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Treatment Outcomes of Blastocysts Thaw-Cycles, Comparing the Presence and Absence of a Corpus Luteum: A Systematic Review and Meta-analysis.

Authors: Joscelyn Gan[1], Genia Rozen[2,3,4], Alex Polyakov[2,3,4]

1 Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Melbourne, Australia 3010

2 Department of Obstetrics and Gynaecology, University of Melbourne, Melbourne, Australia 3010

3 Reproductive Services, Royal Women's Hospital, Melbourne, Australia 3052

4 Melbourne IVF, East Melbourne, Australia 3002

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ABSTRACT

Objective

This study aims to review the literature and perform a meta-analysis to determine if the presence of a corpus luteum has an impact on treatment outcomes in thaw cycles, where blastocyst embryos are transferred.

Design

Systematic review.

Data sources

PUBMED, EMBASE, CENTRAL and CINAHL were searched for papers published between January 2017 and July 27th, 2020. Additional articles were selected from the reference list of the results and previous reviews.

Data Extraction and Synthesis

Three reviewers independently reviewed and extracted data. Any discrepancies were discussed until a consensus was reached. The meta-analysis was conducted though RevMan 5.4.1. Studies were quality assessed with the Cochrane risk of bias tool and the Newcastle Ottawa Scale.

Results

A total of nine publications were included for data-extraction and subsequent meta-analysis. Two studies were randomised control trials, and seven were cohort studies. Both study designs were included in the meta-analysis. Sub-group analysis of the different study designs was performed.

Whilst the rates of positive b-hCG results (RR 1.0, 95% CI 0.95 - 1.05) and clinical pregnancies (RR 1.06, 95% CI 0.96 -1.18) were comparable between the two groups, the rates of live births were higher in thaw-cycles with a corpus luteum (RR 1.14, 95% CI 1.06 - 1.22). Analysis of

pregnancy losses demonstrated that both biochemical pregnancy (early miscarriage) (RR 0.71, 95% CI 0.62 - 0.82) and miscarriages (RR 0.72, 95% CI 0.62 - 0.83) were increased in cycles without a corpus luteum.

Conclusion

Where clinically appropriate, the use of cycle types that have a functional corpus luteum should be favoured. There were several limitations to this study, including a fair to moderate quality of studies and the inherent bias of retrospective cohort studies. Further, high-quality research, particularly randomised controlled trials with blastocysts embryos, is required to further explore these findings.

PROSPERO Registration Number

CRD42020209583

STRENGTHS AND LIMTATIONS

- As the use of blastocysts in thaw cycles is becoming increasingly more common, this review is timely and relevant
- The safety of embryo transfers without a corpus luteum is a growing area of research
- The limitations of this study include the limited number of studies in the area and lack of high quality randomised controlled trials
- Further high-quality studies are required to further explore these findings.

STUDY FUNDING/COMPETING INTEREST(S):

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INTRODUCTION

In recent years, embryo cryopreservation has become a fundamental tool in reproductive medicine. With improvements in the vitrification processes, culture mediums and desire for single embryo transfers (SETs), thaw cycles are becoming more common(1-4). The benefits of embryo verification include the need for fewer ovarian stimulation cycles, as well as an improved cumulative pregnancy(3). In Australia, the proportion of cryopreserved of cryopreserved embryo transfers increased from 47.1% in 2014 to 57.2% in 2018(2). In particular, the cryopreservation of blastocysts for frozen embryo transfer has been an increasingly adopted practice. The European IVF Monitoring Consortium reported that in 2016 more than half of frozen embryo transfers (62.2%) were performed at the blastocysts stage(5). It was also noted that pregnancy rates were higher in the frozen embryo transfers which used blastocyst (39.7%) compared to cleavage staged embryos (28.3%)(5).

Various protocols for endometrial preparation have been developed to assist with thaw-cycles transfers. One of the most widely used methods is the true natural cycle (tNC) or variations of it such as the modified natural cycle (mNC) or the mildly stimulated cycle (SC). These preparation techniques rely on the patient ovulating, either spontaneously, or with the assistance of ovulation induction agents or trigger. These protocols result in the formation of a corpus luteum (CL), which produces endogenous hormonal support for early pregnancy, with or without further luteal phase support with exogenous progesterone. These methods are typically used in normo-ovulatory women and uses no or minimal medications. However, these methods require extensive monitoring, which may be inconvenient for the patient and clinician. These cycles may also result in some degree of unpredictability in terms of embryo transfer timing, with some clinics preferring not to perform embryo transfers on certain days, such as weekends. The artificial cycle (AC) is an alternative method of endometrial preparation which relies on the administration of exogenous estrogen (E2) to induce endometrial proliferation and growth suppression of the dominant follicle, and the subsequent administration of progesterone (P4) to

induce the secretory phase of the endometrium. This protocol aims to mimic the body's physiological process of endometrial priming and maturation. As the AC does not involve ovulation, a CL is not formed during this process and hormone supplementation is continued until placental autonomy is established at 10 to 12 weeks gestation. The AC is typically used in situations where a woman has ovulatory dysfunction and is unable to produce a healthy CL, or in normo-ovulatory women due to its convenience for both the patient and clinician(4, 6).

Previous studies have found that treatment outcomes of tNC and ACs have been comparable(7-9). Some studies, however, have noted that thaw-cycles without a CL may have experienced higher rates of early pregnancy loss. (4, 10, 11). This review aimed to explore these findings further. Trials in reproductive medicine are often small and not adequately powered, hence a meta-analysis is a useful technique to observe trends that may not be obvious with smaller, individual studies(12).

Our objective is to compare the treatment outcomes of blastocyst embryo transfers in thaw cycles with and without a CL.

To our knowledge, this is the first review to specifically look at treatment outcomes of thaw-cycles comparing the presence and absence of a CL. Similarly, to align more closely with the contemporary clinical practices, this review focuses on data from blastocysts transfers only(2).

MATERIALS AND METHODS

Search Strategy

This review was registered with PROSPERO CRD42020209583. We conducted a search on the 27 July 2020, using four databases: PubMed/MEDLINE, EMBASE, CINAHL and Cochrane Central Register of Controlled Trials (CENTRAL). The search strategies were based on an earlier Cochrane systematic review that was published in 2017(7). The search strategy utilised 3 key concepts: endometrial preparation AND frozen embryos AND reproductive outcomes. The detailed search strategy can be found in supplementary file 1. Searches were limited to 2017 to

July 2020 as we looked through the reference lists of studies from previously conducted systematic reviews prior to 2017 for potential additional studies(7, 8). No language restrictions were used in the search. We followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) guidelines(13).

After the removal of 644 duplications, the search yielded 2184 studies. Four additional studies were hand selected from the references of the retrieved articles. The initial search was independently screened based on title and abstract by three reviewers (AP, GR, JG). Any discrepancies were discussed among the three reviewers and a consensus decision was reached.

Inclusion Criteria

To be included, studies had to contain data on blastocyst transfers which utilised thaw cycles involving the presence and absence of a CL. Cycles which involved the presence of a CL included tNC, mNC and mildly SC. Cycles without a CL included ACs with or without gonadotropin-releasing hormone analogue (GnRHa) suppression. Blastocysts were defined as day 5 or 6 embryos(14).

Exclusion Criteria

Studies that included cleavage stage embryos or blastocysts data pooled with cleavage staged embryos were excluded. We also excluded data from donor eggs, or from non-primary sources such as reviews, letters, book chapters and conference abstracts. Papers not written in English but had titles and abstracts available in English were assessed, however no relevant studies were identified.

Outcomes and Definitions

The primary outcome examined was live birth (LB) or ongoing pregnancy rate where LB was not available. Secondary outcomes that were analysed were rates of positive beta-human Chorionic Gonadotropin (b-hCG), clinical pregnancy, biochemical pregnancy, and miscarriage.

Where applicable, we used the definitions agreed upon by the International Glossary on Infertility and Fertility Care, 2017(14). A LB was defined as a birth which demonstrated evidence of life after at least 22 weeks gestation(14). An ongoing pregnancy was defined as a viable pregnancy which reached a gestational age of at least 20 weeks. Due to the low rates of pregnancy loss after 29 weeks gestation (15), ongoing pregnancy rates were included in the analysis of live birth rates. However, we performed a sub-analysis of the studies which reported live births as their primary outcome in addition to the total LB rate which would include ongoing pregnancy rates. A positive b-hCG was defined as a b-hCG of ≥5. Where positive b-hCG was not available, it was calculated through the addition of biochemical pregnancies and clinical pregnancies. The study by Alur-Gupta *et al*.,(2018) (16), did not report clinical pregnancy, hence it was calculated by adding the number of live births, ectopic pregnancies, stillbirths, and spontaneous abortions reported. A clinical pregnancy was defined as a positive b-hCG with evidence of at least one gestational sac on ultrasound, including ectopic pregnancies(14). Biochemical pregnancies were classified as a pregnancy which yielded a positive b-hCG result but did not reach the stage of clinical pregnancy(14). Where biochemical pregnancy was not reported, it was calculated by subtracting the reported clinical pregnancies from the number of positive b-hCG results. Where biochemical pregnancy was not reported, it was calculated by subtracting the reported clinical pregnancies from the number of positive b-hCG results. Similarly, miscarriage referred to any pregnancy that did not progress past 20 weeks gestation. Where therapeutic abortions were reported, those cycles were removed from the analysis. Due to the nature of the studies included, we reported data per thaw cycle, as data per woman was not possible to calculate.

Data Extraction Process

The data was independently extracted by three reviewers (GR, AP, JG) for author/s, year of publication, title of the article, year of trial, study design, number cycles, demographics of

women, positive b-hCG, clinical pregnancy, biochemical pregnancy, miscarriage, live births, or ongoing births where live births were not available. The data was collated by a single reviewer (JG) and any discrepancies were discussed among three reviewers and until a consensus was reached.

Quality Assessment

Included randomised control trials were quality assessed using the Revised Cochrane Risk of Bias Tool for randomised trials (RoB 2)(17). The Newcastle-Ottawa Scale (NOS) for assessing the quality of non-randomised studies in meta-analyses was used to assess cohort studies(18). Both tools were used to assess bias at an individual study level. The quality assessment was used to judge the strength of evidence reported, and to guide our interpretations of the reported findings.

Statistical Analysis

The meta-analysis was performed using RevMan 5.4.1 computer program, The Cochrane Collaboration, 2020(19). Meta-analyses of rates of positive b-hCG, live births, biochemical pregnancy, and miscarriage were conducted with a fixed-effect model where there was low heterogeneity among the studies, and a random-effect model where there was a significant heterogeneity. Heterogeneity was assessed with both the I^2 and X^2 statistic. P-values of X^2 that were <0.05, and I^2 > 50% were considered represent significant heterogeneity. Relative risk with 95% confidence intervals (CI), were used as the principal summary measure. The Mantel-Haenszel method was applied to estimate the pooled effect size. A funnel plot analysis was conducted for each meta-analysis to assess for reporting bias (Supplementary Figure 4).

As we included studies that reported ongoing pregnancy rates where LB rates were not available, we conducted a sub-group analyses which individually looked at LB rates and miscarriages from studies which reported LBs as their primary outcome. Separate analysis grouped by study design is demonstrated in Supplementary Figure 2 and 3.

RESULTS

After the removal of duplicates, the search yielded 2184 articles. After screening by title and abstract, we reviewed 20 full-text and included an additional 4 articles from the reference lists of included articles and previous systematic reviews. We included nine studies in our final quantitative analysis(10, 16, 20-26).Two of which were randomised controlled trials (RCTs)(22, 23) and seven were retrospective cohort studies(10, 16, 20, 21, 24-26). This process is summarized in Figure 1. The final meta-analysis included a total of 6138 cycles with a CL and 3491 cycles without a CL.

A summary of the studies included in the meta-analysis can be found in table 1. The largest study included 3030 cycles by Pakes *et al.,* 2020(10), and the smallest study included 116 cycles by Sheikhi *et al.,* (2018)(23).

The average quality of the studies was rated with a fair to moderate risk of bias.

Table 1: Overview of studies included in a meta-analysis comparing reproductive outcomes in blastocysts transfers using thaw-cycles

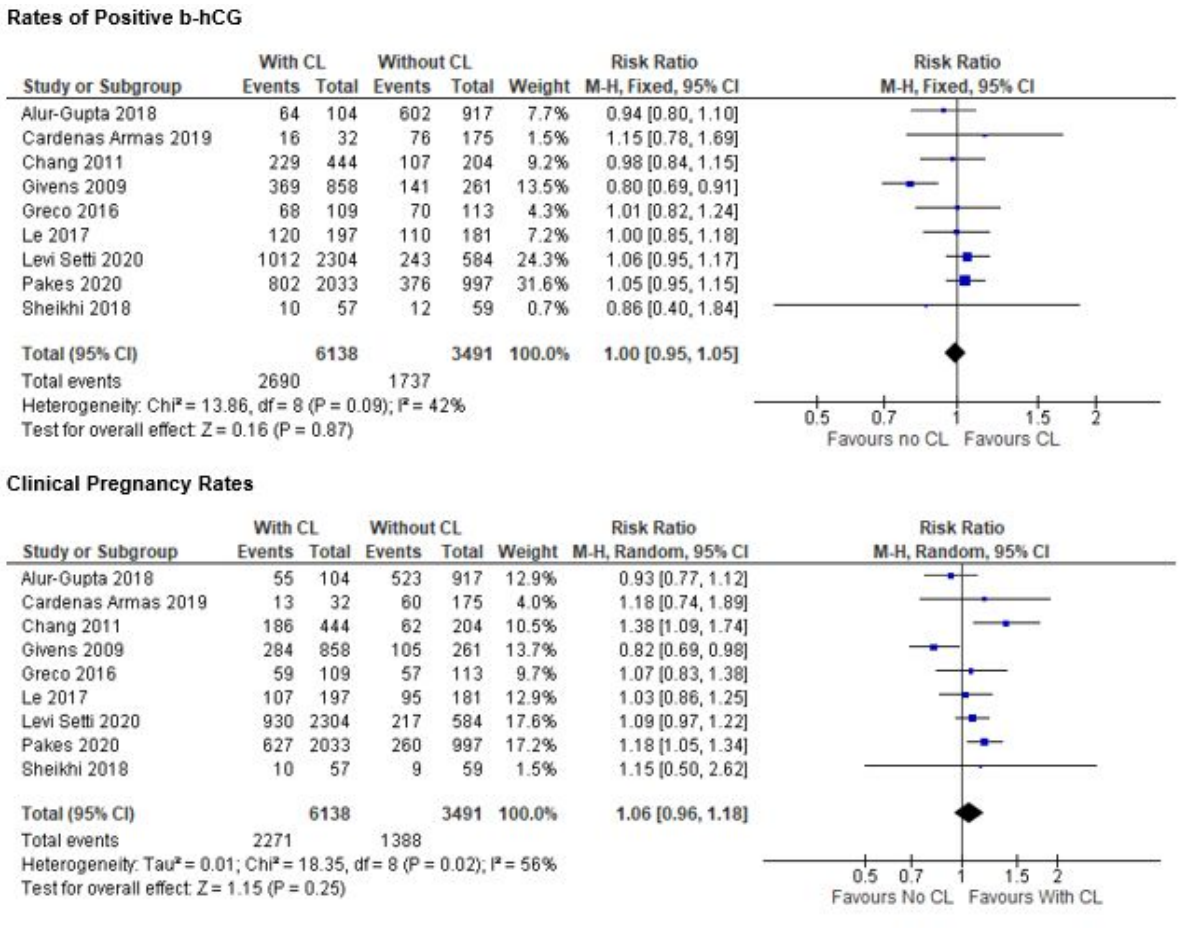
	Study Design				Demographics				Outcomes			
Study	Design	Cycles with blastocysts (n)	Study Period	Allocation	Women (n)	Study population	Mean Age, years (SD)	BMI, kg/m2 (SD)	Positive b-hCG (n)	CP (n)	LB/OP	Quality[a]
Alur-Gupta et al. (2018)(16)	Retrospective Cohort	1021 Cycles (with CL =104, without CL = 917)	2013 - 2017	Clinical judgement	NR	Both normo-ovulatory patients and women with ovulatory dysfunction	NC = 35.6 (3) AC = 35.4 (4)	NC = 23.3 (3.7) AC = 25.2 (5.3)	With CL = 64 Without CL = 602	With CL = 55 Without CL = 523	LB	Fair
Cardenas Armas et al. (2019)(24)	Retrospective Cohort	207 Cycles (with CL = 32; without CL = 175)	2014 - 2017	Preference, cycle characteristics	860	normo-ovulatory patients, no PGT	NC = 36.15 (0.29) AC(Transdermal) = 35.71 (0.17) AC (Oral) = 36.86 (0.19)	NC = 22.1 (2.1) AC(Transdermal) = 21.6 (2.2) AC (Oral) = 23.3 (1.7)	With CL = 16 Without CL = 76	With CL = 13 Without CL = 60	LB	Good
Chang et al. (2011)(21)	Retrospective Cohort	648 Cycles (with CL = 444, without CL = 204)	2007 - 2009	Convenience, Cost	611	normo-ovulatory patients with regular menstruation	NC = 34.2 (3.7) mNC = 33.7 (3.3) AC = 33.7 (3.7)	NC = 20.9 (2.8) mNC = 21.5 (3.5) AC = 20.9 (2.4)	With CL = 229 Without CL = 107	With CL = 186 Without CL = 62	OP	Good
Givens (2009) et al.(20)	Retrospective Cohort	1119 Cycles (with CL = 858, without CL = 261)	2000 - 2006	Clinical judgement	807	Both normo-ovulatory patients and women with ovulatory dysfunction	mNC = 35.1 (4.1) AC = 34.8 (5.0)	NR	With CL = 369 Without CL = 141	With CL = 284 Without CL =105	LB	Fair
Greco (2016) et al.(22)	RCT	222 Cycles (with CL = 109, without CL = 113)	2015	Computer-generated randomization (non-concealed)	236	normo-ovulatory patients, PGT	mNC = 35.2 (3.6) AC + GnRHa = 35.5 (3.8)	mNC = 22.1 (3.1) AC + GnRHa = 22.1 (3.8)	With CL = 68 Without CL = 70	With CL = 59 Without CL = 523	LB	Some concerns
Le (2017) et al.(26)	Retrospective Cohort	378 cycles (with CL 197, without CL = 181)	2006 - 2014	Clinical judgement	428[b]	Both normo-ovulatory patients and women with ovulatory dysfunction	mNC = 34.3 (4.2) AC = 33.3 (4.8)	mNC = 21.3 (5.5) AC = 27.1 (7.0)	With CL= 120 Without CL = 110	With CL = 107 Without CL = 95	LB	Fair
Levi Setti et al. (2020)(25)	Retrospective Cohort	2888 Cycles (with CL = 2304, without CL = 584)[c]	2011 - 2017	Clinical judgement	NR	Both normo-ovulatory patients and women with ovulatory dysfunction; no PGT	NC = 35.4 (4.3) mNC = 35.3 (4.0) AC = 34.4 (4.2)	NC = 21.4 (3.0) mNC = 21.8 (3.0) AC = 22.5 (3.3)	With CL = 1012 Without CL = 243	With CL = 930 Without CL = 217	LB	Fair
Pakes et al. (2020)(10)	Retrospective Cohort	3030 Cycles (with CL = 2033, without CL = 997)	2015 - 2018	Clinical judgement	NR	Both normo-ovulatory patients and women with ovulatory dysfunction; no PGT	NC = 35.56 (0.89) AC = 33.79 (0.14)	NR	With CL = 802 Without CL = 376	With CL = 627 Without CL = 260	LB	Fair
Sheikhi et al. (2018)(23)[d]	RCT	116 Cycles (with CL = 57, without CL = 59)	2015 - 2016	Computer-generated randomization (non-concealed)	123[e]	normo-ovulatory patients, without severe endometriosis	mNC = 29.71 (3.79) mSC = 30.31 (4.58) AC = 30.5 (5.59)	mNC = 26.19 (3.24) mSC = 25.80 (3.29) AC = 25.26 (5.27)	With CL = 10 Without CL = 12	With CL = 10 Without CL = 9	OP	Some concerns

First author stated only. RCT, randomised controlled trial; CL, corpus luteum; NC, natural cycle; mNC, modified natural cycle; AC, artificial cycle; mSC = mildly stimulated cycle; GnRHa, gonadotropin-releasing hormone analogue; PGT, pre-implantation genetic testing; NR, not reported. [a] quality assessed with Cochrane Risk of Bias tool 2 or Newcastle-Ottawa Scale. [b] 66 women excluded due to various reasons. [c] therapeutic abortion cycles excluded. [d] demographic data extracted from table 1 of study (conflicted data reported in written results section) [e]7 women lost to follow-up

Positive b-hCG rates

From the eight studies, a total of 6138 cycles involving a CL were assessed. Of these, 2690 cycles (44%) resulted in a positive b-hCG. In the 3491 cycles without a CL, 1737 (50%) resulted in a positive b-hCG. The individual and combined estimates for positive-hCG are shown in Figure 2. The pooled estimates for positive b-hCG (RR 1.00, 95% CI 0.95 – 1.05) demonstrated no statistically significant difference in rates of positive b-hCG between cycles with and without a CL. Subgroup analysis of positive b-hCG rates by study design are shown in Supplementary Figure 2.

Figure 2: Meta-analysis comparing rates of positive b-hCG, clinical pregnancy and live births in cycles with and without a corpus luteum



CL, Corpus Luteum; CI, Confidence interval

Clinical Pregnancy Rates

Out of the 6138 cycles which involved the presence of a CL, 2271 (37%) progressed to a clinical pregnancy. In the 3491 cycles without a CL, 1388 (40%) progressed to a clinical pregnancy. The individual and combined estimates for clinical pregnancy are shown in Figure 2. The pooled estimates for clinical pregnancy rates (RR 1.06, 95% CI 0.96 – 1.18) demonstrated no statistical difference between the two groups.

Due to the heterogeneity of the studies a random effect model was used. To overcome the statistical heterogeneity of the studies we performed a sensitivity analysis after removing the study by Givens *et al*., (2009) (20) which was the only study to observe a higher clinical pregnancy rate in AC compared to NCs. The results of this are shown in Supplementary Figure 1. The sensitivity analysis showed that live birth rates were statistically higher in the cycles involving the presence of a CL (RR 1.12, 95% CI 1.05 - 1.20).

Based on these two analyses, it can be concluded that the most likely point estimate lays somewhere between 1.06 and 1.12, favouring cycles with CL. The confidence interval of this point estimate may include 1, but there is a clear trend towards cycles with CL resulting in a higher clinical pregnancy rate. While statistical significance may not be demonstrable, this finding is likely to be clinically significant. Subgroup analysis of clinical pregnancy rates by study design is shown in Supplementary Figure 2.

Live Birth Rates

Seven studies reported LB rates as their primary outcome (one prospective randomised trial and five retrospective studies)(10, 16, 20, 22, 24-26). Two studies reported ongoing pregnancy rates as their primary outcome (one prospective randomised trial, and one cohort study)(21, 23) .

Of the 6138 cycles which involved the presence of a CL, 1902 (31%) resulted in a LB or progressed to an ongoing pregnancy. In the 3491 cycles without a CL, 1124 (32%) resulted in a live birth or ongoing pregnancy. The individual and combined estimates for live births are shown

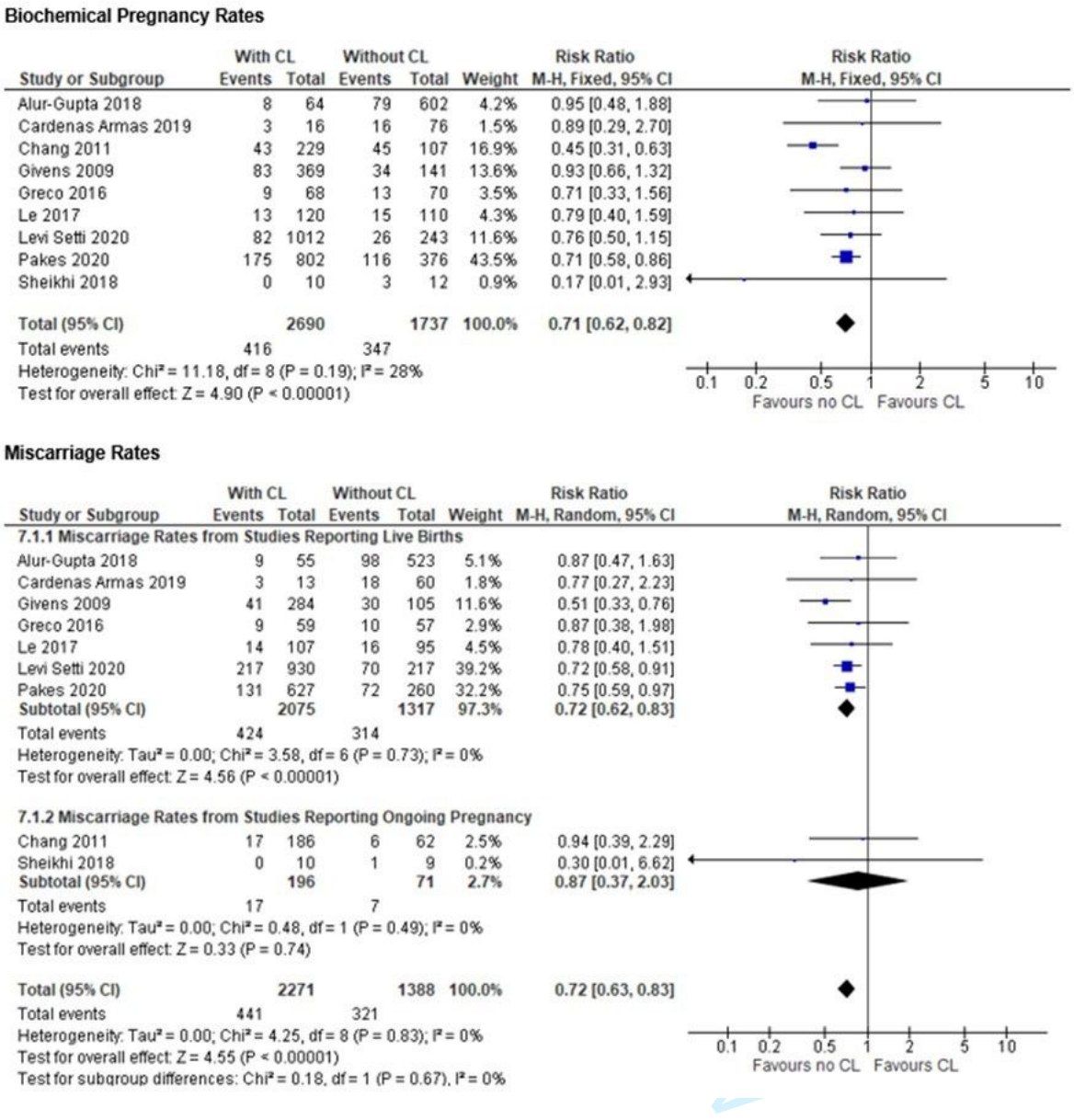
in Figure 2. The pooled estimates for live births (RR 1.14, 95% CI 1.06 - 1.22) demonstrated a statistically significant difference in favour of cycles with a CL. This translates into a clinically significant approximate 14% increase chance of live birth from cycles with a CL.

A subgroup analysis was conducted which looked at studies that only reported LB as their outcome. The results of this can be found in Figure 2. When including only the studies which included LB rates, the estimated live birth rate remained significantly higher in the thaw-cycles with a CL (RR 1.16, 95% CI 1.07 - 1.26). Subgroup analysis of LB rates by study design is shown in Supplementary Figure 2.

Biochemical Pregnancy Rates

In the 2690 positive b-hCG results in the cycles with a CL, 416 (15%) were biochemical pregnancies that did not progress to a clinical pregnancy (i.e., ended in an early miscarriage). In the 1737 positive b-hCG results in the cycles without a CL, 347 (20%) of these resulted in biochemical pregnancies, which likewise did not progress to a clinical pregnancy. The individual and combined estimates for biochemical pregnancies are shown in Figure 3. The estimated biochemical pregnancy rates (RR 0.71, 95% CI 0.62 – 0.82) were significantly lower in the cycles with a CL. Subgroup analysis of biochemical pregnancy rates by study design is shown in Supplementary Figure 3.

Figure 3: Meta-analysis comparing biochemical pregnancy and miscarriage rates in cycles with and without a corpus luteum



CL, Corpus Luteum; CI, Confidence interval

Miscarriage Rates

Of the 2271 clinical pregnancies in the cycles with a CL, 441 (19%) did not progress and resulted in a miscarriage. Of the 1388 clinical pregnancies which resulted from cycles without a CL, 321 clinical pregnancies (23%) did not progress. The individual and combined estimates for biochemical pregnancies are shown in figure 3. The estimated miscarriage rates (RR 0.72, 95% CI 0.63 – 0.83) were statistically lower in the cycles with a CL.

A subgroup analysis was conducted which only included studies which reported LB rates. However, this had no material impact on the results. Subgroup analysis of miscarriage rates by study design is shown in Supplementary Figure 3.

DISCUSSION

This meta-analysis demonstrates that while there were no statistically significant differences in rates of positive b-hCG and clinical pregnancies between thaw cycles with and without a CL, there were statistically higher rates of LBs and lower rates of both early and late pregnancy losses in thaw-cycles in the presence of a CL. This suggests that a CL may not influence initial implantation but may play a significant role in sustaining a pregnancy once an embryo has implanted.

Previous publications have demonstrated conflicting results regarding efficacy of thaw-cycles with and without a CL. The "ANTARCTICA" trial which compared treatment outcomes of mNC to AC protocols did not find any statistical difference in reproductive outcomes among the two groups(6). However, this study did not achieve adequate statistical power to examine the outcomes in question. Furthermore, a large proportion of cleavage stage embryos were included in their data, and data on blastocysts transfers was not clearly separated or analysed. Similarly, a study by Sahin *et al*., (2020), which retrospectively analysed treatment outcomes after mNC and ACs with GnRHa, concluded that LBs rates and pregnancy loss rates were comparable between the two groups(27). However, a statistically greater number of thawed embryos and percentage of blastocysts were transferred in the AC group which may have biased the results to improve the outcomes of the AC. Similarly, a study by Hill *et al*., (2010), demonstrated higher birth rates in the AC compared to the NC group, however, the AC group had more blastocysts transferred which would have likely biased the results to favour the AC(28). A recent Cochrane review was inconclusive regarding its ability to determine an optimal endometrial technique in terms of reproductive outcomes(7). Similar inconclusive results were also observed in other systematic reviews and meta-analyses (8, 29, 30). These studies also included data on cleavage staged embryos, which may not be generalizable to our research question.

Most of the studies included in our analysis were of fair to moderate quality. This is largely due to the possibility of non-comparable groups of women undertaking thaw-cycles involving the presence or absence of a CL. Women with oligo or amenorrhea due to medical conditions like polycystic ovarian syndrome (PCOS), are more likely to undergo the AC for embryo transfer, compared to women with regular menstrual cycles. Women with PCOS may have an increased risk of adverse pregnancy outcomes such as early miscarriage(31), which may be contributing to the observed results. Regarding the RCTs assessed, their quality was affected by the nature of the intervention that makes concealment and blinding challenging to implement. However, as mentioned by a previous Cochrane review, the non-blinding may not affect the measurement of outcomes, which are measured objectively(7).

Previous studies have also noted higher miscarriage rates in cycles without a CL. A large retrospective analysis by Tomás *et al.,* (2012), demonstrated a higher miscarriage rate in the AC cycle group compared to the group receiving the NC protocol(32). Similar findings were observed in the study by Givens *et al.,*(2009)(20). In both these studies, there were a significantly higher proportion of women with PCOS in the AC group, which may have contributed to this result. An older study by Veleva *et al.,* (2008), found that miscarriage rates were higher in the AC group (23.0%) compared to the NCs (11.4%, p-value < 0.0001)(33). However, the BMI of the women in the AC were statistically higher compared to the NC (25.3 ± 5.4, 22.9 ± 3.6, p-value < 0.0001) which may have influenced the miscarriage rate. Similarly, a retrospective study by Guan *et al.,* (2016) (34), which analysed 1482 thawed cleavage-staged embryos noted that women in the NC group experienced significantly lower rates of miscarriage (2.8%) compared to those in the women receiving the AC with GnRHa (14.0%, p-value = 0.003)(34). This may be influenced by the statistically older age of women receiving the AC with GnRHa compared to the women in the NC group. Another retrospective study involving normo-ovulatory women by Cerillo *et al.,* (2017), observed statistically higher miscarriage rates in the women receiving AC (21.2%), compared to the women receiving mNC (12.9%) and the tNC

(11.1%)(35). In a recent retrospective analysis by Liu *et al.,* (2020), which compared mNC and AC protocols in young women with regular menses, it was noted that the women in the AC group exhibited a higher miscarriage rate (13.69%) compared to the mNC arm (8.37%, p value 0.034)(36). Again, as these studies included cleavage-stage embryos their findings may not be generalizable to our research question, which involves data on blastocyst embryos. A recent large retrospective study by Pakes *et al*., (2020) which analysed blastocyst thaw cycles, observed that the AC group experienced a higher pregnancy loss compared to the women in the NC group(10). In this study, women in the AC group were significantly younger and received a higher proportion of good quality day-5 blastocysts compared to the NC which may have biased results to favour the AC, however, the AC group still demonstrated more pregnancy losses compared to the NC group.

There may be several contributing factors influencing this observed increased rate of pregnancy loss in thaw-cycles without a CL. Firstly, we may be disregarding the physiology of the CL. In a recent study,(37) it was observed that cycles without a CL had a significantly lower level of serum progesterone on the day of embryo transfer compared to cycles involving a CL. In the AC, estrogen and P4 only are administered exogenously to provide early pregnancy support. However, it is known that the presence of a CL may alter the concentrations of other hormones in the body such as relaxin(4, 38, 39), indicating that there may be complex interaction between the CL and pregnancy support extending beyond P4 and E2 production. Secondly, as the dosage of P4 is typically a standard dose, with different routes of administration in AC, the amount delivered may be inadequate for optimal luteal support at an individual level. Some studies suggest that serum P4 level may be helpful in guiding the level of supplementation(40-42), however, other studies suggest serum progesterone levels are not well correlated with the intra-uterine levels(43-45). This poor correlation is likely due to the first uterine pass effect(43, 45) and unpredictable levels of progesterone absorption from exogenous vaginal progesterone.

Consequently, some women may not be receiving adequate luteal support, and thus an optimized uterine environment for early pregnancy development may not be achieved.

There have been growing concerns regarding the safety of cycles without a CL. A large retrospective study conducted in Sweden from 2005 to 2015, observed that cycles without a CL were more likely to develop pregnancy-related hypertensive disorders (adjusted odds ratio 1.61, 95% CI 1.22 - 2.10), post-partum haemorrhage (adjusted odds ratio 2.87, 95% CI 2.29 - 2.60), post-term birth (adjusted odds ratio 1.59, 95% CI 1.47 – 2.68) and macrosomia (adjusted odds ratio 1.62, confidence interval 1.03-1.90)(46). Furthermore, a retrospective study conducted in Japan which compared obstetric outcomes of NC and AC embryo transfers found that cycles without a CL exhibited higher rates of pregnancy related hypertensive disorders (adjusted odds ratio 1.43, 95% confidence interval 1.14-1.8) and placenta accreta (adjusted odds ratio, 6.91; 95% CI 2.87 – 16.66) compared to cycles involving the presence of a CL(47). Similar findings have been noted in other studies(48-53). In a recent study which investigated the relation between pregnancy related hypertensive disorders and corpus luteum number, it was noted that pregnancies without a CL did not exhibit the physiologic decline in mean arterial pressure associated with pregnancy(52). This may imply that the presence of a CL may play a vital role in the priming phase of the uterine environment and maternal vasculature for early pregnancy support.

However, in certain circumstances, the use of cycles without a CL may be necessary. Women who are unable to ovulate and hence unable to produce a CL, do not have the option of utilizing the NC or ovulatory induction agents to prime their endometrium. Hence, ACs are still a very import method in frozen embryo transfers.

Strengths of this study included its meta-analysis which has been able to increase the power of individual studies to observe differences that may not have been evident on their own. In addition to this, we limited papers to those that contained data which analysed blastocyst-staged embryos. This narrowed our research question to a particular sub-group of embryo

transfers which is also clinically relevant, with an increasing number of blastocyst transfers observed in clinical practice.

This study has several limitations. Firstly, as most of these studies were of fair to moderate risk of bias due to the nature of the study designs implemented, there is a potential for confounders and selection bias to influence the results. However, most studies had accounted for this by using a multivariate logistic regression to control for confounders. In this study, the Mantel-Haenszel method was used to account for this. Furthermore, as there were less than 10 studies included in the meta-analysis, funnel plots constructed (Supplementary figure 4) had a limited utility in assessing publication bias. The aforementioned heterogeneity of the patient populations studied may also play a factor, with four of the studies only including normo-ovulatory patients, while the other four included women with ovulatory dysfunction in the cycles without a CL. Lastly, due to the ways that the included studies were reported, it was not able to calculate data per woman, which may have been another avenue for bias.

CONCLUSION

As blastocyst thaw cycles are increasingly being utilised worldwide, this review is timely and important. We conclude that cycles involving a CL may be superior to cycles without a CL as they may produce better reproductive outcomes. Furthermore, due to the higher rates of pregnancy loss and potential obstetric complications of AC, CL cycles should be the treatment of choice where clinically appropriate. However, cycles without a CL are still important as they may be necessary for women with irregular or absent periods and for cycles involving donor oocytes. Since the quality of studies included in the analysis is suboptimal, further high-quality research utilizing adequately powered randomised controlled trials involving blastocyst thaw-cycles is urgently required.

LIST OF ABBREVIATIONS

AC – Artificial Cycle

CI – Confidence Intervals

CL – Corpus Luteum

GnRHa - Gonadotropin-Releasing Hormone analogue

LB – Live Birth

mNC – Modified Natural Cycle

PCOS – Polycystic Ovarian Syndrome

SC – Stimulated Cycle

SET – Single Embryo Transfer

tNC - True Natural Cycle

RCT – Randomised Control Trial

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AUTHORS' CONTRIBUTIONS

G.R. and A.P. were involved in the conception and creation of the study design. G.R., A.P., and J.G. wrote the protocol. All authors were involved in the screening of articles for eligibility and data extraction. A.P. provided expertise on statistical analysis. A.P. and J.G. performed the meta-analysis. All authors have contributed significantly to, seen, and approved the final submitted version of the manuscript.

CONFLICTS OF INTEREST

All authors declare no conflicts of interests.

DATA AVAILABILITY

All relevant data to the study is included in the article or in the supplementary materials supplied.

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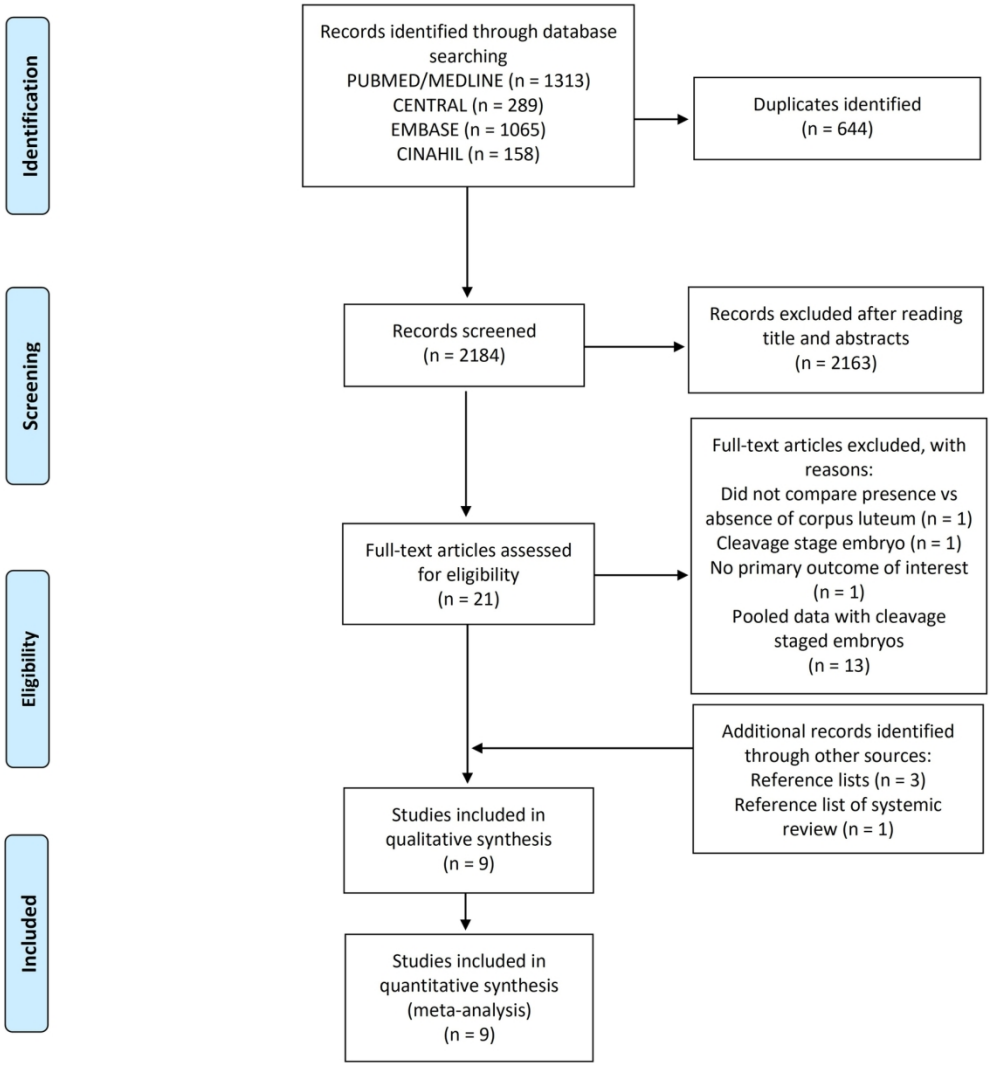


Figure 1: PRISMA Flowchart

159x170mm (300 x 300 DPI)

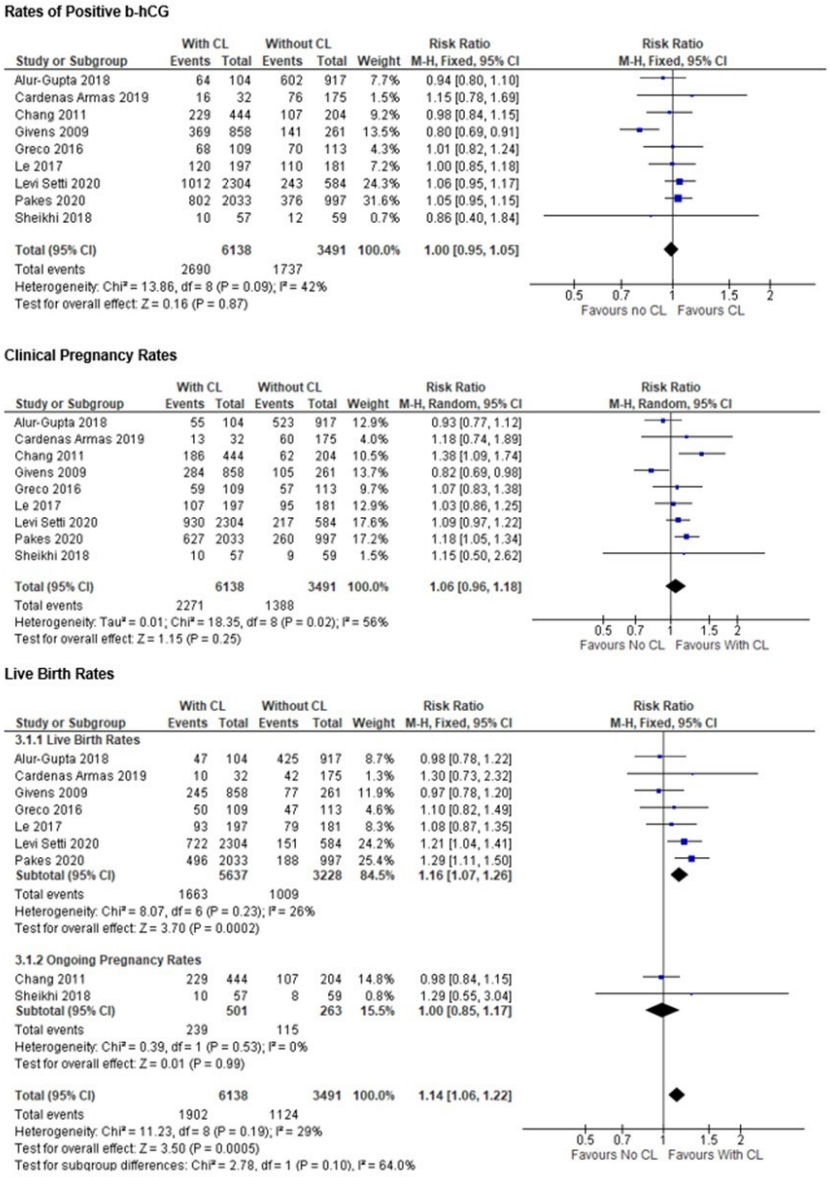


Figure 2: Meta-analysis comparing rates of positive b-hCG, clinical pregnancy, and live births in cycles with and without a corpus luteum

232x326mm (96 x 96 DPI)

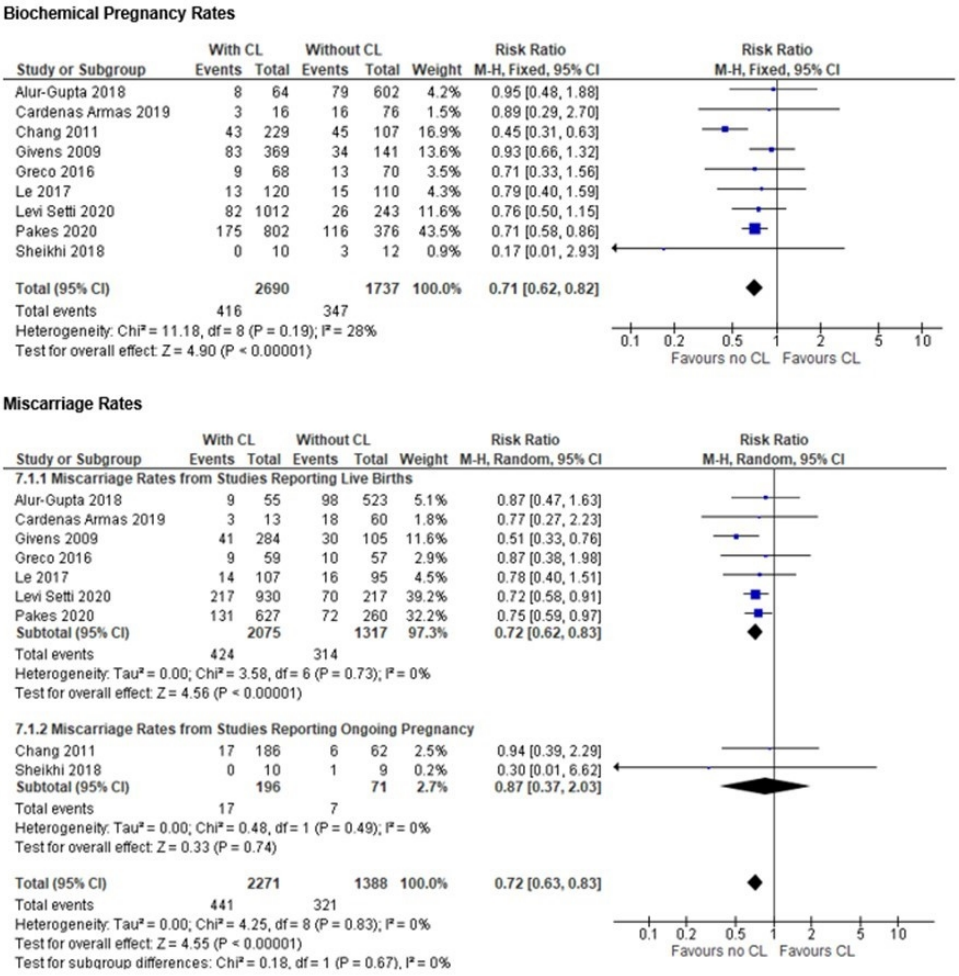


Figure 3: Meta-analysis comparing biochemical pregnancy and miscarriage rates in cycles with and without a corpus luteum

241x238mm (96 x 96 DPI)

Supplementary Files for "Treatment Outcomes of Blastocysts Thaw-Cycles, Comparing the Presence and Absence of a Corpus Luteum: A Systematic Review and Meta-analysis.

Table of Contents

Supplementary File 1 – Search Strategy

PUBMED/MEDLINE

Set	Search	Results
1	Cryopreservation[All Fields]	47,444
2	frozen embryo transfer[All Fields]	3,740
3	Frozen embryo*[All Fields]	8,561
4	frozen-thawed cycle[All Fields]	1,209
5	frozen-thawed embryo transfer[All Fields]	1,457
6	frozen thawed embryos[All Fields]	3,703
7	"FET"[All Fields]	3,577
8	cryopreserved embryos[All Fields]	9,714
9	Cryopreserved-thawed embryos[All Fields]	131
10	vitrification[All Fields]	4,568
11	Vitrified[All Fields]	3,077
12	"vitrified-warmed embryos"[All Fields]	440
13	"frozen-thawed"[All Fields]	5,134
14	embryo vitrification[All Fields]	2,144
15	blastocyst transfer[All Fields]	28,636
16	((((((((((((cryopreservation) OR (frozen embryo transfer)) OR (frozen embryo*)) OR (frozen-thawed cycle)) OR (frozen-thawed embryo transfer)) OR (frozen thawed embryos)) OR (FET)) OR (cryopreserved embryos)) OR (cryopreserved-thawed embryos)) OR (vitrification)) OR (vitrified)) OR (vitrified-warmed embryos)) OR (frozen-thawed)) OR (embryo vitrification)) OR (blastocyst transfer)[All Fields]	81,001
17	endometrial preparation[All Fields]	2,129
18	natural cycle[All Fields]	56,766
19	ovulation induction[All Fields]	16,378
20	modified natural cycle[All Fields]	2,401
21	hormone therapy[All Fields]	659,266
22	Estrogen OR oestrogen OR oestrogens OR estrogens OR oestradiol[All Fields]	286,275
23	progesterone[All Fields]	119,710
24	stimulated cycle[All Fields]	63,307
25	stimulation of endometrium embryo transfer[All Fields]	426
26	artificial cycle	13,886
27	(((((((((endometrial preparation) OR (natural cycle)) OR (ovulation induction)) OR (modified natural cycle)) OR (hormone therapy)) OR (Estrogen or oestrogen or oestrogens or estrogens or estradiol)) OR (progesterone)) OR (stimulated cycle)) OR (stimulation of endometrium embryo transfer)) OR (artificial cycle)[All Fields]	1,012,876
28	((((((((((endometrial preparation) OR (natural cycle)) OR (ovulation induction)) OR (modified natural cycle)) OR (hormone therapy)) OR (Estrogen or oestrogen or oestrogens or estrogens or estradiol)) OR (progesterone)) OR (stimulated cycle)) OR (stimulation of endometrium embryo transfer)) OR (artificial cycle)) AND ((((((((((((((cryopreservation) OR (frozen embryo transfer)) OR	11,974

	(frozen embryo*)) OR (frozen-thawed cycle)) OR (frozen-thawed embryo transfer)) OR (frozen thawed embryos)) OR (FET)) OR (cryopreserved embryos)) OR (cryopreserved-thawed embryos)) OR (vitrification)) OR (vitrified)) OR (vitrified-warmed embryos)) OR (frozen-thawed)) OR (embryo vitrification)) OR (blastocyst transfer))[All Fields]	
29	Pregnancy[All Fields]	987,880
30	live birth*[All Fields]	32,374
31	miscarriage[All Fields]	47,358
32	ongoing pregnancy[All Fields]	8,897
33	clinical pregnancy[All Fields]	190,084
34	chemical pregnancy[All Fields]	45,767
35	(((((pregnancy) OR (live birth*)) OR (miscarriage)) OR (ongoing pregnancy)) OR (clinical pregnancy)) OR (chemical pregnancy) [All Fields]	1,001,238
36	((((((((((endometrial preparation) OR (natural cycle)) OR (ovulation induction)) OR (modified natural cycle)) OR (hormone therapy)) OR (Estrogen or oestrogen or oestrogens or estrogens or estradiol)) OR (progesterone)) OR (stimulated cycle)) OR (stimulation of endometrium embryo transfer)) OR (artificial cycle)) AND ((((((((((((((cryopreservation) OR (frozen embryo transfer)) OR (frozen embryo*)) OR (frozen-thawed cycle)) OR (frozen-thawed embryo transfer)) OR (frozen thawed embryos)) OR (FET)) OR (cryopreserved embryos)) OR (cryopreserved-thawed embryos)) OR (vitrification)) OR (vitrified)) OR (vitrified-warmed embryos)) OR (frozen-thawed)) OR (embryo vitrification)) OR (blastocyst transfer))) AND ((((((pregnancy) OR (live birth*)) OR (miscarriage)) OR (ongoing pregnancy)) OR (clinical pregnancy)) OR (chemical pregnancy)) [All Fields]	7,913
37	animal[All Fields]	6,843,446
48	((((((((((((endometrial preparation) OR (natural cycle)) OR (ovulation induction)) OR (modified natural cycle)) OR (hormone therapy)) OR (Estrogen or oestrogen or oestrogens or estrogens or estradiol)) OR (progesterone)) OR (stimulated cycle)) OR (stimulation of endometrium embryo transfer)) OR (artificial cycle)) AND ((((((((((((((cryopreservation) OR (frozen embryo transfer)) OR (frozen embryo*)) OR (frozen-thawed cycle)) OR (frozen-thawed embryo transfer)) OR (frozen thawed embryos)) OR (FET)) OR (cryopreserved embryos)) OR (cryopreserved-thawed embryos)) OR (vitrification)) OR (vitrified)) OR (vitrified-warmed embryos)) OR (frozen-thawed)) OR (embryo vitrification)) OR (blastocyst transfer))) AND ((((((pregnancy) OR (live birth*)) OR (miscarriage)) OR (ongoing pregnancy)) OR (clinical pregnancy)) OR (chemical pregnancy))) NOT (animal) [All Fields]	6,386
39	((((((((((((endometrial preparation) OR (natural cycle)) OR (ovulation induction)) OR (modified natural cycle)) OR (hormone therapy)) OR (Estrogen or oestrogen or oestrogens or estrogens or estradiol)) OR (progesterone)) OR (stimulated cycle)) OR (stimulation of endometrium embryo transfer)) OR (artificial cycle)) AND ((((((((((((((cryopreservation) OR (frozen embryo transfer)) OR (frozen embryo*)) OR (frozen-thawed cycle)) OR (frozen-thawed	6,375

	embryo transfer)) OR (frozen thawed embryos)) OR (FET)) OR (cryopreserved embryos)) OR (cryopreserved-thawed embryos)))) OR (vitrified-warmed embryos)) OR (frozen-thawed)) OR (embryo vitrification)) OR (blastocyst transfer))) AND ((((((pregnancy) OR (live birth*)) OR (miscarriage)) OR (ongoing pregnancy)) OR (clinical pregnancy)) OR (chemical pregnancy))) NOT (animal) [All Fields]	
40	((((((((((((endometrial preparation) OR (natural cycle)) OR (ovulation induction)) OR (modified natural cycle)) OR (hormone therapy)) OR (Estrogen or oestrogen or oestrogens or estrogens or estradiol)) OR (progesterone)) OR (stimulated cycle)) OR (stimulation of endometrium embryo transfer)) OR (artificial cycle)) AND ((((((((((((((cryopreservation) OR (frozen embryo transfer)) OR (frozen embryo*)) OR (frozen-thawed cycle)) OR (frozen-thawed embryo transfer)) OR (frozen thawed embryos)) OR (FET)) OR (cryopreserved embryos)) OR (cryopreserved-thawed embryos)) OR (vitrification)) OR (vitrified)) OR (vitrified-warmed embryos)) OR (frozen-thawed)) OR (embryo vitrification)) OR (blastocyst transfer))) AND ((((((pregnancy) OR (live birth*)) OR (miscarriage)) OR (ongoing pregnancy)) OR (clinical pregnancy)) OR (chemical pregnancy))) NOT (animal) [All Fields] - from 2017 - 2020	1,089

EMBASE

Set	Search	Results
1	cryopreservation.mp. or cryopreservation/	45195
2	(Cryopreserv$ adj7 embryo$).tw.	5646
3	(Cryopreserv$ adj7 blastocyst$).tw.	1080
4	freezing/ or vitrification/	43414
5	(vitrifi$ adj5 embryo$).tw.	2410
6	(vitrifi$ adj5 blastocyst$).tw.	1803
7	(frozen adj5 embryo$).tw.	5929
8	(freez$ adj5 embryo$).tw.	2056
9	(freez$ adj5 blastocyst$).tw.	367
10	(frozen adj5 blastocyst$).tw.	1032
11	FET.tw.	4837
12	freeze thawing/ or freezing/	45930
13	vitrification/	5997
14	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13	95117
15	exp ovulation induction/	16413
16	((ovar$ adj5 stimula$) or (ovulat$ adj5 induc$)).tw.	26000
17	(endometri$ adj2 prepar$).tw.	1032
18	hormon$ regimen$.tw.	373
19	Clomiphene.tw. or Clomiphene/	11562
20	clomid.tw.	1284
21	(Tamoxifen or Letrozole).tw.	37754
22	aromatase inhibitor$.tw.	11798
23	exp human menopausal gonadotropin/	10498
24	(Menotropin$ or menopausal gonadotrop$ or HMG).tw.	20554
25	exp follitropin/	64748

26	(Follicle Stimulating Hormone or FSH or rFSH or rhFSH).tw.	57786
27	gonadorelin/	38181
28	Gonadotropin Releasing Hormone$.tw.	16215
29	Gonadotrophin Releasing Hormone$.tw.	3366
30	GnRH$.tw.	29904
31	exp estrogen/	300360
32	(?estrogen$ or ?estradiol).tw.	240982
33	exp progesterone/	104475
34	exp Progesterone/ or progesterone.tw.	145928
35	(natural$ adj2 cycle$).tw.	3444
36	(artificial$ adj2 cycle$).tw.	633
37	(cycle$ adj2 regimen$).tw.	670
38	pituitary suppression.tw.	486
39	human menopausal.tw.	2684
40	spontaneous ovulation.tw.	615
41	(HCG adj3 trigger$).tw.	1039
42	(stimulat$ adj3 cycle$).tw.	5831
43	exogenous steroid$.tw.	708
44	exogenous steroid$.tw.	708
45	(hormone adj2 therap$).tw.	41571
46	(endometri$ adj2 stimulat$).tw.	835
47	15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46	605551
48	14 and 47	7970
49	Clinical Trial/	999716
50	Randomized Controlled Trial/	615254
51	exp randomization/	87897
52	Single Blind Procedure/	39662
53	Double Blind Procedure/	177011
54	Crossover Procedure/	64180
55	Placebo/	363424
56	Randomi?ed controlled trial$.tw.	233156
57	Rct.tw.	37946
58	random allocation.tw.	2120
59	randomly allocated.tw.	35898
60	allocated randomly.tw.	2597
61	(allocated adj2 random).tw.	981
62	Single blind$.tw.	25372
63	Double blind$.tw.	216438
64	((treble or triple) adj blind$).tw.	1215
65	placebo$.tw.	315943
66	prospective study/	617823
67	retrospective study/	946322
68	49 or 50 or 51 or 52 or 53 or 54 or 55 or 56 or 57 or 58 or 59 or 60 or 61 or 62 or 63 or 64 or 65 or 66 or 67	3142742
69	case study/	80054
70	case report.tw.	444799
71	abstract report/ or letter/	1155908
72	69 or 70 or 71	1669914

73	68 not 72	3064021
74	(exp animal/ or animal.hw. or nonhuman/) not (exp human/ or human cell/ or (human or humans).ti.)	7230873
75	73 not 74	2969724
76	48 and 75	2373
77	76 – limited 2017 to 2020	1065

Cochrane Register of Controlled Trials (CENTRAL)

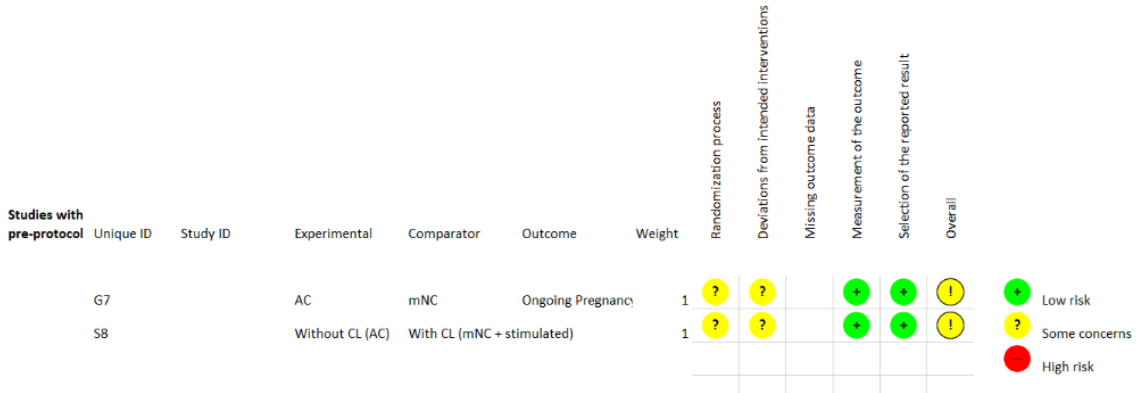
Set	Search	Results
1	((Endometrial Preparation OR Cycle OR Natural Cycle OR Artificial Cycle OR Modified Natural Cycle OR Stimulated Cycle) AND (Pregnancy OR Pregnancy Outcomes OR Clinical Pregnancy OR Live Birth)) – Limited to 2017-2020	289

CINAHL

Set	Search	Results
1	MM Cryopreservation+	1,545
2	TX Cryopreserv* N7 embryo*	792
3	TX Cryopreserv* N7 blastocyst*	80
4	MM Freezing	229
5	TX vitrification N7 embryo*	124
6	TX vitrification N7 blastocyst*	58
7	TX frozen N5 embryo*	1,186
8	TX freez* N5 embryo*	360
9	TX freez* N5 blastocyst*	22
10	TX frozen N5 blastocyst*	128
11	TX FET	1,353
12	(TX FET) AND (S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 OR S9 OR S10 OR S11)	1,353
13	MM ovulation induction	973
14	TX (ovar* N5 stimula*) or (ovulat* N5 induct*)	3,738
15	TX (endometri* N2prepar*)	181
16	MM Clomiphene	250
17	TX Clomiphene or TX clomid	1,128
18	TX Menotropin* or menopausal gonadotrop* or HMG)	3,785
19	MM Follicle-Stimulating Hormone	602
20	TX Follicle Stimulating Hormone or FSH	6,532
21	MM Gonadorelin	989
22	MM Pituitary Hormone Release Inhibiting Hormones	3
23	TX Gonadotrop?in-Releasing Hormone*	344
24	TX GnRH*	2,961
25	MM Estrogens	3,969
26	TX oestrogen or estrogen	46,066
27	MM Progesterone	1,914
28	TX Progesterone	17,782
29	TX natural* N2 cycle*	1,104
30	TX (artificial* N2 cycle*)	137

31	TX (cycle* N2 regimen*)	626
32	TX pituitary suppression	472
33	TX spontaneous* ovulat*	145
34	TX stimulat* N3 cycle	1,335
35	((TX stimulat* N3 cycle OR S13 OR S14 OR S15 OR S16 OR S17 OR S18 OR S19 OR S20 OR S21 OR S22 OR S23 OR S24 OR S25 OR S26 OR S27 OR S28 OR S29 OR S30 OR S31 OR S32 OR S33 OR S34)) AND (S13 OR S14 OR S15 OR S16 OR S17 OR S18 OR S19 OR S20 OR S21 OR S22 OR S23 OR S24 OR S25 OR S26 OR S27 OR S28 OR S29 OR S30 OR S31 OR S32 OR S33 OR S34)	65,832
36	S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7OR S8 OR S9 OR S10 OR S11	4,623
37	(35 AND 36)	2,453
38	MH Clinical Trials+	303,701
39	PT Clinical trial	107,329
40	TX clinic* n1 trial*	393,652
41	TX(singl* n1 blind*) or (singl* n1 mask*)) or TX ((doubl* n1 blind*) or (doubl* n1 mask*)) or TX((tripl* n1 blind*) or (tripl* n1 mask*)) or TX ((trebl* n1 blind*) or (trebl*n1 mask*))	1,177,377
42	TX randomi* control *trial*	298,795
43	MH "Random Assignment"	63,059
44	TX random* allocat*	22,292
45	TX placebo*	125,194
46	MH Placebos	12,837
47	MH Quantitative Studies	27,500
48	TX allocat* random*	22,292
49	S38 OR S39 OR S40 OR S41 OR S42 OR S43 OR S44 OR S45 OR S46 OR S47 OR S48	1,648,483
50	S37 AND S49	817
51	S37 AND S49	225
52	51 – Limited 2017-2020	158

Supplementary File 2 - Supplementary Table 1: Quality of Randomised Controlled Trials using the Revised Cochrane Risk-of-Bias tool 2



Greco 2016:

Domain	Signalling question	Response	Comments
Bias arising from the randomization process	1.1 Was the allocation sequence random?	Y	Two hundred thirty-six patients were included in the study and randomized in two groups according to computer-generated, not cancelled, simple randomization list with allocation assignment. Both the patient and the clinicians were informed of the assigned treatment. Difficult to conceal due to the nature of the intervention.
	1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?	N	
	1.3 Did baseline differences between intervention groups suggest a problem with the randomization process?	N	Baseline characteristics of the patients were not significantly different.
	Risk of bias judgement	**Some concerns**	
Bias due to deviations from intended interventions	2.1 Were participants aware of their assigned intervention during the trial?	Y	Both pateints and clinicians were aware of the assigned intervention. However, due to the nature of the intervention, it would have been difficult to conceal.
	2.2 Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?	Y	
	2.3. [If applicable:] If Y/PY/NI to 2.1 or 2.2: Were important non-protocol interventions balanced across intervention groups?	NA	
	2.4. [If applicable:] Were there failures in implementing the intervention that could have affected the outcome?		
	2.5. [If applicable:] Was there non-adherence to the assigned intervention regimen that could have affected participants' outcomes?	NA	
	2.6. If N/PN/NI to 2.3, or Y/PY/NI to 2.4 or 2.5: Was an appropriate analysis used to estimate the effect of adhering to the intervention?	NA	
	Risk of bias judgement	**Some concerns**	
Bias due to missing outcome data	3.1 Were data for this outcome available for all, or nearly all, participants randomized?	Y	Missing data was accounted for e.g. premature LH surge, inadequate endometrial thickness
	3.2 If N/PN/NI to 3.1: Is there evidence that result was not biased by missing outcome data?	NA	
	3.3 If N/PN to 3.2: Could missingness in the outcome depend on its true value?	NA	
	3.4 If Y/PY/NI to 3.3: Is it likely that missingness in the outcome depended on its true value?	NA	
	Risk of bias judgement		
Bias in measurement of the outcome	4.1 Was the method of measuring the outcome inappropriate?	N	Live birth rates is an appropriate outcome measurement
	4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?	N	Definitions used for the measurement of outcomes was the same in both groups
	4.3 Were outcome assessors aware of the intervention received by study participants?	Y	Probably not, as the outcomes are objective rather than subjective
	4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?	PN	
	4.5 If Y/PY/NI to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?	NA	
	Risk of bias judgement	**Low**	
Bias in selection of the reported result	5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?	Y	
	5.2 ... multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain?	PN	
	5.3 ... multiple eligible analyses of the data?	PN	
	Risk of bias judgement	**Low**	
Overall bias	**Risk of bias judgement**	**Some concerns**	

Sheikhi 2018:

Domain	Signalling question	Response	Comments
Bias arising from the randomization process	1.1 Was the allocation sequence random?	Y	The randomization was done at the start of the cycle using sequential numbering based on a computer-generated list that had been prepared at the Statistics Center of the Babol University of Medical Science and sent to them. Both participants and clinicians were aware of the treatment allocation.
	1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?	N	
	1.3 Did baseline differences between intervention groups suggest a problem with the randomization process?	N	Baseline characteristics were fairly similar across both treatment groups.
	Risk of bias judgement	**Some concerns**	Difficult to implement blinding and concealment due to the nature of the intervention.
Bias due to deviations from intended interventions	2.1 Were participants aware of their assigned intervention during the trial?	Y	Yes, as it is difficult to blind participants and clinicians due to the nature of the intervention
	2.2 Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?	Y	
	2.3. [If applicable:] If Y/PY/NI to 2.1 or 2.2: Were important non-protocol interventions balanced across intervention groups?	NA	
	2.4. [If applicable:] Were there failures in implementing the intervention that could have affected the outcome?	PY	Seven women were lost to follow-up (with explainations)
	2.5. [If applicable:] Was there non-adherence to the assigned intervention regimen that could have affected participants' outcomes?	NA	
	2.6. If N/PN/NI to 2.3, or Y/PY/NI to 2.4 or 2.5: Was an appropriate analysis used to estimate the effect of adhering to the intervention?		
	Risk of bias judgement	**Some concerns**	
Bias due to missing outcome data	3.1 Were data for this outcome available for all, or nearly all, participants randomized?	PY	
	3.2 If N/PN/NI to 3.1: Is there evidence that result was not biased by missing outcome data?	NA	
	3.3 If N/PN to 3.2: Could missingness in the outcome depend on its true value?	NA	
	3.4 If Y/PY/NI to 3.3: Is it likely that missingness in the outcome depended on its true value?	NA	
	Risk of bias judgement		
Bias in measurement of the outcome	4.1 Was the method of measuring the outcome inappropriate?	PN	Live births would have been a better measure of outcome, however as pregnancy loss after 20 weeks is very rare, it is still an appropriate outcome.
	4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?	N	Outcome measurements are objective rather than subjective due to the nature of the study.
	4.3 Were outcome assessors aware of the intervention received by study participants?	Y	
	4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?	PN	Outcome measurements are objective rather than subjective due to the nature of the study.
	4.5 If Y/PY/NI to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?	NA	
	Risk of bias judgement	**Low**	
Bias in selection of the reported result	5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?	PY	
	5.2 ... multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain?	PN	
	5.3 ... multiple eligible analyses of the data?	PN	
	Risk of bias judgement	**Low**	
Overall bias	**Risk of bias judgement**	**Some concerns**	

Supplementary File 3 - Supplementary Table 2: Quality of Observational Studies using the Newcastle-Ottawa Scale

Authors		Alur-Gupta et al, 2018	Cardenas Armas et al, 2019	Chang et al, 2011	Givens et al, 2009	Le et al, 2017	Levi Setti et al, 2020	Pakes et al, 2020
	Item							
A	**Selection**							
	Exposed cohort is truly representative of the average	−	−	+	−	−	−	−
	Selection of the non-exposed cohort from the same community	−	−	+	−	−	−	−
	Exposure ascertained by a secure record or interview	+	+	+	+	+	+	+
	Demonstration of outcome of interest was not present at the start of the study	+	+	+	+	+	+	+
B	**Comparability***							
	Study controls for additional variables	+	+	+	−	+	+	+
C	**Outcome**							
	Follow-up was adequate for outcome to occur	+	+	−	+	+	+	+
	Complete follow-up of all subjects was accounted for	+	+	+	+	+	+	+
	Subjects lost to follow up were unlikely to introduce bias	+	+	+	+	+	+	+
	Score (_/9)	6	7	7	5	6	6	6
	Conversion to AHRQ Standards	fair	good	good	fair	fair	fair	fair

AHRQ, Agency for Healthcare Research and Quality
*Comparability may have up to a maximum of 2 points

Supplementary File 4 - Supplementary Table 3: PRISMA Checklists

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3-4
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	5-6
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	6
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	7
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	7-8
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Supplementary material 1
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	7-8
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	9
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	8-9

Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	9
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	10
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	10
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Supplementary material 9
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	10, 13
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	10
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 1, page 26
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Table 1 & supplementary files
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Refer to figures II & III
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	13-15
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	12, table I & supplementary files
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	12, supplementary files
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	15

Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	16-20
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	16-20
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	22

Supplementary File 5 - Supplementary Table 4: Excluded Studies

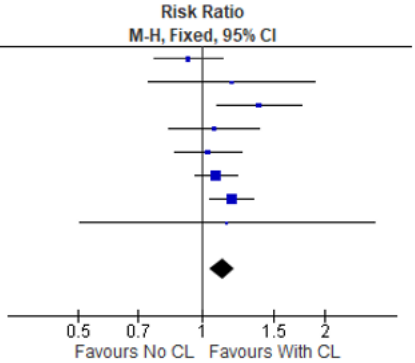
Supplementary Table IV: Excluded studies in a systematic review and meta-analysis of treatment outcomes of blastocysts thaw-cycles, comparing the presence and absence of a corpus luteum

Authors	Study title	Year of Publication	Reason for exclusion
Agha-Hosseini *et al.*	*Natural cycle versus artificial cycle in frozen-Thawed embryo transfer: A randomized prospective trial.*	2018	Data contains both cleavage and blastocyst stage embryos
Al Krayem *et al.*	*Cryo-thawed embryo transfer. Artificial versus natural cycle.*	2018	Data contains both cleavage and blastocyst stage embryos
Cerrillo *et al.*	*Impact of Endometrial Preparation Protocols for Frozen Embryo Transfer on Live Birth Rates.*	2017	Data contains both cleavage and blastocyst stage embryos
Groenewoud *et al.*	*Natural cycle versus artificial cycle in frozen-thawed embryo transfer: A randomized prospective trial*	2018	Data contains both cleavage and blastocyst stage embryos
Groenewoud *et al.*	*A randomized controlled, non-inferiority trial of modified natural versus artificial cycle for cryo-thawed embryo transfer*	2016	Data contains both cleavage and blastocyst stage embryos
Kalem *et al.*	*Natural cycle versus hormone replacement therapy cycle in frozen-thawed embryo transfer*	2018	Data contains both cleavage and blastocyst stage embryos
Kang *et al.*	*Comparison of the clinical outcome of frozen-thawed embryo transfer with and without pretreatment with a gonadotropin-releasing hormone agonist*	2018	Data contains both cleavage and blastocyst stage embryos
Labrosse *et al.*	*Comparison of stimulated versus modified natural cycles for endometrial preparation prior to frozen embryo transfer: a randomized controlled trial.*	2020	Did not compare the presence and absence of a corpus luteum
Liu *et al.*	*Effects of endometrial preparations and transferred embryo types on pregnancy outcome from patients with advanced maternal age.*	2019	Data contains both cleavage and blastocyst stage embryos
Liu *et al.*	*Natural cycle frozen-thawed embryo transfer in young women with regular menstrual cycles increases the live-birth rates compared with hormone replacement treatment: a retrospective cohort study.*	2020	Data contains both cleavage and blastocyst stage embryos
Madani *et al.*	*Live birth rates after different endometrial preparation methods in frozen cleavage-stage embryo transfer cycles: a randomized controlled trial.*	2019	Cleavage stage embryos
Masrour *et al.*	*The study of natural versus hormone replacement therapy cycles in frozen embryo transfer in infertile couples on pregnancy outcome: A double blind randomized control trial.*	2018	Did not contain primary outcomes of interest
Mubarak *et al.*	*A Comparison of the Miscarriage and Live Birth Rate for Frozen Embryo Transfer According to Two Endometrial Preparations: Natural or Primed with Estrogens.*	2019	Data contains both cleavage and blastocyst stage embryos
Peigne *et al.*	*Higher live birth rate with stimulated rather than artificial cycle for frozen-thawed embryo transfer.*	2019	Data contains both cleavage and blastocyst stage embryos
Sahin *et al.*	*Live birth after frozen-thawed embryo transfer: which endometrial preparation protocol is better?*	2020	Data contains both cleavage and blastocyst stage embryos
Samsami *et al.*	*Frozen embryo transfer: Endometrial preparation by letrozole versus hormone replacement cycle: A randomized clinical trial.*	2019	Data contains both cleavage and blastocyst stage embryos

Supplementary File 6 - Supplementary Figure 1: Meta-analysis comparing clinical pregnancy rates in cycles with and without a corpus luteum – sensitivity analysis

Study or Subgroup	With CL Events	With CL Total	Without CL Events	Without CL Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Alur-Gupta 2018	55	104	523	917	10.0%	0.93 [0.77, 1.12]
Cardenas Armas 2019	13	32	60	175	1.7%	1.18 [0.74, 1.89]
Chang 2011	186	444	62	204	7.9%	1.38 [1.09, 1.74]
Greco 2016	59	109	57	113	5.2%	1.07 [0.83, 1.38]
Le 2017	107	197	95	181	9.3%	1.03 [0.86, 1.25]
Levi Setti 2020	930	2304	217	584	32.4%	1.09 [0.97, 1.22]
Pakes 2020	627	2033	260	997	32.6%	1.18 [1.05, 1.34]
Sheikhi 2018	10	57	9	59	0.8%	1.15 [0.50, 2.62]
Total (95% CI)		**5280**		**3230**	**100.0%**	**1.12 [1.05, 1.20]**
Total events	1987		1283			

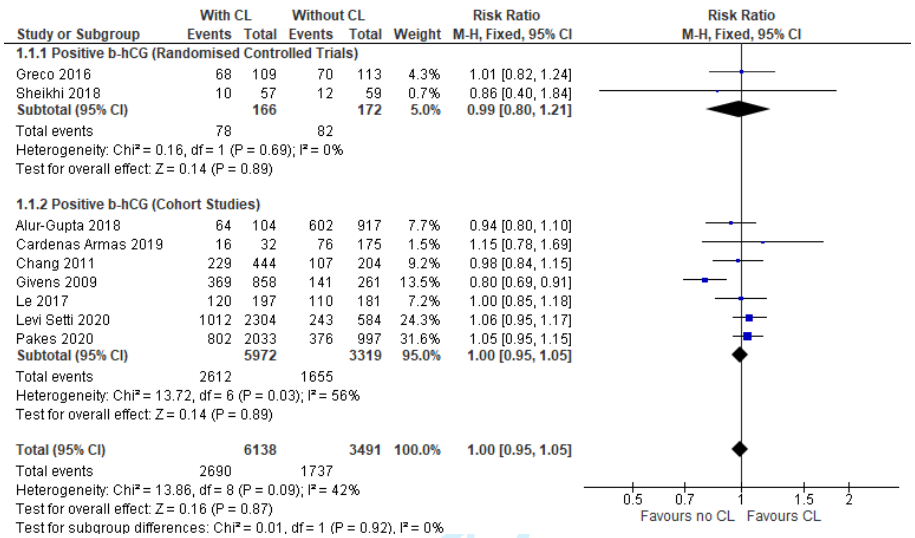
Heterogeneity: Chi² = 8.70, df = 7 (P = 0.27); I² = 20%
Test for overall effect: Z = 3.41 (P = 0.0007)



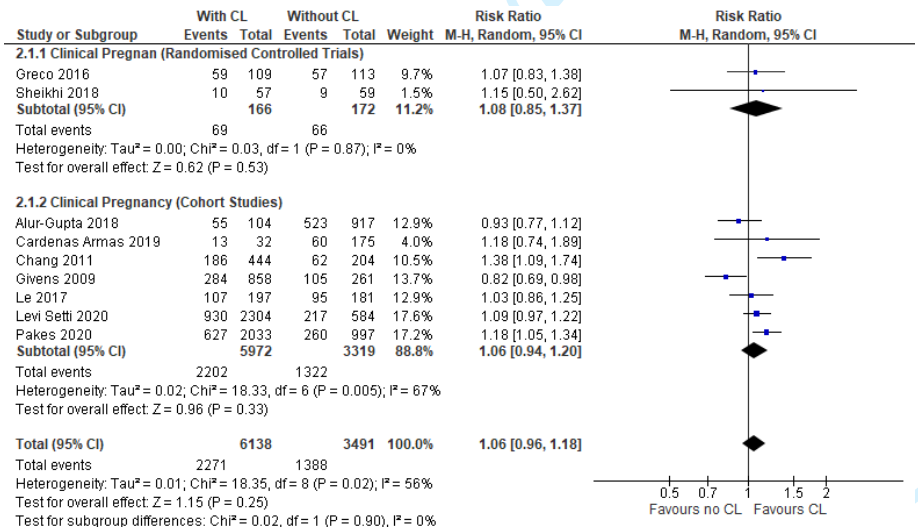
CL, Corpus Luteum; CI, Confidence interval

Supplementary File 7 - Supplementary Figure 2: Meta-analysis comparing rates of positive b-hCG, clinical pregnancy and live births in cycles with and without a corpus luteum – separated by study design

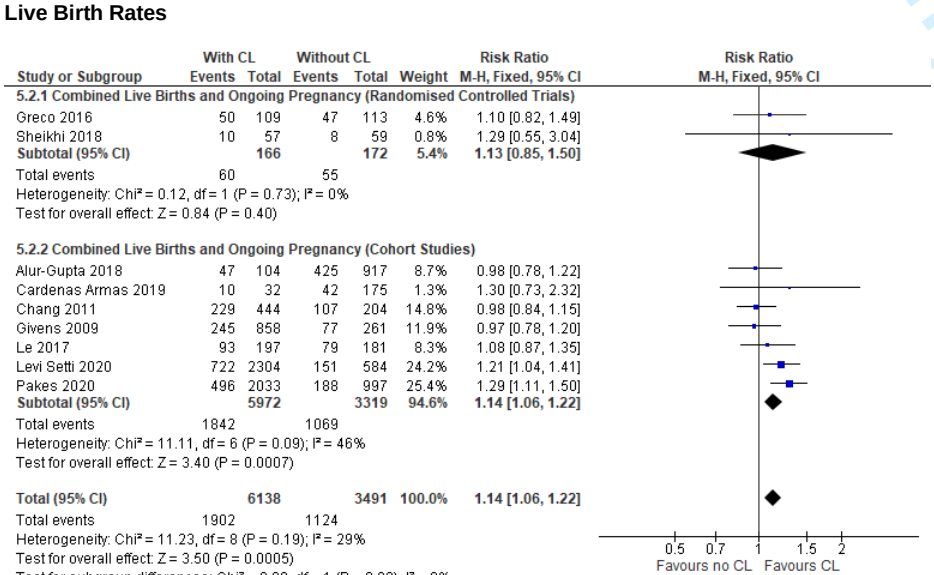
Positive b-hCG Rates



Clinical Pregnancy Rates



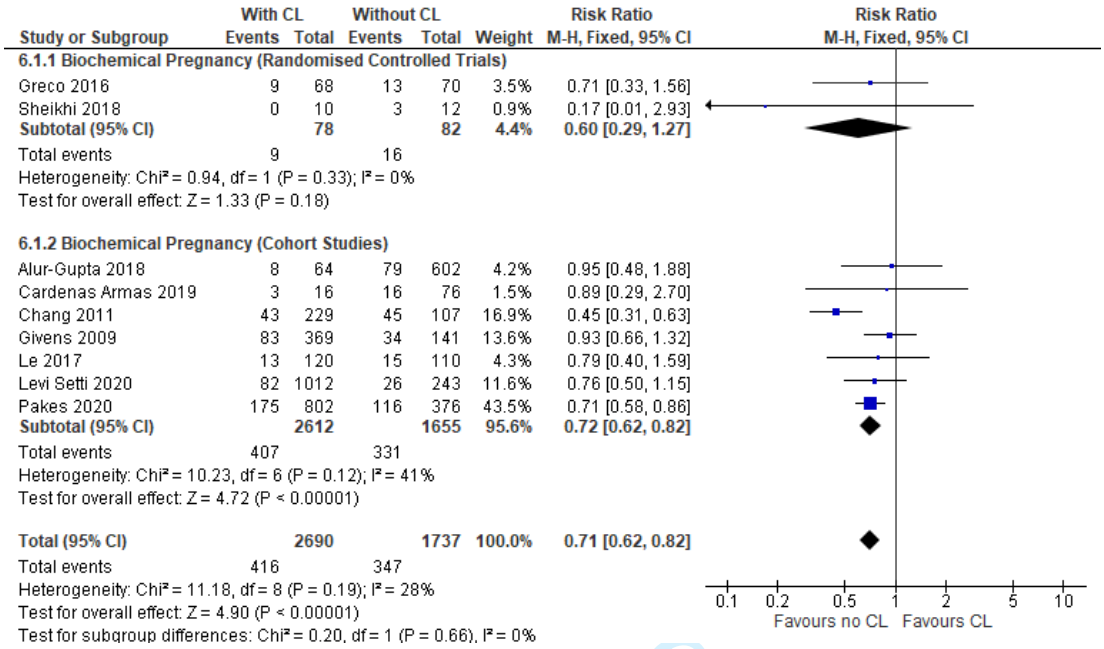
Live Birth Rates



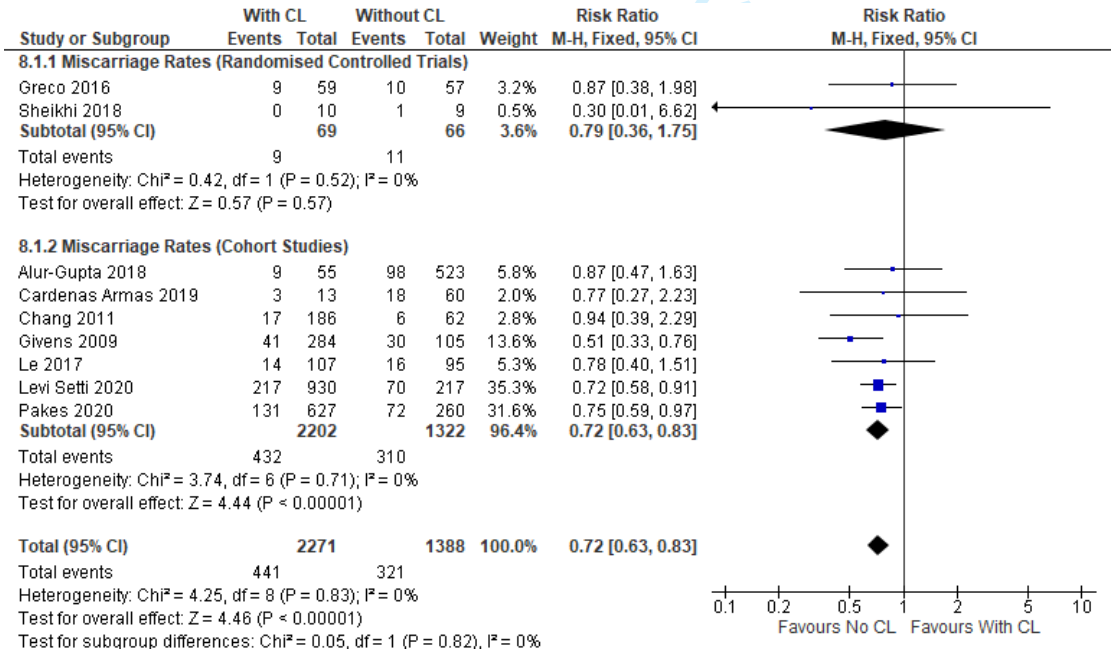
CL, Corpus luteum; CI, Confidence Interval

Supplementary File 8 - Supplementary Figure 3: Meta-analysis comparing rates of pregnancy losses in cycles with and without a corpus luteum – separated by study design

Biochemical Pregnancy Rates (Early Miscarriage)



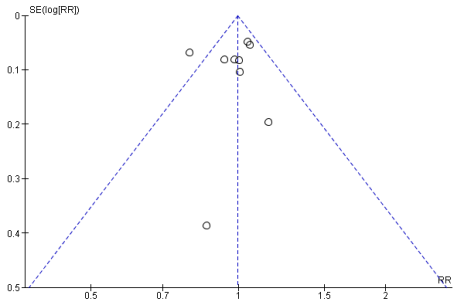
Miscarriage Rates



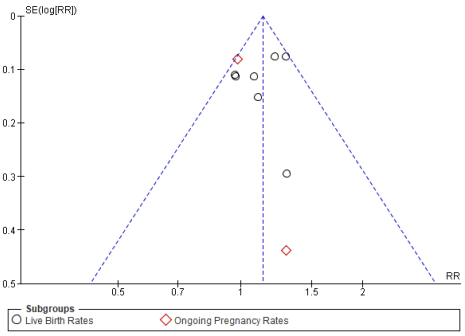
CL, Corpus luteum; CI, Confidence Interval

Supplementary File 9 - Supplementary Figure 4: Funnel Plot Analyses

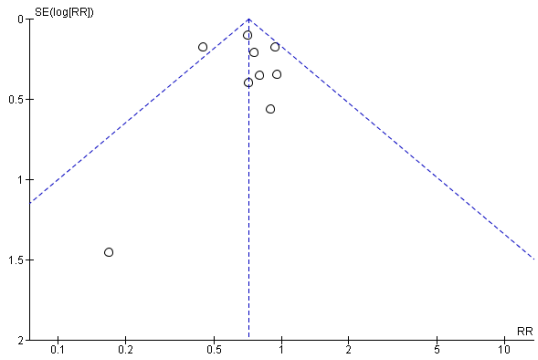
Funnel Plot for Positive b-hCG Rates



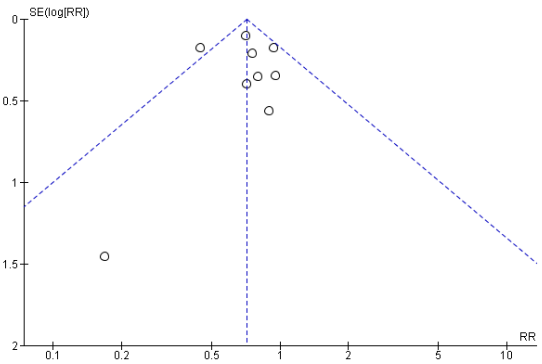
Funnel Plot for Live Birth Rates



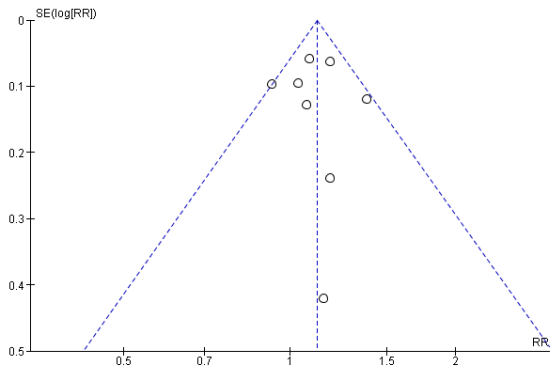
Funnel Plot for Clinical Pregnancy Rates



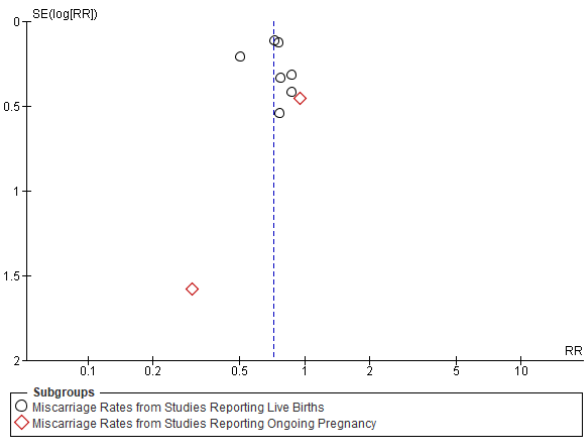
Funnel Plot for Biochemical Pregnancy Rates



Funnel Plot for Clinical Pregnancy Rates – Sensitivity Analysis



Funnel plot for Miscarriage Rates



PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3-5
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	7-8
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	8
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	9
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	9-10
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	9
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Supplementary materials
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	9
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	11
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	10-11
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	11
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	11-12
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	11-12

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	11
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	12
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	12
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table I (separate file to manuscript)
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Table I & supplementary files (separate file to manuscript
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Refer to figures II & III
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	15-17
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	11, table I & supplementary files
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	See supplementary figure 1-3
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	17
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	21
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	22
FUNDING			

PRISMA 2009 Checklist

Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	23

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Treatment Outcomes of Blastocysts Thaw-Cycles, Comparing the Presence and Absence of a Corpus Luteum: A Systematic Review and Meta-analysis.

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Treatment Outcomes of Blastocysts Thaw-Cycles, Comparing the Presence and Absence of a Corpus Luteum: A Systematic Review and Meta-analysis.

Authors: Joscelyn Gan[1], Genia Rozen[2,3,4], Alex Polyakov[2,3,4]

1 Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Melbourne, Australia 3010

2 Department of Obstetrics and Gynaecology, University of Melbourne, Melbourne, Australia 3010

3 Reproductive Services, Royal Women's Hospital, Melbourne, Australia 3052

4 Melbourne IVF, East Melbourne, Australia 3002

Corresponding author email: joscelyn.gan@gmail.com

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ABSTRACT

Purpose

This study aims to review the literature and perform a meta-analysis to determine if the presence of a corpus luteum has an impact on treatment outcomes in thaw cycles, where blastocyst embryos are transferred.

Method

PUBMED, EMBASE, CENTRAL and CINAHL were searched for papers published between January 2017 and July 27th, 2020. Additional articles were selected from the reference list of the results and previous reviews.

Three reviewers independently reviewed and extracted data. The meta-analysis was conducted though RevMan 5.4.1. Studies were quality assessed with the Cochrane risk of bias tool and the Newcastle Ottawa Scale.

Results

Nine publications were included for data-extraction and subsequent meta-analysis. Two studies were randomised control trials, and seven were cohort studies. Sub-group analysis of the different study designs was performed.

Whilst the rates of positive hCG results (RR 1.0, 95% CI 0.95 - 1.05) and clinical pregnancies (RR 1.06, 95% CI 0.96 -1.18) were comparable between the two groups, the rates of live births were higher in thaw-cycles with a corpus luteum (RR 1.14, 95% CI 1.06 - 1.22). Analysis of pregnancy losses demonstrated that both biochemical pregnancy (early miscarriage) (RR 0.71, 95% CI 0.62 - 0.82) and miscarriages (RR 0.72, 95% CI 0.62 - 0.83) were increased in cycles without a corpus luteum.

Conclusion

Where clinically appropriate, the use of cycle types that have a functional corpus luteum should be favoured. There were several limitations to this study, including the quality of studies and the inherent bias of retrospective cohort studies. Further, high-quality research, particularly randomised controlled trials with blastocysts embryos, is required to further explore these findings.

PROSPERO Registration Number: CRD42020209583

STRENGTHS AND LIMTATIONS

- As the use of blastocysts in thaw cycles is becoming increasingly more common, this review is timely and relevant
- The safety of embryo transfers without a corpus luteum is a growing area of research
- The limitations of this study include the limited number of studies in the area and lack of high quality randomised controlled trials
- Further high-quality studies are required to further explore these findings.

ETHICS APPROVAL

This study involves human participants but an Ethics Committee(s) or Institutional Board(s) exempted this study. This is a systematic review, hence review by an ethics committee is not required.

STUDY FUNDING/COMPETING INTEREST(S):

All authors declare no conflicts of interest. This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

INTRODUCTION

In vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) treatments conventionally include a fresh embryo transfer, sometimes followed by one or more cryopreserved embryo transfers in subsequent cycles. Alternatively, all suitable embryos are cryopreserved and transferred in subsequent cycles. In recent years, embryo cryopreservation has become a fundamental tool in reproductive medicine. With improvements in the vitrification processes, culture mediums and desire for single embryo transfers (SETs), thaw cycles are becoming more common(1-4). The benefits of embryo verification include the need for fewer ovarian stimulation cycles, as well as an improved cumulative pregnancy(3). In Australia, the proportion of cryopreserved embryo transfers increased from 47.1% in 2014 to 57.2% in 2018(2). In particular, the cryopreservation of blastocysts for frozen embryo transfer has been an increasingly adopted practice. The European IVF Monitoring Consortium reported that in 2016 more than half of frozen embryo transfers (62.2%) were performed at the blastocysts stage(5). It was also noted that pregnancy rates were higher in the frozen embryo transfers which used blastocyst (39.7%) compared to cleavage staged embryos (28.3%)(5).

Various protocols for endometrial preparation have been developed to assist with thaw-cycles transfers. One of the most widely used methods is the true natural cycle (tNC) or variations of it such as the modified natural cycle (mNC) or the mildly stimulated cycle (SC). These preparation techniques rely on the patient ovulating, either spontaneously, or with the assistance of ovulation induction agents or trigger. These protocols result in the formation of a corpus luteum (CL), which produces endogenous hormonal support for early pregnancy, with or without further luteal phase support with exogenous progesterone. These methods are typically used in normo-ovulatory women and uses no or minimal medications. However, these methods require extensive monitoring, which may be inconvenient for the patient and clinician. These cycles may also result in some degree of unpredictability in terms of embryo transfer timing, with some clinics preferring not to perform embryo transfers on certain days, such as weekends. The artificial cycle (AC) is an alternative method of endometrial preparation which relies on the administration of exogenous estrogen (E2) to induce endometrial proliferation and growth suppression of the dominant follicle, and the subsequent administration of progesterone (P4) to induce the secretory phase of the endometrium. This protocol aims to mimic the body's physiological process of endometrial priming and maturation. As the AC does not involve ovulation, a CL is not formed during this process and hormone supplementation is continued until placental autonomy is established at 10 to 12 weeks gestation. The AC is typically used in situations where a woman has ovulatory dysfunction and is unable to produce a healthy CL, or in normo-ovulatory women due to its convenience for both the patient and clinician(4, 6).

Previous studies have found that treatment outcomes of tNC and ACs have been comparable(7-9). Some studies, however, have noted that thaw-cycles without a CL may have experienced higher rates of early pregnancy loss. (4, 10, 11). This review aimed to explore these findings further. Trials in reproductive medicine are often small and not adequately powered, hence a meta-analysis is a useful technique to observe trends that may not be obvious with smaller, individual studies(12).

Our objective is to compare the treatment outcomes of blastocyst embryo transfers in thaw cycles with and without a CL.

To our knowledge, this is the first review to specifically look at treatment outcomes of thaw-cycles comparing the presence and absence of a CL. Similarly, to align more closely with the contemporary clinical practices, this review focuses on data from blastocysts transfers only(2).

MATERIALS AND METHODS

PICO statement:

Population – women undergoing thaw embryo transfer cycles.

Intervention – thaw cycles which include CL formation and therefore endogenous progesterone production (natural and ovulation induction cycles).

Comparison – thaw cycles that rely solely on exogenous progesterone production (artificial thaw cycles)

Outcomes – Live birth, clinical pregnancy, biochemical pregnancy, pregnancy loss (miscarriage rate)

Clinical Question – Are clinical outcomes of thaw embryo transfer cycles differ, depending on the presence or absence of CL (endogenous progesterone production)?

Patient and Public Involvement

No patient involved.

Search Strategy

This review was registered with PROSPERO CRD42020209583. We conducted a search on the 27 July 2020, using four databases: PubMed/MEDLINE, EMBASE, CINAHL and Cochrane Central Register of Controlled Trials (CENTRAL). The search strategies were based on an earlier Cochrane systematic review that was published in 2017(7). The search strategy utilised 3 key concepts: endometrial preparation AND frozen embryos AND reproductive outcomes. The detailed search strategy can be found in supplementary File 1. Searches were limited to 2017 to July 2020 as we looked through the reference lists of studies from previously conducted systematic reviews prior to 2017 for potential additional studies(7, 8). No language restrictions were used in the search. We followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) guidelines(13).

After the removal of 644 duplications, the search yielded 2184 studies. Four additional studies were hand selected from the references of the retrieved articles. The initial search was independently screened based on title and abstract by three reviewers (AP, GR, JG). Any discrepancies were discussed among the three reviewers and a consensus decision was reached.

Inclusion Criteria

To be included, studies had to contain data on blastocyst transfers which utilised thaw cycles involving the presence and absence of a CL. Cycles which involved the presence of a CL included tNC, mNC and mildly SC. Cycles without a CL

included ACs with or without gonadotropin-releasing hormone analogue (GnRHa) suppression. Blastocysts were defined as day 5 or 6 embryos(14).

Exclusion Criteria

Studies that included cleavage stage embryos or blastocysts data pooled with cleavage staged embryos were excluded. We also excluded data from donor eggs, or from non-primary sources such as reviews, letters, book chapters and conference abstracts.

Outcomes and Definitions

The primary outcome examined was live birth (LB) or ongoing pregnancy rate where LB was not available. Secondary outcomes that were analysed were rates of positive human Chorionic Gonadotropin (hCG), clinical pregnancy, biochemical pregnancy, and miscarriage.

Where applicable, we used the definitions agreed upon by the International Glossary on Infertility and Fertility Care, 2017(14). A LB was defined as a birth which demonstrated evidence of life after at least 22 weeks gestation(14). An ongoing pregnancy was defined as a viable pregnancy which reached a gestational age of at least 20 weeks. Due to the low rates of pregnancy loss after 29 weeks gestation (15), ongoing pregnancy rates were included in the analysis of live birth rates. However, we performed a sub-analysis of the studies which reported live births as their primary outcome in addition to the total LB rate which would include ongoing pregnancy rates. A positive hCG was defined as a hCG of ≥5. Where positive hCG was not available, it was calculated through the addition of biochemical pregnancies and clinical pregnancies. The study by Alur-Gupta *et al*.,(2018) (16), did not report clinical pregnancy, hence it was calculated by adding the number of live births, ectopic pregnancies, stillbirths, and spontaneous abortions reported. A clinical pregnancy was defined as a positive hCG with evidence of at least one gestational sac on ultrasound, including ectopic pregnancies(14). Biochemical pregnancies were classified as a pregnancy which yielded a positive hCG result but did not reach the stage of clinical pregnancy(14). Where biochemical pregnancy was not reported, it was calculated by subtracting the reported clinical pregnancies from the number of positive hCG results. Similarly, miscarriage referred to any pregnancy that did not progress past 20 weeks gestation. Where therapeutic abortions were reported, those cycles were removed from the analysis. Due to the nature of the studies included, we reported data per thaw cycle, as data per woman was not possible to calculate.

Data Extraction Process

The data was independently extracted by three reviewers (GR, AP, JG) for author/s, year of publication, title of the article, year of trial, study design, number cycles, demographics of women, positive hCG, clinical pregnancy, biochemical pregnancy, miscarriage, live births, or ongoing births where live births were not available. The data was collated by a single reviewer (JG) and any discrepancies were discussed among three reviewers and until a consensus was reached.

Quality Assessment

Included randomised control trials were quality assessed using the Revised Cochrane Risk of Bias Tool for randomised trials (RoB 2)(17). The Newcastle-Ottawa Scale (NOS) for assessing the quality of non-randomised studies in meta-analyses was used to assess cohort studies(18). Both tools were used to assess bias at an individual study level. The quality assessment was used to judge the strength of evidence reported, and to guide our interpretations of the reported findings. Results of this can be found in Supplementary File 2 and 3.

Statistical Analysis

The meta-analysis was performed using RevMan 5.4.1 computer program, The Cochrane Collaboration, 2020(19). Meta-analyses of rates of positive hCG, live births, biochemical pregnancy, and miscarriage were conducted with a fixed-effect model where there was low heterogeneity among the studies, and a random-effect model where there was a significant heterogeneity. Heterogeneity was assessed with both the I^2 and X^2 statistic. P-values of X^2 that were <0.05, and $I^2 > 50\%$ were considered represent significant heterogeneity. Relative risk with 95% confidence intervals (CI), were used as the principal summary measure. The Mantel-Haenszel method was applied to estimate the pooled effect size. A funnel plot analysis was conducted for each meta-analysis to assess for reporting bias (Supplementary File 4).

As we included studies that reported ongoing pregnancy rates where LB rates were not available, we conducted a sub-group analyses which individually looked at LB rates and miscarriages from studies which reported LBs as their primary outcome. Separate analysis grouped by study design is shown in Supplementary File 5 and 6 respectively.

RESULTS

After the removal of duplicates, the search yielded 2184 articles. After screening by title and abstract, we reviewed 20 full-text and included an additional 4 articles from the reference lists of included articles and previous systematic reviews. We included nine studies in our final quantitative analysis(10, 16, 20-26).Two of which were randomised controlled trials (RCTs)(22, 23) both of which studied small sample sizes. The remaining seven were retrospective cohort studies(10, 16, 20, 21, 24-26) which followed a much larger sample size. This process is summarized in Figure 1. The final meta-analysis included a total of 6138 cycles with a CL and 3491 cycles without a CL.

A summary of the studies included in the meta-analysis can be found in table 1. The largest study included 3030 cycles by Pakes *et al.,* 2020(10), and the smallest study included 116 cycles by Sheikhi *et al.,* (2018)(23).

The average quality of the studies was rated with a fair to moderate risk of bias.

Table 1: Overview of studies included in a meta-analysis comparing reproductive outcomes in blastocysts transfers using thaw-cycles

	Study Design				Demographics				Outcomes			
Study	Design	Cycles with blastocysts (n)	Study Period	Allocation	Women (n)	Study population	Mean Age, years (SD)	BMI, kg/m2 (SD)	Positive - hCG (n)	CP (n)	LB/OP	Quality[a]
Alur-Gupta et al. (2018)(16)	Retrospective Cohort	1021 Cycles (with CL =104, without CL = 917)	2013 - 2017	Clinical judgement	NR	Both normo-ovulatory patients and women with ovulatory dysfunction	NC = 35.6 (3) AC = 35.4 (4)	NC = 27.2 (3.7) AC = 25.1 (5.3)	With CL = 64 Without CL = 602	With CL = 55 Without CL = 523	LB	Fair
Cardenas Armas et al. (2019)(24)	Retrospective Cohort	207 Cycles (with CL = 32; without CL = 175)	2014 - 2017	Preference, cycle characteristics	860	normo-ovulatory patients, no PGT	NC = 36.15 (0.29) AC(Transdermal) = 35.71 (0.17) AC (Oral) = 36.86 (0.19)	NC = 22.6 (2.1) AC(Transdermal) = 21.6 (2.4) AC (Oral) = 23.3 (1.7)	With CL = 16 Without CL = 76	With CL = 13 Without CL = 60	LB	Good
Chang et al. (2011)(21)	Retrospective Cohort	648 Cycles (with CL = 444, without CL = 204)	2007 - 2009	Convenience, Cost	611	normo-ovulatory patients with regular menstruation	NC = 34.2 (3.7) mNC = 33.7 (3.3) AC = 33.7 (3.7)	NC = 20.7 (2.8) mNC = 20.5 (3.5) AC = 20.7 (2.4)	With CL = 229 Without CL = 107	With CL = 186 Without CL = 62	OP	Good
Givens (2009) et al.(20)	Retrospective Cohort	1119 Cycles (with CL = 858, without CL = 261)	2000 - 2006	Clinical judgement	807	Both normo-ovulatory patients and women with ovulatory dysfunction	mNC = 35.1 (4.1) AC = 34.8 (5.0)	NR	With CL = 369 Without CL = 141	With CL = 284 Without CL =105	LB	Fair
Greco (2016) et al.(22)	RCT	222 Cycles (with CL = 109, without CL = 113)	2015	Computer-generated randomization (non-concealed)	236	normo-ovulatory patients, PGT	mNC = 35.2 (3.6) AC + GnRHa = 35.5 (3.8)	mNC = 22.1 (3.1) AC + GnRHa = 22.1 (3.2)	With CL = 68 Without CL = 70	With CL = 59 Without CL = 523	LB	Some concerns
Le (2017) et al.(26)	Retrospective Cohort	378 cycles (with CL 197, without CL = 181)	2006 - 2014	Clinical judgement	428[b]	Both normo-ovulatory patients and women with ovulatory dysfunction	mNC = 34.3 (4.2) AC = 33.3 (4.8)	mNC = 25.3 (5.5) AC = 27.7 (7.0)	With CL= 120 Without CL = 110	With CL = 107 Without CL = 95	LB	Fair
Levi Setti et al. (2020)(25)	Retrospective Cohort	2888 Cycles (with CL = 2304, without CL = 584)[c]	2011 - 2017	Clinical judgement	NR	Both normo-ovulatory patients and women with ovulatory dysfunction; no PGT	NC = 35.4 (4.3) mNC = 35.3 (4.0) AC = 34.4 (4.2)	NC = 22.8 (3.0) mNC = 21.8 (3.0) AC = 22.5 (3.3)	With CL = 1012 Without CL = 243	With CL = 930 Without CL = 217	LB	Fair
Pakes et al. (2020)(10)	Retrospective Cohort	3030 Cycles (with CL = 2033, without CL = 997)	2015 - 2018	Clinical judgement	NR	Both normo-ovulatory patients and women with ovulatory dysfunction; no PGT	NC = 35.56 (0.89) AC = 33.79 (0.14)	NR	With CL = 802 Without CL = 376	With CL = 627 Without CL = 260	LB	Fair

Sheikhi et al. (2018)(23)[d]	RCT	116 Cycles (with CL = 57, without CL = 59)	2015 - 2016	Computer-generated randomization (non-concealed)	123[e]	normo-ovulatory patients, without severe endometriosis	mNC = 29.71 (3.79) mSC = 30.31 (4.58) AC = 30.5 (5.59)	mNC = 26.19 (3.24) mSC = 25.80 (3.29) AC = 25.36 (5.27)	With CL = 10 Without CL = 12	With CL = 10 Without CL = 9	OP	Some concerns

First author stated only. RCT, randomised controlled trial; CL, corpus luteum; NC, natural cycle; mNC, modified natural cycle; AC, artificial cycle; mSC = mildly stimulated cycle; GnRHa, gonadotropin-releasing hormone analogue; PGT, pre-implantation genetic testing; NR, not reported. [a] quality assessed with Cochrane Risk of Bias tool 2 or Newcastle-Ottawa Scale. [b] 66 women excluded due to various reasons. [c] therapeutic abortion cycles excluded. [d] demographic data extracted from table 1 of study (conflicted data reported in written results section) [e]7 women lost to follow-up

Positive hCG rates

From the eight studies, a total of 6138 cycles involving a CL were assessed. Of these, 2690 cycles (44%) resulted in a positive hCG. In the 3491 cycles without a CL, 1737 (50%) resulted in a positive hCG. The individual and combined estimates for positive-hCG are shown in Figure 2. The pooled estimates for positive hCG (RR 1.00, 95% CI 0.95 – 1.05) showed no statistically significant difference in rates of positive hCG between cycles with and without a CL. Subgroup analysis of positive hCG rates by study design are shown in Supplementary File 5.

Clinical Pregnancy Rates

Out of the 6138 cycles which involved the presence of a CL, 2271 (37%) progressed to a clinical pregnancy. In the 3491 cycles without a CL, 1388 (40%) progressed to a clinical pregnancy. The individual and combined estimates for clinical pregnancy are shown in Figure 2. The pooled estimates for clinical pregnancy rates (RR 1.06, 95% CI 0.96 – 1.18) showed no statistical difference between the two groups.

Due to the heterogeneity of the studies a random effect model was used. To overcome the statistical heterogeneity of the studies we performed a sensitivity analysis after removing the study by Givens *et al*., (2009) (20) which was the only study to observe a higher clinical pregnancy rate in AC compared to NCs. The results of this are shown in Supplementary File 7. The sensitivity analysis showed that live birth rates were statistically higher in the cycles involving the presence of a CL (RR 1.12, 95% CI 1.05 - 1.20).

Based on these two analyses, it can be inferred that the likely point estimate lays somewhere between 1.06 and 1.12, favouring cycles with CL. The confidence interval of this point estimate may include 1, but there is a trend towards cycles with CL resulting in a higher clinical pregnancy rate. While statistical significance may not be demonstrable, this finding may be clinically significant. Subgroup analysis of clinical pregnancy rates by study design is shown in Supplementary File 5.

Live Birth Rates

Seven studies reported LB rates as their primary outcome (one prospective randomised trial and five retrospective studies)(10, 16, 20, 22, 24-26). Two studies reported ongoing pregnancy rates as their primary outcome (one prospective randomised trial, and one cohort study)(21, 23) .

Of the 6138 cycles which involved the presence of a CL, 1902 (31%) resulted in a LB or progressed to an ongoing pregnancy. In the 3491 cycles without a CL, 1124 (32%) resulted in a live birth or ongoing pregnancy. The individual and combined estimates for live births are shown in Figure 2. The pooled estimates for live births (RR 1.14, 95% CI 1.06 - 1.22) showed a statistically significant difference in favour of cycles with a CL. This translates into a clinically significant approximate 14% increase chance of live birth from cycles with a CL.

A subgroup analysis was conducted which looked at studies that only reported LB as their outcome. The results of this can be found in Figure 2. When including only the studies which included LB rates, the estimated live birth rate remained significantly higher in the thaw-cycles with a CL (RR 1.16, 95% CI 1.07 - 1.26). Subgroup analysis of LB rates by study design is shown in Supplementary File 5.

Biochemical Pregnancy Rates

In the 2690 positive hCG results in the cycles with a CL, 416 (15%) were biochemical pregnancies that did not progress to a clinical pregnancy (i.e., ended in an early miscarriage). In the 1737 positive hCG results in the cycles without a CL, 347 (20%) of these resulted in biochemical pregnancies, which likewise did not progress to a clinical pregnancy. The individual and

combined estimates for biochemical pregnancies are shown in Figure 3. The estimated biochemical pregnancy rates (RR 0.71, 95% CI 0.62 – 0.82) were significantly lower in the cycles with a CL. Subgroup analysis of biochemical pregnancy rates by study design is shown in Supplementary File 6.

Miscarriage Rates

Of the 2271 clinical pregnancies in the cycles with a CL, 441 (19%) did not progress and resulted in a miscarriage. Of the 1388 clinical pregnancies which resulted from cycles without a CL, 321 clinical pregnancies (23%) did not progress. The individual and combined estimates for biochemical pregnancies are shown in figure 3. The estimated miscarriage rates (RR 0.72, 95% CI 0.63 – 0.83) were statistically lower in the cycles with a CL.

A subgroup analysis was conducted which only included studies which reported LB rates. However, this had no material impact on the results. Subgroup analysis of miscarriage rates by study design is shown in Supplementary File 6.

DISCUSSION

This meta-analysis demonstrates that while there were no statistically significant differences in rates of positive hCG and clinical pregnancies between thaw cycles with and without a CL, there were statistically higher rates of LBs and lower rates of both early and late pregnancy losses in thaw-cycles in the presence of a CL. This suggests that a CL may not influence initial implantation but may play a significant role in sustaining a pregnancy once an embryo has implanted.

Previous publications have demonstrated conflicting results regarding efficacy of thaw-cycles with and without a CL. The "ANTARCTICA" trial which compared treatment outcomes of mNC to AC protocols did not find any statistical difference in reproductive outcomes among the two groups(6). However, this study did not achieve adequate statistical power to examine the outcomes in question. Furthermore, a large proportion of cleavage stage embryos were included in their data, and data on blastocysts transfers was not clearly separated or analysed. Similarly, a study by Sahin *et al*., (2020), which retrospectively analysed treatment outcomes after mNC and ACs with GnRHa, concluded that LBs rates and pregnancy loss rates were comparable between the two groups(27). However, a statistically greater number of thawed embryos and percentage of blastocysts were transferred in the AC group which may have biased the results to improve the outcomes of the AC. Similarly, a study by Hill *et al*., (2010), demonstrated higher birth rates in the AC compared to the NC group, however, the AC group had more blastocysts transferred which would have likely biased the results to favour the AC(28). A recent Cochrane review was inconclusive regarding its ability to determine an optimal endometrial technique in terms of reproductive outcomes(7). Similar inconclusive results were also observed in other systematic reviews and meta-analyses (8, 29, 30). These studies also included data on cleavage staged embryos, which may not be generalizable to our research question.

Most of the studies included in our analysis were of fair to moderate quality. This is largely due to the possibility of non-comparable groups of women undertaking thaw-cycles involving the presence or absence of a CL. Women with oligo or

amenorrhea due to medical conditions like polycystic ovarian syndrome (PCOS), are more likely to undergo the AC for embryo transfer, compared to women with regular menstrual cycles. Women with PCOS may have an increased risk of adverse pregnancy outcomes such as early miscarriage(31), which may be contributing to the observed results. Regarding the RCTs assessed, their quality was affected by the nature of the intervention that makes concealment and blinding challenging to implement. However, as mentioned by a previous Cochrane review, the non-blinding may not affect the measurement of outcomes, which are measured objectively(7).

Previous studies have also noted higher miscarriage rates in cycles without a CL. A large retrospective analysis by Tomás *et al.,* (2012), demonstrated a higher miscarriage rate in the AC cycle group compared to the group receiving the NC protocol(32). Similar findings were observed in the study by Givens *et al.,*(2009)(20). In both these studies, there were a significantly higher proportion of women with PCOS in the AC group, which may have contributed to this result. An older study by Veleva *et al.,* (2008), found that miscarriage rates were higher in the AC group (23.0%) compared to the NCs (11.4%, p-value < 0.0001)(33). However, the BMI of the women in the AC were statistically higher compared to the NC (25.3 ± 5.4, 22.9 ± 3.6, p-value < 0.0001) which may have influenced the miscarriage rate. Similarly, a retrospective study by Guan *et al.,* (2016) (34), which analysed 1482 thawed cleavage-staged embryos noted that women in the NC group experienced significantly lower rates of miscarriage (2.8%) compared to those in the women receiving the AC with GnRHa (14.0%, p-value = 0.003)(34). This may be influenced by the statistically older age of women receiving the AC with GnRHa compared to the women in the NC group. Another retrospective study involving normo-ovulatory women by Cerillo *et al.,* (2017), observed statistically higher miscarriage rates in the women receiving AC (21.2%), compared to the women receiving mNC (12.9%) and the tNC (11.1%)(35). In a recent retrospective analysis by Liu *et al.,* (2020), which compared mNC and AC protocols in young women with regular menses, it was noted that the women in the AC group exhibited a higher miscarriage rate (13.69%) compared to the mNC arm (8.37%, p value 0.034)(36). Again, as these studies included cleavage-stage embryos their findings may not be generalizable to our research question, which involves data on blastocyst embryos. A recent large retrospective study by Pakes *et al*., (2020) which analysed blastocyst thaw cycles, observed that the AC group experienced a higher pregnancy loss compared to the women in the NC group(10). In this study, women in the AC group were significantly younger and received a higher proportion of good quality day-5 blastocysts compared to the NC which may have biased results to favour the AC, however, the AC group still demonstrated more pregnancy losses compared to the NC group.

There may be several contributing factors influencing this observed increased rate of pregnancy loss in thaw-cycles without a CL. Firstly, we may be disregarding the physiology of the CL. In a recent study,(37) it was observed that cycles without a CL had a significantly lower level of serum progesterone on the day of embryo transfer compared to cycles involving a CL. In the AC, estrogen and P4 only are administered exogenously to provide early pregnancy support. However, it is known that the presence of a CL may alter the concentrations of other hormones in the body such as relaxin(4, 38, 39), indicating that there

may be complex interaction between the CL and pregnancy support extending beyond P4 and E2 production. Secondly, as the dosage of P4 is typically a standard dose, with different routes of administration in AC, the amount delivered may be inadequate for optimal luteal support at an individual level. Some studies suggest that serum P4 level may be helpful in guiding the level of supplementation(40-42), however, other studies suggest serum progesterone levels are not well correlated with the intra-uterine levels(43-45). This poor correlation is likely due to the first uterine pass effect(43, 45) and unpredictable levels of progesterone absorption from exogenous vaginal progesterone. Consequently, some women may not be receiving adequate luteal support, and thus an optimized uterine environment for early pregnancy development may not be achieved.

There have been growing concerns regarding the safety of cycles without a CL. A large retrospective study conducted in Sweden from 2005 to 2015, observed that cycles without a CL were more likely to develop pregnancy-related hypertensive disorders (adjusted odds ratio 1.61, 95% CI 1.22 - 2.10), post-partum haemorrhage (adjusted odds ratio 2.87, 95% CI 2.29 - 2.60), post-term birth (adjusted odds ratio 1.59, 95% CI 1.47 – 2.68) and macrosomia (adjusted odds ratio 1.62, confidence interval 1.03-1.90)(46). Furthermore, a retrospective study conducted in Japan which compared obstetric outcomes of NC and AC embryo transfers found that cycles without a CL exhibited higher rates of pregnancy related hypertensive disorders (adjusted odds ratio 1.43, 95% confidence interval 1.14-1.8) and placenta accreta (adjusted odds ratio, 6.91; 95% CI 2.87 – 16.66) compared to cycles involving the presence of a CL(47). Similar findings have been noted in other studies(48-53). In a recent study which investigated the relation between pregnancy related hypertensive disorders and corpus luteum number, it was noted that pregnancies without a CL did not exhibit the physiologic decline in mean arterial pressure associated with pregnancy(52). This may imply that the presence of a CL may play a vital role in the priming phase of the uterine environment and maternal vasculature for early pregnancy support.

However, in certain circumstances, the use of cycles without a CL may be necessary. Women who are unable to ovulate and hence unable to produce a CL, do not have the option of utilizing the NC or ovulatory induction agents to prime their endometrium. Hence, ACs are still a very import method in frozen embryo transfers.

Strengths of this study included its meta-analysis which has been able to increase the power of individual studies to observe differences that may not have been evident on their own. In addition to this, we limited papers to those that contained data which analysed blastocyst-staged embryos. This narrowed our research question to a particular sub-group of embryo transfers which is also clinically relevant, with an increasing number of blastocyst transfers observed in clinical practice.

This study has several limitations. Firstly, as most of these studies were of fair to moderate risk of bias due to the nature of the study designs implemented, there is a potential for confounders and selection bias to influence the results. However, most studies had accounted for this by using a multivariate logistic regression to control for confounders. In this study, the Mantel-Haenszel method was used to account for this. Furthermore, as there were less than 10 studies included in the meta-analysis, funnel plots constructed (Supplementary File 4) had a limited utility in assessing publication bias. The aforementioned

heterogeneity of the patient populations studied may also play a factor, with four of the studies only including normo-ovulatory patients, while the other four included women with ovulatory dysfunction in the cycles without a CL. Lastly, due to the ways that the included studies were reported, it was not able to calculate data per woman, which may have been another avenue for bias.

CONCLUSION

As blastocyst thaw cycles are increasingly being utilised worldwide, this review is timely and important. We conclude that cycles involving a CL may be slightly superior to cycles without a CL as they may produce marginally better reproductive outcomes. Furthermore, due to the higher rates of pregnancy loss and potential obstetric complications of AC, CL cycles should be the treatment of choice where clinically appropriate. However, cycles without a CL are still important as they may be necessary for women with irregular or absent periods and for cycles involving donor oocytes. As a result of this and the retrospective study design of many of the included studies, it should be noted that the population in whom artificial thaw cycles are performed may have an inherently different, possibly higher risks of pregnancy losses. However, the AC approach is routinely used in many centres and therefore would not be subject to this bias. Since the quality of studies included in the analysis is suboptimal, further high-quality research utilizing adequately powered randomised controlled trials involving blastocyst thaw-cycles is urgently required.

LIST OF ABBREVIATIONS

AC – Artificial Cycle

CI – Confidence Intervals

CL – Corpus Luteum

GnRHa - Gonadotropin-Releasing Hormone analogue

ICSI – intracytoplasmic sperm injection

IVF – In vitro fertilisation

LB – Live Birth

mNC – Modified Natural Cycle

PCOS – Polycystic Ovarian Syndrome

SC – Stimulated Cycle

SET – Single Embryo Transfer

tNC - True Natural Cycle

RCT – Randomised Control Trial

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AUTHORS' CONTRIBUTIONS

G.R. and A.P. were involved in the conception and creation of the study design. G.R., A.P., and J.G. wrote the protocol. All authors were involved in the screening of articles for eligibility and data extraction. A.P. provided expertise on statistical analysis. A.P. and J.G. performed the meta-analysis. All authors have contributed significantly to, seen, and approved the final submitted version of the manuscript.

CONFLICTS OF INTEREST

All authors declare no conflicts of interests.

DATA AVAILABILITY

All relevant data to the study is included in the article or in the supplementary materials supplied.

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FIGURE CAPTIONS

Figure 1: PRISMA Flowchart

Figure 2: Meta-analysis comparing rates of positive hCG, clinical pregnancy, and live births in cycles with and without a corpus luteum

Figure 3: Meta-analysis comparing biochemical pregnancy and miscarriage rates in cycles with and without a corpus luteum

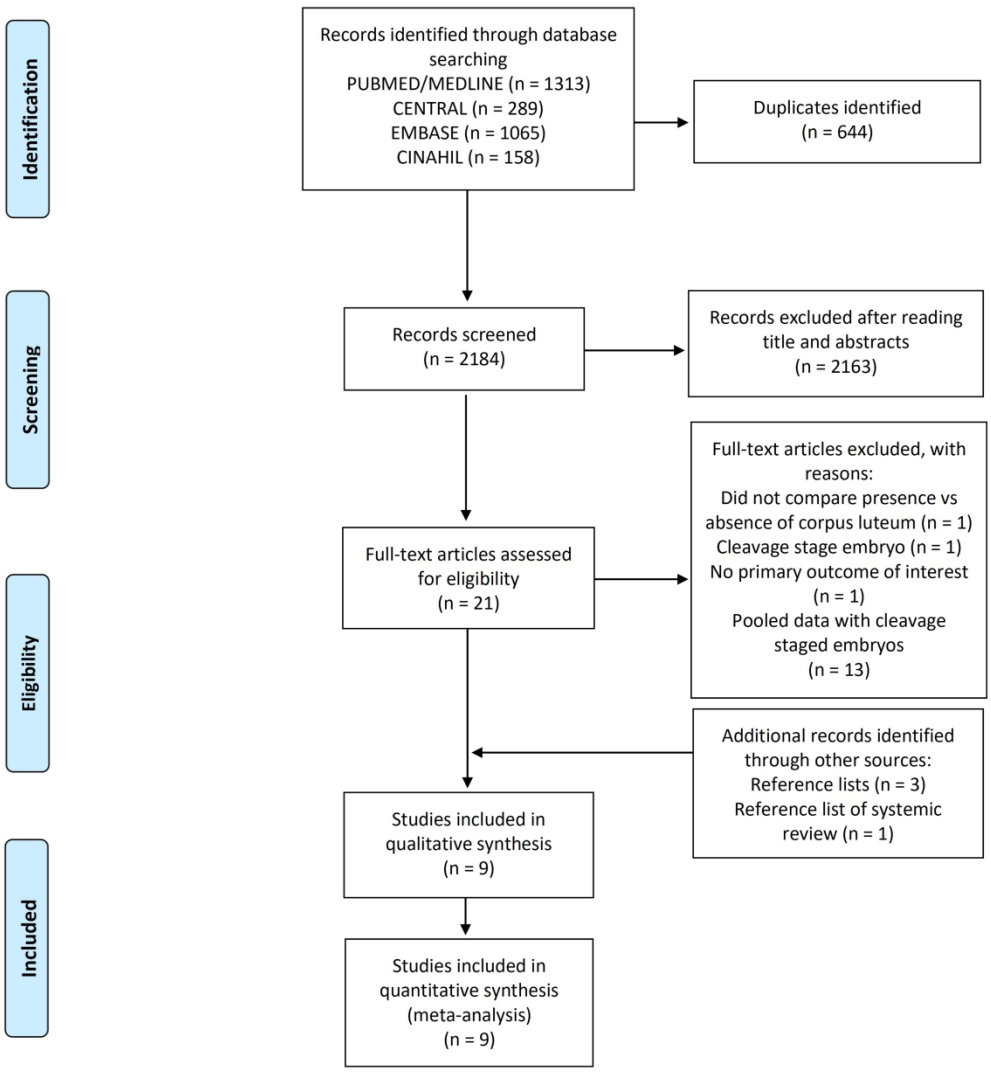


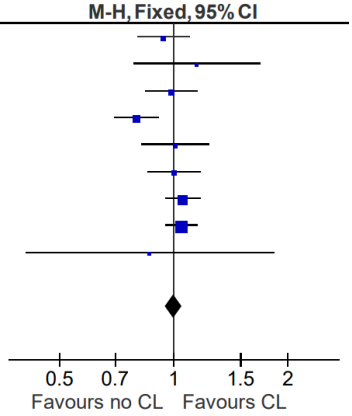
Figure 1: PRISMA Flowchart

159x170mm (600 x 600 DPI)

Rates of Positive hCG

Study or Subgroup	With CL Events	With CL Total	Without CL Events	Without CL Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Alur-Gupta 2018	64	104	602	917	7.7%	0.94 [0.80, 1.10]
Cardenas Armas 2019	16	32	76	175	1.5%	1.15 [0.78, 1.69]
Chang 2011	229	444	107	204	9.2%	0.98 [0.84, 1.15]
Givens 2009	369	858	141	261	13.5%	0.80 [0.69, 0.91]
Greco 2016	68	109	70	113	4.3%	1.01 [0.82, 1.24]
Le 2017	120	197	110	181	7.2%	1.00 [0.85, 1.18]
Levi Setti 2020	1012	2304	243	584	24.3%	1.06 [0.95, 1.17]
Pakes 2020	802	2033	376	997	31.6%	1.05 [0.95, 1.15]
Sheikhi 2018	10	57	12	59	0.7%	0.86 [0.40, 1.84]
Total (95% CI)		**6138**		**3491**	**100.0%**	**1.00 [0.95, 1.05]**
Total events	2690		1737			

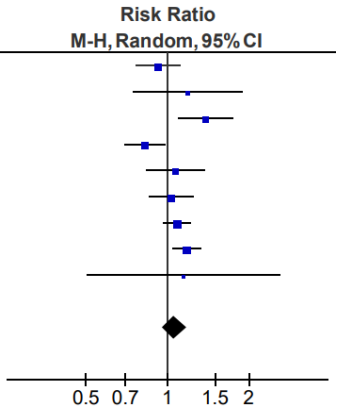
Heterogeneity: Chi² = 13.86, df = 8 (P = 0.09); I² = 42%
Test for overall effect: Z = 0.16 (P = 0.87)



Clinical Pregnancy Rates

Study or Subgroup	With CL Events	With CL Total	Without CL Events	Without CL Total	Weight	Risk Ratio M-H, Random, 95% CI
Alur-Gupta 2018	55	104	523	917	12.9%	0.93 [0.77, 1.12]
Cardenas Armas 2019	13	32	60	175	4.0%	1.18 [0.74, 1.89]
Chang 2011	186	444	62	204	10.5%	1.38 [1.09, 1.74]
Givens 2009	284	858	105	261	13.7%	0.82 [0.69, 0.98]
Greco 2016	59	109	57	113	9.7%	1.07 [0.83, 1.38]
Le 2017	107	197	95	181	12.9%	1.03 [0.86, 1.25]
Levi Setti 2020	930	2304	217	584	17.6%	1.09 [0.97, 1.22]
Pakes 2020	627	2033	260	997	17.2%	1.18 [1.05, 1.34]
Sheikhi 2018	10	57	9	59	1.5%	1.15 [0.50, 2.62]
Total (95% CI)		**6138**		**3491**	**100.0%**	**1.06 [0.96, 1.18]**
Total events	2271		1388			

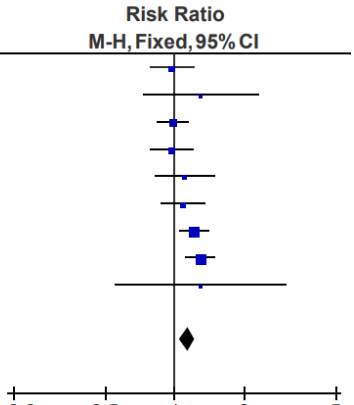
Heterogeneity: Tau² = 0.01; Chi² = 18.35, df = 8 (P = 0.02); I² = 56%
Test for overall effect: Z = 1.15 (P = 0.25)



Live Births and Ongoing Pregnancy Rates

Study or Subgroup	With CL Events	With CL Total	Without CL Events	Without CL Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Alur-Gupta 2018	47	104	425	917	8.7%	0.98 [0.78, 1.22]
Cardenas Armas 2019	10	32	42	175	1.3%	1.30 [0.73, 2.32]
Chang 2011	229	444	107	204	14.8%	0.98 [0.84, 1.15]
Givens 2009	245	858	77	261	11.9%	0.97 [0.78, 1.20]
Greco 2016	50	109	47	113	4.6%	1.10 [0.82, 1.49]
Le 2017	93	197	79	181	8.3%	1.08 [0.87, 1.35]
Levi Setti 2020	722	2304	151	584	24.2%	1.21 [1.04, 1.41]
Pakes 2020	496	2033	188	997	25.4%	1.29 [1.11, 1.50]
Sheikhi 2018	10	57	8	59	0.8%	1.29 [0.55, 3.04]
Total (95% CI)		**6138**		**3491**	**100.0%**	**1.14 [1.06, 1.22]**
Total events	1902		1124			

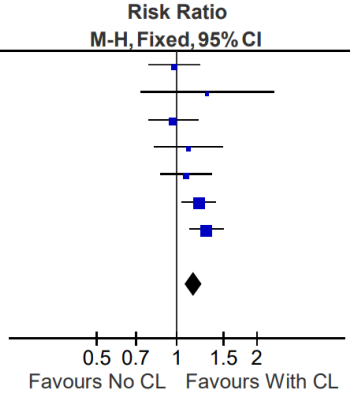
Heterogeneity: Chi² = 11.23, df = 8 (P = 0.19); I² = 29%
Test for overall effect: Z = 3.50 (P = 0.0005)



Live Births Rates Only

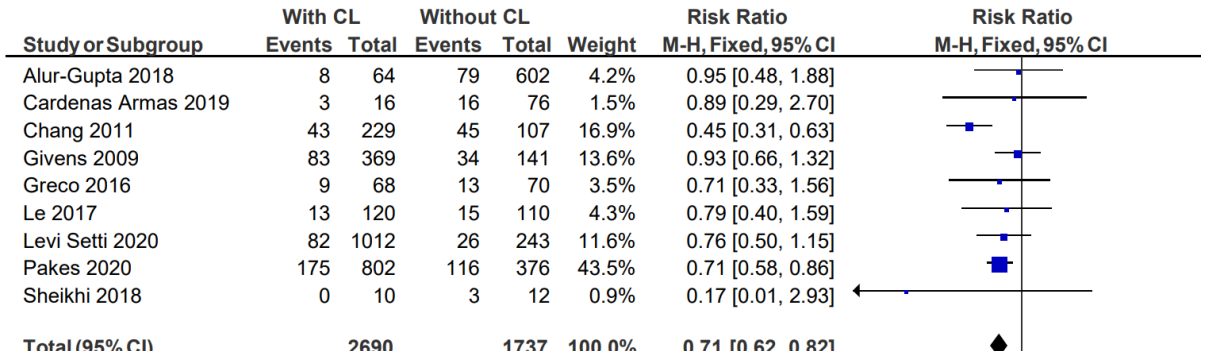
Study or Subgroup	With CL Events	With CL Total	Without CL Events	Without CL Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Alur-Gupta 2018	47	104	425	917	10.3%	0.98 [0.78, 1.22]
Cardenas Armas 2019	10	32	42	175	1.5%	1.30 [0.73, 2.32]
Givens 2009	245	858	77	261	14.1%	0.97 [0.78, 1.20]
Greco 2016	50	109	47	113	5.5%	1.10 [0.82, 1.49]
Le 2017	93	197	79	181	9.8%	1.08 [0.87, 1.35]
Levi Setti 2020	722	2304	151	584	28.7%	1.21 [1.04, 1.41]
Pakes 2020	496	2033	188	997	30.1%	1.29 [1.11, 1.50]
Total (95% CI)		**5637**		**3228**	**100.0%**	**1.16 [1.07, 1.26]**
Total events	1663		1009			

Heterogeneity: Chi² = 8.07, df = 6 (P = 0.23); I² = 26%
Test for overall effect: Z = 3.70 (P = 0.0002)

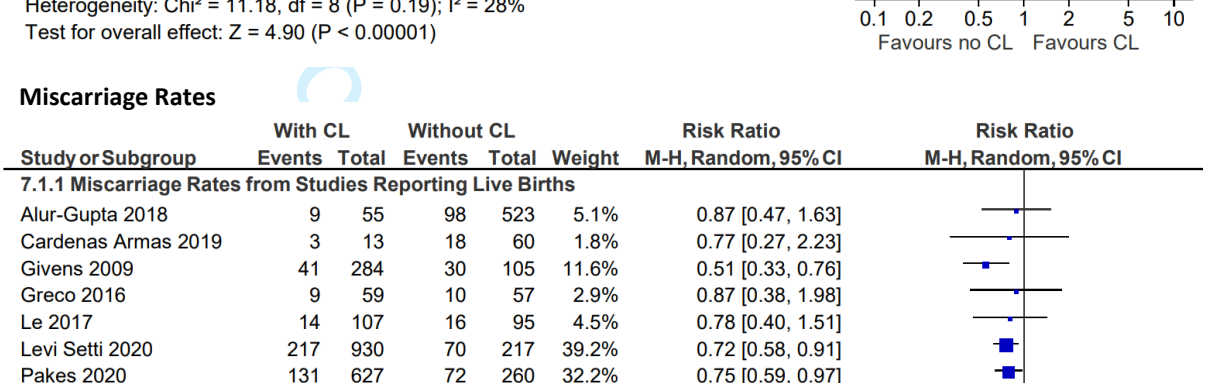


CL, Corpus Luteum; CI, Confidence interval

Biochemical Pregnancy Rates



Miscarriage Rates



CL, Corpus Luteum; CI, Confidence interval

Supplementary Files for "Treatment Outcomes of Blastocysts Thaw-Cycles, Comparing the Presence and Absence of a Corpus Luteum: A Systematic Review and Meta-analysis.

Table of Contents

Supplementary File 1 – Supplementary Table 1: Search Strategy

PUBMED/MEDLINE

Set	Search	Results
1	Cryopreservation[All Fields]	47,444
2	frozen embryo transfer[All Fields]	3,740
3	Frozen embryo*[All Fields]	8,561
4	frozen-thawed cycle[All Fields]	1,209
5	frozen-thawed embryo transfer[All Fields]	1,457
6	frozen thawed embryos[All Fields]	3,703
7	"FET"[All Fields]	3,577
8	cryopreserved embryos[All Fields]	9,714
9	Cryopreserved-thawed embryos[All Fields]	131
10	vitrification[All Fields]	4,568
11	Vitrified[All Fields]	3,077
12	"vitrified-warmed embryos"[All Fields]	440
13	"frozen-thawed"[All Fields]	5,134
14	embryo vitrification[All Fields]	2,144
15	blastocyst transfer[All Fields]	28,636
16	((((((((((((cryopreservation) OR (frozen embryo transfer)) OR (frozen embryo*)) OR (frozen-thawed cycle)) OR (frozen-thawed embryo transfer)) OR (frozen thawed embryos)) OR (FET)) OR (cryopreserved embryos)) OR (cryopreserved-thawed embryos)) OR (vitrification)) OR (vitrified)) OR (vitrified-warmed embryos)) OR (frozen-thawed)) OR (embryo vitrification)) OR (blastocyst transfer)[All Fields]	81,001
17	endometrial preparation[All Fields]	2,129
18	natural cycle[All Fields]	56,766
19	ovulation induction[All Fields]	16,378
20	modified natural cycle[All Fields]	2,401
21	hormone therapy[All Fields]	659,266
22	Estrogen OR oestrogen OR oestrogens OR estrogens OR oestradiol[All Fields]	286,275
23	progesterone[All Fields]	119,710
24	stimulated cycle[All Fields]	63,307
25	stimulation of endometrium embryo transfer[All Fields]	426
26	artificial cycle	13,886
27	(((((((((endometrial preparation) OR (natural cycle)) OR (ovulation induction)) OR (modified natural cycle)) OR (hormone therapy)) OR (Estrogen or oestrogen or oestrogens or estrogens or estradiol)) OR (progesterone)) OR (stimulated cycle)) OR (stimulation of endometrium embryo transfer)) OR (artificial cycle)[All Fields]	1,012,876
28	((((((((((endometrial preparation) OR (natural cycle)) OR (ovulation induction)) OR (modified natural cycle)) OR (hormone therapy)) OR (Estrogen or oestrogen or oestrogens or estrogens or estradiol)) OR (progesterone)) OR (stimulated cycle)) OR (stimulation of endometrium embryo transfer)) OR (artificial cycle)) AND ((((((((((((((cryopreservation) OR (frozen embryo transfer)) OR	11,974

	(frozen embryo*)) OR (frozen-thawed cycle)) OR (frozen-thawed embryo transfer)) OR (frozen thawed embryos)) OR (FET)) OR (cryopreserved embryos)) OR (cryopreserved-thawed embryos)) OR (vitrification)) OR (vitrified)) OR (vitrified-warmed embryos)) OR (frozen-thawed)) OR (embryo vitrification)) OR (blastocyst transfer))[All Fields]	
29	Pregnancy[All Fields]	987,880
30	live birth*[All Fields]	32,374
31	miscarriage[All Fields]	47,358
32	ongoing pregnancy[All Fields]	8,897
33	clinical pregnancy[All Fields]	190,084
34	chemical pregnancy[All Fields]	45,767
35	(((((pregnancy) OR (live birth*)) OR (miscarriage)) OR (ongoing pregnancy)) OR (clinical pregnancy)) OR (chemical pregnancy) [All Fields]	1,001,238
36	((((((((((endometrial preparation) OR (natural cycle)) OR (ovulation induction)) OR (modified natural cycle)) OR (hormone therapy)) OR (Estrogen or oestrogen or oestrogens or estrogens or estradiol)) OR (progesterone)) OR (stimulated cycle)) OR (stimulation of endometrium embryo transfer)) OR (artificial cycle)) AND ((((((((((((((cryopreservation) OR (frozen embryo transfer)) OR (frozen embryo*)) OR (frozen-thawed cycle)) OR (frozen-thawed embryo transfer)) OR (frozen thawed embryos)) OR (FET)) OR (cryopreserved embryos)) OR (cryopreserved-thawed embryos)) OR (vitrification)) OR (vitrified)) OR (vitrified-warmed embryos)) OR (frozen-thawed)) OR (embryo vitrification)) OR (blastocyst transfer))) AND ((((((pregnancy) OR (live birth*)) OR (miscarriage)) OR (ongoing pregnancy)) OR (clinical pregnancy)) OR (chemical pregnancy)) [All Fields]	7,913
37	animal[All Fields]	6,843,446
48	((((((((((endometrial preparation) OR (natural cycle)) OR (ovulation induction)) OR (modified natural cycle)) OR (hormone therapy)) OR (Estrogen or oestrogen or oestrogens or estrogens or estradiol)) OR (progesterone)) OR (stimulated cycle)) OR (stimulation of endometrium embryo transfer)) OR (artificial cycle)) AND ((((((((((((((cryopreservation) OR (frozen embryo transfer)) OR (frozen embryo*)) OR (frozen-thawed cycle)) OR (frozen-thawed embryo transfer)) OR (frozen thawed embryos)) OR (FET)) OR (cryopreserved embryos)) OR (cryopreserved-thawed embryos)) OR (vitrification)) OR (vitrified)) OR (vitrified-warmed embryos)) OR (frozen-thawed)) OR (embryo vitrification)) OR (blastocyst transfer))) AND ((((((pregnancy) OR (live birth*)) OR (miscarriage)) OR (ongoing pregnancy)) OR (clinical pregnancy)) OR (chemical pregnancy))) NOT (animal) [All Fields]	6,386
39	((((((((((endometrial preparation) OR (natural cycle)) OR (ovulation induction)) OR (modified natural cycle)) OR (hormone therapy)) OR (Estrogen or oestrogen or oestrogens or estrogens or estradiol)) OR (progesterone)) OR (stimulated cycle)) OR (stimulation of endometrium embryo transfer)) OR (artificial cycle)) AND ((((((((((((((cryopreservation) OR (frozen embryo transfer)) OR (frozen embryo*)) OR (frozen-thawed cycle)) OR (frozen-thawed	6,375

	embryo transfer)) OR (frozen thawed embryos)) OR (FET)) OR (cryopreserved embryos)) OR (cryopreserved-thawed embryos)))) OR (vitrified-warmed embryos)) OR (frozen-thawed)) OR (embryo vitrification)) OR (blastocyst transfer))) AND ((((((pregnancy) OR (live birth*)) OR (miscarriage)) OR (ongoing pregnancy)) OR (clinical pregnancy)) OR (chemical pregnancy))) NOT (animal) [All Fields]	
40	((((((((((((endometrial preparation) OR (natural cycle)) OR (ovulation induction)) OR (modified natural cycle)) OR (hormone therapy)) OR (Estrogen or oestrogen or oestrogens or estrogens or estradiol)) OR (progesterone)) OR (stimulated cycle)) OR (stimulation of endometrium embryo transfer)) OR (artificial cycle)) AND ((((((((((((((cryopreservation) OR (frozen embryo transfer)) OR (frozen embryo*)) OR (frozen-thawed cycle)) OR (frozen-thawed embryo transfer)) OR (frozen thawed embryos)) OR (FET)) OR (cryopreserved embryos)) OR (cryopreserved-thawed embryos)) OR (vitrification)) OR (vitrified)) OR (vitrified-warmed embryos)) OR (frozen-thawed)) OR (embryo vitrification)) OR (blastocyst transfer))) AND ((((((pregnancy) OR (live birth*)) OR (miscarriage)) OR (ongoing pregnancy)) OR (clinical pregnancy)) OR (chemical pregnancy))) NOT (animal) [All Fields] - from 2017 - 2020	1,089

EMBASE

Set	Search	Results
1	cryopreservation.mp. or cryopreservation/	45195
2	(Cryopreserv$ adj7 embryo$).tw.	5646
3	(Cryopreserv$ adj7 blastocyst$).tw.	1080
4	freezing/ or vitrification/	43414
5	(vitrifi$ adj5 embryo$).tw.	2410
6	(vitrifi$ adj5 blastocyst$).tw.	1803
7	(frozen adj5 embryo$).tw.	5929
8	(freez$ adj5 embryo$).tw.	2056
9	(freez$ adj5 blastocyst$).tw.	367
10	(frozen adj5 blastocyst$).tw.	1032
11	FET.tw.	4837
12	freeze thawing/ or freezing/	45930
13	vitrification/	5997
14	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13	95117
15	exp ovulation induction/	16413
16	((ovar$ adj5 stimula$) or (ovulat$ adj5 induc$)).tw.	26000
17	(endometri$ adj2 prepar$).tw.	1032
18	hormon$ regimen$.tw.	373
19	Clomiphene.tw. or Clomiphene/	11562
20	clomid.tw.	1284
21	(Tamoxifen or Letrozole).tw.	37754
22	aromatase inhibitor$.tw.	11798
23	exp human menopausal gonadotropin/	10498
24	(Menotropin$ or menopausal gonadotrop$ or HMG).tw.	20554
25	exp follitropin/	64748

26	(Follicle Stimulating Hormone or FSH or rFSH or rhFSH).tw.	57786
27	gonadorelin/	38181
28	Gonadotropin Releasing Hormone$.tw.	16215
29	Gonadotrophin Releasing Hormone$.tw.	3366
30	GnRH$.tw.	29904
31	exp estrogen/	300360
32	(?estrogen$ or ?estradiol).tw.	240982
33	exp progesterone/	104475
34	exp Progesterone/ or progesterone.tw.	145928
35	(natural$ adj2 cycle$).tw.	3444
36	(artificial$ adj2 cycle$).tw.	633
37	(cycle$ adj2 regimen$).tw.	670
38	pituitary suppression.tw.	486
39	human menopausal.tw.	2684
40	spontaneous ovulation.tw.	615
41	(HCG adj3 trigger$).tw.	1039
42	(stimulat$ adj3 cycle$).tw.	5831
43	exogenous steroid$.tw.	708
44	exogenous steroid$.tw.	708
45	(hormone adj2 therap$).tw.	41571
46	(endometri$ adj2 stimulat$).tw.	835
47	15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46	605551
48	14 and 47	7970
49	Clinical Trial/	999716
50	Randomized Controlled Trial/	615254
51	exp randomization/	87897
52	Single Blind Procedure/	39662
53	Double Blind Procedure/	177011
54	Crossover Procedure/	64180
55	Placebo/	363424
56	Randomi?ed controlled trial$.tw.	233156
57	Rct.tw.	37946
58	random allocation.tw.	2120
59	randomly allocated.tw.	35898
60	allocated randomly.tw.	2597
61	(allocated adj2 random).tw.	981
62	Single blind$.tw.	25372
63	Double blind$.tw.	216438
64	((treble or triple) adj blind$).tw.	1215
65	placebo$.tw.	315943
66	prospective study/	617823
67	retrospective study/	946322
68	49 or 50 or 51 or 52 or 53 or 54 or 55 or 56 or 57 or 58 or 59 or 60 or 61 or 62 or 63 or 64 or 65 or 66 or 67	3142742
69	case study/	80054
70	case report.tw.	444799
71	abstract report/ or letter/	1155908
72	69 or 70 or 71	1669914

73	68 not 72	3064021
74	(exp animal/ or animal.hw. or nonhuman/) not (exp human/ or human cell/ or (human or humans).ti.)	7230873
75	73 not 74	2969724
76	48 and 75	2373
77	76 – limited 2017 to 2020	1065

Cochrane Register of Controlled Trials (CENTRAL)

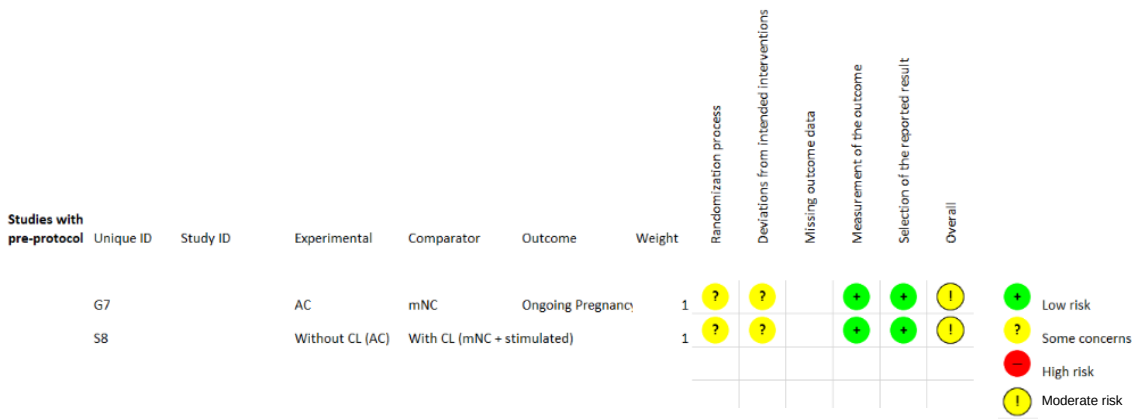
Set	Search	Results
1	((Endometrial Preparation OR Cycle OR Natural Cycle OR Artificial Cycle OR Modified Natural Cycle OR Stimulated Cycle) AND (Pregnancy OR Pregnancy Outcomes OR Clinical Pregnancy OR Live Birth)) – Limited to 2017-2020	289

CINAHL

Set	Search	Results
1	MM Cryopreservation+	1,545
2	TX Cryopreserv* N7 embryo*	792
3	TX Cryopreserv* N7 blastocyst*	80
4	MM Freezing	229
5	TX vitrification N7 embryo*	124
6	TX vitrification N7 blastocyst*	58
7	TX frozen N5 embryo*	1,186
8	TX freez* N5 embryo*	360
9	TX freez* N5 blastocyst*	22
10	TX frozen N5 blastocyst*	128
11	TX FET	1,353
12	(TX FET) AND (S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 OR S9 OR S10 OR S11)	1,353
13	MM ovulation induction	973
14	TX (ovar* N5 stimula*) or (ovulat* N5 induct*)	3,738
15	TX (endometri* N2prepar*)	181
16	MM Clomiphene	250
17	TX Clomiphene or TX clomid	1,128
18	TX Menotropin* or menopausal gonadotrop* or HMG)	3,785
19	MM Follicle-Stimulating Hormone	602
20	TX Follicle Stimulating Hormone or FSH	6,532
21	MM Gonadorelin	989
22	MM Pituitary Hormone Release Inhibiting Hormones	3
23	TX Gonadotrop?in-Releasing Hormone*	344
24	TX GnRH*	2,961
25	MM Estrogens	3,969
26	TX oestrogen or estrogen	46,066
27	MM Progesterone	1,914
28	TX Progesterone	17,782
29	TX natural* N2 cycle*	1,104
30	TX (artificial* N2 cycle*)	137

31	TX (cycle* N2 regimen*)	626
32	TX pituitary suppression	472
33	TX spontaneous* ovulat*	145
34	TX stimulat* N3 cycle	1,335
35	((TX stimulat* N3 cycle OR S13 OR S14 OR S15 OR S16 OR S17 OR S18 OR S19 OR S20 OR S21 OR S22 OR S23 OR S24 OR S25 OR S26 OR S27 OR S28 OR S29 OR S30 OR S31 OR S32 OR S33 OR S34)) AND (S13 OR S14 OR S15 OR S16 OR S17 OR S18 OR S19 OR S20 OR S21 OR S22 OR S23 OR S24 OR S25 OR S26 OR S27 OR S28 OR S29 OR S30 OR S31 OR S32 OR S33 OR S34)	65,832
36	S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7OR S8 OR S9 OR S10 OR S11	4,623
37	(35 AND 36)	2,453
38	MH Clinical Trials+	303,701
39	PT Clinical trial	107,329
40	TX clinic* n1 trial*	393,652
41	TX(singl* n1 blind*) or (singl* n1 mask*)) or TX ((doubl* n1 blind*) or (doubl* n1 mask*)) or TX((tripl* n1 blind*) or (tripl* n1 mask*)) or TX ((trebl* n1 blind*) or (trebl*n1 mask*))	1,177,377
42	TX randomi* control *trial*	298,795
43	MH "Random Assignment"	63,059
44	TX random* allocat*	22,292
45	TX placebo*	125,194
46	MH Placebos	12,837
47	MH Quantitative Studies	27,500
48	TX allocat* random*	22,292
49	S38 OR S39 OR S40 OR S41 OR S42 OR S43 OR S44 OR S45 OR S46 OR S47 OR S48	1,648,483
50	S37 AND S49	817
51	S37 AND S49	225
52	51 – Limited 2017-2020	158

Supplementary File 2 - Supplementary Table 2: Quality of Randomised Controlled Trials using the Revised Cochrane Risk-of-Bias tool 2



Greco 2016:

Domain	Signalling question	Response	Comments
Bias arising from the randomization process	1.1 Was the allocation sequence random?	Y	Two hundred thirty-six patients were included in the study and randomized in two groups according to computer-generated, not cancelled, simple randomization list with allocation assignment. Both the patient and the clinicians were informed of the assigned treatment. Difficult to conceal due to the nature of the intervention.
	1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?	N	
	1.3 Did baseline differences between intervention groups suggest a problem with the randomization process?	N	Baseline characteristics of the patients were not significantly different.
	Risk of bias judgement	**Some concerns**	
Bias due to deviations from intended interventions	2.1 Were participants aware of their assigned intervention during the trial?	Y	Both pateints and clinicians were aware of the assigned intervention. However, due to the nature of the intervention, it would have been difficult to conceal.
	2.2 Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?	Y	
	2.3. [If applicable:] If Y/PY/NI to 2.1 or 2.2: Were important non-protocol interventions balanced across intervention groups?	NA	
	2.4. [If applicable:] Were there failures in implementing the intervention that could have affected the outcome?		
	2.5. [If applicable:] Was there non-adherence to the assigned intervention regimen that could have affected participants' outcomes?	NA	
	2.6. If N/PN/NI to 2.3, or Y/PY/NI to 2.4 or 2.5: Was an appropriate analysis used to estimate the effect of adhering to the intervention?	NA	
	Risk of bias judgement	**Some concerns**	
Bias due to missing outcome data	3.1 Were data for this outcome available for all, or nearly all, participants randomized?	Y	Missing data was accounted for e.g. premature LH surge, inadequate endometrial thickness
	3.2 If N/PN/NI to 3.1: Is there evidence that result was not biased by missing outcome data?	NA	
	3.3 If N/PN to 3.2: Could missingness in the outcome depend on its true value?	NA	
	3.4 If Y/PY/NI to 3.3: Is it likely that missingness in the outcome depended on its true value?	NA	
	Risk of bias judgement		
Bias in measurement of the outcome	4.1 Was the method of measuring the outcome inappropriate?	N	Live birth rates is an appropriate outcome measurement
	4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?	N	Definitions used for the measurement of outcomes was the same in both groups
	4.3 Were outcome assessors aware of the intervention received by study participants?	Y	Probably not, as the outcomes are objective rather than subjective
	4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?	PN	
	4.5 If Y/PY/NI to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?	NA	
	Risk of bias judgement	**Low**	
Bias in selection of the reported result	5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?	Y	
	5.2 ... multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain?	PN	
	5.3 ... multiple eligible analyses of the data?	PN	
	Risk of bias judgement	**Low**	
Overall bias	**Risk of bias judgement**	**Some concerns**	

Sheikhi 2018:

Domain	Signalling question	Response	Comments
Bias arising from the randomization process	1.1 Was the allocation sequence random?	Y	The randomization was done at the start of the cycle using sequential numbering based on a computer-generated list that had been prepared at the Statistics Center of the Babol University of Medical Science and sent to them. Both participants and clinicians were aware of the treatment allocation.
	1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?	N	
	1.3 Did baseline differences between intervention groups suggest a problem with the randomization process?	N	Baseline characteristics were fairly similar across both treatment groups.
	Risk of bias judgement	**Some concerns**	Difficult to implement blinding and concealment due to the nature of the intervention.
Bias due to deviations from intended interventions	2.1 Were participants aware of their assigned intervention during the trial?	Y	Yes, as it is difficult to blind participants and clinicians due to the nature of the intervention
	2.2 Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?	Y	
	2.3. [If applicable:] If Y/PY/NI to 2.1 or 2.2: Were important non-protocol interventions balanced across intervention groups?	NA	
	2.4. [If applicable:] Were there failures in implementing the intervention that could have affected the outcome?	PY	Seven women were lost to follow-up (with explainations)
	2.5. [If applicable:] Was there non-adherence to the assigned intervention regimen that could have affected participants' outcomes?	NA	
	2.6. If N/PN/NI to 2.3, or Y/PY/NI to 2.4 or 2.5: Was an appropriate analysis used to estimate the effect of adhering to the intervention?		
	Risk of bias judgement	**Some concerns**	
Bias due to missing outcome data	3.1 Were data for this outcome available for all, or nearly all, participants randomized?	PY	
	3.2 If N/PN/NI to 3.1: Is there evidence that result was not biased by missing outcome data?	NA	
	3.3 If N/PN to 3.2: Could missingness in the outcome depend on its true value?	NA	
	3.4 If Y/PY/NI to 3.3: Is it likely that missingness in the outcome depended on its true value?	NA	
	Risk of bias judgement		
Bias in measurement of the outcome	4.1 Was the method of measuring the outcome inappropriate?	PN	Live births would have been a better measure of outcome, however as pregnancy loss after 20 weeks is very rare, it is still an appropriate outcome.
	4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?	N	Outcome measurements are objective rather than subjective due to the nature of the study.
	4.3 Were outcome assessors aware of the intervention received by study participants?	Y	
	4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?	PN	Outcome measurements are objective rather than subjective due to the nature of the study.
	4.5 If Y/PY/NI to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?	NA	
	Risk of bias judgement	**Low**	
Bias in selection of the reported result	5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?	PY	
	5.2 ... multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain?	PN	
	5.3 ... multiple eligible analyses of the data?	PN	
	Risk of bias judgement	**Low**	
Overall bias	**Risk of bias judgement**	**Some concerns**	

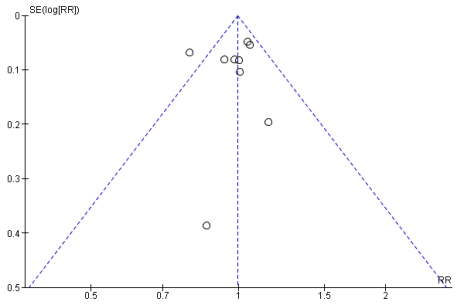
Supplementary File 3 - Supplementary Table 3: Quality of Observational Studies using the Newcastle-Ottawa Scale

Authors		Alur-Gupta et al, 2018	Cardenas Armas et al, 2019	Chang et al, 2011	Givens et al, 2009	Le et al, 2017	Levi Setti et al, 2020	Pakes et al, 2020
	Item							
A	**Selection**							
	Exposed cohort is truly representative of the average	−	−	+	−	−	−	−
	Selection of the non-exposed cohort from the same community	−	−	+	−	−	−	−
	Exposure ascertained by a secure record or interview	+	+	+	+	+	+	+
	Demonstration of outcome of interest was not present at the start of the study	+	+	+	+	+	+	+
B	**Comparability***							
	Study controls for additional variables	+	+	+	−	+	+	+
C	**Outcome**							
	Follow-up was adequate for outcome to occur	+	+	−	+	+	+	+
	Complete follow-up of all subjects was accounted for	+	+	+	+	+	+	+
	Subjects lost to follow up were unlikely to introduce bias	+	+	+	+	+	+	+
	Score (_/9)	6	7	7	5	6	6	6
	Conversion to AHRQ Standards	fair	good	good	fair	fair	fair	fair

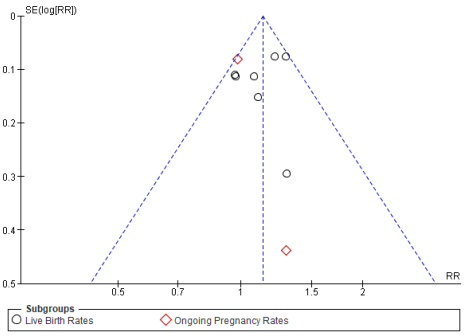
AHRQ, Agency for Healthcare Research and Quality
*Comparability may have up to a maximum of 2 points

Supplementary File 4 - Supplementary Figure 1: Funnel Plot Analyses

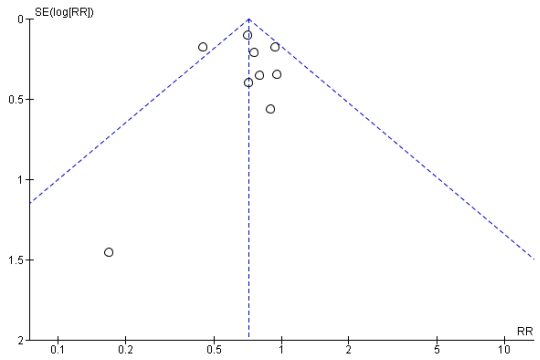
Funnel Plot for Positive hCG Rates



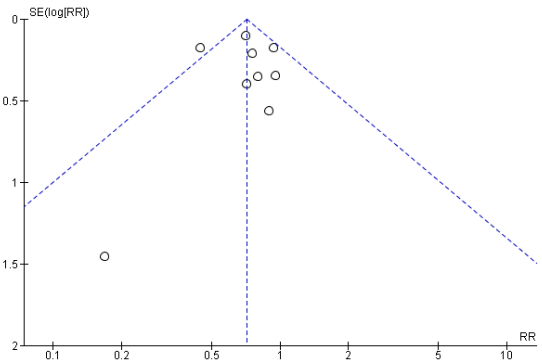
Funnel Plot for Live Birth Rates



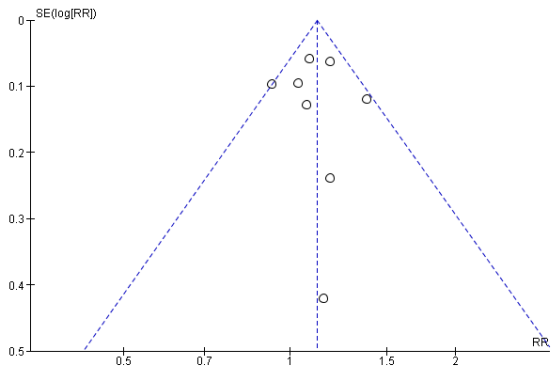
Funnel Plot for Clinical Pregnancy Rates



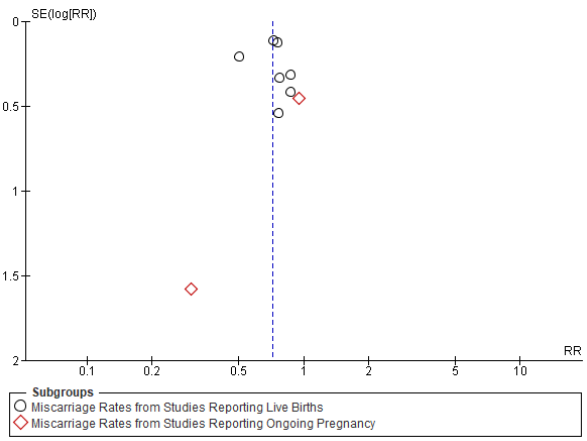
Funnel Plot for Biochemical Pregnancy Rates



Funnel Plot for Clinical Pregnancy Rates – Sensitivity Analysis

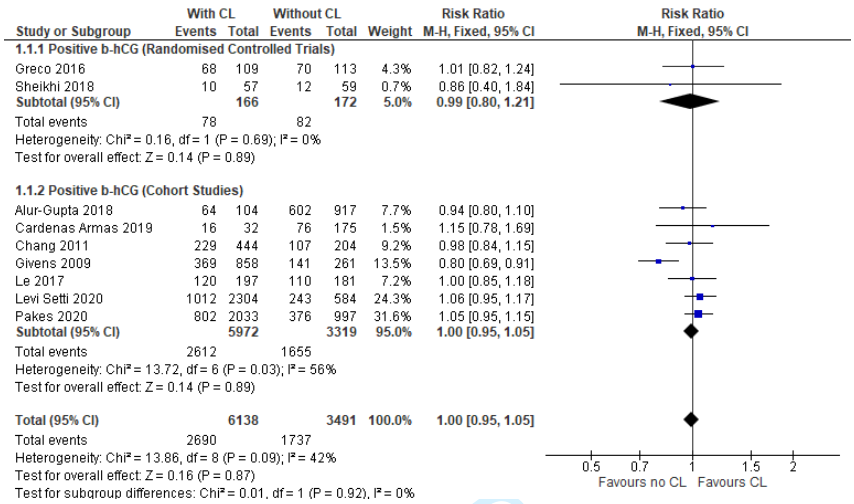


Funnel plot for Miscarriage Rates

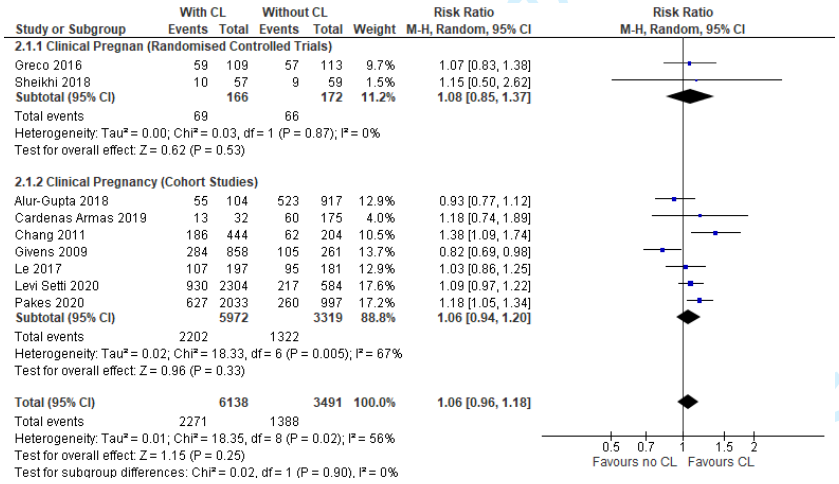


Supplementary File 5 - Supplementary Figure 2: Meta-analysis comparing rates of positive hCG, clinical pregnancy and live births in cycles with and without a corpus luteum – separated by study design

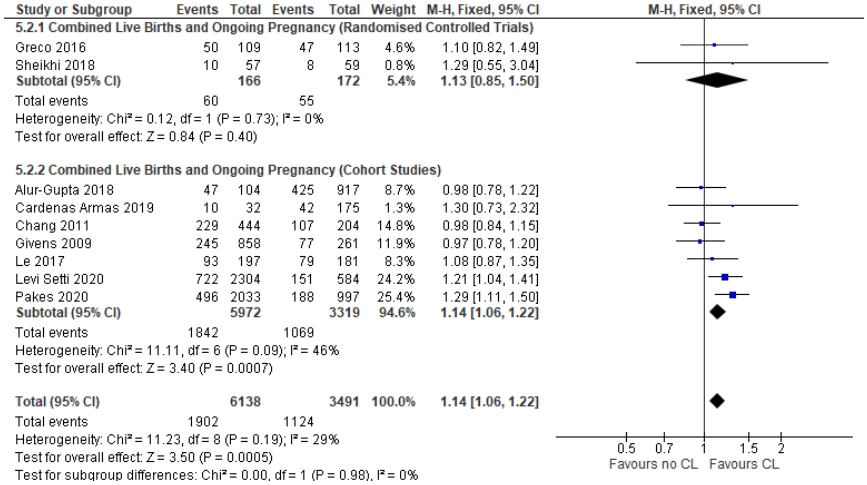
Positive hCG Rates



Clinical Pregnancy Rates



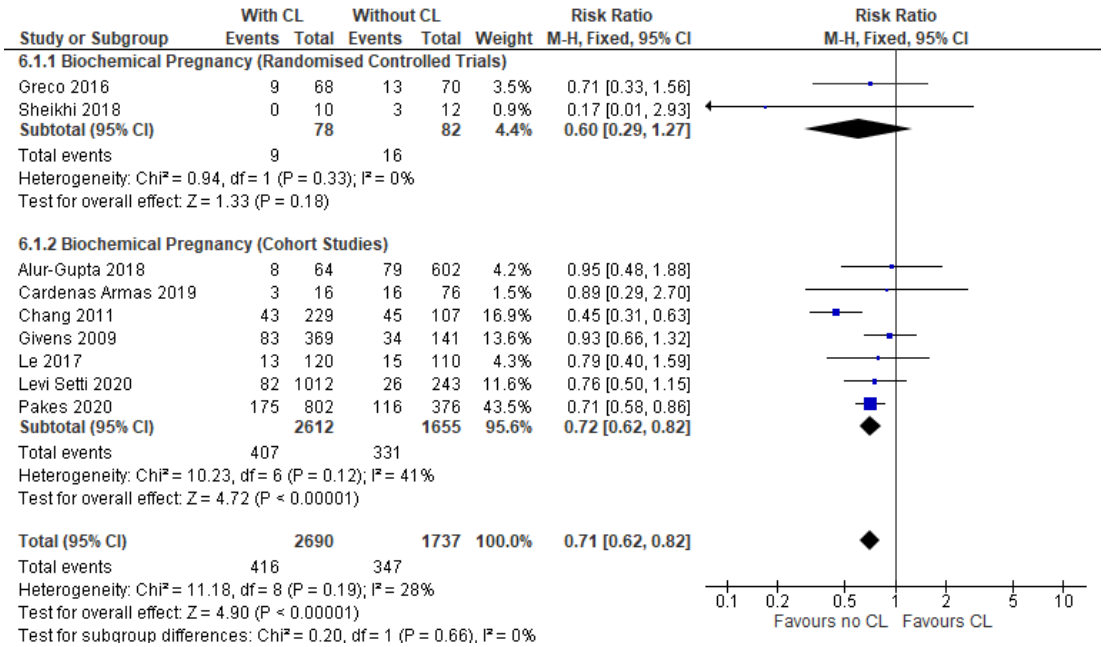
Live Birth Rates



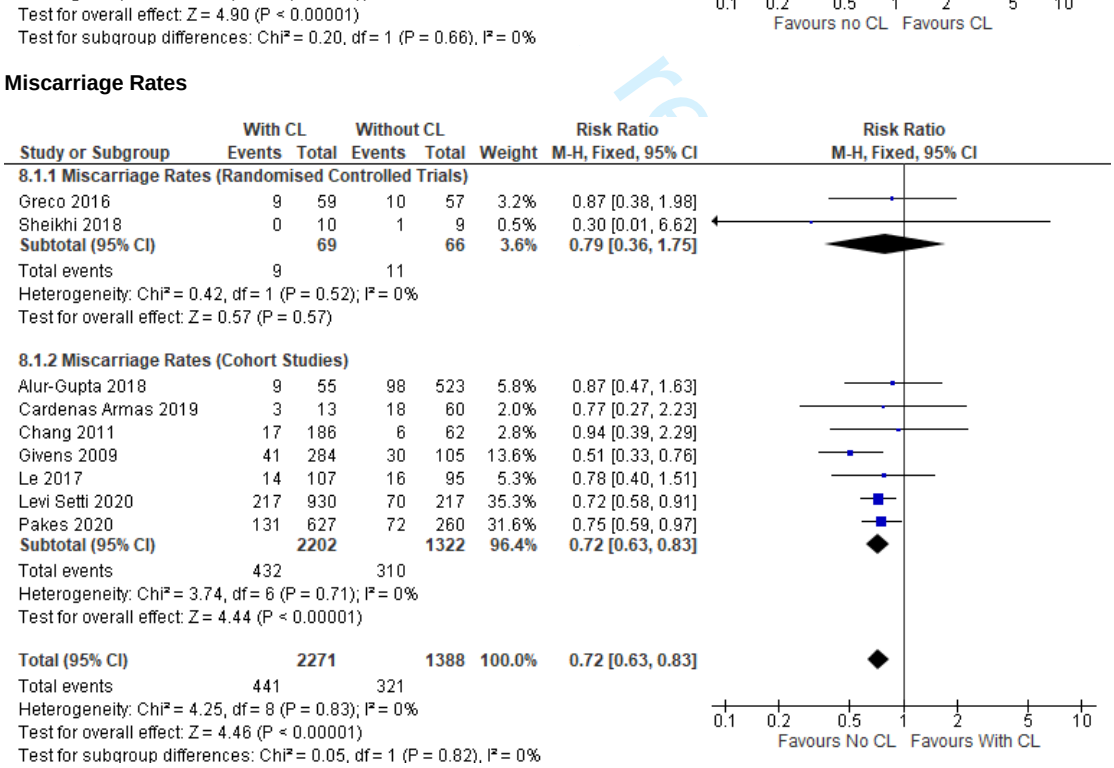
CL, Corpus luteum; CI, Confidence Interval

Supplementary File 6 - Supplementary Figure 3: Meta-analysis comparing rates of pregnancy losses in cycles with and without a corpus luteum – separated by study design

Biochemical Pregnancy Rates (Early Miscarriage)

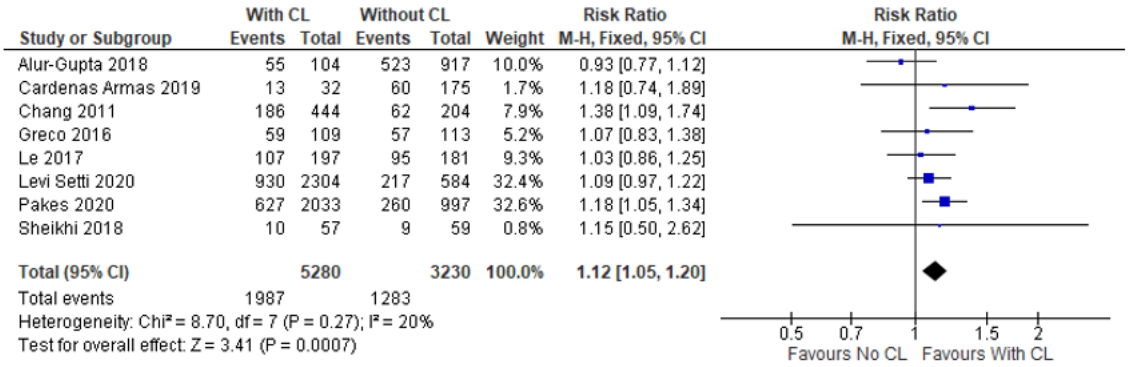


Miscarriage Rates



CL, Corpus luteum; CI, Confidence Interval

Supplementary File 7 - Supplementary Figure 4: Meta-analysis comparing clinical pregnancy rates in cycles with and without a corpus luteum – sensitivity analysis



CL, Corpus Luteum; CI, Confidence interval

PRISMA Checklists

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	**Page 2** (line 22-24)
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	**Page 5** (line 1 to 60) to **page 6** line 1 to 5)
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	**Page 7** (line 2 to 60) to page 8 (line 1 to 7)
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	**Page 8** (line 12 to 23)
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	**Page 8** (line 27)
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	**Page 8** (line 12 to 38; line 52 to 58) to **Page 9** (line 2 to 5)
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	**Page 8** (line 25 to 36)

Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Supplementary file 1
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	**Page 8** (line 44 to 58) to page 9 (line 3 to 5)
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	**Page 10** (line 52 to 50)
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	**Page 9** (line 13 to 50)
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	**Page 10** (line 1 to 11; line 29 to 33)
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	**Page 10** (line 22 to 25)
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	**Page 10** (line 14 to 26)
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	**Page 10** (line 3 to 10; line 24 to 25) Supplementary file 8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	**Page 9** (line 26 to 30; page 10 line 33)
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	**Page 10** (line 37 to 47) **Figure 1**
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	**Table 1** (page 12) **Page 22** (line

			22 to 60) to **Page 23** (line 1 to 17)
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	**Table 1** (page 12); **Supplementary file 2 and 3**
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Refer to **Figures 2 & 3**
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	**Page 14** (line 2 to 10) **Page 15** (line 1 60) **Page 16** (line 1 to 60) **Page 17** (line 1 to 60) **Page 18** (line 1 to 7)
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	**Page 10** (line 3 to 10; line 24 to 25) **Supplementary file 8**
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	**Page 16** (line 12 to 18; line 26; line 48 to 52) **Supplementary file 5**
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	**Page 18** (line 12 to 18

Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	**Page 18** (line 46 to 60) **Page 20** (line 39 to 54)
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	**Page 21** (line 3 to 18)
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	**Page 6** (line 1 to 5)