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Obstetric and neonatal outcomes after natural versus artificial cycle frozen embryo transfer and the role of luteal phase support: a systematic review and meta-analysis

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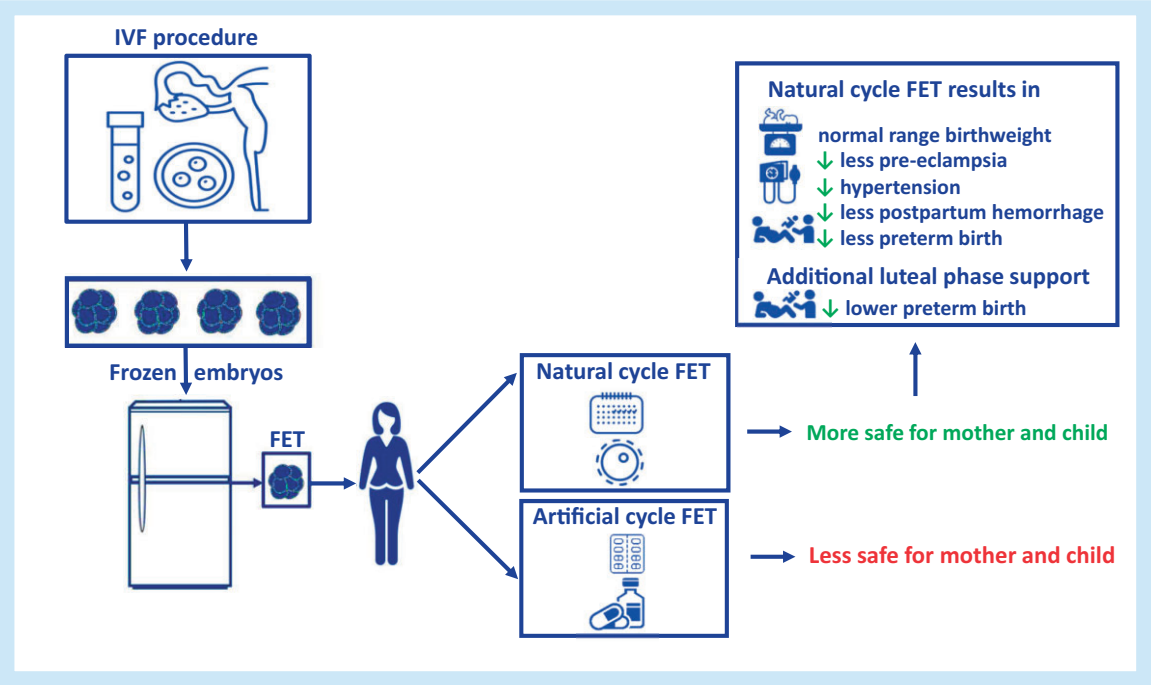
GRAPHICAL ABSTRACT



Natural cycle FET decreases the risk of adverse obstetric and neonatal outcomes compared with artificial cycle FET. FET: frozen embryo transfer.

ABSTRACT

BACKGROUND: The number of frozen embryo transfers (FET) has increased dramatically over the past decade. Based on current evidence, there is no difference in pregnancy rates when natural cycle FET (NC-FET) is compared to artificial cycle FET (AC-FET) in subfertile women. However, NC-FET seems to be associated with lower risk of adverse obstetric and neonatal outcomes compared with AC-FET cycles. Currently, there is no consensus about whether NC-FET needs to be combined with luteal phase support (LPS) or not. The question of how to prepare the endometrium for FET has now gained even more importance and taken the dimension of safety into account as it should not simply be reduced to the basic question of effectiveness.

OBJECTIVE AND RATIONALE: The objective of this project was to determine whether NC-FET, with or without LPS, decreases the risk of adverse obstetric and neonatal outcomes compared with AC-FET.

SEARCH METHODS: A systematic review and meta-analysis was carried out. A literature search was performed using the following databases: CINAHL, EMBASE, and MEDLINE from inception to 10 October 2022. Observational studies, including cohort studies, and registries comparing obstetric and neonatal outcomes between singleton pregnancies after NC-FET and those after AC-FET were sought. Risk of bias was assessed using the ROBINS-I tool. The quality of evidence was evaluated using the Grading of Recommendations Assessment, Development and Evaluation approach. We calculated pooled odds ratios (ORs), pooled risk differences (RDs), pooled adjusted ORs, and prevalence estimates with 95% CI using a random effect model, while heterogeneity was assessed by the I^2.

OUTCOMES: The conducted search identified 2436 studies, 890 duplicates were removed and 1546 studies were screened. Thirty studies (NC-FET n = 56 445; AC-FET n = 57 231) were included, 19 of which used LPS in NC-FET. Birthweight was lower following NC-FET versus AC-FET (mean difference 26.35 g; 95% CI 11.61–41.08, $I^2 = 63\%$). Furthermore NC-FET compared to AC-FET resulted in a lower risk of large for gestational age (OR 0.88, 95% 0.83–0.94, $I^2 = 54\%$), macrosomia (OR 0.81; 95% CI 0.71–0.93, $I^2 = 68\%$), low birthweight (OR 0.81, 95% CI 0.77–0.85, $I^2 = 41\%$), early pregnancy loss (OR 0.73; 95% CI 0.61–0.86, $I^2 = 70\%$), preterm birth (OR 0.80; 95% CI 0.75–0.85, $I^2 = 20\%$), very preterm birth (OR 0.66, 95% CI 0.53–0.84, $I^2 = 0\%$), hypertensive disorders of pregnancy (OR 0.60, 95% CI 0.50–0.65, $I^2 = 61\%$), pre-eclampsia (OR 0.50; 95% CI 0.42–0.60, $I^2 = 44\%$), placenta previa (OR 0.84, 95% CI 0.73–0.97, $I^2 = 0\%$), and postpartum hemorrhage (OR 0.43; 95% CI 0.38–0.48, $I^2 = 53\%$). Stratified analyses on LPS use in NC-FET suggested that, compared to AC-FET, NC-FET with LPS decreased preterm birth risk, while NC-FET without LPS did not (OR 0.75, 95% CI 0.70–0.81). LPS use did not modify the other outcomes. Heterogeneity varied from low to high, while quality of the evidence was very low to moderate.

WIDER IMPLICATIONS: This study confirms that NC-FET decreases the risk of adverse obstetric and neonatal outcomes compared with AC-FET. We estimate that for each adverse outcome, use of NC-FET may prevent 4 to 22 cases per 1000 women. Consequently, NC-FET should be the preferred treatment in women with ovulatory cycles undergoing FET. Based on very low quality of evidence, the risk of preterm birth be decreased when LPS is used in NC-FET compared to AC-FET. However, because of many uncertainties—the major being the debate about efficacy of the use of LPS—future research is needed on efficacy and safety of LPS and no recommendation can be made about the use of LPS.

Keywords: frozen-thawed embryo transfer / artificial cycle / natural cycle / safety / hypertensive disorders of pregnancy / birthweight / luteal phase support

Introduction

It has been more than 30 years since the first successful frozen embryo transfer (FET) (Trounson and Mohr, 1983; Zeilmaker *et al.*, 1984). Nowadays, FET is increasingly applied throughout the world and in 2015 FET accounted for about 40% of all IVF cycles in Europe (De Geyter *et al.*, 2018; ESHRE, 2018; Pereira *et al.*, 2019; European IVF-Monitoring Consortium (EIM) for the European Society of Human Reproduction and Embryology (ESHRE) *et al.*, 2021). The rise of FET is mainly due to improvement in laboratory techniques and use of the freeze-all strategy (Zaat *et al.*, 2021a).

In FET one needs to synchronize the endometrium with the developmental stage of the embryo to facilitate implantation. The two most common ways to prepare the endometrium and to time the FET are artificial cycle FET (AC-FET) and natural cycle FET (NC-FET). In AC-FET exogenous estrogen is administered to develop the endometrium, subsequently progesterone is used to prepare the endometrium and time FET after optional downregulation using GnRH agonist. In NC-FET, there is a natural build-up of the endometrium while using detection of the LH surge to time the embryo transfer.

In spontaneous ovulatory cycles, the resulting corpus luteum (CL) is believed to effectively supply all that is necessary for embryo implantation. When hCG is used for ovulation triggering, its long half-life renders it also as a form of luteal phase support (LPS) (Casper and Yanushpolsky, 2016). A recent meta-analysis concluded—based on low-quality evidence with high heterogeneity in treatment protocols—that progesterone administration for LPS may be beneficial following NC-FET in terms of clinical pregnancy and live birth rates (Mizrachi *et al.*, 2021). A Cochrane review that compared different types of endometrium preparation for FET found comparable effectiveness in terms of pregnancy chance (Glujovsky *et al.*, 2020). Safety issues were not considered in the Cochrane review and no specific analysis was performed on NC-FET with or without LPS. Whether or not women should receive LPS following NC-FET is controversial but, nonetheless, NC-FET with LPS is often used in clinical practice (Weissman, 2020; Mizrachi *et al.*, 2021).

The latest observational data assessing obstetrical and neonatal outcomes after FET suggests higher risk of early pregnancy loss, rates of hypertensive disorders of pregnancy (HDP), gestational diabetes (GDM), placental pathology, postpartum hemorrhage (PPH), higher birthweights, more babies being born as large for gestational age (LGA), and macrosomia in AC-FET cycles compared with NC-FET cycles (Hatoum *et al.*, 2018; Saito *et al.*, 2017; Ginstrom Ernstad *et al.*, 2019; Saito *et al.*, 2019; Wang *et al.*, 2019; von Versen-Hoynck *et al.*, 2019a,b; Wang *et al.*, 2020a,b; Moreno-Sepulveda *et al.*, 2021; Rosalik *et al.*, 2021; Severino and Povoa, 2021; Zaat *et al.*, 2021a; Busnelli *et al.*, 2022; Vinsonneau *et al.*, 2022). In these studies, no analysis was performed on the effectiveness of the use of LPS in NC-FET compared to NC-FET without using LPS. No meta-analyses have been performed on early pregnancy loss in AC-FET compared to NC-FET and in previous meta-analysis, there has not been a stratified analysis on women with or without polycystic ovary syndrome (PCOS). Given the increasing use of FET, it is important to evaluate the safety and effectiveness of all its specific elements. Knowledge on how to prepare the endometrium for FET should not only focus on of optimal pregnancy rates but also on the safest outcome for mother and baby.

With our systematic review, we aim to determine whether NC-FET with or without LPS decreases the risk of adverse obstetric and neonatal outcomes compared with AC-FET.

Methods

This systematic review and meta-analysis was conducted according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The protocol was registered at the International Prospective Register of Systematic Reviews [PROSPERO CRD42020163086].

Literature search and data extraction

A literature search was performed using the following databases: Pubmed, CINAHL, and EMBASE from inception to 10 October 2022. In addition, references of selected articles were examined to identify other relevant studies. Supplementary Data File S1 shows the complete search strategy. Following the search, we have used Covidence systematic review software where duplicates were removed (Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia. Available at www.covidence.org). Two investigators (T.R.Z. and P.K.) independently reviewed titles, abstracts and full text articles, and selected the studies. Any disagreements were resolved by discussion with a third author (M.v.W.) until consensus was reached. Two authors (T.R.Z. and P.K.) independently assessed the quality of the included studies and the quality of the evidence (T.R.Z. and E.K.). Disagreement regarding type and quality of the study was resolved after discussion with the third author (M.v.W.).

Eligibility criteria

Three criteria for inclusion were determined, which all had to be fulfilled. First, the study design was a retrospective cohort study, *post hoc* analysis or follow-up studies of randomized controlled trials (RCTs), because it is not possible to accomplish conditions of prevalence in study designs other than cohort studies. Second, the studies should consist of a study population of women who conceived after NC-FET (including true NC-FET, defined as home-based monitoring of ovulation to time FET and modified NC-FET, defined as ultrasound monitoring and hCG trigger for ovulation to time FET) and a control group of women who conceived after AC-FET. Finally, data had to be available about the obstetric and/or neonatal outcomes. Exclusion criteria were other study designs, studies without control group or studies that did not contain data about obstetric and/or neonatal outcomes. In our research protocol we stated the exclusion criterion: studies including anovulatory women and women with polycystic ovary syndrome (PCOS). However, in the final review and meta-analysis, this exclusion criterion was rejected because of the small number of studies only including ovulatory women. We performed a subgroup analysis on studies that excluded women with PCOS.

Outcome measures

We chose birthweight as the main outcome and report this as: absolute birthweight (g); LGA (birthweight > 90th percentile); macrosomia (as defined by the authors of the included studies); low birthweight (LBW; birthweight < 2500 g); and small for gestational age (SGA; birthweight < p10).

Additional outcomes included:

- Obstetric outcomes
  Obstetric outcomes included: early pregnancy loss (defined as a miscarriage before 20 weeks of gestation expressed per woman with a registered pregnancy, although usually only first trimester pregnancy loss could be extracted); GDM (as defined by the authors of included studies); HDP (including

pregnancy-induced hypertension, pre-eclampsia (PE) and hemolysis, elevated liver enzymes, and low platelets in the blood (HELLP syndrome); all as defined by the authors of included studies); PPH (as defined by the authors of included studies); placenta previa; preterm birth (PTB: delivery <37 weeks of gestation); and very preterm birth (very PTB; delivery <32 weeks of gestation).

- Neonatal outcomes
  Neonatal outcomes were congenital malformations and neonatal mortality.

Assessment of heterogeneity

To ensure that pooling was valid, we assessed the similarity between the eligible studies in their design and clinical characteristics using the I^2 statistic. An I^2 >50% was labeled as marked heterogeneity (Higgins *et al.*, 2003).

Quality and risk of bias assessment

The ROBINS-I bias tool, for assessing the quality of studies in meta-analyses was used to evaluate the included studies (Sterne *et al.*, 2016). The quality of the included studies was evaluated according to the following variables: confounding, selection of participants, classification of intervention, deviations from intended interventions, missing data, measurement of outcomes, and selection of reported results. The included studies were graded as low quality, high quality, or unknown. A risk of bias summary was created in Review Manager software (version 5.4; The Cochrane Collaboration, 2020). The quality of evidence was assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach (Atkins *et al.*, 2004). Quality of evidence was downgraded by one level for serious concerns and by two levels for very serious concerns for risk of bias, inconsistency, indirectness, imprecision, and publication bias. The assessment of bias and grading of evidence was performed independently by two authors. Any disagreements were resolved by discussion or consultation with a third author.

Data analysis

Studies that met the inclusion criteria were selected for analysis. Extracted data from included studies were pooled using StataCorp (2015. Stata Statistical Software: Release 14. College Station, TX, USA: StataCorp LP). We calculated pooled odds ratios (ORs), pooled risk differences (RDs), pooled adjusted ORs, and prevalence estimates with 95% CI using a random effect model, while heterogeneity was assessed by the I^2. We performed stratified analyses on LPS use. As sensitivity analysis, we stratified studies on completely excluding women with PCOS and studies that also included women with PCOS.

Results

Result of the searches

The conducted search identified 2436 studies, then 890 duplicates were removed and 1546 studies were screened based on the abstract. In total, 1509 studies were excluded based on abstract. The remaining 37 studies were considered eligible by at least one of the reviewers. Subsequently, we excluded seven studies by screening of full text. Thirty studies met the inclusion criteria (Nakashima *et al.*, 2013; Lathi *et al.*, 2015; Guan *et al.*, 2016; Cerrillo *et al.*, 2017; Saito *et al.*, 2017; Ernstad *et al.*, 2019; Jing *et al.*, 2019; Saito *et al.*, 2019; von Versen-Hoynck *et al.*, 2019a,b; Bu *et al.*, 2020; Levi Setti *et al.*, 2020; Lin *et al.*, 2020; Makhijani *et al.*, 2020; Pan *et al.*, 2020; Zong *et al.*, 2020; Wang *et al.*, 2020a,b; Aslih *et al.*, 2021; Asserhoj *et al.*, 2021; Hu *et al.*, 2021; Li *et al.*, 2021; Waschkies *et al.*, 2021; Zaat *et al.*, 2021b; Dallagiovanna *et al.*, 2022; Gu *et al.*, 2022; Li *et al.*, 2022; Roelens *et al.*, 2022; Xu *et al.*, 2022; Yang *et al.*, 2022; Zhou *et al.*, 2022). Detailed information about the selection of studies for inclusion is shown in the PRISMA flow diagram in Fig. 1 Characteristics of the included studies are reported in Table 1.

Included studies

Methodology of the included studies

Data from 30 studies (NC-FET n = 56 445; AC-FET n = 57 231) were included in the meta-analysis (Table 1). Five were retrospective register-based cohort studies (Nakashima *et al.*, 2013; Saito *et al.*, 2017; Ernstad *et al.*, 2019; Saito *et al.*, 2019; Asserhoj *et al.*, 2021). Twenty-three were retrospective cohort studies (Lathi *et al.*, 2015; Guan *et al.*, 2016; Jing *et al.*, 2019; Bu *et al.*, 2020; Levi Setti *et al.*, 2020; Lin *et al.*, 2020; Makhijani *et al.*, 2020; Pan *et al.*, 2020; Zong *et al.*, 2020; Wang *et al.*, 2020a,b; Aslih *et al.*, 2021; Hu *et al.*, 2021; Li *et al.*, 2021; Waschkies *et al.*, 2021; Zaat *et al.*, 2021b; Dallagiovanna *et al.*, 2022; Gu *et al.*, 2022; Li *et al.*, 2022; Roelens *et al.*, 2022; Xu *et al.*, 2022; Yang *et al.*, 2022; Zhou *et al.*, 2022). Two were prospective cohort studies (Cerrillo *et al.*, 2017; von Versen-Hoynck *et al.*, 2019a,b). Twenty studies were single-center studies (Lathi *et al.*, 2015; Guan *et al.*, 2016; Cerrillo *et al.*, 2017; Jing *et al.*, 2019; von Versen-Hoynck *et al.*, 2019a,b; Bu *et al.*, 2020; Levi Setti *et al.*, 2020; Makhijani *et al.*, 2020; Zong *et al.*, 2020; Wang *et al.*, 2020a,b; Aslih *et al.*, 2021; Hu *et al.*, 2021; Li *et al.*, 2021; Dallagiovanna *et al.*, 2022; Li *et al.*, 2022; Roelens *et al.*, 2022; Xu *et al.*, 2022; Yang *et al.*, 2022; Zhou *et al.*, 2022). The other 10 studies were multicenter studies (Nakashima *et al.*, 2013; Saito *et al.*, 2017; Ernstad *et al.*, 2019; Saito *et al.*, 2019; Lin *et al.*, 2020; Pan *et al.*, 2020; Asserhoj *et al.*, 2021; Waschkies *et al.*, 2021; Zaat *et al.*, 2021b; Guet *al.*, 2022). Across all studies, data were extracted from national registers or hospitals records (Table 1).

LPS protocols for NC-FET in the included studies

Twenty studies used LPS in NC-FET (Lathi *et al.*, 2015; Cerrillo *et al.*, 2017; Jing *et al.*, 2019; Saito *et al.*, 2019; von Versen-Hoynck *et al.*, 2019a,b; Levi Setti *et al.*, 2020; Lin *et al.*, 2020; Makhijani *et al.*, 2020; Pan *et al.*, 2020; Zong *et al.*, 2020; Wanget *al.*, 2020a,b; Aslih *et al.*, 2021; Hu *et al.*, 2021; Waschkies *et al.*, 2021; Gu *et al.*, 2022; Li *et al.*, 2022; Xu *et al.*, 2022; Yang *et al.*, 2022; Zhou *et al.*, 2022), six studies did not use LPS (Ernstad *et al.*, 2019; Asserhoj *et al.*, 2021; Li *et al.*, 2021; Zaat *et al.*, 2021b; Dallagiovanna *et al.*, 2022; Roelens *et al.*, 2022), and for four studies this was unclear (Nakashima *et al.*, 2013; Guan *et al.*, 2016; Saito *et al.*, 2017; Buet *al.*, 2020). The protocols used for LPS in NC-FET varied widely between studies, as presented in Table 2. The use of LPS in case of pregnancy ranged from 5 to 12 weeks of gestation (Table 2).

Outcomes reported in the included studies

Not all studies demonstrated all pre-defined outcomes, and included outcomes per study are presented in Table 3.

Outcomes

Main outcomes

Birthweight was lower following NC-FET versus AC-FET (MD 26.35 g; 95% CI 11.61–41.08, I^2 = 63%). NC-FET compared to AC-FET resulted in a lower risk of LGA (OR 0.88, 95% 0.83–0.94, I^2 = 54%; RD −0.016, 95% CI −0.024 to −0.008), macrosomia (OR 0.81; 95% CI 0.71–0.93, I^2 = 68%; RD −0.007, 95% CI −0.012 to

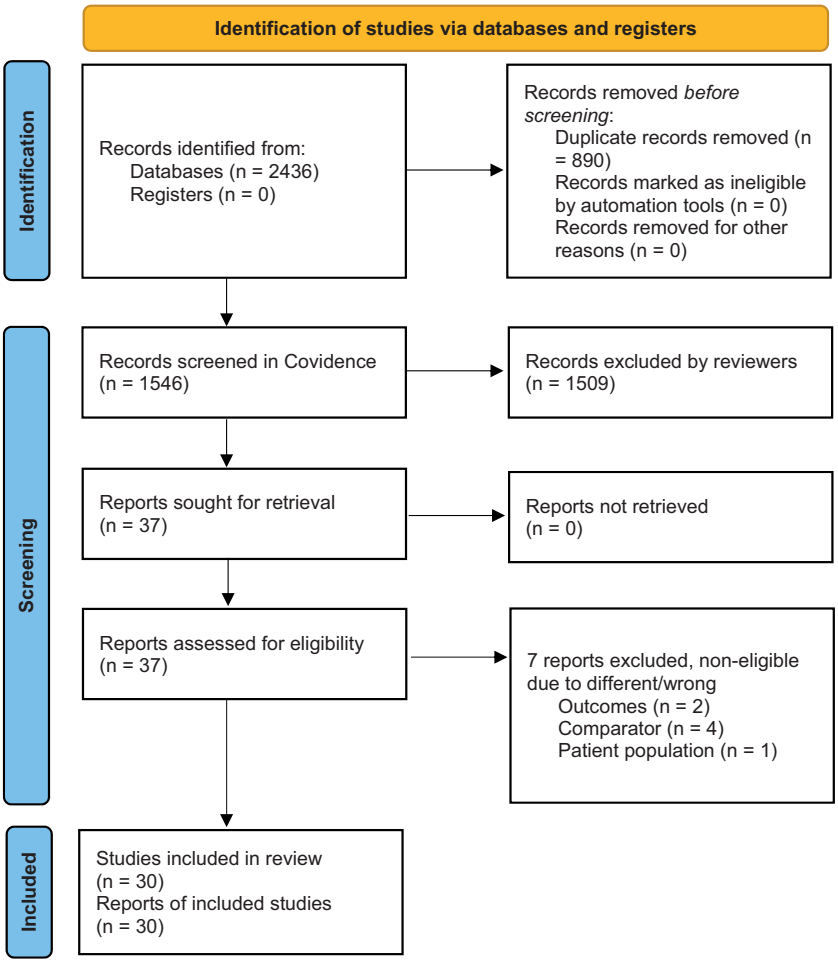


Figure 1. **Prisma flow diagram of the systematic search.**

−0.002), and LBW (OR 0.81, 95% CI 0.77–0.85, $I^2 = 41\%$; RD −0.012, 95% CI −0.018 to −0.005) (Table 4, Fig. 2, Supplementary Figs S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12, S13, S14, S15, S16, S17, S18, S19, S20, S21, S22, S23, S24, S25, S26, S27, and S28). Prevalence estimates for these main outcomes following NC-FET and AC-FET are depicted in Supplementary Table S1.

Stratified analyses on LPS use in NC-FET suggested that compared to AC-FET, NC-FET with LPS decreased PTB risk, while NC-FET without LPS did not (OR 0.75, 95% CI 0.70–0.81).

Pooled adjusted OR resulted in similar estimates (Table 4).

Additional outcomes: obstetric outcomes

NC-FET compared to AC-FET resulted in a lower risk of early pregnancy loss (OR 0.73; 95% CI 0.61–0.86, $I^2 = 71\%$; RD −0.04, 95% CI −0.06 to −0.03). NC-FET compared to AC-FET resulted in a lower risk of PTB (OR 0.80; 95% CI 0.75–0.85, $I^2 = 20\%$; RD −0.015, 95% CI −0.020 to −0.010) and very PTB (OR 0.66, 95% CI 0.53–0.84, $I^2 = 0\%$; RD −0.004, 95% CI −0.007 to −0.001), HDP (OR 0.60, 95% CI 0.50–0.65, $I^2 = 61\%$; RD −0.022, 95% CI −0.031 to −0.020), PE (OR 0.50; 95% CI 0.42–0.60, $I^2 = 44\%$; RD −0.036, 95% CI −0.053 to −0.019), placenta previa (OR 0.84, 95% CI 0.73–0.97, $I^2 = 0\%$; RD −0.002, 95% CI −0.004 to 0.001), and PPH (OR 0.43; 95% CI 0.38–0.48, $I^2 = 53\%$; RD −0.052, 95% CI −0.963 to −0.009).

Pooled adjusted OR resulted in similar estimates. The risk of GDM did not differ between NC-FET and AC-FET (OR 1.01, 95% CI 0.85–1.19, $I^2 = 80\%$; RD 0.000, 95% −0.010 to 0.010) (Table 4, Fig. 3, Supplementary Figs S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, and S12 and S15, S16, S17, S18, S19, S20, S21, S22, S23, S24, S25, and S26).

Prevalence estimates for these outcomes following NC-FET and AC-FET are depicted in Supplementary Table S1.

Additional outcomes: neonatal outcomes

The risk of congenital malformations (OR 0.86; 95% CI 0.66–1.11, $I^2 = 3\%$; RD −0.000, 95% CI −0.010 to 0.010) and neonatal mortality (OR 0.80; 95% CI 0.56–1.13, $I^2 = 10\%$; RD −0.000, 95% CI −0.010 to 0.0010) did not differ between NC-FET compared to AC-FET (Table 4, Supplementary Table S1, Supplementary Figs S12 and S13 and S27 and S28).

Stratified analysis on studies with or without inclusion of women with or without PCOS

We performed subgroup analyses on studies that excluded or included women with PCOS (Table 1), for all outcomes. No statistically significant differences were observed, and the direction of effect was the same, but there were some differences in size of the effect. Compared to studies including women with PCOS, we found that in studies excluding women with PCOS there may be an increased risk of LGA (OR 0.84, 95% CI 0.76–0.92 for studies excluding PCOS versus OR 0.92, 95% CI 0.84–1.01 for studies included women with PCOS) and early pregnancy loss (OR 0.65, 95% CI 0.52–0.81 for studies excluding PCOS versus OR 0.88, 95%

Table 1. Characteristics of included studies.

Study	Country	Study design	Study period	Inclusion criteria*	Exclusion criteria	Embryo stage at transfer (%)	Luteal phase support NC-FET	Study groups (n)
Aslih *et al.* (2021)	Israel	RC	2016–2018	All women with a singleton live birth after FET. *Included women with anovulation and/or PCOS (NC-FET 10.6%; AC-FET 24.2%; P < 0.0001)*	The use of donor oocytes, FET cancel due to endometrial polyps, premature progesterone elevation	Cleavage and blastocyst	Yes	Ovulatory FET including tNC-FET, mNC-FET and sC-FET: (286); AC-FET: (348)
Asserhoj *et al.* (2021)	Denmark	RRBC MC	2006–2014	All women with a singleton live birth after FET. *Included women with anovulation and/or PCOS (NC-FET 2.9%; AC-FET 9.6%)*	The use of donor oocytes or PGT	**Cleavage / Blastocyst** **tNC** 95.2 / 4.8 **mNC** 71.5 / 5.7 **AC** 96.6 / 3.4	No	tNC-FET: (168); mNC-FET: (611); AC-FET: (357)
Wang *et al.* (2020a,b)	China	RC SC	2014–2017	All women with a singleton live birth >28 weeks after FET. *Included women with anovulation and/or PCOS (NC-FET 2.4%; AC-FET 2.4%; P = 0.91).*	The use of donor embryo's, PGT, live-born singletons from twin deliveries with a stillbirth	**Cleavage / Blastocyst** **mNC** 84.5 / 15.5 **AC** 83.1 / 16.9 **sC** 85.4 / 14.6	Yes	mNC-FET: (2224); AC-FET: (4299)
Bu *et al.* (2020)	China	RC SC	2010–2018	All women with a singleton live birth after FET. *Unclear whether women with anovulation and/or PCOS were included.*	Uterus malformation, PGT, oocyte donation, a history of artificial multiple pregnancy reduction/vanish twin	N/A	N/A	NC-FET: (2469); AC-FET: (5998)
Cerrillo *et al.* (2017)	Spain	PC SC	2011–2012	Women with a singleton live birth after FET, age <40, regular cycles (26–35 days) and no more than 2 previous IVF cycles	The use of donor oocytes, PGT, irregular cycles, PCOS, endometriosis stage III/IV	N/A	Yes	tNC-FET: (50); mNC-FET: (68); AC-FET: (93)
Dallagiovanna *et al.* (2022)	Italy	RC SC	2014–2019	All women with a singleton live birth after FET. *Included women with anovulation and/or PCOS (NC-FET 1.0%; AC-FET 26.0%).*	Multiple pregnancies or risk factors for HDP	All frozen blastocysts transfer	No	NC-FET (495); AC-FET (97)
Ernstad *et al.* (2019)	Sweden	RRBC MC	2005–2015	All women with a singleton live birth after FET. *Included women with anovulation and/or PCOS (NC-FET 11.9%; AC-FET 34.0%).*	N/A	**Cleavage / Blastocyst** **tNC** 66.5 / 36.9 **AC** 60.6 / 39.4 **sC** 53.9 / 46.1	No	tNC-FET: (6297); AC-FET: (1446)
Gu *et al.* (2022)	China	RC MC	2016–2019	All women with a singleton live birth after FET delivered after 28 weeks of gestation	Cycles with PGT, vanishing twins, and women with a history of preeclampsia, type 2 diabetes mellitus, prediabetes mellitus, hypertension, and PCOS	All frozen blastocysts transfer	Yes	NC-FET: (499) AC-FET: (900)

(continued)

Table 1. (continued)

Study	Country	Study design	Study period	Inclusion criteria*	Exclusion criteria	Embryo stage at transfer (%)	Luteal phase support NC-FET	Study groups (n)
Guan *et al.* (2016)	China	RC SC	2012–2013	All women with a singleton live birth after FET, regular menstrual cycle	History of recurrent implantation failure or abortion	All vitrified-thawed Day 3 embryo's, stage not reported	N/A	mNC-FET: (427); AC-FET: (794)
Hu *et al.* (2021)	China	RC SC	2013–2019	All women with a singleton live birth after FET. *Included women with anovulation and/or PCOS (NC-FET 6.0%; AC-FET 29.0%).*	The use of donor oocytes or donor sperm, PGT, twin deliveries or neonatal death.	All frozen blastocysts transfer	Yes	tNC-FET: (3790) AC-FET: (2561)
Jing *et al.* (2019)	China	RC SC	2013–2016	Women with a singleton live birth after FET with at least one blastocyst or two cleavage-stage embryos in storage, regular ovulatory cycles, and at most two previous FET	Ovulation disorders, Asherman syndrome, PCOS and uterine malformation	Blastomere / Blastocyst: NC 64.1 / 61.5; AC 35.9 / 38.6	Yes	NC-FET: (8425); AC-FET: (2611)
Lathi *et al.* (2015)	USA	RC SC	2007–2012	All women with a singleton live birth after first attempt FET. *I Unclear whether women with anovulation and/or PCOS were included*	Cycles using embryos cryopreserved at the 2 PN stage or on Day 3 and the use of donor oocytes	All frozen blastocysts transfer	Yes	mNC-FET: (519); AC-FET: (106)
Levi Setti *et al.* (2020)	Italy	RC SC	2011–2017	All women with a singleton live birth after single FET. *Included women with anovulation and/or PCOS (tNC-FET 5.0%; mNC-FET 6.8%; AC-FET 23.9%; P < 0.0001)*	Cycles using PGT or more than one embryo per transfer	All frozen blastocysts transfer	Yes	tNC-FET: (567) mNC-FET: (1749) AC-FET: (585)
Li *et al.* (2021)	China	RC SC	2010–2017	All women with a singleton live birth after FET. *Included women with anovulation and/or PCOS (NC-FET 1.3%; AC-FET 11.6%.*	N/A	Day3 / Day4 / Day5: tNC 73.4 / 15.6 / 11.1; mNC 71.7 / 15.6 / 12.8; AC 72.0 / 16.9 / 11.1	No	tNC-FET: (1921) AC-FET: (1583)
Li *et al.* (2022)	China	RC SC	2018–2020	All women with a singleton live birth after FET.	Women with uterine anatomic abnormalities, donor gametes, PGT, PCOS, chronic hypertension, diabetes, heart disease, foetal anomalies	1599 blastocyst transfer, 594 cleavage stage transfer	Yes	mNC-FET: (314) AC-FET: (1726)
Lin *et al.* (2020)	China	RC MC	2016–2017	Women with a singleton live birth after FET who participated in the previously published RCT with regular cycles undergoing their first IVF cycle	N/A	All frozen blastocysts transfer	Yes	mNC-FET: (513); AC-FET: (287)
Makhijani *et al.* (2020)	USA	RC SC	2013–2018	All women with a singleton live birth after FET, one cycle per participant. *Included women with anovulation and/or PCOS (NC-FET 3.9%; AC-FET 37.3%).*	The use of donor oocytes, cleavage stage embryos or slow freeze embryo's. Multiple pregnancies	All frozen blastocysts transfer	Yes	tNC-FET (384); AC-FET: (391)

(continued)

Table 1. (continued)

Study	Country	Study design	Study period	Inclusion criteria*	Exclusion criteria	Embryo stage at transfer (%)	Luteal phase support NC-FET	Study groups (n)
Nakashima *et al.* (2013)	Japan	RRBC MC	2007–2008	All women with a singleton live birth after FET. *Unclear whether women with anovulation and/or PCOS were included*	The use of frozen-thawed oocytes, gamete intrafallopian transfer, oocyte intrauterine transfer, two-step embryo transfer cycles	Cleavage stage 20.5% Blastocyst stage 79.5% Not stated per group	N/A	tNC-FET: (875); NC-FET: (6244) AC-FET: (6115)
Pan *et al.* (2020)	China	RC MC	2015–2017	Women with a singleton live birth after FET who participated in the previously published RCT, age >20 and ≤35 years, regular cycle (21–35 days), first IVF/ICSI cycle, >5 oocytes retrieved	Uterine anatomic abnormalities, one ovary removed, PCOS, PGT, recurrent miscarriages	All cleavage stage embryo's	Yes	tNC-FET: (683); AC-FET: (225)
Roelens *et al.* (2022)	Belgium	RC SC	2010–2019	All women with a singleton live birth after FET. *Included women with anovulation and/or PCOS (NC-FET 6.5%; AC-FET 41.2%; P < 0.001).*	Cycles with LPS, or FET after ovulation induction	**Day4 / Day5 / Day6** **NC** 31.1 / 54.2 / 14.8 **AC** 30.8 / 54 / 15.2	No	NC-FET: (325) AC-FET: (211)
Saito *et al.* (2017)	Japan	RRBC MC	2013	All women with a singleton live birth >22 weeks after FET from autologous oocytes. *Included women with anovulation and/or PCOS (percentages not stated)*	Multiple pregnancies	**Cleavage / Blastocyst** **NC** 11.7 / 88.3 **AC** 18.5 / 81.5	N/A	tNC-FET: (6287); AC-FET: (10 235)
Saito *et al.* (2019)	Japan	RRBC MC	2014	All women with a singleton live birth after FET from autologous oocytes. *Included women with anovulation and/or PCOS (percentages not stated)*	FET cycles with ovarian stimulation	**Cleavage / Blastocyst** **NC** 21.7 / 75.6 **AC** 31.3 / 64.0	Yes	mNC-FET: (29 760); AC-FET: (75 474)
von Versen-Hoynck *et al.* (2019a,b)	USA	PC SC	Not stated	By administration of the Study Coordinator. *Included women with anovulation and/or PCOS (NC-FET 9.7%; AC-FET 22.3%)*	By administration of the Study Coordinator	N/A	Yes	mNC-FET: (127) AC-FET: (94)
Waschkies *et al.* (2021)	Germany	RC MC	1997–2019	All women with a singleton live birth after FET from autologous oocytes. *Included women with anovulation and/or PCOS (NC-FET 25.0%; AC-FET 20.2%; P = 0.39)*	Multiple pregnancies	N/A	Yes	NC-FET including mNC-FET (22) and sC-FET: (46); AC-FET: (114)

(continued)

Table 1. (continued)

Study	Country	Study design	Study period	Inclusion criteria*	Exclusion criteria	Embryo stage at transfer (%)	Luteal phase support NC-FET	Study groups (n)
Xu *et al.* (2022)	China	RC SC	2016–2021	All women with a singleton live birth after FET, age <43 years, vitrified embryo(s) derived from the first IVF/ICSI cycles. *Included women with irregular cycle (NC-FET 2.5%; AC-FET 25.7%; P < 0.001)*	Cycles with PGT, women with chronic hypertension or diabetes mellitus or with congenital or secondary uterine abnormalities	**Cleavage / Blastocyst** **NC** 78.4 / 21.6 **AC** 77.1 / 22.9	Yes	NC-FET: (959) AC-FET: (2029)
Yang *et al.* (2022)	China	RC SC	2014–2021	Women with a singleton live birth after FET, age ≤40 years at the time IVF treatment	Cycles with oocyte donor, PGT or slow freeze. Women with presence of chromosomal abnormalities, history of uterine surgery, presence of intracavitary lesions	N/A	Yes	tNC-FET: (1783) AC-FET: (550)
Zaat *et al.* (2021b)	Netherlands	RC MC		Women with a singleton live birth after FET who participated in the previously published RCT, age 18–40 years; first, second or third IVF, regular menstrual cycle	Multiple pregnancies	**Cleavage / Blastocyst** **mNC** 94.7 / 5.4 **AC** 97.5 / 2.5	No	mNC-FET: (57); AC-FET: (41)
Wang *et al.* (2020)	China	RC SC	2013–2018	All women with a singleton live birth after FET	The use of frozen-thawed oocytes, age >40, BMI >35 kg/m^2, PCOS, self-history or family history of PE, diagnosis of hypertension, diabetes, renal disease, or abnormal renal function, a history of failure to obtain clinical pregnancy after >3 times FET	All frozen blastocysts transfer	Yes	NC-FET including tNC-FET and mNC-FET: (10 211); AC-FET: (4162)
Zhou *et al.* (2022)	China	RC SC	2017–2020	Women with a singleton live birth after autologous FET, maternal age ≤42 years; BMI <28, regular menstrual cycle (21–35 days).	Multiple pregnancies, congenital uterine malformations, intrauterine adhesions, PCOS. Women with chronic medical conditions that have been associated with adverse pregnancy outcomes	**Cleavage / Blastocyst** **mNC** 41.1 / 58.9 **AC** 38.1 / 61.9 **sC** 38.8 / 61.2	Yes	mNC-FET: (1225) AC-FET: (2136)
Zong *et al.* (2020)	China	RC SC	2015–2018	Women with a singleton live birth >28 weeks after FET, age 20–40	Type II diabetes mellitus, preconceptional hypertension, PCOS, uterine malformation, intrauterine adhesion, the use of donor oocyte or PGT	All frozen blastocysts transfer	Yes	mNC-FET: (4727); AC-FET: (1642)

* The studies of Wang (2020a,b), Gu (2022), and Zong (2020) only included women with live birth after 28 weeks of gestation. N/A: not available; RRBC: retrospective register-based cohort; RC: retrospective cohort; PC: prospective cohort; MC: multiple center; SC: single center; FET: frozen embryo transfer; NC-FET: natural cycle FET (not specified whether true or modified); tNC-FET: true natural cycle FET; mNC-FET: modified natural cycle FET; sC: stimulated cycle FET; AC: artificial cycle FET; PGT: preimplantation genetic testing; PCOS: polycystic ovary syndrome.

Table 2. Luteal phase support during NC-FET in the included studies.

Study	Generic name, dose (brand name/manufacturer)	Administration route	Start of LPS use	Duration of LPS during gestation
Aslih (2021)	Dydrogesterone 10 mg (Duphastone® Abbott, Biologicals); or Micronized progesterone 100 mg (Endometrin®, Ferring); or MVP gel 90 mg (Crinone® 8%, Merck Serono)	Oral Vaginal Vaginal	Not reported	Until 10 weeks of gestation
Wang (2020a,b)	Dydrogesterone 10 mg (Duphastone® Abbott, Biologicals)	Not reported	Third day after hCG injection	Not reported
Cerrillo (2017)	Micronized progesterone 400 mg (Utrogestan® Seid, Barcelona, Spain)	Vaginal	Three or 5 days before FET	Until 5 weeks of gestation
Gu (2022)	Dydrogesterone, dose not reported (not reported) or; Dydrogesterone, dose not reported (not reported) combined with progesterone, dose not reported (not reported)	Oral Oral combined with vaginal	Day of ovulation	Until 10 weeks of gestation
Hu (2021)	Dydrogesterone 20 mg (Duphaston; Solvay Pharmaceuticals BV)	Oral	One day before FET	Not reported
Jing (2019)	Progesterone 600 mg (Duphaston, Abbott Biologicals B.V., The Netherlands)	Vaginal	Two days before FET	Not reported
Lathi (2015)	Progesterone 200 mg (not reported)	Vaginal	mNC-FET: 4 days after hCG injection tNC-FET: 3 days after LH surge	Until 10–12 weeks of gestation
Levi Setti (2020)	Micronized progesterone 200 mg (Prometrium, Rottapharm S.p.a., or Progeffik, Effik Italia S.p.a) or; MVP gel 90 mg (Crinone® 8%, Merck Serono)	Vaginal Vaginal	mNC-FET: 2 days after hCG injection tNC-FET: on day of FET	Not reported
Li (2022)	Progesterone 90 mg (Crinone, Merck Serono, UK) daily or; Dydrogesterone 30 mg (Abbott Biologicals B.V., the Netherlands)	Vaginal Oral	When endometrial thickness reached 7 mm, serum E2 level peaked at 200 pg/ml, and the serum levels of P4 were <1.5 ng/ml	Until 10–12 weeks of gestation
Lin (2020)	Dydrogesterone 30 mg (not reported)	Oral	After day of ovulation	Until 10 weeks of gestation
Makhijani (2020)	Progesterone (Crinone, Merck, Kenilworth, NJ, USA; and Endometrin, Ferring Pharmaceuticals, Parsippany, NJ, USA)	Vaginal	Two days after LH-surge	Not reported
Pan (2020)	Dydrogesterone 20 mg (not reported)	Oral	Day of ovulation	Until 10 weeks of gestation
Saito *et al.* (2019)	Groups: progesterone alone; hCG; progesterone + hCG; estrogen + progesterone; estrogen + progesterone + hCG (not reported)	Not reported	Not reported	Not reported
von Versen-Hoynck (2019a,b)	Estradiol or progesterone (not reported)	Not reported	Not reported	Not reported
Waschkies (2021)	Progesterone 200–300 mg (not reported)	Vaginal	Day of hCG-injection	Until 10–12 weeks of gestation
Xu (2022)	Dydrogesterone 30 mg (Duphastone® Abbott, Biologicals)	Oral	Day of ovulation	Until 10 weeks of gestation
Yang (2022)	Progesterone 80 mg (not reported) Progesterone gel 90 mg (not reported)	Intramuscular Vaginal	After FET	Not reported
Wang (2020)	Progesterone 20–30 mg (not reported)	Not reported	After ovulation	Until 10 weeks of gestation
Zhou (2022)	Dydrogesterone 40 mg (Duphaston; Abbott, OLST, Netherlands)	Oral	On day of ovulation	Not stated
Zong (2020)	Dydrogesterone 30 mg (Duphaston, Abbott Biologicals B.V.)	Oral	On day of ovulation	Until 12 weeks of gestation

FET: frozen embryo transfer; tNC-FET: true natural cycle FET; mNC-FET: modified natural cycle FET; E2: estradiol; P4 progesterone.

Table 3. Outcomes reported per included study.

Study	Birthweight	LGA	Macrosomia	SGA	LBW	EPL	GDM	HDP	PE	PPH	Placenta praevia	PTB	Very PTB	Cong malformations	Neonatal mortality
Aslih (2021)	✓	✓	✗	✗	✗	✓	✓	✓	✗	✗	✗	✓	✗	✗	✗
Asserhoj (2021)	✓	✓	✓	✓	✗	✗	✗	✓	✓	✓	✓	✓	✓	✗	✗
Wang (2020a,b)	✓	✓	✓	✓	✓	✗	✓	✓	✗	✗	✓	✓	✓	✗	✗
Bu (2020)	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✓	✗	x	✗
Cerrillo (2017)	✓	✗	✗	✗	✗	✓	✗	✗	✗	✗	✗	✗	✗	x	✗
Dallagiovanna (2022)	✓	✗	✗	✗	✗	✗	✓	✓	✓	✗	✓	✓	✓	✗	x
Ernstad (2019)	✓	✓	✓	✓	✓	✗	✗	✓	✓	✓	✓	✓	✓	✓	✓
Gu (2022)	✗	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✗	✗
Guan (2016)	✓	✗	✓	✗	✗	✓	✓	✓	✗	✗	✗	✓	✗	✓	✓
Hu (2021)	✓	✓	✓	✓	✓	✗	✓	✓	✗	✗	✓	✓	✓	✓	✗
Jing (2019)	✓	✓	✓	✓	✓	✓	✓	✓	✗	✗	✗	✓	✗	✗	✓
Lathi (2015)	✓	✗	✗	✗	✗	✓	✗	✗	✗	✗	✗	✗	✗	x	✗
Levi Setti (2020)	✓	✓	✗	✗	✗	✓	✗	✗	✗	✗	✗	✗	✗	x	✗
Li (2021)	✓	✓	✗	✓	✗	✓	✓	✓	✓	✗	✗	✓	✗	✗	✗
Li (2022)	✗	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✗	✗	✗
Lin (2020)	✗	✗	✗	✗	✗	✓	✗	✗	✗	✗	✗	✗	✗	x	✗
Makhijani (2020)	✓	✗	✓	✗	✓	✗	✓	✓	✗	✓	✓	✓	✓	✓	✓
Nakashima (2013)	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	x	✗
Pan (2020)	✓	✓	✓	✓	✓	✓	✓	✓	✗	✗	✗	✗	✗	✓	✗
Roelens (2022)	✓	✗	✗	✗	✗	✗	✓	✓	✓	✗	✗	✗	✗	✓	✗
Saito (2017)	✓	✓	✓	✓	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗	✓
Saito *et al.* (2019)	✓	✓	✓	✓	✓	✓	✓	✓	✗	✗	✓	✓	✗	✗	✓
von Versen-Hoynck (2019a,b)	✗	✗	✗	✗	✗	✗	✗	✓	✓	✗	✗	✗	✗	✗	✗
Waschkies (2021)	✓	✓	✓	✓	✓	✗	✓	✓	✗	✓	✗	✓	✓	✓	✓
Xu (2022)	✓	✓	✓	✓	✗	✗	✓	✓	✗	✓	✓	✓	✗	✗	✗
Yang (2022)	✗	✗	✓	✗	✓	✗	✓	✓	✗	✗	✓	✓	✗	✗	✗
Zaat (2021b)	✓	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✗
Wang (2020)	✓	✓	✓	✓	✓	✗	✓	✗	✓	✓	✓	✓	✗	✗	✗
Zhou (2022)	✓	✓	✗	✓	✓	✗	✓	✓	✗	✗	✗	✓	✓	✗	✗
Zong (2020)	✗	✓	✗	✓	✓	✗	✓	✓	✗	✗	✓	✓	✓	✗	✗

LGA: large for gestational age; SGA: small for gestational age; LBW: low birthweight; EPL: early pregnancy loss; GDM: gestational diabetes mellitus; HDP: hypertensive disorders of pregnancy; PE: pre-eclampsia; PPH: postpartum hemorrhage; PTB: preterm birth; cong: congenital.

Table 4. The summary of findings for NC-FET versus AC-FET with the grading of the evidence.

Outcomes	Mean difference (95% CI)			Number of studies	GRADE
Birthweight	26.35 (11.61–41.08)			23 studies	MODERATE
	Pooled odds ratio (95% CI)	**Pooled adjusted odds ratio (95% CI)**	**Absolute risk difference (95% CI)**		
LGA	0.88 (0.83–0.94)	0.87 (0.80–0.93)	−0.016 (−0.024 to −0.008)	17 studies	MODERATE
Macrosomia	0.81 (0.71–0.93)	0.77 (0.69–0.86)	−0.007 (−0.012 to −0.002)	17 studies	LOW
SGA	0.99 (0.90–1.10)	0.97 (0.88–1.06)	−0.001 (−0.006 to −0.004)	16 studies	LOW
LBW	0.79 (0.72–0.87)	0.77 (0.66–0.89)	−0.012 (−0.018 to −0.005)	15 studies	MODERATE
Early pregnancy loss	0.73 (0.61–0.86)	NA	−0.040 (−0.060 to −0.030)	10 studies	LOW
GDM	1.01 (0.85–1.19)	1.02 (0.92–1.14)	0.000 (−0.010 to 0.010)	20 studies	VERY LOW
HDP	0.60 (0.50–0.65)	0.52 (0.47–0.58)	−0.022 (−0.031 to −0.020)	20 studies	MODERATE
PE	0.50 (0.42–0.60)	0.43 (0.37–0.51)	−0.036 (−0.053 to −0.019)	10 studies	MODERATE
PPH	0.43 (0.38–0.48)	0.44 (0.36–0.47)	−0.052 (−0.096 to −0.009)	9 studies	VERY LOW
Placenta previa	0.84 (0.73–0.97)	0.85 (0.66–1.10)	−0.002 (−0.004 to 0.001)	16 studies	MODERATE
PTB	0.80 (0.75–0.85)	0.79 (0.75–0.85)	−0.015 (−0.020 to −0.010)	21 studies	MODERATE
Very PTB	0.66 (0.53–0.84)	0.56 (0.40–0.78)	−0.004 (−0.007 to −0.001)	11 studies	MODERATE
Cong malformations	0.86 (0.66–1.11)	0.99 (0.75–1.30)	0.000 (−0.010 to 0.010)	8 studies	VERY LOW
Neonatal mortality	0.80 (0.56–1.13)	NA	−0.000 (−0.000 to 0.000)	7 studies	VERY LOW
Stratified analysis LPS PTB	0.75 (0.70–0.81)	NA	NA	14 studies	VERY LOW

LGA: large for gestational age; SGA: small for gestational age; LBW: low birthweight; GDM: gestational diabetes mellitus; HDP: hypertensive disorders of pregnancy; PE: pre-eclampsia; PPH: postpartum hemorrhage; PTB: preterm birth; LPS: luteal phase support; NC: natural cycle; AC: artificial cycle; FET: frozen embryo transfer; NA: not available.

CI 0.63–1.23 for studies including women with PCOS) after AC-FET.

In studies excluding women with PCOS, there may be a lower risk of macrosomia (OR 0.91, 95% CI 0.79–1.05 for studies excluding PCOS versus OR 0.76, 95% CI 0.61–0.95 for studies including women with PCOS) after AC-FET, compared to studies including women with PCOS (Supplementary Figs S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12, S13, and S14).

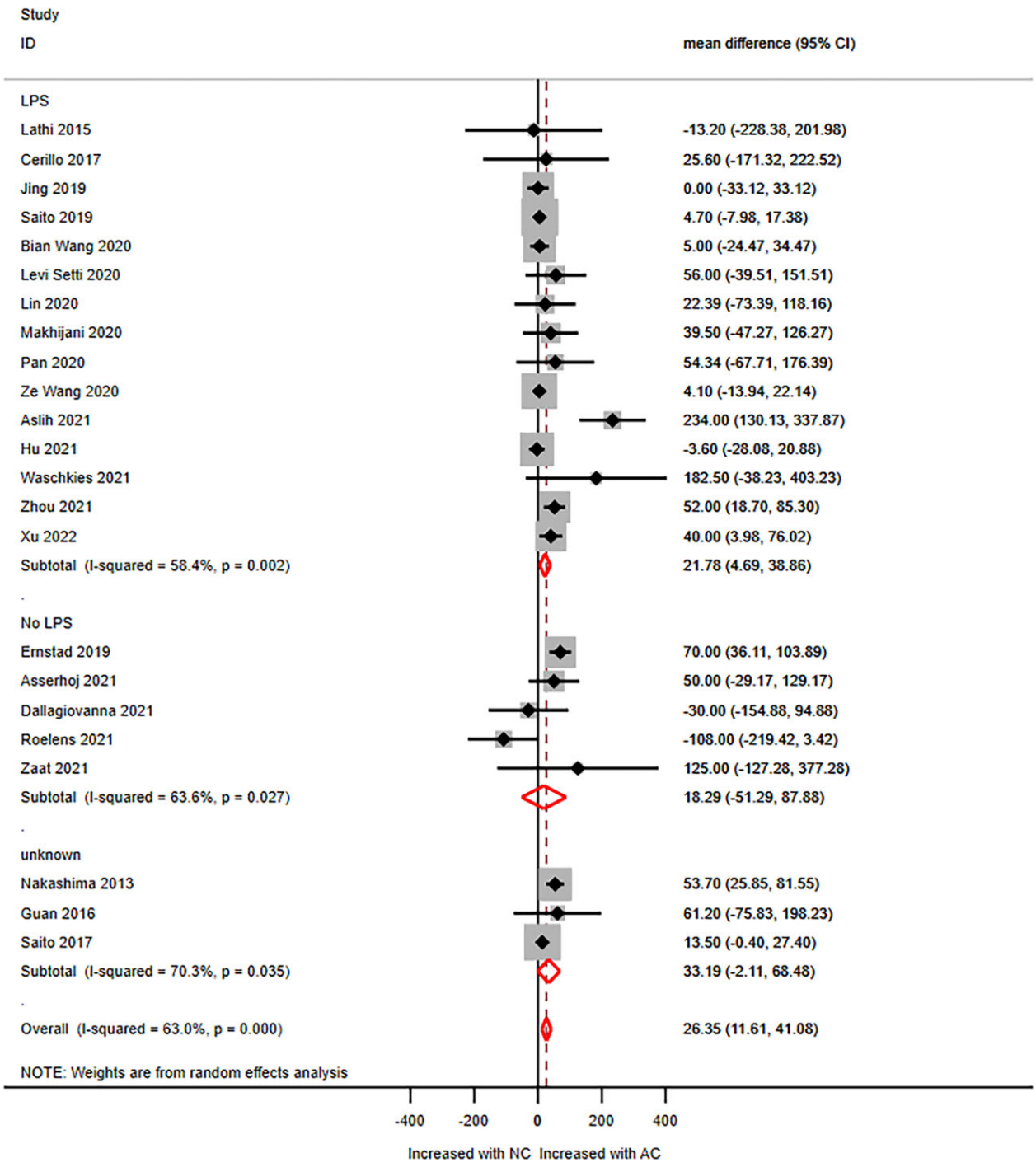


Figure 2. Difference in birthweight for NC-FET versus AC-FET, stratified by luteal phase support use. NC: natural cycle; AC: artificial cycle; LPS: luteal phase support.

Stratified analysis on the use of LPS in NC-FET

Stratified analyses on LPS use in NC-FET suggested that, compared to AC-FET, NC-FET with LPS decreased PTB risk, while NC-FET without LPS did not (OR 0.75, 95% CI 0.70–0.81 with LPS versus OR 0.96, 95% CI 0.82–1.11 without LPS). LPS use did not modify the other outcomes (Table 4, Fig. 4, Supplementary Figs S15, S16, S17, S18, S19, S20, S21, S22, S23, S24, S25, S26, S27, and S28).

Subgroup analysis on true NC-FET versus modified NC-FET

In total, three studies reported on birthweight of babies born after true NC-FET versus modified NC-FET. Birthweight did not differ between true NC-FET when compared with modified NC-FET (MD 44.90 g; 95% CI −186.8–96.9), $I^2 = 70\%$). The significance of the use of LPS in this groups remains to be studied (Fig. 5). Owing to a lack of data, the comparison between true NC-FET versus modified NC-FET could not be pooled for other outcomes.

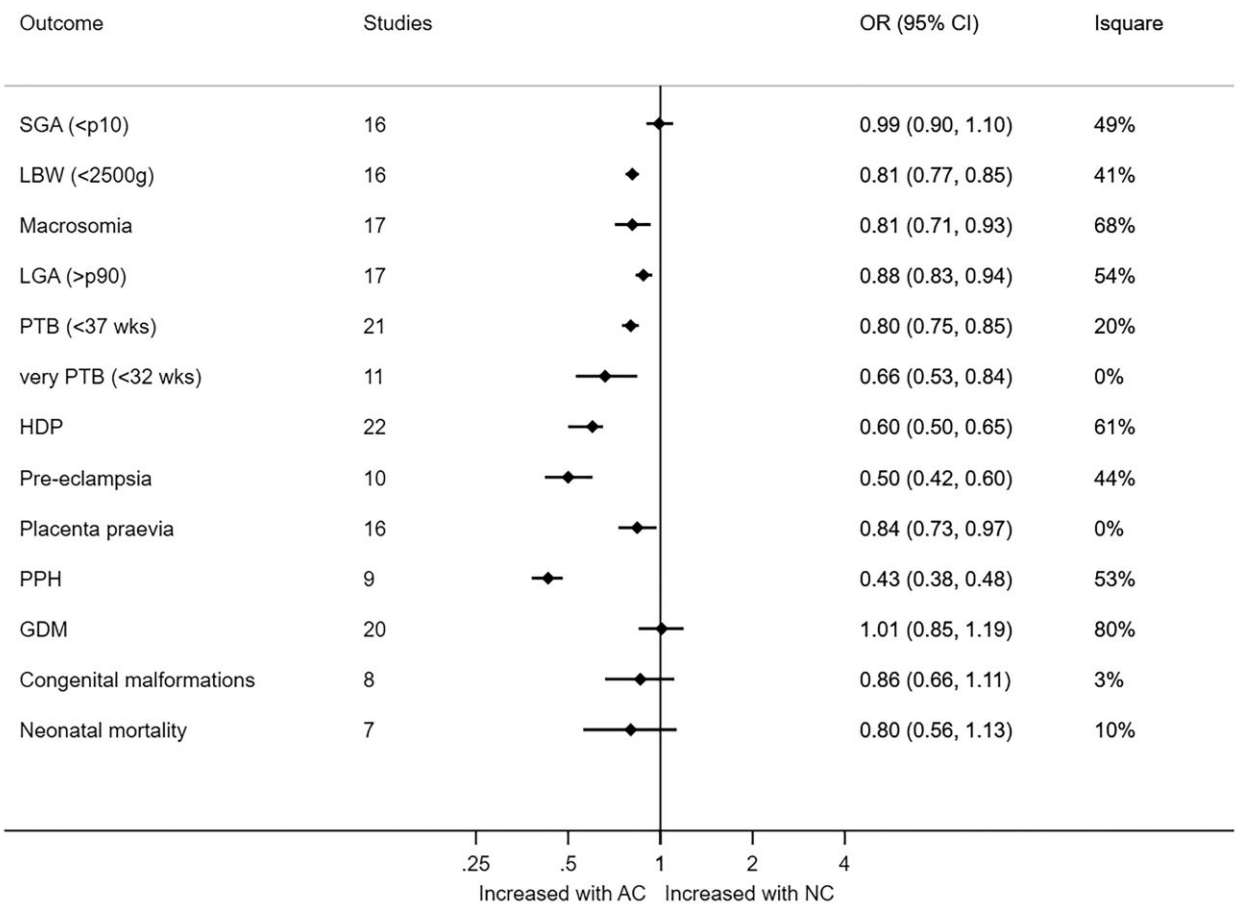


Figure 3. Summary of all averaged secondary outcomes for NC-FET versus AC-FET, expressed as odds ratio with 95% CI. NC: natural cycle; AC: artificial cycle; OR: odds ratio.

Quality and risk of bias assessment

Two of the included studies in the meta-analysis were ranked as having a high risk of bias on the domains of confounding bias, selection bias and reporting bias. One study was ranked as low risk of bias. The other 27 included studies were ranked as moderate risk of bias (Supplementary Fig. S29). The GRADE tool was used for grading the quality of evidence. The quality of evidence ranged from very low to moderate (Table 4).

Discussion

Principal findings

This systematic review and meta-analysis shows an increase in normal range birthweight and a decrease in LGA, macrosomia, LBW, early pregnancy loss, PTB, very PTB, HDP, PE, placenta previa, and PPH in NC-FET compared to AC-FET. Therefore, the risk of adverse obstetric and neonatal outcomes is lower in NC-FET compared with AC-FET.

The use of LPS in NC-FET decreases PTB risk when NC-FET with or without LPS is compared to AC-FET.

The quality of evidence was very low to moderate mainly because this is a review based on observational studies and because of the substantial inter-study heterogeneity obtained, which was assumed to be caused by the variation between study populations.

We estimate that for each adverse outcome the use of NC-FET may prevent 4 to 22 cases per 1000 women with a singleton live birth.

Study strengths

The large sample size of 113 676 live births is a major strength of this study. This is a comprehensive and updated systematic review, which includes analyses of pregnancies following NC-FET and AC-FET. As LPS might have an impact on obstetric and perinatal outcomes, we provided separated analyses of pregnancies resulting from NC-FET with or without LPS. The present systematic review and meta-analysis was carried out in accordance with the PRISMA statement, ensuring high methodological quality. Moreover, the risk of bias of the included studies was assessed using the ROBINS-I tool. The validity of our results is notably improved owing to these factors.

Study limitations

The majority of published articles in this review comprised observational studies. There is a great variety in the included studies in terms of study populations, timeline of the study, development stage of the embryos transferred with FET, freezing protocols, the use of pre-implantation genetic testing, and numbers of embryos transferred. Protocols for LPS can hardly be compared between studies because of the major variety in medication used, starting day of LPS and continuation of LPS in case of gestation (Table 2). Adjustment for relevant confounders was not possible in our main analysis owing to lack of individual patient data. We did perform an adjusted analysis by pooling adjusted ORs of the included studies, resulting in no differences in outcomes (Table 4). It should be noted however that confounders, such as vanishing twins, could not be analyzed and may have influenced the

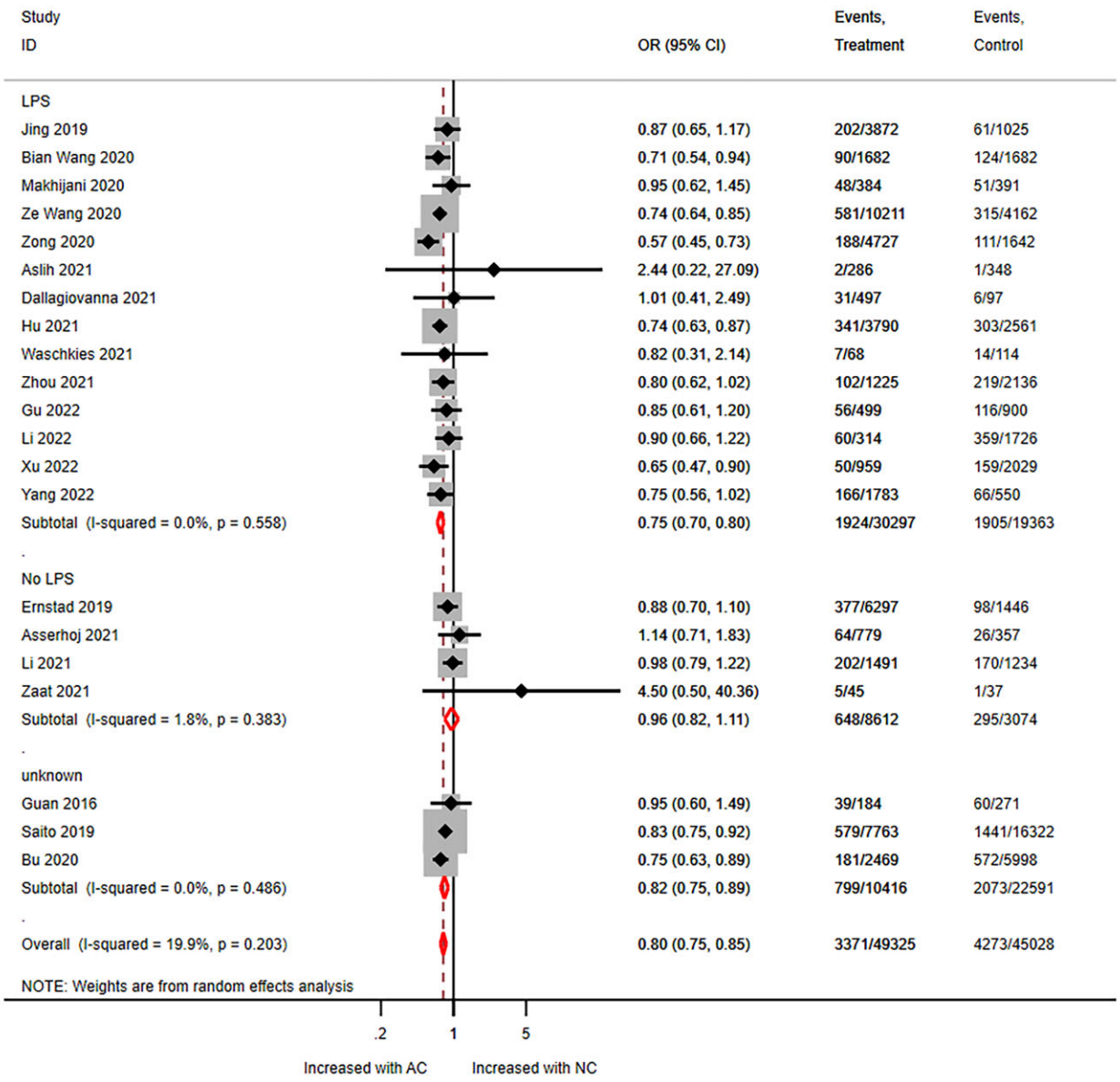


Figure 4. Preterm birth for NC-FET versus AC-FET, stratified by luteal phase support use. NC: natural cycle; AC: artificial cycle; LPS: luteal phase support.

outcomes. In addition, unpublished data as full-text articles and in languages other than English were excluded from the meta-analysis. Clear definitions for some of the outcomes were not reported in all publications. Definitions of GDM, HDP, PE, PPH congenital anomalies, and perinatal mortality were inconsistent across the included studies.

Discrepancies with research protocol

In total, 17 of the included studies also included women with irregular cycles, anovulation and/or PCOS (Table 1). In our research protocol, we stated to exclude women with PCOS/anovulation and revised this during the execution of the study.

This deviation deserves attention because including these women may be associated with a higher risk of perinatal complications, such as HDP, PE, GDM, and PTB, possibly distorting the outcomes of the analyses (Palomba and La Sala, 2016). Therefore, we performed a subgroup analysis on studies that excluded women with PCOS. Although this did not affect our general findings, the results suggest that in studies excluding women with PCOS the RD between NC-FET and AC-FET was larger for LGA and early pregnancy loss and smaller for macrosomia, compared to studies that also included women with PCOS. For the other outcomes, including HDP, PE, GDM, and PTB, no differences were found.

Furthermore, we aimed to include only singleton deliveries in our meta-analysis. Unfortunately, in five studies (Saito *et al.*, 2019; Levi Setti *et al.*, 2020; Pan *et al.*, 2020; Li *et al.*, 2022; Roelens *et al.*, 2022), data were not presented separately for singleton and multiple deliveries. We contacted the authors of these studies for separate data on singleton births. One author responded but was not able to provide data in the short term. The other authors did not respond to our request. The number of multiple deliveries in these studies was comparable between study groups and therefore not likely to have influenced the results of our meta-analysis.

Comparison with other studies

The comparison of our findings with those of three recently published meta-analyses (Moreno-Sepulveda *et al.*, 2021; Rosalik

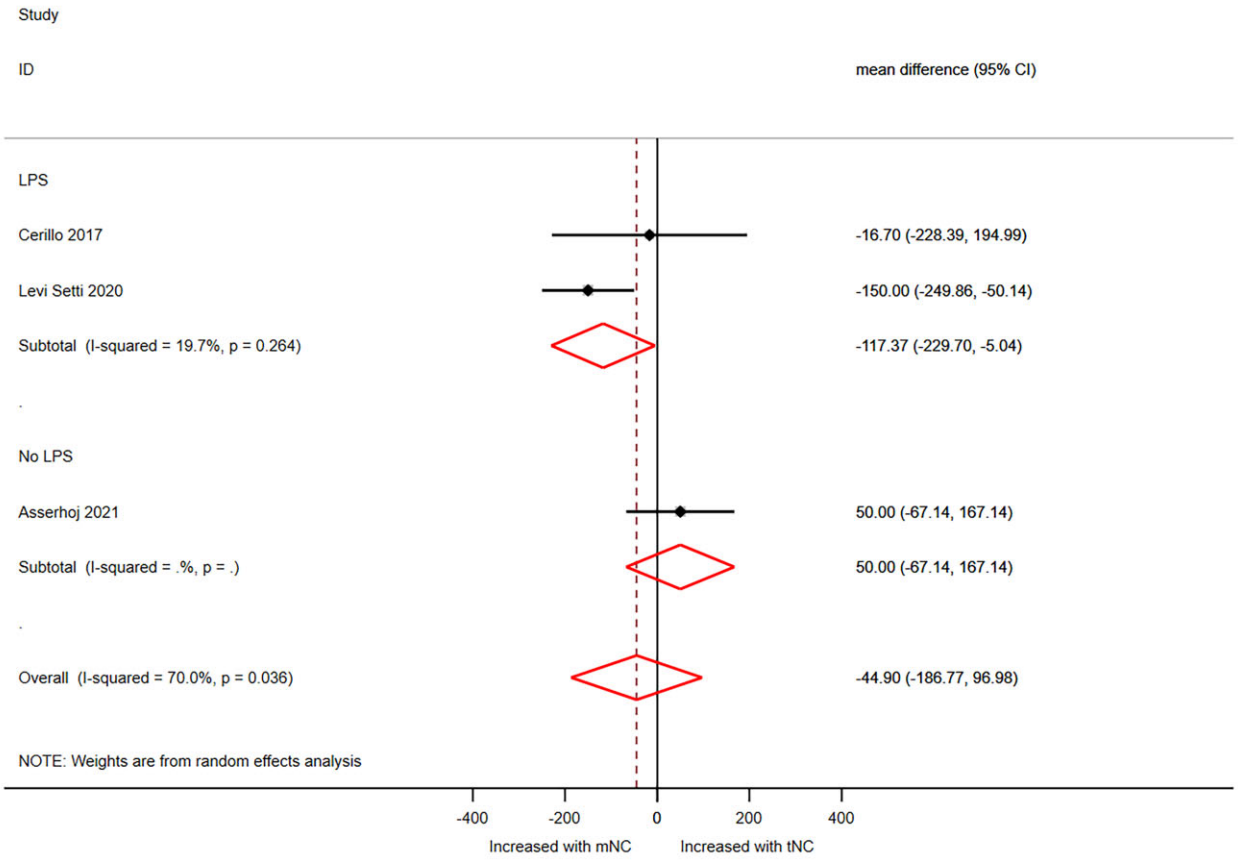


Figure 5. Subgroup analysis on birthweight for true NC-FET versus modified NC-FET. LPS: luteal phase support; NC: natural cycle; FET: frozen embryo transfer; mNC: modified natural cycle; tNC: true natural cycle.

Table 5. Comparison of results with those of three previous meta-analyses.

Outcomes	Present analysis		(Busnelli *et al.*, 2022)		(Moreno-Sepulveda *et al.*, 2021)		(Rosalik *et al.*, 2021)	
	NC-FET	AC-FET	NC-FET	AC-FET	NC-FET	AC-FET	NC-FET	AC-FET
Birthweight	↓	↑	Not reported		Not reported		↓	↑
LGA	↓	↑	↓	↑	↓	↑	↓	↑
Macrosomia	↓	↑	↓	↑	↓	↑	↓	↑
SGA	=	=	=	=	=	=	Not reported	
LBW	↓	↑	=	=	↓	↑	Not reported	
Early pregnancy loss	↓	↑	Not reported		Not reported		Not reported	
GDM	=	=	=	=	=	=	Not reported	
HDP	↓	↑	↓	↑	↓	↑	Not reported	
PE	↓	↑	↓	↑	↓	↑	Not reported	
PPH	↓	↑	↓	↑	↓	↑	Not reported	
Placenta previa	↓	↑	↓	↑	=	=	Not reported	
PTB	↓	↑	↓	↑	↓	↑	Not reported	
Very PTB	↓	↑	↓	↑	Not reported		Not reported	
Cong malformations	=	=	=	=	=	=	Not reported	
Neonatal mortality	=	=	Not reported		=	=	Not reported	
Stratification PCOS	↑ LGA, EPS, very PTB in AC-FET		Not reported		Not reported		Not reported	
Stratification LPS	↓ PTB and placenta previa		Not reported		Not reported		Not reported	

=: no difference; ↑: increased risk; ↓: reduced risk. LGA: large for gestational age; SGA: small for gestational age; LBW: low birthweight; GDM: gestational diabetes mellitus; HDP: hypertensive disorders of pregnancy; PE: pre-eclampsia; PPH: postpartum hemorrhage; PTB: preterm birth; EPS: early pregnancy loss.

et al., 2021; Busnelli *et al.*, 2022) exploring the same topic are reported in Table 5. Busnelli *et al.* (2022) included 19 studies in the meta-analysis, Moreno-Sepulveda *et al.* (2021) included 13 studies and Rosalik *et al.* (2021) included 15 studies, where we included 30 studies. The findings of a decreased risk in LGA and macrosomia in NC-FET compared to AC-FET were found in all four studies (Table 5). The decrease in birthweight after NC-FET compared to AC-FET was also found in the study of Rosalik *et al.* (2021) but not reported in the other two studies (Table 5). Our findings concerning a decrease in the risk of HDP, PE, PPH and PTB after NC-FET compared to AC-FET were also reported by two studies (Moreno-Sepulveda *et al.*, 2021; Busnelli *et al.*, 2022) (Table 5). We found a higher risk of early pregnancy loss after AC-FET compared to NC-FET, and none of the other meta-analyses reported on this

outcome (Table 5). We found no difference in SGA between groups and this was also reported by the studies of Busnelli *et al.* (2022) and Moreno-Sepulveda *et al.* (2021) (Table 5). Our finding of a decrease in LBW after NC-FET compared to AC-FET was in line with the result of Moreno-Sepulveda *et al.*; however, Busnelli *et al.*, did not find a difference in LBW between groups (Table 5). The decrease in risks of placenta previa and very PTB after NC-FET compared to AC-FET was also reported by Busnelli *et al.* (2022) (Table 5). No difference was found in congenital malformations between babies born following NC-FET and those following AC-FET in any meta-analysis (Table 5). No difference in neonatal mortality was found based on our results and the results of Moreno-Sepulveda *et al.* (2021) (Table 5).

None of the other meta-analysis reported on the use of LPS in NC-FET.

Discrepancies among different meta-analyses could be explained by the different criteria used to assess study eligibility and by the varying amount of data covering different periods of time.

Interpretation of the results

Birthweight, large for gestational age, macrosomia, small for gestational age, and low birthweight

For a while now it has been known that babies born from FET have a higher mean birthweight and are more likely to be LGA compared with babies born from fresh embryo transfer (Maheshwari *et al.*, 2018). The biological explanation of this phenomenon is still unknown. It has been hypothesized that epigenetic disturbances during the early embryonic stages, occurring as a result of the freezing and warming procedures, might affect the development of fetal and placental tissues. This may result in asynchrony between the embryo and endometrium and cause disturbance in fetal growth, resulting in an increase in birthweight after FET compared to fresh embryo transfer (Pinborg *et al.*, 2016).

Historically, FET cycles have been scheduled using AC-FET and therefore this type of endometrial preparation has already previously been suggested as a possible confounder for increased birthweight, LGA and macrosomia after FET (Ginstrom Ernstad *et al.*, 2019). Based on our meta-analysis and the meta-analysis of Rosalik *et al.* (2021), we now can conclude that birthweight and the risk of LGA and macrosomia are indeed increased in AC-FET compared to NC-FET.

The exogenous oestrogen and progesterone that is used in AC-FET may affect the endometrium and subsequent placental development. Furthermore, in early pregnancy, progesteron has been described to induce decidualization of endometrial stromal cells, regulate extravillous trophobast (EVT) invasion, and vascular remodeling (Beltrame *et al.*, 2018). However, aberrant progesterone and oestradiol levels in early pregnancy after AC-FET may lead to abnormal invasion of the EVT, impaired spiral artery remodeling and dysfunction of the trophoblast cells (Schatz *et al.*, 2016; Labarrere *et al.*, 2017; Beltrame *et al.*, 2018). This non-physiological increase in steroids during AC-FET in early pregnancy has been linked to PE, abnormal placentation, stillbirth, fetal growth restriction (FGR), and many cases of PTB (Schatz *et al.*, 2016; Labarrere *et al.*, 2017; Beltrame *et al.*, 2018). It is hypothesized that HDP is an etiologically heterogeneous disorder that occurs in at least two subsets, one involving placental dysfunction and FGR, and another with normal or enhanced placental function (Rasmussen and Irgens, 2003; Pinborg *et al.*, 2014). Notably, our results showed that SGA was not different between NC-FET and AC-FET but LBW was increased during AC-FET compared to NC-FET. We hypothesize that AC-FET may more often result in the latter subset of HDP, both clinical and subclinical, causing enhanced placental function owing to very early alterations in implantation and placental development. This hypothesis may explain the increase in birthweight, LGA and macrosomia after AC-FET. However, the majority of the included studies did use progesterone supplementation for LPS in NC-FET, which makes it difficult to tease out the role of progesterone, leaving mainly exogenous estrogen and/or the lack of CL as possible explanations. This needs to be further explored to determine the biological plausibility underlying these adverse outcomes.

Hypertensive disorders of pregnancy and abnormal placentation

As reported in our results and in the other recent meta-analysis, the risk of HDP, PE, and abnormal placentation (placenta previa) is decreased in NC-FET compared to AC-FET. The hypothesised rationale of the biological plausibility is discussed in the previous paragraph. Another possible, or perhaps combined, explanation is the absence of the CL during AC-FET. During AC-FET, estrogen substitution causes suppression of a dominant follicle and therefore no ovulation and CL will appear. Already in 1991, a cohort study showed that relaxin, a vasoactive hormone produced by the CL, was not detected in serum of anovulatory women who conceived with oocyte donation in an artificially prepared endometrium throughout pregnancy (Johnson *et al.*, 1991). In 2019, a group from Stanford-university/University-of-Florida performed a similar study up to 12 weeks pregnancy in ovulatory women who conceived with AC-FET and could not detect relaxin (von Versen-Hoynck *et al.*, 2019a,b). Blood pressure, endothelial function, and the number of circulating endothelial progenitor cells were also affected. The findings of this study support that conception following an artificially prepared endometrium (hence, the lack of CL) has a negative influence on maternal vascular health in early pregnancy compared with pregnancies conceived following NC-FET or natural conception (von Versen-Hoynck *et al.*, 2019a,b). In 2020, a prospective study of two periconception cohorts showed that, during the first trimester, pregnancies conceived in the absence of a CL are characterized by lower circulating renin and prorenin concentrations compared with those conceived naturally (Wiegel *et al.*, 2020). The absence of vasoactive factors produced by the CL in AC-FET led to deficient circulatory adaptations during early gestation and probably led to increased risks of abnormal placentation and HDP (Conrad, 2011; Conrad and Baker, 2013; Conrad *et al.*, 2019; 2019; von Versen-Hoynck *et al.*, 2019a,b; Conrad *et al.*, 2020; Singh *et al.*, 2020; Pereira *et al.*, 2021).

Abnormal placenta invasion in its turn is associated with an increased risk of PTB (Morgan, 2016) and may also be the reason for an increased risk of PPH (Busnelli *et al.*, 2022).

Luteal phase support

Based on our exploratory stratified analysis, the use of LPS in NC-FET decreases the risk of PTB and when NC-FET, with or without LPS, is compared to AC-FET (very low quality of evidence). The other outcomes were not influenced by the use of LPS. We need to take into account that the decrease in risk of PTB may be a spurious finding, given the limited number of included studies and the observational nature of these studies. Also, it should be noted that the protocols used for LPS in our meta-analysis vary widely and are barely comparable, which leads to a high level of clinical heterogeneity. Half of the studies on LPS did not report the duration of LPS in case of pregnancy. It was not clear from

the included studies if PTB had a spontaneous onset or was induced because of pregnancy complications.

To determine a biological rationale for our findings on LPS we searched the literature. For prevention of spontaneous PTB in high-risk women, daily vaginal progesterone or weekly 17-hydroxyprogesterone caproate is recommended from 16 weeks of gestation to 34 weeks of gestation by the guidelines of the National Institute for health and Care Excellence, the American College for Obstetricians and Gynecologists and the International Federation of Gynaecology and Obstetrics ((NICE) NIfHaCE, 2022; American College of Obstetricians and Gynecologists' Committee on Practice Bulletins—Obstetrics, 2021; Shennan *et al.*, 2021). The use of progesterone supplementation limited to the first trimester as a preventative measure for spontaneous PTB has never been studied to our knowledge. Two RCTs included women with recurrent miscarriage and compared the use of vaginal micronized progesterone supplementation to placebo vaginal capsules until 12 and 16–17 completed weeks of gestation, respectively. Progesterone lowered the risk of another miscarriage. The risk of PTB was not reported in this trial but no difference in live birth before 34 weeks of gestation was found (Coomarasamy *et al.*, 2016, 2020).

A recently published systematic review assessed the long-term effect of prenatal progesterone treatment on child development, behavior, and health. All included studies compared progesterone to placebo in second and/or third trimester for the prevention of PTB. The authors concluded that based on the latest evidence there is no effect of prenatal progesterone on child development. Outcomes after first trimester progesterone alone remain unclear (Simons *et al.*, 2021).

The use of LPS following NC-FET may be beneficial in terms of pregnancy rates, based on a low level of evidence (Mizrachi *et al.*, 2021). In clinical practice, NC-FET with LPS is often applied (Weissman, 2020; Mizrachi *et al.*, 2021). Currently, a multicenter RCT is comparing the efficacy of NC-FET with or without LPS in China (trial registration number: ChiCTR2200057498). In terms of safety, LPS after NC-FET has not been assessed thus far. The explanation for the decrease in risk of PTB after NC-FET with LPS remains unclear. Perhaps the use of LPS somehow reduces any alterations in adaption of the cardiovascular system and placental invasion during early pregnancy. Future research on the efficacy and safety of LPS use is of great importance as it remains a major gap in knowledge in the field of ART. Owing to these uncertainties, we cannot make any recommendation about the use of LPS. Future research should focus on the efficacy of LPS in NC-FET and follow-up studies need to investigate any safety issues concerning its use.

To trigger or not to trigger ovulation

The efficacy of triggering ovulation compared to monitoring natural ovulation has been investigated in the Antarctica-2 RCT, which is currently in its follow-up phase (Zaat *et al.*, 2021c).

When hCG is used for ovulation triggering, its long half-life renders it also as a form of LPS (Casper and Yanushpolsky, 2016). This form of LPS has not been proven to be beneficial following NC-FET in terms of clinical pregnancy and live birth rates, based on low quality of evidence (Mizrachi *et al.*, 2021). In terms of safety, we performed a sub-analysis on true NC-FET (natural ovulation) compared to modified NC-FET (triggering ovulation) and did not find any differences in birthweight between groups. For other outcomes this sub-analysis could not be performed owing to lack of data. This question remains to be studied in future research.

Future implications

Implications for clinical practice

The association between the endometrium preparation method for FET and obstetric and neonatal complications merits further attention and awareness in clinical practice in order to optimize the health of both mothers and children after FET. It has been more than 30 years since the first baby was conceived after FET. From the start, FET was performed in an artificially prepared endometrium because its first application was in fresh oocyte donation cycles. A solution for women without oocytes was to apply an 'artificial cycle' to grow the endometrium, in which the natural hormones, as produced in ovulatory women by the CL, are partly substituted with estrogen pills and progesterone vaginal capsules. AC-FET has been a very reliable, effective and predictable protocol for the fertility laboratory and therefore is still popular for FET in ovulatory women. However, the time has come to re-evaluate the use of AC-FET. Many studies, including our meta-analysis, report on increased risks for mother and child in AC-FET compared to NC-FET. The advantage of AC-FET nowadays is the easy alignment of the time point of thawing and transferring embryos with organizational necessities of the IVF laboratory, the treating doctors and the patient, which does not outweigh the disadvantages in terms of adverse obstetric and neonatal outcomes (Zaat *et al.*, 2022). These data on safety outcomes suggest that NC-FET is preferred over AC-FET in ovulatory women.

NC-FET in combination with LPS might be considered, as LPS use may be beneficial in terms of pregnancy rates, did not result in worse safety outcomes and might result in a lower PTB risk. Our recommendations do not concern anovulatory women.

Implications for research

Now that so many studies on safety are available, an individual patient data meta-analysis that includes the original databases of these studies would be welcome. Such an analysis allows to study whether there are differences in safety profile in specific subgroups while adjusting for confounders. This could also provide us with compound adverse outcomes in terms of pregnancy complications.

Concerning implications for future research, the development and use of an extended core outcome set for obstetric and neonatal outcomes in fertility care is needed (Duffy *et al.*, 2017, 2018, 2021). In our meta-analysis, we captured several outcomes, such as early pregnancy loss, HDP, and GDM, based on individual study definition, which introduces heterogeneity. A standardized set of outcomes across studies would facilitate evidence synthesis in meta-analyses and systematic reviews. Furthermore, data on the actual growth during pregnancy of babies born after FET should be collected and analyzed. Previous research demonstrates that crown-rump-length (CRL) is increased in babies after FET and leads to higher birthweight (Zaat *et al.*, 2021b). It would be of great interest to investigate the association between CRL and actual birthweight in a large study in order to look at the risk of FGR after FET. Even babies born with a normal range birthweight could suffer from FGR, which can lead to increased risk of perinatal mortality and morbidity (De Reu *et al.*, 2010; Audette and Kingdom, 2018). FGR is commonly defined as a condition in which the fetus does not reach its intrinsic growth potential (Marijnen *et al.*, 2022). Placental insufficiency, resulting from a variety of placental lesions, is the common underlying pathophysiologic mechanism (Burton and Jauniaux, 2018).

Conclusion

This systematic review and meta-analysis has shown that singletons born from NC-FET might have a lower birthweight and a lower risk of early pregnancy loss, LGA, macrosomia, SGA, LBW, HDP, PE, PPH, and PTB compared to singletons born from AC-FET, based on low to moderate quality of evidence.

In combination with comparable effectiveness of the two approaches, the interpretation is that NC-FET is the preferred treatment in women undergoing FET when the risks of obstetrical complications and potential neonatal complications are considered.

Based on the analysis of current evidence on the effectiveness and safety outcomes reported in this review, NC-FET should be the preferred treatment in women with ovulatory cycles undergoing FET. The difference between NC-FET and AC-FET may be partly related to the use of LPS in NC-FET for the outcome of PTB. This finding warrants further research on the efficacy of LPS before applying LPS in all NC-FET cycles since no head-to-head studies are currently available.

Supplementary data

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

Data availability

The data underlying this article are available in the article and in its online supplementary material.

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Authors' roles

T.R.Z.: involved in study design, data collection, data analysis, and manuscript preparation. E.K.: involved in quality assessments and manuscript preparation. P.K.: involved in data collection, quality assessment, and manuscript preparation. M.G.S.: involved data collection and manuscript preparation. F.M.: involved in study design, data collection, supervision, and manuscript preparation. M.v.W.: involved in study design, data analysis, supervision, and manuscript preparation. All authors contributed to the final version of the manuscript.

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Conflict of interest

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All reported competing interests are outside the submitted work. No other relationships or activities that could appear to have influenced the submitted work. The remaining authors have no conflicts of interest to declare.

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