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Follicular flushing during oocyte retrieval in assisted reproductive techniques (Review)

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[Intervention Review]

Follicular flushing during oocyte retrieval in assisted reproductive techniques

Ektoras X Georgiou[1], Pedro Melo[2], Ying C Cheong[3], Ingrid E Granne[4]

[1]Complete Fertility Centre, Princess Anne Hospital, Southampton, UK. [2]Tommy's National Centre for Miscarriage Research, Institute of Metabolism and Systems Research, University of Birmingham, Birmingham, UK. [3]Human Development and Health Academic Unit, Faculty of Medicine, University of Southampton, Southampton, UK. [4]Nuffield Department of Women's & Reproductive Health, University of Oxford, Oxford, UK

Contact: Ektoras X Georgiou, hgeorgiou@gmail.com.

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ABSTRACT

Background

Follicular aspiration under transvaginal ultrasound guidance is routinely performed as part of assisted reproductive technology (ART) to retrieve oocytes for in vitro fertilisation (IVF). The process involves aspiration of the follicular fluid followed by the introduction of flush, typically culture media, back into the follicle followed by re-aspiration. However, there is a degree of controversy as to whether this intervention yields a larger number of oocytes and is hence associated with greater potential for pregnancy than aspiration only.

Objectives

To assess the safety and efficacy of follicular flushing as compared with aspiration only performed in women undergoing ART.

Search methods

We searched the following electronic databases up to 13 July 2021: the Cochrane Gynaecology and Fertility Specialised Register of Controlled Trials, CENTRAL (containing output from two trial registries and CINAHL), MEDLINE, Embase, and PsycINFO. We also searched LILACS, Google Scholar, and Epistemonikos. We reviewed the reference lists of relevant papers and contacted experts in the field to identify further relevant studies.

Selection criteria

We included randomised controlled trials (RCTs) that compared follicular aspiration and flushing with aspiration alone in women undergoing ART using their own gametes. Primary outcomes were live birth rate and miscarriage rate per woman randomised.

Data collection and analysis

Two review authors independently assessed studies identified by search against the inclusion criteria, extracted data, and assessed risk of bias. A third review author was consulted if required. We contacted study authors as needed. We analysed dichotomous outcomes using Mantel-Haenszel odds ratios (ORs), 95% confidence intervals (CIs), and a fixed-effect model, and we analysed continuous outcomes using mean differences (MDs) between groups presented with 95% CIs. We examined the heterogeneity of studies via the I^2 statistic. We assessed the certainty of evidence using the GRADE approach.

Main results

We included 15 studies with a total of 1643 women. Fourteen studies reported outcomes per woman randomised, and one study reported outcomes per ovary. No studies were at low risk of bias across all domains; the main limitation was lack of blinding. The certainty of the evidence ranged from moderate to very low, and was downgraded for risk of bias, imprecision, and inconsistency.

We are uncertain of the effect of follicular flushing on live birth rate compared to aspiration alone (OR 0.93, 95% CI 0.59 to 1.46; 4 RCTs; n = 467; I^2 = 0%; moderate-certainty evidence). This suggests that with a live birth rate of approximately 30% with aspiration alone, the equivalent live birth rate with follicular flushing lies between 20% and 39%. We are uncertain of the effect of follicular flushing on miscarriage rate compared to aspiration alone (OR 1.98, 95% CI 0.18 to 22.22; 1 RCT; n = 164; low-certainty evidence). This suggests that with a miscarriage rate of approximately 1% with aspiration alone, the equivalent miscarriage rate with follicular flushing lies between 0% and 22%.

We are uncertain of the effect of follicular flushing on oocyte yield (MD −0.47 oocytes, 95% CI −0.72 to −0.22; 9 RCTs; n = 1239; I^2 = 61%; very low-certainty evidence); total number of embryos (MD −0.10 embryos, 95% CI −0.34 to 0.15; 2 RCTs; n = 160; I^2 = 58%; low-certainty evidence); and clinical pregnancy rate (OR 1.12, 95% CI 0.85 to 1.51; 7 RCTs; n = 939; I^2 = 46%; low-certainty evidence). The duration of the retrieval process may be longer with flushing (MD 175.44 seconds, 95% CI 152.57 to 198.30; 7 RCTs; n = 785; I^2 = 87%; low-certainty evidence). It was not possible to perform a meta-analysis for adverse events, although individual studies reported on outcomes ranging from depression and anxiety to pain and pelvic organ injury.

Authors' conclusions

The effect of follicular flushing on both live birth and miscarriage rates compared with aspiration alone is uncertain. Although the evidence does not permit any firm conclusions on the impact of follicular flushing on oocyte yield, total number of embryos, number of cryopreserved embryos, or clinical pregnancy rate, it may be that the procedure itself takes longer than aspiration alone. The evidence was insufficient to permit any firm conclusions with respect to adverse events or safety.

PLAIN LANGUAGE SUMMARY

Follicular flushing during oocyte retrieval in assisted reproductive technology

Review question

We sought to assess the safety and effectiveness of flushing follicles as part of egg collection in women undergoing treatments to help them get pregnant (assisted reproductive technology (ART)).

Background

Couples who have difficulty becoming pregnant naturally may choose to have treatments (interventions) to help them get pregnant. These interventions are known as assisted reproductive technology (ART). One type of ART is in vitro fertilisation (IVF). During IVF, ovarian stimulation is performed using hormones to stimulate multiple eggs to develop within follicles located in each ovary. After ovarian stimulation, a needle guided by ultrasound is inserted into each follicle in order to collect these eggs. Instead of using only suction to obtain the contents of follicles (aspiration), it has been proposed that flushing the follicles after aspiration may lead to collection of more eggs and therefore higher chances of becoming pregnant and having a baby. This technique is called follicular flushing.

Study characteristics

We included 15 studies that randomly assigned a total of 1643 women to follicular aspiration alone or follicular flushing after aspiration. To see if there was a difference between the two techniques, we wanted to look at the main results of live birth rate (number of babies born per 1000 women) and miscarriage rate (number of miscarriages per 1000 women). We carried out a comprehensive search to identify all relevant research in the field in July 2021.

Key results

Four studies reported on the main result of live birth rate. It is uncertain whether follicular flushing has an impact on live birth rate compared with aspiration alone. This suggests that if a live birth rate of approximately 30% is seen with aspiration alone, the equivalent live birth rate with follicular flushing lies between 20% and 39%. One study reported on miscarriage rate, although the certainty of the evidence was low, preventing us from drawing any conclusions with confidence. Nevertheless, the data suggest that if the miscarriage rate is approximately 1% with aspiration alone, the equivalent rate with follicular flushing lies between 0% and 22%.

We are also uncertain of the impact of follicular flushing on the number of eggs retrieved, the number of embryos, or the clinical pregnancy rate compared to aspiration alone. Although the certainty of evidence was low, it appears that follicular flushing takes longer to perform than aspiration alone. The available evidence was insufficient to permit any firm conclusions with respect to adverse events or safety.

More research is needed to find out whether any specific patient groups would benefit from follicular flushing.

Certainty of the evidence

The certainty of evidence for the main outcome of live birth rate was moderate. The certainty of evidence for the other outcomes ranged from very low to low. The main limitations of included studies were lack of blinding (the process of preventing women participating in the trial and research staff from being aware of the intervention used), inconsistency (differences across studies), and imprecision (insufficient data).

Summary of findings 1. Summary of findings table - Aspiration/flush compared to aspiration for women undergoing assisted conception

Aspiration/flush compared to aspiration for women undergoing assisted conception

Patient or population: women undergoing assisted conception
Setting: ART clinic
Intervention: aspiration/flush
Comparison: aspiration

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with aspiration	Risk with aspiration/flush				
Live birth rate - total	301 per 1000	**286 per 1000** (203 to 386)	**OR 0.93** (0.59 to 1.46)	467 (4 RCTs)	⊕⊕⊕⊖ Moderate[a]	Follicular flushing probably has little or no impact on the live birth rate compared to aspiration alone.
Miscarriage rate - total	12 per 1000	**24 per 1000** (2 to 217)	**OR 1.98** (0.18 to 22.22)	164 (1 RCT)	⊕⊕⊖⊖ Low[b]	We are uncertain of the effect of follicular flushing compared to aspiration alone on the miscarriage rate.
Oocyte yield per woman randomised (normally distributed data)	The mean oocyte yield per woman randomised (normally distributed data) was **5.956**	MD **0.47 lower** (0.72 lower to 0.22 lower)	-	1239 (9 RCTs)	⊕⊖⊖⊖ Very low[a,c,d]	We are uncertain of the effect of follicular flushing compared to aspiration alone on oocyte yield.
Duration of oocyte retrieval (normally distributed data; seconds)	The mean duration of oocyte retrieval (normally distributed data; seconds) was **77.14**	MD **175.44 higher** (152.57 higher to 198.3 higher)	-	785 (7 RCTs)	⊕⊕⊖⊖ Low[c,d]	Follicular flushing may increase the duration of oocyte retrieval compared to aspiration alone.
Total number of embryos (normally distributed data)	The mean total number of embryos (normally distributed data) was **1.5**	MD **0.1 lower** (0.34 lower to 0.15 higher)	-	160 (2 RCTs)	⊕⊕⊖⊖ Low[c,d]	We are uncertain of the effect of follicular flushing compared to aspiration alone on the total number of embryos.

Clinical pregnancy rate per woman randomised	293 per 1000	**319 per 1000** (261 to 385)	**OR 1.13** (0.85 to 1.51)	939 (7 RCTs)	⊕⊕⊖⊖ Low[a,c]	We are uncertain of the effect of follicular flushing compared to aspiration alone on the clinical pregnancy rate.
Adverse events (dichotomous data) - total	0 per 1000	**0 per 1000** (0 to 0)	Not estimable	(3 RCTs)	⊕⊕⊖⊖ Low[c,e]	One study reported no differences in patient-reported adverse outcomes (depression, anxiety, and stress). Another study reported higher doses of analgesia required in the follicular flushing group compared with the aspiration alone group. A third study reported no difference in pain scores and no peritoneal infection, pelvic organ injury or significant bleeding in either group.

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the assumed risk in the comparison group and the __relative effect__ of the intervention (and its 95% CI).

CI: confidence interval; **MD:** mean difference; **OR:** odds ratio

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

See interactive version of this table: https://gdt.gradepro.org/presentations/#/isof/isof_question_revman_web_430721461938343401.

[a] Imprecision: wide confidence intervals. Downgraded one level.
[b] Imprecision: few events and wide confidence intervals. Downgraded two levels.
[c] Risk of bias: incorporates at least one open-label study. Downgraded one level.
[d] Inconsistency: high degree of heterogeneity. Downgraded one level.
[e] Imprecision: few events. Downgraded one level.

BACKGROUND

Description of the condition

Assisted reproductive technology (ART) requires the handling of oocytes and embryos outside the woman's body. The technique involves ovarian stimulation, monitoring of follicular growth, oocyte recovery, sperm preparation and insemination, embryo culture, embryo transfer, and luteal support. Other variables, in particular female age, can significantly affect the number of oocytes retrieved and the success rate of ART.

Description of the intervention

Once maturity of the follicles is achieved, human chorionic gonadotropin (hCG) or recombinant luteinising hormone (rLH) is used to trigger oocyte maturation. Oocyte pickup is performed approximately 36 hours later. Technical details of oocyte recovery vary between fertility centres, especially with regard to type of anaesthesia (local, sedation, or general), type of aspiration needle (wide or narrow bore, single or double channel), route of retrieval (transvaginal or abdominal), aspiration alone or aspiration with follicular flushing, type of flushing medium, and the collecting system.

The number of oocytes retrieved is associated with the proportion of good-quality embryos obtained (Vermey 2019), as well as the live birth rate (Toftager 2017; Vaughan 2017). The concept of follicular flushing was introduced with the aim of maximising the number of oocytes recovered (The ESHRE Working Group on Ultrasound in ART 2019). The process involves aspiration of the follicular fluid followed by the introduction of flush, typically culture media, back into the follicle followed by re-aspiration. This process may be repeated several times in a closed system (where the collection tubes are passed to the laboratory after all follicles are punctured) or in an open system (where the embryologist provides simultaneous feedback so that the follicle is rinsed until an oocyte or no cell debris are detected) (The ESHRE Working Group on Ultrasound in ART 2019).

Although the previous version of this review suggested there is little to no benefit of follicular flushing on key outcomes such as live birth and clinical pregnancy rates (Georgiou 2018), the certainty of the evidence was very low to moderate. In addition, there continues to be specific interest on whether there is a benefit to follicular flushing in women who respond poorly to ovarian stimulation (Calabre 2020; Malhotra 2020).

How the intervention might work

The place of follicular flushing during oocyte recovery in ART remains uncertain. The theoretical benefits of flushing could include the possibility of obtaining more oocytes and hence more embryos. It also remains controversial whether this translates into higher pregnancy and live birth rates.

The process of follicular flushing is time-consuming compared to aspiration alone and has been associated with longer operative times (Georgiou 2018), and possibly large doses of anaesthetic and analgesic drugs. It could also mean higher costs from the patient's perspective. At the clinic level, a longer procedure may translate to reduced procedure room availability, increased use of consumables and hence overall poorer resource utilisation. On the molecular level, it is feasible that flushing damages the cumulus complex, although a study by Neyens 2016 did not identify a negative impact of progressively more flushes on embryo quality when compared to aspiration alone.

Why it is important to do this review

The prevalence of infertility and the significant costs of assisted conception make the assessment of ART techniques an imperative to establish which are more effective in terms of attaining a live birth and which are cost-beneficial, with a view towards improving treatment outcomes. This review provides information for women and clinicians and identifies aspects that require future study.

OBJECTIVES

To assess the safety and efficacy of follicular flushing as compared with aspiration only performed in women undergoing assisted reproductive technology (ART).

METHODS

Criteria for considering studies for this review

Types of studies

Randomised controlled trials (RCTs) were eligible for inclusion. We did not include quasi-RCTs. We included cross-over trials only when pre-cross-over data were extractable for analysis. We included conference abstracts and handled these in the same way as full publications.

Types of participants

Participants were women who underwent assisted conception treatment by in vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) using their own gametes.

Types of interventions

We included trials comparing any form of follicular flushing during oocyte retrieval to follicular aspiration alone.

We included trials in which investigators replaced embryos resulting from oocytes derived from mixed groups of flushed and unflushed follicles in the same woman.

To be eligible, trials had to report that all recruited women had undergone only one cycle of treatment within the context of the trial and had had embryos replaced in the uterine cavity in fresh or frozen-thawed cycles. We did not exclude trials where embryo replacement did not take place because of failure of fertilisation or failure of the embryo to divide further (cleavage arrest).

We excluded trials that directly compared different methods of follicular flushing (without an aspiration-only control group).

Types of outcome measures

Primary outcomes

1. Live birth rate per woman randomised, with live birth defined as per the International Committee for Monitoring Assisted Reproductive Technology (ICMART) as "the complete expulsion or extraction from a woman of a product of fertilization, after 22 completed weeks of gestational age; which, after such separation, breathes or shows any other evidence of life, such as heart beat, umbilical cord pulsation or definite

movement of voluntary muscles, irrespective of whether the umbilical cord has been cut or the placenta is attached. A birth weight of 500 grams or more can be used if gestational age is unknown" (Zegers-Hochschild 2017)

2. Miscarriage rate per woman randomised, defined per ICMART as the "spontaneous loss of an intra-uterine pregnancy prior to 22 completed weeks of gestational age" (Zegers-Hochschild 2017)

Secondary outcomes

1. Oocyte yield, defined as number of oocytes retrieved per woman randomised
2. Duration of oocyte retrieval
3. Total number of embryos per woman randomised
4. Number of cryopreserved embryos per woman randomised
5. Clinical pregnancy rate per woman randomised, defined per ICMART as the presence of one or more gestational sacs by ultrasonographic visualisation or definitive clinical signs of pregnancy (Zegers-Hochschild 2017). Of note, this definition incorporates both intrauterine and ectopic pregnancies.
6. Ongoing pregnancy rate per woman randomised, defined as a pregnancy of 12 or more weeks' gestation
7. Adverse events as defined by trialists (patient-reported outcomes and surgical complications including needle blockage, vomiting, and hypotension)

Search methods for identification of studies

We searched from inception of the databases to 13 July 2021 for all published and unpublished RCTs of follicular flushing, without language restrictions and in consultation with the Cochrane Gynaecology and Fertility Group (CGFG) Information Specialist.

Electronic searches

We used the following search strategy to obtain all reports that described (or might have described) RCTs of follicular flushing:

1. Cochrane Gynaecology and Fertility Group (CGF) Specialised Register of Controlled Trials, ProCite platform, searched from inception to 13 July 2021 (Appendix 1);
2. Cochrane Central Register of Controlled Trials (CENTRAL) via the Cochrane Register of Studies Online (CRSO), Web platform, searched from inception to 13 July 2021 (Appendix 2). CENTRAL now contains output from two trial registries, ClinicalTrials.gov (www.clinicaltrials.gov) and the World Health Organization International Clinical Trials Registry Platform (WHO ICTRP) (trialsearch.who.int), and CINAHL (Cumulative Index to Nursing and Allied Health Literature);
3. MEDLINE (Epub Ahead of Print, In-Process & Other Non-Indexed Citations), Ovid platform, searched from 1946 to 13 July 2021 (Appendix 3);
4. Embase, Ovid platform, searched from 1980 to 13 July 2021 (Appendix 4);
5. PsycINFO, Ovid platform, searched from 1806 to 13 July 2021 (Appendix 5).

We planned to combine the MEDLINE search with the Cochrane Highly Sensitive Search Strategy for identifying randomised trials as described in the *Cochrane Handbook for Systematic Reviews of Interventions* Chapter 4, 4.4.7; 4.S1 (Lefebvre 2021). The Embase search is combined with trial filters developed by the Scottish Intercollegiate Guidelines Network (SIGN) (www.sign.ac.uk/what-we-do/methodology/search-filters).

Other electronic sources of trials included the following:

1. LILACS and other Spanish and Portuguese language databases (Latin American and Caribbean Health Science Information database, Web platform, searched from 1982 to 13 July 2021; found in the Virtual Health Library Regional Portal (VHL)) (pesquisa.bvsalud.org/portal/);
2. Google Scholar, Web platform (for recent trials not yet indexed in the major databases);
3. Epistemonikos database (www.epistemonikos.org/), a multilingual database of health evidence.

The searches of 'other electronic sources' described above consisted of simple short keyword searches and checking of the top few hits.

Searching other resources

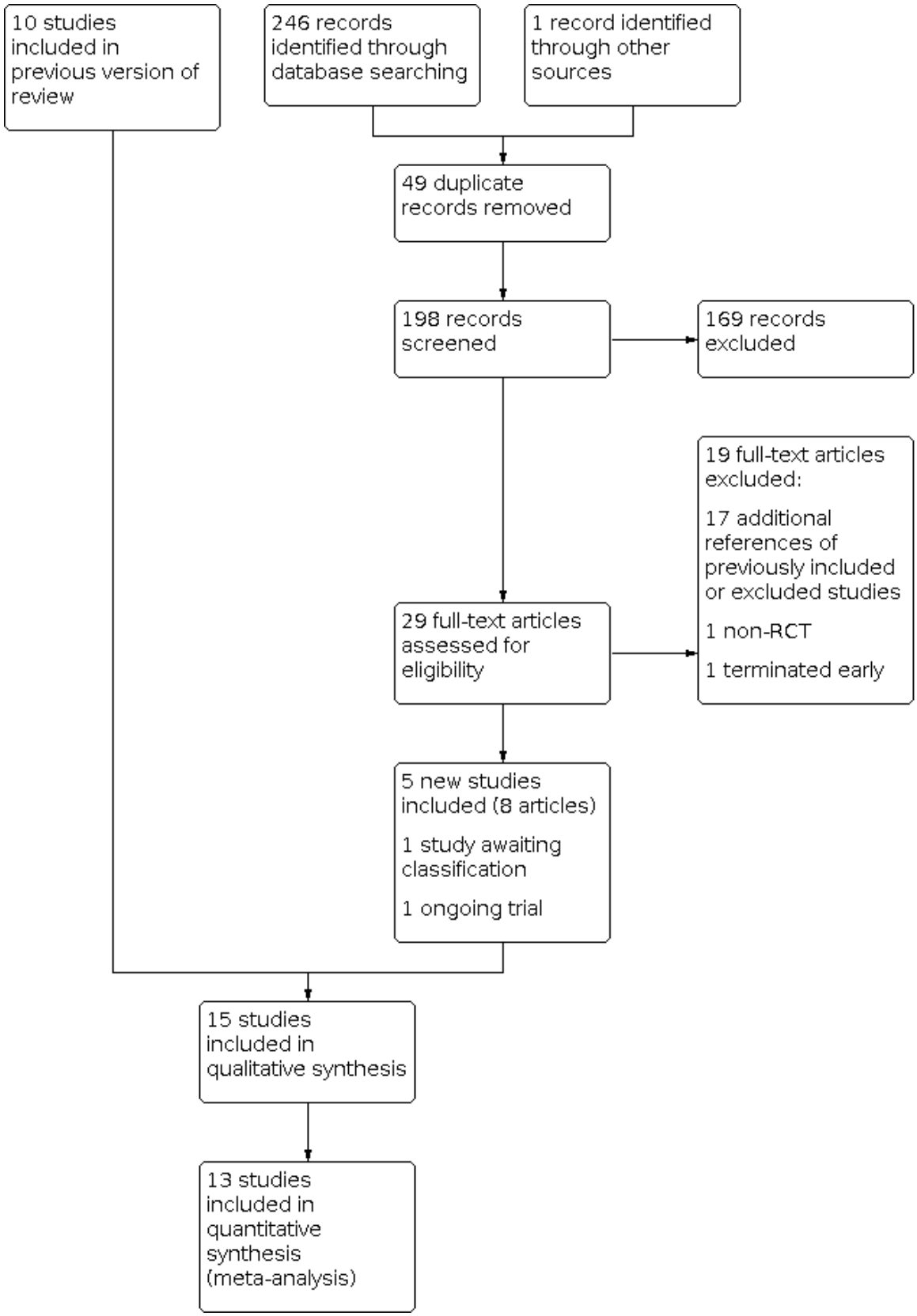
1. We handsearched the reference lists of relevant trials and systematic reviews retrieved by the search and contacted experts in the field to obtain additional data.
2. We handsearched relevant journals and conference abstracts that were not covered in the CGF register, in liaison with the Information Specialist.

Data collection and analysis

Selection of studies

Two review authors (EG and PM) performed an initial screening of titles and abstracts, after which we retrieved the full texts of all potentially eligible studies. Two review authors (EG and PM) independently examined these full texts for compliance with the inclusion criteria (Appendix 6), and selected eligible studies. We corresponded with study investigators as required to clarify study eligibility. Any disagreements were resolved by discussion or through arbitration with a third review author (IG). We documented the selection process using a PRISMA flow diagram (Figure 1).

Figure 1. Study flow diagram.



Data extraction and management

Two review authors (EG and PM) independently extracted data from the eligible studies using the data extraction pro forma that had been designed and pilot-tested by the review authors (Appendix 7), resolving any disagreements by discussion or through arbitration with a third review author (IG). We extracted study characteristics and outcome data. When studies had multiple publications, we collated multiple reports of the same trial under a single study ID with multiple references. We corresponded with study investigators to ask for further data or methods and/or results as required.

Assessment of risk of bias in included studies

Two review authors (EG and PM) independently assessed the included studies for risk of bias using the Cochrane risk of bias assessment tool, which assesses the following domains: selection bias (random sequence generation and allocation concealment); performance bias (blinding of participants and personnel); detection bias (blinding of outcome assessors); attrition bias (incomplete outcome data); reporting bias (selective reporting); and other bias (Higgins 2011a). We assigned judgement as recommended in Section 8.5 of the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011b), resolving any disagreements by discussion or through arbitration with a third review author (IG). We described all judgements fully, presented conclusions in the risk of bias tables, and incorporated this information into our interpretation of review findings by performing sensitivity analyses. With respect to within-trial selective reporting, where identified studies failed to report the primary outcome of live birth, but did report interim outcomes such as pregnancy, we planned to assess whether the interim values were similar to those reported in studies that also reported live birth.

Measures of treatment effect

We performed statistical analysis in accordance with Cochrane guidelines. For dichotomous data (e.g. live births), we used the number of events in each group to calculate Mantel-Haenszel odds ratios (ORs) with 95% confidence intervals (CIs). For continuous data, we calculated mean differences (MDs) between treatment groups and presented these along with 95% CIs. Where data to calculate ORs or MDs were not available, we utilised the most detailed numerical data available. For example, if dichotomous data supplied percentages with sample numbers, we used these to calculate ORs; for continuous data, if alternate measurements of error (e.g. test statistics, P values) were supplied, we used these to calculate CIs.

Unit of analysis issues

The primary analysis was per woman randomised. We summarised in an Additional table data that did not allow valid analysis (e.g. 'per cycle' data, per pregnancy data), but did not include these data in a meta-analysis. We counted a multiple birth as a single live birth event. If we identified any cross-over trials, we would use only first-phase data.

Dealing with missing data

We analysed data on an intention-to-treat basis to the greatest degree possible and attempted to obtain missing data from the original trialists. When these data were unobtainable, we undertook imputation of individual values for live birth only, assuming that live birth did not occur in participants without reported outcomes. We analysed other outcomes using only the available data. Any imputation undertaken was subjected to sensitivity analysis.

When studies reported sufficient detail to allow calculation of MDs but provided no information on associated standard deviation (SD), we assumed that the outcome had an SD equal to the highest SD provided by other studies included within the same analysis.

Assessment of heterogeneity

We used statistical heterogeneity, as ascertained by measurement of the I^2 statistic, to determine whether the clinical and methodological characteristics of included studies were sufficiently similar for meta-analysis. We regarded an $I^2 > 50\%$ as indicative of substantial heterogeneity (Higgins 2021). We explored substantial heterogeneity by conducting the planned subgroup analyses, as detailed below.

Assessment of reporting biases

In view of the difficulty of detecting and correcting for publication bias and other reporting biases, we aimed to minimise the potential for bias by ensuring a comprehensive search for eligible studies and by being alert for duplication of data. We planned that if at least 10 studies were included in the same analysis, we would produce a funnel plot to assess publication bias.

Data synthesis

We presented the primary analysis including trials judged at low risk of selection bias.

Where studies were sufficiently similar, we combined data using a fixed-effect model for the following comparison: flushing versus aspiration only.

We did not stratify data. In meta-analyses, we graphically displayed an increase in the risk of a particular outcome that may be beneficial (e.g. live birth) or detrimental (e.g. miscarriage) to the right of the centre line, and a decrease in the risk of an outcome to the left of the centre line.

Subgroup analysis and investigation of heterogeneity

To determine whether findings differed between studies, we planned to perform the following subgroup analyses for primary outcomes in the case of substantial heterogeneity ($I^2 > 50\%$) and sufficient data.

1. Age: women younger than 40 years old or ≥ 40 years old
2. Poor ovarian reserve: as determined by follicle-stimulating hormone (FSH) levels, anti-Müllerian hormone (AMH) levels, and/or antral follicle count (AFC). We used cutoff values for subgrouping as defined by the trialists or, in cases for which individual data were reported, using the following cutoffs: FSH 10 international units (IU)/mL, AMH 0.8 ng/mL, and AFC < 6 follicles.
3. Poor response to ovarian stimulation: development of fewer than four mature follicles following controlled ovarian stimulation for IVF or ICSI versus normal response; alternatively, poor response as defined by trialists

Where possible, we extracted data on these subgroups directly from the included trials. When these data were not reported, we used mean trial data (e.g. mean trial AMH level) to place the whole trial into one of these subgroups.

Sensitivity analysis

We conducted sensitivity analyses for our primary outcomes to determine whether conclusions were robust to arbitrary decisions made regarding eligibility and analysis. These analyses included consideration of whether review conclusions would have differed if:

1. we included all studies in the analysis (i.e. no restriction to studies considered to be at low risk of selection bias);
2. a random-effects model had been adopted;
3. alternative imputation strategies had been implemented;
4. the summary effect measure had been risk ratio rather than odds ratio.

Summary of findings and assessment of the certainty of the evidence

We prepared summary of findings tables using GRADEpro GDT and Cochrane methods (GRADEpro GDT; Higgins 2021). These tables evaluate the overall certainty of the body of evidence for the main review outcomes (live birth, pregnancy loss, oocyte yield, duration of oocyte retrieval, total number of embryos, clinical pregnancy rate, adverse events) for the main review comparison (follicular flushing versus follicular aspiration alone). We assessed the certainty of the evidence using the five GRADE criteria: risk of bias, consistency of effect, imprecision, indirectness, and publication bias. Two review authors (EG and PM) independently assessed the certainty of the evidence as high, moderate, low, or very low, with any disagreements resolved by discussion with a third review author (IG). We justified, documented, and incorporated all judgements into the reporting of results for each outcome.

RESULTS

Description of studies

Results of the search

For the 2022 update, our electronic search on 13 July 2021 yielded 246 articles. We identified one additional study by manually searching trial registries. After removal of duplicates, we kept 198 articles for screening. Of these, we excluded 169 records that were clearly not relevant. We obtained and reviewed the full texts for the remaining 29 articles, of which 17 were duplicate references of included or excluded studies. Of the remaining 11 articles, two studies were excluded (see Excluded studies) (NCT02277210; Pabuccu 2021); one study has reported during finalisation of this updated review and has been placed in awaiting classification pending assessment of trial methods and data (see Studies awaiting classification) (Ronchetti 2022), and one study is an ongoing trial that had not yet reported its results (see Ongoing studies) (ChiCTR1800016671).

The remaining eight articles included five new trials that provided data on follicular flushing during oocyte retrieval in assisted reproductive cycles (Included studies) (Calabre 2020; de Souza 2021; Kohl Schwartz 2020; Lainas 2018; Malhotra 2020). Along with the 10 studies included in the previous update (Haines 1989; Haydardedeoglu 2011; Haydardedeoglu 2017; Kara 2012; Kingsland 1991; Levens 2009; Mok-Lin 2013; Scott 1989; Tan 1992; von Horn 2017), we included a total of 15 studies in this update, of which 13 were included in quantitative analysis (meta-analysis) (Calabre 2020; de Souza 2021; Haydardedeoglu 2011; Haydardedeoglu 2017; Kara 2012; Kingsland 1991; Kohl Schwartz 2020; Levens 2009; Malhotra 2020; Mok-Lin 2013; Scott 1989; Tan 1992; von Horn 2017). A PRISMA flow diagram is shown in Figure 1.

Included studies

Study design and setting

We included 15 parallel-design RCTs, 14 of which have been published as full articles (Calabre 2020; de Souza 2021; Haines 1989; Haydardedeoglu 2011; Haydardedeoglu 2017; Kara 2012; Kingsland 1991; Kohl Schwartz 2020; Levens 2009; Malhotra 2020; Mok-Lin 2013; Scott 1989; Tan 1992; von Horn 2017), and one as a conference abstract (Lainas 2018). All trials were single-centre studies; three were carried out in the USA (Levens 2009; Mok-Lin 2013; Scott 1989); three in Turkey (Haydardedeoglu 2011; Haydardedeoglu 2017; Kara 2012); two in the UK (Kingsland 1991; Tan 1992); one in Australia (Haines 1989); one in Germany (von Horn 2017); one in France (Calabre 2020); one in India (Malhotra 2020); one in Switzerland (Kohl Schwartz 2020); one in Brazil (de Souza 2021); and one in Greece (Lainas 2018).

Participants

The 15 included studies involved a total of 1643 participants: 827 women in the intervention group, 796 in the control group, and an additional 20 women contributed as both intervention and control, in that one ovary was flushed and the contralateral aspirated only.

Six studies recruited women with poor response to ovarian stimulation (Calabre 2020; Haydardedeoglu 2017; Levens 2009; Malhotra 2020; Mok-Lin 2013; von Horn 2017); each study defined poor ovarian response differently. Levens 2009 defined it as a cumulative follicle count of 4 to 8 follicles greater than or equal to 12 mm with at least 2 follicles greater than 16 mm; Mok-Lin 2013 as 4 or fewer follicles greater than or equal to 12 mm; Haydardedeoglu 2017 as 5 or fewer follicles greater than or equal to 13 mm in size and serum progesterone less than 1.5 ng/mL; von Horn 2017 as 5 or fewer follicles greater than 10 mm; Calabre 2020 as 4 or fewer follicles measuring more than 14 mm on the day of human chorionic gonadotropin (hCG) administration; and Malhotra 2020 as 3 to 5 follicles measuring 14 mm or more on the day of trigger administration. de Souza 2021 did not specifically define their patient cohort as poor responders, but recruited patients with 5 or fewer follicles at 15 to 17 mm, 4 or fewer follicles above 18 mm on trigger day. Apart from poor ovarian response, all participants included in Haydardedeoglu 2017 also had poor ovarian reserve, as defined by an antral follicle count (AFC) less than 6 and an anti-Müllerian hormone (AMH) level less than 0.8 ng/mL. One study recruited patients with tubal damage (Kingsland 1991); one included patients with at least 4 follicles greater than 11 mm on the day of trigger (Lainas 2018); and one included patients with the indication and desire for monofollicular IVF (Kohl Schwartz 2020). Five studies did not specify any inclusion criteria (Haines 1989; Haydardedeoglu 2011; Kara 2012; Scott 1989; Tan 1992).

Two studies excluded patients with poor ovarian response or high ovarian response (Haydardedeoglu 2011; Tan 1992). Various other exclusion factors were reported including natural IVF (Haydardedeoglu 2017; Mok-Lin 2013), absent ovary or ovary(/ies)

predicted to be difficult to access (Kohl Schwartz 2020; von Horn 2017), presence of endometrioma (Haydardedeoglu 2017; Lainas 2018; Malhotra 2020), contraindications to ovary puncture (Calabre 2020), or two previous embryo transfers without pregnancy (Kohl Schwartz 2020). Five studies did not specify any exclusion criteria (de Souza 2021; Haines 1989; Kara 2012; Kingsland 1991; Scott 1989).

Interventions

One study used clomiphene citrate to achieve ovarian hyperstimulation (Haines 1989), and in another study, participants underwent monofollicular IVF defined as a natural cycle with hCG trigger with the option to include clomiphene citrate 25 mg/day from day 6 until trigger to reduce the risk of premature ovulation (Kohl Schwartz 2020).

The other studies employed gonadotropin-releasing hormone agonist in a long-luteal protocol (Kara 2012; Kingsland 1991); an antagonist protocol with or without luteal phase oestradiol priming and with or without five days of clomiphene citrate or letrozole and daily gonadotropin-releasing hormone (GnRH) agonist with ovarian stimulation (Mok-Lin 2013); a long-follicular protocol (Tan 1992); a long-luteal or microdose follicular flare protocol (Levens 2009); a long unspecified protocol (Scott 1989); a mixture of GnRH agonist or antagonist protocols (Calabre 2020; Haydardedeoglu 2011; Haydardedeoglu 2017; Malhotra 2020); or an undefined protocol (de Souza 2021; Lainas 2018; von Horn 2017).

Some studies induced final oocyte maturation with 5000 IU hCG (Calabre 2020; Haines 1989; Kingsland 1991; Kohl Schwartz 2020; von Horn 2017), and others with 10,000 IU hCG (Haydardedeoglu 2017; Kara 2012; Levens 2009; Mok-Lin 2013; Tan 1992). Two studies used the equivalent of 6500 IU hCG (de Souza 2021; Malhotra 2020). Two studies did not specify the dose of hCG used (Haydardedeoglu 2011; Lainas 2018), and in another study it was not clear if hCG was used (Scott 1989).

Two studies used the same type of double-lumen needle: Kingsland 1991 without and Tan 1992 with removal of the inner channel to convert it to a single-channel needle. The other studies used single- or double-lumen needles that were (Calabre 2020; Haydardedeoglu 2011; Haydardedeoglu 2017; Kohl Schwartz 2020; Lainas 2018; Levens 2009; Malhotra 2020; Mok-Lin 2013) or were not (de Souza 2021; Haines 1989; Kara 2012; Scott 1989) standardised for length plus/minus diameter, to control for flow dynamics within the needle. Of note, in one study a higher suction pressure was utilised for the flushing arm compared to the aspiration-alone arm (Malhotra 2020). One study used a 17G Steiner-Tan Needle, which is described as a single-lumen needle surrounded by a plastic tube that allows passage of flushing medium for follicular flushing, and a 17G Gynetics single-lumen needle in the control arm (von Horn 2017).

Four studies used IVF (Kingsland 1991; Kohl Schwartz 2020; Scott 1989; Tan 1992), and three studies used ICSI for fertilisation (Haydardedeoglu 2011; Haydardedeoglu 2017; Kara 2012). Five studies used both IVF and ICSI (Calabre 2020; Levens 2009; Malhotra 2020; Mok-Lin 2013; von Horn 2017). Two studies did not specify how fertilisation occurred (de Souza 2021; Haines 1989; Lainas 2018).

By definition, women in Kohl Schwartz 2020 underwent single embryo transfer. One study transferred up to two embryos (Malhotra 2020); two studies transferred up to three embryos (Kingsland 1991; Tan 1992); and two studies transferred up to four embryos (Haydardedeoglu 2011; Kara 2012). Nine studies did not comment specifically on the number of embryos transferred (Calabre 2020; de Souza 2021; Haines 1989; Haydardedeoglu 2017; Lainas 2018; Levens 2009; Mok-Lin 2013; Scott 1989; von Horn 2017), although the mean number in Haydardedeoglu 2017 was less than two, and in Levens 2009 and Mok-Lin 2013 was less than three.

Outcomes

Primary outcomes

Six studies reported on the primary outcome of live birth rate per woman randomised (Calabre 2020; Haydardedeoglu 2011; Haydardedeoglu 2017; Kohl Schwartz 2020; Malhotra 2020; Mok-Lin 2013).

Two studies reported on the primary outcome of miscarriage rate per woman randomised (Kohl Schwartz 2020; Malhotra 2020).

Secondary outcomes

Nine studies reported on oocyte yield per woman randomised (Calabre 2020; de Souza 2021; Haydardedeoglu 2011; Haydardedeoglu 2017; Kara 2012; Levens 2009; Malhotra 2020; Scott 1989; von Horn 2017), and one study reported oocyte yield per ovary (Lainas 2018). Eleven studies reported on duration of oocyte retrieval (Calabre 2020; Haydardedeoglu 2011; Haydardedeoglu 2017; Kara 2012; Kingsland 1991; Kohl Schwartz 2020; Levens 2009; Malhotra 2020; Mok-Lin 2013; Tan 1992; von Horn 2017).

Four studies reported on the total number of embryos per woman randomised (Calabre 2020; Haydardedeoglu 2017; Malhotra 2020; von Horn 2017); three on the number of cryopreserved embryos per woman randomised (Calabre 2020; Haydardedeoglu 2011; Mok-Lin 2013); seven on clinical pregnancy rate (Haydardedeoglu 2011; Haydardedeoglu 2017; Kara 2012; Kohl Schwartz 2020; Malhotra 2020; Mok-Lin 2013; Tan 1992); four on ongoing pregnancy rate (Kara 2012; Kingsland 1991; Levens 2009; von Horn 2017); one on adverse events including blockage of the needle, vomiting, and hypotension (Tan 1992); one on adverse events including patient depression, anxiety, and stress (von Horn 2017); and one on adverse events including pain, bleeding, peritoneal infection, and pelvic organ injury (Kohl Schwartz 2020).

Author correspondence

For the previous version of this review, we contacted the Haydardedeoglu 2017 and von Horn 2017 authors, and received a response from von Horn 2017. For this update, we contacted the authors of Calabre 2020, de Souza 2021, Kohl Schwartz 2020, Lainas 2018, and Malhotra 2020, and received responses from all authors.

Excluded studies

The previously published version of this systematic review excluded 18 studies (Avila 2013; Aydin 2017; Bagtharia 2005; Biljan 1997; Dean 1997; el Hussein 1992; Faller 2010; Ghosh 2002; Gordon 2002; Khalifa 1999; Knight 2001; Lenz 1987; Mehri 2014; Mendez Lozano 2008; Neyens 2016; Pirrello 2011; Waterstone 1992; Ziebe 2000). We excluded two additional studies in this update.

Of the studies excluded from this update, five were not RCTs (Avila 2013; Aydin 2017; Ghosh 2002; Mehri 2014; Neyens 2016). Two studies incorporated the same population of patients, and we excluded both studies owing to trial author-reported issues regarding study ethics and inclusion criteria (Faller 2010; Pirrello 2011).

Risk of bias in included studies

We assessed risk of bias in all included studies, as shown in Figure 2 and Figure 3. For detailed information, see Characteristics of included studies.

Figure 2. Methodological quality graph: review authors' judgements about each methodological quality item presented as percentages across all included studies.

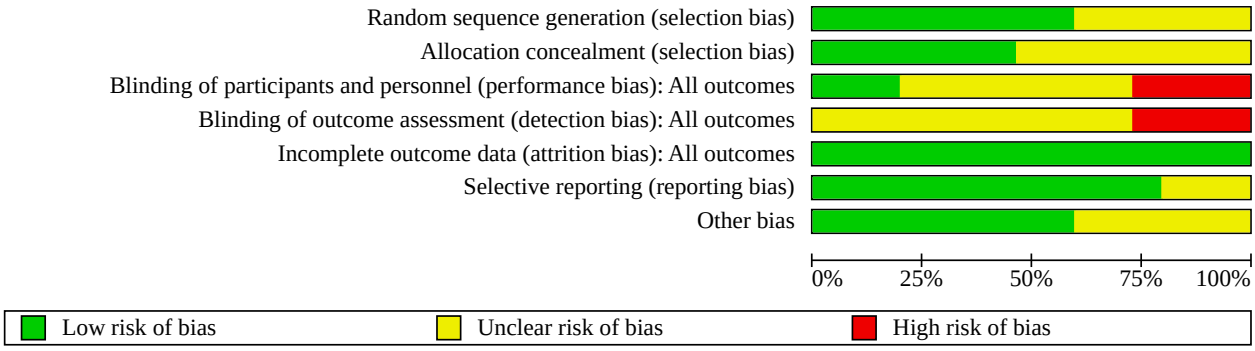
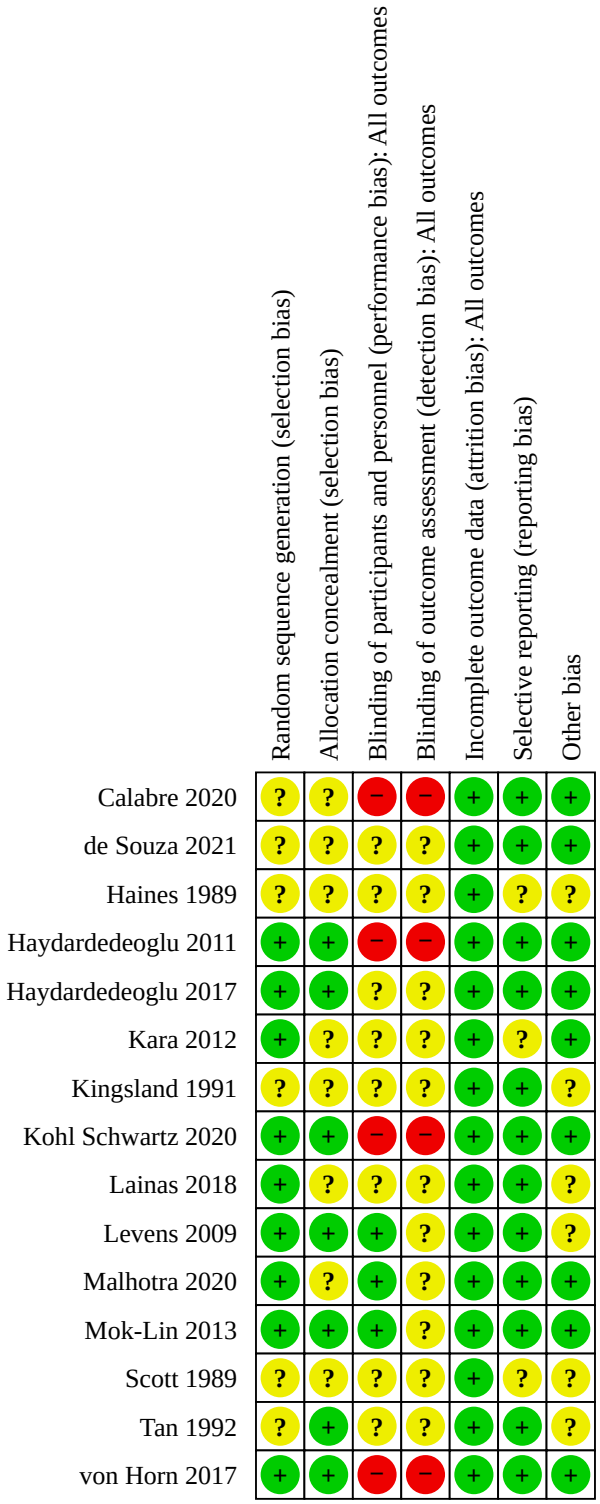


Figure 3. Methodological quality summary: review authors' judgements about each methodological quality item for each included study.



Allocation

Random sequence generation

Nine studies used adequate methods for random sequence generation, such as random numbers tables or computer-generated randomisation sequences, and were hence deemed to be at low risk of bias (Haydardedeoglu 2011; Haydardedeoglu 2017; Kara 2012; Kohl Schwartz 2020; Lainas 2018; Levens 2009; Malhotra 2020; Mok-Lin 2013; von Horn 2017). The remaining six studies did not specify whether or how they performed randomisation and were hence judged to be at unclear risk of bias (Calabre 2020; de Souza 2021; Haines 1989; Kingsland 1991; Scott 1989; Tan 1992).

Allocation concealment

Seven studies reported the use of adequate methods for allocation concealment, such as sequentially numbered, sealed, opaque envelopes, and hence were deemed to be at low risk of bias (Haydardedeoglu 2011; Haydardedeoglu 2017; Kohl Schwartz 2020; Levens 2009; Mok-Lin 2013; Tan 1992; von Horn 2017). Six studies provided no relevant details on allocation concealment and were hence judged to be at unclear risk of bias (de Souza 2021; Haines 1989; Kara 2012; Kingsland 1991; Lainas 2018; Scott 1989). One study reported using numbered, opaque, sealed envelopes, but it was not clear if these were sequentially opened (Calabre 2020), and another study reported using opaque envelopes, but it was not clear if these were numbered and sealed (Malhotra 2020); we judged both of these studies to be at unclear risk of bias.

Blinding

Blinding of participants and personnel (performance bias)

Three studies reported blinding of participants and personnel (Levens 2009; Malhotra 2020; Mok-Lin 2013). Four studies were open-label and were therefore judged to be at high risk of bias (Calabre 2020; Haydardedeoglu 2011; Kohl Schwartz 2020; von Horn 2017). The remaining studies did not report on blinding and were judged to be at unclear risk of bias (de Souza 2021; Haines 1989; Haydardedeoglu 2017; Kara 2012; Kingsland 1991; Lainas 2018; Scott 1989; Tan 1992).

Blinding of outcome assessment (detection bias)

With the exception of Haydardedeoglu 2011, von Horn 2017, Calabre 2020, and Kohl Schwartz 2020, which were open-label and judged to be at high risk of bias, the included studies did not report on blinding of outcome assessors and were therefore judged to be at unclear risk of bias.

Incomplete outcome data

All trials analysed all randomised women.

Selective reporting

Twelve studies reported on a priori outcomes and were judged to be at low risk of bias (Calabre 2020; de Souza 2021; Haydardedeoglu 2011; Haydardedeoglu 2017; Kingsland 1991; Kohl Schwartz 2020; Lainas 2018; Levens 2009; Malhotra 2020; Mok-Lin 2013; Tan 1992; von Horn 2017). The remaining three studies did not include an a priori statement of outcomes to be studied and were hence deemed to be at unclear risk of bias (Haines 1989; Kara 2012; Scott 1989).

Other potential sources of bias

We deemed 10 studies to be at low risk of other bias (Calabre 2020; de Souza 2021; Haydardedeoglu 2011; Haydardedeoglu 2017; Kara 2012; Kohl Schwartz 2020; Levens 2009; Malhotra 2020; Mok-Lin 2013; von Horn 2017). We judged the remaining studies to be at unclear risk of bias owing to lack of information (Haines 1989; Kingsland 1991; Lainas 2018; Scott 1989; Tan 1992).

Effects of interventions

See: **Summary of findings 1** Summary of findings table - Aspiration/flush compared to aspiration for women undergoing assisted conception

1. Follicular flushing versus aspiration alone

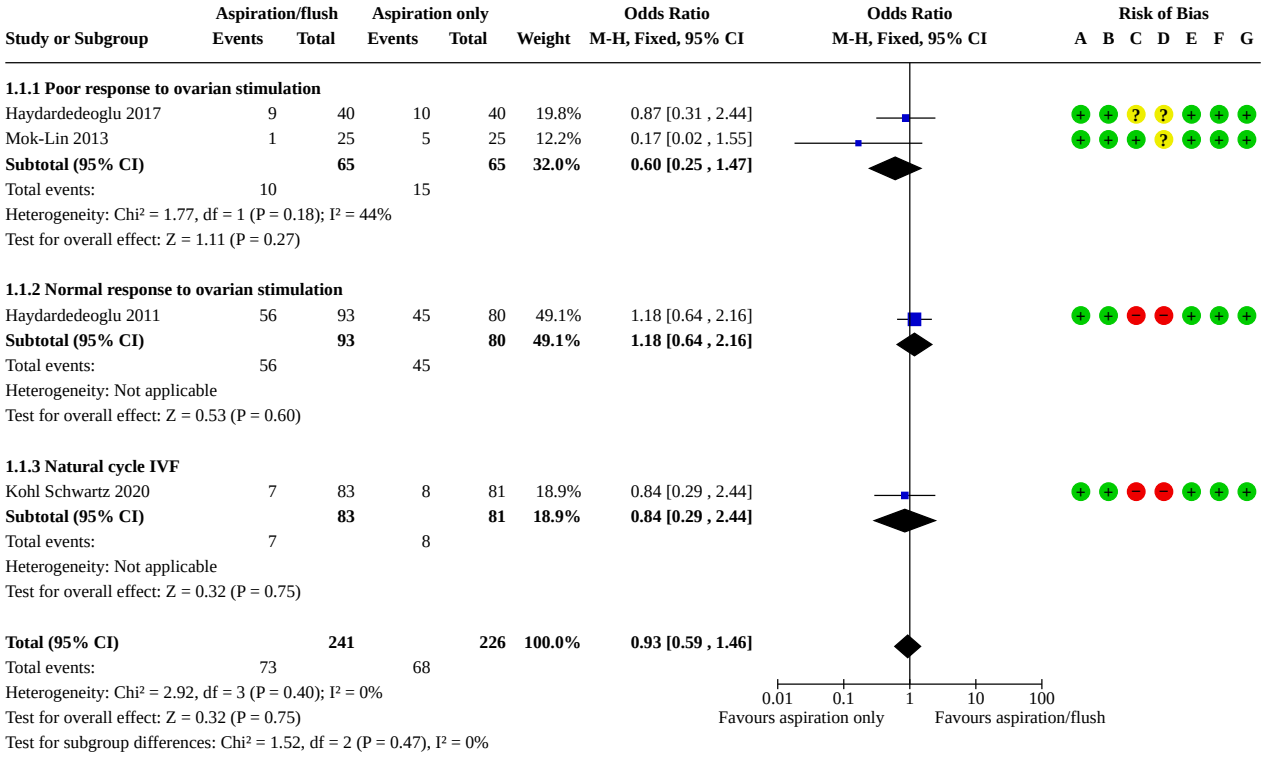
See Summary of findings 1.

Primary outcomes

1.1 Live birth rate

We are uncertain of the effect of follicular flushing on live birth rate compared to aspiration alone (odds ratio (OR) 0.93, 95% confidence interval (CI) 0.59 to 1.46; 4 RCTs; n = 467; I^2 = 0%; moderate-certainty evidence). This suggests that with a live birth rate of approximately 30% (301 per 1000) with aspiration alone, the equivalent live birth rate with follicular flushing lies between 20% and 39% (203 to 386 per 1000). These data are stratified by response to ovarian stimulation: poor or normal response, and in natural cycle IVF. See Analysis 1.1 and Figure 4.

Figure 4. Forest plot of comparison: 1 Follicular flushing, outcome: 1.1 Live birth rate.



Risk of bias legend
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding of participants and personnel (performance bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

Sensitivity analysis without restriction to studies at low risk of selection bias showed similar estimates (OR 1.02, 95% CI 0.70 to 1.49; 6 RCTs; n = 790; I^2 = 0%; moderate-certainty evidence). Sensitivity analysis based on a random-effects model showed estimates similar to those obtained with the fixed-effect model (OR 1.04, 95% CI 0.71 to 1.54; 6 RCTs; n = 790; I^2 = 0%; low-certainty evidence). We did not carry out a sensitivity analysis on alternative imputation strategies, as this was not applicable. Sensitivity analysis based on a risk ratio showed estimates similar to those obtained with the odds ratio effect measure (RR 1.01, 95% CI 0.80 to 1.28; 6 RCTs; n = 790; I^2 = 0%; low-certainty evidence).

1.1.1. Subgroup analysis: age

No studies reported on this outcome.

1.1.2. Subgroup analysis: poor ovarian reserve

No studies reported on this specific comparison. The women included in Haydardedeoglu 2017 had both poor ovarian reserve and poor response to ovarian stimulation. We collectively decided to include them under 'poor response to ovarian stimulation' for the purposes of subgroup analysis.

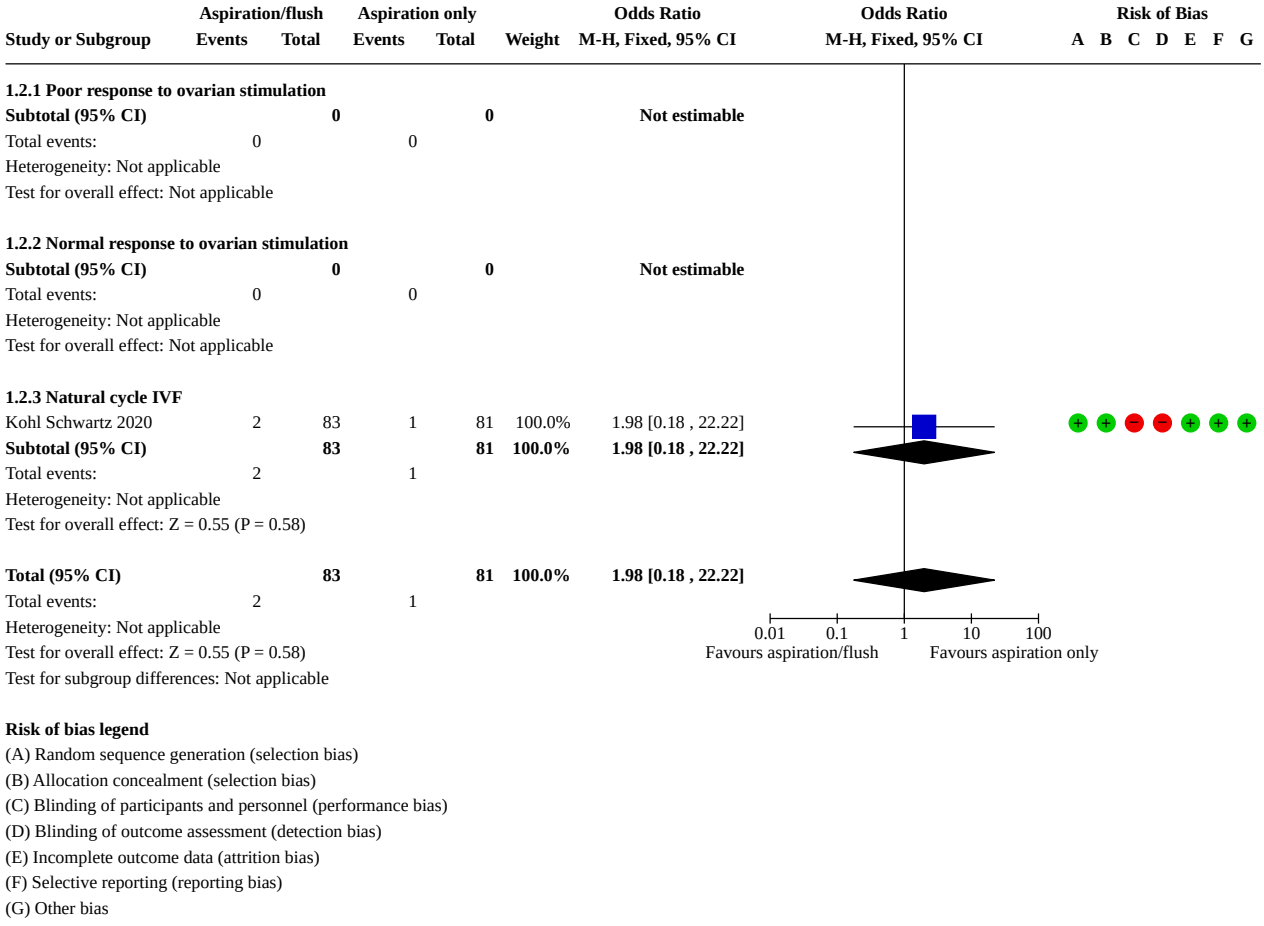
1.1.3. Subgroup analysis: poor response to ovarian stimulation

Follicular flushing has little or no impact on live birth rate amongst participants with poor ovarian response as compared to aspiration alone (OR 1.05, 95% CI 0.60 to 1.82; 4 RCTs; n = 453; I^2 = 13%; moderate-certainty evidence).

1.2. Miscarriage rate

We are uncertain of the effect of follicular flushing on miscarriage rate compared to aspiration alone (OR 1.98, 95% CI 0.18 to 22.22; 1 RCT; n = 164; low-certainty evidence). This suggests that with a miscarriage rate of approximately 1% (12 per 1000) with aspiration alone, the equivalent miscarriage rate with follicular flushing lies between 0% and 22% (2 to 217 per 1000). These data are stratified by response to ovarian stimulation: poor or normal response, and in natural cycle IVF. See Analysis 1.2 and Figure 5.

Figure 5. Forest plot of comparison: 1 Follicular flushing, outcome: 1.2 Miscarriage rate.



Risk of bias legend
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding of participants and personnel (performance bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

Sensitivity analysis without restriction to studies at low risk of selection bias showed similar estimates (OR 4.55, 95% CI 0.77 to 26.98; 2 RCTs; n = 235; I^2 = 0%; low-certainty evidence). Sensitivity analysis based on a random-effects model showed estimates similar to those obtained with the fixed-effect model (OR 3.85, 95% CI 0.59 to 25.05; 2 RCTs; n = 235; I^2 = 0%; low-certainty evidence). We did not carry out a sensitivity analysis on alternative imputation strategies, as this was not applicable. Sensitivity analysis based on a risk ratio showed estimates similar to those obtained with the odds ratio effect measure (RR 4.34, 95% CI 0.76 to 24.04; 2 RCTs; n = 235; I^2 = 0%; low-certainty evidence).

1.2.1. Subgroup analysis: age

No studies reported on this outcome.

1.2.2. Subgroup analysis: poor ovarian reserve

No studies reported on this outcome.

1.2.3. Subgroup analysis: poor response to ovarian stimulation

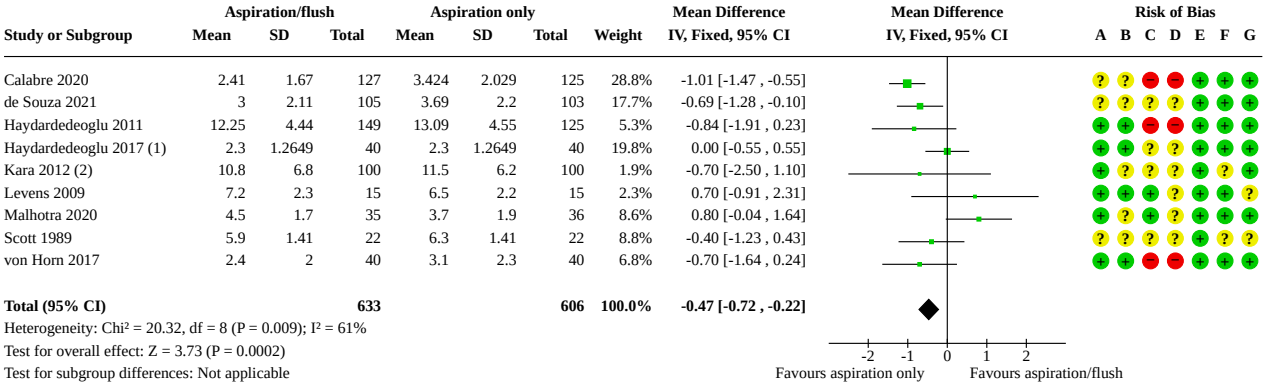
We are uncertain on the effect of follicular flushing on miscarriage rate compared to aspiration alone (OR 10.43, 95% CI 0.54 to 201.32; 1 RCT; n = 71; I^2 = not calculable; low-certainty evidence).

Secondary outcomes

1.3. Oocyte yield

We are uncertain of the effect of follicular flushing on oocyte yield compared to aspiration alone (mean difference (MD) −0.47 oocytes, 95% CI −0.72 to −0.22; 9 RCTs; n = 1239; I^2 = 61%; very low-certainty evidence). See Analysis 1.3 and Figure 6. One of the studies in this analysis reported very small standard deviations (SDs), which varied markedly from those reported in other papers (Haydardedeoglu 2017). We attempted to contact the study authors at the time of the previous update of this review without success. We have assumed the SD to in fact be standard error (SE), and have recalculated this accordingly.

Figure 6. Forest plot of comparison: 1 Follicular flushing, outcome: 1.2 Oocyte yield per woman randomised (normally distributed data).



Heterogeneity: Chi² = 20.32, df = 8 (P = 0.009); I² = 61%
Test for overall effect: Z = 3.73 (P = 0.0002)
Test for subgroup differences: Not applicable

Footnotes
(1) SD recalculated as paper appears to report SE; seeking confirmation from authors
(2) Not entirely clear that data are expressed as +/- SD. Awaiting to hear from authors.

Risk of bias legend
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding of participants and personnel (performance bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

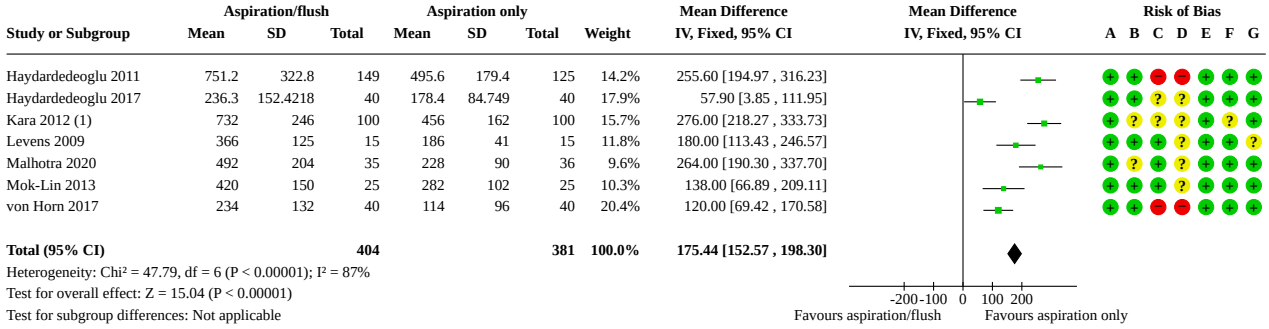
None of the studies providing data that could not be included in the meta-analysis provided any evidence of a difference in oocyte yield between the two groups (Haines 1989; Kingsland 1991; Mok-Lin 2013; Tan 1992). See Analysis 1.4.

Lainas 2018, which included 20 women, randomised at the level of the ovary, whereby one side was flushed and the contralateral aspirated alone, and hence could also not be included in meta-analysis. The authors reported a higher oocyte yield with follicular flushing (74.2%, 95% CI 65.0% to 83.4%) compared to aspiration alone (42.7%, 95% CI 30.0% to 55.5%).

1.4. Duration of oocyte retrieval

The duration of oocyte retrieval may be longer in the aspiration/flush group than in the aspiration-only group (MD 175.44 seconds, 95% CI 152.57 to 198.30; 7 RCTs; n = 785; I^2 = 87%; low-certainty evidence). See Analysis 1.5 and Figure 7. One of the studies in this analysis reported very small SDs, which varied markedly compared with those reported in other papers (Haydardedeoglu 2017). We attempted to contact the study authors at the time of the previous update of this review without success. We have assumed the SD to in fact be SE, and have recalculated this accordingly.

Figure 7. Forest plot of comparison: 1 Follicular flushing, outcome: 1.4 Duration of oocyte retrieval (normally distributed data; seconds).



Footnotes
(1) Not entirely clear that data are expressed as +/- SD. Awaiting to hear from authors.

Risk of bias legend
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding of participants and personnel (performance bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

Sensitivity analysis removing the study reporting markedly different SD, Haydardedeoglu 2017, showed similar results (MD 201.26 seconds, 95% CI 175.83 to 226.30; 6 RCTs; n = 705; I^2= 81%; low-certainty evidence).

All studies providing data that could not be included in the meta-analysis yielded evidence of flushing lasting longer than aspiration alone (Calabre 2020; Kingsland 1991; Kohl Schwartz 2020; Tan 1992). See Analysis 1.6.

One study including 20 women performed randomisation at the ovary level, such that one side was flushed and the other aspirated only (Lainas 2018).

1.5. Total number of embryos

We are uncertain of the effect of follicular flushing compared to aspiration alone on the total number of embryos (MD −0.10 embryos, 95% CI −0.34 to 0.15; 2 RCTs; n = 160; I^2 = 58%; low-certainty evidence). See Analysis 1.7.

We could not include data from two studies in the meta-analysis; these data are summarised in Analysis 1.8.

1.6. Number of cryopreserved embryos

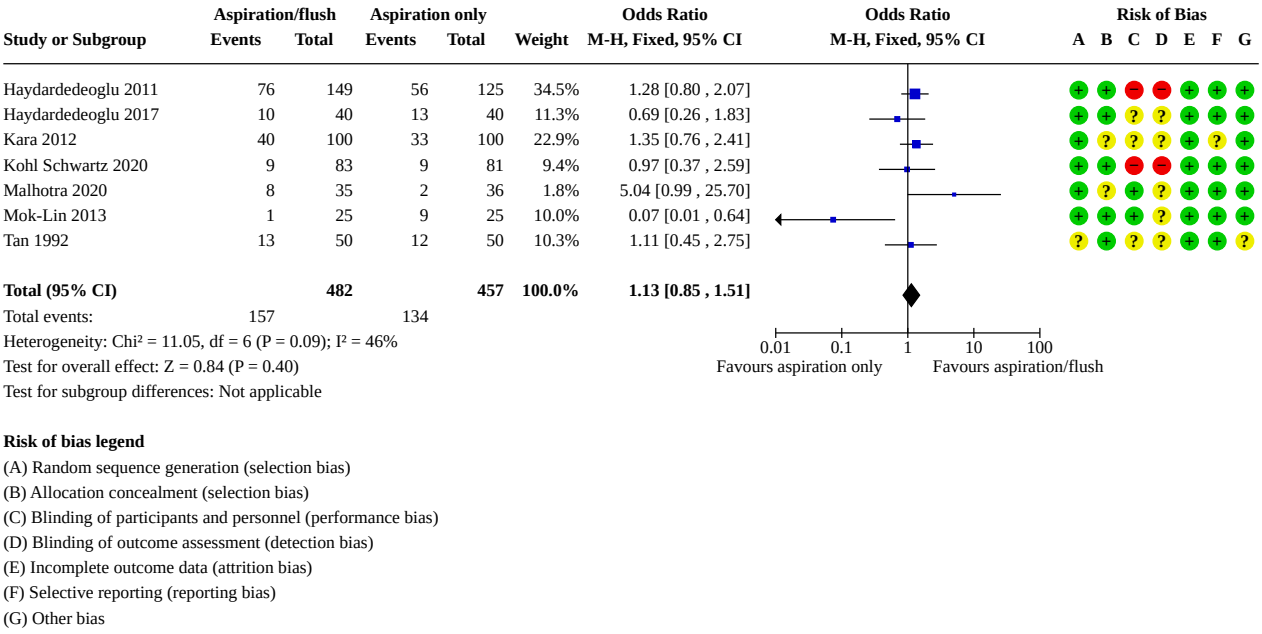
Three studies reported on the number of cryopreserved embryos per woman randomised. However, meta-analysis was not possible, as the mean number in the aspiration/flush group in Mok-Lin 2013 was 0. See Analysis 1.9. The other study in this analysis reported very small SDs, which varied markedly from those reported in other papers (Haydardedeoglu 2017). We attempted to contact the study authors at the time of the previous update of this review without success. We have assumed the SD to in fact be SE, and have recalculated this accordingly.

We could not include data from one study in the meta-analysis; these data are summarised in Analysis 1.10.

1.7. Clinical pregnancy rate

We are uncertain of the effect of follicular flushing compared to aspiration alone on clinical pregnancy rate per woman randomised (OR 1.13, 95% CI 0.85 to 1.51; 7 RCTs; n = 939; I^2 = 46%; low-certainty evidence). See Analysis 1.11 and Figure 8.

Figure 8. Forest plot of comparison: 1 Follicular flushing, outcome: 1.8 Clinical pregnancy rate per woman randomised.



Risk of bias legend

(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding of participants and personnel (performance bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

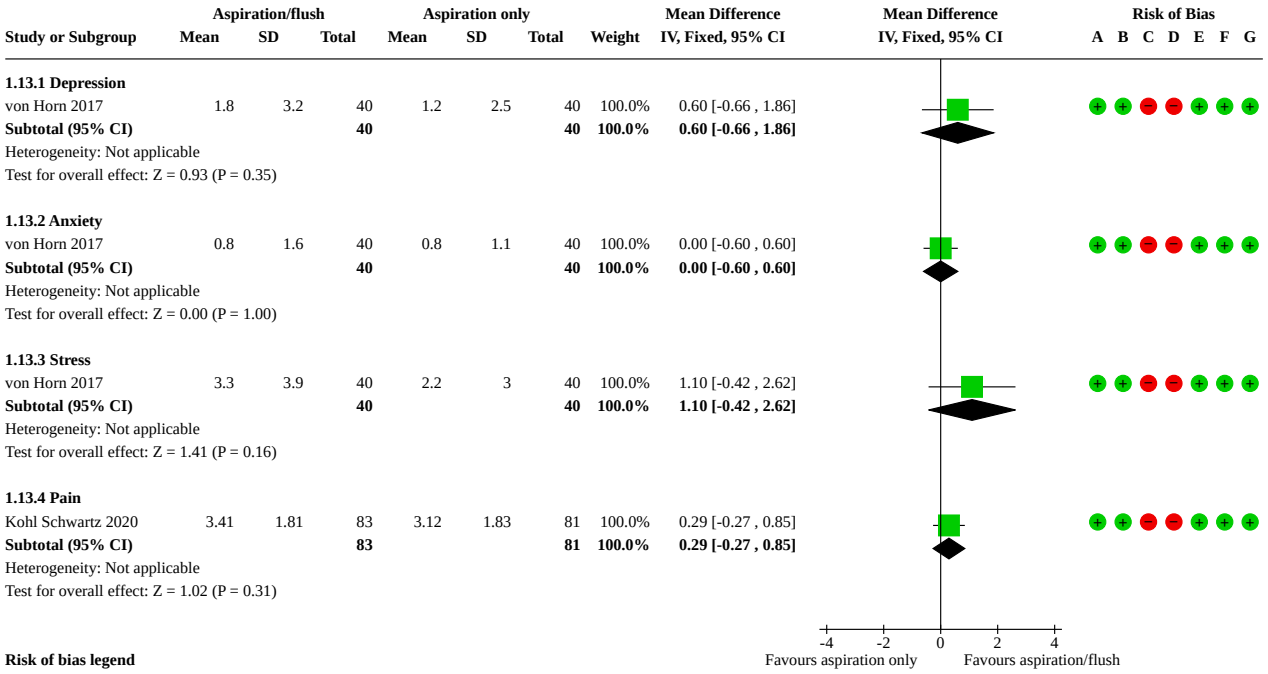
1.8. Ongoing pregnancy rate

We are uncertain of the effect of follicular flushing compared to aspiration alone on ongoing pregnancy rate per woman randomised (OR 1.21, 95% CI 0.73 to 2.02; 4 RCTs; n = 344; I^2 = 0%; low-certainty evidence). See Analysis 1.12.

1.9. Adverse events

von Horn 2017 reported no evidence of a difference on the Depression Anxiety and Stress Scale (DASS)-21 in depression (MD 0.60 points, 95% CI −0.66 to 1.86; 1 RCT; n = 80); anxiety (MD 0.00 points, 95% CI −0.60 to 0.60; 1 RCT; n = 80); or stress (MD 1.10 points, 95% CI −0.42 to 2.62; 1 RCT; n = 80) (moderate-certainty evidence). See Analysis 1.13 and Figure 9.

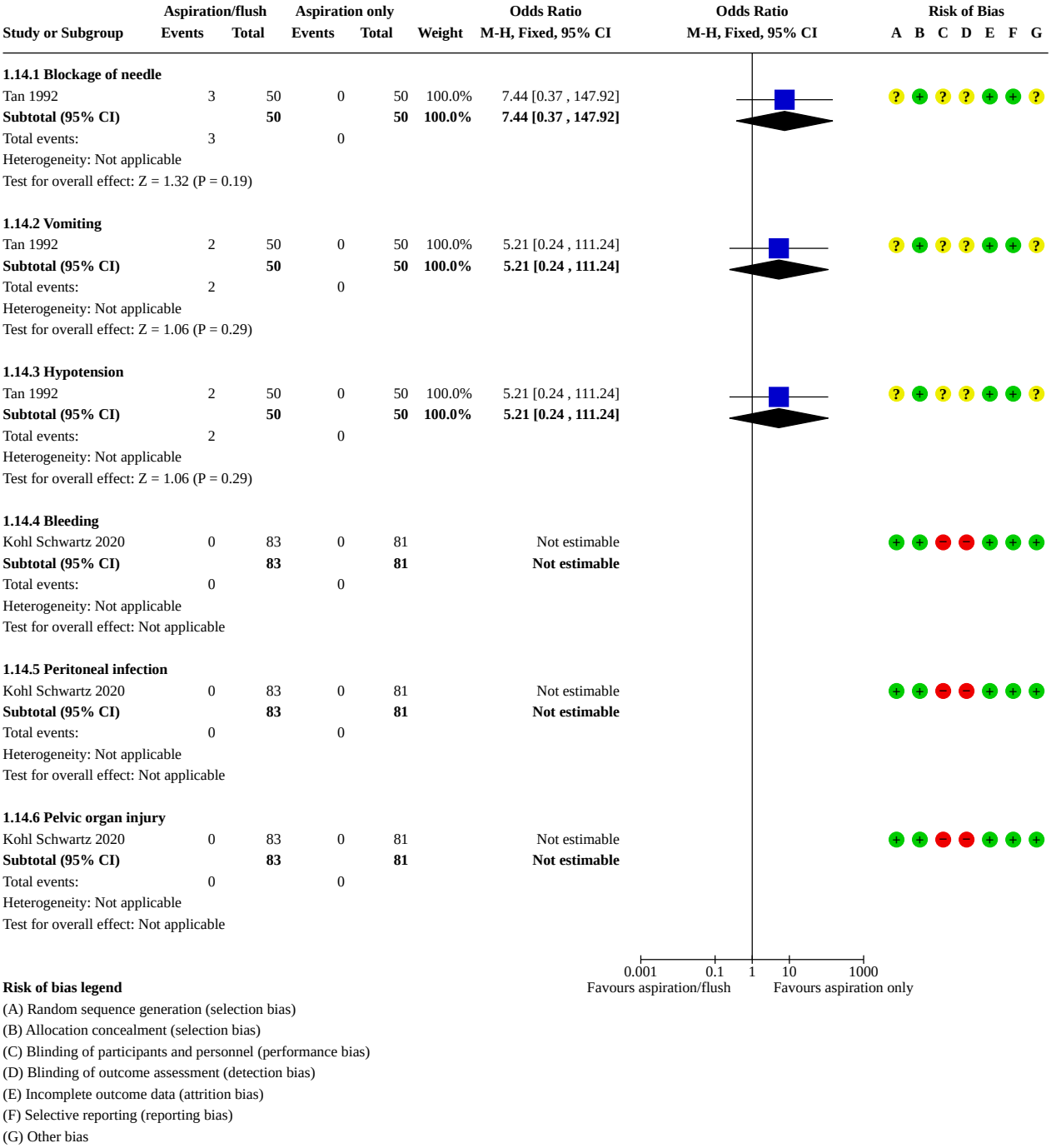
Figure 9. Forest plot of comparison: 1 Follicular flushing, outcome: 1.10 Adverse events (continuous data).



Risk of bias legend
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding of participants and personnel (performance bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

Tan 1992 reported on three adverse events: blockage of the needle (OR 7.44, 95% CI 0.37 to 147.92; 1 RCT; n = 100); vomiting (OR 5.21, 95% CI 0.24 to 111.24; 1 RCT; n = 100); and hypotension (OR 5.21, 95% CI 0.24 to 111.24; 1 RCT; n = 100). We found no evidence of a difference between aspiration/flush compared with aspiration alone for any of these outcomes (Analysis 1.14; Figure 10). Tan 1992 reported that significantly less analgesia was required with the aspiration-alone procedure compared with added flushing (median 50 mg, range 50 to 100 mg for aspiration alone; median 100 mg, range 50 to 100 mg for aspiration/flushing). It should be noted that event rates were low and were derived from a single study, hence caution is advised in interpreting these data.

Figure 10. Forest plot of comparison: 1 Follicular flushing, outcome: 1.11 Adverse events (dichotomous data).



Risk of bias legend
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding of participants and personnel (performance bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

Kohl Schwartz 2020 reported no significant difference in pain scores as assessed on a visual analogue scale (MD 0.29, 95% CI −0.27 to 0.85; 1 RCT; n = 164). See Analysis 1.13 and Figure 9. Furthermore, they reported no instances of significant bleeding, peritoneal infection, or pelvic organ injury. See Analysis 1.14 and Figure 10.

No study provided data on safety.

DISCUSSION

Summary of main results

This Cochrane Review aimed to evaluate the effectiveness of follicular flushing (aspiration/flush) compared with aspiration alone in women undergoing in vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI). Based on moderate-certainty evidence, we are uncertain of the impact of follicular flushing on the primary outcome of live birth. In light of the very

low certainty of the available evidence, we are also uncertain of the impact of follicular flushing on the primary outcome of miscarriage.

Even though oocyte yield appeared to be numerically lower with follicular flushing compared to aspiration alone, we are uncertain of the overall impact of this intervention due to the very low certainty of the evidence. Of note, follicular flushing may increase the duration of oocyte retrieval compared to aspiration alone. We are uncertain of the impact of follicular flushing on the total number of embryos, the total number of cryopreserved embryos, clinical pregnancy rate, and ongoing pregnancy rate. The available evidence was insufficient to permit any firm conclusions regarding adverse events or safety (Summary of findings 1).

Overall completeness and applicability of evidence

Compared to the last version of this review, we noted that more studies are reporting on the key primary outcomes of live birth and miscarriage. No studies reported on this review's subgroup of maternal age. Additionally, only one study focused on women with poor ovarian reserve (Haydardedeoglu 2017); however, as all participants in this study also had a poor response to ovarian stimulation, we decided to include these women in this subgroup, as it represents the more clinically relevant subgroup. Four studies evaluated poor response to ovarian stimulation and noted no difference between groups in live birth rate (Calabre 2020; Haydardedeoglu 2017; Malhotra 2020; Mok-Lin 2013). Of note, Kohl Schwartz 2020 examined the merits of follicular flushing specifically in monofollicular IVF with treatments within a natural cycle or a cycle undergoing stimulation with clomiphene citrate. They reported a higher oocyte yield with follicular flushing, but this did not appear to impact the live birth rate.

Most of the included papers focused on oocyte yield, and seven studies incorporated data that could not be used for meta-analysis (Haines 1989; Kingsland 1991; Mok-Lin 2013; Tan 1992). Nevertheless, these studies reported no change in oocyte yield with follicular flushing. In addition, four studies incorporated data on duration of oocyte retrieval that could not be used for meta-analysis (Calabre 2020; Kingsland 1991; Kohl Schwartz 2020; Tan 1992). These data mirrored the data presented in the meta-analysis, which suggested that follicular flushing may lengthen the procedure duration.

Although the included studies provided few data on adverse events, von Horn 2017 reported no differences between groups in depression, anxiety, or stress; Tan 1992 reported no differences in needle blockage, vomiting, or hypotension; and Kohl Schwartz 2020 reported on pain scores as well as significant bleeding, peritoneal infection, and pelvic organ injury. Data on adverse events should be interpreted with caution, as individual studies were relatively small and event rates low.

Notwithstanding that changes in clinical practice are usually slow to be implemented, the findings of this updated Cochrane Review serve to strengthen the evidence of no benefit to follicular flushing, as least in its current format.

Quality of the evidence

For this review, we identified and included only published data originating from 15 RCTs and incorporating 1643 women. Risk of bias for individual studies is summarised in Figure 2 and Figure 3.

We rated the certainty of evidence using GRADE criteria. The certainty of the evidence ranged from very low to moderate, with evidence downgraded as a result of lack of blinding, imprecision, and inconsistency. Although lack of blinding was a feature of several included studies, and blinding of the operator was not possible, we suggest that this was not essential, as study outcomes were objective. See Summary of findings 1.

Potential biases in the review process

We aimed to reduce the risk of publication bias by conducting systematic searches of multiple databases and trial registries to identify ongoing studies. We contacted trial authors to request further information when applicable, but unfortunately did not receive a response in all cases. Subgroup analysis was not possible for the subgroups of age and poor ovarian reserve owing to lack of data. As prespecified, we performed sensitivity analysis for the primary outcome of live birth. We were unable to construct a funnel plot given the small number of included studies.

Agreements and disagreements with other studies or reviews

Older studies, which were not randomised controlled trials, have suggested that oocyte yield increases with follicular flushing. For example, Bagtharia 2005 found 40% of oocytes in primary aspiration without flushing of the follicle and retrieved up to 82% of oocytes with two flushes and up to 97% with four flushes. Mendez Lozano 2008 observed a 46.8% oocyte recovery rate with aspiration only compared with 84.6% with additional follicular flushing in 165 infertile women with low ovarian reserve who were undergoing 271 consecutive minimal stimulation IVF cycles.

However, data from this systematic review contradict these findings, showing no increase in oocyte yield or in the more clinically relevant outcome of live birth. In addition, recent systematic reviews on the topic are all broadly in agreement with our findings (Levy 2012; Neumann 2018; Roque 2012). All three of these systematic reviews incorporated studies that we have included in this update.

AUTHORS' CONCLUSIONS

Implications for practice

Based on the available evidence, we are uncertain of the effect of follicular flushing on both live birth and miscarriage rates compared with aspiration alone. Although the evidence does not allow for any firm conclusions to be drawn on the impact of follicular flushing on oocyte yield, total number of embryos, number of cryopreserved embryos, clinical pregnancy rate, or ongoing pregnancy rate, it may be that the procedure itself takes longer than aspiration alone. The evidence was insufficient to permit any firm conclusions regarding adverse events or safety.

Implications for research

Although the body of evidence suggestive of no benefit to follicular flushing is growing, further research centred predominantly on population selection and outcomes is required. Study design could be improved by blinding participants, the embryologist, and those assessing outcomes.

Population

As suggested in the previous update of this review, most research so far has not focused on specific populations that may benefit from follicular flushing. Future directions could involve focusing on populations such as older women, women with poor ovarian reserve, and women with a poor response to ovarian stimulation, including the specific situation of monofollicular in vitro fertilisation.

Outcomes

Although more recent studies have focused on the outcome of live birth rate, this remains an underreported outcome. Further research should incorporate this as the primary outcome. In addition, we advise that future studies focus on adverse event reporting including postoperative pain and rarer complications such as infection and visceral injury.

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Calabre 2020

Study characteristics	
Methods	Prospective parallel randomised study
Participants	Country: France Site: Medico-Surgical and Obstetric Center CMCO, Schiltigheim, France

Calabre 2020 *(Continued)*

	Median age: 37 years for control and intervention groups Inclusion: age < 43 years old, 4 or fewer follicles > 14 mm on the day of trigger Exclusion: age > 43 years old, contraindication to ovary puncture, oocyte donor, viral-positive couples, non-French speakers, inability to consent, lack of follicles on day of trigger, weekend oocyte retrieval
Interventions	The stimulation protocol, gonadotropin duration, and dose were selected prior to recruitment to the study based on age, BMI, baseline hormone work-up (FSH, LH, and AMH), and response to any previous stimulation. Either long protocol with GnRH agonists or short protocol with GnRH antagonists was used. Patients who underwent a stimulation protocol for the purpose of IVF and demonstrated 4 follicles measuring more than 14 mm on the day of hCG 5000 administration were recruited on the day of trigger. Participants were randomised into 1 of the following 2 groups. 1. Simple aspiration group (= NO FLUSH): puncture was performed following the department's standard protocol, with a 35-centimetre single-lumen 17-gauge needle (Cook EchoTip Single Lumen Aspiration Needle 1735). The follicular fluid was collected in tubes without differentiating between the follicles. 2. Follicular flushing group (= FLUSH): puncture was performed with aspiration and follicular flushing. A first follicle was aspirated using a 35-centimetre double-lumen 17-gauge needle (Cook EchoTip Double Lumen Aspiration Needle K-OPSD-1735-B-L), and the fluid collected in a tube labelled with the follicle number (here # 1), after which the tube was changed to collect the flush-out from this same follicle using a flushing medium (flushing aspiration of the follicular fluid was performed in all women using a Cook K-MAR-5200 vacuum pump set at −150 mmHg. Following oocyte retrieval, women followed a standard luteal-phase maintenance treatment with vaginally applied 400 mg progesterone. No drug treatment was specifically prescribed for the protocol. Transfer of the embryo(s) took place on day 3 (D3) or day 5 (D5) under ultrasonic guidance. Any supernumerary embryos were frozen if they were of sufficient quality. The participant then followed a daily vaginal progesterone treatment until D21 from oocyte retrieval, at which point a pregnancy test was performed. An ultrasonic investigation was then carried out 6 weeks after transfer to assess whether the pregnancy was progressing.
Outcomes	Number of oocytes retrieved oocytes (mean ± SD) Number of metaphase II oocytes (median + IQR) Duration of oocyte retrieval (minutes, median + IQR) Fertilisation rate (%) Number of transferable embryos (median + IQR) Number of embryo cryopreserved (median + IQR) Live birth rate (n)
Notes	Trial authors contacted (reply awaited).

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No details
Allocation concealment (selection bias)	Unclear risk	Quote: "numbered opaque sealed envelope prepared in advance by the investigating team", unclear if sequential

Calabre 2020 *(Continued)*

Blinding of participants and personnel (performance bias) All outcomes	High risk	Quote: "The study was conduced on an open basis"
Blinding of outcome assessment (detection bias) All outcomes	High risk	Quote: "The study was conduced on an open basis"
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised women were analysed.
Selective reporting (reporting bias)	Low risk	All a priori outcomes were reported.
Other bias	Low risk	Baseline characteristics similar in both groups except oestradiol level on day of hCG administration (lower in the flushing group).

de Souza 2021

Study characteristics	
Methods	Prospective parallel randomised study
Participants	Country: Brazil Site: Fertipraxis, Human Reproduction Center, Rio de Janeiro Mean age ± SD: 39.07 ± 3.88 in the flushing group, 38.11 ± 3.43 in the control group Inclusion: age 34 to 42 years, ≤ 5 follicles 15 to 17 mm during stim, ≤ 4 follicles > 18 mm on hCG day Exclusion: not defined
Interventions	Follicular growth stimulation was initiated between days 2 and 5 of the cycle, with urinary (Menopur, Ferring, Germany) or recombinant gonadotropins (Pergoveris, Merck Serono, Switzerland), with individualised doses that varied from 150 to 300 IU daily, adjusted when necessary according to the assessment of the attending physician and based on ultrasound monitoring of follicular growth. Once the minimum follicular diameter criteria described above were reached, a single dose of 250 µg of rhCG (Ovidrel, Merck-Serono, Switzerland) was administered to induce ovulation and oocyte maturation. The procedure was performed 36 hours after hCG injection, with the participant sedated, with an aspiration needle attached to its own guide, properly fitted to the vaginal transducer. Aspiration was performed by emptying the follicles, in a closed-circuit system using an aspiration pump (Pioneer Pro-Pump OS 483) with pressure set at 90 mmHg. The follicular fluid was directly deposited into a 14-millilitre conical tube. In the follicular flushing group, 17-gauge double-lumen needles were used (Wallace DNS1733); after the first aspiration of each follicle, half buffered medium (PBS, Ingamed) was injected into it, followed by a new aspiration, and the liquid was evaluated by the embryologist to identify the cumulus-oocyte complex. Each follicle was aspirated up to 3 times. For participants in the other group, single-gauge 19-gauge needles (Wallace ONS1733) were used.
Outcomes	Oocyte yield (mean ± SD)
Notes	Trial authors contacted and responses received.
Risk of bias	

de Souza 2021 *(Continued)*

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No details.
Allocation concealment (selection bias)	Unclear risk	No details.
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	No details.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details.
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised women were analysed.
Selective reporting (reporting bias)	Low risk	All a priori outcomes were reported.
Other bias	Low risk	No differences in basal participant characteristics.

Haines 1989

Study characteristics	
Methods	Parallel randomised trial
Participants	Country: Australia Site: Flinders University, Flinders Medical Centre, Adelaide Participants: 36 women undergoing IVF treatment Mean age + SD: not specified Inclusion: not specified Exclusion: not specified
Interventions	Ovarian hyperstimulation was achieved with clomiphene citrate (Clomid, Merrell Dow), 50 mg twice daily on days 5 to 9 of the cycle, and human menopausal gonadotropin (Humegon, Organon), 2 ampoules daily from day 6 and continued according to response. Human chorionic gonadotropin (Profasi, Serono), 5000 IU, was administered when the dominant follicle reached 18 mm in the presence of appropriate oestradiol levels. Oocyte pickup was performed with the woman under intravenous analgesia via a single-lumen (W.A. Cook, Australia; 17G, 23.5 cm; K-OPS-1023-RWH) or double-lumen (W.A. Cook, Australia; 17G, 25 cm; K-OPSD-1725) needle. Flushing was performed up to 5 times. The single-lumen oocyte pickup represented the control group (n = 18), and the double-lumen oocyte pickup represented the intervention group (n = 18).
Outcomes	Number of follicles aspirated (mean + range)

Haines 1989 *(Continued)*

	Fertilisation rate (%)
Notes	No statement regarding competing interests. No declaration of funding source(s), if any

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No details
Allocation concealment (selection bias)	Unclear risk	No details
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	No details
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised women were analysed.
Selective reporting (reporting bias)	Unclear risk	No details
Other bias	Unclear risk	No details

Haydardedeoglu 2011

Study characteristics

Methods	Prospective parallel randomised trial
Participants	Country: Turkey Site: Department of Obstetrics and Gynaecology, Baskent University Adana Participants: 274 women undergoing ICSI treatment Mean age ± SD: 30.58 ± 4.66 in the single-lumen needle group, 30.75 ± 4.96 in the double-lumen needle group Inclusion: not specified Exclusion: women with a poor response (< 6 follicles over 12 mm on the day of hCG), women undergoing a microdose flare protocol, women with a high response (polycystic ovarian syndrome and polycystic ovaries)
Interventions	Participants underwent luteal down-regulation with 1.0 mg leuprolide acetate (Lucrin; Abbott, Istanbul, Turkey) for at least 10 days until day 2 to 3 of menses, at which point baseline ultrasonography and blood tests were carried out. If there were no cysts ≥ 2 cm and E2 levels were < 50 pg/mL, gonadotropin stimulation was performed with 150 to 225 IU gonadotropin (Puregon; Organon, Turkey). E2 monitoring began on the morning of stimulation day 5.

Haydardedeoglu 2011 *(Continued)*

	Other participants underwent a GnRH antagonist cycle with baseline ultrasonography and blood tests. If there were no cysts ≥ 2 cm and the progesterone level was < 1 ng/mL, gonadotropin stimulation was performed with 150 to 225 IU gonadotropin. E2 monitoring began on the morning of stimulation day 5. GnRH antagonist (Orgalutran; Organon) was added on day 6. Ultrasound and E2 monitoring continued until hCG administration criteria were met, i.e. at least 3 follicles with maximum diameter > 17 mm. In the single-lumen needle group (n = 125), a 17-gauge needle (Cook Ireland Ltd, Limerick, Ireland) was used to aspirate the follicles. A 17-gauge needle was used in the double-lumen needle group (n = 149); 2 mL flush medium was injected and aspirated once for each punctured follicle. Oocyte-corona complexes were denuded and ICSI performed after 2 hours of incubation. Embryos were transferred on day 3 with individualised transfer protocols for poor-grade embryos. All participants had luteal support with 90 mg progesterone (8% gel, Crinon; Serono, Istanbul, Turkey) administered vaginally each day after embryo transfer.
Outcomes	Number of retrieved oocytes (mean ± SD) Number of metaphase II oocytes (mean ± SD) Number of germinal vesicles (mean ± SD) Duration of oocyte retrieval (minutes, mean ± SD) Fertilisation rate (%, mean ± SD) Number of transferred embryos (mean ± SD) Biochemical pregnancy rate (mean ± SD) Clinical pregnancy rate (mean ± SD) Live birth rate (mean ± SD) Rate of women hospitalised with ovarian hyperstimulation syndrome (%) Cancellation rate (%)
Notes	Using a baseline live birth rate for normal-responding participants with ICSI of 35% with detectable difference between groups at 5%, a sample size of 1471 participants in each group was required to achieve 0.80 power. Recruitment was terminated after 13 months when it became evident that it would not be possible to recruit this number of participants at a single centre. No statement regarding competing interests. No declaration of funding source(s), if any

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "allocation sequence generated from a random numbers table"
Allocation concealment (selection bias)	Low risk	Quote: "use of consecutively numbered opaque, sealed envelopes"
Blinding of participants and personnel (performance bias) All outcomes	High risk	Quote: "open-label, randomized controlled trial"
Blinding of outcome assessment (detection bias) All outcomes	High risk	Quote: "open-label, randomized controlled trial"

Haydardedeoglu 2011 *(Continued)*

Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised women were analysed.
Selective reporting (reporting bias)	Low risk	All a priori outcomes were reported.
Other bias	Low risk	No differences in basal participant characteristics. No statement regarding conflicts of interest

Haydardedeoglu 2017

Study characteristics	
Methods	Prospective parallel randomised trial
Participants	Country: Turkey Site: Division of Reproductive Endocrinology and IVF Unit, Department of Obstetrics and Gynaecology, Baskent University Adana Participants: 80 women undergoing ICSI treatment Mean age ± SD: 34.3 ± 5.4 in the single-lumen needle group, 36.2 ± 3.9 in the double-lumen needle group Inclusion: women aged 20 to 43 years with poor ovarian response defined as 5 or fewer follicles ≥ 13 mm in size, serum progesterone level < 1.5 ng/mL on the day of hCG administration, and known poor functional ovarian reserve to predict a poor ovarian response to gonadotropin stimulation diagnosed by an AFC < 6 in both ovaries together with an AMH level < 0.8 ng/mL Exclusion: monofollicular ovarian response, natural IVF cycle programme, and presence of ovarian endometrioma
Interventions	Approximately half of poor responders took part in a low-dose luteal GnRH agonist programme consisting of a luteal dose of 0.5 mg leuprorelin acetate (Lucrin; Abbott, Paris, France) until day 2 or 3 following menses. After ovarian suppression was achieved, the dose was reduced to 0.25 mg until the date of 10,000 IU hCG (Pregnyl ampoule; MSD) administration. If there were no cysts ≥ 2 cm and the E2 level was < 50 pg/mL, then gonadotropin stimulation with 300 IU (Puregon; MSD, Oss, the Netherlands) was performed. Ultrasound and blood E2 monitoring continued until administration of 10,000 IU hCG when at least 2 follicles had reached maximum diameter > 17 mm. The GnRH antagonist protocol involved administration of letrozole (Femara; Novartis, Basel, Switzerland) and rFSH (Puregon; MSD). On day 2 or 3 of menses, letrozole 5 mg/d was started and continued for 5 days. Administration of gonadotropins was started on the same day with 150 IU rFSH plus 150 IU pure hMG (Menopur; Ferring Pharmaceuticals, Lozan Saint-Prex, Switzerland). A GnRH antagonist (Orgalutran; MSD) was added to this regimen when the leading follicle had reached 14 mm. Ultrasound and blood E2 monitoring continued until the hCG administration criterion was met, with at least 2 follicles having a maximum diameter of > 17 mm. 4 participants were placed on the FSH + pure hMG/GnRH antagonist protocol, which administered gonadotropins and started on day 2 or 3 of menses with 150 IU rFSH plus 150 IU pure hMG. Orgalutran was added to this regimen when the leading follicle reached 14 mm. After the leading follicle reached > 17 mm, 10,000 IU of hCG and 0.2 mg/mL triptorelin were injected. Allocation sequence was done using a random numbers table to assign participants to single-lumen (direct aspiration) or double-lumen (follicular flushing) needle groups. Consecutively numbered opaque, sealed envelopes were used on the day of oocyte retrieval. All participants were blinded to randomisation for the duration of the study. Clinicians performing the oocyte retrieval procedure were

Haydardedeoglu 2017 *(Continued)*

	notified of treatment allocation on the day of retrieval to record duration of the procedure and were given anaesthetic drug amounts. Transvaginal ultrasound-guided oocyte retrieval was performed 36 hours after trigger under sedation with 1% propofol (Fresenius Kabi, Homburg, Germany). For the single-lumen needle group (n = 40), a 17-gauge needle (Cook Ireland, Limerick, Ireland) was used to aspirate follicles. A 17-gauge needle (Cook Ireland) was also used in the double-lumen needle group (n = 40), and 2 mL was injected into each follicle via a manually pressed syringe containing 10 mL of culture medium warmed to 37 °C and re-aspirated and re-injected 3 times for each punctured follicle. The pressure at which the follicles were aspirated was strictly maintained at 80 mmHg. The oocyte–corona complexes were denuded, and ICSI was performed after a 2-hour incubation. Embryos were transferred on day 3. All participants received luteal support with daily intravaginal 90 mg progesterone (Crinone 8% gel; Merck Serono, Darmstadt, Germany) and 0.1 mg/mL triptorelin on the third day after embryo transfer.
Outcomes	Number of metaphase II oocytes retrieved (mean ± SD) Number of punctured follicles (n) Number of retrieved oocytes (n) Fertilisation rate (%, mean ± SD) Implantation rate (%, mean ± SD) Duration of procedure (seconds, mean ± SD) Total use of anaesthetic (mean ± SD) Clinical pregnancy rate (%) Live birth rate (%)
Notes	No competing interests. Funding by Baskent University Faculty of Medicine

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "Using a random numbers table, the 80 eligible patients were assigned randomly"
Allocation concealment (selection bias)	Low risk	Quote: "using consecutively numbered opaque, sealed envelopes on the day of oocyte retrieval"
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Quote: "All patients were blinded to the randomisation for the duration of the study"
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No statement regarding blinding of personnel assessing outcomes
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised women were analysed.
Selective reporting (reporting bias)	Low risk	All a priori outcomes were reported.

Haydardedeoglu 2017 *(Continued)*

Other bias	Low risk	No differences in basal patient characteristics. No competing interests declared

Kara 2012

Study characteristics	
Methods	Prospective parallel randomised trial
Participants	Country: Turkey Site: Department of Obstetrics and Gynecology, Bozok University Medical Faculty, Yozgat, Turkey Participants: 200 women undergoing ICSI treatment Mean age ± SD: 28.1 ± 5.5 in the aspiration group, 30.1 ± 5.3 in the aspiration/flush group Inclusion: not specified Exclusion: not specified
Interventions	In all participants, the pituitary was down-regulated with 0.5 mg leuprolide acetate (Lucrin, Abbott, USA), starting on the 21st day of the previous cycle. The dose was reduced to 0.25 mg and was continued until the day of hCG injection. Controlled ovarian stimulation was performed with FSH on cycle day 3. The starting FSH dose was 300 IU, and this was individually adjusted on the basis of previous treatment cycles, BMI, and age. Follicular development was monitored with E2 levels and ultrasonographic measurements. When 1 or 2 follicles reached 17 mm, hCG (Pregnyl, Schering-Plough, USA) was administered. Transvaginal ultrasound-guided needle aspiration of follicular fluid was carried out 35 to 36 hours after hCG administration. For the aspiration-only group (group 1), a single-lumen transvaginal oocyte retrieval needle (Otrieva Tapered Ovum Aspiration Needle, K-TIVM-172035-US, Cook Medical, Spencer, IN, USA) was used. In the flushing group (group 2), a double-lumen transvaginal oocyte retrieval needle (Echo Tip Double Lumen Aspiration Needle, K-OPSD-1635-A-L, Cook Medical, Spencer, IN, USA) was used. Flushing was done with 2 mL flush medium. Women were anaesthetised with propofol 1000 mg/mL (Abbott, USA) during the oocyte pickup procedure. All women underwent ICSI. Up to 4 embryos were transferred on day 2, 3, or 5 after oocyte retrieval using Rocket THin wall Transfer set (Rocket Medical, Hingham, MA, USA). Luteal support was provided by vaginal progesterone administration (Crinon 8% vaginal gel, Merck Serono, Switzerland). Progesterone administration was initiated on the oocyte pickup day and continued for 12 days until the day of pregnancy testing. In cases of pregnancy, progesterone was continued until the 12th gestational week. All women were initially randomly numbered, then computer-assisted randomisation was utilised according to the instructions at www.randomization.com.
Outcomes	Number of retrieved oocytes (likely mean, unclear if ± SD) Number of metaphase II oocytes (likely mean, unclear if ± SD) Number of metaphase I oocytes (likely mean, unclear if ± SD) Fertilisation rate (%) Clinical pregnancy rate (%) Ongoing pregnancy rate (%) Cancellation rate (%)

Kara 2012 *(Continued)*

	Duration of procedure (minutes, likely mean, unclear if ± SD)
Notes	Trial authors contacted to clarify whether data consist of mean ± SD (reply awaited). No competing interests. No declaration of funding source(s), if any

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "computer assisted randomization was utilized"
Allocation concealment (selection bias)	Unclear risk	No details
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	No details
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised women were analysed.
Selective reporting (reporting bias)	Unclear risk	No a priori statement regarding outcomes
Other bias	Low risk	No differences in basal participant characteristics. No competing interests declared

Kingsland 1991

Study characteristics	
Methods	Prospective parallel randomised trial
Participants	Country: UK Site: 34 women undergoing IVF Median age: 31 years for group 1, 30.5 years for group 2 Inclusion: aged 35 years or younger with tubal damage as the sole cause of infertility Exclusion: no details
Interventions	Downregulation with long-luteal regimen using buserelin. Ovarian stimulation with hMG, hCG administered when at least 3 follicles > 18 mm diameter Transvaginal ultrasound-guided retrieval via JP6L double-channelled needle

Kingsland 1991 *(Continued)*

	Pain relief: 1 mg lorazepam given orally on the evening before oocyte retrieval and repeated on the morning of aspiration. A single dose of 150 mg pethidine was administered IM 20 minutes before aspiration. No participants required additional anaesthesia. Group 1 had aspiration only vs Group 2 had follicles emptied, then flushed with 10 mL Earle's balanced salt solution (EBSS, Gibco, Paisley, UK) supplemented with pyruvate and bicarbonate and buffered with HEPES if the oocyte was not retrieved in the aspiration. A maximum of 2 mL of fluid was instilled into each follicle at each flush (maximum of 5 flushes per follicle). All oocyte retrievals were done by the same operator. Oocytes were washed once in flushing medium, incubated at 37 °C in 5% carbon dioxide in air, pre-equilibrated 1 mL drops of EBSS supplemented with 0.11 mg/mL sodium pyruvate, 1% sodium bicarbonate, 0.02 mg gentamicin, and 10% IMS.
Outcomes	Number of oocytes obtained (median) Time taken for oocyte retrieval (minutes, median) Fertilisation rate (%) Ongoing pregnancy rate (n)
Notes	No statement regarding competing interests. No declaration of funding source(s), if any

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No details
Allocation concealment (selection bias)	Unclear risk	No details
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	No details
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised women were analysed.
Selective reporting (reporting bias)	Low risk	All a priori outcomes were reported.
Other bias	Unclear risk	No differences in baseline participant characteristics. No statement regarding conflicts of interest

Kohl Schwartz 2020

Study characteristics	
Methods	Prospective parallel randomised study
Participants	Country: Switzerland Site: Division of Gynecological Endocrinology and Reproductive Medicine, Bern University Hospital, University of Bern, Inselspital, Bern Median age: 35 years for control and intervention groups Inclusion: age 18 to 42 years; gonadotropin-free monofollicular IVF and fertilisation via ICSI; regular menstrual cycles; ovaries reachable transvaginally for follicle aspiration; single follicle ≥ 16 mm on the day of oocyte retrieval Exclusion: more than 2 previous embryo transfers without pregnancy, LH surge on trigger day, previous enrolment in the same trial
Interventions	Monofollicular IVF was defined as IVF therapies within the natural menstrual cycle in which women injected only 5000 units of urinary human chorionic gonadotropin to trigger ovulation. In addition, women were allowed to be treated additionally with doses of CC (clomiphene citrate 25 mg/day from day 6 until induction of ovulation) to reduce the risk of premature ovulation. Ovulation was induced 36 h before OPU. On the day of OPU, after confirmation of the presence of a follicle (16 mm) by transvaginal ultrasound scan, women were randomised real-time online to either the follicular flushing or the aspiration-only study arm. Follicles were aspirated with an aspiration pressure of 220 mmHg to achieve a flow rate of 20 to 25 mL/min, which is the value suggested for oocyte retrieval with minimal damage to the COC and zona pellucida, following the manufacturer's suggestion. This was done without anaesthesia or analgesia, using gauge (G) 19 single-lumen needles (NMS Biomedical SA, Praroman, Switzerland). In the aspiration-only group, the needle was retracted after emptying the follicle, whereas in the follicular flushing group, the follicle was aspirated and the needle was left inside the follicle to flush the follicles 5 times with a flushing medium containing heparin (SynVitroVR Flush, Origio, Berlin, Germany). Flushing volume was calculated (sphere formula) based on the size of the follicle. The needle was rinsed at the end of the aspirations. Embryos were transferred at cleavage stage (day 2 or 3 after OPU) under ultrasound guidance.
Outcomes	Live birth rate (n) Miscarriage rate (n) Duration of oocyte retrieval (minutes, median + IQR) Clinical pregnancy rate (n) Adverse event: pain (mean ± SD) Adverse event: bleeding (n) Adverse event: peritoneal infection (n) Adverse event: pelvic organ injury (n)
Notes	Trial authors contacted and responses received.

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**

Kohl Schwartz 2020 *(Continued)*

Random sequence generation (selection bias)	Low risk	Quote: "Block randomisation with block sizes of two, four and six, stratified according to age and stimulation scheme)".
Allocation concealment (selection bias)	Low risk	Author correspondence: "There was a randomized allocation within the database “RedCap”. The randomization was real-time after the sonographic check-up at the day of follicular retrieval."
Blinding of participants and personnel (performance bias) All outcomes	High risk	No blinding
Blinding of outcome assessment (detection bias) All outcomes	High risk	No blinding
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised women were analysed.
Selective reporting (reporting bias)	Low risk	All a priori outcomes were reported.
Other bias	Low risk	Quote: "There were no differences in age and in BMI between the two groups."

Lainas 2018

Study characteristics	
Methods	Prospective parallel randomised study
Participants	Country: Greece Site: unclear if single or multicentre Mean age ± SD: 34.6 ± 4.7 Inclusion: aged < 42 years with intact ovaries, each containing at least 4 follicles > 11 mm on the day of hCG Exclusion: women with endometriotic cysts
Interventions	Oocyte retrieval was performed 35 to 36 hours after hCG by transvaginal ultrasound-guided aspiration using the same double-lumen needle (16 G, Casmed International Ltd, UK) and the same digitally adjusted aspiration vacuum for both ovaries. The right and left ovary from each woman were randomised to be aspirated using either follicular flushing or no flushing.
Outcomes	Oocyte recovery rate (%, 95% confidence interval) Number of COC retrieved (mean, 95% confidence interval) Number of metaphase II oocytes (mean, 95% confidence interval) Number of fertilised oocytes (mean, 95% confidence interval) Maturation rates (mean, 95% confidence interval) Proportion of embryos transferred (mean, 95% confidence interval)

Lainas 2018 *(Continued)*

	Adverse event: bleeding (no units)
Notes	Trial authors contacted and responses received.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "computer-generated randomization list"
Allocation concealment (selection bias)	Unclear risk	No details
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	No details
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised women were analysed.
Selective reporting (reporting bias)	Low risk	All a priori outcomes were reported.
Other bias	Unclear risk	No statement regarding differences in basal patient characteristics.

Levens 2009

Study characteristics

Methods	Prospective randomised study
Participants	Participants: 30 poor responders undergoing ART Site: Walter Reed Army Medical Center ART Program, USA Mean age: 37.1 ± 3.2 and 36.2 ± 3.4 years for single- and double-lumen groups, respectively (P = 0.48) Inclusion: low responders with a cumulative follicle count of 4 to 8 follicles ≥ 12 mm (with at least 2 follicles achieving ≥ 16 mm)
Interventions	Pre-treatment with OCPs during the cycle preceding ovarian stimulation. A combination of rFSH (Gonal-F) and hMG (Repronex, Ferring) was given twice daily. Adequate follicular development was assessed by serial serum E2 ultrasound. hCG 10,000 IU was given, followed by transvaginal oocyte retrieval 34 to 36 hours later. Assignment to single- or double-lumen group was done immediately before oocyte retrieval. Computerised randomisation in blocks of 10 to 20 was used to ensure balanced group size. Concealment was achieved by using sequentially numbered, opaque envelopes that were opened in the operating room after anaesthesia was administered. The length and diameter of retrieval needles were standardised (35 cm, 16G) to control flow dynamics within the needle that may affect oocyte recovery. Cook EchoTip single-lumen (K-J-ANC-16R-35) and double-lumen (K-OPSD-1635-B-S) transvaginal oocyte retrieval needles were used. Suction pressure of 150 to 200 mmHg (provided by Pioneer

Levens 2009 *(Continued)*

	Pro-pump, Genx International, Guilford, CT, USA) was used under direct transvaginal ultrasound guidance (Acuson Sequoia 512 with an 8-megahertz probe). Women in the single-lumen needle group did not undergo saline follicular flushing (direct aspiration), whereas those in the double-lumen group had each aspirated follicle flushed once with 2 mL sterile PBS and subsequently re-aspirated.
Outcomes	Number of oocytes obtained (mean ± SD) Total oocytes mature, maturity (%) Fertilisation rate (%) Implantation rate (%) Ongoing pregnancy rate (%) Retrieval times (seconds, mean ± SD)
Notes	No statement regarding competing interests. No declaration of funding source(s), if any

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "randomly assigned" Quote: "computerised randomization in blocks of 10 and 20 to ensure balanced group size"
Allocation concealment (selection bias)	Low risk	Quote: "Allocation was performed by the Walter Reed Army Medical Center Department of Clinical Investigation and concealed by using sequentially numbered, opaque envelopes that were opened in the operating theater after anesthesia was administered"
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Quote: "the embryologist identifying and collecting the oocytes remained blinded to the group assignments" Quote: "The providers performing the oocyte retrieval remained blinded to the number of oocytes retrieved until the completion of the procedure"
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised women were analysed.
Selective reporting (reporting bias)	Low risk	A priori outcomes were reported.
Other bias	Unclear risk	No differences in basal participant characteristics. No statement regarding competing interests

Malhotra 2020

Study characteristics

Methods	Prospective parallel randomised study

Malhotra 2020 *(Continued)*

Participants	Country: India Site: Reproductive Medicine Unit, All India Institute of Medical Sciences, New Delhi Mean age ± SD: 32 ± 3.9 in the flushing group, 32.5 ± 4.1 in the control group Inclusion: age 22 to 38 years; 3 to 5 follicles ≥ 14 mm on the day of trigger injection, normal uterine cavity Exclusion: ≤ 2 follicles on the day of trigger, ovarian endometrioma
Interventions	All participants underwent controlled ovarian stimulation by long agonist or antagonist protocol. Women who underwent long protocol were started on GnRH-a (leuprolide) 0.5 mg on day 21 of the previous cycle. Once downregulated (serum oestradiol < 40 pg/mL, LH < 3 IU/mL, no follicles > 10 mm, and endometrial thickness < 4 mm), gonadotropin (rFSH - Gonal F; Merck Serono) was started at doses ranging from 375 to 450 IU per day. Women who underwent antagonist protocol were started on rFSH (Gonal F; Merck Serono) from day 2 of the menstrual cycle. Follicle monitoring was started from day 5 of stimulation. GnRH antagonist (Cetrotide; Serono Laboratories) was initiated when lead follicle measured 14 mm. Serial follicle tracking was done to assess ovarian response to stimulation and gonadotropin doses were adjusted accordingly. All women were triggered with rHCG (250 µg, Ovitrel, Merck Serono) when there were at least 2 follicles ≥ 18 mm. The number and the size of all the follicles were documented on the day of the trigger. Serum oestradiol and progesterone were estimated, and women were randomised to undergo follicular flushing (Group A) or direct aspiration (Group B). Oocyte retrieval was done 34 to 36 hours after hCG trigger under short general anaesthesia. In women randomised to the flushing group, a double-lumen needle of 17 gauge was used. Oocyte retrieval was done under transvaginal ultrasound guidance, with a suction of 160 to 180 mmHg. 2 mL of flush with culture medium (Vitrolife Sweden AB, Göteborg, Sweden) was used each time if no oocyte was retrieved at direct aspiration. In case no oocyte was retrieved at first flush, further flushes were done up to a maximum of 3 flushes before moving to the next follicle. In women randomised to direct aspiration, oocyte retrieval was done as the standard procedure using a single-lumen needle of 17 gauge with a suction pressure of 100 to 110 mmHg. Retrieved oocytes were inseminated or injected with husband's spermatozoa by conventional IVF or ICSI. Fertilisation check was done 16 to 18 hours after insemination. All women underwent fresh embryo transfer. Up to a maximum of 2 good-quality embryos were transferred on day 3 or 5 under ultrasound guidance using a soft embryo transfer catheter (Cook's Medical, Sydney, Australia). Luteal support was given in the form of micronised progesterone 100 mg daily IM injections (Injection Susten, Sun Pharma, India). Serum hCG was checked 16 days after embryo transfer, and those with a positive hCG were confirmed for clinical pregnancy by sonography 4 weeks after embryo transfer.
Outcomes	Live birth rate (n) Miscarriage rate (n) Oocyte yield (mean ± SD) Duration of oocyte retrieval (minutes, mean ± SD) Clinical pregnancy rate (n)
Notes	Trial authors contacted and responses received.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "Allocation sequence were generated with help of computer-generated random numbers using STATA software. Block randomisation was done to obtain equal distribution between two groups."

Malhotra 2020 *(Continued)*

Allocation concealment (selection bias)	Unclear risk	Quote: "Allocation concealment was achieved by using an opaque envelope which was opened before the procedure." Unclear if the envelopes were numbered and sealed.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Quote: "Patients and embryologists were blinded."
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	Low risk	Data for all women were reported.
Selective reporting (reporting bias)	Low risk	A priori outcomes were reported.
Other bias	Low risk	No differences in baseline characteristics

Mok-Lin 2013

Study characteristics	
Methods	Prospective parallel randomised study
Participants	Country: USA Site: Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine of Weill Cornell Medical College Participants: 50 women undergoing IVF Mean age ± SD: 39.5 ± 3.2 in the direct aspiration group, 38.2 ± 4.3 in the flushing group Inclusion: poor responders with 4 or fewer follicles ≥ 12 mm Exclusion: women without a planned fresh embryo transfer; undergoing natural IVF; women whose cycles were cancelled before hCG administration; women offered enrolment or randomised in a previous cycle
Interventions	Most poor responders utilised a GnRH antagonist protocol with luteal oestrogen priming. Women were placed on a 0.1-milligram oestradiol patch every other day beginning 8 to 10 days after an LH surge, followed by COH on day 2 of menses. Other protocols included the use of a GnRH antagonist without priming, 5 days of clomiphene citrate or letrozole, and daily subcutaneous 40 µg leuprolide with COH. COH was performed with rFSH and human menopausal gonadotropins. hCG 10,000 IU was administered intramuscularly when 1 to 2 follicles ≥ 17 mm were present. Women were randomised on the day of hCG administration to direct aspiration or flushing. Treatment allocation was performed via a computer-generated randomisation sequence. Allocation was concealed by sequentially numbered, opaque envelopes. All participants were blinded to randomisation. Embryologists were blinded to the allocation scheme. In the direct aspiration group, a 16-gauge single-lumen oocyte retrieval needle (EchoTip ovum aspiration needle; Cook Medical; Bloomington, IN, USA) was used to aspirate follicles with transvaginal ultrasound guidance. In the flushing group, each aspirated follicle was flushed up to 4 times via a manually pressed syringe with 5 mL of culture media warmed to 37 °C and was re-aspirated with a 16-gauge double-lumen needle (EchoTip Double Lumen

Mok-Lin 2013 *(Continued)*

	Aspiration Needle; Cook Medical). All embryos were transferred on day 3, and transferring physicians were blinded to the assigned intervention.
Outcomes	Number of oocytes retrieved (mean ± SD) Anaesthesia time (minutes, mean ± SD) Procedure time (minutes, mean ± SD) Number of mature oocytes (mean ± SD) Number of embryos transferred (mean ± SD) Implantation rate (%) Clinical pregnancy rate (%) Live birth rate (%)
Notes	No competing interests. No external funding for the study

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "Treatment allocation was performed using a computer-generated randomization sequence"
Allocation concealment (selection bias)	Low risk	Quote: "Allocation was performed by the research team and concealed using sequentially numbered, opaque envelopes"
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Quote: "All patients were blinded to the randomization for the duration of the study" Quote: "Determination of need for ICSI and selection of embryos for transfer were performed by embryologists blinded to the allocation scheme" Quote: "All embryos were transferred on Day 3 and transferring physicians were blinded to the assigned intervention"
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No statement regarding blinding of personnel assessing outcome
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised women were analysed.
Selective reporting (reporting bias)	Low risk	All a priori outcomes were reported.
Other bias	Low risk	No differences in basal participant characteristics. No competing interests declared

Scott 1989

Study characteristics

Scott 1989 *(Continued)*

Methods	Prospective parallel randomised study
Participants	Country: USA Participants: 44 women undergoing IVF Median ages of both groups not given. No inclusion or exclusion criteria given.
Interventions	All women underwent gonadotropin stimulation via previously described protocols (in textbook, no details given in the paper). Retrieval with single-lumen needle (n = 22) was done with a Swe-Med needle (outer diameter 1.5 mm, inner diameter 1 mm) (Swe-Med Lab, Frolunda, Sweden). The follicle was aspirated with a hand-held 20-millilitre syringe, and the needle was removed from the participant; this was followed by aspiration of an additional 2 mL of heparinised Dulbecco's solution through the system to wash fluid in the dead space back into the syringe. The double-lumen needle (Swe-Med Lab) had an inner diameter of the aspiration lumen of 1 mm and an outer diameter of 1.6 mm. The follicle was aspirated, then 1 to 3 mL of heparinised Dulbecco's solution was injected into the follicle through the second port. This volume was then aspirated back into the syringe. Lavage was performed 1 more time until the oocyte was recovered, or until the follicle was not re-expanding well, before proceeding to the next follicle. Pain relief: method not mentioned
Outcomes	Number of follicles aspirated and number of oocytes retrieved (mean ± SE) Incidence of fractured zona in both groups (%)
Notes	No statement regarding competing interests. No declaration of funding source(s), if any

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No details
Allocation concealment (selection bias)	Unclear risk	No details
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	No details
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised women were analysed.
Selective reporting (reporting bias)	Unclear risk	Fertilisation rate and clinical pregnancy rate were not described.

Scott 1989 *(Continued)*

Other bias	Unclear risk	No details

Tan 1992

Study characteristics	
Methods	Prospective parallel randomised study
Participants	Country: UK Site: 100 women undergoing IVF treatment at an assisted conception unit Median age was 32 (25 to 42 years) for group 1 and 32.5 (23 to 43 years) for group 2. Inclusion: not specified Exclusion: women who had developed > 25 or < 4 follicles wider than 14-millimetre diameter on the day of hCG administration
Interventions	Long follicular protocol, starting buserelin acetate (Suprefact; Hoechst, Hounslow, UK) was administered intranasally (200 µg 4-hourly) on day 1 or 2 of the menstrual cycle. When serum oestradiol concentration was < 200 pmol/L, human menopausal gonadotropin (Pergonal; Serono, Welwyn Garden City, UK) was started at 2 to 6 ampoules daily. hCG (Profasi; Serano) 10,000 IU was administered when there were at least 4 follicles > 14 mm in diameter, and mean diameter of the largest follicle was > 20 mm. Transvaginal ultrasound-guided follicle aspiration was performed 33 to 38 hours post-hCG as an outpatient procedure. Pain relief was achieved with intravenous pethidine 50 to 100 mg in bolus doses of 25 mg as required. Aspiration via JP6L double-channel needle (Casmed, Cheam, UK). Maximum aspiration pressure of 100 mmHg was used in both groups. Group 1 (aspiration only; n = 50): inner channel of needle removed to convert it to a single-channel needle. Each follicle was aspirated until empty. The probe was moved around until all follicular fluid was aspirated as evidenced by some blood-stained fluid in the tubing. The same procedure was repeated until all follicles > 10 mm had been aspirated from the first ovary. After dead space in the needle was cleared, the procedure was repeated in the second ovary. Group 2 (aspiration and flushing, n = 50): double-channel needle used, and the follicle aspirated through the inner channel. This initial aspirate was termed A1. Once the follicle had been emptied, the collecting tube was changed and, with the valve open, flushing medium was injected until 1.5 mL of fluid had been collected. This was termed A2. A1 and A2 were examined separately, and if no oocyte was observed, the follicle was flushed up to a maximum of 6 times. 1 to 3 embryos were transferred 48 to 72 hours after oocyte recovery.
Outcomes	Number of follicles aspirated and number of oocytes obtained (median + range) Time taken for oocyte aspiration (minutes, median + range) Dose of pethidine required (mg, median + range) Fertilisation rate (%, range) Number of embryos transferred (median + range) Clinical pregnancy rate (%, range)

Tan 1992 *(Continued)*

Notes	No statement regarding competing interests. No declaration of funding source(s), if any

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Unclear as to how randomisation was performed
Allocation concealment (selection bias)	Low risk	Randomised by drawing serially number sealed envelopes
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	No details
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	Low risk	Data for all women were reported.
Selective reporting (reporting bias)	Low risk	A priori outcomes were reported.
Other bias	Unclear risk	No differences in basal participant characteristics. No statement on competing interests

von Horn 2017

Study characteristics

Methods	Prospective parallel randomised study
Participants	Country: Germany Site: Department of Reproductive Endocrinology and Reproductive Medicine, University Hospital of Schleswig-Holstein, Lübeck Participants: 80 women undergoing ICSI treatment Mean age ± SD: 38.7 ± 5.0 in the no-flushing group, 37.5 ± 4.3 in the flushing group Inclusion: BMI > 18 kg/m^2 and < 35 kg/m^2, between the age of 18 and 45 years, presenting with a total ≤ 5 follicles > 10 mm in both ovaries combined at the end of the follicular phase of the treatment cycle Exclusion: 1 ovary absent (e.g. after ovarectomy) or 1/both ovaries foreseeably difficult to puncture (e.g. heterotopic site because of adhesions)
Interventions	IVF protocol used is not described in detail. Final oocyte maturation was induced by 5000 IU urinary hCG as soon as the leading follicle reached a mean diameter of 18 mm or the day thereafter, and oocyte pickup was scheduled 34 to 38 hours thereafter.

von Horn 2017 *(Continued)*

	On the day of the decision to trigger final oocyte maturation, women were randomised to either study group (Steiner-Tan Needle) or control group (Gynetics). The Steiner-Tan needles and the flushing system were provided for free by the manufacturer. Randomisation was performed by 1 of the doctors who performed sonographic monitoring by opening a sealed, opaque, and sequentially numbered envelope containing allocation of the participant. The random sequence was software generated and was produced by 1 of the trial authors. Blocks of 4 were used. In the study group (n = 40), all visible follicles were aspirated with suction pressure of 180 mmHg, then were flushed 3 times under ultrasound. In the control group (n = 40), all visible follicles were aspirated with suction pressure of 180 mmHg. Intracytoplasmic sperm injection was performed as per standard operating procedure.
Outcomes	Number of COC (mean ± SD) and oocyte retrieval rate Number of metaphase II oocytes (mean ± SD) Number of fertilised oocytes (mean ± SD) Proportion of participants undergoing embryo transfer (%) Ongoing pregnancy rate (n) Duration of procedure (minutes, mean ± SD) Depression Anxiety and Stress Scale score (DASS-21) Pain assessment by visual analogue scale 2 hours postprocedure
Notes	Steiner-Tan needles were provided for free by the manufacturer. Study authors declared receiving personal fees.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "The random sequence was software-generated and was produced by one of the authors (G.G.). Blocks of four were used"
Allocation concealment (selection bias)	Low risk	Quote: "sealed, opaque and sequentially numbered envelope containing the allocation of the patient"
Blinding of participants and personnel (performance bias) All outcomes	High risk	Quote: "randomized, controlled, open, superiority trial"
Blinding of outcome assessment (detection bias) All outcomes	High risk	Quote: "randomized, controlled, open, superiority trial"
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomised women were analysed.
Selective reporting (reporting bias)	Low risk	All a priori outcomes were reported.

von Horn 2017 *(Continued)*

Other bias	Low risk	No differences in basal participant characteristics. Competing interests declared

AFC: antral follicle count
AMH: anti-Müllerian hormone
ART: assisted reproductive technology
BMI: body mass index
CC: clomiphene citrate
COC: cumulus-oocyte complex
COH: controlled ovarian hyperstimulation
DASS-21: Depression Anxiety and Stress Scale-21
DLN: double-lumen needle
E2: oestradiol
EBSS: Earle's balanced salt solution
FSH: follicle-stimulating hormone
GnRH: gonadotropin-releasing hormone
GnRH-a: gonadotropin-releasing hormone agonist
hCG: human chorionic gonadotropin
HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
hMG: human menopausal gonadotropin
ICSI: intracytoplasmic sperm injection
IM: intramuscular
IMS: inactivated maternal serum
IQR: interquartile range
IU: international units
IVF: in vitro fertilisation
LH: luteinising hormone
OCPs: oral contraceptive pills
OPU: oocyte pick-up
PBS: phosphate-buffered saline
rFSH: recombinant follicle-stimulating hormone
rHCG: recombinant human chorionic gonadotropin
SD: standard deviation
SE: standard error
SLN: single-lumen needle

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Avila 2013	148 participants divided into 2 groups: 75 allocated to follicular flushing (case), 73 allocated to aspiration only (control). Total oocytes retrieved were 11.80 ± 1.3 in the flushing group vs 9.59 ± 6.1 (P = 0.691) in the control group. Furthermore, no differences were reported in positive pregnancy test (43% and 32%, respectively) or fertilisation rate (55.5% and 55.85%, respectively). Reason for exclusion: retrospective, descriptive study
Aydin 2017	45 poor responders who all underwent aspiration followed by flushing up to 3 times. Trial authors reported an increase in the number of oocytes retrieved with sequential flushing. Reason for exclusion: prospective cohort study, not a randomised study; no aspiration-only group
Bagtharia 2005	All participants had repeated flushing of the follicles. Study compared number of oocytes obtained with each flushing after primary aspiration of the follicle. Study authors concluded that 40% of oocytes were retrieved with primary aspiration without flushing of the follicle, and up to 82% of oocytes were retrieved with 2 flushes and up to 97% with 4 flushes. Reason for exclusion: not an RCT and no control (aspiration-only) group was present

Study	Reason for exclusion
Biljan 1997	35 participants were randomised to have the left or right ovary flushed with heparinised normal saline or heparinised culture medium. Oocytes obtained from each side were cultured separately and were assessed for fertilisation 18 to 21 hours after insemination. From the side flushed with saline, 185 oocytes were collected from 237 follicles, which was not significantly different from 181 oocytes collected from 244 follicles on the side flushed with culture medium (OR 1.23, 95% CI 0.79 to 1.92). No significant difference in fertilisation rates was observed between oocytes obtained after saline (median 71.4%) and culture medium flush (median 75%) (OR 1.08, 95% CI 0.68 to 1.72). Reason for exclusion: no aspiration-only group
Dean 1997	This was an abstract of the same study as Biljan 1997 (published in *Fertility and Sterility* 1997;68:1132-4). Reason for exclusion: duplicate study (under different first trial author)
el Hussein 1992	Study evaluated 100 consecutive patients undergoing 100 cycles of IVF. 4 patients were excluded because their embryos were electively cryopreserved. Study reported an overall oocyte recovery rate of 87.8%. Of 1046 oocytes collected, 40.3% were from initial aspiration (A1), 41.3% from dead space in the collecting system (A2), 13.7% from the first 2-millilitre flush (F1), and 4.7% from the second 2-millilitre flush (F2). Comparable numbers of viable and fertilised oocytes and cleaved, transferred, and frozen embryos in tubes A1 and A2, but all these parameters were significantly lower in tubes F1 and F2 ($P < 0.001$). All these parameters were also significantly higher in F1 compared with F2 ($P < 0.001$), except for numbers of embryos frozen, which showed no difference. Overall pregnancy rate/cycle was 28.1% and pregnancy rate per ET was 31%. No pregnancy was reported in any of the cycles in which embryos originating from F2 were transferred, nor was pregnancy found in cycles in which only embryos from F1 were transferred. Study authors concluded that follicular aspiration together with one 2-millilitre flush maximises the recovery of oocytes that will result in pregnancies. Reason for exclusion: not a randomised study. Aspiration done in all cases.
Faller 2010	Randomised study comparing aspiration alone (39 participants) vs aspiration and flushing (40 participants) in poor responders. In the flushing group, 123 oocytes were collected, whereas 106 were obtained from the aspiration-alone group ($P = 0.06$). No difference was found in fertilisation or pregnancy rates. Reason for exclusion: study authors contacted owing to similarity to another conference abstract with differing participant numbers (Pirrello 2011). Study authors clarified that issues were identified related to study ethics and inclusion criteria.
Ghosh 2002	This is a comparative evaluation comparing aspiration alone (group A, 156 participants) vs repeated follicular flushing (group B, 172 participants) in women with tubal block. Study authors reported oocyte recovery of 5.2 ± 1.1 in group A and 6.2 ± 1.3 in group B. Pregnancy rate was 34.6% in group A and 34.9% in group B; miscarriage rates were 9.2% and 21.6, respectively. Reason for exclusion: not a randomised study
Gordon 2002	A randomised study comparing 2 flushing media (Medicult flushing medium in 25 cases, SynVitroFlush in 22 cases) for follicle irrigation of women undergoing IVF/ICSI treatment. Study authors observed no differences in numbers of oocytes retrieved, fertilisation rates, numbers of embryos replaced, and clinical pregnancy per oocyte collection (2/25 or 8% vs 6/22 or 27.2% for Medicult and SynVitroFlush medium, respectively; $P = 0.052$). Reason for exclusion: no aspiration-only group for comparison
Khalifa 1999	Study included 40 IVF cycles in cases with > 10 follicles. Each case was randomised to the first half of follicles (> 14 mm) flushed with non-heparinised EBSS and the second half with non-heparinised normal saline, or vice versa. 185 oocytes out of 276 follicles (67%) were retrieved when EBSS was used (group I), and 187 out of 284 follicles (65.8%) when normal saline was used (group II). Da-

Study	Reason for exclusion
	ta showed no significant differences in fertilisation (150/185, 81% vs 153/187, 82%; NS), cleavage rates (136/150, 90.6% vs 141/153, 92%; NS), or grade I embryos at 48 hours (74% vs 76%) and 72 hours (68% vs 67%) in groups I and II, respectively. Reason for exclusion: no aspiration-only group for comparison
Knight 2001	A retrospective study involving 1139 cycles of oocyte aspiration only and 1139 cycles of aspiration plus flushing at City West IVF during 1991 to 1993. 23 women had failed collections in each group and were excluded (leaving only 1139 in each group). (Total number of participants in the abstract (2378) did not match that in the text (1139 + 1139 + 23 + 23 = 2324).) Reason for exclusion: historical comparison of aspiration alone and aspiration with additional flushing of each follicle. Not a randomised trial
Lenz 1987	Oocyte collection was done in 53 cases by ultrasonically guided abdominal puncture under local or epidural anaesthesia. After follicle aspiration, 2 to 6 flushes with culture medium were performed with a syringe. A total of 196 oocytes were collected, 84 of which (42.9%) were found in the flushes. Mechanical damage was observed in 5.1% of oocytes. Cleavage rates in mature oocytes (157) after 48 hours in culture were similar in the aspirate group (56.5%) and the flush group (54.2%). 10 clinical pregnancies were reported, corresponding to a pregnancy rate of 18.9%. Reason for exclusion: TAS, not TVS approach, only 1 group of aspiration with flushing
Mehri 2014	One aim of this study was to determine whether oocytes retrieved with or without follicular flushing have different developmental competence. 49 cycles were studied; if an oocyte was not collected with aspiration alone, flushing would be conducted twice. Data showed no difference in oocyte maturity between flushed and not-flushed groups and no differences in fertilisation and cleavage rates. Reason for exclusion: observational study
Mendez Lozano 2008	Study prospectively included 165 infertile women with low ovarian reserve, 20 to 37 years of age, undergoing 271 consecutive minimal stimulation IVF cycles from January 2005 to December 2006. Oocyte retrieval was performed 34 hours after hCG administration, rather than after 36 hours, to avoid risk of possible follicular rupture before aspiration. Follicular fluid was aspirated with a single-channel 16-gauge needle attached to a 10-millilitre syringe. The aspiration needle was kept steady inside the follicle until the oocyte was found and isolated (follicular aspiration group; FA group). In case of negative oocyte recovery, sequential flushings were performed via 10-millilitre syringes filled with 3 mL of Tyrode's salt solution. These oocytes were entered into the follicular flushing group (FF). Data showed 46.8% oocyte recovery with aspiration only, compared with 84.6% with additional follicular flushings. In addition, oocytes retrieved by follicular flushing demonstrated better morphological quality (top-quality embryos 43/75 or 59.7% vs 40/98 or 41.2%; P = 0.01) and implantation outcomes (implantation rate 34.8% vs 20.4%; P = 0.04) for the corresponding embryo compared with those already present in follicular fluid. Reason for exclusion: recruited women underwent > 1 cycle of treatment. Not an RCT, as aspiration was followed by flushing only when no oocyte was obtained
NCT02277210	Study terminated due to poor recruitment. No data available for inclusion.
Neyens 2016	138 patients undergoing IVF underwent aspiration (A) and up to 3 flushes (F1, F2, F3) and were inspected for the presence of OCCs. 91% of OCCs were obtained with aspiration only (A) after 1 flush (F1); significantly more mature oocytes were collected with aspiration only (P = 0.03). Fertilisation rates were similar in all groups. Clinical pregnancy rate and live birth rate were not affected by the first 2 flushes. Reason for exclusion: observational study
Pabuccu 2021	This was a prospective study including infertile women aged between 18 and 42 years with diminished ovarian reserve who had a single follicle > 17 mm on the day of oocyte retrieval. Follicular

Study	Reason for exclusion
	flushing was performed up to 8 times in flushing group using an 17-gauge double-lumen needle. Direct follicular aspiration using a 17-gauge single-lumen needle was performed in direct aspiration group. Total numbers of collected oocytes, metaphase 2 oocytes, fertilisation and pregnancy rates were compared amongst groups. Reason for exclusion: not an RCT; authors describe the study as "quasi-experimental"
Pirrello 2011	A randomised study comparing aspiration alone (36 participants) with aspiration and flushing (38 participants) amongst poor responders. An equivalent number of oocytes was collected in both groups (P = 0.06). Reason for exclusion: we contacted the study authors regarding similarity with another conference abstract (Faller 2010), but with differing participant numbers. Study authors clarified that these data are duplicated in Faller 2010, and that differences in numbers were due to issues related to study ethics and inclusion criteria.
Waterstone 1992	All 50 participants had follicle aspiration with flushing. The origin of each oocyte was established, i.e. whether it had been obtained in the initial part of the aspirate, in the dead space aspirate, in the first to third flushes, or in the fourth to sixth flushes. Trialists concluded that 20% more oocytes were obtained than with aspiration alone. Reason for exclusion: not an RCT; only 1 group included
Ziebe 2000	In 107 IVF/ICSI cycles, Medicult and SynVitro flushing media were prospectively randomised for use in follicle flushing. No adverse effects were noted during oocyte recovery in either of the 2 groups. Average numbers of oocytes collected (8.2 ± 2.8 vs 8.3 ± 2.9), recovery rates (86.8 ± 14.6 vs 82.8 ± 15), cleavage rates (60.7 ± 30.3 vs 61.1 ± 28.2), implantation rates (21.1% vs 18.3%), and ongoing pregnancy rates per completed cycle (27.7% vs 27.5%) were similar with SynVitro and Medicult flushing media, respectively. Reason for exclusion: no aspiration-only group for comparison

A: aspiration
CI: confidence interval
EBSS: Earle's balanced salt solution
ET: embryo transfer
F: flushing
FA: follicular aspiration
FF: follicular flushing
hCG: human chorionic gonadotropin
ICSI: intracytoplasmic sperm injection
IVF: in vitro fertilisation
NS: not significant
OCC: oocyte-cumulus complex
OR: odds ratio
RCT: randomised controlled trial
TAS: transabdominal sonography
TVS: transvaginal sonography

Characteristics of studies awaiting classification *[ordered by study ID]*

Ronchetti 2022

Methods	Prospective open-label randomised controlled trial
Participants	Women aged 18 to 42 years

Ronchetti 2022 *(Continued)*

Interventions	Direct aspiration with SL1 (Cook Single Lumen) or follicular flushing with DL1 (Cook EchoTip Double Lumen)
Outcomes	Primary outcome measure: 1. Oocyte retrieval percentage per aspirated follicles Secondary outcome measures: 1. Time for single oocyte retrieval (minutes) 2. Percentage of mature (MII) oocytes retrieved
Notes	

MII: metaphase II stage

Characteristics of ongoing studies *[ordered by study ID]*

ChiCTR1800016671

Study name	The correlation between follicular flushing and oocyte retrieval in poor ovarian responders undergoing in vitro fertilization
Methods	Randomised parallel controlled trial
Participants	Inclusion criteria: 1. Women undergoing in vitro fertilisation treatment who were diagnosed as poor ovarian response 2. Indications including fallopian tube factor or male factors, or both 3. Presenting no more than 3 follicles with a diameter from 16 to 22 mm and < 5 follicles more than 12 mm at the trigger day Exclusion criteria: 1. BMI above 28 kg/m^2 2. Polycystic ovary syndrome, endometriosis, and immunologic infertility 3. Severe oligospermia or azoospermia 4. Female and male chromosome abnormality 5. Using intracytoplasmic sperm injection for fertilisation 6. Severe adenomyosis, uterine malformations, intrauterine adhesions, and other organic diseases of the uterus or uterine cavity
Interventions	Group 1: aspiration alone Group 2: follicular flushing
Outcomes	Number of oocytes retrieved Recovery rate Number of matured oocytes Fertilisation rate Number of available embryos Clinical pregnancy rate Live birth rate

ChiCTR1800016671 *(Continued)*	Duration of oocyte retrieval Dosage of anaesthetic
Starting date	July 2018
Contact information	Yu Xiao 910 Hengshan Road, Shanghai, China
Notes	Trial authors contacted (reply awaited).

BMI: body mass index

DATA AND ANALYSES

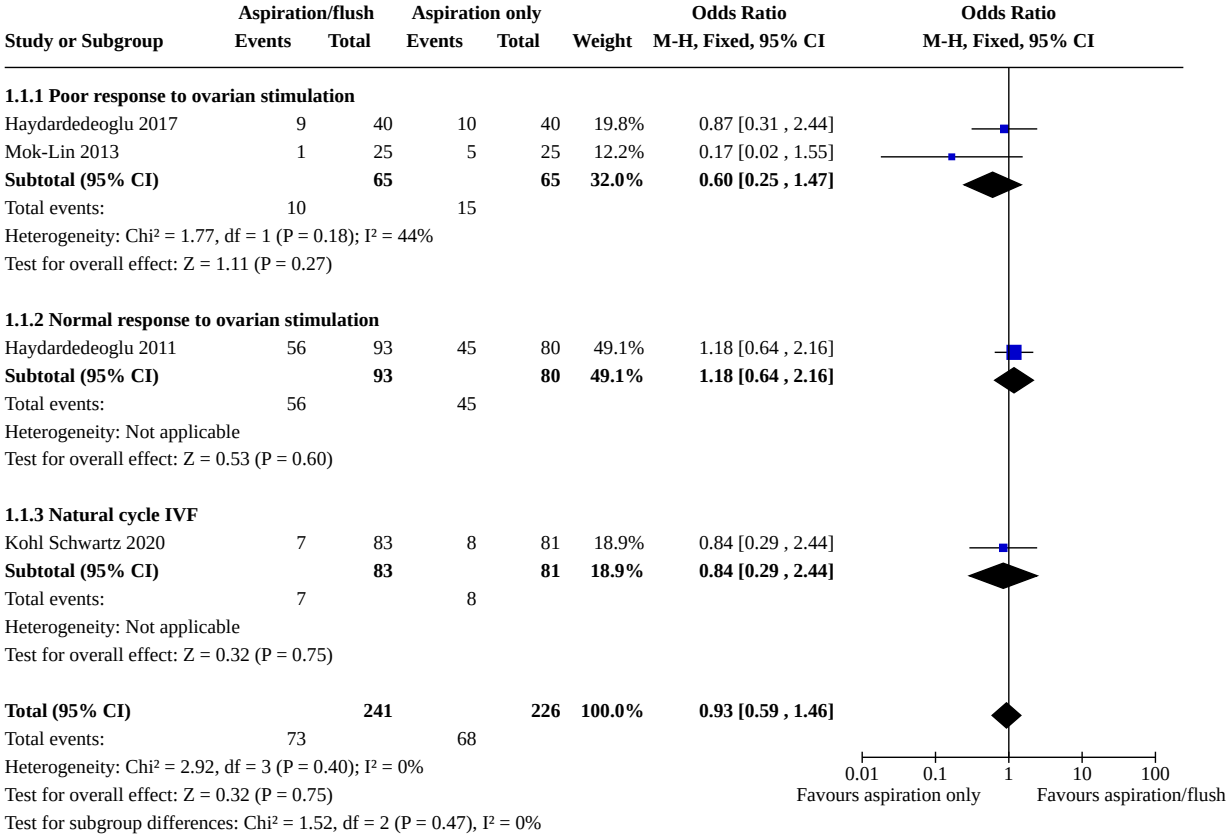
Comparison 1. Follicular flushing

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.1 Live birth rate	4	467	Odds Ratio (M-H, Fixed, 95% CI)	0.93 [0.59, 1.46]
1.1.1 Poor response to ovarian stimulation	2	130	Odds Ratio (M-H, Fixed, 95% CI)	0.60 [0.25, 1.47]
1.1.2 Normal response to ovarian stimulation	1	173	Odds Ratio (M-H, Fixed, 95% CI)	1.18 [0.64, 2.16]
1.1.3 Natural cycle IVF	1	164	Odds Ratio (M-H, Fixed, 95% CI)	0.84 [0.29, 2.44]
1.2 Miscarriage rate	1	164	Odds Ratio (M-H, Fixed, 95% CI)	1.98 [0.18, 22.22]
1.2.1 Poor response to ovarian stimulation	0	0	Odds Ratio (M-H, Fixed, 95% CI)	Not estimable
1.2.2 Normal response to ovarian stimulation	0	0	Odds Ratio (M-H, Fixed, 95% CI)	Not estimable
1.2.3 Natural cycle IVF	1	164	Odds Ratio (M-H, Fixed, 95% CI)	1.98 [0.18, 22.22]
1.3 Oocyte yield per woman randomised (normally distributed data)	9	1239	Mean Difference (IV, Fixed, 95% CI)	-0.47 [-0.72, -0.22]
1.4 Oocyte yield per woman randomised (non-normally distributed data)	4		Other data	No numeric data
1.5 Duration of oocyte retrieval (normally distributed data; seconds)	7	785	Mean Difference (IV, Fixed, 95% CI)	175.44 [152.57, 198.30]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.6 Time taken for procedure (non-normally distributed data)	4		Other data	No numeric data
1.7 Total number of embryos (normally distributed data)	2	160	Mean Difference (IV, Fixed, 95% CI)	-0.10 [-0.34, 0.15]
1.8 Total number of embryos (non-normally distributed data)	2		Other data	No numeric data
1.9 Number of embryos cryopreserved per woman randomised (normally distributed data)	2	324	Mean Difference (IV, Fixed, 95% CI)	-0.44 [-0.94, 0.06]
1.10 Number of embryos cryopreserved per woman randomised (non-normally distributed data)	1		Other data	No numeric data
1.11 Clinical pregnancy rate per woman randomised	7	939	Odds Ratio (M-H, Fixed, 95% CI)	1.13 [0.85, 1.51]
1.12 Ongoing pregnancy rate per woman randomised	4	344	Odds Ratio (M-H, Fixed, 95% CI)	1.21 [0.73, 2.02]
1.13 Adverse events (continuous data)	2		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
1.13.1 Depression	1	80	Mean Difference (IV, Fixed, 95% CI)	0.60 [-0.66, 1.86]
1.13.2 Anxiety	1	80	Mean Difference (IV, Fixed, 95% CI)	0.00 [-0.60, 0.60]
1.13.3 Stress	1	80	Mean Difference (IV, Fixed, 95% CI)	1.10 [-0.42, 2.62]
1.13.4 Pain	1	164	Mean Difference (IV, Fixed, 95% CI)	0.29 [-0.27, 0.85]
1.14 Adverse events (dichotomous data)	2		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
1.14.1 Blockage of needle	1	100	Odds Ratio (M-H, Fixed, 95% CI)	7.44 [0.37, 147.92]
1.14.2 Vomiting	1	100	Odds Ratio (M-H, Fixed, 95% CI)	5.21 [0.24, 111.24]
1.14.3 Hypotension	1	100	Odds Ratio (M-H, Fixed, 95% CI)	5.21 [0.24, 111.24]
1.14.4 Bleeding	1	164	Odds Ratio (M-H, Fixed, 95% CI)	Not estimable
1.14.5 Peritoneal infection	1	164	Odds Ratio (M-H, Fixed, 95% CI)	Not estimable

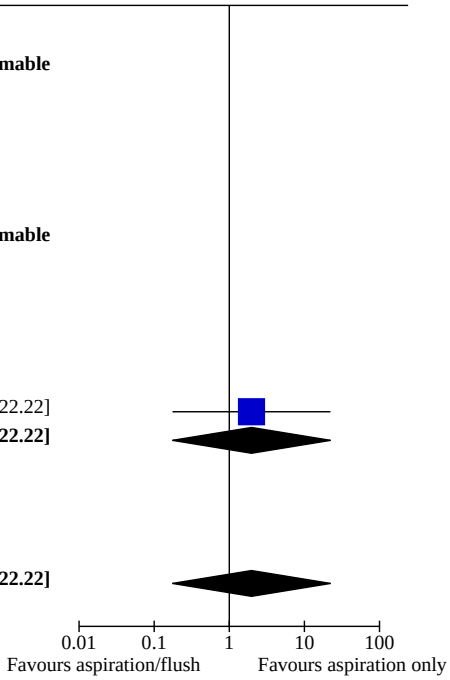
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.14.6 Pelvic organ injury	1	164	Odds Ratio (M-H, Fixed, 95% CI)	Not estimable

Analysis 1.1. Comparison 1: Follicular flushing, Outcome 1: Live birth rate



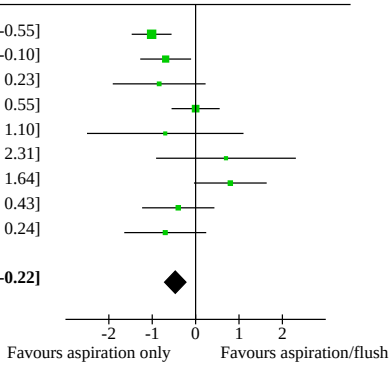
Analysis 1.2. Comparison 1: Follicular flushing, Outcome 2: Miscarriage rate

Study or Subgroup	Aspiration/flush Events	Total	Aspiration only Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
1.2.1 Poor response to ovarian stimulation						
Subtotal (95% CI)		**0**		**0**		**Not estimable**
Total events:	0		0			
Heterogeneity: Not applicable						
Test for overall effect: Not applicable						
1.2.2 Normal response to ovarian stimulation						
Subtotal (95% CI)		**0**		**0**		**Not estimable**
Total events:	0		0			
Heterogeneity: Not applicable						
Test for overall effect: Not applicable						
1.2.3 Natural cycle IVF						
Kohl Schwartz 2020	2	83	1	81	100.0%	1.98 [0.18 , 22.22]
Subtotal (95% CI)		**83**		**81**	**100.0%**	**1.98 [0.18 , 22.22]**
Total events:	2		1			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.55 (P = 0.58)						
Total (95% CI)		**83**		**81**	**100.0%**	**1.98 [0.18 , 22.22]**
Total events:	2		1			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.55 (P = 0.58)						
Test for subgroup differences: Not applicable						



Analysis 1.3. Comparison 1: Follicular flushing, Outcome 3: Oocyte yield per woman randomised (normally distributed data)

Study or Subgroup	Aspiration/flush Mean	SD	Total	Aspiration only Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95% CI
Calabre 2020	2.41	1.67	127	3.424	2.029	125	28.8%	-1.01 [-1.47 , -0.55]
de Souza 2021	3	2.11	105	3.69	2.2	103	17.7%	-0.69 [-1.28 , -0.10]
Haydardedeoglu 2011	12.25	4.44	149	13.09	4.55	125	5.3%	-0.84 [-1.91 , 0.23]
Haydardedeoglu 2017 (1)	2.3	1.2649	40	2.3	1.2649	40	19.8%	0.00 [-0.55 , 0.55]
Kara 2012 (2)	10.8	6.8	100	11.5	6.2	100	1.9%	-0.70 [-2.50 , 1.10]
Levens 2009	7.2	2.3	15	6.5	2.2	15	2.3%	0.70 [-0.91 , 2.31]
Malhotra 2020	4.5	1.7	35	3.7	1.9	36	8.6%	0.80 [-0.04 , 1.64]
Scott 1989	5.9	1.41	22	6.3	1.41	22	8.8%	-0.40 [-1.23 , 0.43]
von Horn 2017	2.4	2	40	3.1	2.3	40	6.8%	-0.70 [-1.64 , 0.24]
Total (95% CI)			**633**			**606**	**100.0%**	**-0.47 [-0.72 , -0.22]**
Heterogeneity: Chi² = 20.32, df = 8 (P = 0.009); I² = 61%								
Test for overall effect: Z = 3.73 (P = 0.0002)								
Test for subgroup differences: Not applicable								



Footnotes

(1) SD recalculated as paper appears to report SE; seeking confirmation from authors

(2) Not entirely clear that data are expressed as +/- SD. Awaiting to hear from authors.

Analysis 1.4. Comparison 1: Follicular flushing, Outcome 4: Oocyte yield per woman randomised (non-normally distributed data)

Oocyte yield per woman randomised (non-normally distributed data)

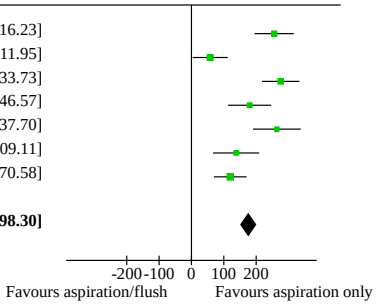
Study	Aspiration/flush	Aspiration only	p value
Haines 1989	Mean oocyte yield: 5.6 (range 2-15)	Mean oocyte yield: 6.8 (range 2-14)	p = 0.22 (NS)

Kingsland 1991	Median oocyte yield: 7	Median oocyte retrieved: 8.5	NS
Mok-Lin 2013	Median oocyte yield: 3 (IQR 2-5)	Median oocyte yield: 4 (IQR 2-6)	p = 0.41
Tan 1992	Median oocyte yield: 9 (range 1-22)	Median oocyte yield 11 (range: 1-24)	NS

Analysis 1.5. Comparison 1: Follicular flushing, Outcome 5: Duration of oocyte retrieval (normally distributed data; seconds)

Study or Subgroup	Aspiration/flush Mean	SD	Total	Aspiration only Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95% CI
Haydardedeoglu 2011	751.2	322.8	149	495.6	179.4	125	14.2%	255.60 [194.97 , 316.23]
Haydardedeoglu 2017	236.3	152.4218	40	178.4	84.749	40	17.9%	57.90 [3.85 , 111.95]
Kara 2012 (1)	732	246	100	456	162	100	15.7%	276.00 [218.27 , 333.73]
Levens 2009	366	125	15	186	41	15	11.8%	180.00 [113.43 , 246.57]
Malhotra 2020	492	204	35	228	90	36	9.6%	264.00 [190.30 , 337.70]
Mok-Lin 2013	420	150	25	282	102	25	10.3%	138.00 [66.89 , 209.11]
von Horn 2017	234	132	40	114	96	40	20.4%	120.00 [69.42 , 170.58]
Total (95% CI)			**404**			**381**	**100.0%**	**175.44 [152.57 , 198.30]**

Heterogeneity: Chi² = 47.79, df = 6 (P < 0.00001); I² = 87%
Test for overall effect: Z = 15.04 (P < 0.00001)
Test for subgroup differences: Not applicable



Footnotes
(1) Not entirely clear that data are expressed as +/- SD. Awaiting to hear from authors.

Analysis 1.6. Comparison 1: Follicular flushing, Outcome 6: Time taken for procedure (non-normally distributed data)

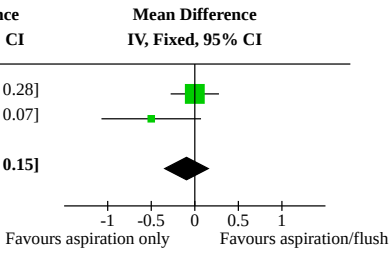
Time taken for procedure (non-normally distributed data)

Study	Aspiration/flush	Aspiration only	p value
Calabre 2020	Median time taken: 10 minutes (IQR 4 minutes)	Median time taken: 7 minutes (IQR 4 minutes)	p < 0.001
Kingsland 1991	Median time taken for procedure: 35 minutes	Median time taken for procedure: 20 minutes	p < 0.001
Kohl Schwartz 2020	Median time taken: 3.28 mins (IQR: 0.76 minutes)	Median time taken: 0.43 minutes (IQR: 0.17 minutes)	p < 0.01
Tan 1992	Median time taken: 30 minutes (range 15 to 70 minutes)	Median time taken: 15 minutes (range 4 to 30 minutes)	p < 0.00001

Analysis 1.7. Comparison 1: Follicular flushing, Outcome 7: Total number of embryos (normally distributed data)

Study or Subgroup	Aspiration/flush Mean	SD	Total	Aspiration only Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95% CI
Haydardedeoglu 2017 (1)	1.5	0.6325	40	1.5	0.6325	40	81.0%	0.00 [-0.28 , 0.28]
von Horn 2017	1	1.2	40	1.5	1.4	40	19.0%	-0.50 [-1.07 , 0.07]
Total (95% CI)			**80**			**80**	**100.0%**	**-0.10 [-0.34 , 0.15]**

Heterogeneity: Chi² = 2.38, df = 1 (P = 0.12); I² = 58%
Test for overall effect: Z = 0.75 (P = 0.45)
Test for subgroup differences: Not applicable



Footnotes
(1) SD recalculated as paper appears to report SE; seeking confirmation from authors

Analysis 1.8. Comparison 1: Follicular flushing, Outcome 8: Total number of embryos (non-normally distributed data)

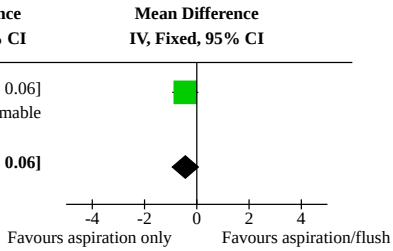
Total number of embryos (non-normally distributed data)

Study	Aspiration/flush	Aspiration only	p value
Calabre 2020	Median + IQR: 1 (2)	Median + IQR: 1 (2)	0.148
Malhotra 2020	Median + IQR: 4 (2)	Median + IQR: 3 (2)	0.073

Analysis 1.9. Comparison 1: Follicular flushing, Outcome 9: Number of embryos cryopreserved per woman randomised (normally distributed data)

Study or Subgroup	Aspiration/flush Mean	SD	Total	Aspiration only Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95% CI
Haydardedeoglu 2011	5.37	2.34	149	5.81	1.86	125	100.0%	-0.44 [-0.94 , 0.06]
Mok-Lin 2013	0	0	25	0.16	0.55	25		Not estimable
Total (95% CI)			**174**			**150**	**100.0%**	**-0.44 [-0.94 , 0.06]**

Heterogeneity: Not applicable
Test for overall effect: Z = 1.73 (P = 0.08)
Test for subgroup differences: Not applicable



Analysis 1.10. Comparison 1: Follicular flushing, Outcome 10: Number of embryos cryopreserved per woman randomised (non-normally distributed data)

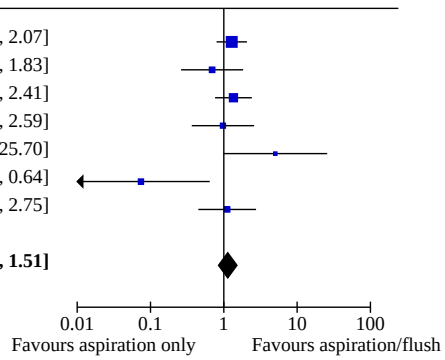
Number of embryos cryopreserved per woman randomised (non-normally distributed data)

Study	Aspiration/flush	Aspiration only	p value
Calabre 2020	Median + IQR: 0 (0)	Median + IQR: 0 (0)	0.224

Analysis 1.11. Comparison 1: Follicular flushing, Outcome 11: Clinical pregnancy rate per woman randomised

Study or Subgroup	Aspiration/flush Events	Total	Aspiration only Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Haydardedeoglu 2011	76	149	56	125	34.5%	1.28 [0.80 , 2.07]
Haydardedeoglu 2017	10	40	13	40	11.3%	0.69 [0.26 , 1.83]
Kara 2012	40	100	33	100	22.9%	1.35 [0.76 , 2.41]
Kohl Schwartz 2020	9	83	9	81	9.4%	0.97 [0.37 , 2.59]
Malhotra 2020	8	35	2	36	1.8%	5.04 [0.99 , 25.70]
Mok-Lin 2013	1	25	9	25	10.0%	0.07 [0.01 , 0.64]
Tan 1992	13	50	12	50	10.3%	1.11 [0.45 , 2.75]
Total (95% CI)		**482**		**457**	**100.0%**	**1.13 [0.85 , 1.51]**
Total events:	157		134			

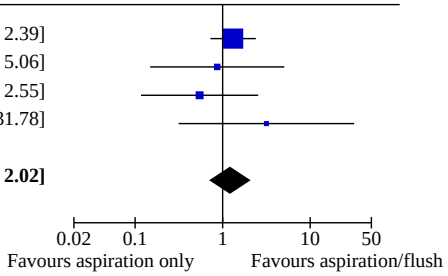
Heterogeneity: Chi² = 11.05, df = 6 (P = 0.09); I² = 46%
Test for overall effect: Z = 0.84 (P = 0.40)
Test for subgroup differences: Not applicable



Analysis 1.12. Comparison 1: Follicular flushing, Outcome 12: Ongoing pregnancy rate per woman randomised

Study or Subgroup	Aspiration/Flush Events	Total	Aspiration only Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Kara 2012	35	100	29	100	70.3%	1.32 [0.73 , 2.39]
Kingsland 1991	3	18	3	16	9.9%	0.87 [0.15 , 5.06]
Levens 2009	4	15	6	15	16.4%	0.55 [0.12 , 2.55]
von Horn 2017 (1)	3	40	1	40	3.4%	3.16 [0.31 , 31.78]
Total (95% CI)		**173**		**171**	**100.0%**	**1.21 [0.73 , 2.02]**
Total events:	45		39			

Heterogeneity: Chi² = 1.91, df = 3 (P = 0.59); I² = 0%
Test for overall effect: Z = 0.73 (P = 0.46)
Test for subgroup differences: Not applicable

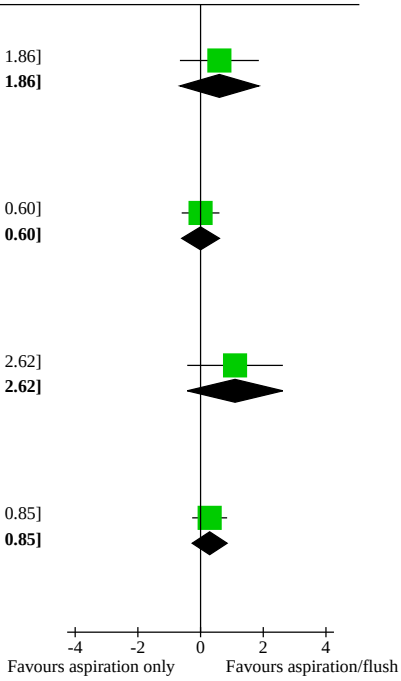


Footnotes

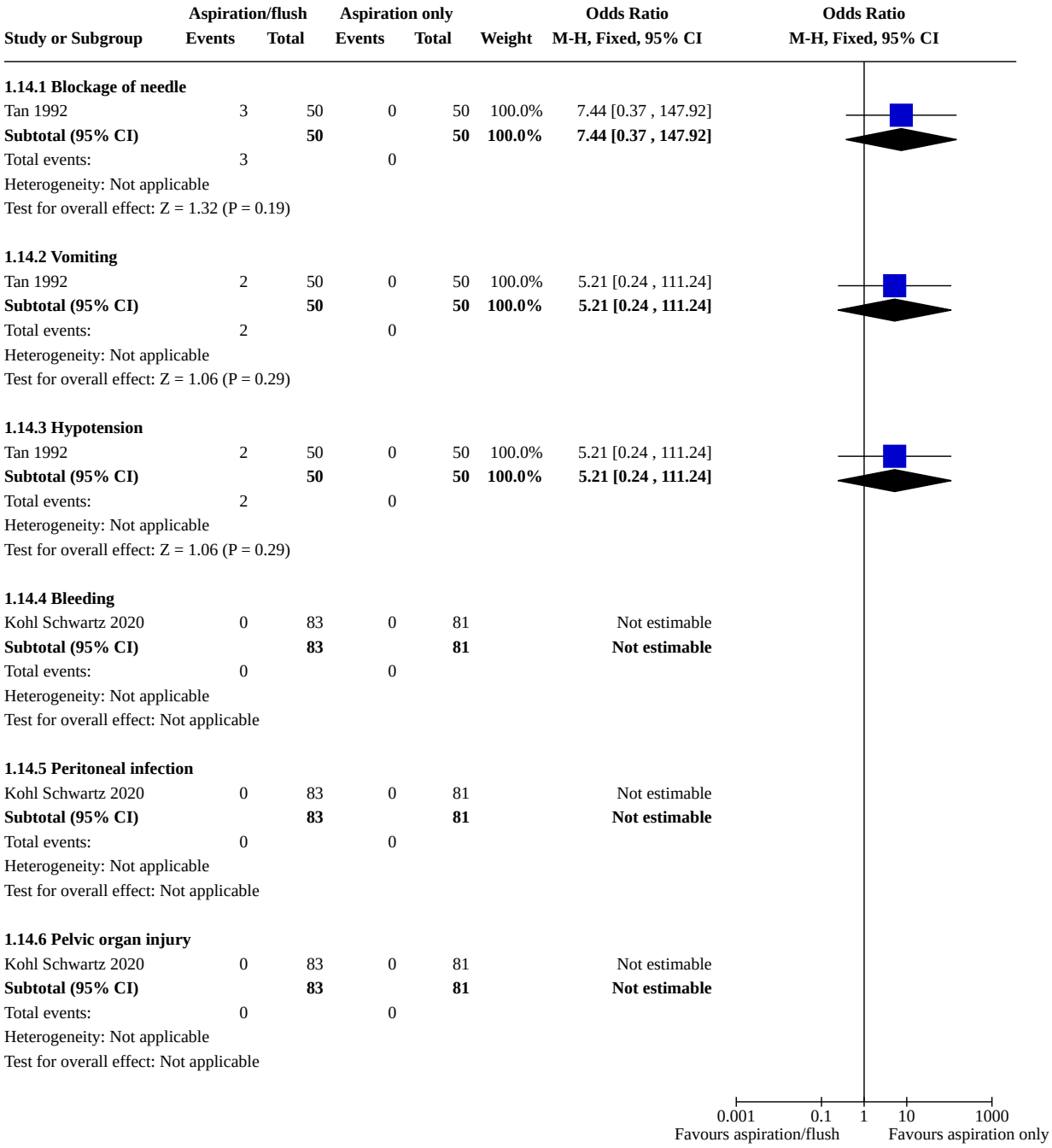
(1) Confirmed with authors that all pregnancies ongoing as they were unable to confirm re live births.

Analysis 1.13. Comparison 1: Follicular flushing, Outcome 13: Adverse events (continuous data)

Study or Subgroup	Aspiration/flush Mean	SD	Total	Aspiration only Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95% CI
1.13.1 Depression								
von Horn 2017	1.8	3.2	40	1.2	2.5	40	100.0%	0.60 [-0.66 , 1.86]
Subtotal (95% CI)			**40**			**40**	**100.0%**	**0.60 [-0.66 , 1.86]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 0.93 (P = 0.35)								
1.13.2 Anxiety								
von Horn 2017	0.8	1.6	40	0.8	1.1	40	100.0%	0.00 [-0.60 , 0.60]
Subtotal (95% CI)			**40**			**40**	**100.0%**	**0.00 [-0.60 , 0.60]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 0.00 (P = 1.00)								
1.13.3 Stress								
von Horn 2017	3.3	3.9	40	2.2	3	40	100.0%	1.10 [-0.42 , 2.62]
Subtotal (95% CI)			**40**			**40**	**100.0%**	**1.10 [-0.42 , 2.62]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.41 (P = 0.16)								
1.13.4 Pain								
Kohl Schwartz 2020	3.41	1.81	83	3.12	1.83	81	100.0%	0.29 [-0.27 , 0.85]
Subtotal (95% CI)			**83**			**81**	**100.0%**	**0.29 [-0.27 , 0.85]**
Heterogeneity: Not applicable								
Test for overall effect: Z = 1.02 (P = 0.31)								



Analysis 1.14. Comparison 1: Follicular flushing, Outcome 14: Adverse events (dichotomous data)



APPENDICES

Appendix 1. Cochrane Gynaecology and Fertility Group Specialised Register search strategy

Searched 13 July 2021

ProCite platform

Keywords CONTAINS "follicular flushing" or "follicular rinsing" or "Flushing" or "flushing media" or "flushing outcome" or "tubal flushing" or "follicle aspiration" or "follicular aspiration" or "Flushing-Outcome" or "Flushing" or Title CONTAINS "follicular flushing" or "follicular

rinsing" or "Flushing" or "flushing media" or "flushing outcome" or "tubal flushing" or "follicle aspiration" or "follicular aspiration" or "Flushing-Outcome" or "flushing media" or "Flushing" (539 records)

Appendix 2. CENTRAL via the Cochrane Register of Studies Online (CRSO) search strategy

Searched 13 July 2021, Issue 7

Web platform

#1 (follic* adj15 flush*):TI,AB,KY 56
#2 (follic* adj15 wash*):TI,AB,KY 9
#3 ((flush* or wash*) adj15 oocyte*):TI,AB,KY 54
#4 (ovar* adj15 flush*):TI,AB,KY 21
#5 (ovar* adj15 wash*):TI,AB,KY 9
#6 flush* adj5 media 12
#7 flush* adj5 medium 23
#8 flush* adj5 ivf 16
#9 flush* adj5 in vitro fertili?ation 6
#10 #1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9 128

Appendix 3. MEDLINE search strategy

Searched from 1946 to 13 July 2021

Ovid platform
1 (follic$ adj15 flush$).tw. (166)
2 (follic$ adj15 wash$).tw. (132)
3 ((flush$ or wash$) adj15 oocyte$).tw. (429)
4 (ovar$ adj15 flush$).tw. (245)
5 (ovar$ adj15 wash$).tw. (340)
6 (flush$ adj7 ivf).tw. (22)
7 (flush$ adj5 in vitro fertili?ation).tw. (7)
8 (flush$ adj5 media$).tw. (258)
9 (flush$ adj5 medium$).tw. (140)
10 or/1-9 (1501)
11 randomized controlled trial.pt. (537286)
12 controlled clinical trial.pt. (94289)
13 randomized.ab. (526400)
14 randomised.ab. (104829)
15 placebo.tw. (225581)
16 clinical trials as topic.sh. (196614)
17 randomly.ab. (361254)
18 trial.ti. (243367)
19 (crossover or cross-over or cross over).tw. (89740)
20 or/11-19 (1450727)
21 exp animals/ not humans.sh. (4859215)
22 20 not 21 (1335740)
23 10 and 22 (134)

Appendix 4. Embase search strategy

Searched from 1980 to 13 July 2021

Ovid platform

1 (follic$ adj15 flush$).tw. (209)
2 (follic$ adj15 wash$).tw. (192)
3 ((flush$ or wash$) adj15 oocyte$).tw. (639)
4 (ovar$ adj15 flush$).tw. (292)
5 (ovar$ adj15 wash$).tw. (503)
6 (flush$ adj5 medium).tw. (174)
7 (flush$ adj5 in vitro fertili?ation).tw. (12)
8 (flush$ adj5 ivf).tw. (27)
9 (flush$ adj5 media).tw. (117)

10 or/1-9 (1837)
11 Clinical Trial/ (996613)
12 Randomized Controlled Trial/ (661483)
13 exp randomization/ (91256)
14 Single Blind Procedure/ (43038)
15 Double Blind Procedure/ (182473)
16 Crossover Procedure/ (67313)
17 Placebo/ (354532)
18 Randomi?ed controlled trial$.tw. (261209)
19 Rct.tw. (42529)
20 random allocation.tw. (2178)
21 randomly allocated.tw. (38516)
22 allocated randomly.tw. (2658)
23 (allocated adj2 random).tw. (829)
24 Single blind$.tw. (26893)
25 Double blind$.tw. (214105)
26 ((treble or triple) adj blind$).tw. (1367)
27 placebo$.tw. (321961)
28 prospective study/ (694760)
29 or/11-28 (2370557)
30 case study/ (79342)
31 case report.tw. (442697)
32 abstract report/ or letter/ (1154778)
33 or/30-32 (1664795)
34 29 not 33 (2313304)
35 10 and 34 (217)

Appendix 5. PsycINFO search strategy

Searched from 1806 to 13 July 2021

Ovid platform

1 (follic$ adj15 flush$).tw. (2)
2 (follic$ adj15 wash$).tw. (0)
3 ((flush$ or wash$) adj15 oocyte$).tw. (1)
4 (ovar$ adj15 flush$).tw. (7)
5 (ovar$ adj15 wash$).tw. (2)
6 or/1-5 (11)
7 random.tw. (62160)
8 control.tw. (467805)
9 double-blind.tw. (23734)
10 clinical trials/ (11946)
11 placebo/ (6045)
12 exp Treatment/ (1101568)
13 or/7-12 (1517916)
14 6 and 13 (3)

Appendix 6. Inclusion criteria

Date	
Assessor	EG PM
First author	
Publication year	

(Continued)

Journal	
Language	
Retrieval	Electronic search Handsearched
Study design	
Q1: Is the study a randomised controlled trial?	Yes No Unclear
If 'no', trial excluded. If yes, then proceed to Q2.	
Participants	
Q2: Are the participants undergoing assisted conception treatment by IVF or ICSI?	Yes No Unclear
Q3: Did study participants use their own gametes?	Yes No Unclear
If 'no' to either Q2 or Q3, trial excluded. If yes, then proceed to Q4.	
Intervention	
Q4: Was the intervention follicular aspiration and flushing versus follicular aspiration alone?	Yes No Unclear
Final decision	
Study included if 'yes' to Q1, Q2, Q3, and Q4	Include Exclude
Reasoning for exclusion	
If 'unclear', action taken	
Both assessors in agreement?	Yes No
If no, outcome of discussion and/or arbitration	

Appendix 7. Data extraction form

Date	
Assessor	EG PM
First author	
Publication year	
Published	Yes No
Language	
Retrieval	Electronic search Handsearched
Study design	
Randomised controlled trial?	Yes No
What type of randomised controlled trial?	Parallel (intervention vs control) Cross-over (participants used as intervention and control groups)
Participant recruitment	Prospective Retrospective Unclear
Participants	
Country	
Site (single or multiple centres, location)	
Age	Mean + SD/median + range Intervention group Control group
Inclusion criteria	
Exclusion criteria	
Power calculation was performed and followed	Yes

(Continued)

		No
		Unclear
Study size		
Number recruited		
Number randomised		
Number excluded		
Number analysed		
Number lost to follow-up		
Interventions		
To include description of the ovarian stimulation protocol (when appropriate), as well as details of follicular aspiration and flushing procedures		
Primary outcomes		
Live birth rate	Occurrence of outcome	Non-occurrence of outcome
Intervention group		
Control group		
Total (by event)		
Miscarriage rate	Occurrence of outcome	Non-occurrence of outcome
Intervention group		
Control group		
Total (by event)		
Secondary outcomes		
Oocyte yield	Occurrence of outcome	Non-occurrence of outcome
Intervention group		
Control group		
Total (by event)		
Duration of oocyte retrieval	Occurrence of outcome	Non-occurrence of outcome
Intervention group		

(Continued)

Control group		
Total (by event)		
Total number of embryos	Occurrence of outcome	Non-occurrence of outcome
Intervention group		
Control group		
Total (by event)		
Number of embryos cryopreserved	Occurrence of outcome	Non-occurrence of outcome
Intervention group		
Control group		
Total (by event)		
Ongoing pregnancy rate	Occurrence of outcome	Non-occurrence of outcome
Intervention group		
Control group		
Total (by event)		
Adverse event:	Occurrence of outcome	Non-occurrence of outcome
Intervention group		
Control group		
Total (by event)		
Adverse event:	Occurrence of outcome	Non-occurrence of outcome
Intervention group		
Control group		
Total (by event)		
Adverse event:	Occurrence of outcome	Non-occurrence of outcome
Intervention group		
Control group		

(Continued)

Total (by event)		
Subgroups:		
Age		
Poor ovarian reserve		
Poor response to ovarian stimulation		
Live birth rate	Occurrence of outcome	Non-occurrence of outcome
Intervention group		
Control group		
Total (by event)		
Miscarriage rate	Occurrence of outcome	Non-occurrence of outcome
Intervention group		
Control group		
Total (by event)		
Risk of bias assessment		
Selection bias	Was the allocation sequence adequately generated? (adequate: computerised random number generator; random numbers table)	Yes No Unclear
	Was participant allocation concealment adequate? (adequate: central computer randomisation; sequentially numbered, sealed, opaque envelopes)	Yes No Unclear
Performance bias	Were participants blinded?	Yes No Unclear
	Were personnel (embryologist) blinded?	Yes No Unclear
Detection bias	Were those assessing outcomes blinded?	Yes No

(Continued)

		Unclear
Attrition bias (incomplete outcome data)	Was loss to follow-up accounted for?	Yes No Unclear
	Was an intention-to-treat analysis performed?	Yes No Unclear
Selective outcome reporting	Are reports of the study free of the suggestion of selective outcome reporting?	Yes No Unclear
Other sources of bias (high risk of bias: commercial funding source, early stopping, baseline imbalances, poor choice of design)		

WHAT'S NEW

Date	Event	Description
9 February 2022	New citation required but conclusions have not changed	The addition of 5 new studies has not led to a change in the live birth rate, but data are now available on miscarriage rate.
9 February 2022	New search has been performed	Updated. Five new citations added (Calabre 2020; de Souza 2021; Kohl Schwartz 2020; Lainas 2018; Malhotra 2020).

HISTORY

Protocol first published: Issue 1, 2004
Review first published: Issue 9, 2010

Date	Event	Description
20 March 2018	New search has been performed	Searches for this update have identified 6 studies (Haines 1989 Haydardedeoglu 2011; Haydardedeoglu 2017; Kara 2012; Mok-Lin 2013; von Horn 2017).
20 March 2018	New citation required but conclusions have not changed	The addition of 6 new studies has not led to a change in the conclusions of the review.
31 March 2010	New search has been performed	Review has had a search run. One new study was identified for the update, and formatting has been amended to include all subheadings for Review Manager 5. Amendments to the original pro-

Date	Event	Description
		tocol have been made, and some outcomes and objectives have been removed.
19 January 2010	New search has been performed	Review completed, no changes to protocol.
2 April 2008	Amended	Converted to new review format
11 November 2003	New citation required and major changes	Substantive amendments

CONTRIBUTIONS OF AUTHORS

E Georgiou was involved in preparing all sections of the review.

P Melo was involved in data extraction for the review.

Y Cheong made substantial editorial amendments to the review.

I Granne was involved in preparing all sections of the protocol and made substantial editorial amendments to the review.

DECLARATIONS OF INTEREST

EG has no interests to declare.

PM has no interests to declare.

YC is a consultant for Complete Fertility, and has received lecture fees from Merck (to April 2021).

IG is a principal investigator on a project grant from Bayer. She declares that she has not received the funds personally and cannot access or control the spending of the moneys.

SOURCES OF SUPPORT

Internal sources

- None, Other

  None

External sources

- None, Other

  None

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

For the 2010 review

The Methods indicated that we planned to compare single versus multiple flushes, and different volumes for flushing, in terms of live births and ongoing pregnancies in women undergoing in vitro fertilisation and intracytoplasmic sperm injection. However, as aspiration and aspiration with flushing do not yield any differences in clinical and ongoing pregnancies, nor in the number of oocytes obtained, this analysis becomes both irrelevant and unnecessary, and we removed the secondary objective from the final review.

We made clinical pregnancy a primary outcome, with ongoing pregnancy.

We removed several secondary outcomes from the protocol, as they did not contribute to the overall aim of the review, or they were potentially biased, as data not could not be analysed per woman randomised. These outcomes included fertilisation rate, rate of embryo cleavage, rates of congenital and chromosomal abnormalities, amount of anaesthetic required, and cost per oocyte retrieval procedure performed.

We added adverse events as a primary outcome.

For the 2018 update

We updated the primary and secondary outcomes. We updated the 'Search methods' and 'Data collection and analysis' sections in keeping with the most recent Cochrane Gynaecology and Fertility Group guidelines.

For the 2022 update

We made no protocol changes for this update.

INDEX TERMS

Medical Subject Headings (MeSH)

*Abortion, Spontaneous [epidemiology]; Fertilization in Vitro; *Oocyte Retrieval [methods]; Pregnancy Rate; Reproductive Techniques, Assisted

MeSH check words

Female; Humans; Pregnancy