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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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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 27 July 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 V.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 controlled trials, and seven were cohort studies. Subgroup analysis of the different study designs was performed. While the rates of positive human chorionic gonadotropin results (relative risk, RR 1.0, 95% CI 0.95 to 1.05) and clinical pregnancies (RR 1.06, 95% CI 0.96 to 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 to 1.22). Analysis of pregnancy losses demonstrated that both biochemical pregnancy (early miscarriage) (RR 0.71, 95% CI 0.62 to 0.82) and miscarriages (RR 0.72, 95% CI 0.62 to 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.

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

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Strengths and limitations of this study

► A strength of this study includes its limitation to include only blastocysts in thaw cycle transfers, which is becoming increasingly more common practice.

► Another strength of this study is the meta-analysis performed, which increased the strength of each individual study to look at trends otherwise not observed.

► The limitations of this study include the limited number of studies in the area and lack of high quality randomised controlled trials.

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medicine. With improvements in the vitrification processes, culture mediums and desire for single embryo transfer, 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 with 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 NC (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 oestrogen (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 10–12 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 (LB), 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 patients involved.

Search strategy
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 used three key concepts: endometrial preparation AND frozen embryos AND reproductive outcomes. The detailed search strategy can be found in online supplemental 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 Preferred Reporting Items for Systematic Reviews and Meta-Analyses 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 and 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 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 on by the International Glossary on Infertility and Fertility

Care, 2017.[14] An 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 LB rates. However, we performed a subanalysis of the studies which reported LBs 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*,[16] did not report clinical pregnancy, hence it was calculated by adding the number of LBs, 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 were independently extracted by three reviewers (GR, AP and 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, LBs, or ongoing births where LBs were not available. The data were collated by a single reviewer (JG) and any discrepancies were discussed among three reviewers and until a consensus was reached.

Quality assessment

Included randomised controlled trials (RCTs) were quality assessed using the Revised Cochrane Risk of Bias Tool for randomised trials (RoB 2).[17] The Newcastle-Ottawa Scale 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 online supplemental files 2 and 3.

Statistical analysis

The meta-analysis was performed using RevMan V.5.4.1 computer programme, The Cochrane Collaboration, 2020.[19] Meta-analyses of rates of positive hCG, LBs,

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 χ^2 statistic. P values of χ^2 that were <0.05, and I^2 >50% were considered represent significant heterogeneity. Relative risk with 95% CIs was 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 (online supplemental file 4).

As we included studies that reported ongoing pregnancy rates where LB rates were not available, we conducted a subgroup 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 online supplemental files 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 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 summarised 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*,[10] and the smallest study included 116 cycles by Sheikhi *et al*.[23]

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

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 (relative risk, RR 1.00, 95% CI 0.95 to 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 online supplemental 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
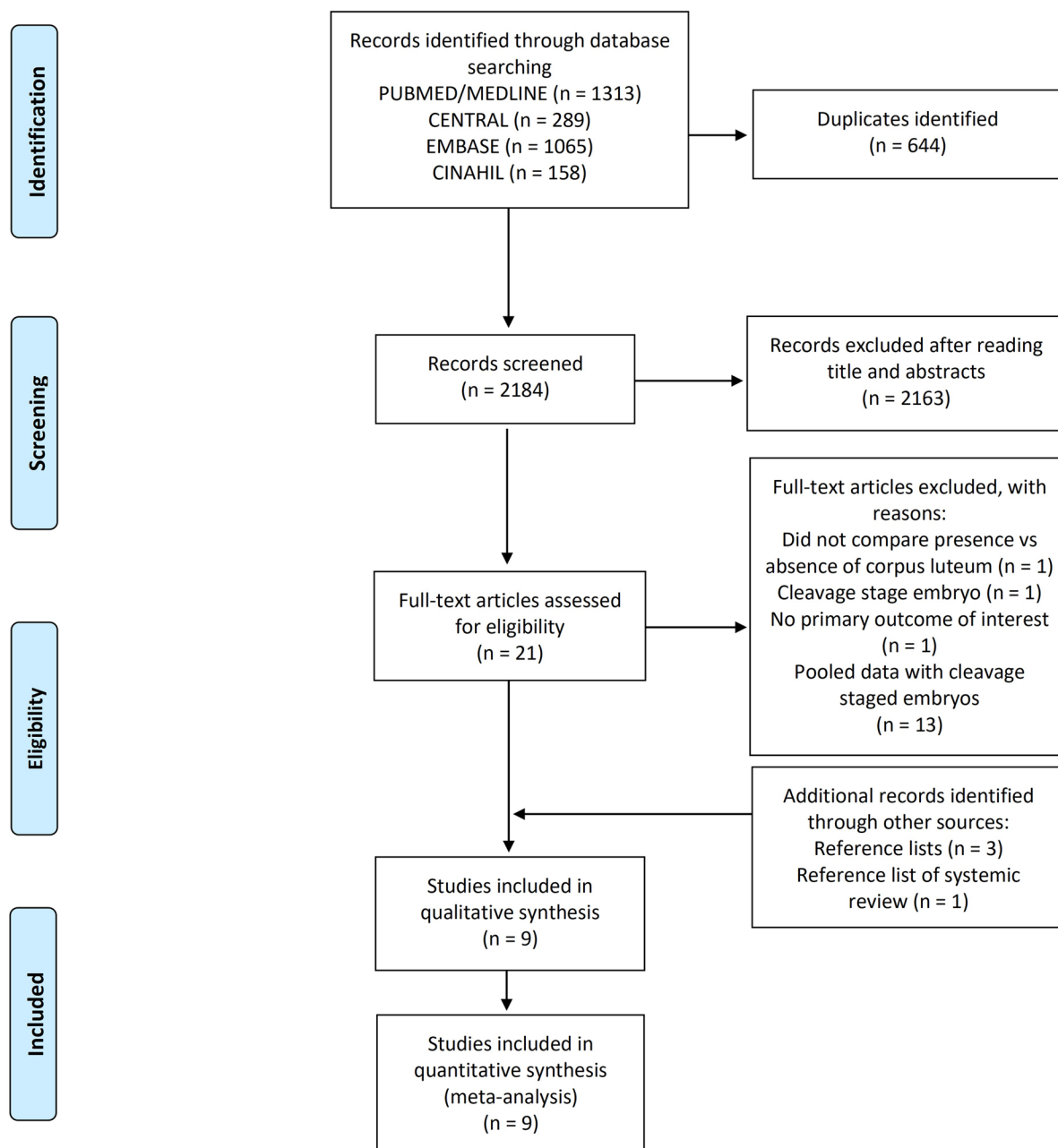
Identification

Records identified through database
searching
PUBMED/MEDLINE (n = 1313)
CENTRAL (n = 289)
EMBASE (n = 1065)
CINAHIL (n = 158)

Duplicates identified
(n = 644)

Screening

Records screened
(n = 2184)

Records excluded after reading
title and abstracts
(n = 2163)

Full-text articles excluded, with
reasons:
Did not compare presence vs
absence of corpus luteum (n = 1)
Cleavage stage embryo (n = 1)
No primary outcome of interest
(n = 1)
Pooled data with cleavage
staged embryos
(n = 13)

Eligibility

Full-text articles assessed
for eligibility
(n = 21)

Additional records identified
through other sources:
Reference lists (n = 3)
Reference list of systemic
review (n = 1)

Included

Studies included in
qualitative synthesis
(n = 9)

Studies included in
quantitative synthesis
(meta-analysis)
(n = 9)

Figure 1 PRISMA flow chart. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

for clinical pregnancy are shown in figure 2. The pooled estimates for clinical pregnancy rates (RR 1.06, 95% CI 0.96 to 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[20] which was the only study to observe a higher clinical pregnancy rate in AC compared with NCs. The results of this are shown in online supplemental file 7. The sensitivity analysis showed that LB rates were statistically higher in the cycles involving the presence of a CL (RR 1.12, 95% CI 1.05 to 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 CI 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 online supplemental file 5.

LB 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

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	Study Period	Cycles with blastocysts (n)	Allocation	Women (n)	Study population	Mean age, years (SD)	BMI, kg/m² (SD)	Positive -hCG (n)	CP (n)	LB/OP	Quality*
Alur-Gupta (2018)[16]	Retrospective Cohort	2013–2017	1021 Cycles (with CL=104, without CL=917)	Clinical judgement	NR	Both normo-ovulatory patients and women with ovulatory dysfunction	NC=35.6 (3) AC=35.4 (4)	NC=23.2 (3.7) AC=25.1 (5.3)	With CL=64 Without CL=602	With CL=55 Without CL=523	LB	Fair
Cardenas Armas (2019)[24]	Retrospective Cohort	2014–2017	207 Cycles (with CL=32; without CL=175)	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 (2.2) AC (Oral)=23.3 (1.7)	With CL=16 Without CL=76	With CL=13 Without CL=60	LB	Good
Chang (2011)[21]	Retrospective Cohort	2007–2009	648 Cycles (with CL=444, without CL=204)	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)[20]	Retrospective Cohort	2000–2006	1119 Cycles (with CL=858, without CL=261)	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)[22]	RCT	2015	222 Cycles (with CL=109, without CL=113)	Computer-generated randomisation (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)[26]	Retrospective Cohort	2006–2014	378 cycles (with CL 197, without CL=181)	Clinical judgement	428†	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 (2020)[25]	Retrospective Cohort	2011–2017	2888 Cycles (with CL=2304, without CL=584)‡	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=21.8 (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

Continued

Table 1 Continued

Study	Study design				Demographics				Outcomes			
	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*
Pakes (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 (2018)[23]§	RCT	116 Cycles (with CL=57, without CL=59)	2015–2016	Computer-generated randomisation (non-concealed)	123¶	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.
*Quality assessed with Cochrane Risk of Bias tool 2 or Newcastle-Ottawa Scale.
†66 women excluded due to various reasons.
‡Therapeutic abortion cycles excluded.
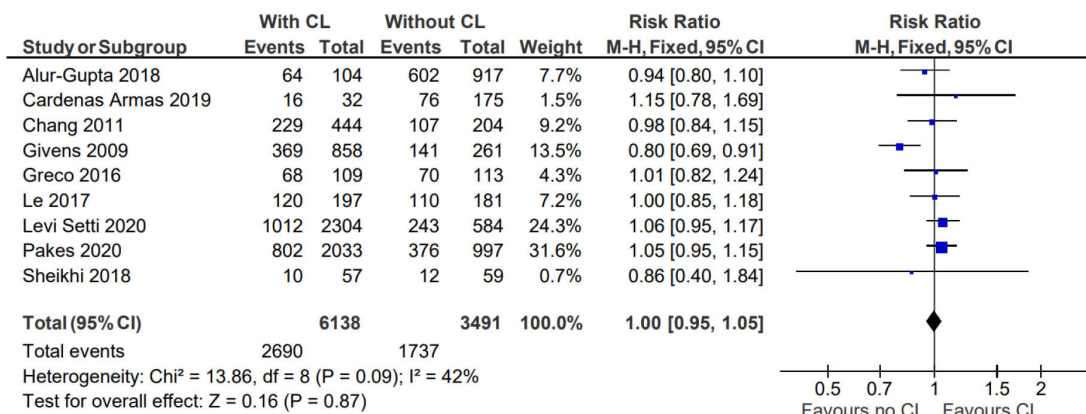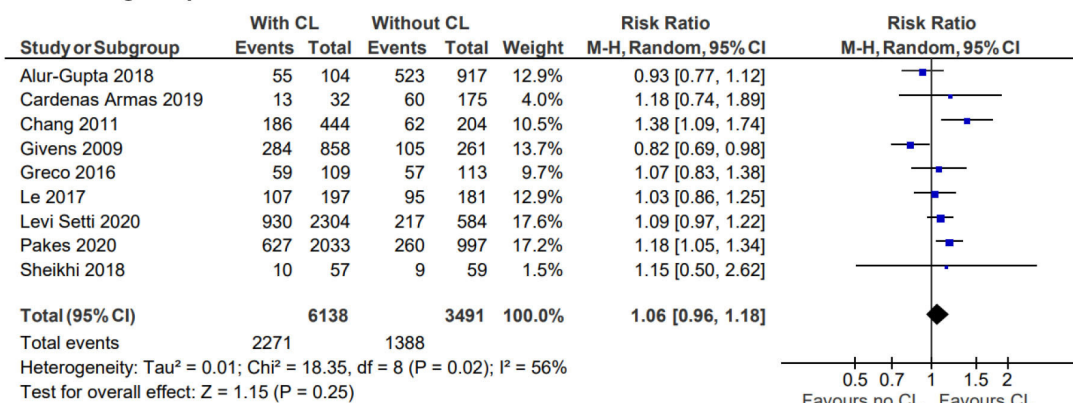§Demographic data extracted from table 1 of study (conflicted data reported in written results section).
¶17 women lost to follow-up.
AC, artificial cycle; CL, corpus luteum; GnRHa, gonadotropin-releasing hormone analogue; LB, live birth; mNC, modified natural cycle; mSC, mildly stimulated cycle; NR, not reported; PGT, preimplantation genetic testing; RCT, randomised controlled trial.
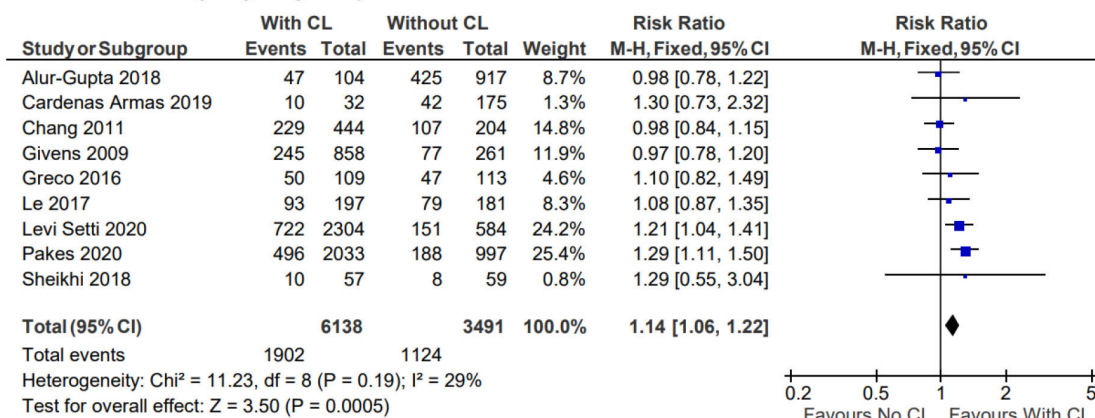
Rates of Positive hCG

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%
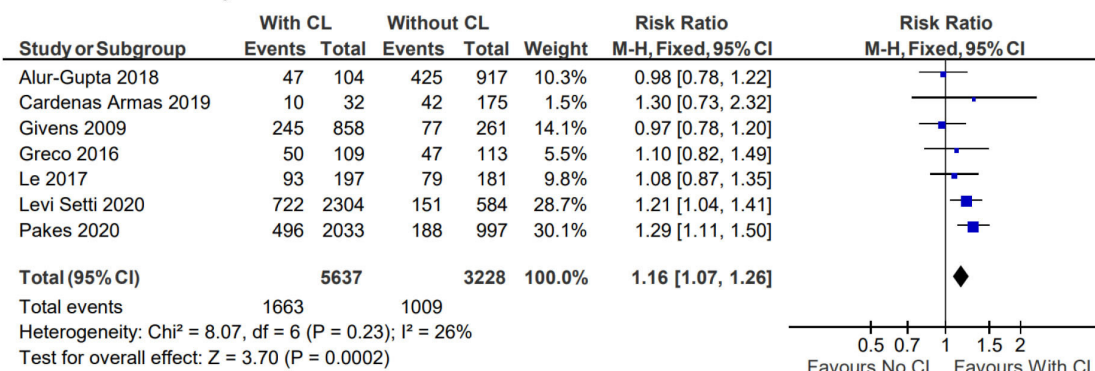Test for overall effect: Z = 3.70 (P = 0.0002)

Figure 2 Meta-analysis comparing rates of positive hCG, clinical pregnancy, and live births in cycles with and without a CL. CL, Corpus Luteum; hCG, human chorionic gonadotropin; M-H, Mantel-Haenszel.

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 an LB or progressed to an ongoing pregnancy. In the 3491 cycles without a CL, 1124 (32%) resulted in an LB or ongoing pregnancy. The individual and combined estimates for LBs are shown in figure 2. The pooled estimates for LBs (RR 1.14, 95% CI 1.06 to 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 LB 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 LB rate remained significantly higher in the thaw cycles with a CL (RR 1.16, 95% CI 1.07 to 1.26). Subgroup analysis of LB rates by study design is shown in online supplemental 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 (ie, 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 to 0.82) were significantly lower in the cycles with a CL. Subgroup analysis of biochemical pregnancy rates by study design is shown in online supplemental 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 to 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 online supplemental 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
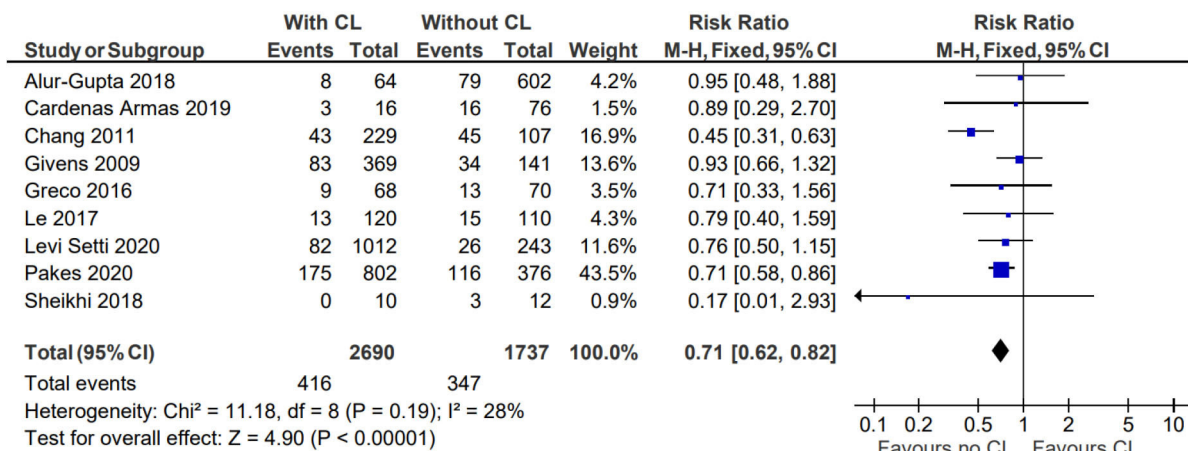
CL may not influence initial implantation but may play a significant role in sustaining a pregnancy once an embryo has implanted.

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, 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, demonstrated higher birth rates in the AC compared with 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 generalisable 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 with 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, demonstrated a higher miscarriage rate in the AC cycle group compared with the group receiving the NC protocol.[32] Similar findings were observed in the study by Givens et al.[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 found that miscarriage rates were higher in the AC group (23.0%) compared with the NCs (11.4%, p<0.0001).[33] However,
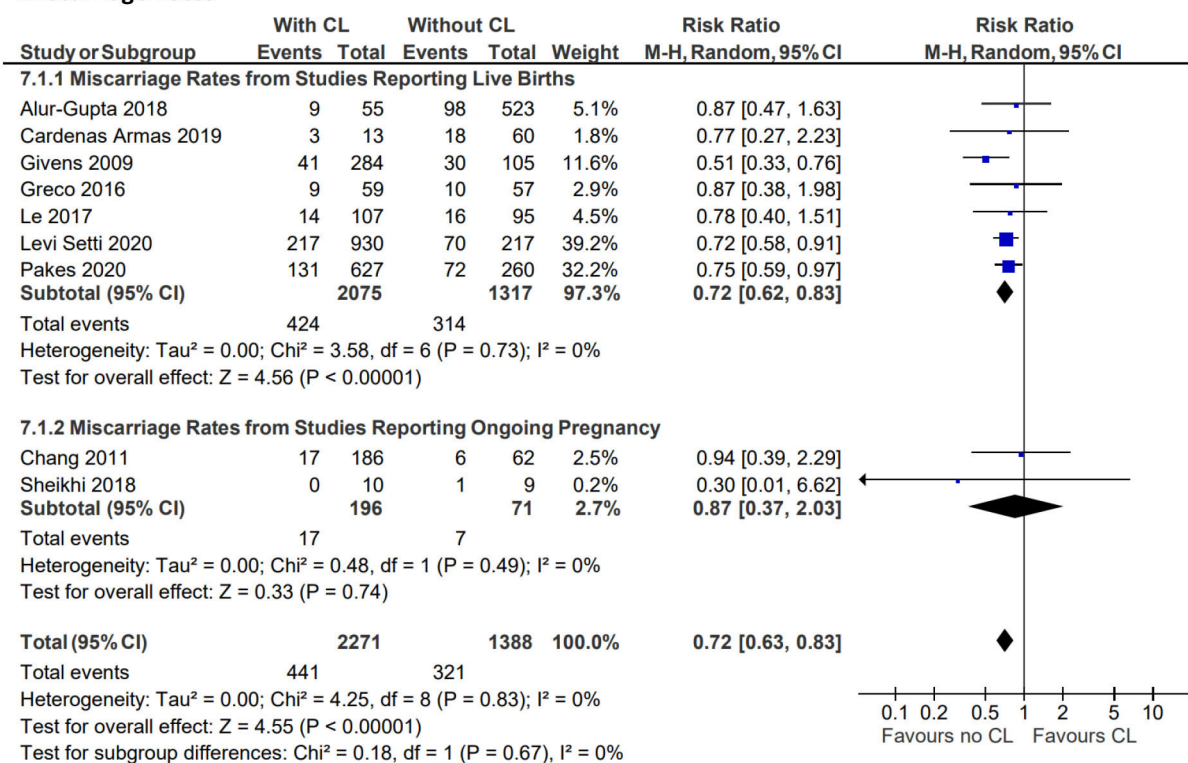
Biochemical Pregnancy Rates

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	8	64	79	602	4.2%	0.95 [0.48, 1.88]
Cardenas Armas 2019	3	16	16	76	1.5%	0.89 [0.29, 2.70]
Chang 2011	43	229	45	107	16.9%	0.45 [0.31, 0.63]
Givens 2009	83	369	34	141	13.6%	0.93 [0.66, 1.32]
Greco 2016	9	68	13	70	3.5%	0.71 [0.33, 1.56]
Le 2017	13	120	15	110	4.3%	0.79 [0.40, 1.59]
Levi Setti 2020	82	1012	26	243	11.6%	0.76 [0.50, 1.15]
Pakes 2020	175	802	116	376	43.5%	0.71 [0.58, 0.86]
Sheikhi 2018	0	10	3	12	0.9%	0.17 [0.01, 2.93]
Total (95% CI)		**2690**		**1737**	**100.0%**	**0.71 [0.62, 0.82]**
Total events	416		347			

Heterogeneity: Chi² = 11.18, df = 8 (P = 0.19); I² = 28%
Test for overall effect: Z = 4.90 (P < 0.00001)

Forest plot scale: 0.1 0.2 0.5 1 2 5 10; Favours no CL / Favours CL

Miscarriage Rates

Study or Subgroup	With CL Events	With CL Total	Without CL Events	Without CL Total	Weight	Risk Ratio M-H, Random, 95% CI
7.1.1 Miscarriage Rates from Studies Reporting Live Births						
Alur-Gupta 2018	9	55	98	523	5.1%	0.87 [0.47, 1.63]
Cardenas Armas 2019	3	13	18	60	1.8%	0.77 [0.27, 2.23]
Givens 2009	41	284	30	105	11.6%	0.51 [0.33, 0.76]
Greco 2016	9	59	10	57	2.9%	0.87 [0.38, 1.98]
Le 2017	14	107	16	95	4.5%	0.78 [0.40, 1.51]
Levi Setti 2020	217	930	70	217	39.2%	0.72 [0.58, 0.91]
Pakes 2020	131	627	72	260	32.2%	0.75 [0.59, 0.97]
Subtotal (95% CI)		**2075**		**1317**	**97.3%**	**0.72 [0.62, 0.83]**
Total events	424		314			

Heterogeneity: Tau² = 0.00; Chi² = 3.58, df = 6 (P = 0.73); I² = 0%
Test for overall effect: Z = 4.56 (P < 0.00001)

Study or Subgroup	With CL Events	With CL Total	Without CL Events	Without CL Total	Weight	Risk Ratio M-H, Random, 95% CI
7.1.2 Miscarriage Rates from Studies Reporting Ongoing Pregnancy						
Chang 2011	17	186	6	62	2.5%	0.94 [0.39, 2.29]
Sheikhi 2018	0	10	1	9	0.2%	0.30 [0.01, 6.62]
Subtotal (95% CI)		**196**		**71**	**2.7%**	**0.87 [0.37, 2.03]**
Total events	17		7			

Heterogeneity: Tau² = 0.00; Chi² = 0.48, df = 1 (P = 0.49); I² = 0%
Test for overall effect: Z = 0.33 (P = 0.74)

	With CL		Without CL			Risk Ratio
Total (95% CI)		**2271**		**1388**	**100.0%**	**0.72 [0.63, 0.83]**
Total events	441		321			

Heterogeneity: Tau² = 0.00; Chi² = 4.25, df = 8 (P = 0.83); I² = 0%
Test for overall effect: Z = 4.55 (P < 0.00001)
Test for subgroup differences: Chi² = 0.18, df = 1 (P = 0.67), I² = 0%

Forest plot scale: 0.1 0.2 0.5 1 2 5 10; Favours no CL / Favours CL

Figure 3 Meta-analysis comparing biochemical pregnancy and miscarriage rates in cycles with and without a CL. CL, corpus luteum; M-H, Mantel-Haenszel.

the BMI of the women in the AC were statistically higher compared with the NC (25.3±5.4, 22.9±3.6, p<0.0001) which may have influenced the miscarriage rate. Similarly, a retrospective study by Guan et al,[34] which analysed 1482 thawed cleavage-staged embryos noted that women in the NC group experienced significantly lower rates of miscarriage (2.8%) compared with those in the women receiving the AC with GnRHa (14.0%, p=0.003).[34] This may be influenced by the statistically older age of women receiving the AC with GnRHa compared with the women in the NC group. Another retrospective study involving normo-ovulatory women by Cerillo et al, observed statistically higher miscarriage rates in the women receiving AC (21.2%), compared with the women receiving mNC (12.9%) and the tNC (11.1%).[35] In a recent retrospective analysis by Liu et al, 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 with the mNC arm (8.37%, p=0.034).[36] Again, as these studies included cleavage-stage embryos their findings may not be generalisable to our research question, which involves data on blastocyst embryos. A recent large retrospective study by Pakes et al which analysed blastocyst thaw cycles, observed that the AC group experienced a higher pregnancy loss compared with 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 with the NC which may have biased results to favour the AC, however, the AC group still demonstrated more pregnancy losses compared with the NC group.

There may be several contributing factors influencing this observed increased rate of pregnancy loss in thaw cycles without a CL. First, 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 with cycles involving a CL. In the AC, oestrogen 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. Second, 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 intrauterine 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 optimised 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 OR 1.61, 95% CI 1.22 to 2.10), postpartum haemorrhage (adjusted OR 2.87, 95% CI 2.29 to 2.60), post-term birth (adjusted OR 1.59, 95% CI 1.47 to 2.68) and macrosomia (adjusted OR 1.62, CI 1.03 to 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 OR 1.43, 95% CI 1.14 to 1.8) and placenta accreta (adjusted OR, 6.91; 95% CI 2.87 to 16.66) compared with 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 CL number, it was noted that pregnancies without a CL did not exhibit the physiological 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 using 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 subgroup of embryo transfers which is also clinically relevant, with an increasing number of blastocyst transfers observed in clinical practice.

This study has several limitations. First, 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 (online supplemental 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 used 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 using adequately powered RCTs involving blastocyst thaw cycles is urgently required.

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