Cochrane Library

Cochrane Database of Systematic Reviews

Semen preparation techniques for intrauterine insemination (Review)

Boomsma CM, Cohlen BJ, Farquhar C

Boomsma CM, Cohlen BJ, Farquhar C.
Semen preparation techniques for intrauterine insemination.
Cochrane Database of Systematic Reviews 2019, Issue 10. Art. No.: CD004507.
DOI: 10.1002/14651858.CD004507.pub4.

www.cochranelibrary.com

WILEY

TABLE OF CONTENTS

[Intervention Review]

Semen preparation techniques for intrauterine insemination

Carolien M. Boomsma[1], Ben J Cohlen[2], Cindy Farquhar[3]

[1]Obstetrics and Gynaecology, Bravis Hospital, Bergen op Zoom, Netherlands. [2]Department of Obstetrics and Gynaecology, Isala Clinics, Location Sophia, Isala Zwolle, Netherlands. [3]Department of Obstetrics and Gynaecology, University of Auckland, Auckland, New Zealand

Contact: Carolien M. Boomsma, Obstetrics and Gynaecology, Bravis Hospital, Boerhaaveplein 1, Bergen op Zoom, 4624 VT, Netherlands. carolienboomsma@hotmail.com, c.m.boomsma@umcutrecht.nl.

Editorial group: Cochrane Gynaecology and Fertility Group.
Publication status and date: Edited (no change to conclusions), published in Issue 11, 2019.

Citation: Boomsma CM, Cohlen BJ, Farquhar C. Semen preparation techniques for intrauterine insemination. *Cochrane Database of Systematic Reviews* 2019, Issue 10. Art. No.: CD004507. DOI: 10.1002/14651858.CD004507.pub4.

ABSTRACT

Background

Semen preparation techniques for assisted reproduction, including intrauterine insemination (IUI), were developed to select the motile morphologically normal spermatozoa. The yield of many motile, morphologically normal spermatozoa might influence treatment choices and therefore outcomes.

Objectives

To compare the effectiveness of three different semen preparation techniques (gradient; swim-up; wash and centrifugation) on clinical outcomes (live birth rate; clinical pregnancy rate) in subfertile couples undergoing IUI.

Search methods

We searched the Cochrane Gynaecology and Fertility Group (CGFG) trials register, CENTRAL, MEDLINE, Embase, Science Direct Database, National Research Register, Biological Abstracts and clinical trial registries in March 2019, and checked references and contacted study authors to identify additional studies.

Selection criteria

We included randomised controlled trials (RCTs) comparing the efficacy in terms of clinical outcomes of semen preparation techniques used for subfertile couples undergoing IUI.

Data collection and analysis

We used standard methodological procedures recommended by Cochrane. The primary review outcomes are live birth rate and clinical pregnancy rate per couple.

Main results

We included seven RCTS in the review; we included six of these, totalling 485 couples, in the meta-analysis. No trials reported the primary outcome of live birth. The evidence was of very low-quality. The main limitations were (unclear) risk of bias, signs of imprecision and inconsistency in results among studies and the small number of studies/participants included.

Swim-up versus gradient technique

Considering the quality of evidence, we are uncertain whether there was a difference between clinical pregnancy rates (CPR) for swim-up versus a gradient technique (odds ratio (OR) 0.83, 95% CI 0.51 to 1.35; I^2 = 71%; 4 RCTs, 370 participants; very low-quality evidence). The results suggest that if the chance of pregnancy after the use of a gradient technique is assumed to be 24%, the chance of pregnancy after

using the swim-up technique is between 14% and 30%. We are uncertain whether there was a real difference between ongoing pregnancy rates per couple (OR 0.39, 95% CI 0.19 to 0.82; heterogeneity not applicable; 1 RCT, 223 participants; very low-quality evidence). Considering the quality of evidence, we are uncertain whether there was a difference between multiple pregnancy rates (MPR) per couple comparing a swim-up versus gradient technique (MPR per couple 0% versus 0%; 1 RCT, 25 participants; very low-quality of evidence). Considering the quality of evidence, we are also uncertain whether there was a difference between miscarriage rates (MR) per couple comparing a swim-up versus gradient technique (OR 0.85, 95% CI 0.28 to 2.59; I^2 = 44%; 3 RCTs, 330 participants; very low-quality evidence). No studies reported on ectopic pregnancy rate, fetal abnormalities or infection rate.

Swim-up versus wash technique

Considering the quality of evidence, we are uncertain whether there is a difference in clinical pregnancy rates after a swim-up technique versus wash and centrifugation (OR 0.41, 95% CI 0.15 to 1.13; I^2 = 55%; 2 RCTs, 78 participants; very low-quality evidence). The results suggest that if the chance of pregnancy after the use of a wash technique is assumed to be 38%, the chance of pregnancy after using the swim-up technique is between 9% and 41%. Considering the quality of evidence, we are uncertain whether there was a difference between multiple pregnancy rates between swim-up technique versus wash technique (OR 0.49, 95% CI 0.02 to 13.28; heterogeneity not applicable; 1 RCT, 26 participants; very low-quality evidence). Miscarriage rate was only reported by one study: no miscarriages were reported in either treatment arm. No studies reported on ongoing pregnancy rate, ectopic pregnancy rate, fetal abnormalities or infection rate.

Gradient versus wash technique

Considering the quality of evidence, we are uncertain whether there is a difference in clinical pregnancy rates after a gradient versus wash and centrifugation technique (OR 1.78, 95% CI 0.58 to 5.46; I^2 = 52%; 2 RCTs, 94 participants; very low-quality evidence). The results suggest that if the chance of pregnancy after the use of a wash technique is assumed to be 13%, the chance of pregnancy after using the gradient technique is between 8% and 46%. Considering the quality of evidence, we are uncertain whether there was a difference between multiple pregnancy rates per couple between the treatment groups (OR 0.33, 95% CI 0.01 to 8.83; very low-quality evidence). Considering the quality of evidence, we are also uncertain whether there was a difference between miscarriage rates per couple between the treatment groups (OR 6.11, 95% CI 0.27 to 138.45; very low-quality evidence). No studies reported on ongoing pregnancy rate, ectopic pregnancy rate, fetal abnormalities or infection rate.

Authors' conclusions

The very low quality of the available evidence means we cannot be certain about the relative effectiveness of the different semen preparation techniques: swim-up versus gradient versus wash and centrifugation technique. No studies reported on live birth rates. We are uncertain whether there is a difference in clinical pregnancy rates, ongoing pregnancy rates, multiple pregnancy rates or miscarriage rates per couple between the three sperm preparation techniques. Further randomised trials are warranted that report live birth data

PLAIN LANGUAGE SUMMARY

Semen preparation techniques for intrauterine insemination

Review question

Cochrane authors reviewed the evidence about the effectiveness of three different sperm preparation techniques (gradient, swim-up, and wash technique) on clinical outcome after intrauterine insemination (IUI).

Background

Semen preparation techniques are used in assisted reproduction to separate sperm which have a normal appearance and move spontaneously from the fluid portion of the semen in which the sperm are suspended. The effectiveness of specific semen preparation techniques for increasing pregnancy rates in subfertile couples undergoing IUI is unknown.

Study characteristics

We found six randomised controlled trials comparing a gradient, swim-up or wash technique, in a total of 485 couples undergoing IUI. The evidence is current to March 2019.

Key results

We are uncertain whether there is a difference in pregnancy outcomes between the three sperm preparation techniques for subfertile couples undergoing IUI. No studies reported on live birth rates.

Swim-up versus gradient technique

Considering the quality of evidence (very low), we are uncertain whether there was a difference between clinical pregnancy rates (CPR) for swim-up versus a gradient technique. The results suggest that if the chance of pregnancy after the use of a gradient technique is assumed to be 24%, the chance of pregnancy after using the swim-up technique is between 14% and 30%. We are uncertain whether there was a

difference between ongoing pregnancy rates per couple, multiple pregnancy rates (MPR) per couple or miscarriage rates (MR) per couple when comparing a swim-up versus gradient technique. The quality of the evidence for these outcomes was very low. No studies reported on ectopic pregnancy rate, fetal abnormalities or infection rate.

Swim-up versus wash technique

Considering the quality of evidence (very low), we are uncertain whether there is a difference in clinical pregnancy rates after a swim-up technique versus wash and centrifugation. The results suggest that if the chance of pregnancy after the use of a wash technique is assumed to be 38%, the chance of pregnancy after using the swim-up technique is between 9% and 41%. Considering the very low-quality evidence, we are uncertain whether there was a difference between multiple pregnancy rates between swim-up technique versus wash technique. Miscarriage rate was only reported by one study: no miscarriages were reported in either treatment arm. No studies reported on ongoing pregnancy rate, ectopic pregnancy rate, fetal abnormalities or infection rate.

Gradient versus wash technique

Considering the quality of evidence (very low), we are uncertain whether there is a difference in clinical pregnancy rates after a gradient versus wash and centrifugation technique. The results suggest that if the chance of pregnancy after the use of a wash technique is assumed to be 13%, the chance of pregnancy after using the gradient technique is between 8% and 46%. Considering the quality of evidence, we are uncertain whether there was a difference between multiple pregnancy rates per couple between the treatment groups. Considering the quality of evidence, we are also uncertain whether there was a difference between miscarriage rates per couple between the treatment groups. No studies reported on ongoing pregnancy rate, ectopic pregnancy rate, fetal abnormalities or infection rate.

Quality of evidence

The quality of the evidence was very low. The main limitations were (unclear) risk of bias, signs of imprecision (small number of studies/ participants included) and inconsistency in results among studies.

SUMMARY OF FINDINGS

Summary of findings for the main comparison. Swim-up technique compared to gradient technique for undergoing intrauterine insemination

Swim-up technique compared to gradient technique for undergoing intrauterine insemination

Patient or population: patients undergoing intrauterine insemination (fresh semen)
Intervention: swim-up technique
Comparison: gradient technique

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No. of participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Gradient technique	Swim-up technique				
Live birth rate per couple	See comment	See comment				No studies reported on this outcome.
Clinical pregnancy rate per couple	**244 per 1000**	**212 per 1000** (142 to 304)	**OR 0.83** (0.51 to 1.35)	370 (4 studies)	⊕⊖⊖⊖ **Very Low** a,b,c, g	
Ongoing pregnancy rate per couple	**234 per 1000**	**107 per 1000** (55 to 201)	**OR 0.39** (0.19 to 0.82)	223 (1 study)	⊕⊖⊖⊖ **Very Low**d,e	
Multiple pregnancy rate per couple	See comment	See comment	Not estimable	25 (1 study)	⊕⊖⊖⊖ **Very Low** d,e	There were no events recorded in either group
Miscarriage rate per couple	**38 per 1000**	**33 per 1000** (11 to 94)	**OR 0.85** (0.28 to 2.59)	330 (3 studies)	⊕⊖⊖⊖ **Very Low** a,f,c	

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **OR:** Odds ratio;

GRADE Working Group grades of evidence
High quality: further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

Very low quality: we are very uncertain about the estimate.

[a] Risk of bias, downgrade 1 level: unclear risk of bias, no adequate explanation for randomisation or allocation concealment or attrition. High risk of performance bias.
[b] Inconsistency, downgrade 1 level: I^2 statistic was 71% indicating substantial statistical heterogeneity and a plausible explanation was not found.
[c] Serious imprecision, downgrade 1 level: there were signs of imprecision concerning wide confidence intervals due to small sample size and small number of events.
[d] Very serious imprecision, downgrade 2 levels: wide confidence interval around effect estimate due to small sample size and small number of/no events.
[e] Evidence based on a single RCT of limited sample size, downgrade 1 level.
[f] Inconsistency, downgrade 1 level: little overlap confidence intervals and moderate statistical heterogeneity.
[g] Other bias, downgrade 1 level: definition of pregnancy not described.

Summary of findings 2. Swim-up technique compared to wash and centrifugation for undergoing intrauterine insemination

Swim-up technique compared to wash and centrifugation for undergoing intrauterine insemination

Patient or population: patients undergoing intrauterine insemination (fresh semen)
Intervention: swim-up technique
Comparison: wash and centrifugation

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No. of participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Wash and centrifugation	Swim-up technique				
Live birth rate per couple	See comment	See comment				No studies reported on this outcome.
Clinical pregnancy rate per couple	**381 per 1000**	**201 per 1000** (85 to 410)	**OR 0.41** (0.15 to 1.13)	78 (2 studies)	⊕⊖⊖⊖ **very low** [a,b,c]	
Ongoing pregnancy rate per couple	See comment	See comment				No studies reported on this outcome.
Multiple pregnancy rate per couple	**63 per 1000**	**32 per 1000** (1 to 470)	**OR 0.49** (0.02 to 13.28)	26 (1 study)	⊕⊖⊖⊖ **very low** [d,e]	
Miscarriage rate per couple	See comment	See comment	Not estimable	20 (1 study)	⊕⊖⊖⊖ **very low** [d,e]	There were no events in either group

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **OR:** Odds ratio

GRADE Working Group grades of evidence
High quality: further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: we are very uncertain about the estimate.

[a] Risk of bias, downgrade 1 level: 1 of the 2 trials did not conceal allocation and there was no blinding. High risk of performance bias.
[b] Inconcistency, downgrade 1 level: Little overlap in confidence intervals. I^2 statistic was 55% indicating substantial statistical heterogeneity and a plausible explanation was not found.
[c] Serious imprecision, downgrade 1 level: there were signs of imprecision concerning wide confidence intervals due to small sample size and small number of events.
[d] Very serious imprecision, downgrade 2 levels: wide confidence interval around effect estimate due to small sample size and small number of/no events.
[e] Downgrade 1 level: Evidence based on a single RCT of limited sample size.

Summary of findings 3. Gradient technique compared to wash and centrifugation for undergoing intrauterine insemination

Gradient technique compared to Wash and centrifugation for undergoing intrauterine insemination

Patient or population: patients with undergoing intrauterine insemination (fresh semen)
Intervention: gradient technique
Comparison: wash and centrifugation

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No. of participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Wash and centrifugation	Gradient technique				
Live birth rate per couple	See comment	See comment				No studies reported on this outcome.
Clinical pregnancy rate per couple	**133 per 1000**	**215 per 1000** (82 to 457)	**OR 1.78** (0.58 to 5.46)	94 (2 studies)	⊕⊖⊖⊖ **very low** [a, b, c, f]	
Ongoing pregnancy rate per couple	See comment	See comment				No studies reported on this outcome.
Multiple pregnancy rate per couple	**63 per 1000**	**22 per 1000** (1 to 371)	**OR 0.33** (0.01 to 8.83)	31 (1 study)	⊕⊖⊖⊖ **very low** [d,e]	

Miscarriage rate per couple	no events (0/16)	2/15 see comment	**OR 6.11** (0.27 to 138.45)	31 (1 study)	⊕⊖⊖⊖ **very low** [d,e]	Corresponding risk not estimable since there were no events in "wash and centrifugation" (0/16).

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **OR:** Odds ratio

GRADE Working Group grades of evidence
High quality: further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: we are very uncertain about the estimate.

[a] Risk of bias, downgrade 1 level: 1 of the 2 trials did not provide adequate details on randomisation or allocation concealment and did not use blinding, abstract. High risk of performance bias.
[b] Inconsistency, downgrade 1 level: I^2 statistic was 52% indicating substantial statistical heterogeneity.
[c] Serious imprecision, downgrade 1 level: there were signs of imprecision concerning wide confidence intervals due to small sample size and small number of events.
[d] Very serious imprecision, downgrade 2 levels: wide confidence interval around effect estimate due to small sample size and small number of/no events.
[e] Downgrade 1 level: evidence based on a single RCT of limited sample size.
[f] Other bias, downgrade 1 level: definition of pregnancy not described.

BACKGROUND

Description of the condition

The success of the treatment of subfertile couples has made substantial progress over the last two decades. Subfertile couples are defined as couples who have tried unsuccessfully to conceive for at least one year despite regular and unprotected sexual intercourse (Evers 2002). Subfertility is a common problem, affecting up to 15% of couples (Evers 2002; Templeton 1990). Demand for infertility treatment is on the rise as increasing numbers of women delay having children till an age when natural female fertility is in decline and there is a raised chance of exposure to sexually transmitted diseases and continually falling sperm counts (Delhanty 2001; Niederberger 2018; Swan 1999), although worldwide the percentage of women seeking treatment has not significantly changed over the last two decades (Mascarenhas 2012). According to a number of high-quality studies, intrauterine insemination (IUI) should be the first choice treatment in case of unexplained and moderate male factor subfertility and an unfavourable prognosis for natural conception (Farquhar 2018; Ombelet 2017).

With the emergence of in vitro fertilization (IVF) with uterine transfer of embryos (IVF-ET), semen preparation techniques were developed to separate motile sperm that are morphologically normal (normal appearance) from seminal plasma (the fluid portion of the semen in which the spermatozoa are suspended) and foreign material. It is known that white blood cells, bacteria and dead spermatozoa produce oxygen radicals that negatively influence the ability of normal spermatozoa to fertilize the egg (Aitken 1994; De Jonge 2002; Parinaud 1997). Reactive oxygen species (ROS) cause peroxidative damage and loss of sperm function, as well as DNA damage in both the nuclear and mitochondrial genomes (WHO 2010). A randomised controlled trial (RCT) of prepared sperm compared to unprepared first split ejaculates showed that semen preparation significantly increased the probability of conception after IUI in a group of couples with male subfertility (Goldenberg 1992). Furthermore, in IUI the use of fresh unprepared semen has been reported to cause uterine cramps and may induce pelvic inflammatory disease, endometritis, cervicitis or vaginitis, as well as an increased likelihood of miscarriage, premature delivery or a malformed fetus (Wang 1991; Yan 1998).

Some research has suggested an association between the probability of conception after IUI and the absolute number of motile sperm that are inseminated. Some retrospective studies have defined a threshold level beyond which pregnancy rates reached a plateau (Berg 1997; Huang 1996a; Khalil 2001; Madbouly 2017). However, the threshold levels found in these studies differed substantially from one to five million motile sperm, which makes these results less useful in practice. One prospective controlled trial demonstrated links between total sperm motility and the probability of conception after IUI (Van Voorhis 2001).

In couples with subfertility, the yield of as many motile, morphologically normal spermatozoa as possible is important as it influences treatment choices and therefore outcomes. A high yield can lead to a preference for IUI or IVF, whereas a lower yield could result in a preference for intracytoplasmic sperm injection (ICSI). ICSI is an IVF procedure in which a single sperm is injected directly into an egg, a procedure that is most commonly used to overcome severe male infertility problems. The treatment outcome after ICSI is not related to the number of available motile sperm.

Description of the intervention

Many sperm preparation procedures are available, but there are three main groups of methods.

Firstly, spermatozoa may be selected on their ability to swim, known as the 'swim-up technique'. This technique is performed by layering culture medium over the liquefied semen. Motile spermatozoa swim up into the culture. The upper part of the layered medium is then carefully removed for further use.

The second method of selecting spermatozoa is by the use of density gradients. The semen sample is pipetted on top of the density column, which is then centrifuged. Density gradient centrifugation separates spermatozoa according to their density. This way you can select the motile, morphologically normal spermatozoa in the solution with the highest concentration of gradient, which is aspirated for further use (WHO 1999). Sperm preparation with the use of density gradient centrifugation has been a standard technique in assisted reproductive techniques. Fresh semen samples have been centrifuged on Percoll gradients in the 40% to 90% range with good recovery (Byrd 1996). In late 1996, Percoll was removed from clinical human use. This product was replaced by silica stabilized with covalently bound hydrophilic silane, marketed under several commercial names.

The third method is the conventional wash method in combination with centrifugation, previously only used for diagnostic procedures. The semen sample is diluted with a medium and centrifuged. Subsequently, the pellet (the bottom part after centrifugation) is resuspended in a small amount of medium and incubated until the time of insemination.

Apart from a simple wash technique, the swim-up technique is the oldest and most commonly used sperm preparation method. It is still used largely in IUI and IVF laboratories around the world. Density gradient techniques are easier to standardize than the swim-up technique and the results are more consistent. Usually, the choice of sperm preparation technique is dictated by the nature of the semen sample. Swim-up technique is often used when semen samples are considered to be largely normal, whereas density gradient techniques can be preferred in male factor infertility because of the greater total number of motile spermatozoa recovered (Henkel 2003; WHO 2010).

How the intervention might work

The aim of semen preparation is to separate the normal sperm from the debris of the ejaculate and, in the case of IUI, to yield as many normal motile spermatozoa as possible. The number of motile sperm after preparation in relation to the total number of motile sperm before preparation is expressed as the recovery rate. Preparation techniques that have higher recovery rates are considered superior for IUI. Sperm preparation techniques may also influence DNA fragmentation. A prospective randomised study was conducted in subfertile patients (unexplained and male factor infertility) to compare basal and post-procedure DNA fragmentation rates in swim-up and gradient techniques. Swim-up method significantly reduces sperm DNA fragmentation rates (Oguz 2018). Current evidence supports the association between

high sperm DNA fragmentation and poor reproductive outcomes for natural conception and intrauterine insemination (Cho 2017).

Although spermatozoa recovery rates might be interesting when you compare different semen preparation techniques, clinicians and prospective parents regard live birth rate as the most important outcome. One type of semen preparation technique might be superior to another in relation to clinical outcome after IUI.

Why it is important to do this review

The comparison of different semen preparation techniques in relation to semen parameters has been the focus of a substantial amount of research. Studies addressing semen parameters after different semen preparation techniques may be less useful since different practitioners have different methods of sperm analysis, resulting in less comparable data. Clinical outcomes are objective and of interest to patients and clinicians. There is, however, no consensus in the literature on this topic. Differences found in individual trials do not always reach significance. It seemed appropriate to perform a meta-analysis combining the results of available randomised controlled trials. This systematic review investigated which semen preparation technique is superior.

OBJECTIVES

To compare the effectiveness of three different semen preparation techniques (gradient; swim-up; wash and centrifugation) on clinical outcomes (live birth rate, clinical pregnancy rate) in subfertile couples undergoing IUI.

METHODS

Criteria for considering studies for this review

Types of studies

We included randomised controlled trials (RCTs) in this review. We assessed the method of randomisation to determine whether each study was truly randomised. We only included studies with a cross-over design in the meta-analysis if the first cycle was randomised and first cycle data were available (prior to crossing-over). We did not include split-sample studies since by design they cannot compare clinical outcomes.

Types of participants

We defined subfertility as couples who have tried unsuccessfully to conceive for at least one year, despite regular and unprotected sexual intercourse (Evers 2002). We placed no restriction on causes of subfertility. We defined unexplained subfertility as infertility for at least one year without any abnormality found at routine fertility check-up (normal results in semen analyses, luteal phase assessment, tubal patency, immunological testing and investigations into uterine anomalies). We did not include normal fertile participants or healthy volunteers.

If essential information about the participants was lacking, we sought more information from the authors.

Types of interventions

Any included study must have made a comparison of the following, in pairs or in a combination of all three techniques:

- A gradient technique
- A swim-up technique
- Wash and centrifugation

We included subfertile couples undergoing IUI. We excluded subfertile couples undergoing other assisted reproduction techniques because of the likelihood of a large difference in the number of motile sperm needed for IUI compared to IVF, ICSI or gamete intrafallopian transfer (GIFT), for example.

Types of outcome measures

Primary outcomes

- Live birth rate (LBR) per couple. Live birth is defined as delivery of a live fetus after 20 completed weeks of gestation.
- Clinical pregnancy rate (CPR) per couple defined as evidence of a gestational sac, confirmed by ultrasound.

Secondary outcomes

- Ongoing pregnancy rate (OPR) per couple. Ongoing pregnancy is defined as evidence of a gestational sac with fetal heart motion at 12 weeks, confirmed with ultrasound.
- Multiple pregnancy rate (MPR) per couple — twins, triplets or higher order — specified if possible (confirmed by ultrasound or delivery).
- Miscarriage rate (MR) per couple, confirmed by ultrasound and pregnancy test or by histology.
- Ectopic pregnancy rate per couple (confirmed by histology).
- Fetal abnormalities per couple.
- Infections per couple.

Search methods for identification of studies

We searched for all published and unpublished RCTs comparing clinical outcomes after a gradient technique, swim-up technique or wash and centrifuge, without language restriction and in consultation with the Cochrane Gynaecology and Fertility Group (CGF) Information Specialist, Marian Showell.

Electronic searches

We searched the following electronic databases for relevant trials.

- The CGFG Specialised Register of Controlled Trials, PROCITE platform (searched 12 March 2019);
- CENTRAL; via the Cochrane Register of Studies Online (CRSO Web platform) (searched 12 March 2019);
- MEDLINE (Epub Ahead of Print, In-Process & Other Non-Indexed Citations) Ovid platform (searched from 1946 to 12 March 2019);
- Embase Ovid platform (searched from 1980 to 12 March 2019);
- PsycINFO Ovid platform (searched from 1806 to 12 March 2019);

We combined the MEDLINE search with the Cochrane Highly Sensitive Search Strategy for identifying randomised trials which appears in the *Cochrane Handbook of Systematic Reviews of Interventions* (Version 5.1.0 chapter 6, 6.4.11). We combined the Embase and PsycINFO searches with trial filters developed by the Scottish Intercollegiate Guidelines Network (SIGN) https://www.sign.ac.uk/search-filters.html.

We searched databases using different search strategies as provided in Appendix 1, Appendix 2, Appendix 3, Appendix 4 and Appendix 5.

Searching other resources

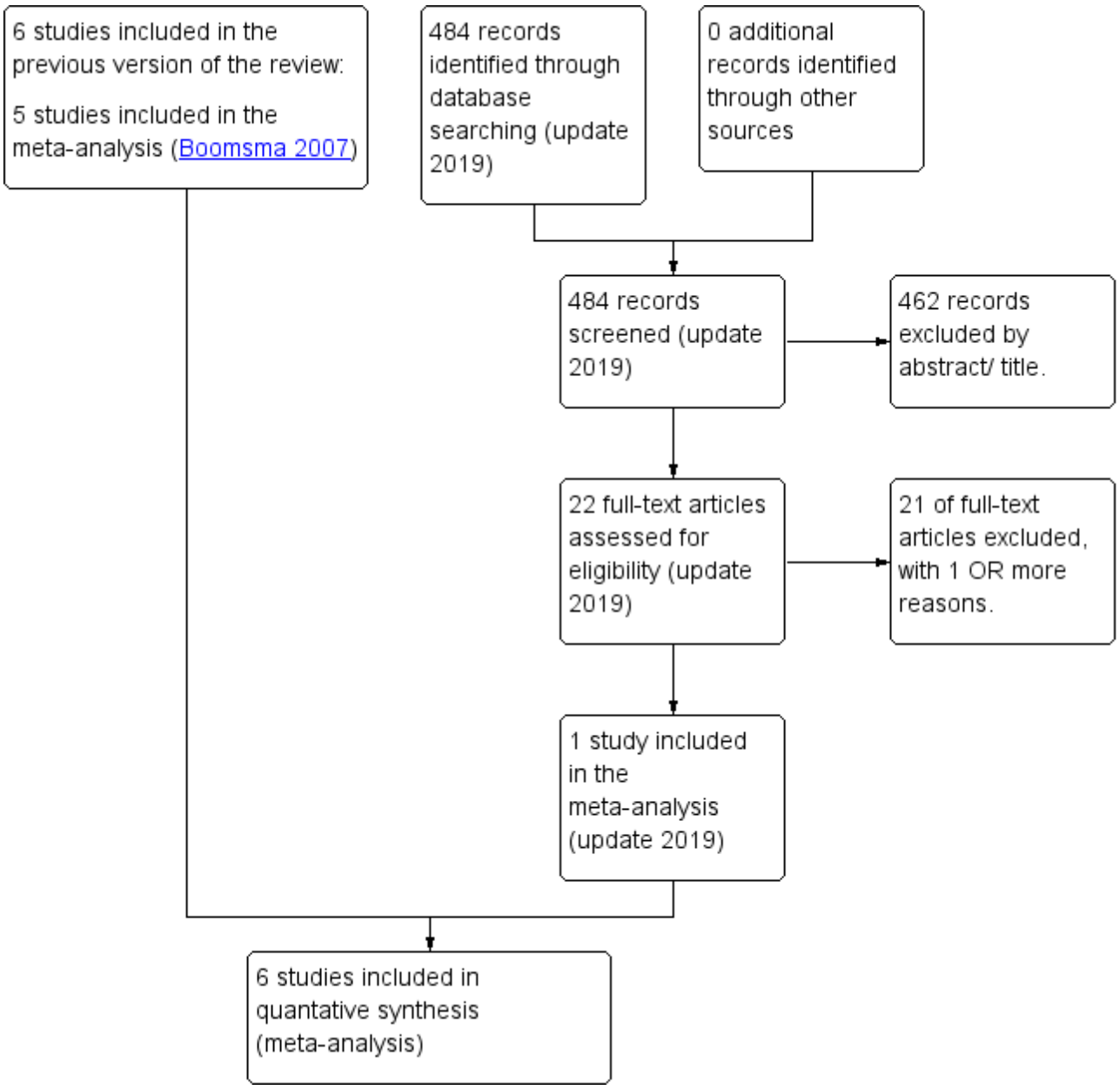
We also handsearched the citation lists of relevant publications, review articles, European Society of Human Reproduction and Embryology (ESHRE) and American Society for Reproductive Medicine (ASRM) abstract books. We conducted a simple search in PubMed and Google in order to identify any trials not yet indexed in the major databases. We searched abstracts of scientific meetings and included studies. We also searched the trial register www.ClinicalTrials.gov (a service of the US National Institutes of Health) for ongoing and registered trials (Appendix 6). In addition we had personal communication with experts and authors in the field.

Data collection and analysis

Selection of studies

After an initial screen of titles and abstracts retrieved by the search, conducted by two authors (Boomsma and Cohlen), we retrieved the full texts of all potentially eligible studies. Two review authors (Boomsma and Cohlen) independently examined these full-text articles for compliance with the inclusion criteria and selected eligible studies. We corresponded with study investigators as required, to clarify study eligibility. We resolved disagreement through discussion or, if required, in consultation with the third author until we reached consensus. If any reports had required translation, we would have described the process used for data collection. We have documented the selection process with a PRISMA flow chart (Figure 1).

Figure 1. PRISMA study flow diagram (updated search 2019)



Data extraction and management

Two review authors independently extracted data from eligible studies using a data extraction form designed and pilot-tested by the authors. We resolved any disagreements by discussion. Data extracted included study characteristics and outcome data (see data extraction form for details, Appendix 7). We corresponded with study investigators for further data on methods, results or both, as required. Data are often presented in a non-standardised format: we included studies irrespective of whether outcomes are reported in a 'usable' way. In multi-arm studies, we excluded data from arms that do not meet eligibility criteria.

Assessment of risk of bias in included studies

Two review authors independently assessed the included studies for risk of bias using the Cochrane 'Risk of bias' assessment tool (Higgins 2011) to assess the following.

- Selection bias (random sequence generation and allocation concealment)
- Performance bias (blinding of participants and personnel)
- Detection bias (blinding of outcome assessment; the primary outcome 'live birth rate/ongoing pregnancy rate' was not, however, susceptible to this kind of bias)
- Attrition bias (describing the completeness of outcome data)
- Reporting bias (selective reporting, such as failure to report outcomes/publication bias)
- Other bias.

We assigned judgements as recommended in the *Cochrane Handbook for Systematic Reviews of Interventions*, Section 8.5. There were no disagreements. We summarised results in the 'Risk of bias' tables for all included studies, and incorporated them into our interpretation of review findings.

Measures of treatment effect

For dichotomous data (e.g. clinical pregnancy rates), we used the numbers of events in the control and intervention groups of each study to calculate odds ratio (OR). We presented 95% confidence intervals for all outcomes. Should data to calculate ORs not have been available, we would have utilised the most detailed numerical data available that may facilitate similar analyses of included studies (e.g. test statistics, P values). We assessed whether the estimates calculated in the review for individual studies were compatible in each case with the estimates reported in the study publications.

Unit of analysis issues

Results from included studies that we excluded from the meta-analysis due to a cross-over design are described in additional tables (Table 1, Table 2). The primary analyses are data per couple randomised; only miscarriage rates per pregnancy were mentioned in the review text. We have only included first-phase data from cross-over trials and we contacted authors when needed.

Dealing with missing data

For included studies, we have noted levels of attrition in the Characteristics of included studies tables. We analysed the data on an intention-to-treat basis as far as possible.

Assessment of heterogeneity

We examined heterogeneity between the results of different studies by inspecting the scatter in the data points and the overlap in their confidence intervals, and more formally by checking the results of the Chi^2 tests. We took an I^2 statistic greater than 50% to indicate substantial statistical heterogeneity (Higgins 2011). Clinical heterogeneity in subfertility cannot be avoided because most centres use their own materials and methods, which can differ in a number of ways. When trials met the inclusion criteria and they had performed the same intervention, we considered it appropriate to pool their results.

Assessment of reporting biases

In view of the difficulty of detecting and correcting for publication bias and other reporting biases, we aimed to minimise their potential impact by ensuring a comprehensive search for eligible studies and by being alert for duplication of data. If there had been 10 or more studies in an analysis, we would have used a funnel plot to explore the possibility of small-study effects (a tendency for estimates of the intervention effect to be more beneficial in smaller studies).

Data synthesis

We combined the data using a fixed-effect model (underlying effect size assumed to be the same for all trials in the analysis) in the following comparisons.

- Swim-up versus gradient technique
- Swim-up versus wash technique
- Gradient versus wash technique

We performed statistical analysis using Review Manager 5 (Review Manager 2014). We considered clinical pregnancy and live birth rates to be positive consequences of treatment; we therefore considered a higher proportion achieving these outcomes to be a benefit. The outcomes of adverse effects (multiple pregnancy, miscarriage, ectopic pregnancy, fetal abnormalities and infections) are negative consequences of treatment and therefore we considered higher numbers to be detrimental. This needs to be taken into consideration when viewing the summary graphs.

Subgroup analysis and investigation of heterogeneity

A priori, it was planned to perform a subgroup analysis to look at the possible contribution of differences in the indication of subfertility (male factor versus other) and type and method of the semen preparation technique. It was planned to perform these analyses if there were more than five trials in each group.

Sensitivity analysis

A priori, we planned to perform a sensitivity analysis to determine whether the conclusions are robust to arbitrary decisions made regarding the eligibility and analysis and to look at the possible contribution of differences in methodological quality of the trials. We would have performed sensitivity analyses by excluding those studies with a high risk of bias. We planned to perform these analyses if there were more than five trials in each group.

Overall quality of the body of evidence: 'Summary of findings' table

We prepared a 'Summary of findings' table using GRADEpro and Cochrane methods. This table evaluates the overall quality of the body of evidence for the main review outcomes (clinical pregnancy rate, ongoing pregnancy rate, multiple pregnancy rate, and miscarriage rate) for the main review comparisons (swim-up versus gradient technique, swim-up versus wash technique, and gradient versus wash technique). Two review authors (Boomsma and Farquhar) assessed the quality of the evidence independently by using GRADE criteria: risk of bias; consistency of effect; imprecision; indirectness; and publication bias.

RESULTS

Description of studies

Results of the search

Sixty-one studies (39 studies after the initial search and 22 studies after the update) were potentially eligible and we retrieved them in full text. Seven studies met our inclusion criteria (of which we included six studies in the meta-analysis). We excluded 54 studies. See study tables: Characteristics of included studies, and Characteristics of excluded studies. A PRISMA flow chart of the results of the initial and updated search is included (Figure 1).

Included studies

Study design and setting

We included six randomised controlled trials in the meta-analysis (Dodson 1998; Grigoriou 2005; Karamahmutoglu 2014; Posada 2005; Soliman 2005; Xu 2000). We included one of the studies in the meta-analysis after contact with the authors of the study (Dodson 1998). This study had a cross-over design but the authors were able to provide initial cycle data, prior to the cross-over. Carrell 1998 was not able to provide data from the initial treatment cycle, and therefore we included this study in the review but excluded it from the meta-analysis. The characteristics and results of these cross-over trials are summarized in Table 1 and Table 2.

Only Karamahmutoglu 2014 and Dodson 1998 performed a power analysis; only Karamahmutoglu 2014 performed it prospectively. Dodson 1998 reported that 700 cycles would have been needed in each treatment arm (power 80%) and they included 153 cycles in total. Karamahmutoglu 2014 reported 280 patients were required (140 in each treatment arm), 223 couples were included in total. Both studies do not describe the reasons for not including the number of patients needed for adequate statistical power.

Participants

The studies included 485 subfertile couples undergoing IUI with subfertility for at least one year. Dodson 1998, Karamahmutoglu 2014, Posada 2005 and Soliman 2005 included women with a variety of causes of infertility. Male factor infertility was excluded by Carrell 1998. Grigoriou 2005 included couples with unexplained infertility. The cause of subfertility in Xu 2000 was male factor infertility only (all semen samples were oligoasthenoteratospermic).

Treatment groups were similar at baseline in Dodson 1998, Karamahmutoglu 2014, Posada 2005. Since Grigoriou 2005 and Xu 2000 lacked details about important prognostic indicators concerning the participants (women's age, duration of infertility), it was unclear whether treatment groups were similar at baseline regarding these indicators. Women's age is an important factor in predicting the success of reproductive treatment (Campana 1996). In Dodson 1998 we were able to extract information about the participants from the raw data supplied by the authors. Soliman 2005, an abstract, did report women's age (32.4 and 34.5 years for the gradient and wash technique respectively).

The studies were performed in different countries: Canada (Soliman 2005), China (Xu 2000), Colombia (Posada 2005), Greece (Grigoriou 2005), Turkey (Karamahmutoglu 2014), and the USA (Dodson 1998).

Interventions

Xu 2000 compared a gradient technique (Percoll) versus swim-up technique versus a real-time separation technique (which was not considered by this review). Grigoriou 2005 compared a wash technique (with exogenous platelet-activating factor) versus a swim-up technique. Soliman 2005 also compared a gradient technique with a wash technique. Dodson 1998 compared the efficacy of wash and centrifugation versus multiple tube swim-up versus a gradient technique (Percoll). Karamahmutoglu 2014 and Posada 2005 compared a swim-up versus a gradient technique. Carrell 1998 compared five different semen preparation techniques: wash technique; swim-up; swim-down; gradient technique; refrigeration and heparin technique. See the table Characteristics of included studies for further details.

The assisted reproductive technique used in all studies was IUI. In Dodson 1998, Grigoriou 2005, Karamahmutoglu 2014, Posada 2005 and Soliman 2005, all women received ovarian hyperstimulation with gonadotropins or clomiphene citrate, or both. Soliman 2005 performed two inseminations per cycle, 24 hours apart. Carrell 1998 included IUI both with and without controlled ovarian hyperstimulation. In Xu 2000 it was not stated whether controlled ovarian hyperstimulation was used.

Outcomes

Primary outcomes

No trials reported the primary outcome 'live birth'. All included studies reported the primary outcome 'clinical pregnancy rate per couple'. However, Xu 2000 and Soliman 2005 only reported pregnancy rates without a definition. Posada 2005 reported clinical pregnancy rates, also without definition. Dodson 1998, Grigoriou 2005 and Karamahmutoglu 2014 defined clinical pregnancy rate by the presence of a gestational sac on ultrasound scan.

Secondary outcomes

Karamahmutoglu 2014 reported ongoing pregnancy rates defined as a viable fetus detected after 12 weeks of pregnancy.

After receiving raw data from Dodson 1998 we were also able to calculate the miscarriage rate and multiple pregnancy rate per couple (first cycle). Karamahmutoglu 2014 and Posada 2005 also reported the miscarriage rate per couple. No other adverse effects were described by the studies.

Excluded studies

Fifty-four studies failed to meet the inclusion criteria for reasons outlined in the table Characteristics of excluded studies. Exclusions, for one or more reasons, were as follows.

We excluded 32 studies as they did not perform a comparison of interest (Abed 2015; Almagor 1993; Aribarg 1995; Bajamonte 1994; Baka 2009; Berteli 2017; Bhakta 2010; Chan 1992; Fazaeli 2018; Fleming 2008; Gentis 2012; Heidari 2016; Huang 1996b; Inaudi 2002; Jalilian 2016; Karlström 1991; Kücük 2008; Mathieu 1988; Menge 1992; Monqaut 2011; Ozturk 2008; Paul 2004; Ragni 1998; Romany 2017; Roth 2018; Siam 2012; Su 1993; Tomari 2017; Tsai 2004; Urry 1988; Zarmakoupis-Zavos 1998; Zavos 1992). Menge 1992, a conference abstract, was not able to provide separate data from the swim-up and Percoll group and, after contact with the authors, this allocation seemed to us to be non-randomised. Urry and colleagues did not provide separate data in their article about the comparison between the swim-up and wash preparation in the 'husband artificial insemination group' (Urry 1988). We did not succeed in contacting the authors to see if separate data were available.

We excluded 17 studies for not using IUI as an assisted reproduction technique (Bajamonte 1994; Chan 1992; Cimino 1990; Guerin 1989; Hammadeh 2001; Heidari 2016; Jaroudi 1993; Leonetti 1995; Levay 1995; Mathieu 1988; Ord 1990; Ricci 2009; Sapienza 1993; Tanphaichitr 1988; Tomari 2017; Van Der Zwalmen 1991; Zech 1993).

We excluded 30 studies for failing to use a randomised design (Almagor 1993; Bajamonte 1994; Berteli 2017; Caccamo 1995; Chan 1992; Cimino 1990; Depypere 1995; Fleming 2008; Guerin 1989; Hammadeh 2001; Heidari 2016; Huang 1996b; Leonetti 1995; Levay 1995; Mathieu 1988; Menge 1992; Morshedi 2003; Oguz 2018; Ohashi 1992; Ord 1990; Remohi 1989; Ren 2004; Ricci 2009; Roth 2018; Su 1993; Tanphaichitr 1988; Tomari 2017; Urry 1988; Van Der Zwalmen 1991; Werlin 1992). Four studies were quasi-randomised (Bajamonte 1994; Morshedi 2003; Tomari 2017; Werlin 1992); and Van Der Zwalmen 1991 and Remohi 1989 failed to describe the design. Two studies were excluded after contact with the authors (Butt 2016; Oguz 2018). Butt 2016 appeared to be a prospective observational study. The method of sperm preparation (density gradient versus swim-up) was dependent on sperm parameters rather than randomised. Oguz 2018 compared a swim-up and gradient technique on sperm DNA fragmentation status of semen samples from patients undergoing IUI. Sperm DNA fragmentation rates were evaluated in two portions of each sample of semen that was prepared with either swim-up or gradient techniques. Afterwards, patients were randomised to swim-up versus gradient technique, and one half of the semen sample was used for IUI. However, clinical data in relation to sperm preparation technique was not available.

Risk of bias in included studies

The risk of bias of the studies is summarised in Figure 2 and Figure 3.

Figure 2. Risk domains: review authors' judgements about each potential risk of bias item for each included study.

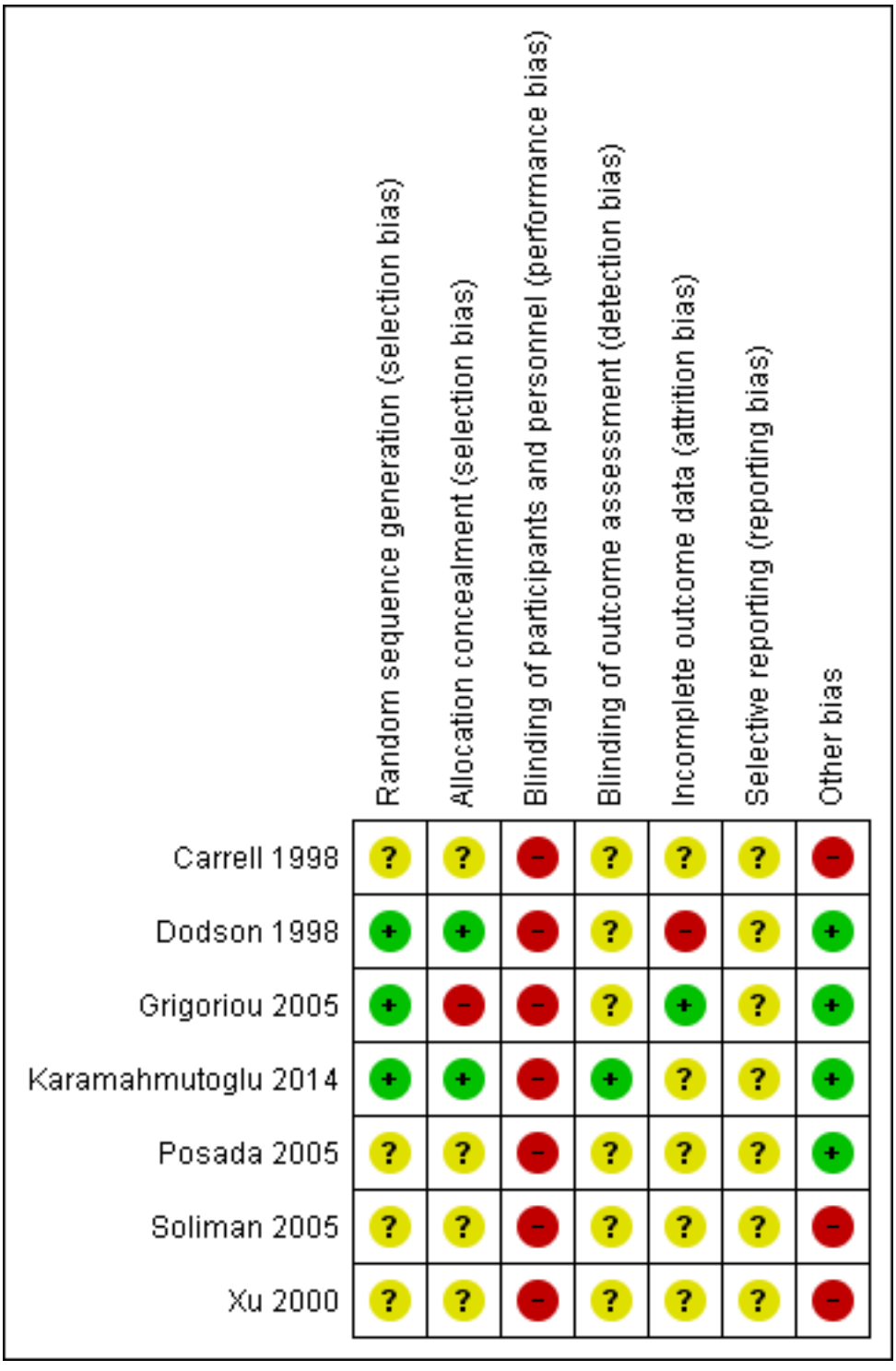
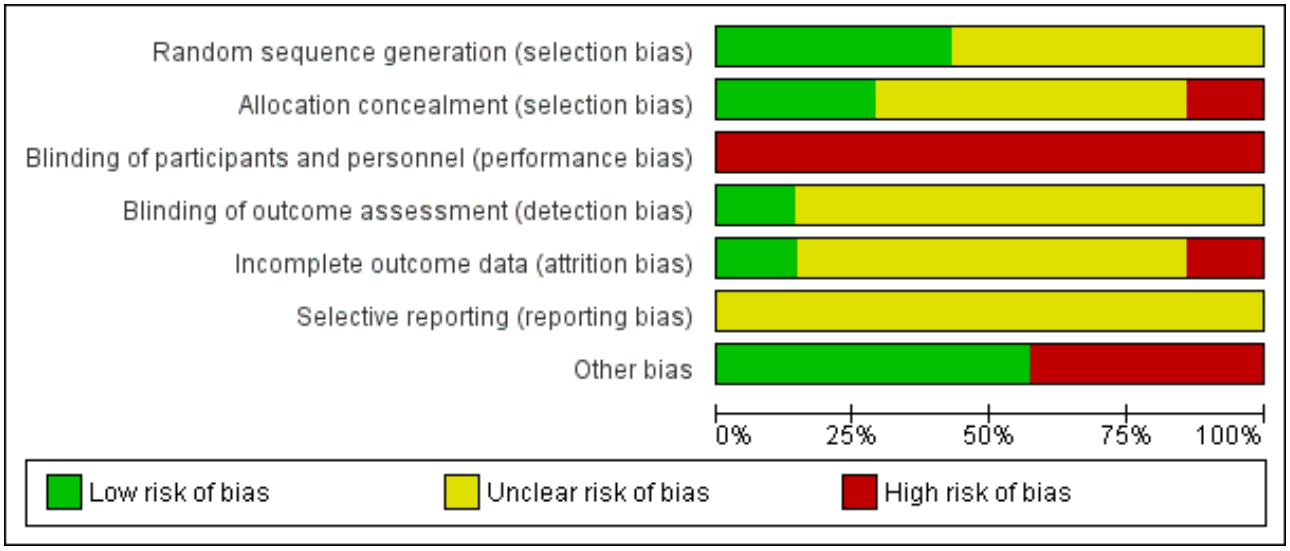


Figure 3. Risk of bias: review authors' judgements about each risk domain presented as percentages across all included studies.



Allocation

Sequence generation

We rated two studies at low risk of selection bias related to sequence generation, as they used computer randomisation (Dodson 1998; Karamahmutoglu 2014). We also rated Grigoriou 2005 at low risk of selection bias since they randomised their patients by a permuted block design from a table with random numbers. Four studies did not describe the method used and we rated them at unclear risk of this bias (Carrell 1998; Posada 2005; Soliman 2005; Xu 2000). We did not succeed in contacting the authors.

Allocation concealment

We rated two studies at low risk of selection bias regarding allocation concealment, as allocation was concealed by keeping the random numbers sequence at the laboratory in a separate location (Dodson 1998; Karamahmutoglu 2014). Four studies failed to describe methods of allocation concealment and we rated these at unclear risk of bias for this domain (Carrell 1998; Posada 2005; Soliman 2005; Xu 2000). We did not succeed in contacting the authors. We rated one study at high risk of selection bias since allocation was not concealed (Grigoriou 2005).

Blinding

Performance bias

In Karamahmutoglu 2014 the patients and the clinicians were blinded from knowledge of which sperm preparation technique was used by keeping the method of sperm preparation restricted to the laboratory staff. In Dodson 1998 study participants were blinded (information provided by the authors), but personnel were not blinded from knowledge of which intervention a participant received. Grigoriou 2005, Posada 2005, Soliman 2005 and Xu 2000 did not report blinding. We considered all studies to be at a high risk of performance bias, since the laboratory staff can easily perform better or worse with either technique according to subjective and subconscious prejudice.

Detection bias

In Karamahmutoglu 2014 outcome assessors were blinded from knowledge of which intervention a participant received; intended blinding was effective. Laboratory staff were not involved in outcome assessment. Overall, therefore, a low risk of detection bias.

We consider risk of detection bias for all other studies (due to knowledge of the allocated interventions by outcome assessors) to be unclear, since the primary review outcomes are not susceptible to bias (clinical pregnancy/live birth rate).

Incomplete outcome data

Dodson 1998 reported four dropouts who achieved a treatment-independent pregnancy during study enrolment; no intention-to-treat analysis was done. They do not report further dropouts (high risk of attrition bias). Grigoriou 2005 reported two dropouts in the swim-up study group; no reason was reported. An intention-to-treat analysis was performed (by imputation of no event) (low risk of attrition bias). The number of cancelled cycles was not stated. Five studies did not report dropouts or loss to follow-up and we judged them to be at unclear risk of attrition bias (Carrell 1998; Karamahmutoglu 2014; Posada 2005; Soliman 2005; Xu 2000).

Selective reporting

We identified no studies at high risk for selective reporting. No studies reported live birth as an outcome; this primary outcome is often not reported in fertility studies, however, because of the need for long follow-up rather than selective reporting bias. None of the studies failed to report outcomes that they planned to in their Methods section. However, data on adverse events were available for only two of the studies (Dodson 1998; Posada 2005). We did not classify the studies which did not report adverse events as at high risk of selective reporting in this review since adverse events are not expected as a result of different semen preparation techniques and the impact of failure to report them is unclear.

It was not useful to use a funnel plot to assess for publication bias, since at most four studies were pooled in any meta-analysis.

Other potential sources of bias

We identified use of inappropriate cross-over design as a source of potential bias in two studies (Carrell 1998; Dodson 1998). The risk of other biases was unclear in two studies (Soliman 2005; Xu 2000), one of which was an abstract (Soliman 2005), as they did not describe their methods in detail. In Xu 2000 it was unclear whether treatment groups were similar at baseline. In addition, the definition of pregnancy was unclear in Posada 2005, Xu 2000 and Soliman 2005, and we assumed it to be a risk of bias. Although Posada 2005 did report clinical pregnancy rates, rather than pregnancy rates.

Effects of interventions

See: **Summary of findings for the main comparison** Swim-up technique compared to gradient technique for undergoing intrauterine insemination; **Summary of findings 2** Swim-up technique compared to wash and centrifugation for undergoing intrauterine insemination; **Summary of findings 3** Gradient technique compared to wash and centrifugation for undergoing intrauterine insemination

1. Swim-up versus gradient technique

We included Dodson 1998, Karamahmutoglu 2014, Posada 2005 and Xu 2000 in this analysis.

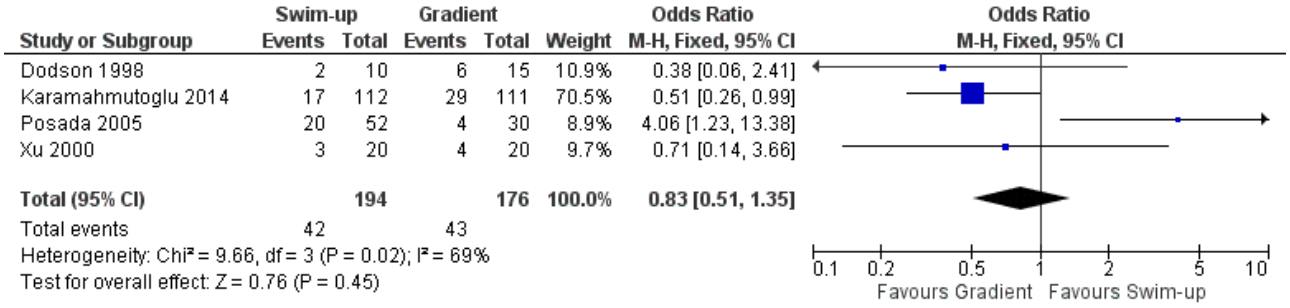
Live birth rate per couple

No studies reported on this outcome.

1.1 Clinical pregnancy rate per couple

Considering the quality of evidence, we are uncertain whether there was a difference between CPR for swim-up versus a gradient technique (PR 22% versus 24% respectively; odds ratio (OR) 0.83, 95% CI 0.51 to 1.35; I^2 = 71%; 4 RCTs, 370 participants; very low-quality evidence). The results suggest that if the chance of clinical pregnancy after the use of a gradient technique is assumed to be 24%, the chance of clinical pregnancy after using the swim-up technique is between 14% and 30%. See Figure 4.

Figure 4. Forest plot of comparison: 1 Swim-up versus gradient technique; fresh semen, outcome: 1.1 Clinical pregnancy rate per couple.



1.2 Ongoing pregnancy rate per couple (secondary outcome)

There were no available data from Dodson 1998, Posada 2005 and Xu 2000. The ongoing pregnancy rate per couple was 11% after swim-up technique versus 23% after gradient technique (Karamahmutoglu 2014). Although there was a significantly higher ongoing pregnancy rate after gradient versus swim-up technique, we are uncertain whether there was a real difference between ongoing pregnancy rates per couple considering the quality of evidence (OR 0.39, 95% CI 0.19 to 0.82; heterogeneity not applicable; 1 RCT, 223 participants; very low-quality evidence).

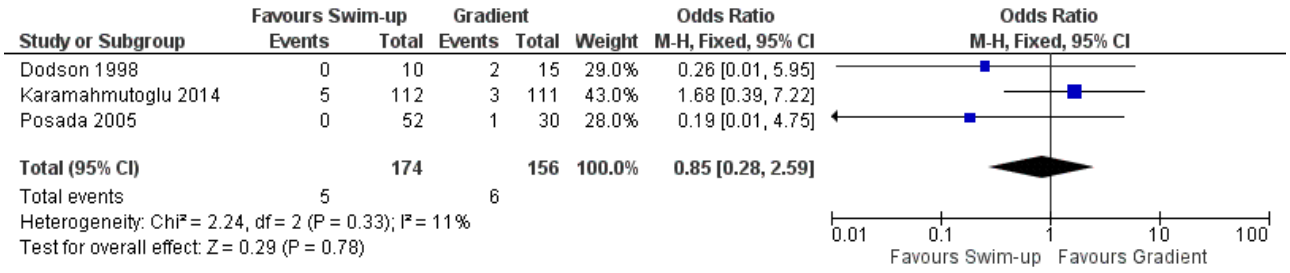
1.3 Multiple pregnancy rate per couple (secondary outcome)

There were no available data from Karamahmutoglu 2014, Posada 2005 and Xu 2000. No multiple pregnancies were observed by Dodson 1998.

1.4 Miscarriage rate per couple (secondary outcome)

Considering the quality of evidence, we are uncertain whether there was a difference between miscarriage rates (MR) per couple comparing a swim-up versus gradient technique (MR per couple 3% versus 4%; OR 0.85, 95% CI 0.28 to 2.59; I^2 = 44%; 3 RCTs, 330 participants; very low-quality evidence). See Figure 5.

Figure 5. Forest plot of comparison: 1 Swim-up versus gradient technique; fresh semen, outcome: 1.4 Miscarriage rate per couple.



Ectopic pregnancy rate per couple (secondary outcome)

No studies reported on this outcome.

Fetal abnormalities per couple (secondary outcome)

No studies reported on this outcome.

Infection rate per couple (secondary outcome)

No studies reported on this outcome.

2. Swim-up versus wash and centrifugation technique

We included Dodson 1998 and Grigoriou 2005 in the analysis.

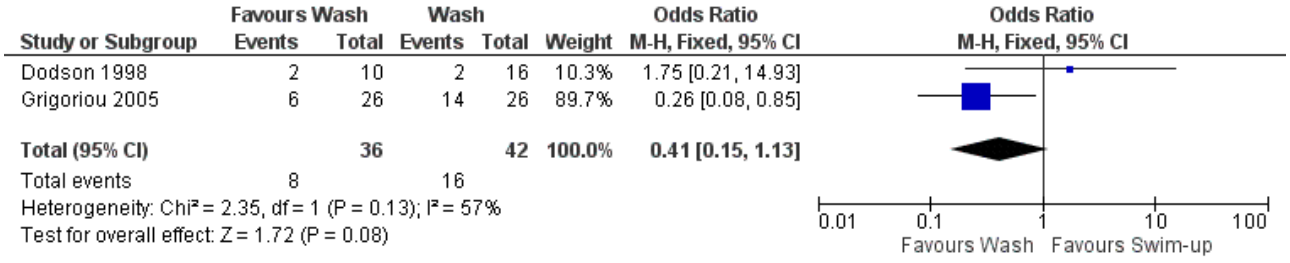
Live birth rate per couple

No studies reported on this outcome.

2.1 Clinical pregnancy rate per couple

Considering the quality of evidence, we are uncertain whether there is a difference between CPR per couple for swim-up versus a wash technique (CPR 22% versus 38% respectively; OR 0.41, 95% CI 0.15 to 1.13; $I^2 = 55\%$; 2 RCTs, 78 participants; very low-quality evidence). The results suggest that if the chance of clinical pregnancy after the use of a wash technique is assumed to be 38%, the chance of clinical pregnancy after using the swim-up technique is between 9% and 41%. See Figure 6.

Figure 6. Forest plot of comparison: 2 Swim-up versus wash and centrifugation; fresh semen, outcome: 2.1 Clinical pregnancy rate per couple.



Ongoing pregnancy rate per couple (secondary outcome)

No studies reported on this outcome.

2.2 Multiple pregnancy rate per couple (secondary outcome)

There were no available data from Grigoriou 2005. Considering the quality of evidence, we are uncertain whether there was a difference between multiple pregnancy rates between treatment groups (OR 0.49, 95% CI 0.02 to 13.28; heterogeneity not applicable; 1 RCT, 26 participants; very low-quality evidence). The multiple pregnancy rate per couple was 0% versus 6.3%, respectively (Dodson 1998). One triplet pregnancy was observed after the wash technique.

2.3 Miscarriage rate per couple (secondary outcome)

There were no available data from Grigoriou 2005. In Dodson 1998, after both techniques the miscarriage rate per couple was 0%.

Ectopic pregnancy rate per couple (secondary outcome)

No studies reported on this outcome.

Fetal abnormalities per couple (secondary outcome)

No studies reported on this outcome.

Infection rate per couple (secondary outcome)

No studies reported on this outcome.

3. Gradient versus wash and centrifugation technique

Dodson 1998 and Soliman 2005 were included in the analysis.

Live birth rate per couple

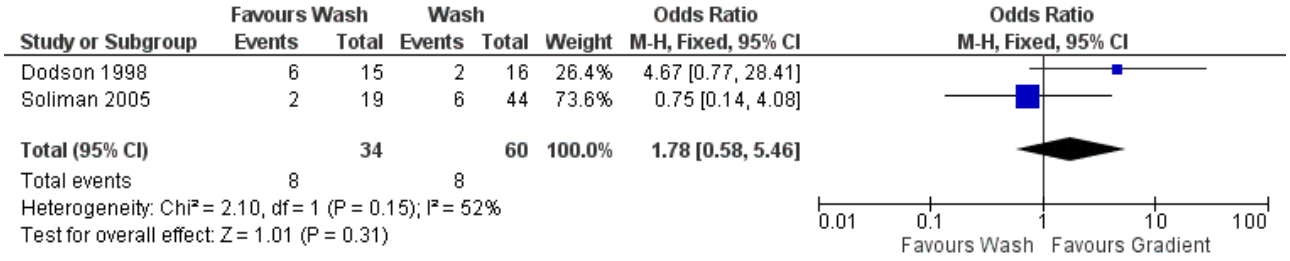
No studies reported on this outcome.

3.1 Clinical pregnancy rate per couple

Considering the quality of evidence, we are uncertain whether there was a difference between CPR for gradient technique versus a wash technique (CPR 24% versus 13%, respectively; OR 1.78, 95% CI 0.58 to 5.46; $I^2 = 52\%$; 2 RCTs, n = 94; very low-quality evidence). The results suggest that if the chance of clinical pregnancy after the use of a wash technique is assumed to be 13%, the chance of clinical

pregnancy after using the gradient technique is between 8% and 46%. See Figure 7.

Figure 7. Forest plot of comparison: 3 Gradient technique versus wash and centrifugation; fresh semen, outcome: 3.1 Clinical pregnancy rate per couple.



Ongoing pregnancy rate per couple (secondary outcome)

No studies reported on this outcome.

3.2 Multiple pregnancy rate per couple (secondary outcome)

There were no available data from Soliman 2005. Considering the quality of evidence, we are uncertain whether there was a difference between multiple pregnancy rates per couple between the treatment groups (OR 0.33, 95% CI 0.01 to 8.83; very low-quality evidence). The multiple pregnancy rate per couple was 0% versus 6%, respectively. One triplet pregnancy was recorded after the wash technique (Dodson 1998).

3.3 Miscarriage rate per couple (secondary outcome)

There were no available data from Soliman 2005. Considering the quality of evidence, we are uncertain whether there was a difference between miscarriage rates per couple between the treatment groups (OR 6.11, 95% CI 0.27 to 138.45; very low-quality evidence). The miscarriage rate per couple was 10% (miscarriage rate per pregnancy 30%) versus 0%, respectively (Dodson 1998).

Ectopic pregnancy rate per couple (secondary outcome)

No studies reported on this outcome.

Fetal abnormalities per couple (secondary outcome)

No studies reported on this outcome.

Infection rate per couple (secondary outcome)

No studies reported on this outcome.

Overall there was no clear evidence which semen preparation technique was superior. No studies provided information on laboratory time and costs per preparation technique. Summaries of our findings are provided in the Summary of findings for the main comparison, Summary of findings 2, and Summary of findings 3.

Heterogeneity results of included studies

We examined heterogeneity between the results of the different studies by inspecting the scatter in the data points and the overlap in their confidence intervals, and more formally by checking the results of the Chi² tests. We took an I^2 measurement greater than 50% as an indicator of substantial statistical heterogeneity. Considering the results of the meta-analysis, there was a large overlap in confidence intervals. There was, however, a large difference in the direction of effect. Meta-analysis of the pregnancy results after swim-up technique versus gradient technique (I^2 = 71%), and swim-up versus wash technique (I^2 = 55%) and wash technique versus gradient technique (I^2 = 52%) showed indication of substantial heterogeneity. This may partly be explained by heterogeneity in the sperm preparation procedures among the different trials, which are not standardised. Care must be taken in the interpretation of the Chi² test in these meta-analyses though, since it has low power when studies have small sample sizes and are few in number. We could perform no sensitivity analyses to explore the heterogeneity.

DISCUSSION

Summary of main results

The aim of this review was to compare the effectiveness of three different semen preparation techniques (gradient; swim-up; wash and centrifugation) on clinical outcomes (live birth rate; clinical pregnancy rate) in subfertile couples undergoing IUI.

The first conclusion that can be drawn from this systematic review is that large, high-quality randomised controlled trials comparing the effectiveness of a gradient, swim-up or wash and centrifugation technique, alone or in combination, are lacking. No studies reported on live birth rates. We identified only seven RCTs which compared a gradient technique versus a swim-up technique or a wash technique for IUI (Carrell 1998; Dodson 1998; Grigoriou 2005; Karamahmutoglu 2014; Posada 2005; Soliman 2005; Xu 2000). We identified one cross-over RCT, which we excluded from the meta-analysis but included in the review (Carrell 1998), since we could not extract data prior to crossing over.

In conclusion, we are uncertain whether there is a difference in pregnancy outcomes between the three sperm preparation techniques for subfertile couples undergoing IUI. The quality of evidence was very low. The main limitations were (unclear) risk of bias (unclear reporting), signs of imprecision and inconsistency in results among studies and the lack of power.

Overall completeness and applicability of evidence

The increasing availability of therapeutic choices resulting from advances in subfertility research poses a problem in trying to determine whether these options are equally effective in clinical care. In 2010 the World Health Organization (WHO) published a WHO laboratory manual for the examination and processing of

human semen (WHO 2010). The manual describes the choices for sperm preparation, which is dictated by the nature of the semen sample (Canale 1994). For example, the direct swim-up technique is often used when the semen sample is considered to be largely normal; whereas in cases of severe oligozoospermia, teratospermia or asthenospermia, density gradients are usually preferred because of the greater total number of motile spermatozoa recovered. Wash and centrifugation is not widely used, and is only thought to be suitable for normospermic specimens (WHO 2010). We have not been able to perform subgroup analyses to investigate this research question. The WHO laboratory manual also advises that each laboratory should determine after rigorous pre-clinical testing the optimal centrifugal force and centrifugation time necessary to increase the chance of recovering the maximum number of spermatozoa (WHO 2010).

We included six studies in the meta-analysis, of which only one study was less than 10 years old. The relevance of these older studies in current laboratory practice can be questioned. However, in general, laboratory procedures of sperm preparation have not significantly changed in the last two decades. Media used have been more standardised, though. A simple two-step discontinuous density-gradient preparation method is most widely applied. A number of commercial products are available for making density gradients suitable for semen processing (WHO 2010). Percoll was previously used in ART; since 1996, however, it has only been used for research purposes due to concerns about its safety. Xu 2000 and Dodson 1998 both used Percoll gradient. Research demonstrated, however, that the new products appear to be as effective as Percoll for the recovery of good, progressively motile sperm (Centola 1998).

The reason for the absence of current studies is unknown; and we could identify none ongoing in trial registers either. There is a wide practice variation of used methods and outcomes in IUI in fertility laboratories (Lemmens 2018). Although there is a lack of evidence for their efficacy, many laboratories seem to prefer density gradient techniques which are easier to use and standardize than the swim-up technique (WHO 2010). The lack of new studies may also be the result of the ongoing debate about the value of IUI (Lemmens 2017). The British National Institute for Health and Care Excellence (NICE) guideline for infertility treatment (NICE 2013) strongly reduced the indications for IUI with IVF/ICSI as a first line treatment in the majority of cases.

The studies we identified were not sufficient to address the objectives of this review, due to statistical heterogeneity among the studies and methodological limitations.

Quality of the evidence

Only randomised controlled trials were included in this meta-analysis. The quality of the evidence was very low. The main limitations in the evidence were unclear risk of bias, signs of inconsistency and (very) serious imprecision. Only two of the seven included studies used and described an adequate method of allocation concealment. In addition to the main limitations, there was a lack of blinding in most studies and high risk of performance bias in all studies. None of the studies reported live birth, which is the outcome most relevant to subfertile couples; and data on adverse events were available for only three of the studies.

In addition, the number of studies was low. One study dominated the results (Karamahmutoglu 2014). However, even this study with the largest study population seriously lacked power. Many fertility trials lack power. A prospective power calculation should always be performed, although the calculated sample size in most cases will be prohibitively large. Accruing this number of participants would require several years or a multi-centre design to complete the trial. In both cases, this would increase clinical heterogeneity (Daya 2001), but might also ensure that studies more closely resemble the heterogeneity of daily practice. Only one of the trials performed an intention-to-treat analysis. The performance of this analysis minimizes an exclusion bias. A strategy to minimize this bias is to conduct the randomisation as late as possible in the study design; the dictum of 'select subjects early but randomise late' is particularly relevant in subfertility research (Daya 2001).

Potential biases in the review process

We assume the risk of bias in review design to be minimal; it was predefined and objective. We assume the bias in locating studies to be minimal, since the search was not limited to language or timeframe, and was conducted in multiple literature sources. We minimised the risk of bias in selecting studies by using two independent reviewers throughout the screening and data collection process, which reduces reviewer bias. The quality of the studies was assessed by two independent reviewers according to GRADE criteria.The risk of bias in synthesising studies is also assumed to be low. There was no selective outcome reporting. We published the study protocol in advance to promote transparency.

Agreements and disagreements with other studies or reviews

The results of this review are in agreement with other evidence from studies or reviews. Many studies on the efficacy of sperm preparation techniques focus on sperm recovery rates. The direct swim-up technique generally recovers a lower number of motile sperm compared to density gradient techniques (Butt 2016; Ng 1992; WHO 2010). This outcome is not relevant to subfertile couples, however, and may not reflect clinical outcome. Firstly, the sperm preparation technique aims not only to recover a high number of morphologically normal and motile sperm, but also to eliminate any factors detrimental to fertilization and prostaglandins, and to perform sperm capacitation. Dodson 1998 and Butt 2016 showed that potential critical differences in sperm isolation and recovery for IUI yield no benefit in cycle fecundity. This may be due to all methods surpassing a low threshold number of motile sperm for conception, or all methods recover a subset of sperm capable of achieving fertilization with no benefit of additional sperm (Dodson 1998).

AUTHORS' CONCLUSIONS

Implications for practice

The very low quality of the available evidence means we cannot be certain about the relative effectiveness of the different semen preparation techniques: swim-up versus gradient versus wash and centrifugation technique. No studies reported on live birth rates. We are uncertain whether there is a difference in clinical pregnancy rates, ongoing pregnancy rates, multiple pregnancy rates or miscarriage rates per couple between the three sperm preparation techniques. Further randomised trials are warranted that report live birth data. This meta-analysis was restricted to three types of sperm preparation but other techniques are available.

Implications for research

More research needs to be performed on this topic as firm conclusions cannot be drawn from the literature available. In addition to large RCTs, the results from thorough phase II research with semen parameters as an outcome would have substantial meaning for optimising the techniques. These type of studies are suitable for 'within participant' comparisons (such as Ricci 2009).

It may be interesting to combine a split sample study on semen parameters at initial semen analysis (at fertility check-up) and subsequently randomise semen preparation techniques (in the treatment cycle) to investigate whether the type of preparation needs to be individualized according to semen parameters after different preparation techniques.

Studies should report clinically relevant outcomes, such as ongoing pregnancy or preferably live birth rate per woman, rather than per cycle. Yet most research in the fertility field focuses on fertilisation rates, recovery rates and embryo development. Many fertility trials lack adequate reporting of methodology. The methods of randomisation and allocation concealment should be reported (Vail 2003). Adherence to the recommendations in the guideline for reporting clinical trials (CONSORT) would create a massive improvement. Because of a large range of factors contributing to the outcome in fertility research, we recommend a clear definition of the population, inclusion and exclusion criteria and a comparison of these factors in the treatment groups. In addition, the methodology of semen preparation needs to be standardised in order to allow appropriate comparison.

ACKNOWLEDGEMENTS

The authors would like to thank several people for helping in the construction of this review, namely Michelle Proctor, Review Group Coordinator of the Cochrane Gynaecology and Fertility Group (CGF); Ruth Buist for retrieval of trials and Sue Hall for helping to collect trials we needed, and for their positive support; and Anne Lethaby for her statistical advice.

We would also like to thank Dr M Merrilees and Dr MHJM Curfs and Dr AMM Wetzels for their advice on laboratory techniques and A Vail and J Deeks for their advice on the statistical analysis. A special thanks to Dr WC Dodson, Dr R Menkveld, Dr Prakash for putting such an effort into getting the information we asked for and for always replying to the numerous e-mails we sent. A 'thank you' is also extended to Dr E Makrakis, Dr M Bajamonte, Dr J Check, Dr WCL Ford, Dr M Hammadeh, Dr A Menge, Dr M Morshedi, Dr A Oliva, Dr U Punjabi, Dr P Serafini, Dr C Srisombut, Dr B Storey, Dr F Butt and Dr L Werlin, who provided additional information on their trials. We acknowledge the contribution of Professor Maas Jan Heineman to previous versions of this review. We acknowledge the peer reviewers for their valuable suggestions: Selma Mourad, Astrid Cantineau, and Andy Vail.

REFERENCES

References to studies included in this review

Carrell 1998 *{published data only}*

* Carrell DT, Kuneck PH, Peterson CM, Hatasaka HH, Jones KP, Campbell BF. A randomized, prospective analysis of five sperm preparation techniques before intrauterine insemination of husband sperm. *Fertility and Sterility* 1998;**69**(1):122-6.

Dodson 1998 *{published and unpublished data}*

* Dodson WC, Moessner J, Miller J, Legro RS, Gnatuk CL. A randomized comparison of the methods of sperm preparation for intrauterine insemination. *Fertility and Sterility* 1998;**70**(3):574-5.

Grigoriou 2005 *{published data only}*

Grigoriou O, Makrakis E, Konidaris S, Hassiakos D, Papadias K, Baka S, et al. Effect of sperm treatment with exogenous platelet-activating factor on the outcome of intrauterine insemination. *Fertility and Sterility* 2005;**83**(3):618-21.

Karamahmutoglu 2014 *{published data only}*

Karamahmutoglu H, Erdem A, Erdem M, Mutlu MF, Bozkurt N, Oktem M, et al. The gradient technique improves success rates in intrauterine insemination cycles of unexplained subfertile couples when compared to swim-up technique; a prospective randomized study. *Journal of Assisted Reproduction and Genetics* 2014;**31**(9):1139-45.

Posada 2005 *{published data only}*

Posada MN, Azuero AM, Arango AM, Raigosa GC, Cano JF, Perez AL. Sperm washing with swim-up versus gradients in intra uterine insemination (IUI): results of a prospective randomized study comparing pregnancy rates and costs. Fertility and Sterility Abstract book 61st ASRM meeting. 2005; Vol. 84 Suppl 1:361.

Soliman 2005 *{published data only}*

Soliman S, Goyal A. RCT comparing two different sperm preparations for intrauterine insemination. Fertility and Sterility, Abstractbook 61st ASRM meeting. 2005; Vol. 84 Suppl 1:156.

Xu 2000 *{published data only}*

* Xu L, Lu RK, Chen L, Zheng YL. Comparative study on efficacy of three sperm-separation techniques. *Asian Journal of Andrology* 2000;**2**(2):131-4.

References to studies excluded from this review

Abed 2015 *{published data only}*

Abed F, Zadehmodarres S. A comparative study of swim-up and upstream methods for isolating sperm cell for intra uterine insemination. *International Journal of Women's Health and Reproduction Sciences* 2015;**3**(2):103-6.

Almagor 1993 *{published data only}*

* Almagor M, Benbenishti D, Rosenak D, Mogle Y, Yaffe H. Simultaneous swim-up/swim-down of sperm in assisted reproduction procedures. *Journal of Assisted Reproduction and Genetics* 1993;**10**(4):261-5.

Aribarg 1995 *{published data only}*

Aribarg A, Sukcharoen N. Intrauterine insemination of washed spermatozoa for treatment of oligozoospermia. *International Journal of Andrology* 1995;**18 suppl 1**:62-6.

Bajamonte 1994 *{published data only}*

* Bajamonte M, Ruvolo G, Cimino C, Cittadini E. Advantages of treatment with human follicular fluid in the management of severely dyspermic patients in human in vitro fertilization programs. *Acta Europaea Fertilitatis* 1994;**25**(2):87-92.

Baka 2009 *{published data only}*

Baka S, Grigoriou O, Hassiakos D, Konidaris SW, Papadias K, Makrakis E. Treatment of sperm with platelet activating factor does not improve intrauterine insemination outcomes in unselected cases of mild male factor infertility: a prospective double blind randomized crossover study. *Urology* 2009;**74**(5):1025-8.

Berteli 2017 *{published data only}*

Berteli TS, Da Broi MG, Martins WP, Ferriani RA, Navarro PA. Magnetic-activated cell sorting before density gradient centrifugation improves recovery of high-quality spermatozoa. *Andrology* 2017;**5**(4):776-82.

Bhakta 2010 *{published data only}*

Bhakta R, Kumar P, Adiga SK, Kalthur G. A randomized controlled study to evaluate the cost-effectiveness in sperm extraction using carbon dioxide and carbon dioxide free system in relation to intrauterine insemination pregnancy. *Journal of Human Reproductive Sciences* 2010;**3**(1):8-10.

Butt 2016 *{published data only}*

Butt F, Arshad Chohan M. Comparative efficacy of density gradient and swim-up methods of semen preparation in intrauterine insemination cycles. *Journal of the Pakistan Medical Association* 2016;**66**(8):932-7.

Byrd 1994 *{published data only}*

Byrd W, Drobnis EZ, Kutteh WH, Marshburn P, Carr BR. Intrauterine insemination with frozen donor sperm: a prospective randomized trial comparing three different sperm preparation techniques. *Fertility and Sterility* 1994;**62**(4):850-6.

Caccamo 1995 *{published data only}*

Caccamo A, Scarduelli C, Tesoriere G, Giametta P, Lombroso GC. Comparison of the Percoll and swim-up methods for sperm preparation for normal semen samples. *Human Reproduction* 1995;**10**(Abstractbook 2):114.

Chan 1992 *{published data only}*

Chan P, Tredway D, Su B, Corselli J, Pang S. A double method sperm wash for artificial insemination. *Archives of Andrology* 1992;**29**(1):43-8.

Cimino 1990 *{published data only}*

* Cimino C, Cittadini E, Gattuccio F. New developments in the treatment of male sub-fertility by means of assisted fecundation techniques. *Acta Europaea Fertilitatis* 1990;**21**(6):281-8.

Depypere 1995 *{published data only}*

* Depypere H, Milingos S, Comhaire F. Intrauterine insemination in male subfertility: a comparative study of sperm preparation using a commercial percoll kit and conventional sperm wash. *European Journal of Obstetrics, Gynecology, and Reproductive Biology* 1995;**62**(2):225-9.

Fazaeli 2018 *{published data only}*

Fazaeli H, Davoodi F, Kalhor N, Tabatabaii Qomi R. The effect of supernatant product of adipose tissue derived mesenchymal stem cells and density gradient centrifugation preparation methods on pregnancy in intrauterine insemination cycles: An RCT. *International Journal of Reproductive Biomedicine (Yazd, Iran)* 2018;**16**(3):299-308.

Fleming 2008 *{published data only}*

Fleming SD, Ilad RS, Griffin AM, Wu Y, Ong KJ, Smith HC, et al. Prospective controlled trial on an electrophoretic method of sperm preparation for assisted reproduction: comparison with density gradient centraifugation. *Human Reproduction (Oxford, England)* 2008;**23**(12):2646-51.

Gentis 2012 *{published data only}*

Gentis RK, Siebert I, Kruger TF, de beer-Windt ML. Implementation of an office-based semen preparation method (SEP-D Kit) for intra-uterine insemination (IUI): A controlled randomised study to compare the IUI pregnancy outcome between a routine (swim-up) and the Sep-D Kit method. *South African Journal of Obstetrics and Gynaecology* 2012;**18**(2):54-5.

Guerin 1989 *{published data only}*

* Guerin JF, Mathieu C, Lornage J, Pinatel MC, Boulieu D. Improvement of survival and fertilizing capacity of human spermatozoa in an IVF programme by selection on discontinuous Percoll gradients. *Human Reproduction* 1989;**4**(7):798-804.

Hammadeh 2001 *{published data only}*

Butt F, Chohan MA. Comparative efficacy of density gradient and sim-up methods of semen preparation in intrauterine insemination cycles. *Journal of the Pakistan Medical Association* 2016;**66**(8):932-7.

* Hammadeh ME, Kuhnen A, Amer AS, Rosenbaum P, Schmidt W. Comparison of sperm preparation methods: effect on chromatin and morphology recovery rates and their consequences on the clinical outcome after in vitro fertilization embryo transfer. *International Journal of Andrology* 2001;**24**(6):360-8.

Heidari 2016 *{published data only}*

Heidari M, Darbandi M, Darbandi S, Shani S, Lakpour N, Sadeghi MR. Comparative study of sperm quality on swim up and upstream processing techniques. ISERB conference abstract. *Journal of Reproduction & Infertility* 2016;**17**:110-1.

Huang 1996b *{published data only}*

Huang HY, Lai YM, Lee CL, Chang MY, Chang SY, Soong YK. Improved sperm recovery rate with Percoll gradient method in intrauterine insemination. *Chang Gung Medical Journal* 1996;**19**(1):24-9.

Inaudi 2002 *{published data only}*

Inaudi P, Petrilli S, Joghtapour A, Trusso P, Petraglia F. Reduction of steps in the preparation of motile sperm for intra uterine insemination does not reduce efficacy of the procedure: Simplified one-step swim-up method versus classic swim-up. *Human Reproduction* 2002;**17**(5):1288-91.

Jalilian 2016 *{published data only}*

Jalilian N, Bakhtiyari M, Keshavarzi F, Nankali A, Rezavand N, Veisi F, et al. Improvement in sperm quality and increased pregnancy rate after intrauterine insemination. *Acta Medica Mediterranea* 2016;**32**:941-5.

Jaroudi 1993 *{published data only}*

Jaroudi KA, Carver-Ward JA, Hamilton CJCM, Sieck UV, Sheth KV. Percoll semen preparation enhances human oocyte fertilization in male-factor infertility as shown by a randomized cross-over study. *Human Reproduction* 1993;**8**(9):1438-42.

Karlström 1991 *{published data only}*

Karlström PO, Bakos O, Bergh T, Lundkvist Ö. Intrauterine insmeination and comparison of two methods of sperm preparation. *Human Reproduction* 1991;**6**(3):390-5.

Kücük 2008 *{published data only}*

* Kücük T, Sozen E, Buluç B. Effect of heat-induced hypermotility on pregnancy rate in intrauterine insemination for male factor infertility associated with asthenospermia: a prospective, randomized, controlled study. *Journal of Assisted Reproduction and Genetics* 2008;**25**(6):235-8.

Leonetti 1995 *{published data only}*

* Leonetti T, Causio F, Cagnazzo G. Techniques of sperm selection and in vitro fertilization results. *Acta Europaea Fertilitatis* 1995;**26**(1):31-4.

Levay 1995 *{published data only}*

Levay PF, Fourie FR, Bezuidenhout C, Koch A. Effectiveness of various sperm processing methods in removing seminal plasma from insemination media. *Human Reproduction* 1995;**10**(8):2056-60.

Mathieu 1988 *{published data only}*

* Mathieu C, Guerin JF, Gille Y, Pinatel MC, Lornage J, Boulieu D. Isolation of spermatozoa using Percoll gradients; it's value for in vitro fertilization (IVF) [Separation des spermatozoides en gradient de Percoll: interet pour la fecondation in vitro (FIV)]. *Journal de Gynecologie, Obstetrique et Biologie de la Reproduction* 1988;**17**(2):237-41.

Menge 1992 *{published data only}*

* Menge AC, Hurd WW, Randolph JF, Ansbacher R, Hiner MR, Grady E, et al. The effect of TEST-Yolk buffer on intrauterine insemination pregnancy rates. Fertility and Sterility. Abstracts

from the Meeting of the American Society for Reproductive Medicine. 1992; Vol. 58 Suppl 155:P140.

Monqaut 2011 *{published data only}*

Monqaut AL, Zavaleta C, Lopez G, Lafuente R, Brassesco M. Use of high-magnification microscopy for the assessment of sperm recovered after two different sperm processing methods. *Fertility and Sterility* 2011;**95**(1):277-80.

Morshedi 2003 *{published data only}*

* Morshedi M, Duran HE, Taylor S, Oehninger S. Efficacy and pregnancy outcome of two methods of semen preparation for intrauterine insemination: a prospective randomized study. *Fertility and Sterility* 2003;**79**(Suppl 3):1625-32.

Morshedi M, Taylor S, Duru K, Montgomery C, Barroso G, Oehninger S. A prospective randomized study comparing Isolate™-processed versus washed semen in intrauterine insemination (IUI) therapy using husband's sperm: efficiency and pregnancy outcome. Fertility and Sterility. 1999; Vol. 72 Suppl 1:217.

Oguz 2018 *{published data only}*

Oguz Y, Guler I, Erdem A, Mutlu MF, Gumuslu S, Oktem M, et al. The effect of swim-up and gradient sperm preparation techniques on deoxyribonucleic acid (DNA) fragmentation in subfertile patients. *Journal of Assisted Reproduction and Genetics* 2018;**35**(6):1083-9.

Ohashi 1992 *{published data only}*

* Ohashi K, Saji F, Wakimoto A, Kato M, Tsutsui T, Tanizawa O. Preparation of oligozoospermic and/ or asthenozoospermic semen for intrauterine insemination using the Spermprep semen filtration column. *Fertility and Sterility* 1992;**57**(4):866-70.

Ord 1990 *{published data only}*

* Ord T, Patrizio P, Marello E, Balmaceda JP, Asch RH. Mini-Percoll: a new method of semen preparation for IVF in severe male factor infertility. *Human Reproduction* 1990;**5**(8):987-9.

Ozturk 2008 *{published data only}*

* Ozturk TN, Pekel A, Arikan OY, Bayrak O, Kafali H. One wash or two washes: effects of sperm wash processing by density gradient centrifugation on intrauterine insemination outcomes. Abstract. *Human Reproduction* 2008;**23**(Suppl 1):i119.

Paul 2004 *{published data only}*

Paul JB, Seidemann EL, Wbster BW, Cochran RA. Influence of different gradient separation protocols on percent motile spermatozoa recovery. Fertility and Sterility. 2004; Vol. 82 Suppl:268.

Ragni 1998 *{published data only}*

Ragni G, De Lauretis L, D'Ambrogio G, Pellegrini S, Maggioni P, Vegetti W, et al. Prospective randomized comparison of test yolk buffer preincubation and swim-up standard preparation in intrauterine insemination. Human Reproduction. 1996; Vol. Abstract book 12th Annual Meeting of the ESHRE:122.

Ragni G, De Lauretis L, D'Ambrogio G, Pellegrini S, Maggioni P, Vegetti W, et al. Semen preparation by standard swim-up versus swim-up with test yolk buffer incubation in intrauterein insemination: a randomized study. *Human Reproduction* 1998;**13**(7):1859-63.

Remohi 1989 *{published data only}*

* Remohi J, Gastaldi C, Patrizio P, Gerli S, Ord T, Asch RH. Intrauterine insemination and controlled ovarian hyperstimulation in cycles before GIFT. *Human Reproduction* 1989;**4**(8):918-20.

Ren 2004 *{published data only}*

Ren SS, Sun GH, Ku CH, Chen DC, Wu GJ. Comparison of four methods for sperm preparation for IUI. *Archives of Andrology* 2004;**50**(3):139-43.

Ricci 2009 *{published data only}*

Ricci G, Perticarari S, Boscolo R, Montico M, Guaschino S, Presani G. Semen preparation methods and sperm apoptosis: swim-up versus gradient-density centrifugation technique. *Fertility and Sterility* 2009;**91**(2):632-8.

Romany 2017 *{published data only}*

Romany L, Rivera Egea R, Meseguer M, Aparicio-Ruiz B, Remohi J, Garrido N. Magnetic activated sorting selection (MACS) of non-apoptotic sperm (NAS) improves ongoing pregnancy rates in homologous intrauterine insemination (IUI). ASRM conference abstract. *Fertility and Sterility* 2017;**108**(Suppl 1):e128-e129.

Roth 2018 *{published data only}*

Roth F, Wusk B, Redolfi R, Haeberlin F. Semen processing-simultaneous swim-up vs swim-down technique: Re-evaluation of the benefits. *Journal of Assisted Reproduction and Genetics* 2018;**35**:2099.

Sapienza 1993 *{published data only}*

* Sapienza F, Verheyen G, Tournaye H, Janssens R, Pletincx I, Derde M, et al. An auto-controlled study in in-vitro fertilization reveals the benefit of Percoll centrifugation to swim-up in the preparation of poor-quality semen. *Human Reproduction* 1993;**8**(11):1856-62.

Siam 2012 *{published data only}*

* Siam EM. Pregnancy outcome after IUI for male and idiopathic infertility using a new simplified method for sperm preparation. *Middle East Fertility Society Journal* 2012;**17**(1):30-6.

Su 1993 *{published data only}*

Su BC, Chan PJ, Tredway DR, Pang SC. Clinical pregnancy rate after the double method wash and intrauterine insemination. *Archives of Andrology* 1993;**31**(1):63-7.

Tanphaichitr 1988 *{published data only}*

* Tanphaichitr N, Agulnick A, Seibel M, Taymor M. Comparison of the in vitro fertilization rate by human sperm capacitated by multiple-tube swim-up and Percoll gradient centrifugation. *Journal of in Vitro Fertilization and Embryo Transfer* 1988;**5**(3):119-22.

Tomari 2017 *{published data only}*

Tomari H, Honjo K, Nagata Y. Evaluation of a new sperm density gradient and sperm wash for assisted reproductive technology. ASRM conference abstract. *Fertility and Sterility* 2017;**108**:e243.

Tsai 2004 *{published data only}*

Tsai YC, Lin MY, Chen SH, Kang CY, Tsai YT, Lin YT, et al. Comparing the clinical outcomes of intrauterine insemination by two different density gradient preparation methods. *Journal of the Chinese Medical Association : JCMA* 2004;**67**(4):168-71.

Urry 1988 *{published data only}*

Urry RL, Middleton RG, Jones K, Poulson M, Worley R, Keye W. Artificial insemination: a comparison of pregnancy rates with intrauterine versus cervical insemination and washed sperm versus swim-up sperm preparations. *Fertility and Sterility* 1988;**49**(6):1036-8.

Van Der Zwalmen 1991 *{published data only}*

* Van Der Zwalmen P, Bertin-Segal G, Geerts L, Debauche C, Schoysman R. Sperm morphology and IVF pregnancy rate: comparison between Percoll gradient centrifugation and swim-up procedures. *Human Reproduction* 1991;**6**(4):581-8.

Werlin 1992 *{published data only}*

Werlin L, Mayer J, Kramer T, Le N, Elwood V, Westerhout S, et al. A prospective randomized study of two methods of semen preparation for intrauterine insemination (IUI): a significant difference in the clinical pregnancies between Percoll (PW) and sperm wash (SW). Fertility and Sterility. Abstracts from the American Society for Reproductive Medicine. 1992; Vol. 58:Suppl 108. P215.

Zarmakoupis-Zavos 1998 *{published data only}*

Zarmakoupis-Zavos PN, Zavos PM. Can the method of sperm preparation for intrauterine insemination affect subsequent pregnancy rates? Comparison between the SpermPrep (TM) and the traditional double wash method. *Middle East Fertility Society Journal* 1998;**3**:2.

Zavos 1992 *{published data only}*

Zavos PM, Centola GM. Methods of semen preparation for intrauterine insemination and subsequent pregnancy rates. *Tohoku Journal of Experimental Medicine* 1992;**168**(4):583-90.

Zech 1993 *{published data only}*

* Zech H, Moschig M, Weiss P, Zech J. Comparison of sperm preparation techniques with regard to sperm recovery, sperm survival and fertilization rate in vitro in normal and pathological ejaculates [Vergleich von praparationstechniken in bezug auf die spermatozoen-endkonzentration und -uberlebenszeit sowie die fertilisierungsrate in vitro bei normalen und pathologischen ejaculaten]. *Geburtshilfe und Frauenheilkunde* 1993;**53**(10):715-20.

Additional references

Aitken 1994

Aitken J, Fisher H. Reactive oxygen species generation and human spermatozoa: the balance of benefit and risk. *BioEssays* 1994;**16**(4):259-67.

Berg 1997

Berg U, Brucker C, Berg FD. Effect of motile sperm count after swim-up on outcome of intrauterine insemination. *Fertility and Sterility* 1997;**67**(4):747-50.

Byrd 1996

Byrd W. Processing of human semen for insemination: comparison of methods. In: GM Centola, K Ginsburg editor(s). Evaluation and treatment of the infertile male. Cambridge: Cambridge University Press, 1996:89-107.

Campana 1996

Campana A, Sakkas D, Stalberg A, Bianchi PG, Comte I, Pache T, et al. Intrauterine insemination: evaluation of the results according to the woman's age, sperm quality, total sperm count per insemination and life table analysis. *Human Reproduction* 1996;**11**(4):732-6.

Canale 1994

Canale D, Giorgi PM, Gasperini M, Pucci E, Barletta D, Gasperi M, Martino E. Inter and intra-individual variability of sperm morphology after selection with three different techniques: layering, swimup from pellet and Percoll. *Journal of Endocrinological Investigation* 1994;**17**(9):729-32.

Centola 1998

Centola GM, Herko R, Andolina E, Weisensel S. Comparison of sperm separation methods: effect on recovery, motility, motion parameters, and hyperactivation. *Fertility and Sterility* 1998;**70**(6):1173-5.

Cho 2017

Cho CL, Agarwal A. Role of sperm DNA fragmentation in male factor infertility: a systematic review. *Arab Journal of Urology* 2017;**16**(1):21-34.

Daya 2001

Daya S. Pitfalls in the design and analysis of efficacy trials in subfertility. *Human Reproduction* 2003;**18**(5):1005-9.

De Jonge 2002

De Jonge C. The clinical value of sperm nuclear DNA assessment. *Human Fertility* 2002;**5**(2):51-3.

Delhanty 2001

Delhanty JDA. Preimplantation genetics: an explanation for poor human fertility?. *Annals of Human Genetics* 2001;**65**(Pt 4):331-8.

Evers 2002

Evers JL. Female subfertility. *Lancet* 2002;**360**(9327):151-9.

Farquhar 2018

Farquhar CM, Liu E, Armstrong S, Arroll N, Lensen S, Brown J. Intrauterine insemination with ovarian stimulation versus expectant management for unexplained infertility (TUI): a pragmatic, open-label, randomised, controlled, two-centre trial. *Lancet* 2018;**391**(10119):441-50.

Goldenberg 1992

Goldenberg, M, Rabinovici J, Bider D, Lunenfeld B, Blankstein J, Weissenberg R. Intra-uterine insemination with prepared sperm vs. unprepared first split ejaculates. A randomized study. *Andrologia* 1992;**24**(3):135-40.

Henkel 2003

Henkel RR, Schill WB. Sperm preparation for ART. *Reproductive Biology and Endocrinology : RB&E* 2003;**1**:108.

Higgins 2011

Higgins JP, Green S, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011). The Cochrane Collaboration, 2011. Available from handbook.cochrane.org.

Huang 1996a

Huang HY, Lee CL, Lai YM, Chang MY, Wang HS, Chang SY, et al. The impact of the total motile sperm count on the success of intrauterine insemination with husband's spermatozoa. *Journal of Assisted Reproduction and Genetics* 1996;**13**(1):56-63.

Khalil 2001

Khalil MR, Rasmussen PE, Erb K, Laursen SB, Rex S, Westergaard LG. Homologous intrauterine insemination. An evaluation of prognostic factors based on a review of 2473 cycles. *Acta Obstetricia et Gynecologica Scandinavica* 2001;**80**(1):74-81.

Lemmens 2017

Lemmens L, Kos S, Beijer C, Braat DD, Nelen WL, Wetzels AM. Techniques used for IUI: is it time for a change?. *Human Reproduction* 2017;**32**(9):1835-45.

Lemmens 2018

Lemmens L, Kos S, Beijer C, Braat DD, Jonker MA, Nelen WL, Wetzels AM. Optimization of laboratory procedures for intrauterine insemination: survey of methods in relation to clinical outcome. *Andrology* 2018;**6**(5):707-13.

Madbouly 2017

Madbouly K, Isa A, Habous M, Almannie R, Abu-Rafea B, Binsaleh S. Postwash total motile sperm count: should it be included as a standard male infertility work up. *Canadian Journal of Urology* 2017;**24**(3):8847-52.

Mascarenhas 2012

Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA. National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. *PLoS Medicine* 2012;**9**(12):e1001356.

Ng 1992

Ng FL, Liu DY, Baker HW. Comparison of Percoll, mini-Percoll and swim-up methods for sperm preparation from abnormal semen samples. *Human Reproduction (Oxford, England)* 1992;**7**(2):261-6.

NICE 2013

Nice: National Institute for Health and Care Excellence. Fertility problems: assessment and treatment [Clinical guideline [CG156]]. 2013, update 2017.

Niederberger 2018

Niederberger C, Pellicer A, Cohen J, Gardner DK, Palermo GD, O'Neill CL, et al. Forty years of IVF. *Fertility and Sterility* 2018;**110**(2):185-324.

Oguz 2018

Oguz Y, Guler I, Erdem A, Mutlu MF, Gumuslu S, Oktem M, et al. The effect of swim-up and gradient sperm preparation techniques on deoxyribonucleic acid (DNA) fragmentation in subfertile patients. *Journal of Assisted Reproduction and Genetics* 2018;**35**(6):1083-9.

Ombelet 2017

Ombelet W. The revival of intrauterine insemination: evidence-based data have changed the picture. *Facts, Views & Vision in ObGyn* 2017;**9**(3):131-2.

Parinaud 1997

Parinaud J, Le Lannou D, Vieitez G, Griveau JF, Milhet P, Richoilley G. Enhancement of motility by treating spermatozoa with an antioxidant solution (Sperm-Fit) following ejaculation. *Human Reproduction* 1997;**12**(11):2434-6.

Review Manager 2014 [Computer program]

Nordic Cochrane Centre, The Cochrane Collaboration. Review Manager 5 (RevMan 5). Version 5.3. Copenhagen: Nordic Cochrane Centre, The Cochrane Collaboration, 2014.

Swan 1999

Swan SH, Elkin EP. Declining semen quality: can the past inform the present?. *BioEssays* 1999;**21**(7):614-21.

Templeton 1990

Templeton A, Fraser C, Thompson B. The epidemiology of infertility in Aberdeen. *BMJ* 1990;**301**(6744):148-52.

Vail 2003

Vail A, Gardener E. Common statistical errors in the design and analysis of subfertility trials. *Human Reproduction* 2003;**18**(5):1000-4.

Van Voorhis 2001

Van Voorhis BJ, Barnett M, Sparks AE, Syrop CH, Rosenthal G, Dawson J. Effect of the total motile sperm count on the efficacy and cost-effectiveness of intrauterine insemination and in vitro fertilization. *Fertility and Sterility* 2001;**75**(4):661-8.

Wang 1991

Wang FN. Assisted Human Reproductive Technology. Washington DC: Hemisphere Publishing Co, 1991.

WHO 1992

World Health Organization. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. Cambridge: Cambridge University Press, 1992.

WHO 1999

World Health Organization. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. Cambridge: Cambridge University Press, 1999.

WHO 2010

World Health Organization. WHO laboratory manual for the examination and processing of human semen. 5th Edition. WHO press, 2010.

Yan 1998

Yan RY. Practical Eugenics. 2nd Edition. Beijing: People Health Publishing House, 1998.

References to other published versions of this review

Boomsma 2007

Boomsma CM, Heineman MJ, Cohlen BJ, Farquhar C. Semen preparation techniques for intrauterine insemination. Cochrane Database of Systematic Reviews. John Wiley & Sons, Ltd, 2007, issue 4. [DOI: 10.1002/14651858.CD004507.pub3; CD004507]

* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

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

Carrell 1998

Methods	Cross-over RCT. Stated random, but no details. Single-centre. Concealment of allocation, blinding, number of dropouts or cancelled cycles, intention-to-treat analysis, power calculation: not stated.
Participants	363 couples. Cause of infertility: variety. Progressive motile sperm count < 20 million excluded.
Interventions	5 sperm preparation techniques: wash, swim-up, swim-down, gradient, and refrigeration/heparin. IUI with or without COH (gonadotropins/cc).
Outcomes	Primary outcomes • Live birth rate not reported. • Swim-up, CPR 13%. Gradient, CPR 13%. Wash, CPR 9%. Secondary outcomes • OPR, MR and MPR not reported.
Notes	Cross-over study design. We have contacted the authors; they were unable to provide the data of the first treatment cycle.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Stated as randomised, no further details.
Allocation concealment (selection bias)	Unclear risk	Method of allocation concealment not described.
Blinding of participants and personnel (performance bias) All outcomes	High risk	Study participants and personnel were not blinded from knowledge of which intervention a participant received.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Outcome assessors were not blinded from knowledge of which intervention a participant received. However, risk of detection bias due to knowledge of the

Carrell 1998 *(Continued)*

		allocated interventions by outcome assessors may be low due to primary outcomes which are not susceptible to bias.
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Dropouts or loss to follow-up not stated. Duration of follow-up not stated.
Selective reporting (reporting bias)	Unclear risk	Does not report adverse events.
Other bias	High risk	Cross-over design. First cycle was randomised; however first cycle data were not available.

Dodson 1998

Methods	Cross-over RCT (by computer). Participants were randomised, samples not equally divided. Single centre. Concealment of allocation: good (list at laboratory). Single blinded (participant). Duration of follow-up: not stated. Groups similar regarding important indicators at baseline: estradiol level, follicles, cause of subfertility, age. Number of dropouts: not stated. 4 women achieved treatment independent pregnancies during study enrolment. Power calculation: performed (retrospectively, > 700 cycles/arm needed with power of 0.8 in cross-over design). Cancelled cycles, cancellation criteria, intention-to-treat analysis: not stated.
Participants	41 couples, 41 fresh semen samples. Quality: mixed. Age of women: 28 to 40 (mean 31.6) yrs. Duration subfertility > 1 yr. Cause infertility: 49% unexplained, 6% male subfertility, 33% endometriosis, 13% pelvic adhesions. Previous fertility treatment: not stated. Exclusion criteria: oligomenorrhoea, severe oligospermia, donor semen, female anatomic distortion reproductive tract, bilateral tubal occlusion. Inclusion criteria: not stated.
Interventions	3 preparation techniques. 1) WASH: 1:1 Ham's F-10, 10 min. 150 × g centrifugation, pellet resuspended, 10 min. 150 × g centrifugation, pellet resuspended. 2) SWIM-UP: multiple tube (4) 1:1 medium, 10 min 150 × g centrifugation, supernatant discarded. Overlayed with medium, 45 min incubation. Top removed +wash, 10 min 150 × g centrifugation. 3) GRADIENT: 90%/45% Percoll, 20 min 300 × g centrifugation, pellet washed, 10 min 150 × g centrifugation. ART: single IUI. 0.5 ml volume. Number IUI: 1. COH: all women, gonadotropins/hCG. Analysis by: not stated.
Outcomes	Primary outcomes • Live birth rate: not reported. • Swim-up, CPR/couple: 20% (2/10). Gradient: CPR/couple: 40% (6/15). Wash: CPR/couple: 13 % (2/16). Secondary outcomes • MR/couple Swim-up/Wash: 0%, Gradient: 10% (2/15). MR/pregnancy, Gradient: 30% (2/6). • MPR/couple Swim-up/Gradient: 0%. Wash, 6% (1/16). 1 triplet. PR/cycle: see additional table 02. • OPR not reported.
Notes	Cross-over study: only initial cycle was included in meta-analysis in both sections. All-cycle results are reported in Table 02 of Additional tables. Author provided additional information and data from which outcomes were calculated.

Risk of bias

Bias	Authors' judgement	Support for judgement

Dodson 1998 *(Continued)*

Random sequence generation (selection bias)	Low risk	Assigned randomly from a computer-generated random sequence.
Allocation concealment (selection bias)	Low risk	Allocation was concealed by keeping the random numbers sequence at the laboratory in a separate location.
Blinding of participants and personnel (performance bias) All outcomes	High risk	Study participants were blinded. Personnel were not blinded from knowledge of which intervention a participant received. The laboratory staff can easily perform better or worse with either technique according to subjective and subconscious prejudice.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Personnel were not blinded from knowledge of which intervention a participant received. However, risk of detection bias due to knowledge of the allocated interventions by outcome assessors may be low due to primary outcomes which are not susceptible to bias.
Incomplete outcome data (attrition bias) All outcomes	High risk	They reported 4 dropouts who achieved a treatment independent pregnancy, no intention-to-treat analysis was done. They do not report further dropouts.
Selective reporting (reporting bias)	Unclear risk	Unclear risk.
Other bias	Low risk	Cross-over design. However, first cycle data were included only, which were randomised.

Grigoriou 2005

Methods	Parallel design. Randomised by permuted block design from table with random numbers. Allocation not concealed. No blinding. 2 dropouts in swim-up study arm; reason: not stated. Groups similar regarding important indicators at baseline. Intention-to-treat analysis, power calculation: not stated. Single centre study.
Participants	52 couples. Age women: 30.6 ± 3.1 yrs, men: 34.1 ± 5.3 yrs. Duration subfertility > 1 yr. Cause of infertility: unexplained. Semen quality: normal (WHO criteria).
Interventions	2 preparation techniques. 1) WASH with PAF: 10 min. 400 × g centrifugation, treated with PAF in Cook medium for 15 minutes. Washed free. 2) SWIM-UP: direct swim-up with sperm washing medium (Cook). Inseminated sperm standardized to a volume of 0.5 ml, and a count of 20 million progressive motile sperm. ART: IUI. Number IUI: 1 to 3. COH: 100 mg clomiphene citrate day 3 to 7. 0.5 ml volume. Number IUI: 1. COH: all women: gonadotropins/hCG. Analysis by: Student's t-test, Kruskal Wallis, Fisher's exact test.
Outcomes	Primary outcomes • Live birth rate: not reported. • Wash +PAF: CPR/couple: 22 % (14/63), Swim-up, CPR/couple: 9% (6/70). Secondary outcomes • OPR, MR and MPR not reported.
Notes	Cross-over after 3 cycles. Only first 3 cycles included in the meta-analysis. IUI standardised to a volume of 0.5 ml, and a count of 20 million progressive motile sperm.

Grigoriou 2005 *(Continued)*

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomised by a permuted block design from a table with random numbers.
Allocation concealment (selection bias)	High risk	Allocation was not concealed.
Blinding of participants and personnel (performance bias) All outcomes	High risk	Study participants and personnel were not blinded from knowledge of which intervention a participant received.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Outcome assessors were not blinded from knowledge of which intervention a participant received. However, risk of detection bias due to knowledge of the allocated interventions by outcome assessors may be low due to outcomes which are not susceptible to bias.
Incomplete outcome data (attrition bias) All outcomes	Low risk	2 dropouts in the swim-up study group, no reason was reported. An intention-to-treat analysis has been performed (by imputation of no event).
Selective reporting (reporting bias)	Unclear risk	Does not report adverse events.
Other bias	Low risk	None identified.

Karamahmutoglu 2014

Methods	Parallel design. Randomised by a computer-generated randomisation table. Allocation was concealed by keeping the random numbers sequence at the laboratory, blinded for the clinicians and participants. No dropouts reported. Groups similar regarding important indicators at baseline. Intention-to-treat analysis: not stated. Power calculation: performed. Single centre study.
Participants	223 couples, fresh semen samples. Quality: normal; initial sperm count 5 to 15 million/ml. Age of women: 20 to 40 (mean) yrs. Duration subfertility > 1 yr. Cause of infertility: unexplained infertility, mild male infertility (initial sperm count 5 to 15 million/ml). Previous fertility treatment: excluded Exclusion criteria: oligomenorrhoea, endocrine disorders,prior ovarian surgery, moderate to severe endometriosis, ovarian cysts, oligospermia. Inclusion criteria: unexplained infertility, mild male infertility (initial sperm count 5 to 15 million/ml), age of women: 20 to 40 yrs, regular ovulatory menstrual cycle, basal FSH levels < 15 IU/L, bilateral tubal patency, normospermic according to WHO criteria.
Interventions	2 preparation techniques. 1) GRADIENT: Sperm Grad-125 was used as a gradient solution. 90%/40% gradient, centrifugation, pellet resuspended, centrifugation. 2) SWIM-UP: 1:1 dilute of medium. centrifugate. Supernatant extracted. Incubation at an angle of 45º. ART: IUI. Number of cycles per patient: 223 couples underwent 338 cycles. COH: rFSH 75-100 IU, hCG and luteal support. Number IUI per cycle: 1.
Outcomes	Primary outcomes • Live birth rate not reported.

Karamahmutoglu 2014 *(Continued)*

- Swim-up, CPR/couple: 15% (17/112). Gradient: CPR/couple: 26% (29/111).
- Swim-up, OPR/couple: 11% (12 /112). Gradient: OPR/couple: 23% (26 /111).

Secondary outcomes

- MR/couple Swim-up: 5% (5/112), Gradient: 3% (3/111).
- MR/pregnancy Swim-up: 29% (5/17), Gradient: 10% (3/29).
- MPR not reported.

Notes	Data in the text of the article are not correct (switched around); in the table they are correct. This was verified with the authors.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Assigned randomly from a computer-generated random sequence.
Allocation concealment (selection bias)	Low risk	Allocation was concealed by keeping the random numbers sequence at the laboratory, blinded for the clinicians.
Blinding of participants and personnel (performance bias) All outcomes	High risk	Study participants and clinicians were blinded from knowledge of which intervention a participant received. Intended blinding was effective. The laboratory staff can easily perform better or worse though with either technique according to subjective and subconscious prejudice.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Outcome assessors were blinded from knowledge of which intervention a participant received. Intended blinding was effective. Laboratory staff is not involved in outcome assessment.
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Dropouts or loss to follow-up not stated. Duration of follow-up 12 weeks.
Selective reporting (reporting bias)	Unclear risk	Miscarriage rates were not reported but can be extracted from data of clinical and ongoing pregnancies. No other adverse events reported.
Other bias	Low risk	None identified.

Posada 2005

Methods	Stated random, but no details. Design: parallel, single-centre. Concealment of allocation, blinding, number of dropouts or cancelled cycles, intention-to-treat analysis, power calculation: not stated.
Participants	82 couples. Age of women < 38 yrs. Mean age swim-up: 32.06 ± 3.7 yrs, gradient 32.37 ± 4.0 yrs. Cause of infertility: variety. No or moderate male factor. Duration subfertility: not stated. Baseline similarity: good.
Interventions	2 preparation techniques. 1) GRADIENT: not described 2) SWIM-UP: not described ART: IUI. Number IUI: swim-up 1.51 ± 0.81, gradient 1.67 ± 0.86. COH: CC and/or gonadotropins. Number IUI: 1.
Outcomes	Primary outcomes • Live birth rate not reported.

Posada 2005 *(Continued)*

	• Gradient, CPR/couple: 13% (4/30). Swim-up, CPR/couple: 39% (20/52). Secondary outcomes • MR/couple Swim-up 0%. Gradient: 3%. • OPR and MPR not reported.
Notes	Abstract. Big difference in results. Preparation techniques not described.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Stated as randomised, no further details. Treatment groups were similar at baseline.
Allocation concealment (selection bias)	Unclear risk	Allocation concealment not described.
Blinding of participants and personnel (performance bias) All outcomes	High risk	Study participants and personnel were not blinded from knowledge of which intervention a participant received.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Outcome assessors were not blinded from knowledge of which intervention a participant received. However, risk of detection bias due to knowledge of the allocated interventions by outcome assessors may be low due to outcomes which are not susceptible to bias.
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Dropouts or loss to follow-up not stated. Duration of follow-up not stated.
Selective reporting (reporting bias)	Unclear risk	Unclear risk.
Other bias	Low risk	None identified.

Soliman 2005

Methods	Stated random, but no details. Ratio 2:1 (wash versus gradient). Design: parallel (1 cycle), single-centre. Concealment of allocation, blinding, number of dropouts or cancelled cycles, intention-to-treat analysis, power calculation: not stated.
Participants	63 couples. Age of women: gradient 32.4 yrs, wash 34.5 yrs. Cause, duration of infertility: not stated. Semen quality: not stated.
Interventions	2 preparation techniques. 1) GRADIENT: 90%/45% gradient, centrifuge, pellet resuspended, centrifuge. 2) WASH: wash with medium, centrifuge at higher speed, supernatant discarded, pellet resuspended, centrifugation, mixed with medium. ART: IUI. Number of cycles per patient: 1. COH: not stated. Number IUI per cycle: 2.
Outcomes	Primary outcomes • Live birth rate not reported. • Gradient, PR/couple: 11% (2/17). Wash, PR/couple 14% (6/44).

Soliman 2005 *(Continued)*

	Secondary outcomes • OPR, MPR and MR not reported.
Notes	Abstract. Definition of pregnancy was not described.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Stated as randomised, no further details.
Allocation concealment (selection bias)	Unclear risk	Concealment of allocation was not stated.
Blinding of participants and personnel (performance bias) All outcomes	High risk	Study participants and personnel were not blinded from knowledge of which intervention a participant received.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Outcome assessors were not blinded from knowledge of which intervention a participant received. However, risk of detection bias due to knowledge of the allocated interventions by outcome assessors may be low due to outcomes which are not susceptible to bias.
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Dropouts or loss to follow-up not stated. Duration of follow-up not stated.
Selective reporting (reporting bias)	Unclear risk	Unclear risk.
Other bias	High risk	Abstract. Not enough data on methodological assessment. Definition of pregnancy was not described.

Xu 2000

Methods	Parallel design. Stated randomised, no further details. Concealment allocation, blinding, duration of follow-up, dropouts/cancelled cycles: not stated. Groups similar regarding important indicators at baseline: not stated. Intention-to-treat analysis, power calculation: not stated. Single-centre study. Samples were not equally divided.
Participants	40 couples. Age of women: not stated. Age of men: 24 to 43 yrs (for all 140 men). Duration of subfertility: not stated. All 140 men did not have children 2 to 13 yrs after marriage. Cause of subfertility: women were healthy and gynaecologically normal. Male factor subfertility, all semen samples were oligoasthenoteratospermic, no donor semen. Previous fertility treatment: not stated. Exclusion criteria: not stated. Inclusion criteria: not stated.
Interventions	Semen preparation techniques: swim-up and gradient (Percoll). Performance semen preparation techniques: as described in (WHO 92). ART: IUI. Number IUI: swim-up 1 to 3, gradient 1 to 3 (average 2.5). COH: not stated. Cancellation criteria: not stated.
Outcomes	Primary outcomes • Live birth rate not reported. • Swim-up: CPR/couple: 15% (3/20). Gradient: n = 20; CPR/couple: 20 % (4/20).

Xu 2000 *(Continued)*

	Secondary outcomes • OPR, MR and MPR not reported.
Notes	Definition of pregnancy was not described.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Stated as randomised, no further details.
Allocation concealment (selection bias)	Unclear risk	Concealment of allocation was not stated.
Blinding of participants and personnel (performance bias) All outcomes	High risk	Study participants and personnel were not blinded from knowledge of which intervention a participant received.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Outcome assessors were not blinded from knowledge of which intervention a participant received. However, risk of detection bias due to knowledge of the allocated interventions by outcome assessors may be low due to outcomes which are not susceptible to bias.
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Dropouts or loss to follow-up: not stated. Duration of follow-up: not stated.
Selective reporting (reporting bias)	Unclear risk	Unclear risk.
Other bias	High risk	A lot of important information was not reported in the article (e.g. baseline similarity of groups). Definition of pregnancy was not described.

ART: assisted reproductive technique
COH: controlled ovarian hyperstimulation
CC: clomiphene citrate
CPR: clinical pregnancy rate
hCG: human chorionic gonadotropins
MR: miscarriage rate
MPR: multiple pregnancy rate
OPR: ongoing pregnancy rate
PAF: platelet activating factor
(r)FSH: (recombinant) follicle stimulating hormone
yr(s): year(s)

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

Study	Reason for exclusion
Abed 2015	No comparison of interest: swim-up technique versus upstream method.
Almagor 1993	Not a randomised controlled trial. No comparison of interest. Type of intervention: swim-up, swim-down versus gradient.

Study	Reason for exclusion
Aribarg 1995	No comparison of interest.
Bajamonte 1994	Not a randomised controlled trial. After contact with the authors the method of randomisation appeared to be quasi-randomised. Use of assisted reproduction technique other then IUI. No comparison of interest: the swim-up technique used is modified by 20 minute sperm incubation period in hFF.
Baka 2009	No comparison of interest: to evaluate the effect of exogenous platelet-activating factor (PAF) on clinical outcome. Cross-over design.
Berteli 2017	No comparison of interest: MACS and density gradient technique. Not a randomised controlled trial. No clinical data.
Bhakta 2010	No comparison of interest: carbon dioxide versus no carbon dioxide.
Butt 2016	After contact with the authors it appeared to be a prospective observational study comparing density gradient technique and swim-up technique. The method of preparation was based on semen parameters rather than randomisation.
Byrd 1994	Participants were fertile women undergoing donor inseminations.
Caccamo 1995	Not a randomised controlled trial.
Chan 1992	No outcome of interest, no use of IUI, not a randomised controlled trial.
Cimino 1990	Retrospective design. Not a randomised controlled trial. Use of assisted reproduction technique other then IUI.
Depypere 1995	Not a randomised controlled trial. Centre A used wash procedure, centre B used gradient technique.
Fazaeli 2018	No comparison of interest: density gradient technique versus SPAS (supernatant product of adipose tissue derived mesenchymal stem cells).
Fleming 2008	No comparison of interest: density gradient technique versus an electrophoretic method. Not a randomised controlled trial. No clinical outcome.
Gentis 2012	No comparison of interest: two types of swim-up techniques.
Guerin 1989	Not a randomised controlled trial. Use of assisted reproduction technique other then IUI.
Hammadeh 2001	Not a randomised controlled trial. Use of assisted reproduction technique other then IUI.
Heidari 2016	No comparison of interest: swim up and upstream method. Not a randomised controlled trial. Split sample study. No clinical data.
Huang 1996b	Not a randomised controlled trial.
Inaudi 2002	No comparison of interest: 2 types of swim-up technique.
Jalilian 2016	Stated as randomised, however described as a matched control study. 3 methods of sperm preparation by a gradient technique, p50-p40-80 and with a swim-up variance. We did not succeed in contacting the authors to clarify the materials and methods.
Jaroudi 1993	Use of assisted reproduction technique other then IUI.

Study	Reason for exclusion
Karlström 1991	No comparison of interest: two types of swim-up techniques.
Kücük 2008	No comparison of interest: gradient technique with or without heat induced hypermotility.
Leonetti 1995	Not a randomised controlled trial. Use of assisted reproduction technique other then IUI.
Levay 1995	No IUI, no randomised controlled trial.
Mathieu 1988	Use of assisted reproduction technique other then IUI. No comparison of interest (the abstract reports Percoll is more efficient than swim-up, however in the article Percoll was used and no control group). Not a randomised controlled trial.
Menge 1992	This trial, conference abstract, compares two different types of medium. In one group they use either swim-up or gradient technique. After contact with the authors this appeared not to be randomised and they could not provide the separate data in this group.
Monqaut 2011	No comparison of interest: use of high-magnification microscopy for sperm assessment.
Morshedi 2003	Quasi-randomised controlled study (according to the day of the month). The study design is crossover. We found a conference abstract and article describing the same trial.
Oguz 2018	No clinical outcome, only semen parameters. We contacted the authors, they could not provide data on clinical outcomes in relation to sperm preparation technique.
Ohashi 1992	Not a randomised controlled trial. The different semen preparation techniques were used alternately, all in the same sequence.
Ord 1990	Not a randomised controlled trial. Use of assisted reproduction technique other then IUI.
Ozturk 2008	No comparison of interest: gradient technique with one versus two washes.
Paul 2004	No comparison of interest: four different gradient techniques.
Ragni 1998	No comparison of interest: two types of swim-up techniques.
Remohi 1989	This study was primary about IUI and GIFT (gamete intrafallopian tube transfer), but they reported they examined no significant difference in pregnancy rates between gradient and swim-up. We contacted the authors, but they were not able to provide the data. The study also had an unclear study design.
Ren 2004	Not a randomised controlled trial.
Ricci 2009	Not a randomised controlled trial. No clinical outcome, only semen parameters.
Romany 2017	No comparison of interest: swim-up technique with or without magnetic activated sorting selection (MACS).
Roth 2018	No comparison of interest: swim-up versus swim-down technique. Not a randomised controlled trial.
Sapienza 1993	Use of assisted reproduction technique other then IUI.
Siam 2012	No comparison of interest: 2 types of swim-up technique.
Su 1993	Not a randomised controlled trial, no comparison of interest: 2 types of wash techniques.

Study	Reason for exclusion
Tanphaichitr 1988	Not a randomised controlled trial. The two preparation techniques were used alternately among the participants. Use of assisted reproduction technique other then IUI.
Tomari 2017	Quasi-randomised controlled study. No comparison of interest: two types of density gradient techniques. Use of assisted reproduction technique other then IUI: IVF/ICSI.
Tsai 2004	No comparison of interest: 2 types of density gradient techniques.
Urry 1988	Not a randomised controlled trial (use of a randomised protocol, but allocation to a protocol is not stated to be randomised). Cross-over design. We did not succeed in contacting the authors.
Van Der Zwalmen 1991	Use of assisted reproduction technique other then IUI. Unclear study design.
Werlin 1992	Abstract. Excluded after contact with the authors: quasi-randomised design. The authors gave no further details. Parallel group study design.
Zarmakoupis-Zavos 1998	No comparison of interest: wash technique versus filtration technique.
Zavos 1992	No comparison of interest wash technique versus sperm prep filtration.
Zech 1993	Not a randomised controlled trial. Use of assisted reproduction technique other then IUI.

.

DATA AND ANALYSES

Comparison 1. Swim-up versus gradient technique, fresh semen

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Clinical pregnancy rate per couple	4	370	Odds Ratio (M-H, Fixed, 95% CI)	0.83 [0.51, 1.35]
2 Ongoing pregnancy rate	1	223	Odds Ratio (M-H, Fixed, 95% CI)	0.39 [0.19, 0.82]
3 Multiple pregnancy rate per couple	1	25	Odds Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
4 Miscarriage rate per couple	3	330	Odds Ratio (M-H, Fixed, 95% CI)	0.85 [0.28, 2.59]

Analysis 1.1. Comparison 1 Swim-up versus gradient technique, fresh semen, Outcome 1 Clinical pregnancy rate per couple.

Study or subgroup	Swim-up n/N	Gradient n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
Dodson 1998	2/10	6/15		10.95%	0.38[0.06,2.41]
Karamahmutoglu 2014	17/112	29/111		70.45%	0.51[0.26,0.99]
Posada 2005	20/52	4/30		8.9%	4.06[1.23,13.38]
Xu 2000	3/20	4/20		9.69%	0.71[0.14,3.66]
Total (95% CI)	**194**	**176**		**100%**	**0.83[0.51,1.35]**
Total events: 42 (Swim-up), 43 (Gradient)					
Heterogeneity: Tau^2=0; Chi^2=9.66, df=3(P=0.02); I^2=68.95%					
Test for overall effect: Z=0.76(P=0.45)					

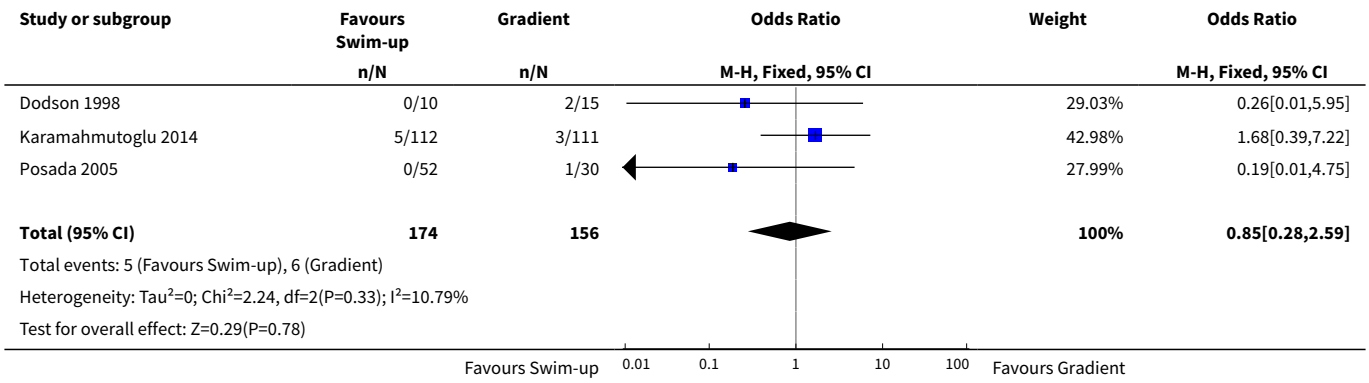
Analysis 1.2. Comparison 1 Swim-up versus gradient technique, fresh semen, Outcome 2 Ongoing pregnancy rate.

Study or subgroup	Favours Gradient n/N	Gradient n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
Karamahmutoglu 2014	12/112	26/111		100%	0.39[0.19,0.82]
Total (95% CI)	**112**	**111**		**100%**	**0.39[0.19,0.82]**
Total events: 12 (Favours Gradient), 26 (Gradient)					
Heterogeneity: Not applicable					
Test for overall effect: Z=2.47(P=0.01)					

Analysis 1.3. Comparison 1 Swim-up versus gradient technique, fresh semen, Outcome 3 Multiple pregnancy rate per couple.

Study or subgroup	Favours Swim-up n/N	Gradient n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
Dodson 1998	0/10	0/15			Not estimable
Total (95% CI)	**10**	**15**			**Not estimable**
Total events: 0 (Favours Swim-up), 0 (Gradient)					
Heterogeneity: Not applicable					
Test for overall effect: Not applicable					

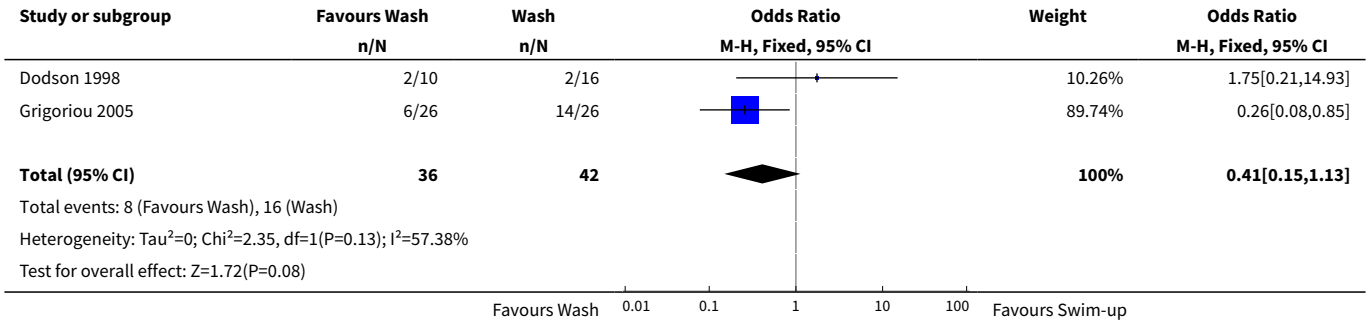
Analysis 1.4. Comparison 1 Swim-up versus gradient technique, fresh semen, Outcome 4 Miscarriage rate per couple.



Comparison 2. Swim-up versus wash and centrifugation, fresh semen

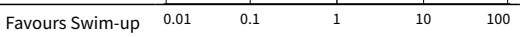
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Clinical pregnancy rate per couple	2	78	Odds Ratio (M-H, Fixed, 95% CI)	0.41 [0.15, 1.13]
2 Multiple pregnancy rate per couple	1	26	Odds Ratio (M-H, Fixed, 95% CI)	0.49 [0.02, 13.28]
3 Miscarriage rate per couple	1	20	Odds Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]

Analysis 2.1. Comparison 2 Swim-up versus wash and centrifugation, fresh semen, Outcome 1 Clinical pregnancy rate per couple.



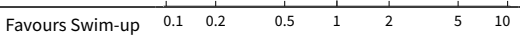
Analysis 2.2. Comparison 2 Swim-up versus wash and centrifugation, fresh semen, Outcome 2 Multiple pregnancy rate per couple.

Study or subgroup	Favours Swim-up n/N	Wash n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
Dodson 1998	0/10	1/16		100%	0.49[0.02,13.28]
Total (95% CI)	**10**	**16**		**100%**	**0.49[0.02,13.28]**
Total events: 0 (Favours Swim-up), 1 (Wash)					
Heterogeneity: Tau^2=0; Chi^2=0, df=0(P<0.0001); I^2=100%					
Test for overall effect: Z=0.42(P=0.67)					



Analysis 2.3. Comparison 2 Swim-up versus wash and centrifugation, fresh semen, Outcome 3 Miscarriage rate per couple.

Study or subgroup	Favours Swim-up n/N	Wash n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
Dodson 1998	0/10	0/10			Not estimable
Total (95% CI)	**10**	**10**			**Not estimable**
Total events: 0 (Favours Swim-up), 0 (Wash)					
Heterogeneity: Not applicable					
Test for overall effect: Not applicable					

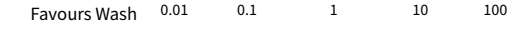


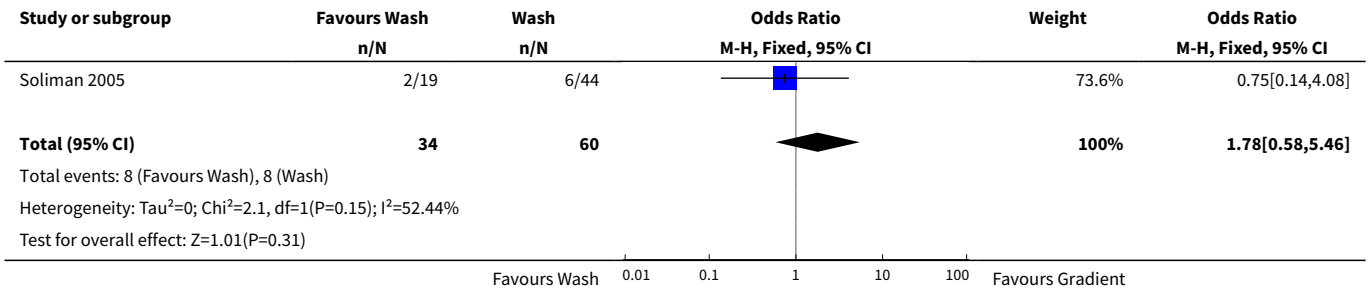
Comparison 3. Gradient technique versus wash and centrifugation, fresh semen

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Clinical pregnancy rate per couple	2	94	Odds Ratio (M-H, Fixed, 95% CI)	1.78 [0.58, 5.46]
2 Multiple pregnancy rate per couple	1	31	Odds Ratio (M-H, Fixed, 95% CI)	0.33 [0.01, 8.83]
3 Miscarriage rate per couple	1	31	Odds Ratio (M-H, Fixed, 95% CI)	6.11 [0.27, 138.45]

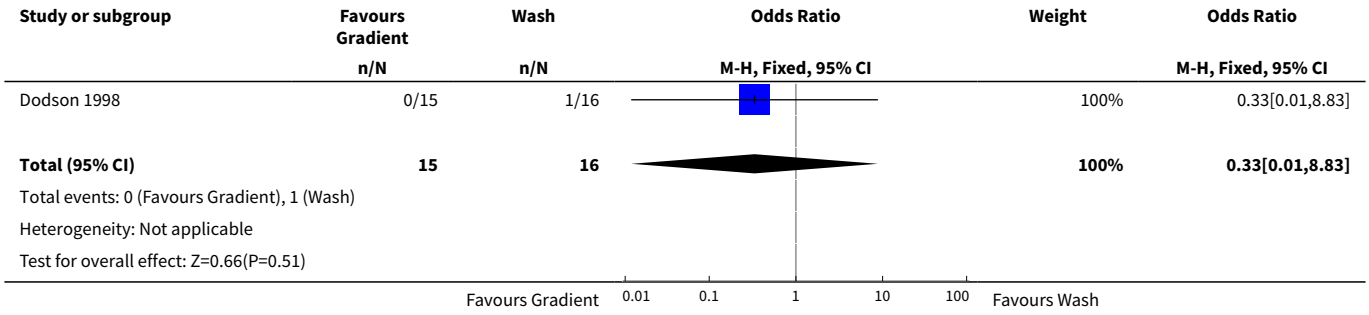
Analysis 3.1. Comparison 3 Gradient technique versus wash and centrifugation, fresh semen, Outcome 1 Clinical pregnancy rate per couple.

Study or subgroup	Favours Wash n/N	Wash n/N	Odds Ratio M-H, Fixed, 95% CI	Weight	Odds Ratio M-H, Fixed, 95% CI
Dodson 1998	6/15	2/16		26.4%	4.67[0.77,28.41]

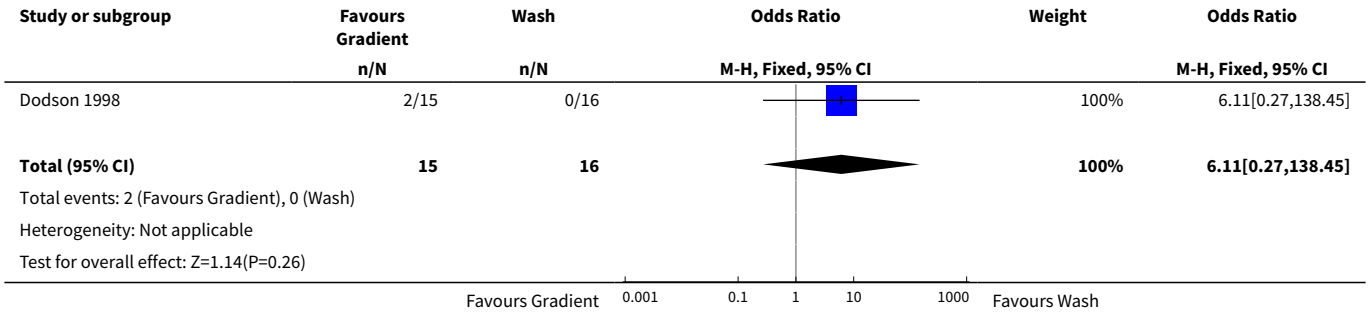




Analysis 3.2. Comparison 3 Gradient technique versus wash and centrifugation, fresh semen, Outcome 2 Multiple pregnancy rate per couple.



Analysis 3.3. Comparison 3 Gradient technique versus wash and centrifugation, fresh semen, Outcome 3 Miscarriage rate per couple.



ADDITIONAL TABLES

Table 1. Characteristics of cross-over RCTs excluded from meta-analysis

Study ID	Allocation Score	Methods	Participants	Interventions	Outcomes
Carrell 1998	B	Stated random, but no details. Design: cross-over, multi-cen-	363 women: 558 cycles in the 3 methods of interest. Age of women, dura-	3 preparation techniques (out of 5 described). 1) Sperm wash: 8 to 10 ml. medium (Ham's F-10), 10 min. 400 × g centrifugation. Su-	Clinical pregnancy rate (CPR)/cycle, Miscarriage

Table 1. Characteristics of cross-over RCTs excluded from meta-analysis *(Continued)*

	tre. Concealment of allocation, blinding, number of dropouts or cancelled cycles, intention-to-treat analysis, power calculation: all not stated.	tion subfertility: not stated. Cause: unexplained/(fe)male related disorders. Exclusion criteria: oligoasthenozoospermic semen samples after preparation. Inclusion criteria: not stated.	pernatant decanted, pellet resuspended. 2) Swim-up: 2× washed, resuspended. Medium layered on top. Incubation 1h. Top removed. 3) Gradient: 1x wash + resuspension. Percoll, (35%/ 90%).15 min 400 × g centrifugation. 90% layer washed. Single IUI. 2.5 ± 0.3 inseminations per women. 124 women: 50 to 200 mg. CC day 5 to 9 or no COH. 239 women: gonadotropin/hCG.		rate (MR)/ pregnancy, Live birth rate (LBR)/ cycle

Table 2. Results from cross-over RCTs excluded from meta-analysis

Study ID	Sample Size	Gradient technique	Swim-up	Wash and centrifuge	Conclusion	Statistical analysis
Carrell 1998	558 cycles	CPR/cycle: 16% (33/204), LBR/ cycle: 13% (26/204), MR/pregnancy: 21% (7/33)	CPR/cycle: 15% (29/197), LBR/cycle: 13% (26/197), MR/ pregnancy: 10 % (3/29)	CPR/cycle: 9% (14/157), LBR/cycle: 7% (11/157), MR/ pregnancy: 21% (3/14)	CPR/cycle wash-method significantly lower than Swim-up/Percoll ($P < 0.05$), LBR/cycle wash-method significantly lower than Swim-up/Percoll ($P < 0.05$). No other significant differences.	CPR/cycle and MR/pregnancy: ×2 analysis and Fisher's exact test. Statistical significance $P < 0.05$.

APPENDICES

Appendix 1. Cochrane Gynaecology and Fertility Group (CGFG) search string

ProCite platform

Searched 12 March 2019

Keywords CONTAINS "intrauterine" or "Intrauterine Insemination" or "IUI" or "artificial insemination" or "insemination" or "insemination, intrauterine" or "insemination-utero tubal" or Title CONTAINS "intrauterine" or "Intrauterine Insemination" or "IUI" or "artificial insemination" or "insemination" or "insemination, intrauterine" or "insemination-utero tubal"

AND

Keywords CONTAINS "sperm gradient separation protocols" or "sperm extraction techniques" or "sperm preparation" or "sperm preparation techniques" or "sperm selection techniques" or "sperm separation" or "sperm stimulation" or "sperm-swim up" or "semen preparation" or "percoll gradients" or "isolate" or "washed sperm" or "centrifugation" or "centrifuge" or "mini percoll" or Title CONTAINS "sperm gradient separation protocols" or "sperm extraction techniques" or "sperm preparation" or "sperm preparation techniques" or "sperm selection techniques" or "sperm separation" or "sperm stimulation" or "sperm-swim up" or "semen preparation" or "percoll gradients" or "isolate" or "washed sperm" or "centrifugation" or "centrifuge" or "mini percoll"

(108 records)

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

Web platform

Searched 12 March 2019

#1 MESH DESCRIPTOR Insemination, Artificial EXPLODE ALL TREES 357

#2 iui:TI,AB,KY 557
#3 insemination*:TI,AB,KY 1210
#4 #1 OR #2 OR #31 291
#5 MESH DESCRIPTOR Centrifugation, Density Gradient EXPLODE ALL TREES 41
#6 (sperm* adj2 prepar*):TI,AB,KY 90
#7 (semen adj2 prepar*):TI,AB,KY 28
#8 (sperm* adj2 separat*):TI,AB,KY 22
#9 gradient*:TI,AB,KY 2908
#10 (swim up):TI,AB,KY 96
#11 (swim down):TI,AB,KY 5
#12 wash:TI,AB,KY 4157
#13 centrifug*:TI,AB,KY 858
#14 percoll:TI,AB,KY 84
#15 (semen adj2 separat*):TI,AB,KY 1
#16 (semen adj2 treatment*):TI,AB,KY 31
#17 (sperm* adj2 treatment*):TI,AB,KY 258
#18 isolate*:TI,AB,KY 11422
#19 spermprep*:TI,AB,KY 12
#20 #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 19353
#21 #4 AND #20 126

Appendix 3. MEDLINE search strategy

OVID platform

Searched from 1946 to 12 March 2019

1 exp insemination, artificial/ or exp insemination, artificial, heterologous/ or exp insemination, artificial, homologous/ (11349)
2 insemination.tw. (14902)
3 iui.tw. (1626)
4 AI.tw. (22622)
5 assisted reproducti*.tw. (13486)
6 or/1-5 (52014)
7 exp Centrifugation, Density Gradient/ (35553)
8 (sperm$ adj5 prepar$).tw. (1971)
9 (semen adj5 prepar$).tw. (447)
10 (sperm$ adj3 separation$).tw. (381)
11 gradient.tw. (162844)
12 swim up.tw. (1130)
13 swim down.tw. (19)
14 (wash or washing or washed).tw. (64123)
15 centifug$.tw. (15)
16 centrifug$.tw. (58338)
17 percoll.tw. (5703)
18 (semen adj3 separation$).tw. (21)
19 (semen adj5 treatment$).tw. (781)
20 (sperm$ adj5 treatment$).tw. (4644)
21 (isolate$ or isolation).tw. (1247577)
22 spermprep$.tw. (25)
23 (MiniPercoll$ or SpermPrep$).tw. (27)
24 or/7-23 (1508562)
25 randomized controlled trial.pt. (477274)
26 controlled clinical trial.pt. (92948)
27 randomized.ab. (436415)
28 placebo.tw. (201223)
29 clinical trials as topic.sh. (186186)
30 randomly.ab. (306719)
31 trial.ti. (195181)
32 (crossover or cross-over or cross over).tw. (79428)
33 or/25-32 (1230277)
34 (animals not (humans and animals)).sh. (4521762)
35 33 not 34 (1130365)
36 6 and 24 and 35 (235)

Appendix 4. Embase search strategy

OVID platform

Searched from 1980 to 12 March 2019

1 exp artificial insemination/ (16251)
2 insemination.tw. (15979)
3 AI.tw. (29900)
4 iui.tw. (3003)
5 assisted reproducti*.tw. (20631)
6 or/1-5 (68195)
7 exp density gradient centrifugation/ or exp centrifugation/ (44894)
8 (sperm$ adj5 prepar$).tw. (2292)
9 (semen adj5 prepar$).tw. (612)
10 (sperm$ adj3 separation$).tw. (452)
11 gradient.tw. (180054)
12 swim up.tw. (1485)
13 swim down.tw. (27)
14 (wash or washing or washed).tw. (78652)
15 centifug$.tw. (30)
16 centrifug$.tw. (65775)
17 percoll.tw. (6312)
18 (semen adj3 separation$).tw. (28)
19 (semen adj5 treatment$).tw. (1016)
20 (sperm$ adj5 treatment$).tw. (5591)
21 (isolate$ or isolation).tw. (1383093)
22 spermprep$.tw. (37)
23 MiniPercoll$.tw. (4)
24 or/7-23 (1671303)
25 6 and 24 (6111)
26 Clinical Trial/ (941365)
27 Randomized Controlled Trial/ (531339)
28 exp randomization/ (81256)
29 Single Blind Procedure/ (33837)
30 Double Blind Procedure/ (154640)
31 Crossover Procedure/ (58050)
32 Placebo/ (315856)
33 Randomi?ed controlled trial$.tw. (195432)
34 Rct.tw. (31056)
35 random allocation.tw. (1837)
36 randomly.tw. (397170)
37 randomly allocated.tw. (31562)
38 allocated randomly.tw. (2395)
39 (allocated adj2 random).tw. (797)
40 Single blind$.tw. (22042)
41 Double blind$.tw. (187579)
42 ((treble or triple) adj blind$).tw. (899)
43 placebo$.tw. (278389)
44 prospective study/ (499260)
45 or/26-44 (2197281)
46 case study/ (59199)
47 case report.tw. (362638)
48 abstract report/ or letter/ (1037233)
49 or/46-48 (1449907)
50 45 not 49 (2147262)
51 (exp animal/ or animal.hw. or nonhuman/) not (exp human/ or human cell/ or (human or humans).ti.) (5631078)
52 50 not 51 (1997756)
53 25 and 52 (527)

Appendix 5. PsycINFO search strategy

OVID platform

Searched from 1806 to 12 March 2019

1 exp Reproductive Technology/ (1729)
2 artificial insemination.tw. (253)
3 intrauterine insemination.tw. (25)
4 iui.tw. (35)
5 intra uterine insemination.tw. (2)
6 or/1-5 (1886)
7 (sperm$ adj2 prepar$).tw. (7)
8 (semen adj2 prepar$).tw. (1)
9 (sperm$ adj2 separation$).tw. (3)
10 gradient.tw. (7691)
11 (swim up or swim down).tw. (8)
12 centrifug$.tw. (1141)
13 percoll.tw. (14)
14 (semen adj2 treatment$).tw. (4)
15 (sperm adj2 treatment$).tw. (11)
16 isolate$.tw. (35076)
17 (wash or washing or washed).tw. (2511)
18 or/7-17 (46145)
19 6 and 18 (18)

Appendix 6. ClinicalTrials.gov

Web platform

Searched 12 March 2019

search terms: (intrauterine OR Intrauterine Insemination OR IUI OR artificial insemination OR insemination) AND (sperm gradient separation protocols OR sperm extraction techniques OR sperm preparation OR sperm-swim up OR sperm wash OR gradient technique)

study type: interventional studies

Appendix 7. Data extraction form

Type of participants

- Age of women and men and other demographic information
- Cause and duration of subfertility
- Previous fertility treatment
- Condition of semen
- Fresh or cryopreserved semen
- Semen quality: normal, subnormal, mixed (according to WHO 1992)

Types of interventions

- What assisted reproductive technique was used? IUI or other
- In combination with controlled ovarian hyperstimulation (COH)
- Which semen preparation technique was used? Swim-up, density gradient, wash and centrifugation
- Number of cycles per woman

Types of outcome measures

- Clinical pregnancy rate per couple or woman
- Live birth rate per couple or woman
- Additional outcomes

WHAT'S NEW

Date	Event	Description
21 November 2019	Amended	Amended text in author's conclusions, plain language summary and discussion.

HISTORY

Protocol first published: Issue 4, 2003
Review first published: Issue 3, 2004

Date	Event	Description
12 March 2019	New search has been performed	We updated the search. We identified 1 new study to be included in the review (Karamahmutoglu 2014). Converted to new review format.
12 March 2019	New citation required but conclusions have not changed	The addition of 1 new study did not lead to changes in conclusions.
5 August 2011	New search has been performed	Converted to new review format. Updated search. No new studies were identified.
2 July 2007	New citation required and conclusions have changed	Substantive amendment

CONTRIBUTIONS OF AUTHORS

CM Boomsma prepared the manuscript. CM Boomsma and BJ Cohlen performed the selection of studies for inclusion. CM Boomsma and C Farquhar assessed the quality of the evidence using GRADE criteria. All authors were involved in concept and study design.

DECLARATIONS OF INTEREST

CM Boomsma: none known.

BJ Cohlen: none known.

C Farquhar: none known.

SOURCES OF SUPPORT

Internal sources

- University of Auckland, New Zealand.

External sources

- Marco Polo Fonds, Netherlands.
- Groninger Universiteits Fonds, Netherlands.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

After identifying the studies eligible for inclusion in the meta-analysis, we made some changes to the protocol for this review. Initially we intended to include studies investigating clinical outcomes after IUI, IVF or GIFT. We decided to limit the review to IUI due to a large difference in the amount and quality of sperm needed for IUI compared to IVF and GIFT.

It is the intention of the review authors that a new search for RCTs will be performed every five years and we will update the review accordingly.

NOTES

None

INDEX TERMS

Medical Subject Headings (MeSH)

*Sperm Motility; Centrifugation, Density Gradient; Insemination, Artificial [*methods]; Randomized Controlled Trials as Topic; Semen; Specimen Handling [methods]; Sperm Count; Spermatozoa [*physiology]

MeSH check words

Humans; Male