: live birth rate; BDR: birth defect rate; OPR: ongoing pregnancy rate; MR: miscarriage rate; EMT: endometrial thickness.

[a] Quality scores for the methodology used in non-RCT studies were assessed by the modified Newcastle-Ottawa scale. High-quality studies were RCTs or non-RCT studies with quality scores of more than 5.

[b] Additional HMG at a dose of 75 IU daily was supplemented on day 7.

[c] If the diameter of dominant follicles was < 16 mm on day 10, additional HMG 150 IU daily was supplemented.

[d] If the diameter of dominant follicles was < 14 mm on day 10, additional HMG 75 IU daily was supplemented, with incremental doses of 37.5 IU if needed.

ing the effect of letrozole and CC for FET was found to meet the inclusion criteria. ▶ **Table 1** displays the characteristics of included studies.

The average quality of the included studies was low. Of the 10 included studies, only 1 study was an RCT (level of evidence: 2b) [20]. The other studies included 1 prospective cohort study [21], 7 retrospective cohort studies [22–28] and 1 case-control study (level of evidence: 2b–4) [29]. The risk of bias in the included studies is shown in Supplemental Table **S1** and Supplemental Table **S2**.

Results of meta-analysis

Letrozole versus NC

A total of 6 studies involving 48037 FET cycles were included for comparisons between letrozole and NC [21–23, 25, 27, 29]. No statistical differences were identified between the two protocols with regard to CPR (OR 1.24, 95% CI: 0.69–2.24) (▶ **Fig. 2**), LBR (OR 1.18, 95% CI: 0.60–2.32) (▶ **Fig. 3**), OPR (OR 0.75, 95% CI: 0.55–1.03), MR (OR 1.14, 95% CI: 0.77–1.68), BDR (OR 0.98, 95% CI: 0.60–1.61), or EMT (WMD −0.27, 95% CI; −0.62 to 0.08) (▶ **Table 2**).

Letrozole was supplemented with HMG in only one study by Guo et al. [23]. Nevertheless, there were no significant differences in CPR (OR 0.96, 95% CI: 0.64–1.46) (▶ **Fig. 2**), LBR (OR 0.92, 95% CI: 0.60–1.42) (▶ **Fig. 3**), OPR (OR 0.89, 95% CI: 0.58–1.36), MR (OR 1.18, 95% CI: 0.54–2.56), and EMT (WMD 0.12, 95% CI: −0.43 to 0.67) between letrozole plus HMG and NC in the aforementioned study (▶ **Table 2**). BDR was not estimated as no birth defect was observed in either arm of the study. In the other 5 studies, HMG was not used in the letrozole arm [21, 22, 25, 27, 29]. When letrozole alone was compared with NC, the OR did not differ significantly for CPR (OR 1.30, 95% CI: 0.68–2.52) (▶ **Fig. 2**), LBR (OR 1.24, 95% CI: 0.59–2.63) (▶ **Fig. 3**), MR (OR 1.13, 95% CI: 0.72–1.77), or BDR (OR 0.98, 95% CI: 0.60–1.61) and there was no significant difference in WMD with regard to EMT (WMD −0.36, 95% CI: −0.75 to 0.04) (▶ **Table 2**). Based on the single study by Guan et al., the OR for OPR was calculated to be 0.64 (95% CI: 0.43–0.97) for the letrozole alone protocol, favouring NC [22].

Letrozole versus AC

A total of 8 studies representing 75598 cycles were included for comparisons between letrozole and AC [22–29]. Pooled analysis revealed that the two regimens were comparable with regard to CPR (OR 1.46, 95% CI: 0.87–2.44) (▶ **Fig. 2**), LBR (OR 1.39, 95% CI: 0.77–2.52) (▶ **Fig. 3**), OPR (OR 1.24, 95% CI: 0.95–1.63), MR (OR 0.75, 95% CI: 0.51–1.10), BDR (OR 1.39, 95% CI: 0.84–2.28) and EMT (WMD 0.57, 95% CI: −0.04 to 1.18) (▶ **Table 2**).

▶ **Table 2** Results of meta-analysis including subgroup analysis and sensitivity analysis.

Outcomes of interest	Studies (n)	Cycles (n)		WMD/OR (95% CI)	p	I^2 (%)
		Letrozole	Other regimens			
Letrozole versus NC						
Clinical pregnancy rate	6	3497	44540	1.24 (0.69, 2.24)	0.48	97
▪ Letrozole plus HMG	1	142	233	0.96 (0.64, 1.46)	0.86	NA
▪ Letrozole alone	5	3355	44307	1.30 (0.68, 2.52)	0.43	97
▪ Exclusion of Tatsumi et al. [27]	5	1088	3070	0.98 (0.78, 1.24)	0.91	52
Live birth rate	6	3497	44540	1.18 (0.60, 2.32)	0.63	97
▪ Letrozole plus HMG	1	142	233	0.92 (0.60, 1.42)	0.72	NA
▪ Letrozole alone	5	3355	44307	1.24 (0.59, 2.63)	0.57	98
▪ Exclusion of Tatsumi et al. [27]	5	1088	3070	0.92 (0.70, 1.21)	0.53	63
Ongoing pregnancy rate	2	274	660	0.75 (0.55, 1.03)	0.07	13
▪ Letrozole plus HMG	1	142	233	0.89 (0.58, 1.36)	0.58	NA
▪ Letrozole alone	1	132	427	0.64 (0.43, 0.97)	**< 0.05**	NA
Miscarriage rate	3	279	1211	1.14 (0.77, 1.68)	0.50	0
▪ Letrozole plus HMG	1	70	117	1.18 (0.54, 2.56)	0.68	NA
▪ Letrozole alone	2	209	1094	1.13 (0.72, 1.77)	0.59	0
Birth defect rate	3	1358	11375	0.98 (0.60, 1.61)	0.94	0
▪ Letrozole plus HMG	1	70	117	Not estimable[a]	NA	NA
▪ Letrozole alone	2	1288	11258	0.98 (0.60, 1.61)	0.94	0
Endometrial thickness	4	973	3015	−0.27 (−0.62, 0.08)	0.13	85
▪ Letrozole plus HMG	1	142	233	0.12 (−0.43, 0.67)	0.67	NA
▪ Letrozole alone	3	831	2782	−0.36 (−0.75, 0.04)	0.08	89
▪ Exclusion of Li et al. [29]	3	614	2498	−0.40 (−0.75, −0.06)	**< 0.05**	68
Letrozole versus AC						
Clinical pregnancy rate	8	4752	70846	1.46 (0.87, 2.44)	0.15	97
▪ Letrozole plus HMG	3	1418	1093	1.30 (0.80, 2.12)	0.28	73
▪ Letrozole alone	5	3334	69753	1.48 (0.79, 2.80)	0.22	97
▪ Exclusion of Tatsumi et al. [27]	7	2343	4003	1.18 (0.96, 1.44)	0.11	59
▪ PCOS population	2	1276	926	1.48 (0.54, 4.10)	0.45	84
Live birth rate	6	4618	70674	1.39 (0.77, 2.52)	0.27	98
▪ Letrozole plus HMG	2	1378	1017	1.15 (0.98, 1.36)	0.09	0
▪ Letrozole alone	4	3240	69657	1.53 (0.72, 3.28)	0.27	98
▪ Exclusion of Tatsumi et al. [27]	5	2209	3831	1.16 (0.96, 1.40)	0.12	51
Ongoing pregnancy rate[b]	5	1644	1983	1.24 (0.95, 1.63)	0.12	53
▪ Letrozole plus HMG	3	1418	1093	1.26 (0.87, 1.82)	0.22	55
▪ Letrozole alone	2	226	890	1.30 (0.64, 2.62)	0.47	75
▪ PCOS population	2	1276	926	1.53 (0.67, 3.50)	0.32	77
Miscarriage rate[b]	5	1074	1727	0.75 (0.51, 1.10)	0.15	38
▪ Letrozole plus HMG	1	745	524	0.52 (0.35, 0.78)	**< 0.05**	NA
▪ Letrozole alone	4	329	1203	0.91 (0.63, 1.32)	0.62	0
Birth defect rate	3	1358	16086	1.39 (0.84, 2.28)	0.20	0
Endometrial thickness	7	2343	4003	0.57 (−0.04, 1.18)	0.07	97
▪ Letrozole plus HMG	3	1418	1093	1.18 (0.45, 1.92)	**< 0.05**	88
▪ Letrozole alone	4	925	2910	0.15 (−0.53, 0.83)	0.66	96
▪ Exclusion of Sibai et al. [26]	6	2249	3907	0.79 (0.19, 1.39)	**< 0.05**	97

Continued next page

▶ **Table 2** Results of meta-analysis including subgroup analysis and sensitivity analysis. *(Continued)*

Outcomes of interest	Studies (n)	Cycles (n)		WMD/OR (95% CI)	p	I^2 (%)
		Letrozole	Other regimens			
Letrozole versus AC + GnRH-a[c]						
Clinical pregnancy rate	3	297	279	1.11 (0.78, 1.59)	0.54	6
▪ Letrozole plus HMG	1	50	50	1.11 (0.46,2.68)	0.82	NA
▪ Letrozole alone	2	247	229	1.13 (0.66, 1.95)	0.65	53
Live birth rate	3	297	279	1.18 (0.82, 1.68)	0.38	0
▪ Letrozole plus HMG	1	50	50	0.82 (0.34, 1.97)	0.66	NA
▪ Letrozole alone	2	247	229	1.26 (0.85, 1.88)	0.24	0
Birth defect rate	1	50	50	Not estimable	NA	NA
Endometrial thickness	2	182	179	−0.24 (−0.84, 0.36)	0.44	81
▪ Letrozole plus HMG	1	50	50	0.02 (−0.12, 0.16)	0.78	NA
▪ Letrozole alone	1	132	129	−0.60 (−1.12, −0.08)	**< 0.05**	NA
Letrozole versus HMG[d]						
Clinical pregnancy rate	2	380	1 258	1.46 (0.29, 7.21)	0.65	90
▪ Letrozole plus HMG	1	40	32	3.55 (1.33, 9.42)	**< 0.05**	NA
▪ Letrozole alone	1	340	1 226	0.69 (0.54, 0.88)	**< 0.05**	NA
Live birth rate	1	340	1 226	0.67 (0.52, 0.86)	**< 0.05**	NA
Ongoing pregnancy rate	1	40	32	4.50 (1.62, 12.48)	**< 0.05**	NA
Miscarriage rate	1	155	673	1.02 (0.60, 1.74)	0.94	NA
Endometrial thickness	2	380	1258	0.01 (−1.69, 1.70)	0.99	96
▪ Letrozole plus HMG	1	40	32	0.90 (0.28, 1.52)	**< 0.05**	NA
▪ Letrozole alone	1	340	1 226	−0.83 (−1.03, −0.63)	**< 0.05**	NA

NC: natural cycles; AC: artificial cycles; AC + GnRH-a: artificial cycles with gonadotropin-releasing hormone agonist; HMG: human menopausal gonadotropin; PCOS: polycystic ovary syndrome; CI: confidence interval; WMD: weighted mean difference; OR: odds ratio; NA: not applicable.

[a] OR of birth defect rate was not estimable as no birth defect was reported for either arm of the study.

[b] Outcomes of sensitivity analysis were not displayed as heterogeneity was not reduced and the initial outcome was not reversed by sensitivity analysis.

[c] Miscarriage rate and birth defect rate were not reported.

[d] Birth defect rate was not reported.

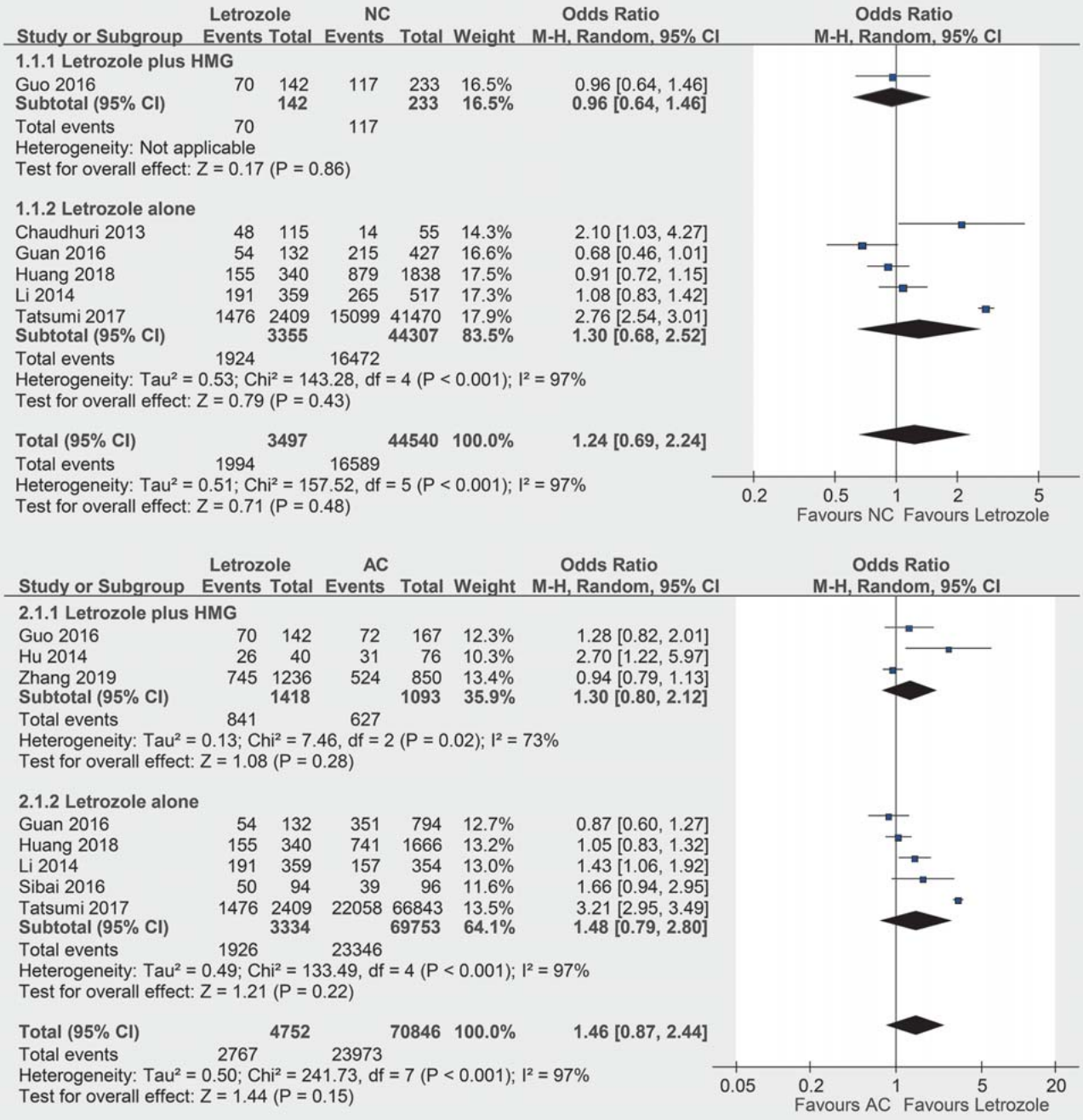
HMG was added to the letrozole cycle in 3 studies [23, 24, 28], while no HMG was administered in the letrozole arm in 5 studies [22, 25 – 27, 29]. When letrozole plus HMG was compared with AC, there was no statistically significant difference in CPR (OR 1.30, 95% CI: 0.80–2.12) (▶ **Fig. 2**), LBR (OR 1.15, 95% CI: 0.98–1.36) (▶ **Fig. 3**), OPR (OR 1.26, 95% CI: 0.87–1.82) and EMT (WMD 1.18, 95% CI: 0.45–1.92), and a statistically significantly lower MR (OR 0.52, 95% CI: 0.35–0.78) was observed for letrozole plus HMG (▶ **Table 2**). BDR was not estimated, as it was only reported in one study which observed no birth defects. The pooled estimates for CPR (OR 1.48, 95% CI: 0.79–2.80) (▶ **Fig. 2**), LBR (OR 1.53, 95% CI: 0.72–3.28) (▶ **Fig. 3**), OPR (OR 1.30, 95% CI: 0.64–2.62), MR (OR 0.91, 95% CI: 0.63–1.32), BDR (OR 1.39, 95% CI: 0.84–2.28), and EMT (WMD 0.15, 95% CI: −0.53 to 0.83) showed no statistically significant differences between letrozole alone and AC (▶ **Table 2**).

It is also noteworthy that only two articles clearly stated that their study populations were PCOS patients [24, 28], while the other articles did not clearly differentiate between patients. This meant that we were unable to perform subgroup analyses for women with or without a PCOS diagnosis. Therefore, we only used the aforementioned two articles to analyse the effect of a letrozole protocol for FET in women with PCOS. No significant differences were observed between letrozole plus HMG and AC with regard to CPR (OR 1.48, 95% CI: 0.54–4.10) and OPR (OR 1.53, 95% CI: 0.67–3.50) in women with a diagnosis of PCOS (▶ **Table 2**).

Letrozole versus AC + GnRH-a

A total of 3 studies representing a total of 3314 cycles were included for comparisons between letrozole and AC + GnRH-a [20 – 22]. No significant differences were identified with respect to CPR (OR 1.11, 95% CI: 0.78–1.59) (▶ **Fig. 4**), LBR (OR 1.18, 95% CI: 0.82–1.68) (▶ **Fig. 5**) and EMT (WMD −0.24, 95% CI: −0.84 to 0.36) (▶ **Table 2**). One study reported BDR, but no birth defects were observed in the two groups (▶ **Table 2**) [22].

In one study, HMG was added to the letrozole cycle [20]. In this study, CPR (OR 1.11, 95% CI: 0.46–2.68) (▶ **Fig. 4**), LBR (OR 0.82, 95% CI: 0.34–1.97) (▶ **Fig. 5**) and EMT (WMD 0.02, 95% CI: −0.12–0.16) were found to be comparable for the letrozole plus HMG and the AC + GnRH-a groups (▶ **Table 2**). In another two

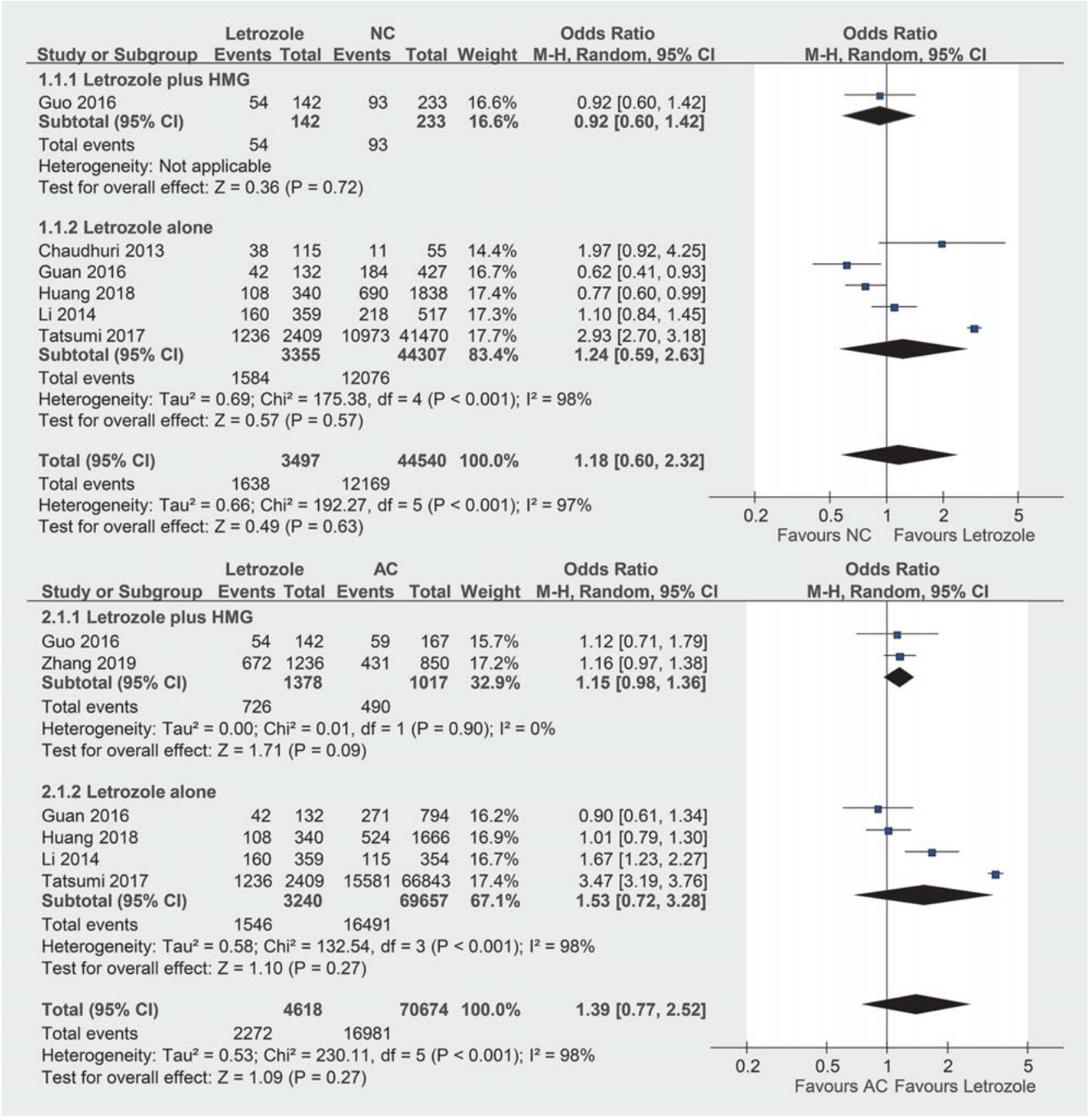


▶ **Fig. 2** Forest plots comparing clinical pregnancy rates of letrozole with NC and AC.

studies, no HMG supplementation was used in the letrozole arm [21,22]. When letrozole alone was compared with AC + GnRH-a, the ORs for CPR and LBR were calculated to be 1.13 (95% CI: 0.66–1.95) (▶ **Fig. 4**) and 1.26 (95% CI: 0.85–1.88) (▶ **Fig. 5**), respectively. However, the results for EMT were found to be poor in the letrozole alone group (WMD −0.60, 95% CI: −1.12 to −0.08) (▶ **Table 2**).

Letrozole versus HMG

Two studies representing 1638 cycles were included to compare outcomes for letrozole with those for HMG [24,25]. There were no evident differences between the groups with regard to CPR (OR 1.46, 95% CI: 0.29–7.21) (▶ **Fig. 4**), MR (OR 1.02, 95% CI: 0.60–1.74) and EMT (WMD 0.01, 95% CI: −1.69 to 1.70) (▶ **Table 2**). A statistically higher OPR (OR 4.50, 95% CI: 1.62–12.48) was reported for the letrozole group in one study [24], while the other study reported a statistically lower LBR (OR 0.67, 95% CI: 0.52–0.86) [25] (▶ **Fig. 5**, **Table 2**).

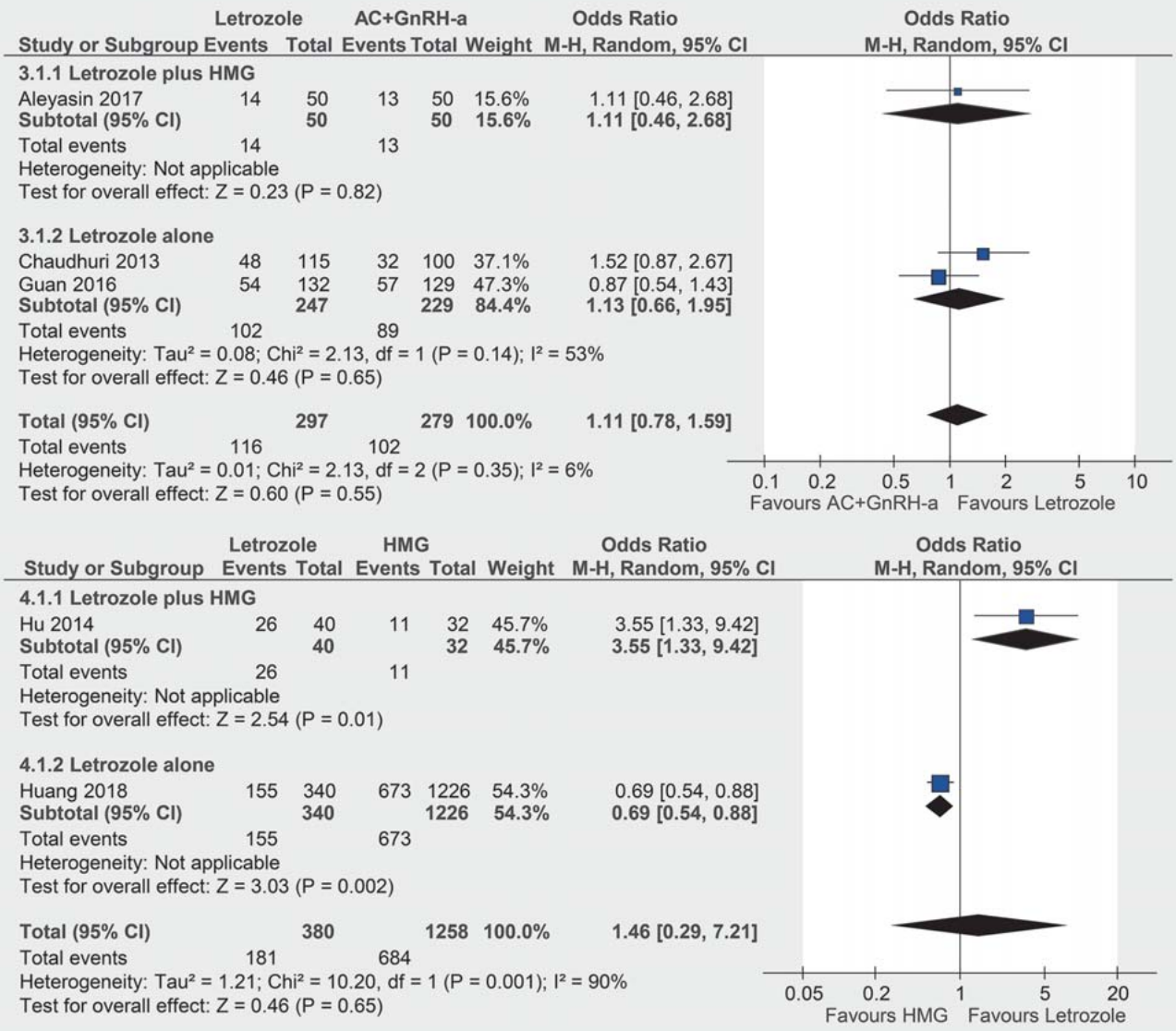


▶ **Fig. 3** Forest plots comparing live birth rates of letrozole with NC and AC.

The study by Hu et al. used HMG supplementation [24], while no HMG was administered in the study by Huang et al. [25] According to the findings of Hu et al., a statistically higher CPR (OR 3.55, 95% CI: 1.33–9.42) (▶ **Fig. 4**), OPR (OR 4.50, 95% CI: 1.62–12.48) and greater EMT (WMD 0.90, 95% CI: 0.28–1.52) were observed in the letrozole plus HMG group (▶ **Table 2**). However, Hu et al. did not report on LBR or MR. Based on the results of Huang et al., the letrozole alone group had a statistically lower CPR (OR 0.69, 95% CI: 0.54–0.88) (▶ **Fig. 4**), and LBR (OR 0.67, 95% CI: 0.52–0.86) (▶ **Fig. 5**) and poor EMT (WMD −0.83, 95% CI: −1.03 to −0.63), and there were significant differences between the two groups with regard to MR (OR 1.02, 95% CI: 0.60–1.74) (▶ **Table 2**). Huang et al. did not report on OPR.

Publication bias

▶ **Fig. 6 a** shows the funnel plots for the CPR of letrozole and NC. ▶ **Fig. 6 b** displays the funnel plots for the CPR of letrozole and AC. These asymmetric funnel plots suggest a publication bias.

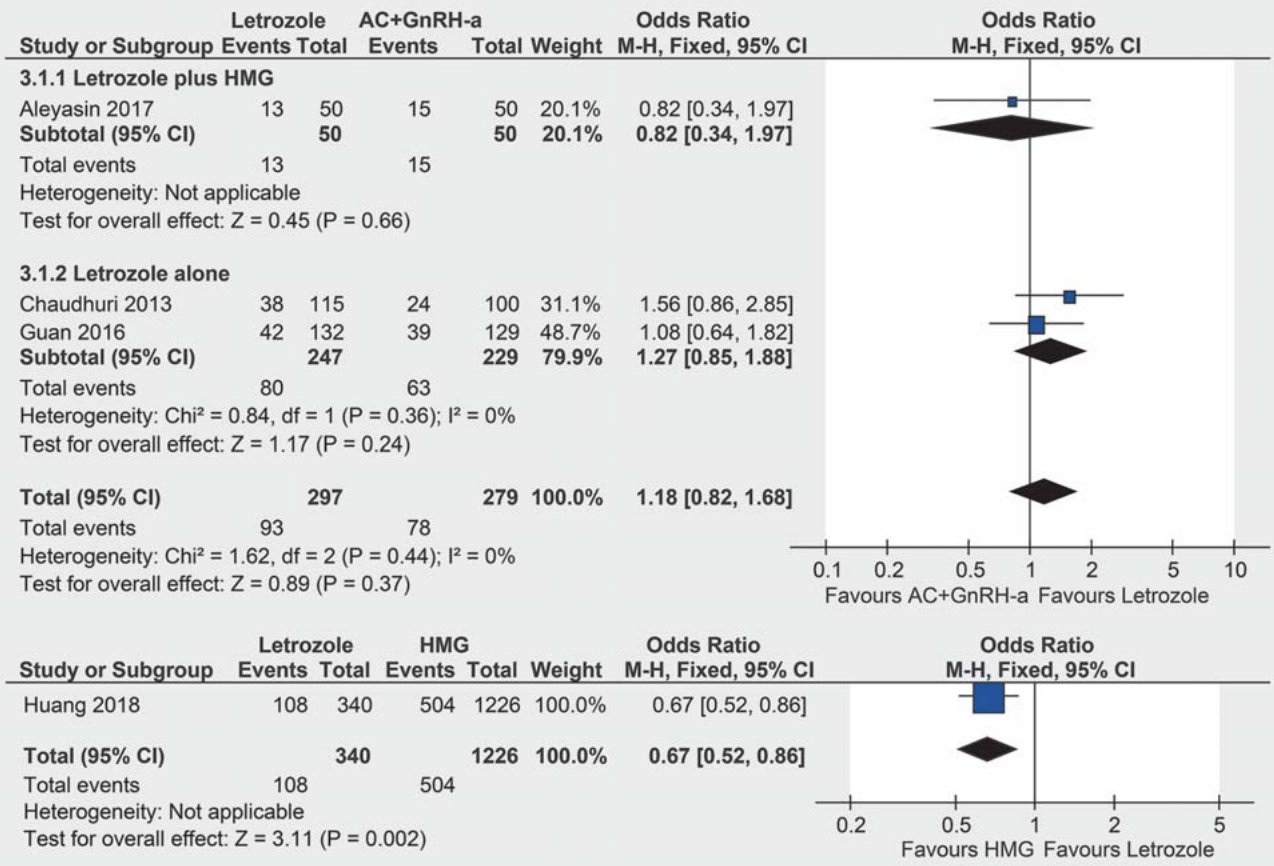


▶ **Fig. 4** Forest plots comparing clinical pregnancy rates of letrozole with AC + GnRH-a and HMG.

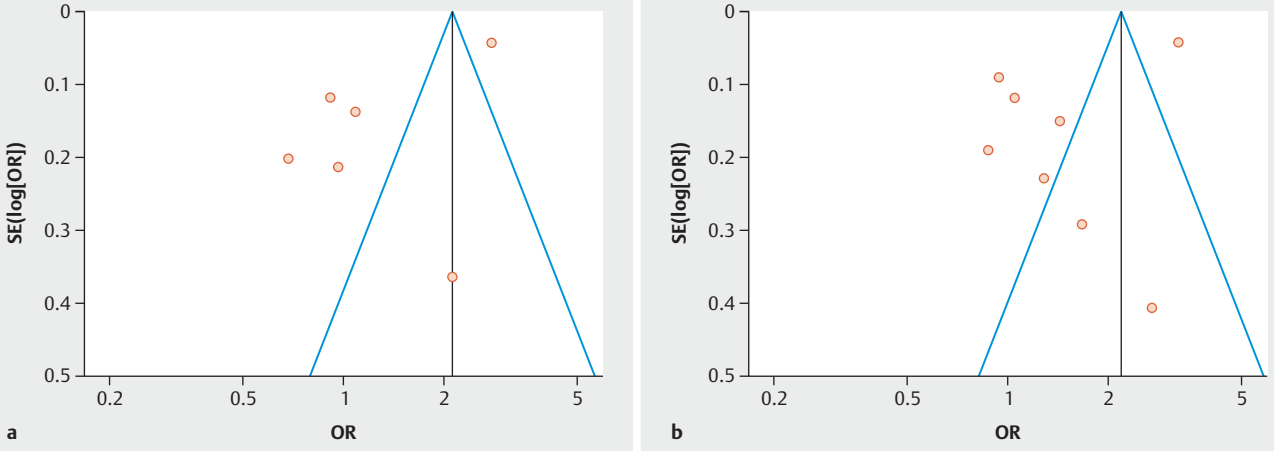
Sensitivity analysis

As significant heterogeneity was observed in this study, we performed a sensitivity analysis for comparisons of more than 3 studies (▶ **Table 2**). The heterogeneity of the studies comparing the CPR and LBR with letrozole with those with NC was noted to be significantly decreased after the study by Tatsumi et al. [27] was omitted, with I^2 decreasing from 97% to 52% and from 97% to 63%, respectively. Similarly, when the CPR and LBR with letrozole were compared with CPR and LBR with AC, exclusion of the study by Tatsumi et al. significantly decreased the heterogeneity of the studies, with I^2 decreasing from 97% to 59% and from 98% to 51%, respectively. The merged results after the exclusion of the study by Tatsumi et al. did not lead to a reversal of the original results. While omission of the study by Li et al. [29] led to a decrease in heterogeneity in the studies comparing EMT with letrozole and EMT with NC, a reversal of the pooled result was also observed (before: WMD −0.27, 95% CI: −0.62 to 0.08, p = 0.13, I^2 = 85%; after: WMD −0.40, 95% CI: −0.75 to −0.06, p = 0.02, I^2 = 68%). Although considerable heterogeneity was observed when EMT with letrozole was compared with EMT with AC, sensitivity analysis did not decrease heterogeneity, and a recalculation of the merged results after omitting the study by Sibai et al. [26] showed a significant reversal of the original results (before: WMD 0.57, 95% CI: −0.04 to 1.18, p = 0.07, I^2 = 97%; after: WMD 0.79, 95% CI: 0.19–1.39, p = 0.01, I^2 = 97%).

When OPR and MR with letrozole was compared with OPR and MR with AC, sensitivity analyses additionally revealed that heterogeneity was not reduced and initial outcomes were not reversed. In the remaining comparisons, the number of included studies were no more than 3, and no sensitivity analyses were therefore performed.



▶ **Fig. 5** Forest plots comparing live birth rates of letrozole with AC + GnRH-a and HMG.



▶ **Fig. 6** Funnel plots for the clinical pregnancy rates of **a** letrozole and NC; **b** letrozole and AC.

Discussion

This meta-analysis assessed the efficacy and safety of letrozole as an endometrial preparation protocol for FET. Pooled analysis revealed that letrozole produces similar clinical effects as NC and AC (with and without GnRH-a suppression), with no significant differences in clinical pregnancy rates, live birth rates, and birth defect rates. The use of letrozole in FET could lead to similar clinical pregnancy rates but lower live birth rates compared to HMG, which warrants more high-quality studies for confirmation.

If it is assumed that embryo quality does not differ between protocols, then appropriate endometrial preparation is recognised as a key factor to improve pregnancy rates in FET. The most commonly used methods for endometrial preparation are NC and AC [4]. NC is usually recommended and used in patients who ovulate regularly. However, early accidental ovulation still frequently occurs, resulting in a high cycle cancellation rate for NC [30]. The other widely applied method, AC, is used for patients with irregular menstruation. With AC, the time of administering exogenous oestradiol and progesterone and the quantities used can be carefully planned, making clinical arrangements more accurate and flexible. In addition, early ovulation induced by a spontaneous LH surge can be avoided by combining AC with GnRH-a [31]. However, AC is expensive and usually requires daily injections, which can be tedious for both medical staff and patients [32]. Moreover, the use of oestrogen at supraphysiological doses has also been found to decrease endometrial receptivity, increase the risk of thrombosis and cancer, and lead to adverse perinatal outcomes [33 – 35].

The present meta-analysis demonstrates that there were no significant differences in CPR, LBR and EMT when letrozole was compared with NC and AC (with and without GnRH-a suppression). In addition, the miscarriage rate for letrozole was found to be comparable to that of NC and AC. Cortinez et al. observed similar EMT rates and endometrial morphology in a group receiving letrozole treatment compared to NC [13]. Furthermore, Ganesh et al. reported that letrozole induces higher expression of integrin in the endometrial epithelium than NC, which improves the endometrial environment [14]. The superiority of letrozole could also be due to activation of the Wnt/B-catenin pathway [36, 37]. The aforementioned findings and results indicate that letrozole tends to exert no adverse effects on endometrial receptivity and has similar transplantation outcomes as NC and AC. Moreover, the use of letrozole avoids the side effects caused by high doses of oestrogen, as letrozole administration is closer to natural physiological processes and can be used both in patients with and those without regular menstrual cycles.

Letrozole, HMG and CC are all used in ovarian stimulation protocols where endometrial preparation is achieved by stimulating follicular growth and which are especially suitable for patients with infrequent ovulation and irregular cycles. However, only a few studies have compared the effects of endometrial preparation on FET cycles. We retrieved the only two related studies which compared letrozole with HMG in FET. Although pooled analysis demonstrated that letrozole did not exhibit any obvious differences to HMG with regard to CPR and EMT, the results of the two studies were significantly different. Hu et al. indicated that letrozole plus HMG resulted in a higher CPR and EMT than HMG [24], while Huang et al. showed that the CPR, LBR and EMT were lower after the administration of letrozole alone compared to HMG [25]. In line with Huang et al., a previous meta-analysis demonstrated that in women with unexplained subfertility undergoing IUI, a letrozole alone protocol resulted in a thinner EMT than treatment with gonadotrophins [38]. So far, definite conclusions cannot be drawn based on only these two studies. However, compared to letrozole, HMG has the drawbacks of an increased risk of OHSS and the necessity of performing repeat injections, neither of which cannot be ignored in clinical practice and making it unnecessary to conduct more searches to compare the clinical consequences in FET.

CC is well-known for its antagonistic effect on oestrogen receptors and adverse effect on endometrial receptivity during ovulation induction compared to letrozole [39, 40]. Unfortunately, only one RCT compared the two in FET cycles, reporting a significantly improved endometrial thickness in the letrozole group compared to the CC group [41]. Pregnancy was not achieved in either group, possibly due to the limited sample size (n = 10 in the letrozole group and n = 9 in the CC group). When more studies are available, comparisons of letrozole with CC in FET can be added to future meta-analyses.

As letrozole can elevate the expression of follicle-stimulating hormone (FSH) receptors in granulosa, which in turn enhances susceptibility to gonadotropins and helps to reduce the dosages of gonadotropins [42], it is worth investigating whether a combination of letrozole and HMG could achieve a better clinical efficacy than letrozole alone in FET. Our subgroup analyses revealed that both letrozole alone and letrozole plus HMG resulted in CPR and LBR similar to those of NC and AC. Notably, a lower MR was observed for letrozole plus HMG compared with AC. Moreover, letrozole alone led to a lower OPR than with NC and a thinner EMT than with AC + GnRH-a, which was not observed for letrozole plus HMG. However, whether letrozole plus HMG offers more promising outcomes than letrozole alone in FET still remains to be elucidated, as the subgroup analyses were based on a very limited number of studies, and the specific combinations of letrozole and HMG used in the studies varied (▶ **Table 1**).

PCOS patients are prone to develop multiple follicles and suffer from OHSS during ART, rendering FET the best choice in most cases. Strikingly, letrozole has the ability to promote monofollicular development and reduce the incidence of OHSS in PCOS patients undergoing ART [10, 43, 44]. Therefore, we propose a hypothesis that emphasises the benefits of letrozole as the better option to prepare the endometrium in PCOS patients. However, pooled analysis of two studies suggests that letrozole has no advantages over AC with regard to CPR and OPR in PCOS patients undergoing FET. This could be explained by the fact that FET focuses more on endometrial growth, while controlled ovarian stimulation cycles such as IUI and IVF affect both endometrial thickness and the number of induced oocytes. Despite the limited evidence because of the limited number of studies, we believe that this is still a novel aspect, and should lead to more studies exploring the effects of letrozole on PCOS patients during FET cycles.

In theory, a short-term administration of letrozole in the early follicular stage should not increase the fetal malformation rate, as

the short half-life (45 min) ensures its clearance prior to embryo implantation [45, 46]. An oral abstract reported an increase in the rates of cardiac and musculoskeletal malformations in fetuses from women who underwent letrozole treatment [47]; however, there were some methodological problems with the study [48]. In addition, accumulating evidence from studies have also reported the opposite results. For example, Tulandi et al. reported that there were no differences in the overall rates of congenital malformations between letrozole and CC, while congenital cardiac malformations were significantly lower in the letrozole group (0.2 % vs. 1.8 %, p = 0.02) [49]. Moreover, Tastumi et al. found that, compared with NC, letrozole was able to reduce the risk of miscarriage without increasing the risk of major congenital anomalies or adverse neonatal outcomes [50]. Likewise, the results of the current study found no significant differences in BDR when letrozole was compared with NC or AC, suggesting that letrozole is at least as safe as the two mainstream protocols in FET. However, it is unclear whether there are differences in BDR between letrozole and HMG, as only one study without observed birth defects was included.

Moreover, patients often complain about AC and HMG cycles in clinical practice, not only because of the high costs but also because of the uncomfortable and painful injections. As a low-cost and non-invasive solution, letrozole is definitely more patient-friendly and could improve the compliance of already cash-strapped patients.

However, the current study has its own limitations.

Firstly, only a limited number of studies were included in this meta-analysis, and they were generally of low quality. Most of the included studies were observational studies, except for one RCT. In addition, a case-control study was included in our meta-analysis, which generally does not meet the inclusion criteria in other meta-analyses.

Secondly, 90 % (9/10) of the included studies were carried out in Asia. English language restrictions were adopted as part of the selection criteria. Therefore, the patients involved in the studies may not fully represent the situation in other parts of the world, which may lead to publication bias in this study.

Thirdly, we observed significant heterogeneity in this meta-analysis. Using sensitivity analysis, we found that the study of Tatsumi et al. [27] accounted for the heterogeneity in the comparisons between letrozole and NC or AC with regard to CPR and LBR. A possible reason for this could be the significant inconsistencies in basic characteristics between groups, such as younger patient age, greater possibility of blastocyst transfer, and different luteal-phase support methods in the letrozole group. Excluding the study of Tatsumi et al. [27] did not lead to a reversal of the outcomes, demonstrating the stability of the results. The study by Li et al. [29] accounted for the heterogeneity in the comparison of EMT between letrozole and NC, with opposite outcomes observed after excluding this study, suggesting that this result is instable. The case-controlled design of this study could explain the variability. When EMT was compared for letrozole and AC, sensitivity analyses failed to determine the source of the heterogeneity, which could be due to the differences in sample size, patient allocation, letrozole dosage, etc. We were unable to perform subgroup analyses on these methodological details, as they were not always clearly stated in some studies. Meanwhile, exclusion of the study by Sibai et al. [26] led to a reversal of the outcome in the comparison of EMT between letrozole and AC. After a thorough review of the literature, we found that the criteria for patient allocation were not stated, and it was also unclear whether there was a difference in EMT between groups in previous FET cycles.

In conclusion, this is the first systematic review and meta-analysis assessing the role of letrozole for endometrial preparation in FET cycles. Letrozole administration had comparable CPR and LBR to those for NC, AC, and AC + GnRH-a, and did not lead to increased BDR compared to NC or AC. These findings indicate that letrozole could be a promising and convenient option for endometrial preparation in FET, with equivalent safety outcomes for infants. However, our results should be interpreted with caution due to the insufficient and poor quality of the included studies. More research is warranted to compare the clinical efficacy of letrozole with HMG regimens for FET, and it remains to be elucidated whether letrozole alone or a combined administration of letrozole and HMG would lead to the better FET outcomes.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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