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## Semen preparation techniques for intrauterine insemination

Boomsma, Carolien M.; Heineman, M. J.; Cohlen, B. J.; Farquhar, C.

*Published in:*  
Cochrane Database of Systematic Reviews

*DOI:*  
[10.1002/14651858.CD004507.pub3](https://doi.org/10.1002/14651858.CD004507.pub3)

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2007

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Boomsma, C. M., Heineman, M. J., Cohlen, B. J., & Farquhar, C. (2007). Semen preparation techniques for intrauterine insemination. *Cochrane Database of Systematic Reviews*, (4), [004507].  
<https://doi.org/10.1002/14651858.CD004507.pub3>

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## Semen preparation techniques for intrauterine insemination (Review)

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Semen preparation techniques for intrauterine insemination.  
*Cochrane Database of Systematic Reviews* 2007, Issue 4. Art. No.: CD004507.  
DOI: 10.1002/14651858.CD004507.pub3.

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[Intervention Review]

# Semen preparation techniques for intrauterine insemination

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**Editorial group:** Cochrane Gynaecology and Fertility Group.

**Publication status and date:** New search for studies and content updated (no change to conclusions), published in Issue 10, 2011.

**Citation:** Boomsma CM, Heineman MJ, Cohlen BJ, Farquhar C. Semen preparation techniques for intrauterine insemination. *Cochrane Database of Systematic Reviews* 2007, Issue 4. Art. No.: CD004507. DOI: 10.1002/14651858.CD004507.pub3.

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## ABSTRACT

### Background

Semen preparation techniques for assisted reproduction, including intrauterine insemination (IUI), were developed to separate the motile morphologically normal spermatozoa. Leucocytes, bacteria and dead spermatozoa produce oxygen radicals that negatively influence the ability to fertilize the egg. The yield of many motile, morphologically normal spermatozoa might influence treatment choices and therefore outcomes.

### Objectives

To compare the effectiveness of gradient, swim-up, or wash and centrifugation semen preparation techniques on clinical outcomes in subfertile couples undergoing intrauterine insemination (IUI).

### Search methods

We searched the Menstrual Disorders and Subfertility Group Trials Register (August 2011), MEDLINE (1966 to August 2011), EMBASE (1980 to August 2011), Science Direct Database (1966 to August 2011), Cochrane Central Register of Controlled Trials (CENTRAL) (*The Cochrane Library* 2011, Issue 7), National Research Register (2000 to 2011), Biological Abstracts (2000 to August 2011), CINAHL (1982 to August 2011) and reference lists of relevant articles. We also contacted experts and authors in the field.

### Selection criteria

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

### Data collection and analysis

Two review authors independently assessed trial quality and extracted data. Study authors were contacted for additional information.

### Main results

Five RCTs, including 262 couples in total, were included in the meta-analysis (Dodson 1998; Grigoriou 2005; Posada 2005; Soliman 2005; Xu 2000). Xu 2000 compared all three techniques. Soliman 2005 compared a gradient versus a wash technique. Dodson 1998 and Posada 2005 compared a gradient technique versus a swim-up technique, whereas Grigoriou 2005 compared swim-up versus a wash technique. No trials reported the primary outcome of live birth.

There was no evidence of a difference between pregnancy rates (PR) for swim-up versus a gradient technique (PR 30.5% versus 21.5% respectively; Peto odds ratio (OR) 1.57, 95% CI 0.74 to 3.32). A swim-up technique versus wash and centrifugation also showed no significant difference in PR (PR 22.2% versus 38.1% respectively; Peto OR 0.41, 95% CI 0.15 to 1.10). Two studies compared a gradient versus wash centrifugation technique (PR 23.5% versus 13.3%; Peto OR 1.76, 95% CI 0.57 to 5.44). There was no evidence of a difference in the miscarriage rate (MR) in two studies comparing a swim-up versus gradient technique (MR 0% versus 6.7%; Peto OR 0.13, 95% CI 0.01 to 1.33).

### **Authors' conclusions**

There is insufficient evidence to recommend any specific semen preparation technique. Large, high quality randomised controlled trials comparing the effectiveness of a gradient, swim-up and wash and centrifugation technique on clinical outcomes are lacking. Further randomised trials are warranted.

## **PLAIN LANGUAGE SUMMARY**

### **Semen preparation techniques for intrauterine insemination**

The effectiveness of specific semen preparation techniques for increasing pregnancy rates in subfertile couples undergoing intrauterine insemination (IUI) is unknown.

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. It is known that white blood cells, bacteria and dead sperm in semen can impair fertilization of the egg. This review found that there is insufficient evidence to recommend any specific semen preparation technique for subfertile couples undergoing intrauterine insemination (a procedure which places sperm directly into the uterus) as there were no differences in pregnancy rates using the different techniques. More research is needed.

## SUMMARY OF FINDINGS FOR THE MAIN COMPARISON *[Explanation]*

Swim-up technique compared to gradient technique for undergoing intrauterine insemination						
<b>Patient or population:</b> patients undergoing intrauterine insemination (fresh semen)						
<b>Intervention:</b> swim-up technique						
<b>Comparison:</b> 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				
Pregnancy rate per couple	215 per 1000	301 per 1000 (169 to 477)	OR 1.57 (0.74 to 3.32)	147 (3 studies)	⊕○○○ very low <sup>1,2</sup>	
Miscarriage rate per couple	67 per 1000	9 per 1000 (1 to 87)	OR 0.13 (0.01 to 1.33)	107 (2 studies)	⊕⊕⊕○ moderate <sup>3</sup>	
Multiple pregnancy rate per couple	See comment	See comment	Not estimable	25 (1 study)	⊕⊕⊕○ moderate <sup>4</sup>	There were no events recorded 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% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; 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.

<sup>1</sup> Two of the trials did not use blinding, nor was there adequate explanation for randomisation or allocation concealment or attrition in these two studies.

<sup>2</sup> I square statistic was 64% indicating significant heterogeneity

<sup>3</sup> One of the trials failed to provide adequate details on randomisation, allocation concealment, and attrition. There was no evidence of blinding.

<sup>4</sup> Evidence based on a single trial

## 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 coital exposures (sexual intercourse) (Evers 2002). Involuntary 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 (Delhanty 2001) and there is a raised chance of exposure to sexually transmitted diseases and continually falling sperm counts (Swan 1999).

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). 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 intrauterine insemination (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 (Yan 1998; Wang 1991).

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). 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 di-

rectly 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

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. However, although spermatozoa recovery rates are interesting when you compare different semen preparation techniques, clinicians and prospective parents regard live birth rate as the most important outcome.

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). 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.

### How the intervention might work

One type of semen preparation technique might be superior to another in relation to clinical outcome after IUI. 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. In the past, clinical studies have concentrated on the use of Percoll, but research demonstrated that the new products appear to be as effective as Percoll for the recovery of good, progressively motile sperm (Centola 1998).



## 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. However, there is 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

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, pregnancy rate) in subfertile couples undergoing IUI.

## METHODS

### Criteria for considering studies for this review

#### Types of studies

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

#### Types of participants

Subfertility was defined as couples who have tried unsuccessfully to conceive for at least one year, despite regular and unprotected coital exposures (Evers 2002). A variety of causes for subfertility were included, such as unexplained subfertility, male subfertility (as defined by WHO 1992), mild endometriosis, cervical factor and ovulatory dysfunction. Unexplained subfertility was defined 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, postcoital testing, immunological testing and investigations into uterine anomalies). We did not include fertile participants or healthy volunteers.

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

#### Types of interventions

Any included study must have made a comparison of:

- a swim-up technique, and/or
- a gradient technique, and/or
- wash and centrifugation.

Subfertile couples undergoing intrauterine insemination (IUI) were included. Subfertile couples undergoing other assisted reproduction techniques were excluded because of the likelihood of a large difference in the number of motile sperm needed for IUI compared to in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI) or gamete intrafallopian transfer (GIFT), for example.

#### Types of outcome measures

##### Primary outcomes

Live birth rate (LBR) per couple

Pregnancy rate (PR) per couple (established by pregnancy test) or clinical pregnancy rate (CPR) when confirmed by a fetal heart activity detectable on an ultrasound scan

##### Secondary outcomes

Ongoing pregnancy rate per couple (over 12 weeks of gestation)

Multiple pregnancy rate (MPR) per couple or pregnancy, twins, triplets or higher order, specified if possible (confirmed by ultrasound or delivery)

Miscarriage rate (MR) per couple or intrauterine pregnancy, confirmed by ultrasound and pregnancy test or by histology

Ectopic pregnancy rate per couple or pregnancy (confirmed by histology)

Fetal abnormalities per couple or pregnancy

Infections per couple

#### Search methods for identification of studies

All randomised controlled trials comparing clinical outcomes after a gradient technique, swim-up technique or wash and centrifuge were obtained using the following search strategy.

#### Electronic searches

The Menstrual Disorders and Subfertility Group Trials Register was searched for any relevant trials (August 2011). This register is based on regular searches of MEDLINE, EMBASE, CINAHL, PsycINFO and CENTRAL, the handsearching of 20 relevant journals and conference proceedings and searches of several key grey

literature sources. European Society of Human Reproduction and Embryology (ESHRE) and American Society for Reproductive Medicine (ASRM) abstract books were also handsearched. The register was searched for any trials on semen preparation techniques. A full description is given in the Group's module on *The Cochrane Library*.

The following electronic databases were searched.

Cochrane Central Register of Controlled Trials (CENTRAL) (*The Cochrane Library* 2011, Issue 7);

MEDLINE (1966 to August 2011);

EMBASE (1980 to August 2011);

Science Direct Database (1966 to August 2011);

National Research Register (a register of ongoing and recently completed research projects funded by the United Kingdom's National Health Service); as well as entries from the Medical Research Council's Clinical Trials Register (2000 to 2011);

Biological Abstracts: journal articles (1990 to August 2011); reports, reviews and meetings (2000 to August 2011);

CINAHL (1982 to August 2011).

The databases were searched using different search strategies as provided in [Appendix 1](#), [Appendix 2](#), [Appendix 3](#), [Appendix 4](#) and [Appendix 5](#).

### Searching other resources

The citation lists of relevant publications, review articles, abstracts of scientific meetings and included studies were searched.

Personal communication to experts and authors in the field.

There were no language restrictions on any of the searches.

### Data collection and analysis

Information on study characteristics, data collection and methodological quality of all selected studies was assessed independently by two review authors (CM Boomsma and MJ Heineman). Discrepancies were resolved through discussion or, if required, in consultation with the third author. Full consensus was reached. If necessary, additional information on trial methodology and original trial data were sought from the authors of trials which appeared to meet the eligibility criteria. The following data were extracted from the included studies and presented in the table 'Characteristics of included studies'.

#### 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

#### Assessment of risk of bias in included studies

Two authors independently assessed risk of bias for each study using the criteria outlined in the *Cochrane Handbook for Systematic Reviews of Interventions*. Results were summarised in the risk of bias tables for all included studies.

Eligibility and risk of bias was assessed on the following criteria:

#### Random sequence generation

Describes the method used to generate the allocation sequence. Biased allocation to interventions would result in a selection bias.

Method of randomisation:

Truly randomised (e.g. by computer, or random number tables, or drawing lots).

Quasi-randomised (e.g. by hospital number or date of birth), these studies are not included in the meta-analysis.

Not clear (e.g. stated but not further described).

#### Allocation concealment

Describes the method used to conceal the allocation sequence. Biased allocation to interventions due to inadequate concealment of allocations prior to assignment would also result in a selection and performance bias.

- Good quality of concealment of allocation

Yes, (e.g. sealed in opaque envelopes, computerized allocation in a non-participating centre).

Unclear (not stated).

No, (e.g. open list of random numbers, open envelopes, tables).

#### Blinding of participants and personnel

Bias due to knowledge of the allocated interventions by participants and personnel during the study can result in a performance or detection bias.

### Blinding of outcome assessment

Bias due to knowledge of the allocated interventions by outcome assessors can result in detection bias. However, the primary outcome (ongoing) pregnancy rate was not susceptible to this kind of bias.

### Incomplete outcome data

Attrition bias is a kind of selection bias caused by attrition (loss of participants), it included drop-outs, protocol deviators, withdrawals. This type of study is not susceptible to protocol deviators or withdrawals. However, the drop out rates (<10%?) and reasons, and selective loss to follow up were assessed for each study. In addition, the number and reason of cancelled cycles was assessed (<10%?). Was an intention to treat analysis performed? What was the duration of follow-up?

### Selective reporting

Within-study selective reporting bias applies to the failure to report outcomes within studies. This bias was assessed by considering whether individual studies reported all relevant and expected outcomes.

Publication bias is a form of reporting bias referring to the review as a whole rather than individual studies. It refers to the phenomenon by which trials with positive results are more likely to be published (and thus identified) than trials with negative results (Begg 1989). A way to detect such a bias is the construction of a funnel plot, plotting sample size versus effects size. In the absence of bias the graph is symmetrical. The number of trials needed to construct a plot is arbitrarily minimal 4 studies.

### Other sources of bias

Other sources of potential bias were assessed

### Measures of treatment effect

For dichotomous data, results for each included study were expressed as an odds ratio (OR) with 95% confidence interval (CI) and combined for meta-analysis with RevMan software using the Peto odds ratio (OR).

### Unit of analysis issues

Results from included studies that were excluded from the meta-analysis due to a cross-over design are described in additional tables (Table 1, Table 2).

### Dealing with missing data

For included studies, we have noted levels of attrition in the 'Characteristics of included studies' tables.

### Assessment of heterogeneity

Heterogeneity between the results of different studies was examined 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<sup>2</sup> tests. 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.

### Data synthesis

The pregnancy and live birth rates were considered positive consequences of treatment. Therefore a higher proportion achieving these outcomes was considered a benefit. The outcomes of adverse effects (multiple pregnancy, miscarriage, ectopic pregnancy, fetal abnormalities and infections) are negative consequences of treatment and therefore higher numbers were considered to be detrimental. This needs to be taken into consideration when viewing the summary graphs. In order to make these results easier to interpret and compare with the other trials, we calculated several results from the data in each trial. We calculated standard deviations (SDs) where standard errors (SEMs) were presented. If results in a trial were presented in a graph, we extracted data from the graphs. When data were presented in different subgroups (not of interest) we calculated a pooled mean and a pooled SD for the entire group. When we performed these calculations we reported the method in the notes section of the additional tables.

### 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, it was planned to perform a sensitivity analysis to look at the possible contribution of differences in methodological quality of the trials, by excluding those studies with poor allocation concealment or high levels of missing data. It was planned to perform these analyses if there were more than five trials in each group.

## RESULTS

### Description of studies

The included and excluded studies are described in the tables of included and excluded studies.

## Results of the search

Thirty-seven studies were identified as providing data comparing the effectiveness of two or three of the different semen preparation techniques (gradient, swim-up, wash and centrifugation) on pregnancy rates or live birth rates in subfertile couples undergoing IUI.

## Included studies

Five randomised controlled trials were included in the meta-analysis (Dodson 1998; Grigoriou 2005; Posada 2005; Soliman 2005; Xu 2000), including 262 participants in total. These studies were reviewed in detail, see table 'Characteristics of included studies'. One of the included studies was included 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, therefore this study was included in the review but excluded from the meta-analysis. The characteristics and results of these cross-over trials are summarized in Table 1 and Table 2.

## Outcomes

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

## 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). The semen preparation techniques were performed as described in WHO 1992. Grigoriou 2005 compared a wash technique (with exogenous platelet-activating factor) versus a swim-up technique. Dodson 1998 compared the efficacy of wash and centrifugation versus multiple tube swim-up versus a gradient technique (Percoll), see the table 'Characteristics of included studies' for further details. Posada 2005 compared a swim-up versus a gradient technique. The techniques were not further described. Soliman 2005 compared a gradient technique with a wash technique, see the table 'Characteristics of included studies' for further details. Carrell 1998 compared five different semen preparation techniques: wash technique, swim-up, swim-down, gradient technique, refrigeration and heparin technique. The assisted reproductive technique used in all studies was IUI. In Grigoriou 2005, Posada 2005, Soliman 2005 and Xu 2000

the women received one to three cycles with IUI. Only the first IUI cycle was included in Dodson 1998. In Xu 2000 it was not stated whether controlled ovarian hyperstimulation was used. In Dodson 1998, Grigoriou 2005, 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.

## Participants

Xu 2000 included 40 participants. Dodson 1998 included 41 participants (not published), with 16 (wash), 10 (swim-up) and 15 (gradient) participants in each treatment arm. Posada 2005 included 82 women. Soliman 2005 included 63 women who were randomised in a ratio of 2:1 (wash to gradient). Dodson 1998, Posada 2005 and Soliman 2005 included women with a variety of causes of infertility. Grigoriou 2005 included 52 couples with unexplained infertility. The cause of subfertility in Xu 2000 was male factor infertility, all semen samples were oligoasthenoteratospermic. In Xu 2000 the age of the women was not stated (men 24 to 43 years). The duration of subfertility was not stated in Xu 2000 (although all men did not have children 2 to 13 years after marriage); Posada 2005 and Soliman 2005, Dodson 1998 and Grigoriou 2005 included women with subfertility for at least one year. Exclusion criteria in Dodson 1998 were described as follows: oligomenorrhoea, severe oligospermia, donor semen, female anatomic distortion of the reproductive tract and bilateral tubal occlusion. Carrell 1998 included 363 couples in total (204 cycles using a gradient technique, 197 cycles a swim-up technique and 157 cycles a wash and centrifuge technique) with a variety of causes for their infertility. Male factor infertility was excluded by Carrell 1998.

Xu 2000 lacked details about important prognostic indicators concerning the participants (for example women's age). It was unclear whether both 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. Both treatment groups were similar at baseline in Dodson 1998, Posada 2005, and Grigoriou 2005. Soliman 2005, an abstract, did report women's age (32.4 and 34.5 years for the gradient and wash technique respectively).

## Excluded studies

Thirty-three studies failed to meet the inclusion criteria for one or more reasons outlined in the table 'Characteristics of excluded studies'. Twelve studies were excluded as they did not perform a comparison of interest (Almagor 1993; Bajamonte 1994; Baka 2009; Bhakta 2010; Fleming 2008; Kucuk 2008; Mathieu 1988; Menge 1992; Monqaut 2011; Ozturk 2008; Paul 2004; Urry

1988). 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 appeared to be non-randomised. Urry 1988 did not provide separate data in his article about the comparison between the swim-up and wash preparation in the 'husband artificial insemination group'. We did not succeed in contacting the authors to see if separate data were available. Bajamonte 1994 used a modified swim-up technique (by a 20 minute sperm incubation period in human follicular fluid). Paul 2004 compared four different gradient techniques. Eleven studies were excluded for not using IUI as an assisted reproduction technique (Cimino 1990; Guerin 1989; Hammadeh 2001; Jaroudi 1993;

Leonetti 1995; Ord 1990; Ricci 2009; Sapienza 1993; Tanphai. 1988; VDZwalmen 1991; Zech 1993). Seven studies were excluded for failing to use a randomised design. Four studies were not randomised (Depypere 1995; Huang 1996b; Ohashi 1992; Ren 2004); two studies were quasi-randomised (Morshedi 2003; Werlin 1992) and Remohi 1989 failed to describe the design.

### Risk of bias in included studies

The risk of bias of the studies is summarised in Figure 1 and Figure 2.

**Figure 1. Risk of bias: review authors' judgements about each risk domain presented as percentages across all included studies.**

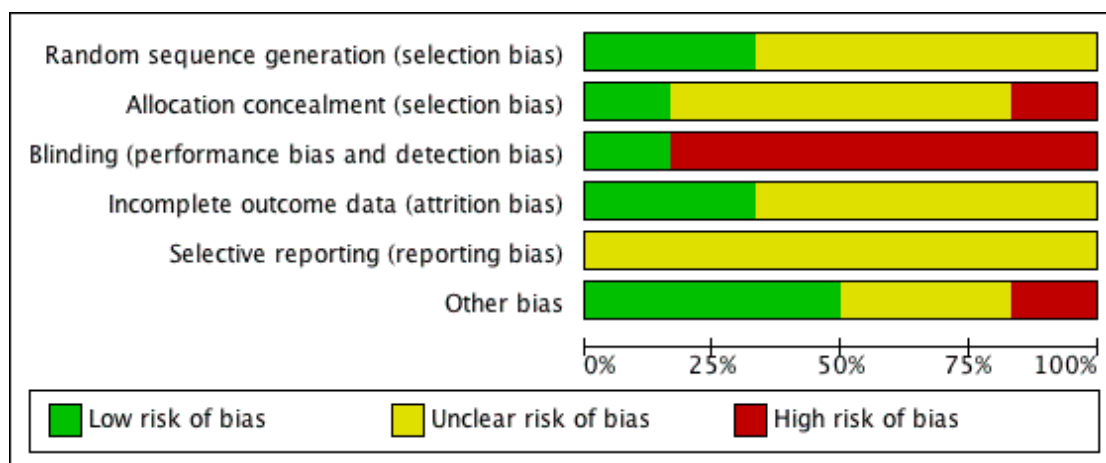


Figure 2. Risk domains: review authors' judgements about each potential risk of bias item for each included study.

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding (performance bias and detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Carrell 1998	?	?	-	?	?	-
Dodson 1998	+	+	+	+	?	+
Grigoriou 2005	+	-	-	+	?	+
Posada 2005	?	?	-	?	?	+
Soliman 2005	?	?	-	?	?	?
Xu 2000	?	?	-	?	?	?

### Allocation

Posada 2005, Soliman 2005 and Xu 2000 reported random allocation of semen samples to one of the preparation techniques. Concealment of allocation was not stated. We did not succeed in contacting the authors. Dodson 1998 used an adequate method of randomisation (assigned randomly from a computer-generated random sequence of numbers on the day of human chorionic gonadotropin (hCG) injection) and allocation was concealed by keeping the random numbers sequence at the laboratory in a separate location. Grigoriou 2005 randomised their patients by a permuted block design from a table with random numbers. Allocation was not concealed.

### Blinding

Grigoriou 2005, Posada 2005, Soliman 2005 and Xu 2000 did not report blinding. In Dodson 1998 only the participants were blinded.

### Incomplete outcome data

Dodson 1998 reported only drop-outs from the study before randomisation due to spontaneous pregnancies. Grigoriou 2005 reported two drop-outs in the swim-up study group, no reason was reported. An intention-to-treat analysis was performed (by imputation of no event). The number of cancelled cycles was not stated. No included study undertook a valid prospective power calculation. Dodson 1998 performed a power analysis on cycles rather than number of participants. They reported that 700 cycles would have been needed in each treatment arm (power 80%) and they included 153 cycles in total.

### Selective reporting

No studies were identified as high risk for selective reporting. No studies reported live birth as an outcome, however this primary outcome is often not reported in fertility studies, 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). The studies which did not report adverse events were not classified in this review as at high risk of selective reporting 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 as only two studies were pooled in the meta-analysis.

### Other potential sources of bias

Use of cross-over data was identified as a source of potential bias in one study (Carrell 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 both treatment groups were similar at baseline.

### 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](#)

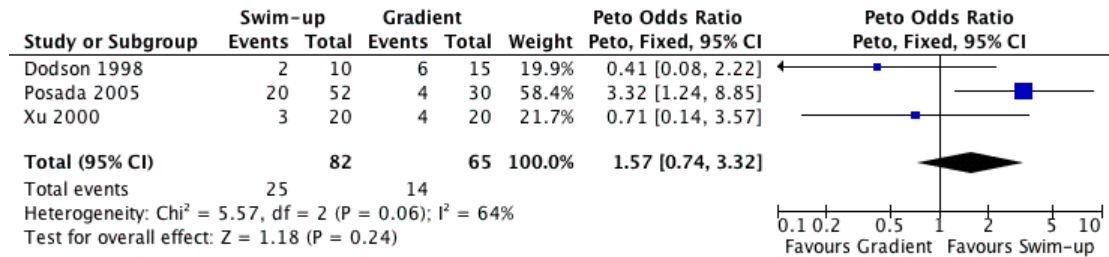
### Swim-up technique versus gradient technique

#### *Pregnancy rate*

The meta-analysis did not show evidence of a difference in the effectiveness of a swim-up versus gradient technique on pregnancy rates per couple (Peto OR 1.57, 95% CI 0.74 to 3.32), including 128 participants. The pregnancy rate per couple was 30.4% versus 21.5%, respectively. See [Figure 3](#).



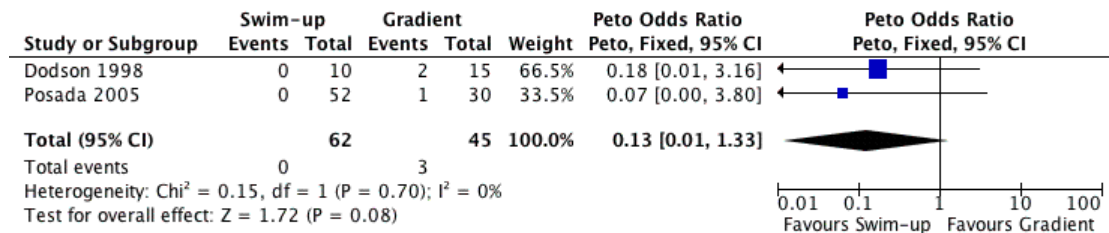
**Figure 3. Forest plot of comparison: I Swim-up versus gradient technique; fresh semen, outcome: I.1 Pregnancy rate per couple.**



### Miscarriage rate

There were no available data from Xu 2000. There was no evidence of a difference in the miscarriage rate per couple between the treatment groups (Peto OR 0.13, 95% CI 0.01 to 1.33). The miscarriage rate per couple was 0% versus 6.7%, respectively. See Figure 4.

**Figure 4. Forest plot of comparison: I Swim-up versus gradient technique; fresh semen, outcome: I.2 Miscarriage rate per couple.**



### Pregnancy rate

#### Multiple pregnancy rate

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

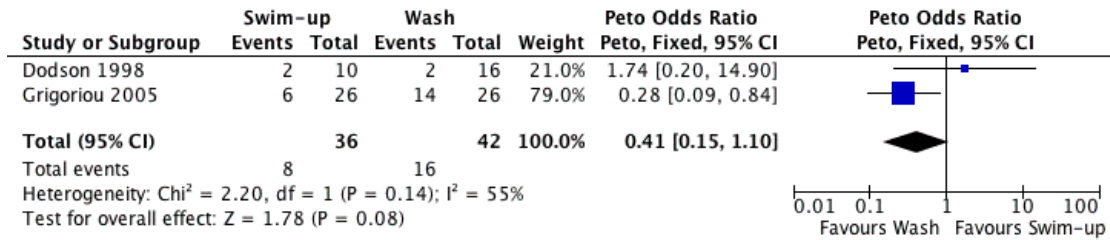
#### Swim-up technique versus wash and centrifugation

Dodson 1998 and Grigoriou 2005 were included in the analysis.

The meta-analysis did not show evidence of a difference in the effectiveness of a swim-up versus wash technique on pregnancy rates per couple (Peto OR 0.41, 95% CI 0.15 to 1.10), including 78 participants. The pregnancy rate per couple was 22.2% versus 38.1%, respectively. See Figure 5.



**Figure 5. Forest plot of comparison: 2 Swim-up versus wash and centrifugation; fresh semen, outcome: 2.1 Pregnancy rate per couple.**



was observed after the wash technique.

### Miscarriage rate

In [Dodson 1998](#), after both techniques the miscarriage rate per couple was 0%.

### Multiple pregnancy rate

In [Dodson 1998](#) there was no evidence of a difference in the multiple pregnancy rate per couple between the treatment groups (Peto OR 0.20, 95% CI 0.00 to 11.06). The multiple pregnancy rate per couple was 0% versus 6.3%, respectively. One triplet pregnancy

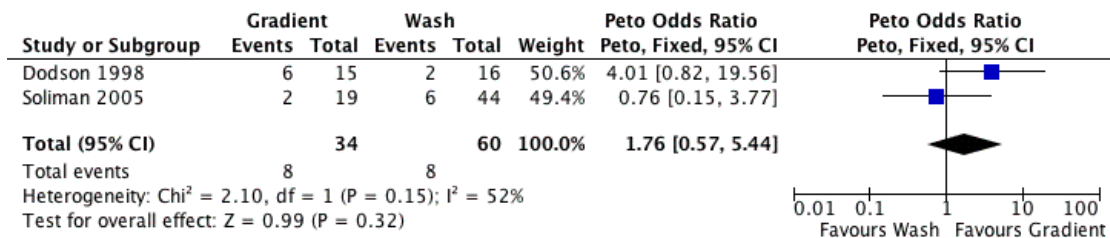
### Gradient technique versus wash and centrifugation

[Dodson 1998](#) and [Soliman 2005](#) were included in the analysis.

### Pregnancy rate

There was no evidence of a difference in the pregnancy rate per couple between the treatment groups (Peto OR 1.76, 95% CI 0.57 to 5.44). The pregnancy rate per couple was 23.5% versus 13.3%, respectively. See [Figure 6](#).

**Figure 6. Forest plot of comparison: 3 Gradient technique versus wash and centrifugation; fresh semen, outcome: 3.1 Pregnancy rate per couple.**



### Miscarriage rate

There were no available data from [Soliman 2005](#). In [Dodson 1998](#) there was no evidence of a difference in the miscarriage rate per couple between the treatment groups (Peto OR 8.48, 95% CI 0.51 to 142.39). The miscarriage rate per couple was 10.3% (miscarriage rate per pregnancy 30.3%) versus 0%, respectively.

### Multiple pregnancy rate

There were no available data from [Soliman 2005](#). In [Dodson 1998](#) there was no evidence of a difference in the multiple pregnancy rate per couple between the treatment groups (Peto OR 0.14, 95% CI 0.00 to 7.28). The multiple pregnancy rate per couple was 0% versus 6.3%, respectively. One triplet pregnancy was recorded after the wash technique.

Overall there was no clear evidence which semen preparation tech-

nique was superior. No studies provided information on laboratory time and costs per preparation technique. A summary of findings is provided in the 'Summary of findings' table.

#### **Heterogeneity results of included studies**

Heterogeneity between the results of the different studies was examined by inspecting the scatter in the data points and the overlap in their confidence intervals, and more formally by checking the

results of the  $\text{Chi}^2$  tests. Considering the results of pregnancy rates per couple after the different semen preparation techniques, there was a large overlap in confidence intervals. However there was a large difference in the direction of effect. The  $\text{Chi}^2$  tests did not show significant statistical heterogeneity. Care must be taken in the interpretation of the  $\text{Chi}^2$  test in these meta-analyses since it has low power when studies have small sample sizes or are few in number.

## ADDITIONAL SUMMARY OF FINDINGS *[Explanation]*

Swim-up technique compared to wash and centrifugation for undergoing intrauterine insemination						
<b>Patient or population:</b> patients undergoing intrauterine insemination (fresh semen)						
<b>Intervention:</b> swim-up technique						
<b>Comparison:</b> 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 centrifuga- tion	Swim-up technique				
<b>Pregnancy rate per couple</b>	<b>381 per 1000</b>	<b>201 per 1000</b> (85 to 404)	<b>OR 0.41</b> (0.15 to 1.1)	78 (2 studies)	⊕⊕○○ <b>low</b> <sup>1,2</sup>	
<b>Miscarriage rate per couple</b>	See comment	See comment	Not estimable	20 (1 study)	⊕⊕⊕○ <b>moderate</b> <sup>3</sup>	There were no events in either group
<b>Multiple pregnancy rate per couple</b>	<b>62 per 1000</b>	<b>13 per 1000</b> (0 to 424)	<b>OR 0.2</b> (0 to 11.06)	26 (1 study)	⊕⊕○○ <b>low</b> <sup>3,4</sup>	

\*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% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; 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.

<sup>1</sup> One of the trials did not conceal allocation and there was no blinding

<sup>2</sup> I square statistic was 55% indicating significant heterogeneity

<sup>3</sup> Evidence based on a single trial

<sup>4</sup> Wide confidence intervals indicate some imprecision

Gradient technique compared to Wash and centrifugation for undergoing intrauterine insemination						
<b>Patient or population:</b> patients with undergoing intrauterine insemination (fresh semen)						
<b>Intervention:</b> gradient technique						
<b>Comparison:</b> 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 centrifuga- tion	Gradient technique				
Pregnancy rate per couple	133 per 1000	213 per 1000 (81 to 456)	OR 1.76 (0.57 to 5.44)	94 (2 studies)	⊕⊕○○ low <sup>1,2</sup>	
Miscarriage rate per couple	0 per 1000	0 per 1000 (0 to 0)	OR 8.48 (0.51 to 142.39)	31 (1 study)	⊕⊕○○ low <sup>3,4</sup>	
Multiple pregnancy rate per couple	62 per 1000	9 per 1000 (0 to 327)	OR 0.14 (0 to 7.28)	31 (1 study)	⊕⊕○○ low <sup>3,4</sup>	

\*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% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).  
**CI:** Confidence interval; **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.

<sup>1</sup> One of the two trials did not provide adequate details on randomisation or allocation concealment and did not use blinding.

<sup>2</sup> I square statistic was 52% indicating some heterogeneity.

<sup>3</sup> Wide confidence intervals were indicative of a lack of precision

<sup>4</sup> Evidence is based on a single trial

## DISCUSSION

### Summary of main results

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. The aim of this review was to investigate which semen preparation technique is superior in clinical outcome.

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, and reporting clinical outcomes (pregnancy rate or live birth rate) are lacking. Only six RCTs (Carrell 1998; Dodson 1998; Grigoriou 2005; Posada 2005; Soliman 2005; Xu 2000) which compared a gradient technique versus a swim-up technique or a wash technique for IUI were identified. All six studies reported pregnancy rates. However, only Dodson 1998 compared the miscarriage and multiple pregnancy rates. Miscarriage rates were also reported by Posada 2005. Other outcomes (live birth rate, ongoing pregnancy, ectopic pregnancy, infections, fetal abnormalities rate per couple and laboratory time) were not reported by any of the included studies.

One cross-over RCT was identified, which was excluded from the meta analysis but included in the review (Carrell 1998) since data prior to crossing over could not be extracted. Carrell 1998 found inferior results in clinical outcomes after a wash technique versus a swim-up or gradient technique.

In conclusion, the meta-analysis did not show evidence of a difference in the effectiveness of a swim-up versus gradient or wash technique on pregnancy rates per couple. Firm conclusions cannot be drawn from the trials included in this review due to both unclear reporting of the methodology and lack of power.

### Quality of the evidence

Only randomised controlled trials were included in this meta-analysis. Only one of the six included studies used and described an adequate method of allocation concealment. The studies included in the review were carried out over a long period of time (22 years). The lack of blinding in most studies may be acceptable in terms of the overall quality since the outcomes are objective. 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 two of the studies.

As noted above, the studies were limited by unclear reporting of methodology and lack of power. However, 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

None identified

### Agreements and disagreements with other studies or reviews

In 2010 the WHO published a WHO laboratory manual for the examination and processing of human semen (WHO 2010). The manual describes the choices for sperm preparation. It 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.

## AUTHORS' CONCLUSIONS

### Implications for practice

Considering the clinical outcomes after IUI, there are insufficient data from RCTs to recommend any of the three semen preparation techniques over each other (swim-up, gradient, wash and centrifugation).

### Implications for research

More research needs to be performed on this topic as firm conclusions can not be drawn from the literature available. Next to large RCTs the results from thorough phase II research with semen parameters as an outcome would have substantial meaning for optimizing 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, a clear definition of the population, inclusion and exclusion criteria and a comparison of these factors in the treatment groups is recommended. In addition, the methodology of semen preparation needs to be standardised in order to allow appropriate comparison. Since all three techniques seem to be equally effective an analysis of laboratory time and costs would be of value.

## 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 Menstrual Disorders and Subfertility Group (MDSG); Ruth Buist for retrieval of trials and Sue Hall for helping to collect trials we needed and their positive support; Anne Lethaby for her statistical advice. We would also like to thank Dr M Merrilees and Dr MHJM. Curfs 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 and Dr P Prakash for putting such an effort into getting the information we asked for and for always replying to the numerous e-mails we sent. Also a thank you is 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 and Dr L Werlin, who provided additional information on their trials.

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\* 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 drop-outs or cancelled cycles, intention-to-treat analysis, power calculation: not stated
Participants	363 couples. Cause 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	Swim-up, PR 13.2%. Gradient, 12.7%. Wash, 8.9%.
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 (performance bias and detection bias) All outcomes	High risk	Not blinded.
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Drop-outs 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 follow up: not stated. Groups similar regarding important indicators at baseline: estradiol level, follicles, cause subfertility, age: yes. Number drop-outs: 4 (naturally pregnant: counted as non-pregnant). 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 women: 28-40 (mean 31.6) yrs. Duration subfertility >1year. 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. 150xg centrifug Pellet resusp, 10 min. 150xg centrifug, pellet resusp. 2) SWIM-UP: multiple tube (4) 1:1 medium, 10 min 150xg centrifug, supernatant discarded. Overlaid with medium, 45 min incubation. Top removed +wash, 10 min 150xg centrifug. 3) GRADIENT: 90%/45% Percoll, 20 min 300xg centrifug, pellet washed, 10 min 150xg centrifug. Ass reprod technique: single IUI. 0.5 ml volume. Number IUI: 1. COH: all women, gonadotropins/hCG. Analysis by: not stated.
Outcomes	Swim-up, PR/couple: 20% (2/10). Gradient: PR/couple: 40% (6/15). Wash: PR/couple: 12.5 % (2/16). MR/couple Swim-up/Wash: 0%, Gradient: 10.3% (2/15). MR/pregnancy, Gradient: 30.3% (2/6). MPR/couple Swim-up/Gradient: 0%. Wash, 6.25% (1/16). 1 triplet. PR/ cycle: see additional table 02
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
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 (performance bias and detection bias) All outcomes	Low risk	Only patients were blinded.

**Dodson 1998** (Continued)

Incomplete outcome data (attrition bias) All outcomes	Low risk	2 drop-outs from the study before randomisation due to spontaneous pregnancies
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 drop outs 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 > 1year. Cause infertility: unexplained. Semen quality: normal (WHO criteria)	
Interventions	2 preparation techniques. 1) WASH with PAF: 10 min. 400xg centrifug, 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: 1-3. COH: 100 mg clomiphene citrate day 3-7. 0.5 ml volume. Number IUI: 1. COH: all women: gonadotropins/ hCG. Analysis by: Student's t-test, Kruskal Wallis, Fisher's exact	
Outcomes	Wash +PAF: PR/couple: 22.2 % (14/63), Swim-up, PR/couple: 8.6% (6/70)	
Notes	Cross-over after 3 cycles. Only first three cycles included in the meta-analysis. IUI standardised to a volume of 0.5 ml, and a count of 20 million progressive motile sperm	

**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 (performance bias and detection bias) All outcomes	High risk	Not blinded.

**Grigoriou 2005** (Continued)

Incomplete outcome data (attrition bias) All outcomes	Low risk	2 drop-outs 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.

**Posada 2005**

Methods	Stated random, but no details. Design: parallel, single-centre. Concealment of allocation, blinding, number of drop outs or cancelled cycles, intention-to-treat analysis, power calculation: not stated	
Participants	82 couples. Age women < 38 yrs. Mean age swim-up: 32.06 ± 3.7 yrs, gradient 32.37 ± 4.0 yrs. Cause 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	Gradient, PR/couple: 13.33% (4/30). Swim-up, PR/couple: 38.5% (20/52). MR/couple Swim-up 0%. Gradient: 3,33%	
Notes	Abstract. Big difference in results. Preparation techniques not described	

***Risk of bias***

<b>Bias</b>	<b>Authors' judgement</b>	<b>Support for judgement</b>
Random sequence generation (selection bias)	Unclear risk	Stated as randomised, no further details. Both treatment groups were similar at baseline
Allocation concealment (selection bias)	Unclear risk	Allocation concealment not described.
Blinding (performance bias and detection bias) All outcomes	High risk	Not blinded.
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Drop-outs or loss to follow up not stated. Duration of follow up not stated
Selective reporting (reporting bias)	Unclear risk	Unclear risk.

Posada 2005 (Continued)

Other bias	Low risk	None identified.
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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 drop-outs or cancelled cycles, intention-to-treat analysis, power calculation: not stated
Participants	63 couples. Age 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, centrifug, pellet resuspended, centrifug. 2) WASH: wash with medium, centrifug at higher speed, supernatant discarded, pellet resuspended, centrifug, mixed with medium. ART: IUI. Number of cycles per patient: 1. COH: not stated. Number IUI per cycle: 2
Outcomes	Gradient, PR/couple: 10.5% (2/17). Wash, PR/couple 13.6% (6/44)
Notes	Abstract.

*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 (performance bias and detection bias) All outcomes	High risk	Not blinded.
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Drop-outs or loss to follow up not stated. Duration of follow up not stated
Selective reporting (reporting bias)	Unclear risk	Unclear risk
Other bias	Unclear risk	Abstract. Not enough data on methodological assessment.

Methods	Parallel design. Stated randomised, no further details. Concealment allocation, blinding, duration follow up, drop-outs/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: 24-43 years (for all 140 men). Duration of subfertility: not stated. All 140 men did not have children 2-13 years 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). Ass reprod technique: IUI. Number IUI: swim-up 1-3, gradient 1-3 (average 2.5). COH: not stated. Cancellation criteria: not stated	
Outcomes	Swim-up: PR/couple: 15% (3/20). Gradient: n=20; PR/couple: 20 % (4/20)	
Notes		
<b><i>Risk of bias</i></b>		
<b>Bias</b>	<b>Authors' judgement</b>	<b>Support for judgement</b>
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 (performance bias and detection bias) All outcomes	High risk	Not blinded.
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Drop-outs or loss to follow up not stated. Duration of follow up not stated
Selective reporting (reporting bias)	Unclear risk	Unclear risk.
Other bias	Unclear risk	A lot of important information was not reported in the article (e.g. baseline similarity of groups)

PAF: platelet activating factor.

COH: controlled ovarian hyperstimulation

CC: clomiphene citrate

PR: pregnancy rate

MR: miscarriage rate



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

Study	Reason for exclusion
<a href="#">Almagor 1993</a>	Not a randomised controlled trial. No comparison of interest. Type of intervention: swim-up, swim-down versus gradient
<a href="#">Bajamonte 1994</a>	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 than IUI. No comparison of interest: the swim-up technique used is modified by 20 min sperm incubation period in hFF
<a href="#">Baka 2009</a>	No comparison of interest: to evaluate the effect of exogenous platelet-activating factor (PAF) on clinical outcome. Cross-over design
<a href="#">Bhakta 2010</a>	No comparison of interest: carbon dioxide versus no carbon dioxide
<a href="#">Byrd 1994</a>	Participants were fertile women undergoing donor inseminations
<a href="#">Caccamo 1995</a>	Not a randomised controlled trial.
<a href="#">Cimino 1990</a>	Retrospective design. Not a randomised controlled trial. Use of assisted reproduction technique other than IUI
<a href="#">Depypere 1995</a>	Not a randomised controlled trial. Centre A used wash procedure, centre B used gradient technique
<a href="#">Fleming 2008</a>	No comparison of interest: density gradient technique versus an electrophoretic method. Not a randomised controlled trial. No clinical outcome
<a href="#">Guerin 1989</a>	Not a randomised controlled trial. Use of assisted reproduction technique other than IUI
<a href="#">Hammadeh 2001</a>	Not a randomized controlled trial. Use of assisted reproduction technique other than IUI
<a href="#">Huang 1996b</a>	Not a randomised controlled trial.
<a href="#">Jaroudi 1993</a>	Use of assisted reproduction technique other than IUI.
<a href="#">Kucuk 2008</a>	No comparison of interest: gradient technique with or without heat induced hypermotility
<a href="#">Leonetti 1995</a>	Not a randomised controlled trial. Use of assisted reproduction technique other than IUI
<a href="#">Mathieu 1988</a>	Use of assisted reproduction technique other than 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
<a href="#">Menge 1992</a>	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

(Continued)

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 cross-over. We found a conference abstract and article describing the same trial
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 than 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 1996	No comparison of interest: two types of swim-up techniques.
Remohi 1989	This study was primary about IUI and GIFT, 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
Sapienza 1993	Use of assisted reproduction technique other than IUI.
Tanphai. 1988	Not a randomised controlled trial. The two preparation techniques were used alternately among the participants. Use of assisted reproduction technique other than IUI
Urry 1988	Not a randomised controlled trial (use of a randomised protocol, but allocation to a protocol is not stated to be randomized). Cross-over design. We did not succeed in contacting the authors
VDZwalmen 1991	Use of assisted reproduction technique other than 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
Zech 1993	Not a randomised controlled trial. Use of assisted reproduction technique other than 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 Pregnancy rate per couple	3	147	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.57 [0.74, 3.32]
2 Miscarriage rate per couple	2	107	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.13 [0.01, 1.33]
3 Multiple pregnancy rate per couple	1	25	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.0 [0.0, 0.0]

### 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 Pregnancy rate per couple	2	78	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.41 [0.15, 1.10]
2 Miscarriage rate per couple	1	20	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.0 [0.0, 0.0]
3 Multiple pregnancy rate per couple	1	26	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.20 [0.00, 11.06]

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

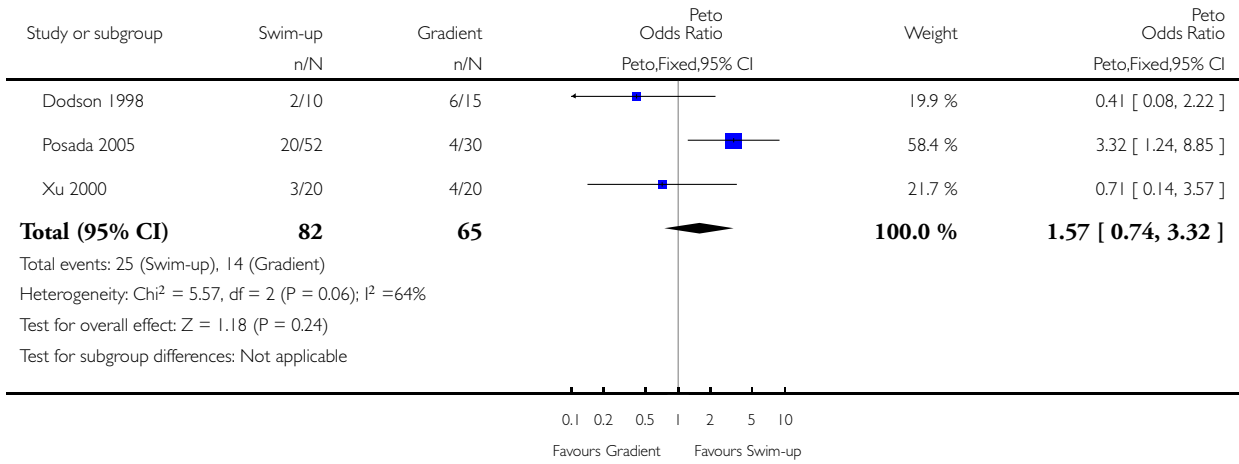
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Pregnancy rate per couple	2	94	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.76 [0.57, 5.44]
2 Miscarriage rate per couple	1	31	Peto Odds Ratio (Peto, Fixed, 95% CI)	8.48 [0.51, 142.39]
3 Multiple pregnancy rate per couple	1	31	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.14 [0.00, 7.28]

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

Review: Semen preparation techniques for intrauterine insemination

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

Outcome: 1 Pregnancy rate per couple

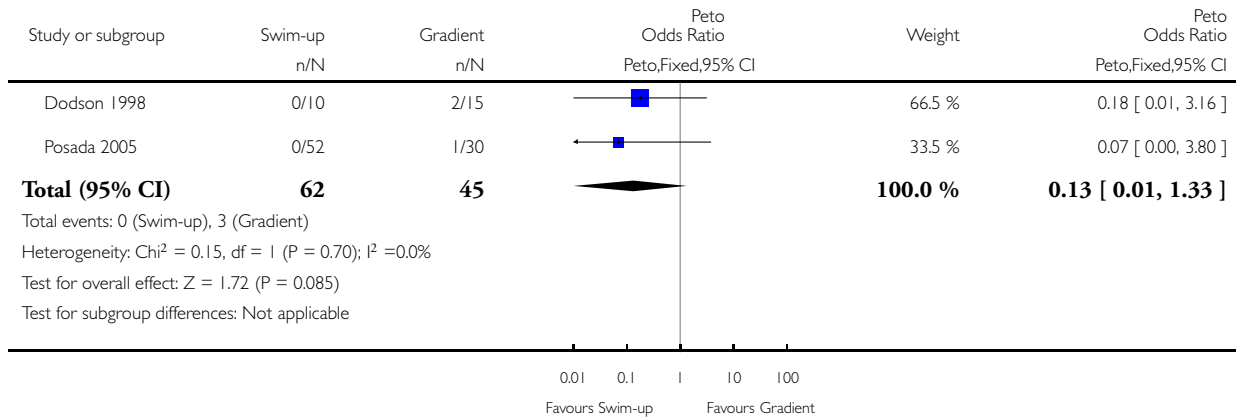


### Analysis 1.2. Comparison 1 Swim-up versus gradient technique, fresh semen, Outcome 2 Miscarriage rate per couple.

Review: Semen preparation techniques for intrauterine insemination

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

Outcome: 2 Miscarriage rate per couple

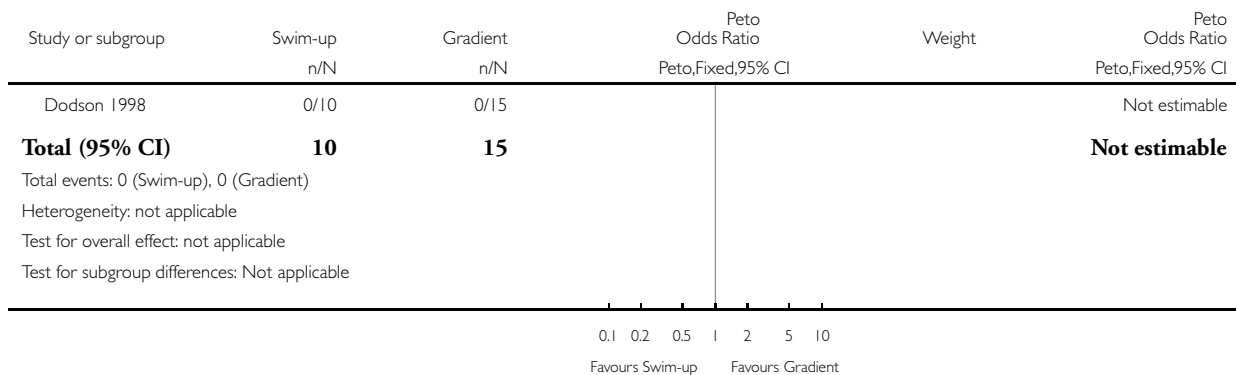


### Analysis 1.3. Comparison 1 Swim-up versus gradient technique, fresh semen, Outcome 3 Multiple pregnancy rate per couple.

Review: Semen preparation techniques for intrauterine insemination

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

Outcome: 3 Multiple pregnancy rate per couple

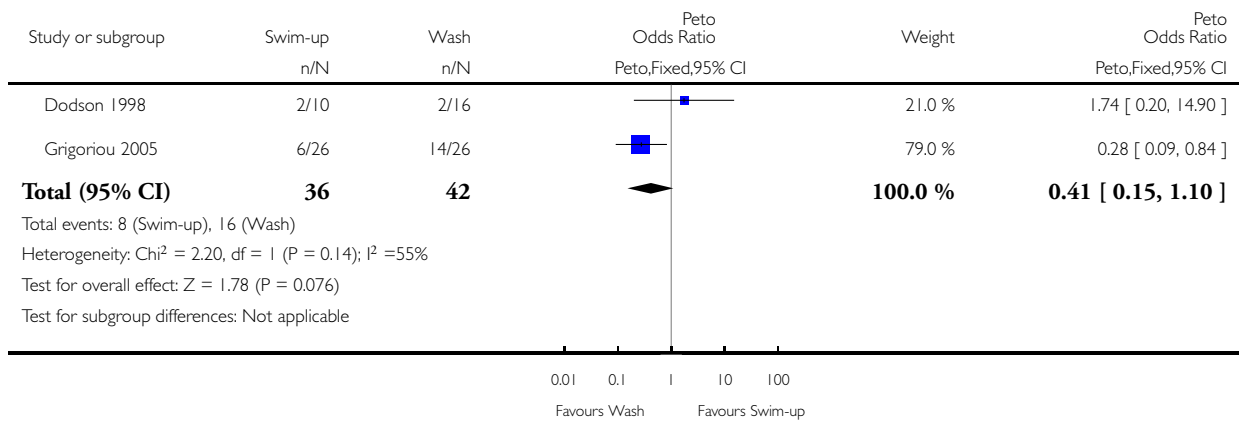


## Analysis 2.1. Comparison 2 Swim-up versus wash and centrifugation, fresh semen, Outcome 1 Pregnancy rate per couple.

Review: Semen preparation techniques for intrauterine insemination

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

Outcome: 1 Pregnancy rate per couple

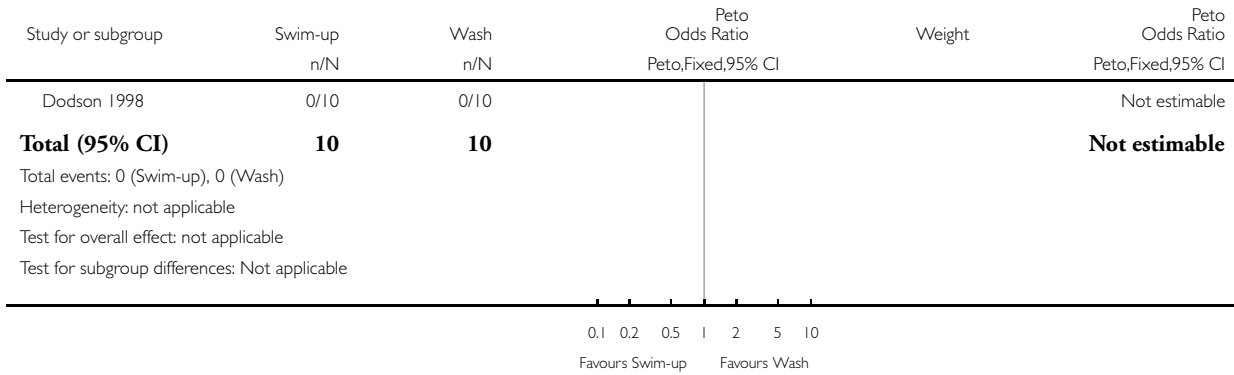


**Analysis 2.2. Comparison 2 Swim-up versus wash and centrifugation, fresh semen, Outcome 2 Miscarriage rate per couple.**

Review: Semen preparation techniques for intrauterine insemination

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

Outcome: 2 Miscarriage rate per couple

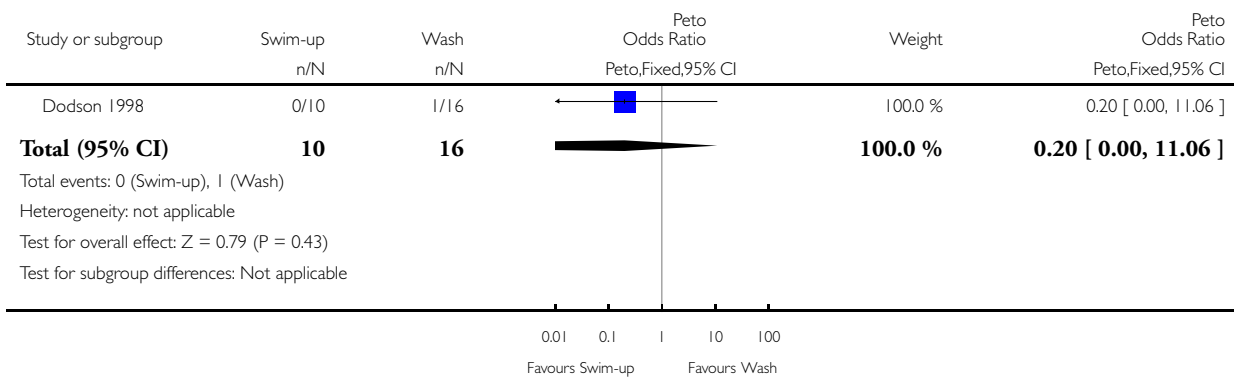


**Analysis 2.3. Comparison 2 Swim-up versus wash and centrifugation, fresh semen, Outcome 3 Multiple pregnancy rate per couple.**

Review: Semen preparation techniques for intrauterine insemination

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

Outcome: 3 Multiple pregnancy rate per couple

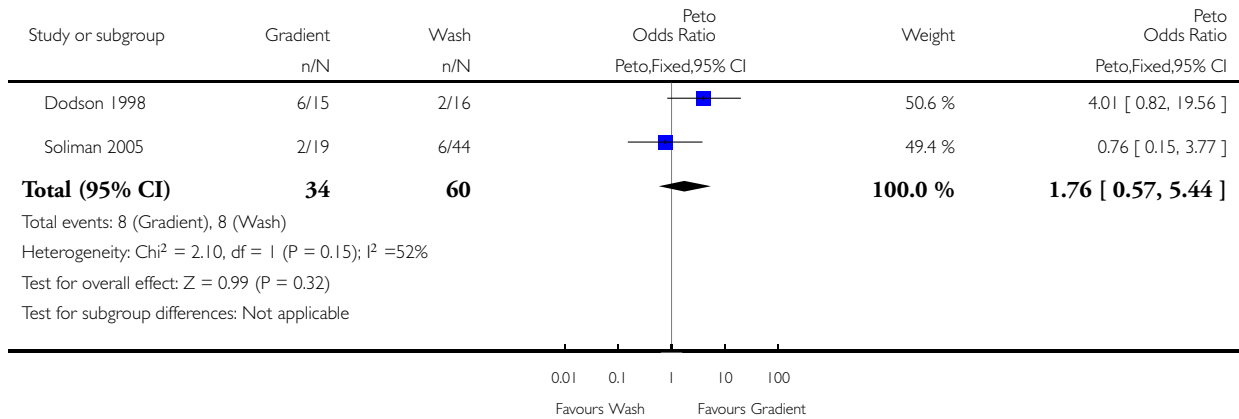


### Analysis 3.1. Comparison 3 Gradient technique versus wash and centrifugation, fresh semen, Outcome 1 Pregnancy rate per couple.

Review: Semen preparation techniques for intrauterine insemination

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

Outcome: 1 Pregnancy rate per couple

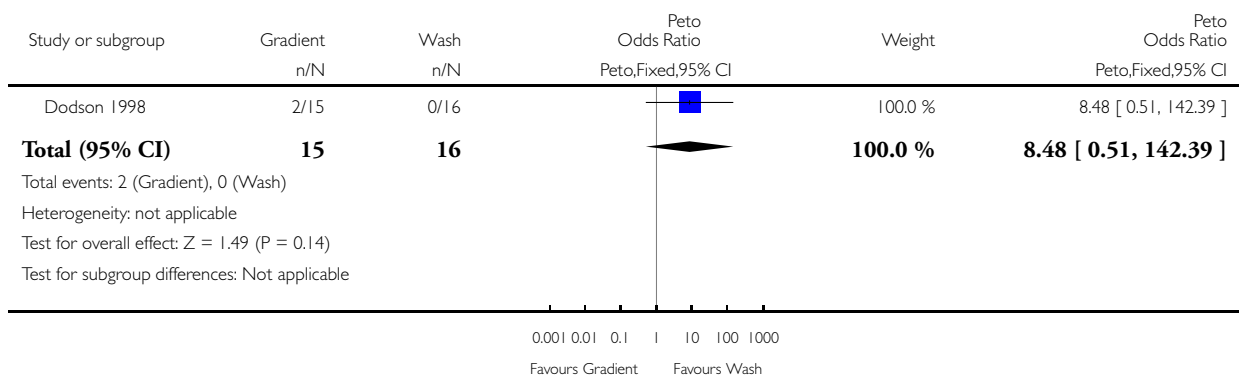


### Analysis 3.2. Comparison 3 Gradient technique versus wash and centrifugation, fresh semen, Outcome 2 Miscarriage rate per couple.

Review: Semen preparation techniques for intrauterine insemination

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

Outcome: 2 Miscarriage rate per couple



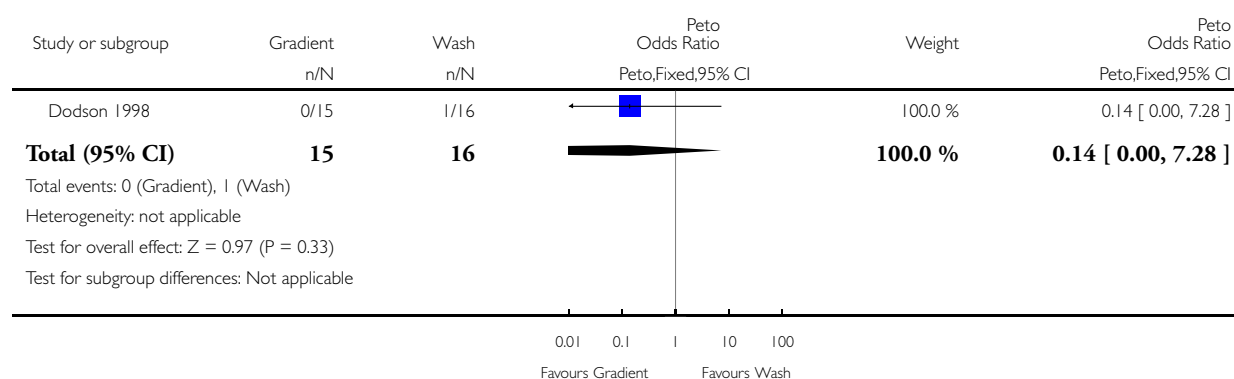


### Analysis 3.3. Comparison 3 Gradient technique versus wash and centrifugation, fresh semen, Outcome 3 Multiple pregnancy rate per couple.

Review: Semen preparation techniques for intrauterine insemination

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

Outcome: 3 Multiple pregnancy rate per couple



## ADDITIONAL TABLES

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

Study ID	Allocation Score	Methods	Participants	Interventions	Outcomes
<a href="#">Carrell 1998</a>	B	Stated random, but no details. Design: cross-over, multi-centre. Concealment of allocation, blinding, number of drop-outs or cancelled cycles, intention-to-treat analysis, power calculation: all not stated	363 women: 558 cycles in the 3 methods of interest. Age women, duration subfertility: not stated. Cause: unexplained/ (fe)male related disorders. Exclusion criteria: oligoasthenozoospermic semen samples af-	3 preparation techniques (out of 5 described) . 1) Sperm wash: 8-10 ml. medium (Ham's F-10), 10 min. 400x g centrifug. Supernatant decanted, pellet resusp. 2) Swim-up: 2x washed, resusp. Medium layered	Pregnancy rate (PR) / cycle, Miscarriage rate (MR)/ pregnancy, Live birth rate (LBR)/ cycle

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

			ter preparation. Inclusion criteria: not stated	on top. Incubation 1h. Top removed. 3) Gradient: 1x wash+ re-susp. Percoll, (35%/90%).15 min 400x g centrifug. 90% layer washed. Single IUI. 2.5 ± 0.3 inseminations per women. 124 women: 50-200 mg. CC day 5-9 or no COH. 239 women: gonadotropin/ hCG	
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**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	PR/ cycle: 16.1% (33/204), LBR/ cycle: 12.7% (26/204), MR/ pregnancy: 21.2% (7/33)	PR/ cycle: 14.7% (29/197), LBR/ cycle: 13.2 (26/197), MR/ pregnancy: 10.3 % (3/29)	PR/ cycle: 8.9% (14/157), LBR/ cycle: 7.0% (11/157), MR/ pregnancy: 21.4% (3/14)	PR/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	PR/cycle and MR/pregnancy: X2 analysis and Fisher's exact test. Statistical significance P<0.05

## APPENDICES

### Appendix 1. MEDLINE search strategy

- 1 exp insemination, artificial/ or exp insemination, artificial, heterologous/ or exp insemination, artificial, homologous/ (9043)
- 2 artificial insemination.tw. (4156)
- 3 intrauterine insemination.tw. (1436)
- 4 iui.tw. (929)
- 5 intra uterine insemination.tw. (135)
- 6 insemination.tw. (10578)
- 7 or/1-6 (14473)
- 8 exp Centrifugation, Density Gradient/ (36309)
- 9 (sperm\$ adj2 prepar\$).tw. (938)
- 10 (semen adj2 prepar\$).tw. (175)
- 11 (sperm\$ adj2 separation\$).tw. (206)
- 12 gradient.tw. (107632)
- 13 (swim up or swim down).tw. (861)
- 14 wash.tw. (11148)
- 15 centrifug\$.tw. (13)
- 16 centrifug\$.tw. (45497)
- 17 percoll.tw. (5212)
- 18 (semen adj2 separation\$).tw. (11)
- 19 (semen adj2 treatment\$).tw. (193)
- 20 (sperm adj2 treatment\$).tw. (542)
- 21 swim-up.tw. (855)
- 22 (wash\$ adj2 semen).tw. (107)
- 23 (wash\$ adj2 sperm\$).tw. (942)
- 24 isolate\$.tw. (767817)
- 25 spermprep\$.tw. (25)
- 26 mini-percoll.tw. (21)
- 27 or/8-26 (925051)
- 28 27 and 7 (1112)
- 29 randomized controlled trial.pt. (303000)
- 30 controlled clinical trial.pt. (82944)
- 31 randomized.ab. (216350)
- 32 placebo.tw. (130491)
- 33 clinical trials as topic.sh. (151888)
- 34 randomly.ab. (160249)
- 35 trial.ti. (93119)
- 36 (crossover or cross-over or cross over).tw. (50062)
- 37 or/29-36 (736426) 38 exp animals/ not humans.sh. (3582756) 39 37 not 38 (680983) 40 28 and 39 (79)

### Appendix 2. MDSG search string

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”

### **Appendix 3. CENTRAL search strategy**

1 exp insemination, artificial/ or exp insemination, artificial, heterologous/ or exp insemination, artificial, homologous/ (245)  
2 artificial insemination.tw. (52)  
3 intrauterine insemination.tw. (343)  
4 iui.tw. (249)  
5 intra uterine insemination.tw. (25)  
6 insemination.tw. (550)  
7 or/1-6 (605)  
8 exp Centrifugation, Density Gradient/ (36)  
9 (sperm\$ adj2 prepar\$).tw. (91)  
10 (semen adj2 prepar\$).tw. (24)  
11 (sperm\$ adj2 separation\$).tw. (21)  
12 gradient.tw. (1356)  
13 (swim up or swim down).tw. (155)  
14 wash.tw. (2256) 15 centrifug\$.tw. (0) 16 centrifug\$.tw. (367)  
17 percoll.tw. (84) 18 (semen adj2 separation\$).tw. (0)  
19 (semen adj2 treatment\$).tw. (44)  
20 (sperm adj2 treatment\$).tw. (82)  
21 swim-up.tw. (155)  
22 (wash\$ adj2 semen).tw. (3)  
23 (wash\$ adj2 sperm\$).tw. (44)  
24 isolate\$.tw. (6272)  
25 spermprep\$.tw. (12)  
26 mini-percoll.tw. (6)  
27 or/8-26 (10433)  
28 27 and 7 (92)

### **Appendix 4. EMBASE search strategy**

1 exp artificial insemination/ (10479)  
2 artificial insemination.tw. (3878)  
3 intrauterine insemination.tw. (1733)  
4 iui.tw. (1219)  
5 intra uterine insemination.tw. (199)  
6 insemination.tw. (10565)  
7 or/1-6 (15578)  
8 exp density gradient centrifugation/ or exp centrifugation/ (47655)  
9 (sperm\$ adj2 prepar\$).tw. (987) 10 (semen adj2 prepar\$).tw. (203)  
11 (sperm\$ adj2 separation\$).tw. (214)  
12 gradient.tw. (105850)  
13 (swim up or swim down).tw. (961) 14 wash.tw. (12243)  
15 centrifug\$.tw. (24) 16 centrifug\$.tw. (42890)  
17 percoll.tw. (5321) 18 (semen adj2 separation\$).tw. (12)  
19 (semen adj2 treatment\$).tw. (184)  
20 (sperm adj2 treatment\$).tw. (551)  
21 swim-up.tw. (955)  
22 (wash\$ adj2 semen).tw. (110)  
23 (wash\$ adj2 sperm\$).tw. (918)

- 24 isolate\$.tw. (775185)
- 25 spermprep\$.tw. (33)
- 26 mini-percoll.tw. (22)
- 27 or/8-26 (940812)
- 28 27 and 7 (1198)

## Appendix 5. PsycINFO search strategy

- 1 exp Reproductive Technology/ (1015)
- 2 artificial insemination.tw. (196)
- 3 intrauterine insemination.tw. (9)
- 4 iui.tw. (15)
- 5 intra uterine insemination.tw. (0)
- 6 insemination.tw. (502)
- 7 or/1-6 (1320)
- 8 (sperm\$ adj2 prepar\$).tw. (7)
- 9 (semen adj2 prepar\$).tw. (1)
- 10 (sperm\$ adj2 separation\$).tw. (1)
- 11 gradient.tw. (3972)
- 12 (swim up or swim down).tw. (6)
- 13 wash.tw. (468)
- 14 centrifug\$.tw. (0)
- 15 centrifug\$.tw. (812)
- 16 percoll.tw. (4)
- 17 (semen adj2 separation\$).tw. (0)
- 18 (semen adj2 treatment\$).tw. (2)
- 19 (sperm adj2 treatment\$).tw. (5)
- 20 swim-up.tw. (4)
- 21 (wash\$ adj2 semen).tw. (0)
- 22 (wash\$ adj2 sperm\$).tw. (0)
- 23 isolate\$.tw. (20333)
- 24 spermprep\$.tw. (0)
- 25 mini-percoll.tw. (0)
- 26 or/8-25 (25489)
- 27 7 and 26 (15)

## WHAT'S NEW

Last assessed as up-to-date: 4 August 2011.

Date	Event	Description
5 August 2011	New search has been performed	Converted to new review format. Updated search. No new studies were identified

## HISTORY

Protocol first published: Issue 4, 2003

Review first published: Issue 3, 2004

Date	Event	Description
2 July 2007	New citation required and conclusions have changed	Substantive amendment

## CONTRIBUTIONS OF AUTHORS

CM Boomsma has prepared the manuscript. CM Boomsma and MJ Heineman have performed the selection of studies for inclusion. C Farquhar and BJ Cohlen were involved in concept and study design.

## DECLARATIONS OF INTEREST

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, some changes were made 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.

### *Timeline*

It is the intention of the review authors that a new search for RCTs will be performed every two years and the review updated 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