Time of insemination culture and outcomes of in vitro fertilization: a systematic review and meta-analysis

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BACKGROUND: Conflicting results have been reported regarding the technique of brief insemination used in IVF. The aim of this meta-analysis was to determine if better clinical outcomes of IVF are associated with a brief co-incubation of gametes than with a standard overnight co-incubation.

METHODS: A computerized search was conducted of the published literature of four databases, using search terms related to gamete, time of co-incubation and outcome measure. Eligible studies compared outcomes of IVF with a brief co-incubation of gametes to that of a control group of standard insemination and reported rates of live birth (primary outcome), normal fertilization, polyspermy, good quality embryos, implantation, clinical pregnancy or ongoing pregnancy (secondary outcomes). A total of 11 studies were included in the meta-analysis. Pooled risk ratios (RRs) and 95% confidence intervals (CIs) were calculated for the data. Statistical heterogeneity was tested using Cochran $Q$ and $I^2$ values.

RESULTS: Brief co-incubation of gametes was associated with significantly higher rates of clinical pregnancy (RR: 1.84, 95% CI: 1.24–2.73) and ongoing pregnancy (RR: 1.73, 95% CI: 1.27–2.33) than standard insemination. Brief co-incubation of gametes was associated also with a significantly
higher rate of implantation (RR: 1.80, 95% CI: 1.43–2.26) than standard insemination. However, the rates of normal fertilization (RR: 0.98, 95% CI: 0.93–1.02), good quality embryos (RR: 1.24, 95% CI: 1.0–1.53) and polyspermy (RR: 0.84, 95% CI: 0.7–1.01) were not significantly different with brief co-incubation of gametes compared with standard insemination.

**CONCLUSIONS:** Reduced gamete exposure time may be associated with beneficial outcomes. Drawbacks inherent to the quality of several studies limit the quality of the available evidence. Adequately powered randomized controlled studies need to be performed to evaluate the efficacy of brief insemination.

**Key words:** IVF / co-incubation of gametes / insemination time / meta-analysis / clinical outcomes

### Introduction

In standard IVF, oocytes are exposed to a large number of spermatozoa for 16–24 h (Trounson, 1994). Several studies have evaluated the potential deleterious effects of this long co-incubation on oocytes and sperm (Bergh et al., 1996; Walters, 1996; Ferriani et al., 1998; Free et al., 1998; Dirnfeld et al., 1999; Graham et al., 1999; Hammitt et al., 1999; Swenson et al., 2000; Bungum et al., 2006; Huang et al., 2011). The long period of co-incubation may create high levels of reactive oxygen species (ROS) which can adversely affect the quality of the embryos (Gianaroli et al., 1996a). Exposure to ROS results in hardening of the zona pellucida and can impair the implantation capacity of embryos (Dirnfeld et al., 1999, 2003; Waldenstrom et al. 1993). Successful fertilization of a human oocyte requires only one sperm to penetrate through the cumulus cells, zona pellucida and oolemma. Gianaroli et al. (1996b) reported that ~15 spermatozoa were observed inside the cumulus mass after a 15 min co-incubation and the number did not change significantly after 4 h. These findings imply that long-term co-incubation of oocytes and sperm may not be necessary and may even be harmful.

Some reports suggest that a sperm–oocyte exposure time of 1–6 h improves IVF outcomes (Gianaroli et al., 1996a; Dirnfeld et al., 1999, 2003; Kattera and Chen, 2003). However, other studies report no such advantage with a short insemination time (Lundqvist et al., 2001; Chen and Kattera, 2003; Lange et al., 2008). Studies of the rates of fertilization, polyspermy (fertilized oocytes with more than two pronuclei (2PN)), good quality embryos, implantation, clinical pregnancy and ongoing pregnancy following short-term insemination have reported contradictory results.

The objective of this study was to compare the effects of reduced (1–6 h) and longer (16–22 h) insemination times on IVF outcome. This was achieved by conducting a systematic review and meta-analysis of published studies on the topic.

### Methods

#### Selection criteria

Studies included in this review were all human randomized controlled trials (RCTs) reporting outcomes of IVF and comparing short-term insemination times with a control group of standard insemination. A minimum of one the following outcomes were documented: fertilization rate or normal fertilization rate (fertilized oocytes with 2PN); polyspermy rate or abnormal fertilization rate (more than 2PN); good embryo rate or high score embryo rate; implantation rate; clinical pregnancy rate; ongoing pregnancy rate or live birth rate. Only published material was included to ensure the quality of studies.

Short-term insemination was defined as oocytes withdrawn from the insemination medium after 1–6 h exposure to spermatozoa. There were two methods: either (i) oocytes were rinsed, washed gently and then cultured in a fresh medium or (ii) oocytes were removed from cumulus cells with surrounding corona cells left intact during washing.

In protocol (i), the cumulus cells, including degenerating cumulus cells, were not detached from the oocytes. In protocol (ii), most of the cumulus cells were removed while rinsing the oocytes.

Oocytes in the control group (standard insemination) were left to incubate with spermatozoa for 16–24 h.

#### Search strategy for identification of studies

For this systematic review, we screened PubMed, Embase, Google web search and the Cochrane central register for controlled trials published prior to August 2012, using the keywords: ‘short co-incubation, long co-incubation, brief insemination, reduced co-incubation, prolonged co-incubation, extended culture, shortened culture, shortened exposure, short insemination, extended co-incubation, reduced culture, prolonged culture’ in-combination with ‘oocyte, egg, sperm, spermatozoa and gamete’. Studies reporting a minimum of one outcome about fertilization rate; polyspermy rate; good embryo rate; implantation rate; clinical pregnancy rate; ongoing pregnancy rate or live birth rate using short-term insemination were eligible for this review. Studies reporting results of rescue ICSI using short-term insemination were also eligible for this review (Nagy et al., 1993, 2006; Chen et al., 1995; Chen and Kattera, 2003). The reference lists of all searched primary studies, review articles, citation lists of relevant publications, abstracts of major scientific meetings (for example, European Society of Human Reproduction and Embryology and American Society for Reproductive Medicine) and related studies were checked to identify additional relevant citations. No language limitations were applied.

Studies were excluded from the systematic review and the meta-analyses if the enrolled subjects had an intervention other than brief insemination. When two studies were reported by the same authors within 1 year, the smaller study was excluded from this meta-analysis in order to avoid overlap of patients and double counting (Gianaroli et al., 1996b). Trials were excluded if they reported the fertilization rate only as the mean ± SD of oocytes fertilized (Swenson et al., 2000) as no data extraction could be performed. All authors of studies included in this meta-analysis were requested to supply missing data and details of their studies. Unfortunately, only one author supplied the requested information (Zhang et al., 2009).

#### Data extraction

Information from each study was extracted independently by two reviewers (Zhang and Gao), using a standardized data extraction form. General characteristics of the study (author, year of publication, location, design, study group, maternal age, total sample size, sperm concentration for co-insemination with oocytes), characteristics of the short-term insemination and control group (criteria, selection), methodology (intervention definition, method of randomization, allocation concealment) and results were recorded when available, and double checked. Disagreements were resolved by discussion with all authors.
The primary outcome measure chosen for the current meta-analysis was live birth rate. Secondary outcome measures included clinical pregnancy rate, ongoing pregnancy rate, implantation rate, good quality embryo rate, polyspermy rate and normal fertilization rate.

Live birth was defined as the delivery of a live fetus after 20 completed weeks of gestation. Ongoing pregnancy was defined as the presence of a gestational sac with fetal heart activity at 12 weeks, confirmed by ultrasound. Clinical pregnancy was defined as the presence of a gestational sac identified by ultrasound examination.

For the determination of live birth rate, clinical pregnancy rate and ongoing pregnancy rate, the denominator was the number of patients who received embryo transfer. For the determination of good quality embryo rate, the denominator was the number of embryos transferred. For the determination of polyspermy rate and normal fertilization rate, the denominator was the number of oocytes used for insemination.

**Quantitative data synthesis**

Dichotomous data were extracted from the individual studies. Statistical heterogeneity between studies was identified using Cochran Q and I² tests. Depending on whether homogeneity was accepted or rejected, a fixed or random effect model was used to compute the combined risk ratio (RR) and its 95% confidence interval (CI). Forest plots were constructed to compare the results of brief and standard insemination times. The meta-analysis was conducted using Stata 12.0 (Statcorp, USA). The presence of publication bias was tested using the Begg’s test. The final results of this meta-analysis were assessed by grade table using Gradepro 3.6 (available from the Cochran website).

**Results**

**Search results**

The search strategy identified 283 potentially relevant studies (Fig. 1). A total of 14 papers met our inclusion criteria. In these 14 included papers, 12 were published in English, 1 in German and 1 in Chinese. After group discussion, two papers (Al-Hasani et al., 1984; Chen and Kattera, 2003) were excluded because the method design was case-control. Two papers were reported by the same authors within 1 year. One study with a small sample size was excluded from analysis (Gianaroli et al., 1996b). Finally, 11 eleven studies were included in the meta-analysis.

We first combined all studies together to calculate the pooled RR of the outcomes, regardless of the method of short-term insemination intervention. A subgroup analysis was then performed based on the method of short-term insemination. Subgroup analysis was performed to evaluate the different methods used in brief insemination (insemination time and cumulus cells removed or retained) when possible. If the two evaluations were not consistent, a meta-regression was performed to detect the cause. One study (Lin et al., 2000) compared the use of 1 h brief insemination and 3 h brief insemination. In order to prevent the potential confounding effect of time interval, we evaluated this study as two trials to calculate the effect of different insemination time. Sensitivity analyses were performed after adjusting for randomization method (patients and oocytes).

**Quality of included studies**

A study with the highest quality for the purpose of this review was of prospective design with sample sizes based upon a power calculation. The ideal study had no significant difference in female age between the short-term insemination and standard insemination groups, and poor responders to ovarian stimulation were not included. Table I shows the characteristics of the included studies in terms of study design, female age and characterization of the insemination groups. Supplementary data, Table SI displays the study populations and quality of studies included in this meta-analysis.

One high-quality RCT with a low risk of bias compared brief insemination with standard insemination in women attempting to become pregnant (Kattera and Chen, 2003). This RCT had adequate sequence generation and was free of early stopping.

Four RCTs with a moderate risk of bias compared brief insemination with standard insemination (Gianaroli et al., 1996a; Dirinfeld et al., 1999, 2003). One of these RCTs used gametes as the unit of the study and was defined as moderate risk of bias because they assessed the developing embryos that were transferred blindly and this procedure seemed reproducible (Lin et al., 2000).

Six RCTs with a high risk of bias compared brief insemination with standard insemination of oocytes (Coskun et al., 1998; Quinn et al., 1998; Boone and Johnson, 2001; Lundqvist et al., 2001; Lange et al., 2008; Zhang et al., 2009). We defined these RCTs as high risk of bias because the randomization of oocytes was performed blindly and not reproducible.

**Primary outcome**

The results of this meta-analysis for both primary and secondary outcomes were assessed using a grade table (Supplementary data, Table SI).

**Live birth rate**

One study included in this meta-analysis reported live birth data for 26 cycles after brief insemination and for 35 cycles after standard IVF. There was no evidence of a significant difference in live birth rates (RR: 1.35, 95% CI: 0.62–2.91) (Lundqvist et al., 2001).

**Secondary outcomes**

**Clinical pregnancy rate**

Two studies included in the meta-analysis reported clinical pregnancy data for 151 cycles after brief insemination and for 155 cycles after standard IVF. A significant increase in the clinical pregnancy rate was observed in the brief insemination group (RR: 1.84, 95% CI: 1.24–2.73) (Fig. 2). There was no significant group heterogeneity (test for heterogeneity, Q = 0.55, df = 1, I² = 0%, fixed effect model). No publication bias was identified in these studies (Begg’s test, P = 0.492).

**Ongoing pregnancy rate**

Two studies included in the meta-analysis reported ongoing pregnancy data for 234 cycles after brief insemination and for 211 cycles after standard IVF. A significant increase in the ongoing pregnancy rate was observed in the brief insemination group (RR: 1.73, 95% CI: 1.27–2.33) (Fig. 3). There was no significant group heterogeneity (test for heterogeneity, Q = 0.06, df = 1, I² = 0%, fixed effect model). No publication bias was observed in these studies (Begg’s test, P = 0.776).

**Implantation rate**

Four studies included in the meta-analysis reported implantation data for 894 transferred embryos undergoing brief insemination and 940
undergoing standard IVF. A significant increase in the implantation rate was observed in the brief insemination group (RR: 1.80, 95% CI: 1.43–2.26) (Supplementary data, Fig. S1). There was no significant group heterogeneity (test for heterogeneity, $Q = 1.96, df = 3, I^2 = 0\%$, fixed effect model). No publication bias was observed in these studies (Begg’s test, $P = 0.174$).

**Good quality embryo rate**

Eight studies included in the meta-analysis reported embryo quality data for 2253 cleaved 2PN zygotes after brief insemination and 2610 cleaved 2PN zygotes after standard IVF. There was no evidence of a significant difference in the rate of good quality embryos (RR: 1.24, 95% CI: 1.0–1.53) (Supplementary data, Fig. S2). There was significant heterogeneity between these groups (test for heterogeneity, $Q = 83.32, df = 7, I^2 = 91.6\%$, randomized effect model).

**Normal fertilization rate**

Seven studies in this meta-analysis reported fertilization data on 3380 oocytes undergoing brief insemination and 3580 undergoing standard IVF. There was no evidence of a significant difference in rate of normal fertilization (RR: 0.98, 95% CI: 0.93–1.02) (Supplementary data, Fig. S3). There was significant between study heterogeneity (randomized effect model, $Q = 13.13, df = 6, I^2 = 54.3\%$). No publication bias was observed in these studies (Begg’s test, $P = 0.665$).

**Polyspermy rate**

Eight studies included in the meta-analysis reported polyspermy data for 3545 fertilized oocytes undergoing brief insemination and 3721 undergoing standard insemination. There was no significant difference in the polyspermy rate (RR: 0.84, 95% CI: 0.7–1.01) (Supplementary data, Fig. S4). There was significant between study heterogeneity ($Q = 11.19, df = 7$,
Table I  Characteristics of studies included in the meta-analysis.

<table>
<thead>
<tr>
<th>Study</th>
<th>Randomized methods</th>
<th>Methods of brief insemination</th>
<th>Study groups</th>
<th>Maternal age</th>
<th>Sperm concentration</th>
<th>Total sample size</th>
<th>Outcome measure</th>
<th>Risk of bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kattera and Chen (2003)</td>
<td>Randomized by patient</td>
<td>Cumulus cell removed</td>
<td>2 h (subgroup by male factor) 20 h</td>
<td>2 h: 35.4 ± 4.1 20 h: 35.1 ± 3.9</td>
<td>2 h: 20–30 × 10^3 spermatozoa and 1 × 10^5 spermatozoa per oocyte 20 h: 20–30 × 10^3 spermatozoa per oocyte</td>
<td>2 h: total 130 patients (1105 oocytes) 20 h: 129 patients (1200 oocytes)</td>
<td>Normal fertilization rate Abnormal fertilization rate Good quality embryo rate Implantation rate</td>
<td>Low</td>
</tr>
<tr>
<td>Dirnfeld et al. (1999)</td>
<td>Randomized by patient</td>
<td>Cumulus cell retained</td>
<td>1 h 16–24 h</td>
<td>1 h: 32.8 ± 3.8 16–24 h: 33.2 ± 4.2</td>
<td>Both 20–50 × 10^3 per oocyte</td>
<td>1 h: 72 patients (732 oocytes) 16–24 h: 86 patients (822 oocytes)</td>
<td>Fertilization rate Good quality embryo rate Implantation rate Pregnancy rate</td>
<td>Moderate</td>
</tr>
<tr>
<td>Lange et al. (2008)</td>
<td>Randomized by oocyte</td>
<td>Cumulus cell retained</td>
<td>1 h 18 h</td>
<td>15 × 10^3 spermatozoa per ml</td>
<td>Total 240 patients 1 h: 1888 oocytes 18 h: 926 oocytes</td>
<td>Normal fertilization rate D2/D3 good quality embryo rate</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>Quinn et al. (1998)</td>
<td>Randomized by oocyte</td>
<td>Cumulus cell removed</td>
<td>1 h 18 h</td>
<td>31.9 ± 2.3</td>
<td>Both 25–40 spermatozoa per drop 1 h: 114 oocytes 18 h: 101 oocytes</td>
<td>Fertilization rate 3PN rate Morphological score of embryos on D2/D3</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>Dirnfeld et al. (2003)</td>
<td>Randomized by patient</td>
<td>Cumulus cell removed</td>
<td>2 h overnight</td>
<td>2 h: 30 ± 4.5 Overnight: 30 ± 6.5</td>
<td>Both 20–50 × 10^3 per oocyte 2 h: 10 patients (30 oocytes) Overnight: 13 patients (79 oocytes)</td>
<td>Good quality embryo rate Zona thickness</td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td>Lundqvist et al. (2001)</td>
<td>Randomized by oocyte</td>
<td>Cumulus cell removed</td>
<td>2 h 18 h</td>
<td>32 (25–40)</td>
<td>Both 2.5 × 10^5 spermatozoa per dish 2 h: 26 patients (488 oocytes) 18 h: 35 patients (504 oocytes)</td>
<td>Normal fertilization rate Polyspermy rate Good quality embryo rate Implantation rate Positive hCG rate Clinical pregnancy rate</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>Gianaroli et al. (1996a)</td>
<td>Randomized by patient</td>
<td>Cumulus cell removed</td>
<td>1 h (subgroup by male factor) 16 h</td>
<td>1 h: 32.7 ± 3 16 h: 32 ± 3.4</td>
<td>Both 2–10 × 10^3 spermatozoa per microdroplets 1 h: 85 patients (595 oocytes) 16 h: 82 patients (555 oocytes)</td>
<td>Normal fertilization rate Polyspermy rate Implantation rate Clinical pregnancy rate Aborting rate Ongoing pregnancy rate Good quality embryo rate</td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td>Lin et al. (2000)</td>
<td>Randomized by oocyte</td>
<td>Cumulus cell retained</td>
<td>1 h 3 h 16–18 h</td>
<td>NA</td>
<td>Both 50–100 × 10^3 spermatozoa per oocyte 1 h: 8 patients (34 oocytes) 3 h: 14 patients (78 oocytes) 16–18 h: 22 patients (216 oocytes)</td>
<td>Normal fertilization rate 3PN rate Good quality embryo rate</td>
<td>Moderate</td>
<td></td>
</tr>
</tbody>
</table>

Continued
$I^2 = 37.4\%$, fixed effect model). No publication bias was observed in these studies (Begg’s test, $P = 0.602$).

**Subgroup analysis: method of brief insemination**

**Live birth rate/ongoing pregnancy rate**
The treatment intervention used in the studies reporting live birth rate and ongoing pregnancy rate was cumulus cell removal. Therefore, no subgroup analysis was required.

**Clinical pregnancy rate**
A separate analysis was performed for brief insemination using retained cumulus cells (RR: 1.63, 95% CI: 1.01–2.63) and removed cumulus cells (RR: 2.22, 95% CI: 1.13–4.37). A significant increase in the clinical pregnancy rate was observed in both subgroups, compared with standard insemination.

**Implantation rate**
A significant increase in the implantation rate was observed in the subgroup of removed cumulus cells (RR: 1.84, 95% CI: 1.42–2.38). There was no significant difference in implantation rate in the cumulus cell retained subgroup (RR: 1.65, 95% CI: 1.01–2.73), compared with standard insemination.

**Good quality embryo rate**
There was no significant increase in the rate of good quality embryos after retention of cumulus cells (RR: 1.24, 95% CI: 0.95–1.62) or removal of cumulus cells (RR: 1.24, 95% CI: 0.88–1.74), compared with standard insemination.

**Normal fertilization rate**
Analysis of brief insemination with retention of cumulus cells (RR: 0.94, 95% CI: 0.89–1.02) and removal of cumulus cells (RR: 0.99, 95% CI: 0.93–1.05) showed no benefit over standard insemination.

**Polyspermy rate**
Analysis of brief insemination with retention of cumulus cells (RR: 0.94, 95% CI: 0.64–1.38) and removal of cumulus cells (RR: 0.81, 95% CI: 0.66–1) showed no benefit over standard insemination.

**Subgroup analysis: time of brief insemination**

**Live birth rate/clinical pregnancy rate**
The time of brief insemination reported in hours was obtained from studies reporting live birth rate and clinical pregnancy rate, but no subgroup analysis was required.

**Ongoing pregnancy rate**
A significant increase in the rate of ongoing pregnancy was observed after a 2 h of brief insemination (RR: 1.69, 95% CI: 1.22–2.34). A report using 1 h of brief insemination (RR: 1.87, 95% CI: 0.86–4.06) showed no significant difference from standard insemination.

**Implantation rate**
A significant increase in the rate of implantation was observed in reports evaluating either 1 h brief insemination (RR: 1.71, 95% CI: 1.16–2.51) or 2 h brief insemination (RR: 1.86, 95% CI: 1.4–2.46).
Good quality embryo rate
The rate of good quality embryos was reported for 1 h brief insemination (RR: 1.16, 95% CI: 0.9–1.5), 2 h brief insemination (RR: 1.54, 95% CI: 1.04–2.26), 3 h brief insemination (RR: 1.03, 95% CI: 0.86–1.23) and 4 h brief insemination (RR: 0.93, 95% CI: 0.75–1.16).

Normal fertilization rate
The rate of normal fertilization was reported for 1 h brief insemination (RR: 0.97, 95% CI: 0.9–1.05), 2 h brief insemination (RR: 0.98, 95% CI: 0.94–1.03), 3 h brief insemination (RR: 0.99, 95% CI: 0.89–1.11) and 4 h brief insemination (RR: 0.93, 95% CI: 0.77–1.13). No benefit of brief insemination was observed.

Meta-regression
To further investigate the impact of the different reported methods on the rates of implantation, good quality embryos, polyspermy and normal fertilization, meta-regression analyses were performed. RR, using variable rates as the dependent variable, and methods and insemination time intervals as the independent variables, were determined. There was no evidence that the method used or the insemination time was a confounding factor in this subgroup analysis.

Sensitivity analysis: randomization units
A sensitivity analysis was performed based on randomization. Meta-analyses were repeated after excluding all studies where randomized oocytes were likely to be a confounding factor. Seven studies were excluded because the randomization was not based on patients (Coskun et al., 1998; Quinn et al., 1998; Lin et al., 2000; Boone and Johson, 2001; Lundqvist et al., 2001; Lange et al., 2008; Zhang et al., 2009).

Live birth rate/clinical pregnancy rate/ongoing pregnancy rate
The randomization used in the studies reporting live birth rate, clinical pregnancy rate and ongoing pregnancy rate was per patient. Therefore, no sensitivity analysis was required.

Implantation rate
Three studies reported data for 842 transferred embryos after brief insemination and 870 after standard IVF. A significant increase in the rate of implantation was observed in the brief insemination group (RR: 1.89, 95% CI: 1.48–2.42).

Good quality embryo rate
Three studies were available to evaluate the rate of good quality embryos after sensitivity analysis. Data were reported for 1251 cleaved 2PN...
zygotes after brief insemination and 1430 after standard IVF. Brief insemination showed a significant increase in the rate of good quality embryos compared with standard IVF (RR: 1.75, 95% CI: 1.6–1.92).

**Polyspermy rate**
Two studies reported 1700 fertilized oocytes treated with brief insemination and 1728 with standard insemination. No significant difference in the RR of polyspermy was observed (RR: 0.85, 95% CI: 0.68–1.06).

**Normal fertilization rate**
Data from six studies were pooled and data on 1700 oocytes treated with brief insemination and 1755 treated with standard IVF were reported. There was no difference in the rate of normal fertilization (RR: 1.02, 95% CI: 0.98–1.06) in these studies.

**Discussion**

**Summary of evidence**
This systematic review and meta-analysis demonstrated that brief insemination was associated with increased rates of clinical pregnancy (RR: 1.84, 95% CI: 1.24–2.73) and ongoing pregnancy (RR: 1.73, 95% CI: 1.27–2.33). Unfortunately, only one study included in this meta-analysis reported the live birth rate (RR: 1.35, 95% CI: 0.62–2.91) and this study had a high risk of bias (Lundqvist et al., 2001). Thus, limitations in methodology and small sample size reduced the reliability of findings related to live birth rate. While treatment-orientated success rates can act as a surrogate for the desired outcome, it is important that more distal patient-orientated outcomes, such as live birth rates, be reported. Future studies should include live birth rates.

Poor methodological design of small studies and inadequate analysis can reduce the usefulness of reported studies in evaluating clinical pregnancy and ongoing pregnancy rate. Only one study (Kattera and Chen, 2003) calculated the sample size before testing was performed. None of the included studies (Gianaroli et al., 1996a; Dirnfeld et al., 1999; Kattera and Chen, 2003) used allocation concealment and blinding, and the studies appeared to have used selective reporting.

This meta-analysis detected a significantly higher implantation rate (RR: 1.80, 95% CI: 1.43–2.26) but no difference in polyspermy rate (RR: 0.84, 95% CI: 0.71–1.01) after brief insemination.

Furthermore, we found no advantage of brief insemination in normal fertilization rate, in contrast to a previous report (Gianaroli et al., 1996a). The difference between that report and other studies may be different sample sizes or methods used, as that study was responsible for the heterogeneity ($I^2 = 54.3\%$, $P < 0.05$) observed in this meta-analysis.

The rate of development of good quality embryos may be a less objective indicator of IVF outcome because there is no universally accepted definition. Nevertheless, this study demonstrated no advantage for brief insemination in good quality embryo rate.

We performed subgroup analyses for brief insemination methods and different brief insemination times to try to account for the discrepancy found in these factors. Brief insemination methods and different brief insemination times did not appear to be independent variables in the brief insemination protocol. A sensitivity analysis was also performed, and exclusion of studies with randomization of oocyte rather than patients had little effect on the outcomes of the meta-analysis.

![Figure 3](image-url)
**Strength and limitations of the study**

All studies included in this meta-analysis shared the common design of comparing a group of patients or gametes after brief insemination with a group using standard overnight insemination. One high quality trial and one moderate quality trial were included in the ongoing pregnancy rate and clinical pregnancy rate evaluations. The quality of the evidence was moderate due to the absence of blinding in these studies.

The normal fertilization rate and good quality embryo rate were less reliable because there was statistical evidence for heterogeneity between the studies with these outcomes. The heterogeneity in the normal fertilization rate finding was most likely due to one study (Gianaroli et al., 1996a) that reported a positive effect on normal fertilization rate (RR: 1.09, 95% CI: 1.01–1.18), but had a small sample size. This study employed a brief insemination using an intervention protocol that required delicate detachment of any digested cumulus cells. We hypothesize that detachment of the digested cumulus cells may not have removed spermatozoa already combined with the zona pellucida. This could allow completion of fertilization. The good quality embryo rate was likely affected by the less objective measurements and small sample sizes of the included studies.

**Mechanisms of the observed effects and confounding factors**

The success rate of any outcome of IVF is not independent of other outcomes and is a reflection of previous stages in oocyte treatment (Winston and Handyside, 2007). The exact mechanisms by which brief insemination may exert a positive effect on IVF outcome are not known. Several factors may be important at different stages of IVF. Previous research suggests that gamete contact and fertilization occurs within 20 min of coitus in mammals. An average of 15 spermatozoa has been observed in vitro in the cumulus after 15 min of gamete contact (Wasserman, 1987, 1988; Gianaroli et al., 1996b). Longer co-incubation may release high levels of ROS, causing a decrease in membrane fluidity (Aitken and Clarkson, 1987) and DNA fragmentation (Twigg et al., 1998).

A potential confounding factor influencing the outcome of IVF is whether the cumulus cells of oocytes are removed or retained after brief insemination. The advantage of the second protocol was that oocytes were observed to be fertilized 2–4 h after exposure to spermatozoa, and the second polar body was released in 90% of fertilized oocytes by 6 h (Plachot et al., 1986; Nagy et al., 1994; Payne et al., 1997). The embryologist determined whether release of the second polar body occurred by 6 h after initial insemination and performed rescue ICSI on those oocytes in which a second polar body was not evident. It is thought (Ménézo and Barak, 2000) this modified removal protocol may improve embryo quality. However, the mechanical stress from the denuding pipette used in the cumulus cell removal procedure may adversely affect the zygote. The zygote is especially vulnerable soon after fertilization because the spindle and microtubules are active at this time (Wang et al., 2001a, b; Wang and Keefe, 2002). The benefit of cumulus and corona co-culture with the pronucleate oocytes has been previously described (Mansour et al., 1994; Quinn, 1994; Tao et al., 2004), and partial cumulus removal before insemination has been reported to decrease the normal fertilization rate and Day 3 embryo quality in humans (Tao et al., 2004). Cumulus cells maintain the oocyte under meiotic arrest (Eppig, 1989).

We found higher polyspermy rates in oocytes removed from the cumulus cells 6 h post-insemination compared with those removed 20 h after insemination (Xiong et al., 2011). In an attempt to account for this heterogeneity, subgroup analysis was performed using the brief insemination protocol that retained or removed the cumulus cells during media change. Subgroup analyses showed that use of the denuding pipette during cumulus cell removal was not harmful to the clinical pregnancy or implantation rate.

Time-lapse cinematography has demonstrated sperm penetrate the zona pellucida after 1.5 ± 0.2 h and the equatorial segment of the sperm head is incorporated with the oocyte membrane after 2.4 ± 2.2 h (Mio and Maeda, 2008). The sperm–oocyte exposure time was identified as a likely cause of clinical heterogeneity between studies (Rehman et al., 1994; Korchar and King, 1998; Korchar et al., 1999). Since the definition of a short time varied widely between studies in this meta-analysis, a subgroup analysis was performed. The subgroups of 1, 2, 3 or 4 h incubations had small sample sizes in each time group which prevented detection of significant differences.

Another potential confounding factor in this study was the numbers of spermatozoa added to the medium containing the oocytes. An excess number of spermatozoa in the insemination medium can lead to detrimental effects on human embryo viability (Dumoulin et al., 1992). We believe a standard protocol should exist for how many spermatozoa are appropriate for optimal IVF.

**Clinical implications**

This meta-analysis found brief insemination may improve pregnancy rates and some laboratory surrogates for pregnancy. The method and time of brief insemination used in daily laboratory practice requires more detailed study.

Time-lapse cinematography or more detailed monitoring may be useful in evaluating brief insemination. These techniques allow detailed and dynamic analysis of the development of human preimplantation embryos and could identify the time-points of different embryonic stages. Previously, brief insemination has been evaluated only for 1–6 h after insemination. The aim of brief insemination is to reduce potential deleterious effects of long-term incubation of oocytes and sperm. Time-lapse cinematography could be helpful in identifying these harmful changes.

Despite the potential advantages, the use of short insemination requires cells to be processed by embryologists during evening or later shifts. Two teams of embryologists are generally needed to implement brief insemination.

**Conclusions**

This meta-analysis of short insemination in IVF has demonstrated reduced gamete exposure time to be beneficial to clinical outcome. Drawbacks inherent to the quality of several studies, such as lack of allocation concealment and no blinding, limit the quality of the available evidence.

A potential confounding effect on the evaluation of laboratory methods is the methodology used, such as the use of oocytes or embryos as the randomized unit. We found that if the observational unit was patients, significantly positive effects were also observed in the implantation rate and good quality embryo rate. We do not have
any proof that these effects were spurious. Nevertheless, these effects must be interpreted with caution.

To better evaluate these issues, future studies should randomize patients, control for the removal of cumulus cells of oocytes in brief in-semination protocols and standardize the sperm numbers added to the medium containing the oocyte. The live birth rate should be reported in every future RCT. This outcome has not been adequately evaluated in previous studies.

Supplementary data
Supplementary data are available at http://humupd.oxfordjournals.org/.

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Authors’ roles
X.D.Z. designed the study, analyzed and interpreted the data and drafted the manuscript. J.X.L. selected the articles and revised the manuscript. Y.G. selected the articles and retrieved the data. W.H. selected the articles and revised the manuscript. S.X. selected the articles and revised the manuscript. W.W.L. selected the articles and supervised the study. All the authors approved the final version of the manuscript.

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Conflict of interest
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