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META-ANALYSIS ***Embryology***

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 these 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.

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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 (Marangos *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 (Alberio *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 remodelling 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; Tóth *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 Ca^{2+} 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/isrctn/); 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 I^2 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

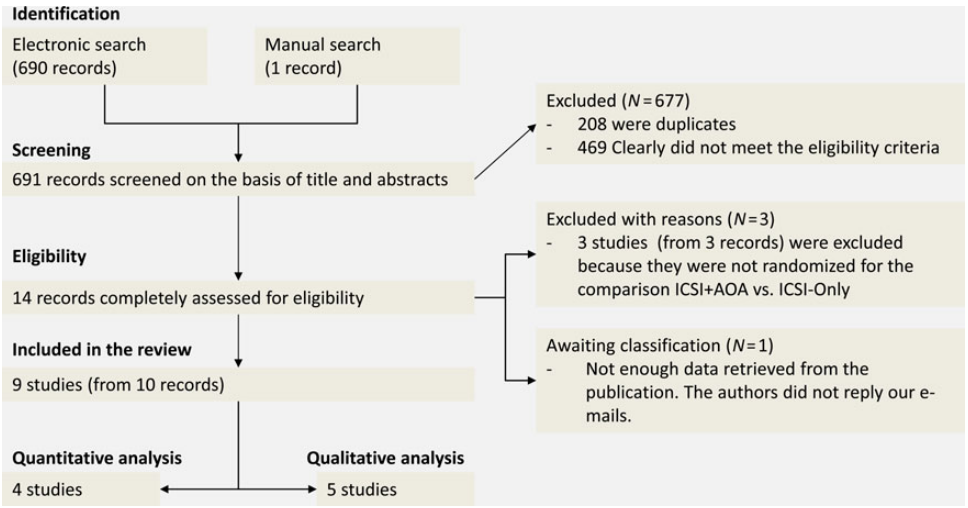

Figure 1 Flowchart of the study selection.

included in the woman-based review (Ebner *et al.*, 2004; Eftekhar *et al.*, 2013). In the other seven studies, the oocytes were randomized and these studies were included in the oocyte-based review.

Four studies evaluated AOA due to fertilization failure after using conventional ICSI. Two studies evaluated couples with history of fertilization failure in a previous ICSI cycle (Baltaci *et al.*, 2010; Vanden Meerschaut *et al.*, 2012). Two studies evaluated AOA as a rescue method for oocytes that had failed to fertilize 16–24 h after ICSI in the current cycle (Zhang *et al.*, 1999; Manipalviratn *et al.*, 2006).

The remaining five studies evaluated AOA for reasons other than fertilization failure. One study included frozen-thawed oocytes that did not mature after *in vitro* maturation (Liu *et al.*, 2013), and four studies included oocytes from women with any indication to ICSI (Ebner *et al.*, 2004; Mansour *et al.*, 2009; Eftekhar *et al.*, 2013). These five studies were not considered to be similar enough to be pooled and their results were reported only in the main text for completeness.

In terms of AOA method, four studies used an electrical activating chamber (Zhang *et al.*, 1999; Manipalviratn *et al.*, 2006; Mansour *et al.*, 2009; Baltaci *et al.*, 2010) and one study used a mechanical method for AOA (Ebner *et al.*, 2004). Three studies used a chemical method: two used calcium ionophore (Vanden Meerschaut *et al.*, 2012; Eftekhar *et al.*, 2013) and one exposed oocytes to ethanol 7% (Liu *et al.*, 2013). All studies compared ICSI-AOA with conventional ICSI. In the women-based review only one of the two included studies reported the primary outcome on-going pregnancy, while in the oocyte-based review five from the seven included studies reported the proportion of oocytes that fertilized and reached cleavage stage on Day 2–3.

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 I Characteristics of the included studies on artificial oocyte activation (AOA) in women.

	Oocyte-based review							Woman-based review	
Study	**Baltaci *et al.* (2010)**	**Ebner *et al.* (2004)**	**Liu *et al.* (2013)**	**Manipalviratn *et al.* (2006)**	**Mansour *et al.* (2009)**	**Vanden Meerschaut et al. (2012)**	**Zhang *et al.* (1999)**	**Ebner *et al.* (2004)**	**Eftekhar *et al.* (2013)**
Country	Turkey	Austria	China	Thailand	Egypt	Belgium	EUA	Austria	Iran
Funding sources	NR	NR	NR	NR	NR	NR	NR	NR	NR
Period of enrolment	Jan 2005 to Sep 2007	2003	Feb 2009 to Dec 2009	Jan 2004 to Sep 2004	Aug 2005 to Jan 2006	Jan 2006 to Dec 2011	NR	2003	Apr 2012 to Dec 2012
Conflicts of interest	None declared.	None declared.	None declared.	None declared.	None declared.	None declared.	None declared.	None declared.	None declared.
Inclusion criteria	All oocytes from consecutive women who had one previous TFF undergoing ICSI within the study period.	All mature oocytes from consecutive women undergoing ICSI with good ovarian response.	Frozen-thawed failed-matured oocytes from women that underwent ICSI in the study period.	Oocytes from ICSI with ejaculated spermatozoon that failed to fertilize after 24 h.	All oocytes from couples in which men had totally abnormal sperm morphology or totally immotile sperms.	Couples with a history of failed or low fertilization following conventional ICSI in whom MOAT was >84%.	Oocytes from ICSI that failed to fertilize after 16 h.	Oocytes from women < 40 years, tubal or unexplained infertility and <4 previous cycles.	All oocytes from couples with teratozoospermic men.
Exclusion criteria	None	None	None	Oocytes damaged or that underwent IVM.	≤6 oocytes retrieved.	None	None	None	Oocytes with abnormal pronuclei, immature, malformed, or postmature
Type of oocyte activation	Electrical activating chamber (BTX Electro-cell manipulator)	Mechanical activation	Exposure to ethanol 7% for 6 minutes	Electrical activating chamber (BTX Electro-cell manipulator)	Electrical activating chamber (BTX Electro-cell manipulator)	Calcium ionophore exposure and CaCl injection.	Electrical activating chamber (BTX Electro-cell manipulator)	Mechanical activation	Calcium ionophore.
Women included	21	24	NR	69	241	14	NR	92	38
Oocytes included	211	277	274	100	3075	193	104	–	–
Embryos transferred	–	–	–	–	–	–	–	1.9 ± 0.6 versus 2.1 ± 0.6	10.6 ± 5.3 versus 9.1 ± 4.1
Age (years)	33.2 ± 3.1	33.0 ± 4.6	30,4 ± 5,0	NR	29,4 ± 4,8	31,6 ± 3,73	25–42	33.1 ± 3.9 versus 32.2 ± 4.4	28.8 ± 3.4 versus 29.9 ± 4.5

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.

		Selection bias		Performance and detection bias		Attrition bias		Selective reporting		Other bias	
	Study	Risk	Explanation	Risk	Explanation	Risk	Explanation	Risk	Explanation	Risk	Explanation
Woman-based review	Ebner *et al.* (2004)	H	Cycles were allocated alternatively.	L	Blinding was not important for reproductive outcomes.	L	No loss of follow-up.	H	Did not report live birth or miscarriage.	H	Cycles were allocated instead of women, did not report how many women re-entered the study.
	Eftekhar *et al.* (2013)	L	A computer based randomization list was used	L	Blinding was not important for reproductive outcomes.	L	No loss of follow-up.	L	Not suspected.	L	Not suspected.
Oocyte-based review	Baltaci *et al.* (2010)	U	Method of random sequence allocation was not described.	H	No blinding.	L	No loss of follow-up.	L	Not suspected.	L	None
	Ebner *et al.* (2004)	H	Split-body RCT but oocytes were not properly randomized.	H	No blinding.	L	No loss of follow-up.	L	Not suspected.	L	None
	Liu *et al.* (2013)	U	Method of random sequence allocation was not described.	H	No blinding.	L	No loss of follow-up.	L	Not suspected.	L	None
	Manipalviratn *et al.* (2006)	H	Split-body RCT but oocytes were not properly randomized.	H	No blinding.	L	No loss of follow-up.	L	Not suspected.	L	None
	Mansour *et al.* (2009)	U	Method of random sequence allocation was not described	L	The embryologist who checked for signs of fertilization was blinded to the allocation.	L	No loss of follow-up.	H	Number of embryos not reported.	L	None
	Vanden Meerschaut *et al.* (2012)	H	Split-body RCT but oocytes were not properly randomized.	H	No blinding.	L	No loss of follow-up.	H	Number of embryos not reported.	L	None
	Zhang *et al.* (1999)	U	Method of random sequence allocation was not described.	H	No blinding.	L	No loss of follow-up.	L	Not suspected.	L	None

U, unclear risk of bias (in yellow); H, high risk of bias (in red); L, low risk of bias (in green).

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.

Outcome	Absolute risk		Relative effect RR (95% CI)	Oocytes (studies)	Observed effect	Quality of the evidence
	ICSI-only	ICSI-AOA				
Previous fertilization failure						
Embryos at cleavage stage (Day 2–3)	11.4%	61.8%	5.4 (3.0–9.9)	211 oocytes (1)	Benefit	Very low[a]
Embryos at blastocyst stage (Day 5–6)			Not reported by the included studies.			
Rescue method						
Embryos at cleavage stage (Day 2–3)	8.8%	75.7%	8.7 (2.3–32.8)	204 oocytes (2)	Benefit	Very low[b]
Embryos at blastocyst stage (Day 5–6)	0%	4.7%	2.0 (0.1–35.0)	104 oocytes (1)	*	Very low[a]

RR, risk ratio; CI, confidence interval.
*The estimate was very imprecise, not being possible to ascertain whether ICSI-AOA is related to harm, no effect or benefit.
[a]Quality of evidence was downgraded because of very serious imprecision, serious inconsistency of the results and high risk of bias of the included studies.
[b]Downgraded because of very serious imprecision, and because evidence comes from only one small study deemed at high risk of bias.

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, $I^2 = 93\%$, Fig. 3); 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, $I^2 = 29\%$, 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, $I^2 = 53\%$, 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 cleavage 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

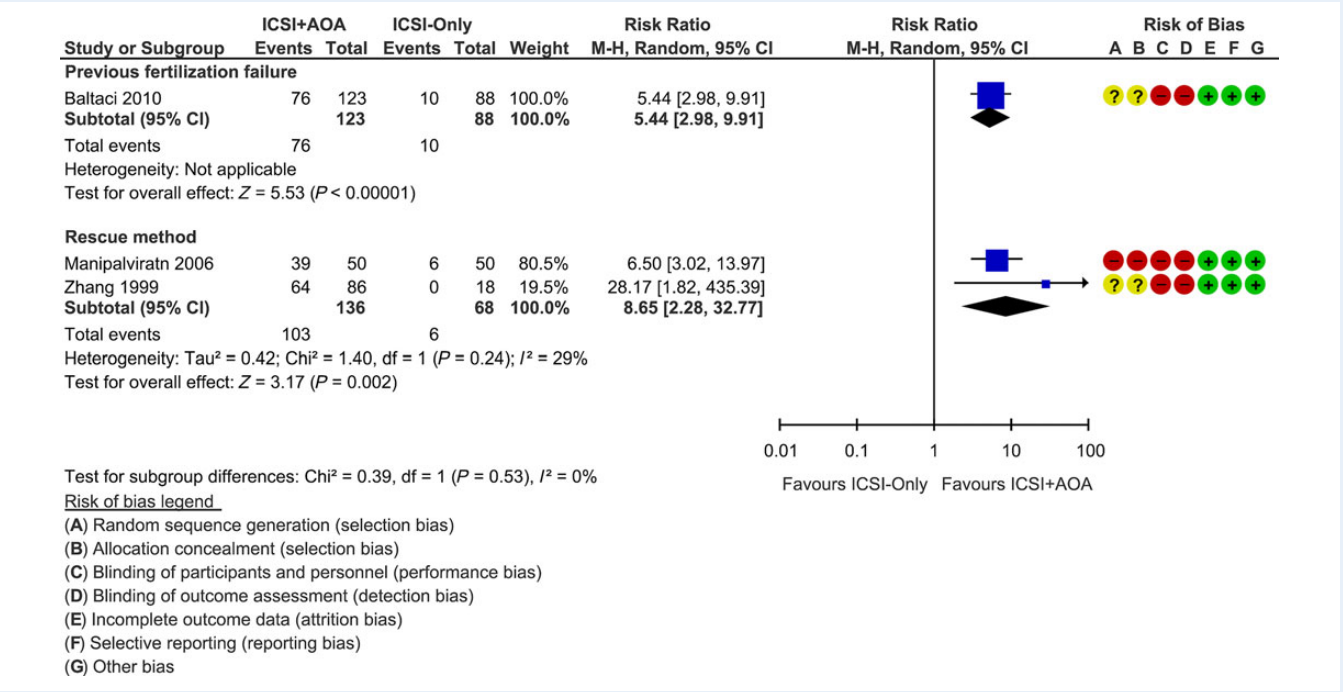

Figure 2 Forest plot for embryos at cleavage stage per oocyte randomized reported by the studies included in the oocyte-based review.

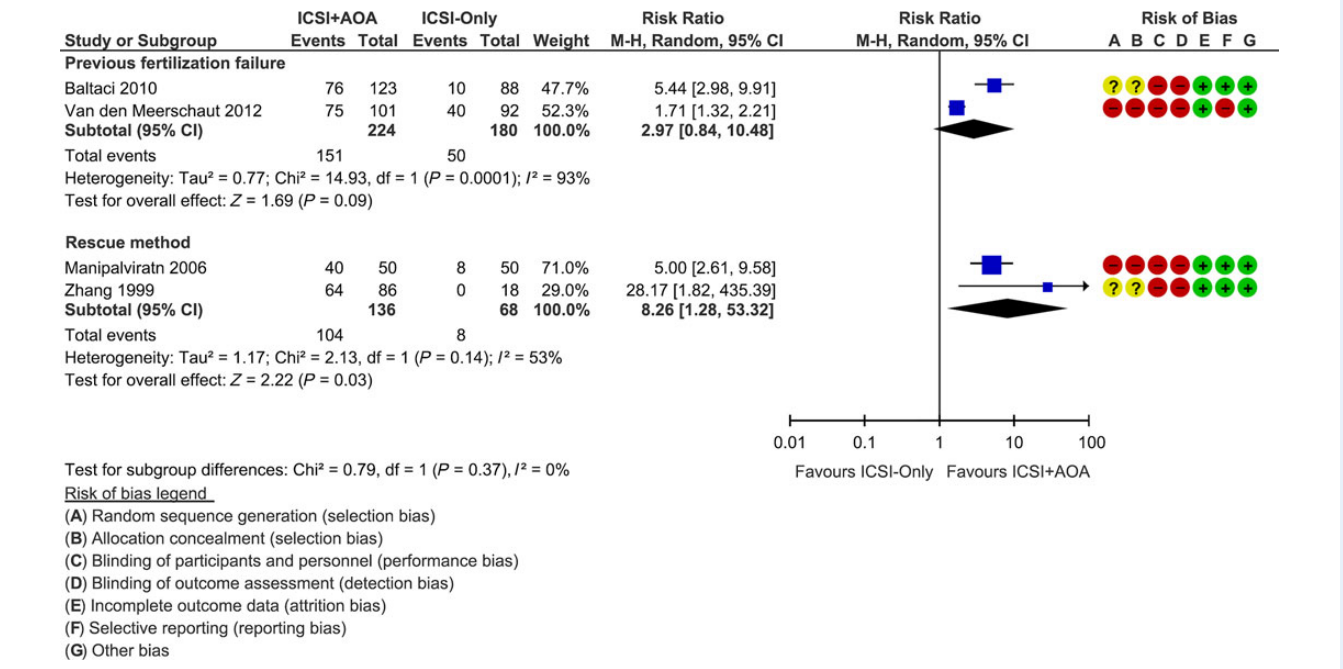

Figure 3 Forest plot for fertilization per oocyte randomized reported by the studies included in the oocyte-based review.

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; Manipalviratn *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: GM508 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; Chen *et al.*, 2010; Kyono *et al.*, 2012; 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; Manipalviratn *et al.*, 2005, 2006; 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

procedures into the clinical setting without robust evidence from high quality clinical research and trials. Although the aim of these innovations, and ICSI-AOA is just one example, is to improve reproductive outcome it is important to adhere to the principles of evidence-based medicine in order to assess their effectiveness and safety and avoid the introduction of technology with no clinical benefit or, even worse, potentially adverse outcomes (Harper *et al.*, 2012; Evers, 2015).

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 and data extraction, and revised the manuscript. E.T. contributed to the extraction 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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