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ABSTRACT

Background
Ultrasound guided transvaginal aspiration of oocytes has replaced other methods of oocyte retrieval for in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). However, there is controversy over whether flushing yields a larger number of oocytes and a higher potential for pregnancy than aspiration only.

Objectives
To determine whether follicular aspiration and flushing increases live birth or ongoing pregnancy rates and the number of oocytes over aspiration alone in women undergoing IVF and ICSI.

Search strategy
We searched the Menstrual Disorders and Subfertility Group Specialised Register of controlled trials, MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials (CENTRAL), PsycINFO and the citation lists of relevant publications (to April 2010).

Selection criteria
Randomised controlled trials that compared follicular aspiration and flushing with aspiration alone were included. Trials were excluded if the flushing method comparison was confounded by comparisons of other methods.

Data collection and analysis
Eligible studies were assessed for methodological quality. For dichotomous data, odds ratios (OR) and 95% confidence intervals (CI) were calculated. For continuous data, mean differences were reported. The heterogeneity of the studies was examined by using statistical tests of homogeneity and the I² statistic.

Main results
No studies reported on the primary outcome of live birth. There was no evidence (3 studies, 164 patients) to suggest an association between follicular aspiration and flushing and ongoing or clinical pregnancy per woman randomised (OR 1.17, 95% CI 0.57 to 2.38). There was no evidence of a difference in adverse events reported between follicular aspiration and flushing and aspiration only. There
was no evidence of significant differences in increased oocyte yield per woman randomised (1 study, 44 patients). Without flushing the operative time was significantly shorter, by 3 to 15 minutes (3 studies, \(P < 0.001\)) and the dose of pethidine required was significantly less (50 mg versus 100 mg, \(P < 0.00001\)).

**Authors’ conclusions**

There is no evidence that follicular aspiration and flushing is associated with improved clinical or ongoing pregnancy rates, nor an increase in oocyte yield. The operative time is significantly longer and more opiate analgesia is required for pain relief during oocyte retrieval. There is a lack of evidence regarding the effect of follicular aspiration and flushing on live birth rates in the identified data.

**PLAIN LANGUAGE SUMMARY**

**Follicular flushing during oocyte retrieval in assisted reproductive technology**

Flushing of the follicles during egg collection is not useful, as it prolongs the operating time and increases the need for pain relief without increasing the chances of a pregnancy or increasing the number of eggs recovered.
<table>
<thead>
<tr>
<th>Outcome</th>
<th>Studies</th>
<th>Participants</th>
<th>Statistical Method</th>
<th>Effect Estimate (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 Clinical pregnancy rate per woman randomised</td>
<td>3</td>
<td>164 women</td>
<td>Odds Ratio (M-H, Fixed, 95% CI)</td>
<td>1.17 [0.57, 2.38]</td>
</tr>
<tr>
<td>1.2 Ongoing pregnancy per woman randomised</td>
<td>2</td>
<td>64 women</td>
<td>Odds Ratio (M-H, Fixed, 95% CI)</td>
<td>1.82 [0.56, 5.93]</td>
</tr>
<tr>
<td>1.3 Oocyte yield</td>
<td>3</td>
<td>2089 oocytes</td>
<td>Odds Ratio (M-H, Fixed, 95% CI)</td>
<td>1.12 [0.91, 1.37]</td>
</tr>
<tr>
<td>1.4 Fertilization rate</td>
<td>2</td>
<td>314 oocytes</td>
<td>Odds Ratio (M-H, Fixed, 95% CI)</td>
<td>1.26 [0.80, 1.98]</td>
</tr>
<tr>
<td>1.5 Implantation rate</td>
<td>1</td>
<td>58 women</td>
<td>Odds Ratio (M-H, Fixed, 95% CI)</td>
<td>2.78 [0.66, 11.80]</td>
</tr>
<tr>
<td>1.6 Maturity of oocytes obtained</td>
<td>1</td>
<td>244 oocytes</td>
<td>Odds Ratio (M-H, Fixed, 95% CI)</td>
<td>0.98 [0.54, 1.77]</td>
</tr>
<tr>
<td>1.7 Time taken for the procedure</td>
<td>3</td>
<td>Aspiration with flushing</td>
<td>Aspiration only</td>
<td>P value</td>
</tr>
<tr>
<td></td>
<td></td>
<td>median 35 min</td>
<td>median 20 minutes</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean time 366 ± 125 sec</td>
<td>Mean time 186 ± 41 sec</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>median 30 min (range 15-70 min)</td>
<td>median 15 min (range 4-30 min)</td>
<td>P&lt;0.00001</td>
</tr>
<tr>
<td>1.8 Pain relief (dose of pethidine required)</td>
<td>1</td>
<td>Aspiration with flushing</td>
<td>Aspiration only</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Median 100 mg</td>
<td>Median 50 mg</td>
<td>P&lt;0.00001</td>
</tr>
<tr>
<td>1.9 Adverse events</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.9.1 Blockage of needle</td>
<td>1</td>
<td>100 women</td>
<td>Odds Ratio (M-H, Fixed, 95% CI)</td>
<td>7.44 [0.37, 147.92]</td>
</tr>
<tr>
<td>1.9.2 Vomiting</td>
<td>1</td>
<td>100 women</td>
<td>Odds Ratio (M-H, Fixed, 95% CI)</td>
<td>5.21 [0.24, 111.24]</td>
</tr>
<tr>
<td>1.9.3 Hypotension</td>
<td>1</td>
<td>100 women</td>
<td>Odds Ratio (M-H, Fixed, 95% CI)</td>
<td>5.21 [0.24, 111.24]</td>
</tr>
</tbody>
</table>
### BACKGROUND

**Description of the condition**

Assisted reproductive technology (ART) requires the handling of oocytes and embryos outside the woman’s body. The technique involves ovarian stimulation, monitoring of follicular growth, oocyte recovery, sperm preparation and insemination, embryo culture, embryo transfer and luteal support.

**Description of the intervention**

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

The number of embryos obtained is dependent on the number of oocytes retrieved (Wood 2000). To maximize the number of oocytes recovered, follicular aspiration followed by one 2-ml flush has been suggested (el Hussein 1992). Waterstone and Parson (1992) reported that the use of double-lumen needles and flushing resulted in 20% more oocytes (Waterstone 1992). On the contrary, other studies found no difference in the number of oocytes collected, fertilization rates, embryo quality or pregnancy rates (Kingsland 1991; Tan 1992; Knight 2001). It was suggested that aspiration without flushing reduced the operative time and decreased the amount of anaesthetic required (Tan 1992).

**How the intervention might work**

The place of follicular flushing during oocyte recovery in ART is still uncertain. The pros of flushing include the possibility of obtaining more oocytes, and subsequently more embryos. Whether this translates into a higher pregnancy rate and live births remains unknown. The cons of flushing are a longer operative time and larger amounts of required anaesthetics and analgesics. From a patient’s perspective, it could also mean higher costs. Moreover, anaesthetics such as propofol could have detrimental effects on embryos, at least in the mouse model (Janssenwillen 1997; Tatone 1998). Flushing could also remove some of the follicular cells that might have an important endocrine luteal support function.

**Why it is important to do this review**

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

### OBJECTIVES

To determine whether follicular flushing improves the live birth and ongoing pregnancy rates and increases the number of oocytes obtained in women undergoing in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI).

### METHODS

**Criteria for considering studies for this review**

**Types of studies**

Randomised controlled trials (RCTs) were eligible for inclusion if they compared a group undergoing follicular aspiration with the addition of flushing with a control group undergoing follicular aspiration alone, during the process of transvaginal oocyte retrieval for IVF or ICSI.

Crossover trials were included only when the pre-crossover data were extractable for analysis. Trials were excluded if the comparison of the flushing method was confounded by a comparison of other methods, such as type of anaesthesia, route of oocyte retrieval,
type of aspiration needles, types of flushing media and the embryo transfer technique.
For the trial to be included in the meta-analysis, all recruited women had undergone only one cycle of treatment within the context of the trial and had embryos replaced in the uterine cavity in fresh or frozen-thawed cycles. Women were not excluded if embryo replacement did not take place because of a failure of fertilization or the embryo failed to divide further (cleavage arrest).

Types of participants
Participants were women who underwent assisted conception treatment by IVF or ICSI using their own gametes and participated in a trial of follicular flushing during oocyte retrieval.

Types of interventions
Trials were included if they investigated any form of follicular flushing during oocyte retrieval. The effects of follicular flushing were compared to a control group in which flushing was not performed.
Trials replacing embryos resulting from oocytes that were derived from mixed groups of flushed and unflushed follicles in the same woman were included. Sensitivity analysis of inclusion or exclusion of these trials was performed, where appropriate.
Trials directly comparing different methods of follicular flushing (without a no-flushing control group) were also included but they were analysed and reported separately.

Types of outcome measures

Primary outcomes
- Live birth rate, defined as the number of live births per woman randomised
- Ongoing pregnancy or clinical pregnancy rate, defined as the number of clinical pregnancies that were still ongoing at the end of the study; clinical pregnancies were defined as the number of sonologically detected fetal heart pulsations per woman randomised
- Adverse events, including the miscarriage rate per woman randomised and the complication rate for the surgical procedure and during the flushing procedure

Secondary outcomes
- Oocyte yield, defined as the number of oocytes retrieved per woman randomised
- Number of embryo cryopreserved per woman randomised
- Duration of oocyte retrieval
- Volume of culture medium used to flush the follicles

Search methods for identification of studies

Electronic searches
All reports which described (or might have described) randomised controlled trials of follicular flushing were obtained using the following search strategy.
(1) The Menstrual Disorders and Subfertility Group (MDSG) Specialised Register of controlled trials was searched for any trials with follicular flushing in the title, abstract or keywords sections (Appendix 1). See the Review Group Module for more details on the make up of the Specialised Register.
(2) The following electronic databases were searched using Ovid software:
   - Menstrual Disorders and Subfertility Group Specialised Register (01.01.09 to 07.04.10) Appendix 1
   - Ovid The Cochrane Central Register of Controlled Trials (CENTRAL) (01.01.09 to 07.04.10) Appendix 4
   - Ovid MEDLINE (01.01.09 to 07.04.10) Appendix 2
   - Ovid EMBASE (01.01.09 to 07.04.10) Appendix 3
   - EMBASE is only searched one year back as the UKCC has hand searched EMBASE to this point and these trials are already in CENTRAL.
   - Ovid PSYCHINFO (01.01.09 to 07.04.10). There were no results for the PSYCINFO search limited from 2009-2010. Appendix 5
The MEDLINE search was combined with the Cochrane highly sensitive search strategy for identifying randomised trials which appears in the Cochrane Handbook of Systematic Reviews of Interventions (Version 5.0.2 chapter 6, 6.4.11)
The EMBASE and CINAHL searches are combined with trial filters developed by the Scottish Intercollegiate Guidelines Network (SIGN) http://www.sign.ac.uk/methodology/filters.html#random
(4) Other databases listing ongoing or recently completed trials, such as CentreWatch and the National Research Register, were also searched for any trials on follicular flushing.

Searching other resources
(1) The citation lists of relevant publications, review articles, abstracts of scientific meetings and included studies were searched.
(2) Letters were sent to experts within the field, pharmaceutical companies producing the products being reviewed, and authors of conference abstracts to identify unpublished trials of follicular flushing.
(3) Handsearches for RCTs for inclusion in the the MDSG Specialised Register are completed in the relevant journals therefore these were searched for any trials on follicular flushing.
Data collection and analysis

Selection of studies
Two review authors (SW, TV) independently scanned titles and abstracts identified from the searches. Potentially relevant trials were selected and independently assessed for inclusion by these review authors using an inclusion and exclusion form. Disagreements were resolved by consensus or through arbitration by a third review author (JB).

Data extraction and management
Data extraction was performed independently by two review authors (SW, TV). Where studies had multiple publications, the main trial was used as the reference and additional details supplemented from the additional sources identified. The review authors corresponded with the authors of the primary studies in order to clarify methodological and data queries.

Assessment of risk of bias in included studies
All assessments of trial quality and data extraction were performed independently by two review authors (SW, TV) using forms that were designed for the review. The trials were evaluated using the Cochrane risk of bias assessment tool (refer to Characteristics of included studies) to assess the following.

Sequence generation (e.g. low risk: investigators used random number tables, computer-generated random numbers, shuffling cards; high risk: sequence generated from date of birth, hospital or clinic record number).

Allocation concealment (e.g. low risk: central allocation, sequentially numbered sealed opaque envelopes; high risk: open number allocation schedule, alternation or rotation).

Blinding (e.g. low risk: blinding of participants or key study personnel or both, use of placebo; low risk: incomplete or no blinding, comparison group with no treatment).

Attrition bias (e.g. low risk: no missing outcome data; high risk: attrition equal to or greater than 20%).

Selective outcome reporting and other potential sources of bias (e.g. low risk: study protocol available; high risk: not all primary outcomes were reported, outcomes reported were not pre-specified).

Additional information was recorded for trial design and setting, trial participants, interventions and outcomes.

Any discrepancies in quality assessment or data extraction were resolved by consensus during discussions with the third review author (JB).

Additional information on trial methodology and actual original trial data were sought from the authors of trials that appeared to meet the eligibility criteria but had aspects of methodology that were unclear, or where the data were in a form unsuitable for meta-analysis.

Measures of treatment effect
Statistical analysis was performed in accordance with the guidelines for statistical analysis developed by The Cochrane Collaboration (Higgins 2009). For dichotomous data (for example live births), results for each trial were expressed as odds ratios (OR) with 95% confidence intervals (CI) and these were then combined for meta-analysis on RevMan 5.0 software using a random-effects model. For continuous data, mean differences between treatment groups were calculated.

Unit of analysis issues
The primary analysis was conducted as per woman randomised. Data reported in a form that did not enable valid analysis (that is per cycle rather than per woman, where women undertook more than one cycle) were summarised in the review but did not contribute to a meta-analysis.

Dealing with missing data
The data were analysed on an intention-to-treat basis, as far as possible, and attempts were made to obtain missing data from primary authors.

Assessment of heterogeneity
Heterogeneity between the results of different trials was examined using the I^2 statistic (Higgins 2009). An I^2 statistic greater than 50% is indicative of substantial heterogeneity. Where heterogeneity was detected, subgroup or sensitivity analysis was conducted to attempt to explain this.

Assessment of reporting biases
In view of the difficulty in detecting and correcting for publication bias and other reporting biases, the authors aimed to minimise the potential for bias by searching multiple databases and grey literature.
Where 10 or more trials were identified, a funnel plot was produced.

Data synthesis
The data from the primary studies was combined using a random-effects model in the comparison: follicular aspiration + flushing versus aspiration only.

Data were not stratified. An increase in the odds of a particular outcome which may be beneficial (for example live birth) or detrimental (for example adverse effects) were displayed graphically in the meta-analyses to the right of the centre line and a decrease in the odds of an outcome was to the left of the centre line.
Subgroup analysis and investigation of heterogeneity
Where heterogeneity was identified, differences in trial design were investigated through the following subgroups.
(a) Age: women below or over the age of 37 years.
(b) Follicle stimulating hormone (FSH) levels: women with normal or high early proliferative phase FSH levels. If individual data were reported, a cut-off value of 10 iu/l or more was used for subgrouping.
(c) Repeated implantation failure: following the first or multiple cycles of IVF or ICSI, or both.
(d) Poor response to ovarian stimulation: when less than three mature follicles developed following controlled ovarian stimulation for IVF or ICSI.
(e) Number of follicular flushes: single or multiple.
Where possible, data on these subgroups were extracted directly from the included trials. Where not reported, the mean trial data (for example, the mean trial FSH level) was used to place the whole trial in one of the subgroups.

Sensitivity analysis
Where required, sensitivity analyses were undertaken to examine the stability of results in relation to:
(a) adequacy of allocation concealment, by removing those trials with unclear or inadequate allocation concealment;
(b) adequacy of the randomisation process, by removing those trials with no stated method of randomisation or where the method was unclear.
Although all potential trials might be statistically homogeneous, differences in clinical parameters can be considerable (clinical heterogeneity). These differences were taken into account when analysing and interpreting the pooled results. Clinical heterogeneity in subfertility cannot be avoided because most centres use their own materials and methods, which can vary in a number of parameters. When trials meet the inclusion criteria and they have performed the same intervention, we considered it appropriate to pool their results.

Updating
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.

RESULTS

Description of studies
See: Characteristics of included studies; Characteristics of excluded studies; Characteristics of studies awaiting classification.

Results of the search
Sixteen trials providing data on follicular flushing during oocyte retrieval in assisted reproductive cycles were identified (Lenz 1987; Scott 1989; Kingsland 1991; el Hussein 1992; Tan 1992; Waterstone 1992; Biljan 1997; Dean 1997; Khalifa 1999; Ziebe 2000; Knight 2001; Ghosh 2002a; Gordon 2002; Bagtharia 2005; Mendez Lozano 2008; Levens 2009).

Included studies
Four randomised studies (Scott 1989; Kingsland 1991; Tan 1992; Levens 2009) met the inclusion criteria and were included in the final analysis. In one additional study, presented as a conference abstract, there was a lack of clarity as to whether the trial was randomised or not and the method of allocation to groups was unclear. The authors have been contacted (Ghosh 2002).
The four included studies had a total of 208 patients. Three studies were small, consisting of only 34 (Kingsland 1991), 30 (Levens 2009) and 44 women (Scott 1989). Only one study (Tan 1992) was of moderate size (100 women). Three studies were conducted in patients undergoing IVF at university hospitals in the United Kingdom (Tan 1992; Kingsland 1991) or the USA (Scott 1989). One study was conducted in an Army Medical Center in the USA (Levens 2009) and specifically involved low responders, defined as those women with a cumulative follicle count of 4 to 8 follicles ≥ 12 mm and at least two follicles achieving ≥ 16 mm. The mean ages of the low responders were 37.1 ± 3.2 and 36.2 ± 3.4 years for the single and double lumen groups, respectively (Levens 2009). They were older than the women in the other two studies, which had a median age of 31 and 30.5 years (Kingsland 1991) and 32 (25 to 42 years) and 32.5 years (23 to 43 years) (Tan 1992) in the aspiration only and aspiration with flushing groups, respectively. The age of the women was not specified in one study (Scott 1989). All the studies employed gonadotropin releasing hormone agonist in a long-luteal protocol (Kingsland 1991), long-follicular protocol (Tan 1992), long-luteal or microdose follicular flare protocol (Levens 2009) or long unspecified protocol (Scott 1989). Two studies used the same type of double-lumen needles, without (Kingsland 1991) or with (Tan 1992) the removal of the inner channel to convert it into a single-channel needle. The other two studies used single or double-lumen needles that were (Levens 2009) or were not (Scott 1989) standardised for the length and diameter, to control for the flow dynamics within the needle.

Excluded studies
One study was excluded because the design was retrospective (Knight 2001), five studies were not randomised controlled trials (Lenz 1987; el Hussein 1992; Waterstone 1992; Bagtharia 2005; Mendez Lozano 2008), four studies (Biljan 1997; Khalifa 1999; Ziebe 2000; Gordon 2002) compared two flushing media and had no aspiration-only group. One study (Dean 1997) was an an
abstract for a scientific meeting that was later published in full under a different first author (Biljan 1997), which was excluded.

**Risk of bias in included studies**

For further details refer to Figure 1 and Figure 2.

**Figure 1. Methodological quality graph: review authors’ judgements about each methodological quality item presented as percentages across all included studies.**
**Allocation**

Allocation concealment was done using sequentially numbered and sealed envelopes in two studies (Tan 1992; Levens 2009) but was not stated in the other two studies.

**Blinding**

Blinding of participants and researchers was reported by Levens 2009. None of the remaining included studies reported on blinding.

**Incomplete outcome data**

All the women randomised were analysed.

**Selective reporting**

All of the studies reported on the a priori outcomes.

**Other potential sources of bias**

No other sources of bias were identified in this review.

**Effects of interventions**

See: Summary of findings for the main comparison Summary Table

**Live birth**

No studies reported on the outcome of live birth.
Ongoing and clinical pregnancy per woman randomised

Ongoing and clinical pregnancies per woman randomised were reported in three studies (Kingsland 1991; Tan 1992; Levens 2009) in a total of 164 women. There was no evidence to suggest that follicular aspiration plus flushing of the follicles increased the chance of ongoing and clinical pregnancy when compared with aspiration alone (OR 1.17, 95% CI 0.57 to 2.38) (Figure 3).

Figure 3. Forest plot of comparison: 1 Follicular flushing, outcome: 1.5 Clinical pregnancy rate per woman randomised.

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>aspiration only</th>
<th>aspiration/flushing</th>
<th>Odd Ratio M.H. Fixed, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kingsland 1991</td>
<td>3</td>
<td>18</td>
<td>1.15 (0.20, 6.74)</td>
</tr>
<tr>
<td>Levens 2009</td>
<td>0</td>
<td>15</td>
<td>2.07 (0.52, 13.84)</td>
</tr>
<tr>
<td>Tan 1992</td>
<td>12</td>
<td>50</td>
<td>0.90 (0.30, 2.72)</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>41</td>
<td>83</td>
<td>1.17 (0.57, 2.38)</td>
</tr>
<tr>
<td>Total events</td>
<td>21</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Heterogeneity: Ch² = 1.03, df = 2 (P = 0.52), I² = 9%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: Z = 0.43 (P = 0.67)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Adverse events

None of the studies reported on the outcome of miscarriage. One study (Tan 1992) reported on three adverse events: blockage of the needle (OR 7.44, 95% CI 0.37 to 147.92), vomiting (OR 5.21, 95% CI 0.24 to 111.24) and hypotension (OR 5.21, 95% CI 0.24 to 111.24). There was no evidence of a difference between follicular aspiration plus flushing compared with follicular aspiration alone for any of these outcomes (Figure 4). In the study by Tan 1992 it was reported that there was significantly less analgesia required with the aspiration alone procedure compared with the addition of flushing (median 50 mg, range 50 to 100 mg for follicular aspiration alone; median 100 mg, range 50 to 100 mg for follicular aspiration plus flushing).
Figure 4. Forest plot of comparison: 1 Follicular flushing, outcome: 1.3 Adverse events.

### Oocyte yield

Data regarding oocyte retrieval were presented in three studies (Kingsland 1991; Tan 1992; Levens 2009). However, the data were presented as per cycle and not per woman randomised and therefore could not be used in the meta-analysis due to unit of analysis issues. Scott 1989 reported a non-significant difference between single and double-lumen needle used for aspiration and flushing (OR 0.4, 95% CI -0.43 to 1.43). Tan 1992 made the interesting remark that a surgeon who knew he was going to flush the follicle could be less cautious in emptying the follicle as completely compared with when it was going to aspirated only. They proposed that surgeon who started flushing the moment the follicle appeared empty could find a high proportion of oocytes in the flushing fluid.

### Number of embryo cryopreserved

There were no data relating to this outcome.

### Duration of procedure

Three studies (Kingsland 1991; Tan 1992; Levens 2009) reported showing a significant reduction in operating time in the aspiration group only compared with the flushing group. Two of these studies reported median data only (Kingsland 1991; Tan 1992). Levens 2009 reported a mean retrieval time of 366 ± 125 seconds for follicular aspiration plus flushing compared with 186 ± 41 seconds for follicular aspiration alone (P < 0.001). Kingsland 1991 reported a median time of 35 minutes for the follicular aspiration plus flushing group and 20 minutes for the follicular aspiration group (P < 0.001). Tan 1992 reported a median duration of 30 (range 15 to 70) minutes for the follicular aspiration plus flushing group and 15 (range 4 to 30) minutes for the follicular aspiration alone group.

### Volume of culture medium

Kingsland 1991 reported that a total of 10 ml flushing medium was used in the procedure (maximum of 2 ml at each flush) and (Scott 1989) reported 2 to 3 ml of fluid used for flushing, with one or more washes. This has implications for cost effectiveness.

### Discussion

#### Summary of main results

There were no studies which reported on the outcome of live birth. There was no evidence to support the use of the addition of flushing to follicular aspiration in oocyte retrieval. Aspiration plus flushing resulted in additional theatre time and analgesia requirements. Adverse effects were poorly reported overall. Refer to the Summary of findings for the main comparison for further details.

#### Overall completeness and applicability of evidence
The number of eligible studies that evaluated the potential beneficial effect of follicular aspiration and flushing was limited. The studies failed to follow women up to obtain data on live births. Flushing medium is relatively expensive and may not be an option for all facilities, or women, internationally. There are potential differences in technique and operator experience which may affect results, such as the techniques of oocyte aspiration, which were not controlled or described in the trials in this review. For example, in a recent retrospective study (Dahl 2009) it was shown that retrievals that utilised follicle curetting (gently and rapidly rotating the needle in a clockwise and counter-clockwise fashion inside the follicle after complete aspiration of the follicular fluid) could significantly increase the number of oocytes retrieved over aspiration without curetting (13.9 ± 0.6 versus 11.4 ± 0.6 oocytes; P = 0.003).

Another possibility is that the vacuum is usually deactivated after the needle exits the follicle in the aspiration-only group. In this manner, follicular fluid from the next follicle will flush any oocyte remaining in the dead space into the collection tube. However, the vacuum has to be deactivated just before flushing and while the needle is still inside the follicle in the aspiration with flushing group. Theoretically the negative pressure inside the follicle can suck the oocyte back (Horne 1996). This oocyte will, therefore, be either recovered in the flushing fluid or lost.

Quality of the evidence
Four randomised trials were available for analysis, comprising a total of 208 women. Moreover, the size of these trials was small (<60 women per group), their methodological quality appears to be variable, and only a limited number of trials provided data for individual outcomes. With these limitations one should be very cautious in concluding that there is no benefit in the flushing of the follicles after aspiration.

Potential biases in the review process
There are some flaws in the presentation of the data in terms of unit of analysis issues, where data are presented per cycle rather than per woman randomised. Only one study reported on the adverse effects associated with the procedure. The authors of the present review have attempted to minimise bias by conducting a rigorous search of the literature, using published and unpublished sources.

Agreements and disagreements with other studies or reviews
Studies that performed routine flushing after aspiration of the follicles reported that additional oocytes could always be obtained. For example, el Hussein 1992 obtained 40.3% of collected oocytes from the initial aspiration, 41.3% from the dead space in the collecting system, 13.7% in the first 2-ml flush, and 4.7% from the second 2-ml flush. Waterstone 1992 concluded that 20% more oocytes were obtained than with aspiration alone in 50 patients who had follicle aspiration with flushing. Bagharia 2005 found 40% of the oocytes in the primary aspiration without flushing of the follicle, while up to 82% of oocytes were retrieved with two flushes and 97% retrieved in up to four flushes. Mendez Lozano 2008 observed 46.8% oocyte recovery rate with aspiration only, compared with 84.6% with additional follicular flushing in 165 infertile women with low ovarian reserve, undergoing 271 consecutive minimal stimulation IVF cycles. The findings from this review found no support for an increase in oocyte retrieval although this is based on a single trial providing data per woman randomised. The data obtained in the studies above may be misinterpreted if they are reported per cycle rather than per woman randomised.

Authors’ conclusions
Implications for practice
The available data do not support routine flushing of the follicles after aspiration. This approach does not increase the chance of clinical pregnancy or the number of oocytes obtained. There is evidence that aspiration with flushing prolongs the retrieval time and may increase the requirement for analgesics during oocyte retrieval.

Implications for research
Up to now, there has been no study that compares the effect of follicle aspiration against aspiration and flushing in terms of live births or miscarriages per woman randomised. Further studies should include details on the technique of aspiration and flushing, such as follicle curetting, the completeness of follicle emptying before flushing. The advantage of follicle aspiration and flushing in the retrieval of immature oocytes for in vitro maturation (IVM) with ICSI and natural cycle IVF deserves further study.

Acknowledgements
We wish to thank the MDSG editorial group for their patience and kind support.

EC Edi-Osagie: involved in reviewing all sections of the protocol.
References to studies included in this review

Kingsland 1991 *(published data only)*

Levens 2009 *(published data only)*

Scott 1989 *(published data only)*

Tan 1992 *(published data only)*

References to studies excluded from this review

Bagtharia 2005 *(published data only)*

Biljan 1997 *(published data only)*

Dean 1997 *(published data only)*

el Hussein 1992 *(published data only)*


Gordon 2002 *(published data only)*

Khalifa 1999 *(published data only)*
Khalifa EAM, Buraidah KFSH. Routine use of normal saline as flushing media has no impact on fertilization, embryo development and pregnancy rates in assisted reproductive technologies. *Fertility and Sterility* 1999;72 Suppl 1:193–4.

Knight 2001 *(published data only)*

Lenz 1987 *(published data only)*

Mendez Lozano 2008 *(published data only)*

Waterstone 1992 *(published data only)*

Ziebe 2000 *(published data only)*

References to studies awaiting assessment

Ghosh 2002 *(published data only)*

Additional references

Dahl 2009

el Hussein 1992
Follicular flushing during oocyte retrieval in assisted reproductive techniques (Review)

Higgins 2009

Horne 1996

Janssenwillen 1997

Kingsland 1991

Knight 2001

Tatone 1998

Waterstone 1992

Wood 2000

* Indicates the major publication for the study

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Characteristics of included studies  (ordered by study ID)

Kingsland 1991

<table>
<thead>
<tr>
<th>Methods</th>
<th>Prospective randomised trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants</td>
<td>Country: UK</td>
</tr>
<tr>
<td></td>
<td>Site: 34 women undergoing IVF</td>
</tr>
<tr>
<td></td>
<td>Median age: 31 years for group 1 and 30.5 years for group 2</td>
</tr>
<tr>
<td></td>
<td>Inclusion: aged 35 years or less with tubal damage as the sole cause of infertility</td>
</tr>
<tr>
<td></td>
<td>Exclusion: no details</td>
</tr>
<tr>
<td>Interventions</td>
<td>Down regulation with long luteal regimen using buserelin. Ovarian stimulation with human menopausal gonadotrophin, HCG administered when at least three follicles &gt;18mm diameter</td>
</tr>
<tr>
<td></td>
<td>Transvaginal ultrasound guided retrieval using JP6L double channeled needle</td>
</tr>
<tr>
<td></td>
<td>Pain relief: 1 mg of lorazepam given orally on the evening before oocyte retrieval and repeated on the morning of aspiration. A single dose of 150 mg pethidine was administered IM 20 minutes prior to aspiration. No patients required additional anaesthesia</td>
</tr>
<tr>
<td></td>
<td>Group 1 had aspiration only versus Group 2 had follicles emptied and then flushed with 10 ml Earle's balanced salt solution (EBS, Gibco, Paisley, UK) supplemented with pyruvate, bicarbonate and buffered with HEPES, if the oocyte was not retrieved in the aspiration. A maximum of 2ml of fluid was instilled into each follicle at each flush (a maximum of five flushes per follicle)</td>
</tr>
<tr>
<td></td>
<td>All oocytes retrievals were done by the same operator. Oocytes were washed once in flushing medium, incubated at 37 degrees in 5% CO₂ in air, pre-equilibrated 1 ml drops of EBS solution supplemented with 0.11 mg/ml sodium pyruvate, 1% sodium bicarbonate, 0.02 mg gentamicin and 10% IMS</td>
</tr>
<tr>
<td>Outcomes</td>
<td>Number of oocytes obtained</td>
</tr>
<tr>
<td></td>
<td>Time taken for oocyte retrieval</td>
</tr>
<tr>
<td></td>
<td>Fertilization rate and pregnancy rate</td>
</tr>
<tr>
<td>Notes</td>
<td>Authors’ judgement</td>
</tr>
<tr>
<td></td>
<td>Description</td>
</tr>
<tr>
<td>Adequate sequence generation?</td>
<td>Unclear</td>
</tr>
<tr>
<td>Allocation concealment?</td>
<td>Unclear, no details</td>
</tr>
<tr>
<td>Blinding?</td>
<td>No</td>
</tr>
<tr>
<td>All outcomes</td>
<td>None</td>
</tr>
</tbody>
</table>

Risk of bias
### Kingsland 1991

(Continued)

<table>
<thead>
<tr>
<th>Incomplete outcome data addressed?</th>
<th>Yes</th>
<th>All women randomised were analysed</th>
</tr>
</thead>
</table>

| Free of selective reporting?      | Yes | All a priori outcomes were reported |

### Levens 2009

<table>
<thead>
<tr>
<th>Methods</th>
<th>Prospective randomised study</th>
</tr>
</thead>
</table>

| Participants                      | Patients: 30 poor responders undergoing ART  
Site: Walter Reed Army Medical Center ART Program, USA  
Mean age 37.1 ± 3.2 and 36.2 ± 3.4 years for single and double lumen groups, respectively (P=0.48)  
Inclusion criteria: low responders with a cumulative follicle count of 4-8 follicles ≥ 12 mm (with at least 2 follicles achieving >16 mm) |
|-----------------------------------|------------------------------|

<table>
<thead>
<tr>
<th>Interventions</th>
<th>Pre-treatment with OCPs during the cycle preceding ovarian stimulation. A combination of rFSH (Gonal-F) and hMG (Repronex, Ferring) were given twice daily. Adequate follicular development assessed by serial serum E2 ultrasound. hCG 10,000 IU given, followed by transvaginal oocyte retrieval 34-36 hrs later. Assignment to single or double lumen group was done immediately before oocyte retrieval. Computerised randomisation in blocks of 10-20 was used to ensure balanced group size. Concealment achieved by using sequentially numbered, opaque envelopes that were opened in the OR after anaesthesia was administered. The length and diameter of retrieval needles were standardized (35 cm, 16G) to control the flow dynamics within the needle that may affect oocyte recovery. Cook Echotip single-lumen (K-J-ANC-16R-35) and double lumen (K-OPSD-1635-B-S) transvaginal oocyte retrieval needles were used. Suction pressure of 150-200 mm Hg (provided by Pioneer Pro-pump, GenX International, CT) was used under direct transvaginal ultrasound guidance (Accuson Sequoia 512 with an 8 MHz probe). Those in the single-lumen needle group did not undergo saline follicular flushing (direct aspiration), whereas those in the double-lumen group had each aspirated follicle flushed once with 2 mL sterile PBS and subsequently re-aspirated</th>
</tr>
</thead>
</table>

| Outcomes                          | Number of oocytes obtained, recovery (%)  
Total oocytes mature, maturity (%)  
Fertilization rate (%) and implantation rate (%)  
Number of ongoing pregnancies (%)  
Retrieval times (mean ± SD) |
|-----------------------------------|------------------------------|

<table>
<thead>
<tr>
<th>Notes</th>
<th></th>
</tr>
</thead>
</table>

### Risk of bias

<table>
<thead>
<tr>
<th>Item</th>
<th>Authors’ judgement</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adequate sequence generation?</td>
<td>Yes</td>
<td>“randomly assigned” “computerised randomization in blocks of 10 and 20 to ensure balanced group size”</td>
</tr>
</tbody>
</table>
Levens 2009  (Continued)

<table>
<thead>
<tr>
<th>Allocation concealment?</th>
<th>Yes</th>
<th>&quot;Allocation was performed by the Walter Reed Army Medical Center Department of Clinical Investigation and concealed by using sequentially numbered, opaque envelopes that were opened in the operating theater after anesthesia was administered&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blinding?</td>
<td>Yes</td>
<td>Subjects unaware of treatment allocation, embryologists identifying and collecting the oocytes remained blinded to the group assignment, clinicians performing oocyte retrieval remained blinded to the number of oocytes retrieved until the completion of the procedure</td>
</tr>
<tr>
<td>Incomplete outcome data addressed?</td>
<td>Yes</td>
<td>All women randomised were analysed</td>
</tr>
<tr>
<td>Free of selective reporting?</td>
<td>Yes</td>
<td>A priori outcomes were reported</td>
</tr>
</tbody>
</table>

Scott 1989

<table>
<thead>
<tr>
<th>Methods</th>
<th>Prospective randomised study</th>
</tr>
</thead>
</table>
| Participants | Country: USA  
Patients: 44 patients undergoing IVF  
Median ages of both groups not given  
No inclusion or exclusion criteria given |
| Interventions | All patients underwent gonadotropin stimulation, using previously described protocols (in textbook, no details given in the paper)  
Retrieval with single lumen needle (SLN) (n=22) was done using Swe-Med needle (outer diameter 1.5 mm, inner diameter 1 mm). The follicle was aspirated with a hand-held 20-ml syringe, remove the needle from the patient, and then aspirate an additional 2 ml of heparinised Dulbecco’s solution through the system to wash the fluid in the dead space back into the syringe  
The double-lumen needle (DLN; Swe-Med Lab, Frolunda, Sweden) had an inner diameter of the aspiration lumen of 1 mm and the outer diameter was 1.6 mm. The follicle was aspirated and then 1-3 ml of heparinised Dulbecco’s solution was injected into the follicle through the second port. This volume was then aspirated back into the syringe. The lavage was performed one more time until the oocyte was recovered or until the follicle was not reexpanding well, before proceeding to the next follicle  
Pain relief: method not mentioned. |
| Outcomes | Number of follicles aspirated and number of oocytes retrieved  
Incidence of fractured zona in both groups |
| Notes | Risk of bias |
### Scott 1989  
(Continued)

<table>
<thead>
<tr>
<th>Item</th>
<th>Authors’ judgement</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adequate sequence generation?</td>
<td>Unclear</td>
<td>Not described</td>
</tr>
<tr>
<td>Allocation concealment?</td>
<td>Unclear</td>
<td>Not described</td>
</tr>
<tr>
<td>Blinding?</td>
<td>Unclear</td>
<td>No</td>
</tr>
<tr>
<td>Incomplete outcome data addressed?</td>
<td>Yes</td>
<td>All women randomised were analysed</td>
</tr>
<tr>
<td>Free of selective reporting?</td>
<td>Unclear</td>
<td>Fertilization rate and clinical pregnancy rate not described</td>
</tr>
</tbody>
</table>

### Tan 1992

<table>
<thead>
<tr>
<th>Methods</th>
<th>Prospective randomised study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants</td>
<td>Country: UK</td>
</tr>
<tr>
<td></td>
<td>Site: 100 women undergoing IVF treatment at an assisted conception unit</td>
</tr>
<tr>
<td></td>
<td>Median age for Group 1 was 32 (25 to 42 years), and for Group 2 was 32.5 years (23 to 43 years)</td>
</tr>
<tr>
<td></td>
<td>Exclusion: women who had developed &gt;25 or &lt;4 follicles wider than 14 mm diameter on the day of hCG administration</td>
</tr>
<tr>
<td>Interventions</td>
<td>Long follicular protocol, starting buserelin acetate (Suprefact; Hoechst, Hounslow, UK) administered intranasally (200 µg 4 hourly) on day 1 or 2 of the menstrual cycle. When serum estradiol concentration was &lt; 200 pmol/L, human menopausal gonadotropin (Pergonal; Serono, Welwyn Garden City, UK) was started at 2-6 ampoules daily. HCG (Profasi; Serano) 10,000 IU was administered when there were at least 4 follicles &gt; 14 mm diameter and the mean diameter of the largest follicle was &gt;20mm</td>
</tr>
<tr>
<td></td>
<td>Transvaginal US guided follicle aspiration was performed 33-38 hours post hCG as an outpatient procedure. Pain relief achieved using IV pethidine 50-100 mg in bolus doses of 25 mg as required</td>
</tr>
<tr>
<td></td>
<td>Aspiration using JP6L double-channel needle (Casmed, Cheam, UK). A maximum aspiration pressure of 100 mm Hg was used in both groups</td>
</tr>
<tr>
<td></td>
<td>Group 1 (Aspiration only; n=50): inner channel of needle removed to convert it into a single channel needle. Each follicle aspirated until empty. The probe was moved around until all follicular fluid was aspirated as evidenced by some blood stained fluid in the tubing. The same procedure was repeated until all follicles &gt;10 mm had been aspirated from the first ovary. After clearing the dead space in the needle the procedure was repeated in the second ovary</td>
</tr>
<tr>
<td></td>
<td>Group 2 (Aspiration and flushing, n=50): double channel needle was used and the follicle aspirated through the inner channel. This initial aspirate was termed A1. Once the follicle had been emptied the collecting tube was changed and, with the valve open, flushing medium was injected until 1.5 ml of fluid had been collected. This was termed A2. A1 and A2 were examined separately and if no oocyte was observed the follicle was flushed...</td>
</tr>
</tbody>
</table>
up to a maximum of six times
One to three embryos were transferred 48 to 72 hours after oocyte recovery

<table>
<thead>
<tr>
<th>Outcomes</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of follicles aspirated</td>
<td></td>
</tr>
<tr>
<td>and number of oocytes obtained</td>
<td></td>
</tr>
<tr>
<td>Time taken for oocyte aspiration</td>
<td></td>
</tr>
<tr>
<td>Dose of pethidine required</td>
<td></td>
</tr>
<tr>
<td>Fertilization rate and clinical</td>
<td></td>
</tr>
<tr>
<td>pregnancy rate</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Notes</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk of bias</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Item</th>
<th>Authors' judgement</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adequate sequence generation?</td>
<td>Unclear</td>
<td>Unclear as to how randomisation was performed</td>
</tr>
<tr>
<td>Allocation concealment?</td>
<td>Yes</td>
<td>Randomised by drawing serially number sealed envelopes</td>
</tr>
<tr>
<td>Blinding? All outcomes</td>
<td>No</td>
<td>Not blinded</td>
</tr>
<tr>
<td>Incomplete outcome data addressed?</td>
<td>Yes</td>
<td>Data recorded for all women</td>
</tr>
<tr>
<td>Free of selective reporting?</td>
<td>Yes</td>
<td>a priori outcomes reported</td>
</tr>
</tbody>
</table>

Characteristics of excluded studies  [ordered by study ID]

<table>
<thead>
<tr>
<th>Study</th>
<th>Reason for exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bagtharia 2005</td>
<td>All patients had repeated flushing of the follicles. The study compared the number</td>
</tr>
<tr>
<td></td>
<td>of oocytes obtained with each flushing after primary aspiration of the follicle.</td>
</tr>
<tr>
<td></td>
<td>They concluded that 40% of the oocytes were retrieved with the primary aspiration</td>
</tr>
<tr>
<td></td>
<td>without flushing of the follicle, while up to 82% of oocytes were retrieved with</td>
</tr>
<tr>
<td></td>
<td>two flushes and 97% retrieved in up to 4 flushes</td>
</tr>
<tr>
<td></td>
<td>The study was excluded as it was not an RCT and no control (aspiration only) group</td>
</tr>
<tr>
<td></td>
<td>was present</td>
</tr>
<tr>
<td>Biljan 1997</td>
<td>35 patients were randomised either to have the left or right ovary flushed with</td>
</tr>
<tr>
<td></td>
<td>heparinised normal saline or heparinised culture medium. Oocytes obtained from each</td>
</tr>
<tr>
<td></td>
<td>side were cultured separately and assessed for fertilization 18-21 hours after</td>
</tr>
<tr>
<td></td>
<td>insemination. From the side flushed with saline, 185 oocytes were collected from</td>
</tr>
<tr>
<td></td>
<td>237 follicles, which was not significantly different from 181 oocytes collected from</td>
</tr>
<tr>
<td></td>
<td>244 follicles on the side flushed with culture medium (OR 1.23, 95% CI 0.79-1.92).</td>
</tr>
<tr>
<td></td>
<td>No significant difference observed in fertilization rates between oocytes obtained</td>
</tr>
<tr>
<td></td>
<td>after saline (median 71.4%) and culture medium flush (median 75%) (OR 1.08, 95% CI</td>
</tr>
<tr>
<td></td>
<td>0.68-1.72). Reason for excluding from meta-analysis: no aspiration only group</td>
</tr>
<tr>
<td>Study (Year)</td>
<td>Methodology</td>
</tr>
<tr>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Dean 1997</td>
<td>This was an abstract of the same study as Biljan MM et al (1997), that was published in Fertility and Sterility 1997;68:1132-4</td>
</tr>
<tr>
<td>el Hussein 1992</td>
<td>The study evaluated 100 consecutive patients undergoing 100 cycles of IVF. Four patients were excluded because their embryos were electively cryopreserved. The study reported an overall oocyte recovery rate of 87.8%. Of the 1046 oocytes collected, 40.3% were from the initial aspiration (A1), 41.3% were from the dead space in the collecting system (A2), 13.7% were in the first 2-ml flush (F1), and 4.7% were from the second 2-ml flush (F2). There were comparable numbers of viable and fertilized oocytes and cleaved, transferred and frozen embryos in tubes A1 and A2, but all these parameters were significantly lower in tubes F1 and F2 (P&lt;0.0001). All these parameters were also significantly higher in F1 compared with F2 (P&lt;0.0001), except for the numbers of embryos frozen, in which there was no difference. The overall pregnancy rate/cycle was 28.1% and the pregnancy rate per ET was 31%. There was no pregnancy in any of the cycles in which embryos originating from F2 were transferred, nor was there pregnancy in cycles in which only embryos from F1 were transferred. They concluded that follicular aspiration together with one 2-ml flush maximises the recovery of oocytes that will result in pregnancies</td>
</tr>
<tr>
<td>Gordon 2002</td>
<td>A randomised study comparing 2 flushing media (Medicult flushing medium 25 cases and SynVitroFlush 22 cases) for follicle irrigation of women undergoing IVF/ICSI treatment. The study observed no differences in the number of oocytes retrieved, fertilization rates, number of embryos replaced, and clinical pregnancy per oocyte collection (2/25 or 8% versus 6/22 or 27.2% for Medicult and SynVitroFlush medium respectively; P=0.052)</td>
</tr>
<tr>
<td>Khalifa 1999</td>
<td>Forty IVF cycles in cases with &gt;10 follicles were included in the study. Each case was randomised to have the first half of follicles (&gt;14 mm) flushed with non-heparinised EBSS and the 2nd half with non-heparinised normal saline, or vice versa. One hundred and eighty five oocytes out of 276 follicles (67%) were retrieved when EBSS was used (Group I) and 187 out of 284 follicles (65.8%) when normal saline was used (Group II). There was no significant differences in fertilization (150/185, 81% versus 153/187, 82%; NS), cleavage rates (136/150, 90.6% versus 141/153, 92%; NS), grade I embryos at 48 hr (74% versus 76%) and 72 hr (68% versus 67%) in group I and II, respectively</td>
</tr>
<tr>
<td>Knight 2001</td>
<td>A retrospective study, involving 1139 cycles of oocyte aspiration only and 1139 cycles of aspiration plus flushing, at City West IVF, during 1991-1993. Twenty-three women had failed collections in each group and were excluded (leaving only 1139 in each group). (Total number of subjects in the abstract (2378) did not match that in the text (1139+1139+23+23=2324))</td>
</tr>
<tr>
<td>Lenz 1987</td>
<td>Oocyte collection was done in 53 cases by ultrasonically guided abdominal puncture under local or epidural anaesthesia. After follicle aspiration, 2-6 flushes with culture medium was performed using a syringe. A total of 196 oocytes were collected, 84 of which (42.9%) were found in the flushes. Mechanical damage was observed in 5.1% of the oocytes. Cleavage rates in mature oocytes (157) after 48 hr in culture were similar in the aspirate group (56.5%) and the flush group (54.2%). Ten clinical pregnancies were obtained, corresponding to a pregnancy rate of 18.9%</td>
</tr>
</tbody>
</table>
The study prospectively included 165 infertile women with low ovarian reserve, 20-37 years of age, undergoing 271 consecutive minimal stimulation IVF cycles from January 2005 to December 2006. Oocyte retrieval was performed 34 hr after HCG administration, rather than 36 hr, to avoid the risk of possible follicular rupture before aspiration. Follicular fluid was aspirated using a single channel 16-gauge needle attached to a 10-ml syringe. The aspiration needle was kept steady inside the follicle until the oocyte was found and isolated (follicular aspiration group; FA group). In case of negative oocyte recovery, sequential flushings were performed using 10-ml syringes filled with 3 ml of Tyrode’s salt solution. These oocytes were entered in the follicular flushing group (FF). The study observed 46.8% oocyte recovery rate with aspiration only, compared with 84.6% with additional follicular flushings. In addition, oocytes retrieved by follicular flushing demonstrated a better morphological quality (top quality embryos 43/75 or 59.7% versus 40/98 or 41.2%; P=0.01) and implantation outcome (implantation rate 34.8% versus 20.4%; P=0.04) for the corresponding embryo than those already present in the follicular fluid.

Reason for exclusion: recruited women underwent >1 cycle of treatment. Not an RCT, as aspiration was followed by flushing only when no oocyte was obtained.

All 50 patients had follicle aspiration with flushing. The origin of each oocyte was established whether it had been obtained in the initial part of the aspirate, in the dead space aspirate, in the first to third flushes, or in the fourth to sixth flushes. They concluded that 20% more oocytes were obtained than aspiration alone.

Reason for exclusion: not an RCT, only 1 group was present.

In 107 IVF/ICSI cycles, Medicult and SynVitro flushing media were prospectively randomised for use in follicle flushing. No adverse effects were noted during oocyte recovery in any of the two groups. The average number of oocytes collected (8.2 ± 2.8 versus 8.3 ± 2.9), recovery rates (86.8 ± 14.6 versus 82.8 ± 15), cleavage rates (60.7 ± 30.3 versus 61.1 ± 28.2), implantation rates (21.1% versus 18.3%) and ongoing pregnancy rates per completed cycle (27.7% versus 27.5%) were similar in the SynVitro and Medicult flushing media, respectively.

Reason for exclusion: no aspiration-only group for comparison.

Characteristics of studies awaiting assessment  [ordered by study ID]

Ghosh 2002

Methods

The methods are unclear: 156 women were allocated to one group and 172 to another but the method of allocation was not detailed. Authors have been contacted and a response is awaited.

Participants

328 women with tubal blockage, age 25-35 years, normal cycling, normogonadotrophic, normopro lactinaemic and euthyroid.

Interventions

Group A was aspirated only once versus Group B where oocyte recovery was performed with repeated follicular flushing.

Outcomes

Oocyte recovery, pregnancy rate, adverse events, miscarriage rate.

Notes

Authors contacted.
## DATA AND ANALYSES

### Comparison 1. Follicular flushing

<table>
<thead>
<tr>
<th>Outcome or subgroup title</th>
<th>No. of studies</th>
<th>No. of participants</th>
<th>Statistical method</th>
<th>Effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Clinical/ongoing pregnancy rate per woman randomised</td>
<td>3</td>
<td>164</td>
<td>Odds Ratio (M-H, Fixed, 95% CI)</td>
<td>1.17 [0.57, 2.38]</td>
</tr>
<tr>
<td>2 Oocyte retrieval per woman randomised</td>
<td>1</td>
<td>44</td>
<td>Mean Difference (IV, Fixed, 95% CI)</td>
<td>0.40 [-0.43, 1.23]</td>
</tr>
<tr>
<td>3 Adverse events</td>
<td>1</td>
<td></td>
<td>Odds Ratio (M-H, Random, 95% CI)</td>
<td>Subtotals only</td>
</tr>
<tr>
<td>3.1 Blockage of needle</td>
<td>1</td>
<td>100</td>
<td>Odds Ratio (M-H, Random, 95% CI)</td>
<td>7.44 [0.37, 147.92]</td>
</tr>
<tr>
<td>3.2 Vomiting</td>
<td>1</td>
<td>100</td>
<td>Odds Ratio (M-H, Random, 95% CI)</td>
<td>5.21 [0.24, 111.24]</td>
</tr>
<tr>
<td>3.3 Hypotension</td>
<td>1</td>
<td>100</td>
<td>Odds Ratio (M-H, Random, 95% CI)</td>
<td>5.21 [0.24, 111.24]</td>
</tr>
<tr>
<td>4 Time taken for procedure</td>
<td>Other data</td>
<td></td>
<td></td>
<td>No numeric data</td>
</tr>
<tr>
<td>5 Pain relief required during procedure</td>
<td>Other data</td>
<td></td>
<td></td>
<td>No numeric data</td>
</tr>
</tbody>
</table>

### Analysis 1.1. Comparison 1 Follicular flushing, Outcome 1 Clinical/ongoing pregnancy rate per woman randomised.

Review: Follicular flushing during oocyte retrieval in assisted reproductive techniques

Comparison: 1 Follicular flushing

Outcome: 1 Clinical/ongoing pregnancy rate per woman randomised

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>aspiration only</th>
<th>aspiration/flushing</th>
<th>Odds Ratio M-H Fixed 95% CI</th>
<th>Weight</th>
<th>Odds Ratio M-H Fixed 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kingsland 1991</td>
<td>3/16</td>
<td>3/18</td>
<td>16.4 %</td>
<td>1.15 [0.20, 6.74]</td>
<td></td>
</tr>
<tr>
<td>Levens 2009</td>
<td>6/15</td>
<td>3/15</td>
<td>12.9 %</td>
<td>2.67 [0.52, 13.66]</td>
<td></td>
</tr>
<tr>
<td>Tan 1992</td>
<td>12/50</td>
<td>13/50</td>
<td>70.7 %</td>
<td>0.90 [0.36, 2.22]</td>
<td></td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td><strong>81</strong></td>
<td><strong>83</strong></td>
<td><strong>100.0 %</strong></td>
<td><strong>1.17 [0.57, 2.38]</strong></td>
<td></td>
</tr>
</tbody>
</table>

Total events: 21 (aspiration only), 19 (aspiration/flushing)
Heterogeneity: Chi² = 1.30, df = 2 (P = 0.52); I² =0.0%
Test for overall effect: Z = 0.43 (P = 0.67)
Analysis 1.2. Comparison 1 Follicular flushing, Outcome 2 Oocyte retrieval per woman randomised.

Review: Follicular flushing during oocyte retrieval in assisted reproductive techniques

Comparison: 1 Follicular flushing

Outcome: 2 Oocyte retrieval per woman randomised

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>Single lumen needle</th>
<th>Double lumen needle</th>
<th>Mean Difference</th>
<th>Weight</th>
<th>Mean Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scott 1989</td>
<td>N=22, Mean(SD)=6.3 (1.4)</td>
<td>N=22, Mean(SD)=5.9 (1.4)</td>
<td>0.40 [-0.43, 1.23]</td>
<td>100.0%</td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>N=22, Mean=6.3 (1.4)</td>
<td>N=22, Mean=5.9 (1.4)</td>
<td>0.40 [-0.43, 1.23]</td>
<td>100.0%</td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: not applicable
Test for overall effect: Z = 0.95 (P = 0.34)
Test for subgroup differences: Not applicable

Analysis 1.3. Comparison 1 Follicular flushing, Outcome 3 Adverse events.

Review: Follicular flushing during oocyte retrieval in assisted reproductive techniques

Comparison: 1 Follicular flushing

Outcome: 3 Adverse events

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>aspiration/flushing</th>
<th>aspiration only</th>
<th>Odds Ratio</th>
<th>Weight</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n/N</td>
<td>n/N</td>
<td>M-H,Random</td>
<td>95% CI</td>
<td>M-H,Random</td>
</tr>
<tr>
<td>1 Blockage of needle</td>
<td>3/50</td>
<td>0/50</td>
<td>7.44 [0.37, 147.92]</td>
<td>100.0%</td>
<td>7.44 [0.37, 147.92]</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>50</td>
<td>50</td>
<td>100.0 %</td>
<td>7.44 [0.37, 147.92]</td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: not applicable
Test for overall effect: Z = 1.32 (P = 0.19)

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>aspiration/flushing</th>
<th>aspiration only</th>
<th>Odds Ratio</th>
<th>Weight</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n/N</td>
<td>n/N</td>
<td>M-H,Random</td>
<td>95% CI</td>
<td>M-H,Random</td>
</tr>
<tr>
<td>2 Vomiting</td>
<td>2/50</td>
<td>0/50</td>
<td>5.21 [0.24, 111.24]</td>
<td>100.0%</td>
<td>5.21 [0.24, 111.24]</td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>50</td>
<td>50</td>
<td>100.0 %</td>
<td>5.21 [0.24, 111.24]</td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: not applicable
Test for overall effect: Z = 1.06 (P = 0.29)

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>aspiration/flushing</th>
<th>aspiration only</th>
<th>Odds Ratio</th>
<th>Weight</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n/N</td>
<td>n/N</td>
<td>M-H,Random</td>
<td>95% CI</td>
<td>M-H,Random</td>
</tr>
<tr>
<td>3 Hypotension</td>
<td>0/50</td>
<td>0/50</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Continued ...)

Follicular flushing during oocyte retrieval in assisted reproductive techniques (Review) 23
Copyright © 2010 The Cochrane Collaboration. Published by John Wiley & Sons, Ltd.
### Analysis 1.4. Comparison 1 Follicular flushing, Outcome 4 Time taken for procedure.

<table>
<thead>
<tr>
<th>Study</th>
<th>Follicular Flushing</th>
<th>Aspiration only</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kingsland 1991</td>
<td>Time taken median 35 minutes</td>
<td>Time taken median 20 minutes</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Levens 2009</td>
<td>Mean retrieval time 366 ± 125 seconds</td>
<td>Mean retrieval time 186 ± 41 seconds</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Tan 1992</td>
<td>Time taken median of 30 minutes (range 15 to 70 minutes)</td>
<td>Time taken for procedure median 15 minutes (range 4 to 30 minutes)</td>
<td>P&lt;0.00001</td>
</tr>
</tbody>
</table>

### Analysis 1.5. Comparison 1 Follicular flushing, Outcome 5 Pain relief required during procedure.

<table>
<thead>
<tr>
<th>Study</th>
<th>Follicular aspiration/flushing</th>
<th>Aspiration alone</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tan 1992</td>
<td>Median 100 mg (range 50 to 100mg)</td>
<td>Median 50 mg pethidine required (range 50-100mg)</td>
<td>P&lt;0.00001</td>
</tr>
</tbody>
</table>
APPENDICES

Appendix 1. MDSG search strategy

Keywords CONTAINS “follicular flushing” or “follicular rinsing” or “Flushing” or “flushing media” or “flushing outcome” or Title CONTAINS “follicular flushing” or “follicular rinsing” or “Flushing” or “flushing media” or “flushing outcome”

Appendix 2. MEDLINE and Biological Abstracts search strategy

Database: Ovid MEDLINE(R) 01.01.09 to 07.04.10

Search Strategy:

1 (follic$ adj5 flush$).tw. (43)
2 (follic$ adj5 wash$).tw. (29)
3 ((flush$ or wash$) adj5 oocyte$).tw. (137)
4 (ovar$ adj5 flush$).tw. (45)
5 (ovar$ adj5 wash$).tw. (73)
6 or/1-5 (303)
7 randomized controlled trial.pt. (283434)
8 controlled clinical trial.pt. (80324)
9 randomized.ab. (192822)
10 placebo.tw. (119559)
11 clinical trials as topic.sh. (147253)
12 randomly.ab. (140063)
13 trial.ti. (83435)
14 (crossover or cross-over or cross over).tw. (44259)
15 or/7-14 (672637)
16 (animals not (humans and animals)).sh. (3363922)
17 15 not 16 (621220)
18 6 and 17 (7)
19 (2009$ or 2010$).ed. (904191)
20 18 and 19 (1)
21 from 20 keep 1 (1)

Appendix 3. EMBASE search strategy

EMBASE 01.01.09 to 07.04.10

1 (follic$ adj5 flush$).tw. (32)
2 (follic$ adj5 wash$).tw. (28)
3 ((flush$ or wash$) adj5 oocyte$).tw. (97)
4 (ovar$ adj5 flush$).tw. (35)
5 (ovar$ adj5 wash$).tw. (62)
6 or/1-5 (230)
7 (2009$ or 2010$).em. (913202)
8 6 and 7 (12)
9 Clinical Trial/ (588293)
10 Randomized Controlled Trial/ (184888)
11 exp randomization/ (27823)
12 Single Blind Procedure/ (9242)
13 Double Blind Procedure/ (77062)
14 Crossover Procedure/ (22728)
Appendix 4. CENTRAL (The Cochrane Library)

Database: EBM Reviews - Cochrane Central Register of Controlled Trials <1st Quarter 2010>
Search Strategy:

1 (follic$ adj5 flush$).tw. (11)
2 (follic$ adj5 wash$).tw. (6)
3 ((flush$ or wash$) adj5 oocyte$).tw. (6)
4 (ovar$ adj5 flush$).tw. (6)
5 (ovar$ adj5 wash$).tw. (1)
6 or/1-5 (21)
7 limit 6 to yr="2009 -Current" (1)
8 from 7 keep 1 (1)

Appendix 5. PsycINFO

Database: PsycINFO <1806 to April Week 1 2010>
Search Strategy:

1 (follic$ adj5 flush$).tw. (1)
2 (follic$ adj5 wash$).tw. (0)
3 ((flush$ or wash$) adj5 oocyte$).tw. (0)
4 (ovar$ adj5 flush$).tw. (1)
5 (ovar$ adj5 wash$).tw. (1)
6 or/1-5 (3)
7 from 6 keep 1-3 (3)
WHAT'S NEW

Last assessed as up-to-date: 30 March 2010.

<table>
<thead>
<tr>
<th>Date</th>
<th>Event</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>31 March 2010</td>
<td>New search has been performed</td>
<td>This review has had a search run. One new study was identified in the update and the formatting has been amended to include all the subheadings for RevMAN 5. There have been amendments also to the original protocol, some outcomes and objectives have been removed</td>
</tr>
</tbody>
</table>

HISTORY

Protocol first published: Issue 1, 2004

Review first published: Issue 9, 2010

<table>
<thead>
<tr>
<th>Date</th>
<th>Event</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 January 2010</td>
<td>New search has been performed</td>
<td>Review completed, no changes to protocol</td>
</tr>
<tr>
<td>2 April 2008</td>
<td>Amended</td>
<td>Converted to new review format.</td>
</tr>
<tr>
<td>11 November 2003</td>
<td>New citation required and major changes</td>
<td>Substantive amendment</td>
</tr>
</tbody>
</table>

CONTRIBUTIONS OF AUTHORS

T Vutyavanich: involved in preparing all sections of the protocol and review.

S Wongtra-ngan: involved in preparing sections on background, objectives, methods, the protocol and results of the review.

J Brown made substantial editorial amendments to the review.

DECLARATIONS OF INTEREST

None known
SOURCES OF SUPPORT

Internal sources
- none, Not specified.

External sources
- none, Not specified.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

In the protocol, we planned to compare single versus multiple flushes, and different volumes for flushing, in terms of live births and ongoing pregnancies in women undergoing IVF and ICSI. However, as aspiration and aspiration with flushing do not yield any difference in clinical and ongoing pregnancies, or in the number of oocytes obtained, this analysis becomes both irrelevant and unnecessary and the secondary objective was removed from the final review.

Clinical pregnancy has been moved to a primary outcome, with ongoing pregnancy.

A number of secondary outcomes have been removed from the protocol as they do not contribute to the overall aim of the study, or they represent methodological issues of bias where data are not attributable to per woman randomised. These outcomes are: fertilisation rate, rates of embryo cleavage, rates of congenital and chromosomal abnormalities, amount of anaesthetic required, cost per oocyte retrieval procedure.

The outcome of adverse events has been added as a primary outcome.

INDEX TERMS

Medical Subject Headings (MeSH)
*Ovarian Follicle; Fertilization in Vitro; Oocyte Retrieval [*methods]; Pregnancy Rate; Randomized Controlled Trials as Topic; Sperm Injections, Intracytoplasmic; Therapeutic Irrigation; Time Factors

MeSH check words
Female; Humans; Pregnancy