Artificial oocyte activation to improve reproductive outcomes in women with previous fertilization failure: a systematic review and meta-analysis of RCTs

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STUDY QUESTION: In couples with previous fertilization failure, are reproductive outcomes improved using ICSI followed by artificial oocyte activation (ICSI-AOA) compared with conventional ICSI?

SUMMARY ANSWER: There is insufficient evidence available from RCTs to judge the efficacy and safety of ICSI-AOA for couples with previous fertilization failure.

WHAT IS KNOWN ALREADY: In cases with previous low fertilization rates or total fertilization failure using ICSI due to sperm-related, oocyte activation deficiency, several methods of AOA have been described, which employ mechanical, electrical or chemical stimuli. Reported fertilization and pregnancy rates appear to be improved after ICSI-AOA compared with conventional ICSI; however, the small studies performed to date make it difficult to assess the clinical efficacy or safety of AOA.

STUDY DESIGN, SIZE, AND DURATION: The present systematic review and meta-analysis identified RCTs that compared ICSI-AOA and conventional ICSI. The last electronic search was conducted in August 2014 and there was no limitation regarding language, publication date, or publication status. We included studies that randomized either oocytes or women and included them in two different parts of this review: a women-based review and an oocyte-based review. For the women-based review, the primary outcome of effectiveness was live birth per randomized woman and the primary outcome for safety was congenital anomalies per clinical pregnancy. For the oocyte-based review, the primary outcome was embryo formation per oocyte randomized.

PARTICIPANTS/MATERIALS, SETTING, AND METHODS: Record screening and data extraction were performed independently by two authors and risk of bias was assessed by three authors. The effects of ICSI-AOA compared with conventional ICSI were summarized as risk ratio (RR) and the precision of the estimates was evaluated by the 95% confidence interval (CI).

MAIN RESULTS AND THE ROLE OF CHANCE: A total of 14 articles were assessed for eligibility and 9 included in the meta-analysis: 2 studies comprised the woman-based review (n = 168 women) and 7 studies the oocyte-based review (n = 4234 oocytes). Only four studies evaluated AOA due to fertilization failure after conventional ICSI: these were included in the quantitative analysis. In two studies evaluating couples with a history of fertilization failure in a previous cycle, ICSI-AOA was associated with an increase in the proportion of cleavage stage embryos (RR 5.44, 95% CI 2.98–9.91) and top/high quality cleavage stage embryos (RR 10.02, 95% CI 2.45–40.95). There was no evidence of effect on fertilization rate (RR 2.97, 95% CI 0.84–10.48). In the two studies that evaluated ICSI-AOA as a rescue method for unfertilized oocytes after conventional ICSI, ICSI-AOA was associated with an increase in fertilization (RR 8.26, 95% CI 1.28–53.32, P = 0.03) and cleavage
rates (RR 8.65, 95% CI 2.28–32.77) although there was no significant effect on the likelihood of blastocyst formation (RR 1.97, 95% CI 0.11–34.99). The remaining five studies evaluated ICSI-AOA for reasons other than fertilization failure and were excluded.

**LIMITATIONS AND REASONS FOR CAUTION:** The majority of the studies were not considered to be similar enough for meta-analysis due to different AOA methods and patient inclusion criteria, thus limiting the possibility of pooling studies and achieving a more robust conclusion. Only two studies examined ICSI-AOA in couples with previous fertilization failure, and only one of those included couples with proven male-related, oocyte activation deficiency, which is the primary indication for AOA. The resulting evidence was considered to be of very low quality and should be interpreted with caution.

**WIDER IMPLICATIONS OF THE FINDINGS:** There is insufficient evidence available from the currently available RCTs to judge the efficacy or safety of ICSI-AOA on key reproductive outcomes in couples with previous fertilization failure. Such interventions should be further examined by well-designed RCTs before the introduction of ICSI-AOA as a standard treatment.

**STUDY FUNDING/COMPETING INTEREST(S):** No funding was obtained. No competing interests to declare.

**REGISTRATION NUMBER:** PROSPERO CRD42014007445.

**Key words:** ionophore / artificial oocyte activation / fertilization failure / ICSI

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**Introduction**

ICSI has allowed the achievement of pregnancy through the injection of a single spermatozoon into the cytoplasm of a mature oocyte for couples with male factor infertility (Palermo et al., 1992). However, complete or nearly complete fertilization failure, when at least three or more mature oocytes are available, still occurs in 1–5% of ICSI cycles (Kashir et al., 2010; Nasr-Esfahani et al., 2010).

Although the exact cause may vary, failure of oocyte activation is thought to be an important cause of fertilization failure following conventional ICSI (Vanden Meerschaut et al., 2014b). Oocyte activation is a complex and spatial-temporal, regulated process induced by a series of intracellular calcium oscillations from endoplasmic reticulum stores as a result of sperm entry into the ooplasm (Tesarik et al., 1994). The calcium rise begins a few minutes after sperm–oocyte fusion following conventional IVF (Lawrence et al., 1997), while it is triggered immediately after the introduction of single spermatozoon into the ooplasm during ICSI due to the calcium influx from the surrounding injection medium (Vanden Meerschaut et al., 2014b). Calcium oscillations continue during the process of fertilization but their amplitude and frequency progressively decline, in a species-specific manner, until they stop at the time of pronuclei formation (Maragos et al., 2003; Ducibella and Fissore, 2008).

It is generally accepted that the rises in calcium play a key role in triggering all downstream nuclear and cytoplasmic changes in fertilized oocytes, leading to successful oocyte activation and the onset of embryogenesis (Miyazaki and Ito, 2006; Ramadan et al., 2012). Consequently, artificial oocyte activation (AOA) methods aim to reproduce this through inducing artificial calcium rises (Alberto et al., 2001). Sperm-specific phospholipase C-zeta (PLCζ), located in the peri-nuclear theca of spermatozoa, appears to be the major factor responsible for inducing intracellular calcium oscillations via an inositol-1,4,5-triphosphate (IP3)-mediated pathway (Saunders et al., 2002; Kashir et al., 2010; Ramadan et al., 2012). Consequently, it is proposed that failure of oocyte activation, and thus failed fertilization, are usually a result of sperm-related deficiency in the PLCζ cascade (Yoon et al., 2012; Nomikos et al., 2013), without excluding the possibility that incomplete nuclear and/or cytoplasmic oocyte maturation may inhibit the response to sperm PLCζ (Swain and Pool, 2008). It has been suggested that oocytes become progressively competent allowing full activation and normal development during their arrest at the metaphase II (MII) stage following the extrusion of the first polar body, with the proportion of normally activated fertilized oocytes gradually increasing with prolongation of the duration of MII arrest (Balakier et al., 2004).

Oocyte activation comprises a number of cytological processes, including zona pellucida remodeling through the cortical reaction as a measure to prevent polyspermy, resumption of meiosis, decondensation of the sperm nucleus, formation of female and male pronuclei, maternal mRNA and protein accumulation, post-translational modifications, and cytoskeleton rearrangements (Ducibella and Fissore, 2008; Horner and Wolfner, 2008). Animal models suggest normal oocyte activation and embryonic development are largely dependent not only on a rise in intracellular calcium but rather a complex interplay between the number, frequency, amplitude, and duration of calcium oscillations (Vanden Meerschaut et al., 2014b). The specific pattern of calcium oscillation during the activation period is thought to have long-term effects on subsequent pre- and post-implantation events, such as gene expression, methylation status, and possibly development to term (Ducibella et al., 2002; Ozil et al., 2006; Töth et al., 2006; Ducibella and Fissore, 2008). Although comparable human data are currently lacking, the above findings highlight the importance of calcium oscillation in embryonic development and pregnancy outcome. In contrast, chemical and electrical methods of AOA, used in human assisted reproductive technology (ART), induce an aberrant calcium rise that includes a single surge without subsequent oscillations (Swann and Ozil, 1994; Vanden Meerschaut et al., 2014b). This raises concerns regarding the safety and physiological relevance of AOA and requires further clinical evaluation.

Several methods have been described in the literature to overcome human oocyte activation failure by the induction of AOA. These methods, which employ either mechanical (Tesarik et al., 2002; Ebner et al., 2004), electrical (Yanagida et al., 1999) or chemical stimuli (Borges et al., 2009a; Kyono et al., 2012), aim to initiate artificial Ca²⁺ rises in the oocyte cytoplasm. ICSI followed by AOA (ICSI-AOA) is therefore primarily intended for patients with male-related oocyte activation deficiency, which may be diagnosed using heterologous ICSI models (Heindryckx et al., 2005, 2008; Vanden Meerschaut et al., 2012). Reported fertilization and pregnancy rates appear to be improved after ICSI-AOA compared with conventional ICSI (ICSI-only); however, the small studies performed so far makes it difficult to assess the clinical
efficacy and safety of AOA. Identification and evaluation of the whole body of evidence would facilitate more robust conclusions.

Our objective was to identify, appraise and summarize the current evidence on the efficiency of ICSI-AOA compared with ICSI-only in patients with fertilization failure, by conducting a systematic review of the literature and a meta-analysis of any suitable randomized trials.

Methods

Protocol and registration

The protocol for this review was registered at PROSPERO (CRD42014007445).

Eligibility criteria

Only RCTs that compared ICSI-AOA with ICSI-only were considered eligible. We included studies that randomized either oocytes or women but they were analysed separately as a women-based review and an oocyte-based review.

Information sources

We searched for RCTs in the following electronic databases from their inception: Cochrane Central Register of Controlled Trials (CENTRAL); Cumulative Index to Nursing and Allied Health Literature (CINAHL) (www.ebscohost.com/cinahl/); Literatura Latino-Americana e do Caribe em Ciências da Saúde (LILACS); Medical Literature Analysis and Retrieval System Online (MEDLINE); PsycINFO; and Scopus. We searched for study protocols and ongoing trials in the following trials registers: ClinicalTrials.gov (www.clinicaltrials.gov); Current Controlled Trials (www.controlled-trials.com/issctrn/); and World Health Organization International Clinical Trials Registry Platform (WHO-ICTRP) (www.who.int/trialsearch/Default.aspx). We searched for conference proceedings in Web of Science (http://apps.webofknowledge.com/) and for grey literature in the Open Grey (www.opengrey.eu/). The following terms were used, adjusting for each database as necessary: (activat*) AND ((Intracytoplasmic Sperm Injection*) OR (ICSI) OR (in vitro fertilization) OR (in vitro fertilisation) OR (IVF) OR (embryo transfer) OR (blastocyst)) AND ((trial) OR (random*)). Additionally, we hand-searched the reference list from included trials and similar reviews.

Study selection

The records were screened independently by two review authors (IAS and MLSL) and full-texts were obtained when necessary; disagreements were solved by consulting a third author (WPM). Authors corresponded with study investigators to clarify study eligibility when required. There was no limitation regarding language, publication date or publication status.

Summary measures

The effects of the intervention were summarized as risk ratio (RR) and the precision of the estimates was evaluated by the 95% confidence interval (CI). We considered the clinical relevance of all comparisons taking into account the precision of the estimates: RR between 0.91 and 1.1 was considered as no relevant effect; 0.83–0.91 or 1.1–1.2 was considered a small effect, 0.67–0.83 or 1.2–1.5 a moderate effect, 0.5–0.63 or 1.5–2.0 a large effect, <0.5 or >2.0 a very large effect. We planned to determine the number needed to treat for an additional beneficial outcome or the number needed to harm for an additional harmful outcome, when a significant benefit or harm was observed.

Synthesis of results

The results were combined for meta-analysis using Review Manager 5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) in a random-effects model because the intervention varied among the studies. Heterogeneity was assessed by the I² statistic. The data from primary studies were combined comparing ‘ICSI-AOA’ versus ‘ICSI-only’ in two strata: ‘Previous fertilization failure’ and ‘Rescue method’, accordingly with the clinical indication for AOA. An increase in the risk of a particular outcome associated with ICSI-AOA, which may be beneficial (e.g. fertilization, embryo formation, live birth) or detrimental (e.g. miscarriage), was displayed graphically in the meta-analyses to the right of the centre line and a decrease in the risk of an outcome to the left of the centre line.

Data collection process, data items, risk of bias in individual studies, risk of bias across studies, additional analyses and overall quality of the evidence

See Supplementary Data.

Results

Study selection

The last electronic search was conducted on the 25 August 2014, resulting in a total of 690 records: CENTRAL = 49; CINAHL = 3; LILACS = 0; MEDLINE = 176; PsycINFO = 0; Scopus = 266; Web of science = 192; ClinicalTrials.gov = 4; Current Controlled-Trials = 0; WHO International Trials Registry Platform = 0. Additionally, we included 1 record by hand-searching the reference list of included studies. From the 691 records, we removed 677 records after reading titles and abstracts: 208 records were duplicates and 469 clearly did not meet eligibility criteria.

We further examined 14 records for eligibility: three studies (from 3 records) were excluded because they were not randomized for the comparison ICSI-AOA versus ICSI-Only (Nasr-Esfahani et al., 2008; Borges et al., 2009a; Kyono et al., 2012); one study is still awaiting classification because we could not retrieve enough information from the publication and we were not able to contact the authors through e-mail requests (Razavi et al., 2012); and 9 studies (from 10 records) were included in this review.

Two studies were included in the woman-based review (Ebner et al., 2004; Eftekhar et al., 2013); and seven studies were included in the oocyte-based review (Zhang et al., 1999; Ebner et al., 2004; Manipalviratn et al., 2006; Mansour et al., 2009; Baltaci et al., 2010; Vanden Meerschaut et al., 2012; Liu et al., 2013). The study flow diagram is shown in Fig. 1.

Regarding the difference between the number of records and studies, two studies had two records each (Zhang et al., 1997, 1999; Manipalviratn et al., 2005, 2006), two studies, one randomizing women and the other randomizing oocytes, were reported in one full text article (Ebner et al., 2004); and the other six studies had one record each (Mansour et al., 2009; Baltaci et al., 2010; Razavi et al., 2012; Vanden Meerschaut et al., 2012; Eftekhar et al., 2013; Liu et al., 2013).

Study characteristics

The characteristics of the nine studies included are reported in Table I. In two studies women were randomized allowing the assessment of patient-centred clinical reproductive outcomes; those studies were...
Risk of bias in the included studies

All risk of bias judgments of the studies included in the quantitative analysis are presented in Table II. Only one study was deemed at low risk of selection bias (Eftekhar et al., 2013). Four studies were considered to be at high risk of selection bias; three from the oocyte-based review were split-body RCTs but oocytes were not properly randomized (Ebner et al., 2004; Manipalviratn et al., 2006; Vanden Meerschaut et al., 2012), and one from the women-based review allocated cycles alternatively (Ebner et al., 2004). Four studies did not describe properly the method used for randomization and were deemed at unclear risk of selection bias (Zhang et al., 1999; Mansour et al., 2009; Baltaci et al., 2010; Liu et al., 2013). We considered that blinding was important both to the selection of the oocytes and to the assessment of embryo development in the oocyte-based review because of the relatively subjective nature of the systems of classification; thus only one study was considered at low risk of performance and detection bias (Mansour et al., 2009) with the other six considered at high risk (Zhang et al., 1999; Ebner et al., 2004; Manipalviratn et al., 2006; Baltaci et al., 2010; Vanden Meerschaut et al., 2012; Liu et al., 2013). Due to the objective nature of the medical treatment and reproductive outcomes, blinding was considered not to be relevant for the women-based review.

All studies were considered to be at low risk of attrition bias and only two at high risk of selective reporting bias: one did not report the number of embryos formed in the oocyte-based review (Vanden Meerschaut et al., 2012) and one did not report live birth or miscarriage, just clinical pregnancy in the women-based review (Ebner et al., 2004). The other studies were at low risk of selective reporting. Only one study was deemed at high risk of other bias: it was included in the women-based review and randomized cycles instead of women but did not report how many women re-entered the study. There was insufficient detail reported in one study to properly judge the risk of bias (Razavi et al., 2012).

Effects of the intervention

Main results are summarized in Table III.

Women-based review

Two studies that randomized women and evaluated patient-centred reproductive outcomes were included in this part of the review; neither included women with previous fertilization failure.
Table 1 Characteristics of the included studies on artificial oocyte activation (AOA) in women.

<table>
<thead>
<tr>
<th>Study</th>
<th>Oocyte-based review</th>
<th>Woman-based review</th>
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<tr>
<td></td>
<td><strong>Study</strong></td>
<td><strong>Age (years)</strong></td>
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<tr>
<td></td>
<td><strong>Baltaci et al. (2010)</strong></td>
<td><strong>33.2 ± 3.1</strong></td>
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<td></td>
<td><strong>Ebner et al. (2004)</strong></td>
<td><strong>33.0 ± 4.6</strong></td>
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<td></td>
<td><strong>Liu et al. (2013)</strong></td>
<td><strong>30.4 ± 5.0</strong></td>
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<td></td>
<td><strong>Manipalviratn et al. (2006)</strong></td>
<td><strong>NR</strong></td>
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<tr>
<td></td>
<td><strong>Mansour et al. (2009)</strong></td>
<td><strong>29.4 ± 4.8</strong></td>
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<td></td>
<td><strong>Vanden Meerschaut et al. (2012)</strong></td>
<td><strong>31.6 ± 3.73</strong></td>
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<td></td>
<td><strong>Zhang et al. (1999)</strong></td>
<td><strong>25–42</strong></td>
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<td></td>
<td><strong>Ebner et al. (2004)</strong></td>
<td><strong>31.1 ± 3.9</strong></td>
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<td></td>
<td><strong>Eftekhar et al. (2013)</strong></td>
<td><strong>32.2 ± 4.4</strong></td>
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<td></td>
<td></td>
<td><strong>1.9 ± 0.6 versus</strong></td>
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<td></td>
<td><strong>2.1 ± 0.6</strong></td>
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<td></td>
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<td><strong>10.6 ± 5.3 versus</strong></td>
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<td></td>
<td><strong>9.1 ± 4.1</strong></td>
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<tr>
<td></td>
<td></td>
<td><strong>28.8 ± 3.4 versus</strong></td>
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<td></td>
<td></td>
<td><strong>29.9 ± 4.5</strong></td>
</tr>
</tbody>
</table>

Embryos and age presented as mean ± SD.
NR, not reported; IVM, in vitro maturation; MOAT, mouse oocyte activation test; TFF, total fertilization failure.
Table II  Judgements about risk of bias of included study.

<table>
<thead>
<tr>
<th>Study</th>
<th>Selection bias</th>
<th>Performance and detection bias</th>
<th>Attrition bias</th>
<th>Selective reporting</th>
<th>Other bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woman-based review</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Ebner et al. (2004)</td>
<td>H</td>
<td>Cycles were allocated alternately.</td>
<td>L</td>
<td>Blinding was not important for reproductive outcomes.</td>
<td>L</td>
</tr>
<tr>
<td>Eftekhar et al. (2013)</td>
<td>L</td>
<td>A computer based randomization list was used</td>
<td>L</td>
<td>Blinding was not important for reproductive outcomes.</td>
<td>L</td>
</tr>
<tr>
<td>Oocyte-based review</td>
<td></td>
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<tr>
<td>Baltaci et al. (2010)</td>
<td>U</td>
<td>Method of random sequence allocation was not described</td>
<td>H</td>
<td>No blinding.</td>
<td>L</td>
</tr>
<tr>
<td>Ebner et al. (2004)</td>
<td>H</td>
<td>Split-body RCT but oocytes were not properly randomized.</td>
<td>H</td>
<td>No blinding.</td>
<td>L</td>
</tr>
<tr>
<td>Liu et al. (2013)</td>
<td>U</td>
<td>Method of random sequence allocation was not described</td>
<td>H</td>
<td>No blinding.</td>
<td>L</td>
</tr>
<tr>
<td>Manipalviratn et al. (2006)</td>
<td>H</td>
<td>Split-body RCT but oocytes were not properly randomized.</td>
<td>H</td>
<td>No blinding.</td>
<td>L</td>
</tr>
<tr>
<td>Mansour et al. (2009)</td>
<td>U</td>
<td>Method of random sequence allocation was not described</td>
<td>L</td>
<td>The embryologist who checked for signs of fertilization was blinded to the allocation.</td>
<td>H</td>
</tr>
<tr>
<td>Vanden Meerschaut et al. (2012)</td>
<td>H</td>
<td>Split-body RCT but oocytes were not properly randomized.</td>
<td>H</td>
<td>No blinding.</td>
<td>H</td>
</tr>
<tr>
<td>Zhang et al. (1999)</td>
<td>U</td>
<td>Method of random sequence allocation was not described</td>
<td>H</td>
<td>No blinding.</td>
<td>L</td>
</tr>
</tbody>
</table>

U, unclear risk of bias (in yellow); H, high risk of bias (in red); L, low risk of bias (in green).
Partner with abnormal sperm morphology. One study including 38 women evaluated the effect of chemical AOA in couples with teratozoospermia (Eftekhar et al., 2013). Although the observed results were better in the ICSI-AOA group, there was no significant difference on on-going pregnancy (31.6% versus 15.8%, ICSI-AOA versus regular ICSI respectively, RR 2.0, 95% CI 0.58–6.85) and clinical pregnancy (36.8 versus 15.8%, RR 2.33, 95% CI 0.71–7.70). The authors did not report the occurrence of congenital anomalies or miscarriage.

Unselected population. One study evaluated the application of mechanical AOA as an adjuvant therapy for all women undergoing ICSI during the study period (Ebner et al., 2004). The results for clinical pregnancy were quite similar between groups (32.6% versus 30.4%, ICSI-AOA versus regular ICSI respectively, RR 1.07, 95% CI 0.59–1.96). The authors did not report the occurrence of congenital anomalies or the number of miscarriages.

Oocyte-based review
Seven studies randomized oocytes and were included in this part of the review. They evaluated a variety of clinical situations.

Previous fertilization failure using ICSI. Two studies were included and underwent meta-analysis (Baltaci et al., 2010; Vanden Meerschaut et al., 2012). They evaluated different methods for AOA; electrical activation was used in one study (Baltaci et al., 2010) and chemical activation using calcium ionophore (calcimycin) combined with CaCl injection in the other (Vanden Meerschaut et al., 2012). Although only evaluated by one study (Baltaci et al., 2010), ICSI-AOA was associated with an increase in the proportion of embryos achieving cleavage stage (RR 5.44, 95% CI 2.98–9.91, P < 0.0001, 1 RCT, 211 oocytes, Fig. 2) and in the proportion of top/high quality embryos on cleavage stage (RR 10.02, 95% CI 2.45–40.95, P = 0.001, 1 RCT, 211 oocytes). However, there is no evidence of effect on fertilization rate (RR 2.97, 95% CI 0.84–10.48, P = 0.09, 2 RCTs, 404 oocytes). This was the only outcome reported by both studies.

Rescue method for unfertilized oocytes. Two studies were included for meta-analysis (Zhang et al., 1999; Manipalviratn et al., 2006). Both used electrical activation. ICSI-AOA was associated with an increase in the proportion of embryos achieving cleavage stage (RR 8.65, 95% CI 2.28–32.77, P = 0.002, 2 RCTs, 204 oocytes, I² = 29%, Fig. 2) and also with an increase in fertilization rate (RR 8.26, 95% CI 1.28–53.32, P = 0.03, 2 RCTs, 204 oocytes, I² = 53%, Fig. 3). However, there was no significant effect on the likelihood of achieving embryos at blastocyst stage (RR 1.97, 95% CI 0.11–34.99, 1 RCT, 104 oocytes). No study reported top/high quality embryos at cleavage or blastocyst stages.

Immature oocytes. One study evaluated 274 frozen-thawed failed-matured oocytes from women that underwent ICSI in the study period (Liu et al., 2013). They used a chemical activation method and there was no evidence of effect on the proportion of embryos achieving cleavage stage (RR 1.02, 95% CI 0.75–1.39, P = 0.88), on the fertilization rate (RR 0.92, 95% CI 0.76–1.11, P = 0.38), on the proportion of embryos achieving blastocyst stage (RR 17.00, 95% CI 0.99–291.84, P = 0.05), and on the likelihood of achieving top/high quality embryos at blastocyst stage (RR 7.90, 95% CI 0.43–145.29, P = 0.16). However, they observed an increase in the likelihood of achieving top/high quality embryos at blastocyst stage as no high quality embryo was observed in the regular ICSI group (RR 28.96, 95% CI 1.75–477.91, P = 0.02).

Partner with abnormal sperm. One study evaluated all oocytes from 241 couples in which men had totally abnormal sperm morphology or totally immotile sperms, encompassing 3075 oocytes; they used the electrical activation method (Mansour et al., 2009). Although they observed a statistically significant increase in fertilization rate (RR 1.12, 95% CI 1.06–1.18, P < 0.001), the observed effect was too small and was not considered as being clinically relevant.

Oocytes from unselected patients. One study (Ebner et al., 2004) evaluated the effect of mechanical activation on all 3075 mature oocytes from 241 consecutive women undergoing ICSI with good ovarian response. They aimed to assess a possible application of AOA as a routine method to improve conventional ICSI outcome. However, there was no evidence of effect on the proportion of embryos achieving cleavage stage (RR Table III Summary of findings of RCTs on the effect of ICSI-AOA compared with ICSI-only in oocytes of women with previous fertilization failure, and as a rescue method in oocytes that failed to fertilize.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Absolute risk ICSI-only</th>
<th>Absolute risk ICSI-AOA</th>
<th>Relative effect RR (95% CI)</th>
<th>Oocytes (studies)</th>
<th>Observed effect</th>
<th>Quality of the evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous fertilization failure</td>
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<tr>
<td>Embryos at cleavage stage (Day 2–3)</td>
<td>11.4%</td>
<td>61.8%</td>
<td>5.4 (3.0–9.9)</td>
<td>211 oocytes (1)</td>
<td>Benefit</td>
<td>Very low&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
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<td>Embryos at blastocyst stage (Day 5–6)</td>
<td>0%</td>
<td>4.7%</td>
<td>2.0 (0.1–35.0)</td>
<td>104 oocytes (1)</td>
<td>*</td>
<td>Very low&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rescue method</td>
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<tr>
<td>Embryos at cleavage stage (Day 2–3)</td>
<td>8.8%</td>
<td>75.7%</td>
<td>8.7 (2.3–32.8)</td>
<td>204 oocytes (2)</td>
<td>Benefit</td>
<td>Very low&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Embryos at blastocyst stage (Day 5–6)</td>
<td>0%</td>
<td>4.7%</td>
<td>2.0 (0.1–35.0)</td>
<td>104 oocytes (1)</td>
<td>*</td>
<td>Very low&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>The estimate was very imprecise, not being possible to ascertain whether ICSI-AOA is related to harm, no effect or benefit.

<sup>b</sup>Quality of evidence was downgraded because of very serious imprecision, serious inconsistency of the results and high risk of bias of the included studies.

<sup>c</sup>Downgraded because of very serious imprecision, and because evidence comes from only one small study deemed at high risk of bias.
1.08, 95% CI 0.94–1.24, \( P = 0.29 \), on fertilization rate (RR 1.08, 95% CI 0.94–1.24, \( P = 0.29 \)), or on the likelihood of achieving top/high quality embryos at cleavage stage (RR 1.09, 95% CI 0.67–1.78, \( P = 0.74 \)). This study did not follow all embryos until blastocyst stage.

**Risk of bias across studies**

Publication bias was not suspected; however, this analysis was suboptimal, since the funnel plot analysis was not performed due to the inclusion of only seven studies.
Discussion

The present review showed that there is currently not sufficient evidence to support that ICSI-AOA is beneficial for couples with fertilization failure. The majority of the studies were not considered to be similar enough to validate meta-analysis, due to different AOA methods and patient inclusion criteria, thus limiting the possibility of pooling studies together and achieving a more robust conclusion. Only two studies examined ICSI-AOA in couples with previous fertilization failure (Baltaci et al., 2010; Vanden Meerschaut et al., 2012) whilst two other studies applied ICSI-AOA as a ’rescue’ method for unfertilized oocytes (Zhang et al., 1999; Vanden Meerschaut et al., 2006). The quality of the evidence coming from these studies is of very low quality (Table III), such that we are still uncertain of the effect of the intervention. None of the included studies evaluating couples with fertilization failure have actually randomized participants, only the oocytes, which precludes evaluation of the most important reproductive outcomes, such as live birth, clinical pregnancy, congenital anomalies and miscarriage. Although ’rescue’ AOA of unfertilized MII oocytes may be applicable in some clinical settings, and was included in the quantitative analysis of the present review, adverse changes in oocyte quality as a result of aging must be carefully considered (Segers et al., 2008; Miao et al., 2009). Therefore, it must be highlighted that rescue AOA should not be used routinely, and only after detailed counselling of patients regarding the lack of evidence of its safety.

The main AOA methods employ either chemical, mechanical, or electrical stimuli, mainly aiming to initiate artificial Ca^{2+} rises in the oocyte cytoplasm. The application of these methods in clinical embryology has been previously reviewed (Kashir et al., 2010; Nasr-Esfahani et al., 2010; Vanden Meerschaut et al., 2014b). Chemical oocyte activation appears to be the most popular method in human ART. Remarkably, however, despite the fact that the majority of published AOA studies in the context of human ART employ chemical activation, the present meta-analysis identified only three RCTs with sibling oocytes (Vanden Meerschaut et al., 2012; Eftekhar et al., 2013; Liu et al., 2013). The most common reagents used for chemical activation are calcium ionophores, such as ionomycin (Moaz et al., 2006; Heindryckx et al., 2008; Nasr-Esfahani et al., 2008; Razavi et al., 2012) and calcimycin (A23187) (Borges et al., 2009a,b, Montag et al., 2012; Vanden Meerschaut et al., 2012), including a commercially available calcimycin solution: GMS08 Cult-Active; Gynemed, Germany (Ebner et al., 2012, 2015b). Calcium ionophores are lipid-soluble molecules that transport calcium ions across the oocyte cell membrane, inducing a single transient surge in intracellular calcium concentration, without however being accompanied by subsequent calcium oscillations that occur during normal oocyte activation (Swann and Ozil, 1994). Exposure to calcium ionophore may be used in conjunction with CaCl injection together with the spermatozoon at the time of ICSI (Vanden Meerschaut et al., 2012). Strontium chloride, which has also been employed as an AOA agent (Yanagida et al., 2006; Marchetti et al., 2010; Yang et al., 2012), is able to elicit calcium oscillations and not just a single surge like calcium ionophores, but its efficiency as an activating agent for human oocytes is unclear (Vanden Meerschaut et al., 2014b).

Mechanical oocyte activation entails advancing the microinjection pipette during the ICSI procedure and aspirating peripheral cytoplasm, followed by deposition of the aspirated cytoplasm and the spermatozoon in the centre of the oocyte. The cytoplasm in the periphery of the oocyte is thought to be rich in mitochondria with high inner membrane potential and high metabolic ATP activity. Therefore, the method aims to accumulate peripheral mitochondria, and thus increase energy sources, in the site of subsequent pronuclear formation (Ebner et al., 2004).

During electrical activation, the direct current voltage causes rearrangement of the proteins of the cell membrane, leading to the formation of pores that allow the influx of extracellular calcium (Yanagida et al., 1999; Zhang et al., 1999; Mansour et al., 2009). Similarly to calcium ionophores, oocyte activation is induced by a single calcium rise that decreases again without subsequent calcium oscillations (Vanden Meerschaut et al., 2014b).

Fertilization failure following ICSI is primarily attributed to unsuccessful oocyte activation but may be due to less common causes, including defective sperm DNA decondensation, aberrant pronuclear development, oocyte spindle defects, reduced oocyte yield and quality, severe forms of teratozoospermia, such as globozoospermia, and technical problems (Flaherty et al., 1998; Kang et al., 2005; Dam et al., 2007; Swain and Pool, 2008). Therefore, identification of a sperm-related or oocyte-related deficiency is important for the clinical management of these couples. It has been proposed that heterologous ICSI of patient’s sperm in mouse oocytes (mouse oocyte activation test; MOAT) (Heindryckx et al., 2005, 2008) may serve as a diagnostic test for patients with previous fertilization failure using ICSI. It has been suggested that not all patients might benefit from AOA, but only those with sperm-related activation deficiency, as opposed to patients with a suspected oocyte-related deficiency in whom fertilization failure may not be overcome with AOA (Vanden Meerschaut et al., 2012).

Extremely limited data are available regarding the association between AOA in human ART and potential adverse health outcomes in children born and miscarriage rates. No such information was reported in any of the RCTs included in the present meta-analysis. A recent follow-up study of 21 children born following ionomycin activation reported reassuring outcomes regarding obstetric and neonatal outcomes, birth defects, as well as neurodevelopmental and behavioural outcomes (Vanden Meerschaut et al., 2014a). In addition, a recent study using a commercially available calcium ionophore solution (calcimycin A23187) reported 28% live birth, and 35 children born, one of which had a congenital malformation at birth (Ebner et al., 2015a). Neonatal data were reported from 5 children born following strontium oocyte activation (Kyono et al., 2008), and 22 babies born following calcimycin or strontium exposure (Takisawa et al., 2011). Overall, the number of children followed is too small to allow any meaningful conclusions regarding the safety of the various AOA methods. One should keep in mind that even if ICSI-AOA improves fertilization and embryo formation rates, this should not be extrapolated to an increased pregnancy rate as, aside from the exposure effect to the AOA stimuli, extra manipulation is also required and the minimization of the time required for this and better control of the temperature and pH of the oocytes have been associated with improved outcomes (Garrisi et al., 1993; Picinato et al., 2014). Therefore, new studies should randomize women, not oocytes, to AOA and examine live birth in order to ascertain whether AOA is of benefit for couples with previous fertilization failure. However, one should consider that fertilization failure after ICSI is not common, making it very difficult to design and conduct such a methodologically precise RCT.

The rapid evolution of ART has seen the introduction of several unproven treatments as well numerous diagnostic tests and laboratory...
Conclusions
There is insufficient evidence available from existing RCTs to judge the effect of ICSI-AOA on reproductive outcomes. There is a need for more RCTs on patients with previous fertilization failure in order to confirm its effectiveness and safety in terms of live birth rates and health of children born following the intervention. Such trials will be limited by the relative infrequency of failed fertilization following ICSI.

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

Authors’ roles
I.A.S. performed literature search, record screening, extraction and interpretation of data, and drafted the manuscript. C.O.N. contributed to the extraction, analysis and interpretation of data and revised the manuscript. M.L.S.L. performed literature search, record screening, extraction and data extraction, and revised the manuscript. E.T. contributed to the construction and interpretation of data and revised the manuscript. N.R.-F. contributed to the interpretation of data and revised the manuscript. W.P.M. performed literature search, contributed to record screening and data extraction, performed statistical analysis and interpretation of data and revised the manuscript. All authors approved the final version of the manuscript.

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Conflict of interest
None declared.

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