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Artificial oocyte activation in patients with fertilization failure using calcium ionophores: a meta-analysis on reproductive outcomes.

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Abstract

Purpose

To evaluate the clinical efficacy and safety of artificial oocyte activation using calcium ionophores on reproductive and perinatal outcomes in couples with a history of fertilization failure following ICSI.

Methods

A systematic search was conducted in PubMed for English-language studies published between 2003 and 2025. Studies comparing AOA-treated groups using calcium ionophores versus non-treated ICSI controls were included. Subgroup analyses were performed based on study design, type of ionophore (ionomycin vs. calcimycin), and fertilization failure subtype (total vs. low fertilization). Risk of bias was assessed using Cochrane RoB 2 and ROBINS-I tools.

Results

Twenty-one studies met inclusion criteria. ICSI-AOA using calcium ionophores significantly increased fertilization rate, clinical pregnancy rate, and live birth rate. Miscarriage rate was reduced. Subgroup analyses indicated that ionomycin was more effective than calcimycin in improving fertilization, and that benefits were also observed for total and low fertilization failure. No significant differences were observed in neonatal outcomes or malformation rates. Risk of bias was moderate to high, and no evidence of publication bias was detected.

Conclusion

Calcium ionophore-mediated activation significantly enhances reproductive outcomes in ICSI cycles in patients with fertilization failure, without increasing miscarriage or adverse neonatal outcomes. Their selective use could prevent cycle cancellation and reduce the emotional and financial burden associated with repeated ICSI failures. However, given the variability in ionophore protocols and experimental design, further randomized studies are warranted to standardize dosing, timing, and indications before universal clinical adoption.

Introduction

Intracytoplasmic sperm injection (ICSI) enables the direct introduction of a spermatozoon into the oocyte cytoplasm. First performed in 1992, it has since become the most widely used technique in assisted reproduction cycles [1]. Although originally developed to address severe male factor infertility, such as severe oligozoospermia or obstructive azoospermia [2], ICSI is widely used even in non-severe male factor cases. It is, in fact, used in 70% of the assisted reproduction techniques (ART) cycles reported in Europe [3].

ICSI typically yields fertilization rates ranging from 70% to 80% [4]. The Vienna consensus addressing the KPI values in ICSI cases describe competency and benchmark values between > 65% and > 85% respectively [5].

Nevertheless, approximately 1% to 5% of ICSI cycles result in total or partial fertilization failure (< 30% fertilization rate) [5]. The underlying causes are diverse and may include oocyte activation deficiency (OAD), sperm head decondensation failure due to high levels of maturation promoting factor (MPF) in the ooplasm, transcriptomic defects in the oocyte or technical errors during microinjection, among others [6]. Notably, OAD accounts for approximately 40% to 60% of fertilization failure in ICSI cycles [7].

Oocyte activation is a calcium-dependent signaling process triggered by the sperm-specific phospholipase C zeta (PLCζ), which induces intracellular Ca^{2+} oscillations required for meiosis resumption and pronuclei formation [8–12]. PLCζ deficiency is a major contributor to fertilization failure [13, 14].

Over the past two decades, ICSI has been combined with artificial oocyte activation (AOA) techniques to overcome OAD [15]. AOA consists in the artificial induction of Ca^{2+} release into the ooplasm to compensate for defective sperm-induced activation. Several AOA methods are available, including calcium ionophores, strontium chloride ($SrCl_2$), electrical stimulation and mechanical approaches [16]. Among these, calcium ionophores are the most widely adopted due to their relatively low cytotoxicity [17]. Specifically, ionomycin and calcimycin (A23187) are the most common and known ionophores [18]. These agents bypass the physiological mechanism by promoting Ca^{2+} permeability in oocyte membranes [19]. However, no standardized clinical protocol for calcium ionophore use currently exists, resulting in significant variation among centers in terms of concentration, exposure time, and type of ionophore employed (20). Moreover, there is no universally accepted medical indication for AOA, and it has historically been applied in a wide range of scenarios, including fertilization failure [7, 21, 22], rescue of unfertilized oocytes [23], and poor embryonic development [24, 25].

To date, few studies have rigorously evaluated the clinical efficacy of calcium ionophores in improving reproductive outcomes, and the available literature includes poor sample sizes and highly heterogeneous study designs [26]. Yet, AOA is recommended by ESHRE in cases of fertilization failure or low fertilization rate [27].

Therefore, the aim of this meta-analysis is to assess the efficacy of calcium ionophores on reproductive outcomes specifically in couples with a history of fertilization failure. Through subgroup analyses, we aim to provide insight into the utility of ionophores across different fertilization failure subtypes. To our knowledge, this will be the first meta-analysis to compare outcomes in different study designs, to evaluate the two main types of ionophores, and to examine the potential impact of AOA on perinatal outcomes.

Materials and methods

Search strategy

This meta-analysis was registered in the PROSPERO database under the registration number CRD42024613512 and was conducted in accordance with the PRISMA 2020 guidelines [28]. The literature

search was performed using databases such as PubMed and Google Scholar. Both prospective and retrospective studies published between 2003 and January 2025 were included, using search keywords such as ICSI, fertilization failure, calcium ionophores, calcimycin, A23187, ionomycin, and artificial oocyte activation in their relevant combinations. The reference lists of the reviewed articles were also screened to identify additional eligible studies. Only studies published in English were considered.

Study selection and eligibility criteria

Studies were initially identified in the databases by title by one reviewer, with duplicate records being removed. The second screening was based on abstracts, excluding review articles and those that did not address the primary objectives of this meta-analysis. Finally, full-text articles meeting the eligibility criteria were assessed. The inclusion criteria were as follows: (1) studies comparing ICSI-AOA using calcium ionophores (ionomycin or calcimycin) with a control group undergoing ICSI without AOA; (2) studies involving couples with fertilization failure, either total or low (defined as < 30% fertilization rate); and (3) studies reporting at least one of the primary outcomes. The exclusion criteria were: (1) studies analyzing the effect of rescue AOA or focused on couples without fertilization failure; (2) studies focusing on patients with specific conditions (e.g., globozoospermia, PCOS, azoospermia); (3) studies conducted in non-human species; and (4) studies involving patients of advanced maternal age (> 37 years).

Data extraction and outcomes of interest

Data were extracted by the reviewer as follows: study context (author, year of publication, study design, type of control group and country of origin), protocol-related variables (type of ionophore, concentration, exposure time), patient characteristics (age, type of fertilization failure, number of mature oocytes in each group, and semen source), and fertility-related outcomes. These included the primary outcomes of the meta-analysis (fertilization rate, clinical pregnancy rate, miscarriage rate, and live birth rate) and secondary outcomes (embryo quality, birthweight, birth length, and perinatal malformations). If a study reported data for total fertilization failure (TFF) separately from low fertilization failure (LFF), data extraction was performed as if they were independent outcomes.

Outcomes were defined as follows. Fertilization rate was defined as the number of correctly fertilized oocytes (presence of two pronuclei at 18–20 hours post-ICSI) per number of metaphase II (MII) oocytes. Clinical pregnancy rate was defined as the number of clinical pregnancies per embryo transfer (ET) cycle. Miscarriage rate was defined as the number of miscarriages per clinical pregnancy. Live birth rate was defined as the number of live births per ET cycle. The high-quality embryo rate was defined as the number of A- or B-grade embryos (either on day 3 or at the blastocyst stage), according to the ASEBIR consensus and the Gardner grading scale respectively, per cleaved embryo.

Bias assessment and statistical analysis

A quality assessment of non-randomized studies was conducted using the ROBINS-I tool from the Cochrane Library [29]. The only randomized controlled trial included in this meta-analysis was evaluated according to the RoB 2 guidelines, from the same library [30]. Publication bias was assessed and illustrated using funnel plots for each analysis.

The effect of AOA compared to the control group was measured using odds ratios (OR) with 95% confidence intervals (CI). Statistical analysis was performed using Review Manager Version 5.0. The Mantel-Haenszel random-effects model was preferred, assuming high heterogeneity among studies. Exceptionally, analyses that included a cell with a value of 0 were processed using the Peto method. Subgroup analyses were structured as follows: (i) by type of fertilization failure (total or low); (ii) by type of calcium ionophore used (ionomycin or calcimycin); and (iii) by type of control group (previous cycle of the same couple, intracohort oocyte comparison, or interpatient comparison).

Results

Study selection and study characteristics

A total of 105 studies were identified through both electronic and manual searches. Seven were excluded due to duplication, and 42 were discarded based on title screening. During abstract screening, 23 studies were excluded: four did not report any of the primary outcomes of this meta-analysis, and 19 were reviews. Of the 33 full-text articles assessed for eligibility, six were excluded for defining fertilization failure as a fertilization rate below 50% instead of below 30%. Additional four studies were excluded because they involved patients without fertilization failure (e.g., patients with poor embryo quality or undergoing rescue AOA), and two studies were excluded due to missing data on the number of mature oocytes. Ultimately, 21 studies met the inclusion criteria: four case reports, ten retrospective studies, six prospective studies, and one randomized controlled trial (RCT). Figure 1 illustrates the study selection process.

Two studies focused exclusively on couples with TFF [31, 32], while nine investigated only cases of LFF [7, 22, 33–39]. Four studies included both types of fertilization failure, with results reported separately for each group [4, 40–42]. The remaining seven studies included patients with fertilization rates below 30%, without specifying whether failure was total or partial [15, 21, 43–46]. In one study, the type of fertilization failure was not specified; however, all male partners had a mutation in the PLCζ gene [45]. Study characteristics and detailed information are summarized in Table 1.

Overall primary outcomes

Only 4 out of the 21 included studies did not report fertilization rates [35, 38, 43, 45]. Fixed-effect model analysis (Fig. 2) showed that the ICSI-AOA group was associated with a significantly higher fertilization rate, with an OR of 2.81 (95% CI: 2.65–2.97). A similar significant effect was observed for the ICSI-AOA group in clinical pregnancy rate (OR 2.83, 95% CI: 1.40–5.70, Fig. 3) and live birth rate (OR 2.59, 95% CI: 1.30–5.17, Fig. 4), both assessed using random-effects models.

For miscarriage rate, the random-effects analysis revealed an association between the ICSI-AOA group and a lower miscarriage rate (OR 0.50, 95% CI: 0.25–0.99, Fig. 5). Among all outcomes, miscarriage rate was the least reported, with only 7 of the 21 studies including relevant data [7, 15, 22, 35, 38, 44, 45].

Subgroup analysis of primary outcomes

The subgroup analysis based on the type of fertilization failure revealed that ICSI-AOA treatment showed a positive effect on fertilization rate in both the TFF (OR 16.94, 95% CI: 14.03–20.46) and LFF (OR 2.35, 95% CI: 2.21–2.49) subgroups. However, the effect appeared to be more pronounced in the TFF group (OR 16.94 vs. OR 2.35) (Fig. 6). Since none of the studies reported clinical pregnancy, miscarriage, or live birth rates in the TFF group, there were not enough data to draw conclusions from these subgroup analyses for the remaining three primary outcomes (Supplementary Fig. 1–3).

Table 1
Study characteristics of included articles.

Author and year	Country	Study type	Mean age	Control group type	Sample size	Protocol	Sperm source
Eldar-Geva et al., 2003	Israel	Case report	28	Previous cycle	AOA: 57 MII oocytes Control: 18 MII oocytes	Calcimycin 10µM 1h after ICSI for 7 min	Ejaculated
Heindryckx et al., 2005	Belgium	Prospective	NR	Previous cycle	LFF AOA: 97 MII oocytes LFF Control: 118 MII oocytes TFF AOA: 51 MII oocytes	Ionomycin 10µM 30 min after ICSI for 10 min (twice)	Ejaculated
Terada et al., 2009	Japan	Case report	31	Previous cycle	AOA: 48 MII oocytes Control: 45 MII oocytes	Ionomycin 5µM 30 min after ICSI for 5 min	Ejaculated (fresh & thawed)
Isachenko et al., 2010	Germany	Case report	30	Intracohort (sibling oocytes)	AOA: 6 MII oocytes Control: 7 MII oocytes	Non specified calcium ionophore 10µM right after ICSI for 20 min	Ejaculated
Author and year	**Country**	**Study type**	**Mean age**	**Control group type**	**Sample size**	**Protocol**	**Sperm source**
Vanden Meerschaut et al., 2012	Belgium	Prospective	31,6 ± 3,73	Intracohort (sibling oocytes)	LFF AOA: 68 MII oocytes LFF Control: 64 MII oocytes	Ionomycin 10µM 30 min after ICSI for 10 min	Ejaculated

MII: metaphase II; LFF: low fertilization failure; TFF: total fertilization failure; AOA; artificial oocyte activation

Author and year	Country	Study type	Mean age	Control group type	Sample size	Protocol	Sperm source
					TFF AOA: 33 MII oocytes TFF Control: 28 MII oocytes		
Montag et al., 2012	Germany	Retrospective	35,6 ± 3,9	Previous cycle	LFF AOA: 363 MII oocytes LFF Control: 373 MII oocytes TFF AOA: 231 MII oocytes TFF Control: 114 MII oocytes	Calcimycin 10µM right after ICSI for 15 min	Ejaculated
Yoon et al., 2013	Korea	Retrospective	36,6 ± 5,14	Previous cycle	AOA: 784 MII oocytes Control: 441 MII oocytes	Calcimycin 10µM 30 min after ICSI for 30 min	Ejaculated or testicular
Author and year	**Country**	**Study type**	**Mean age**	**Control group type**	**Sample size**	**Protocol**	**Sperm source**
Deemeh et al., 2014	Iran	Prospective cohort study	31,2 ± 4,7	Non treated couples	NR	Ionomycin 10µM 30 min after ICSI for 10 min	Ejaculated or testicular
Kim et al., 2014	Korea	Prospective	31,75	Previous cycle	AOA: 158 MII oocytes Control: 70 MII oocytes	Calcimycin 10µM right after ICSI for 30 min	Ejaculated

MII: metaphase II; LFF: low fertilization failure; TFF: total fertilization failure; AOA; artificial oocyte activation

Author and year	Country	Study type	Mean age	Control group type	Sample size	Protocol	Sperm source
Ebner et al., 2015	Austria/ Germany	Prospective multicentre	33,9 ± 4,1	Previous cycle	AOA: 884 MII oocytes Control: 1108 MII oocytes	Calcimycin 10µM right after ICSI for 15 min	NR
Kim et al., 2015	Korea	Case report	32	Previous cycle	AOA: 23 MII oocytes Control: 11 MII oocytes	Calcimycin 10µM 30 min after ICSI for 30 min	Ejaculated
Miller et al., 2016	Israel	Retrospective	31,3 ± 5,15	Non treated couples	NR	Calcimycin 10µM right after ICSI for 10 min	NR
Mateizel et al., 2018	Belgium	Retrospective	32,3 ± 3,9	Previous cycle	NR	Calcimycin 10µM right after ICSI for 15 min or ionomycin 30 min after ICSI for 7 min	Ejaculated or testicular
Author and year	**Country**	**Study type**	**Mean age**	**Control group type**	**Sample size**	**Protocol**	**Sperm source**
Fawzy et al., 2018	Egypt	RCT	32,2 ± 5,2	Non treated couples	AOA: 1310 MII oocytes Control: 1180 MII oocytes	Calcimycin 10µM right after ICSI for 20 min	Ejaculated (fresh & thawed)
Bonte et al., 2019	Belgium	Retrospective	32,9	Previous cycle	AOA: 1743 MII oocytes Control: 1884 MII oocytes	Ionomycin 10µM 30 min after ICSI for 10 min	Ejaculated

MII: metaphase II; LFF: low fertilization failure; TFF: total fertilization failure; AOA; artificial oocyte activation

Author and year	Country	Study type	Mean age	Control group type	Sample size	Protocol	Sperm source
Li et al., 2019	China	Prospective cohort study	31,28 ± 3,67	Intracohort (sibling oocytes)	AOA: 262 MII oocytes Control: 251 MII oocytes	Ionomycin 10µM 30 min after ICSI for 5 min (twice)	NR
Lv et al., 2020	China	Retrospective	33,6 ± 2,35	Previous cycle	AOA: 3291 MII oocytes Control: 3277 MII oocytes	Calcimycin 5µM right after ICSI for 15 min	NR
Martínez et al., 2021	Spain	Retrospective	24,78 ± 2,36	Non treated couples	NR	Ionomycin 10µM 30 min after ICSI for 7 min	Ejaculated
Author and year	**Country**	**Study type**	**Mean age**	**Control group type**	**Sample size**	**Protocol**	**Sperm source**
Zhang et al., 2023	China	Retrospective	29,7 ± 3,2	Non treated couples	AOA: 883 MII oocytes Control: 2671 MII oocytes	Calcimycin 10µM right after ICSI for 10 min	Ejaculated or testicular
Ruan et al., 2023	China	Retrospective	33,86 ± 4,98	Previous cycle	LFF AOA: 78 MII oocytes LFF Control: 68 MII oocytes TFF AOA: 138 MII oocytes TFF Control: 123 MII oocytes	Ionomycin 10µM 30 min after ICSI for 10 min	NR

MII: metaphase II; LFF: low fertilization failure; TFF: total fertilization failure; AOA; artificial oocyte activation

Author and year	Country	Study type	Mean age	Control group type	Sample size	Protocol	Sperm source
Akashi et al., 2023	Japan	Retrospective multicentre	34,65 ± 3,7	Previous cycle	AOA: 1036 MII oocytes Control: 722 MII oocytes	Calcimycin or ionomycin right after ICSI for 30 min	Ejaculated

MII: metaphase II; LFF: low fertilization failure; TFF: total fertilization failure; AOA; artificial oocyte activation

Regarding the type of ionophore used, only fertilization rate showed a statistically significant positive association, both in the calcimycin subgroup (OR 1.96, 95% CI: 1.83–2.10) and the ionomycin subgroup (OR 6.83, 95% CI: 6.09–7.65) (Fig. 7). The other primary outcomes showed trends consistent with the overall analysis results, but their CI ranged across the value 1, and therefore these results were not statistically significant (Supplementary Fig. 4–6).

Subgroup analysis by type of control in the experimental design revealed the following. For fertilization rate, studies using previous cycle controls and intracohort controls both showed a positive association with ICSI-AOA (OR 4.25, 95% CI 3.07–4.54 and OR 2.33, 95% CI: 1.73–3.14, respectively). In contrast, studies using untreated independent couples as controls showed a negative association (OR 0.82, 95% CI: 0.73–0.96). However, only two of the 17 studies included in the fertilization rate analysis used this type of control (Fig. 8).

For clinical pregnancy and live birth rates, the number of studies using interpatient controls was higher than for intrapatient ones, the latter being represented by a single study [39]. The previous cycle control subgroup yielded results similar to those observed for fertilization rate. However, the treatment effect in the untreated couples control subgroup was not statistically significant (95% CI: 0.46–2.45 and 0.41–1.71, respectively) (Fig. 9).

In the subgroup analysis for miscarriage rate, a statistically significant negative correlation was observed in the previous cycle control subgroup (OR 0.18, 95% CI: 0.09–0.36), while the untreated couples control subgroup showed no significant effect (OR 0.81, 95% CI: 0.53–1.24) (Supplementary Fig. 7).

Secondary outcomes

The number of studies reporting data on perinatal characteristics was low, with no more than five studies included in each sub-analysis of the secondary outcomes. The effect of ICSI-AOA treatment was found to be significantly associated with improved embryo quality, both for day-3 embryos (OR 1.51, 95% CI: 1.06–2.14) and for blastocysts (OR 1.43, 95% CI: 1.28–1.60) (Fig. 10).

Regarding the perinatal characteristics of babies born from the ICSI-AOA group, only two studies reported birth length [39, 43], and three additional studies provided data on birth weight [35, 38, 46]. Neither variable

showed a statistically significant effect of calcium ionophore treatment (95% CI: −0.19–1.29 and 95% CI: −40.81-139.84, respectively) (Fig. 11). The analysis of perinatal malformations included only two studies with estimable data [35, 38], and yielded similar results (OR 0.55, 95% CI: 0.21–1.45) (Supplementary Fig. 8).

Risk of bias

All case reports were rated as having a high risk of bias, according to the ROBINS-I tool from the Cochrane Library [29]. This is mainly due to the inability to control confounding factors given the small sample sizes of this type of study. The only randomized controlled trial was rated as having a moderate risk of bias according to the RoB 2 tool [30].

The risk of bias analysis for all studies is shown in Fig. 12. All studies are included in a funnel plot, which displays symmetry and is therefore interpreted as showing no evidence of publication bias (Fig. 13).

Discussion

Main findings

This meta-analysis, including 21 studies, demonstrates that calcium ionophores significantly improve fertilization, clinical pregnancy and live birth rates in couples with previous fertilization failure, without increasing miscarriage rate. Embryo quality at both cleavage and blastocyst stages also appeared higher in the ionophore group, and available evidence did not show differences in birth weight, length, or congenital malformations in the 805 children analyzed. Subgroup analyses suggested that couples with TFF benefited most, and that ionomycin may be more effective than calcimycin.

Interpretation

AOA with calcium ionophores is the most widely adopted rescue strategy to overcome fertilization failure after ICSI. Our results support previous meta-analyses [26, 47, 48], but also expand the evidence by focusing exclusively on couples with fertilization failure, avoiding heterogeneous populations such as developmental arrest or severe male factor infertility. This strengthens the clinical relevance of the conclusions.

It is important to note that OAD can originate either from the sperm or the oocyte [49]. Mutations in oocyte-specific genes such as WEE2, TUBB8, or PATL2 have been identified as causes of female infertility [14]. In such scenarios, calcium ionophores are ineffective, as the problem does not lie within the Ca^{2+} dependent signaling pathway. Alternative AOA approaches have been explored to address these genetic defects, such as the microinjection of cRNA [50]. Conversely, fertilization failure due to sperm-related factors, including mutations in ACTL7, PLCZ1 or DPY19, has been successfully treated using calcium ionophores [51, 52]. In fact, one of the studies included in this meta-analysis specifically analyzed couples with fertilization failure in which the male partner carried a PLCZ1 mutation [45].

One of the potential risks associated with AOA using calcium ionophores was the possibility of chromosomal abnormalities or perinatal malformations. Recently, it has been demonstrated that the ploidy of embryos resulting from ICSI cycles with calcium ionophores is not affected by the treatment, nor are

gestational age or weight at birth [25, 46]. Our results are consistent with these findings, as neither birthweight, nor length, nor the occurrence of perinatal malformations were significantly different in the ionophore group. However, the robustness of these outcomes is lower compared to that of reproductive outcomes, as the number of studies contributing data to these analyses is considerably smaller.

Similarly, fears of epigenetic alterations, raised mainly by animal studies, have not been substantiated in humans [24]. Minor imprinting differences described at early stages appear to normalize by the blastocyst stage, suggesting limited long-term risk [53]. Nevertheless, larger studies with long-term follow-up are required to confirm safety.

The effect of ionomycin appeared greater than calcimycin, consistent with reports in both clinical and experimental settings [54]. Although biochemical differences between these ionophores may contribute, protocol variability across studies should also be considered as a confounder [55]. Our analysis also suggests that interpatient controls may underestimate treatment effects compared with intracohort or previous-cycle designs, as differences in baseline infertility etiology between groups are likely.

The use of calcium ionophores as AOA agents should be restricted to cases where it is truly necessary. However, there is currently no commercially available calcium ionophore product that is specifically designed and certified for use in IVF as a medical device.

Moreover, accurate diagnosis of OAD is still underdeveloped, and this treatment is currently being applied to patients for whom it may not be appropriate. Genetic screening for mutations in both male and female partners has enabled the identification of OAD cases [14]. Both heterologous and homologous diagnostic tests exist, depending on whether non-human biological material is involved. One of the most used heterologous diagnostic tests is the mouse oocyte activation test (MOAT). Other tests have been developed to assess intracellular Ca^{2+} signaling via fluorescence, such as the M-OCA (heterologous) [40] and H-OCA (homologous) [56]. However, these assays require fluorescent staining, and efforts are being made to replace them with less invasive methods, such as particle image velocimetry (PIV) [57]. All of these tests can help determine which partner is the source of fertilization failure, thereby supporting a more targeted application of AOA and minimizing unnecessary exposure of gametes to chemical agents.

Limitations and strengths

This study is the first meta-analysis exclusively focused on couples with fertilization failure. The use of calcium ionophores as an AOA treatment has increased in recent years; however, there is still no standardized protocol among clinics, leading to inherent clinical variability whose confounding effect remains unknown.

The design of this study has several strengths. Firstly, it includes outcomes spanning the entire ICSI cycle, from fertilization to live birth rate. We found that the positive effect of ionophores begins at the fertilization stage and extends to improvements in live birth. Secondly, our subgroup analysis provides new insight into the treatment's effects in specific patient populations. These analyses revealed evidence that patients with TFF benefit more significantly than those with LFF, and similarly, that ionomycin is more effective than calcimycin. The analysis of the type of control used in the studies is a novel aspect in the field of calcium

ionophore research. This control analysis further supports that interpatient controls tend to yield non-significant results when compared to previous cycle controls. Thirdly, cases with zero event data were analyzed using Peto's correction method, contributing to the statistical reliability of the findings. Finally, a funnel plot analysis revealed no evidence of publication bias among the studies included in this meta-analysis.

Nevertheless, several limitations apply. Only one randomized trial was available due to the scarcity of such studies and our eligibility criteria. Fertilization failure is a relatively rare outcome, which limits the feasibility of large randomized trials. With less strict inclusion and exclusion criteria, a meta-analysis of randomized trials was published recently [48]. However, heterogeneity in stimulation protocols, timing, exposure, and ionophore concentration still introduces clinical variability that cannot be fully accounted for. Standardized protocols would improve comparability across centers. Finally, the origin of fertilization failure is frequently unknown, which could mask differential responses to ionophore treatment. Even so, ESHRE has recently published that Ca^{2+} ionophore can be considered for use for cases of complete activation failure, very low fertilization rate or globozoospermia [27].

Conclusion

The consistent improvement in fertilization and downstream outcomes supports the use of calcium ionophores as an adjuvant treatment for couples experiencing fertilization failure. These results are reassuring regarding reproductive and perinatal safety, although long-term follow-up data remain limited. Given the lack of benefit in cases of oocyte-related activation deficiency, routine use in all ICSI cycles is not warranted. Instead, calcium ionophores should be considered selectively, ideally following diagnostic evaluation to identify patients most likely to benefit.

Calcium ionophores as artificial oocyte activation agents significantly improve reproductive outcomes in couples with fertilization failure, with no evidence of increased miscarriage or perinatal risk. The benefits appear greater in total fertilization failure and when ionomycin is used. However, until stronger evidence and standardized protocols are available, treatment should be reserved for carefully selected patients rather than applied indiscriminately. As the ROBINS-I guide showed high risk of bias especially in the first two domains, further research should prioritize well-designed multicenter trials with standardized protocols to minimize heterogeneity and confirm efficacy. Also last but not least there is a need of commercially available calcium ionophore product specifically designed and certified for use in IVF.

Declarations

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Consent for publication: Not applicable.

Ethical approval: This article was approved by the institutional ethics committee under the number 2503-VLC-035-MD.

Data availability: All data analyzed in this meta-analysis are available within the article and its supplementary materials. Data will also be made available to the editors of the journal for review or query upon request.

Disclosure statement: The authors declare no conflicts of interest related to this work.

Attestation statement: Data regarding any of the subjects in the study has not been previously published.

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Figures

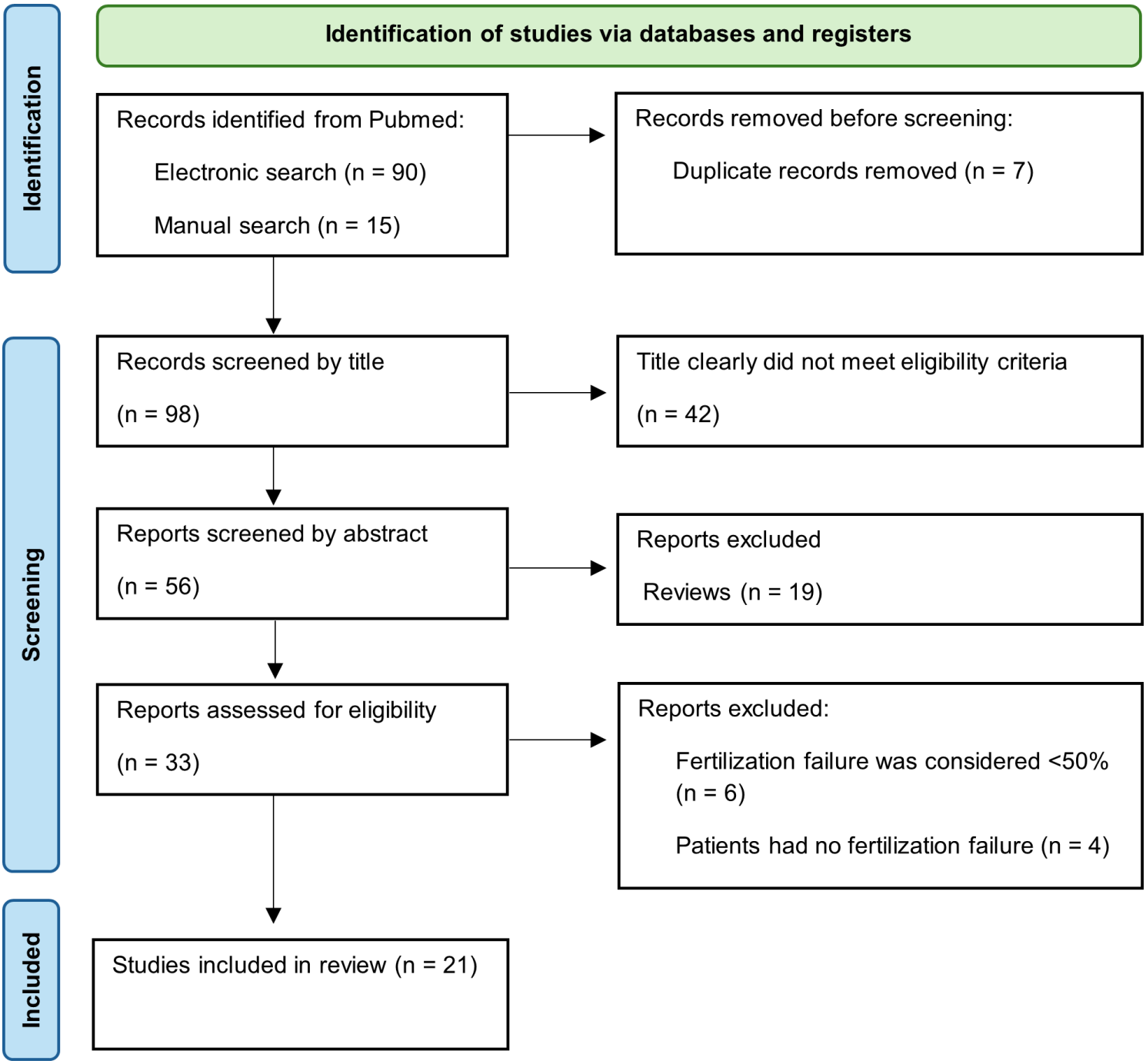


Figure 1

PRISMA 2020 flow diagram of included studies in the meta-analysis

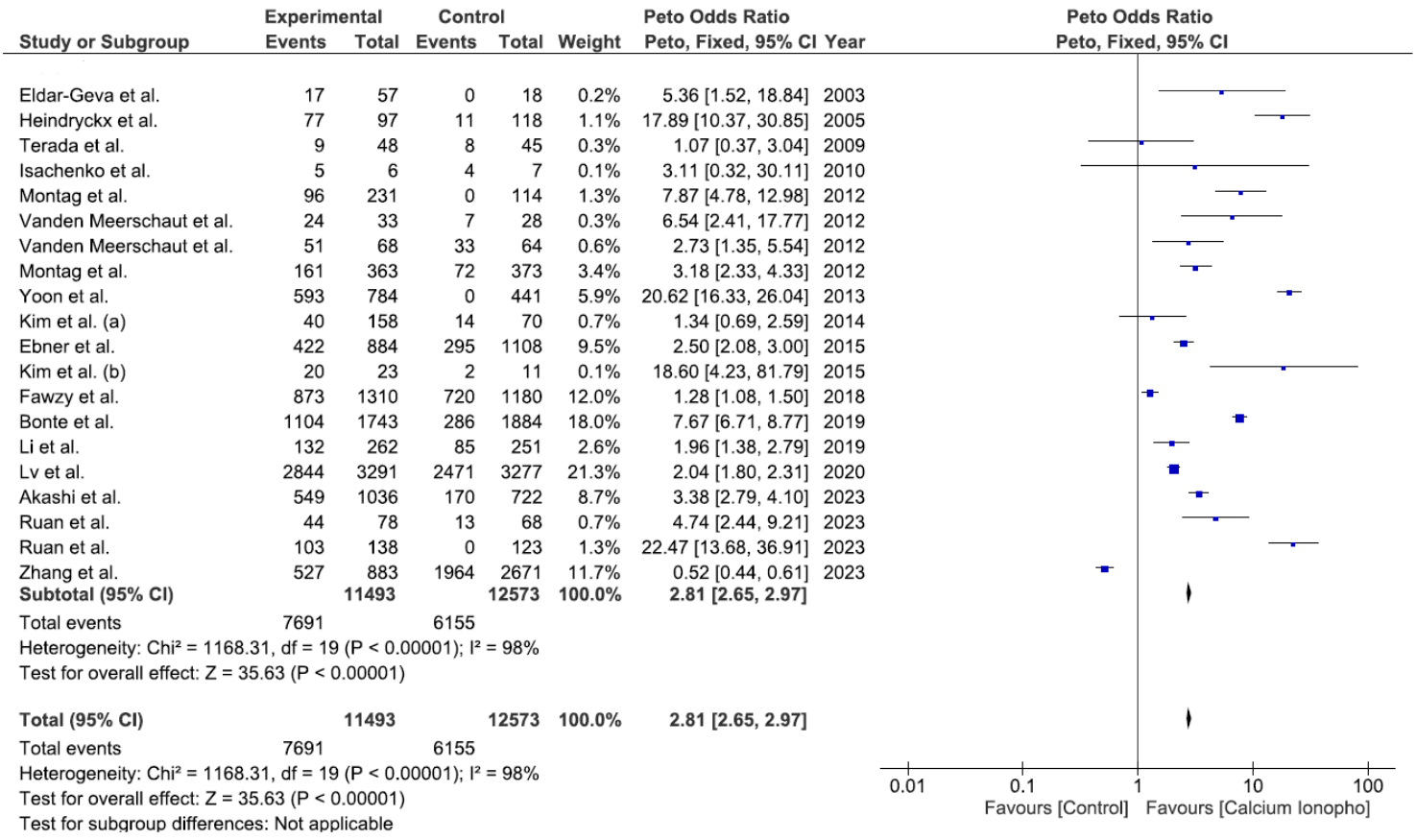


Figure 2

Overall fertilization rate forest plot.

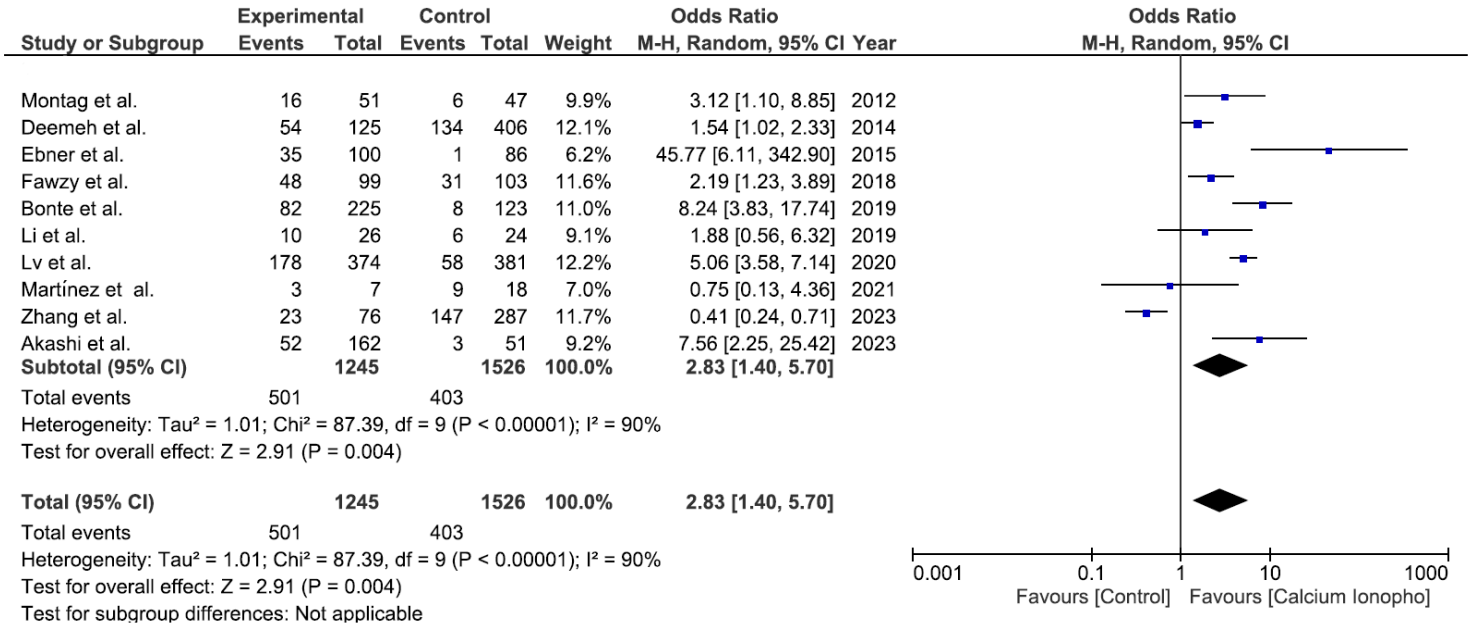


Figure 3

Overall clinical pregnancy rate forest plot.

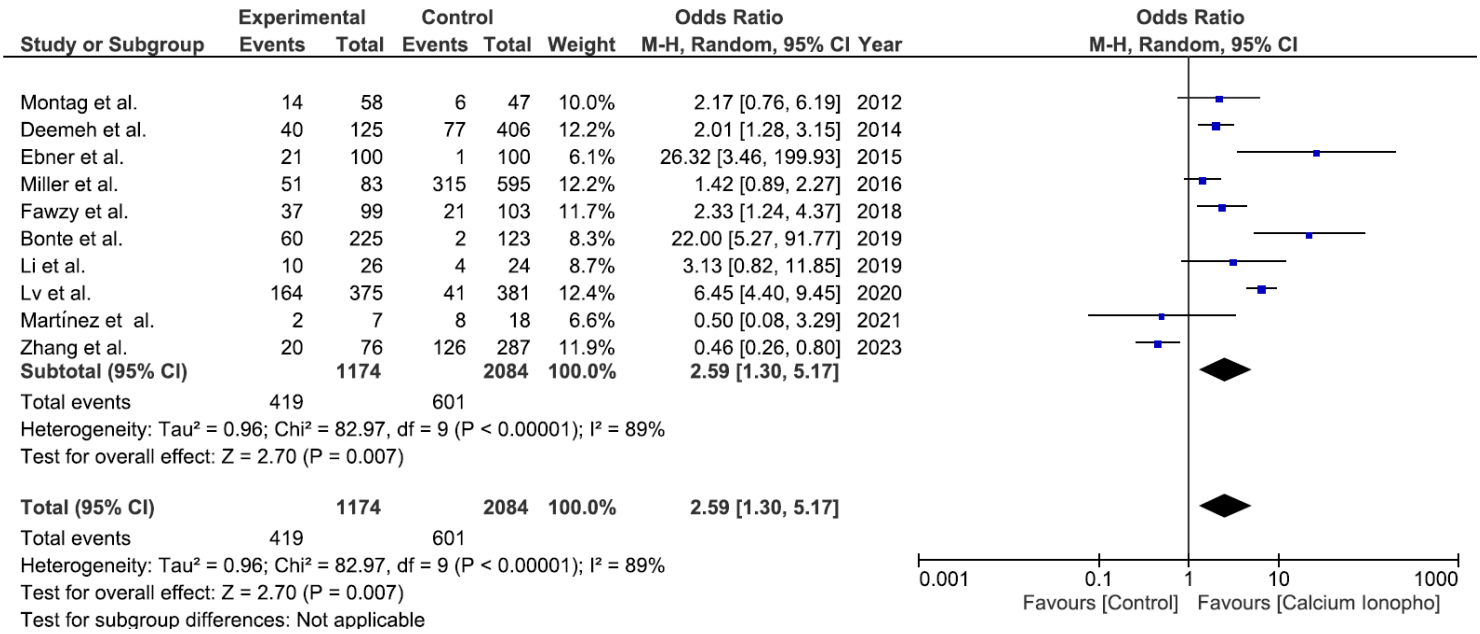


Figure 4

Overall live birth rate forest plot.

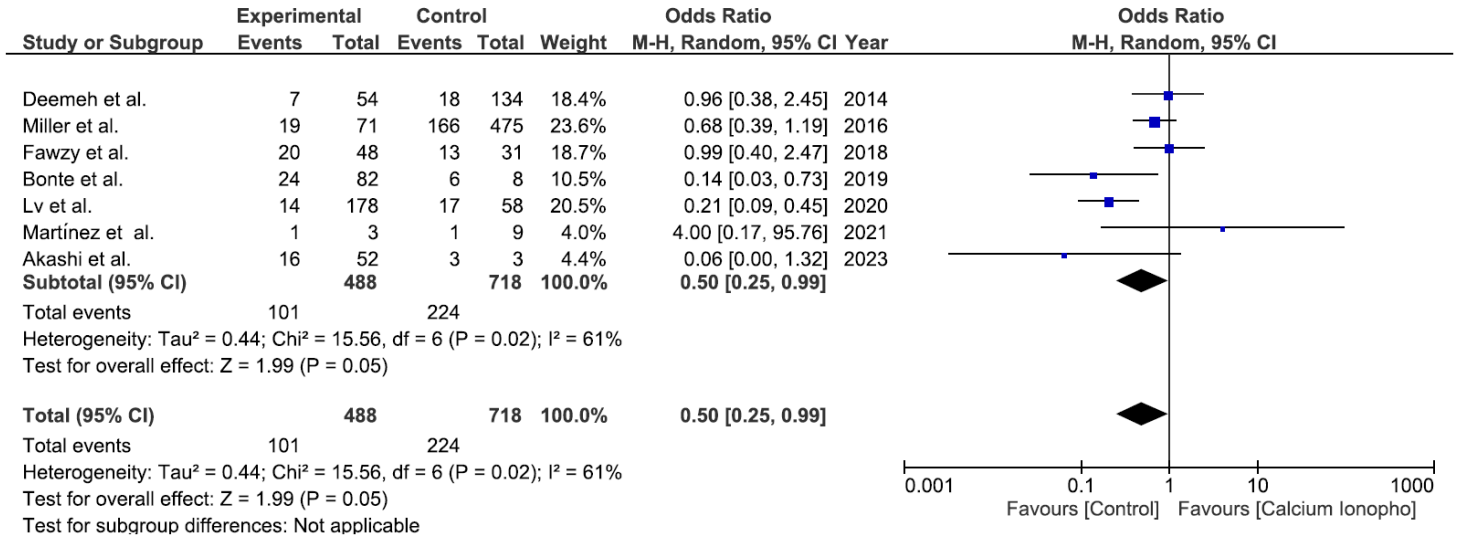


Figure 5

Overall miscarriage rate forest plot.

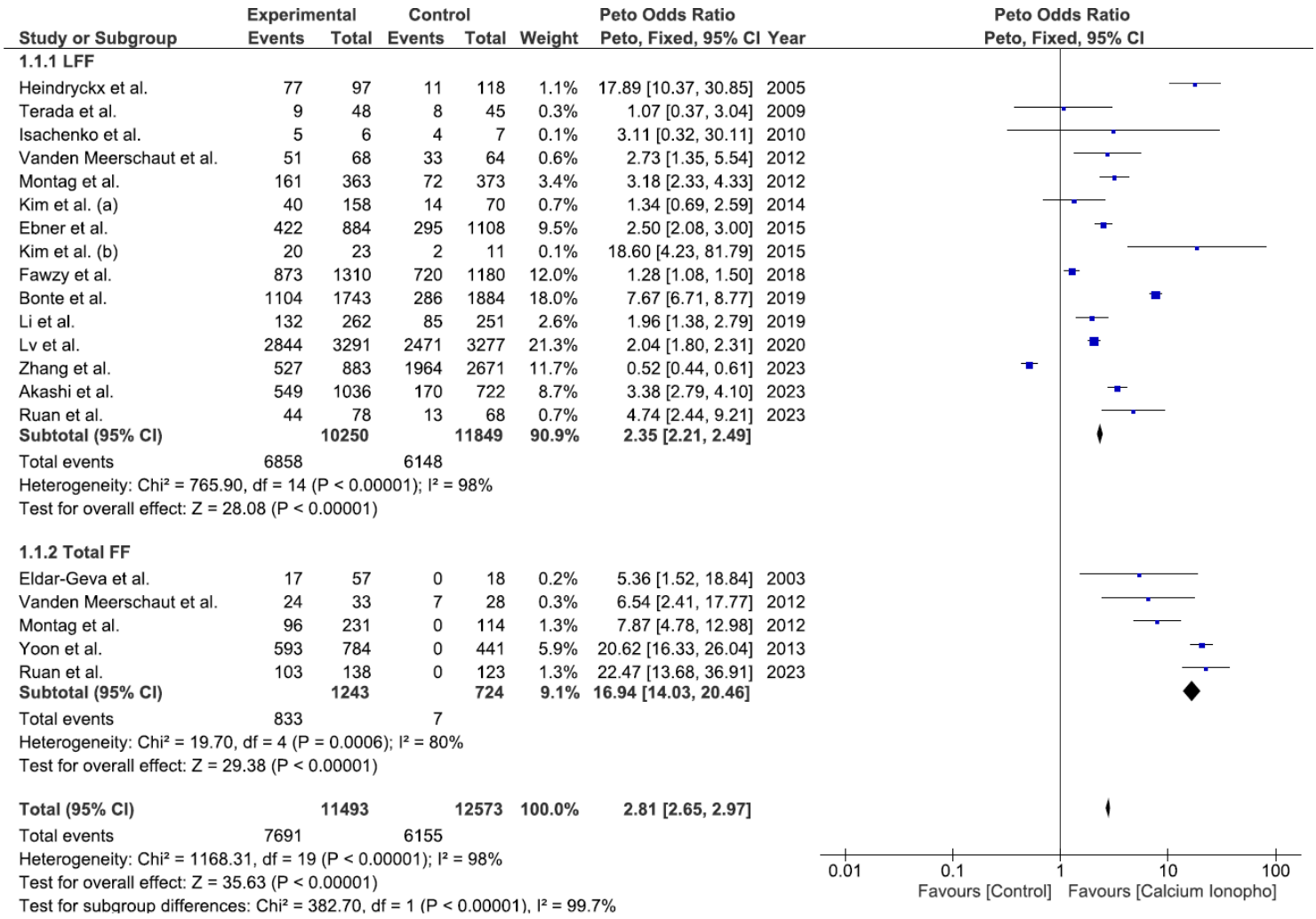


Figure 6

Forest plot for fertilization rate according to the type of fertilization failure.

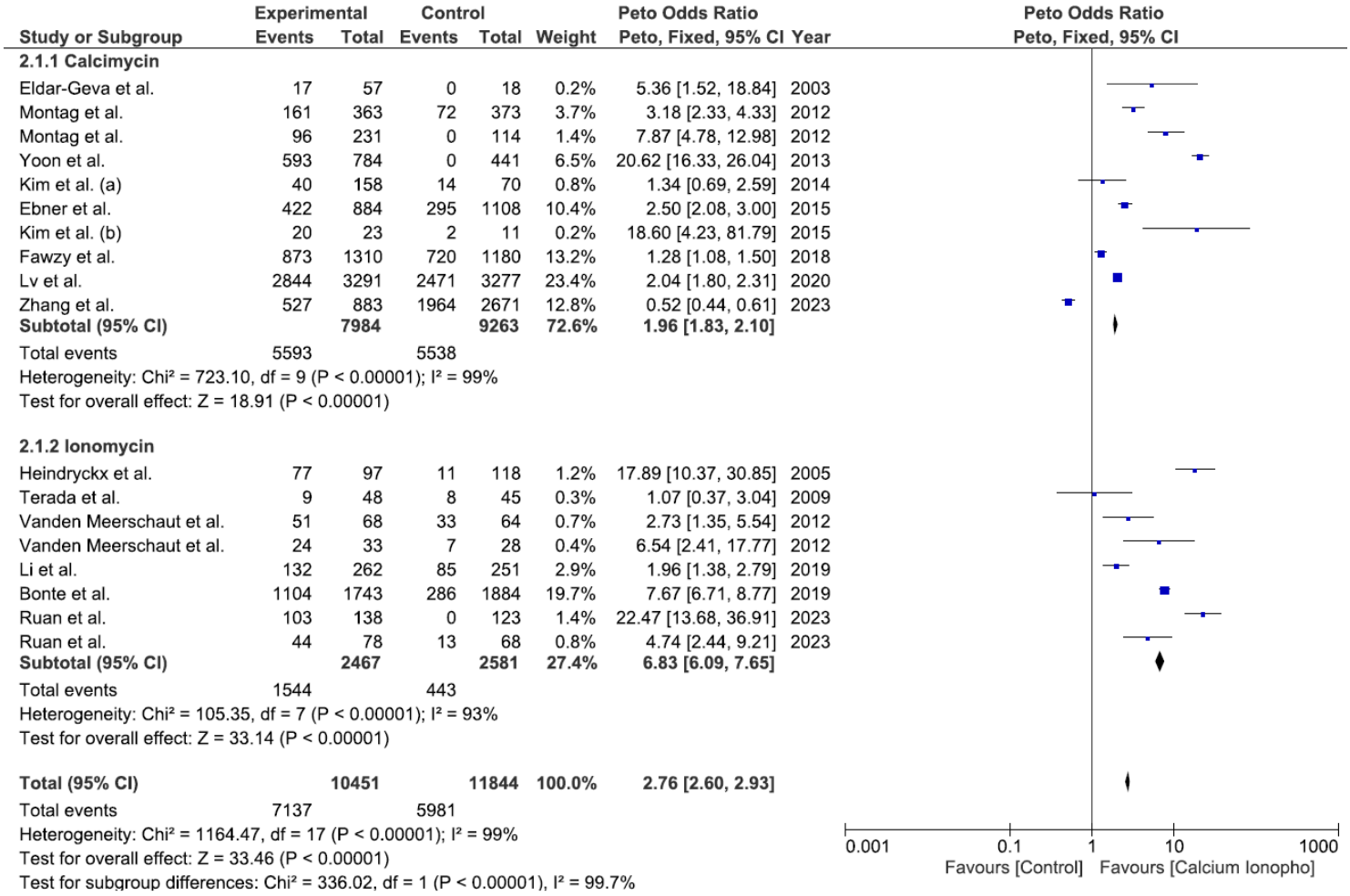


Figure 7

Forest plot for ionophore type subgroup analysis of fertilization rate.

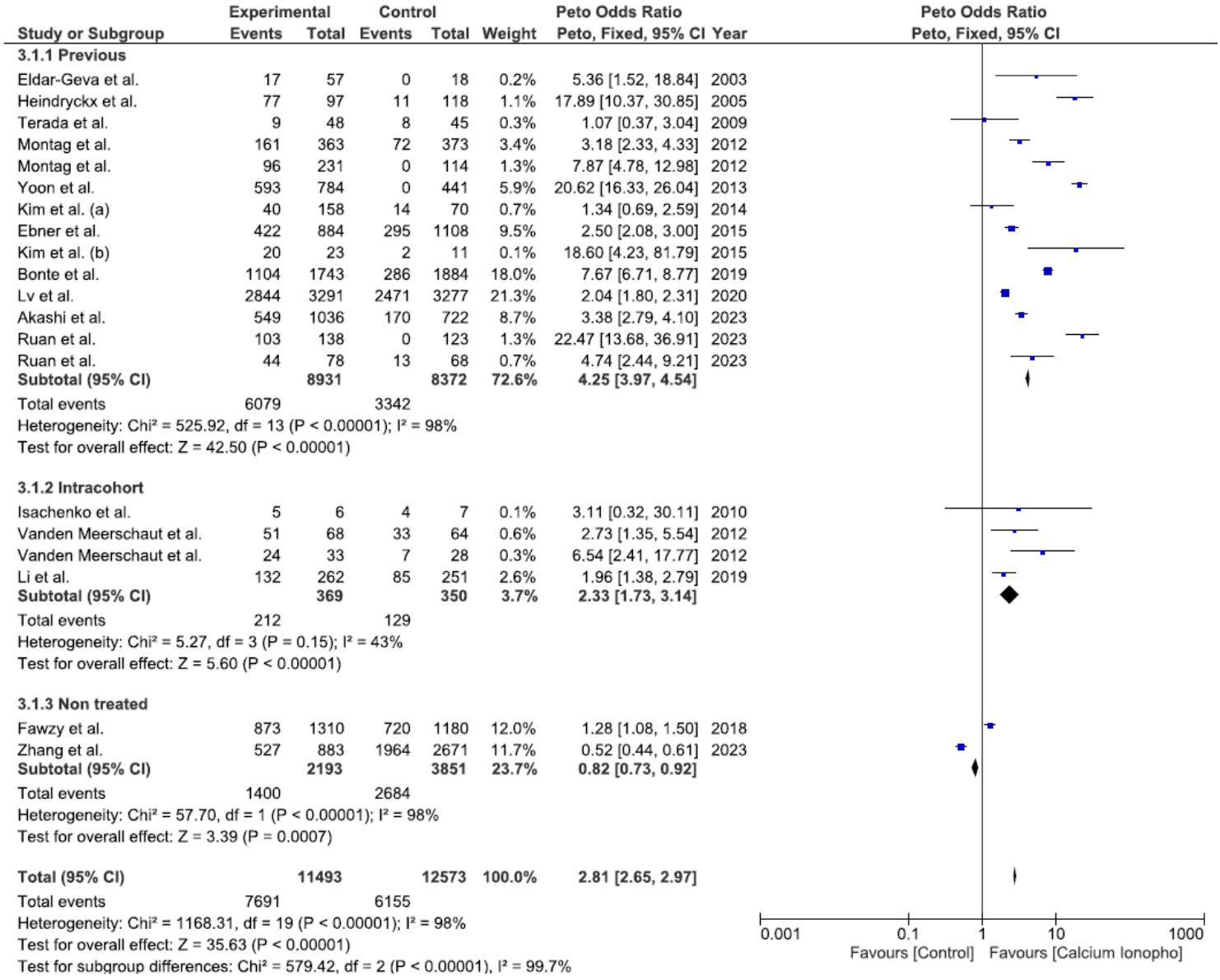
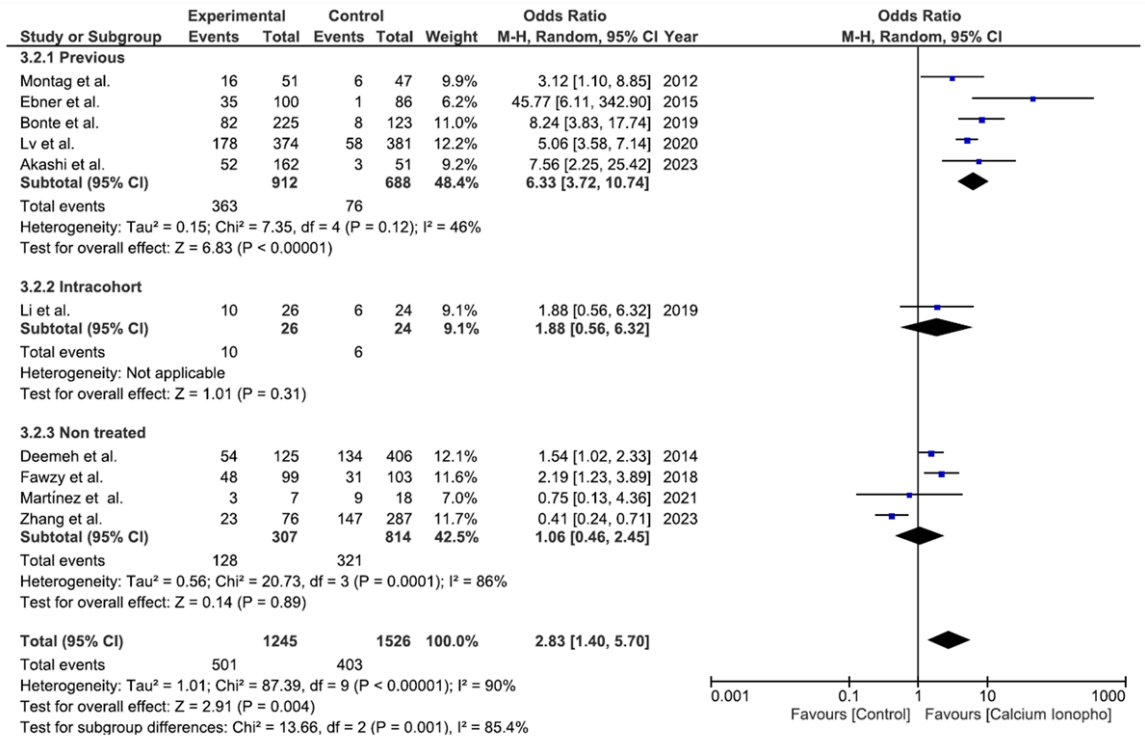


Figure 8

Forest plot for control type subgroup analysis of fertilization rate.



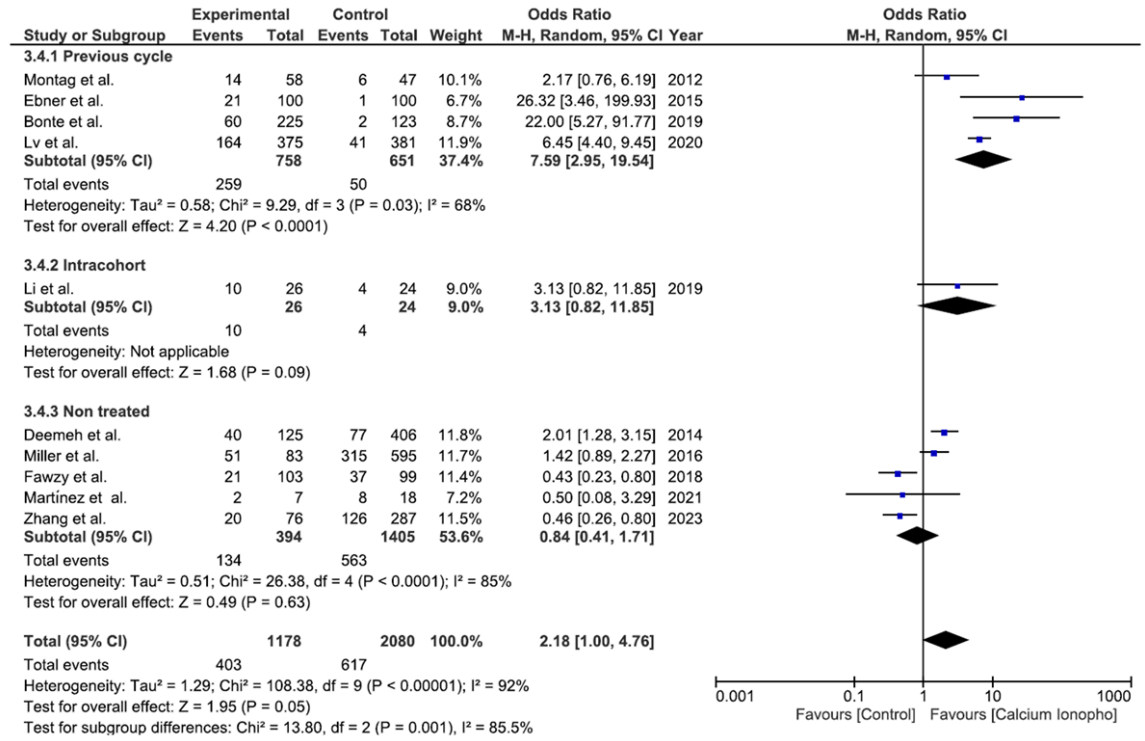


Figure 9

Forest plot for control type subgroup analysis of clinical pregnancy rate (up) and live birth rate (down).

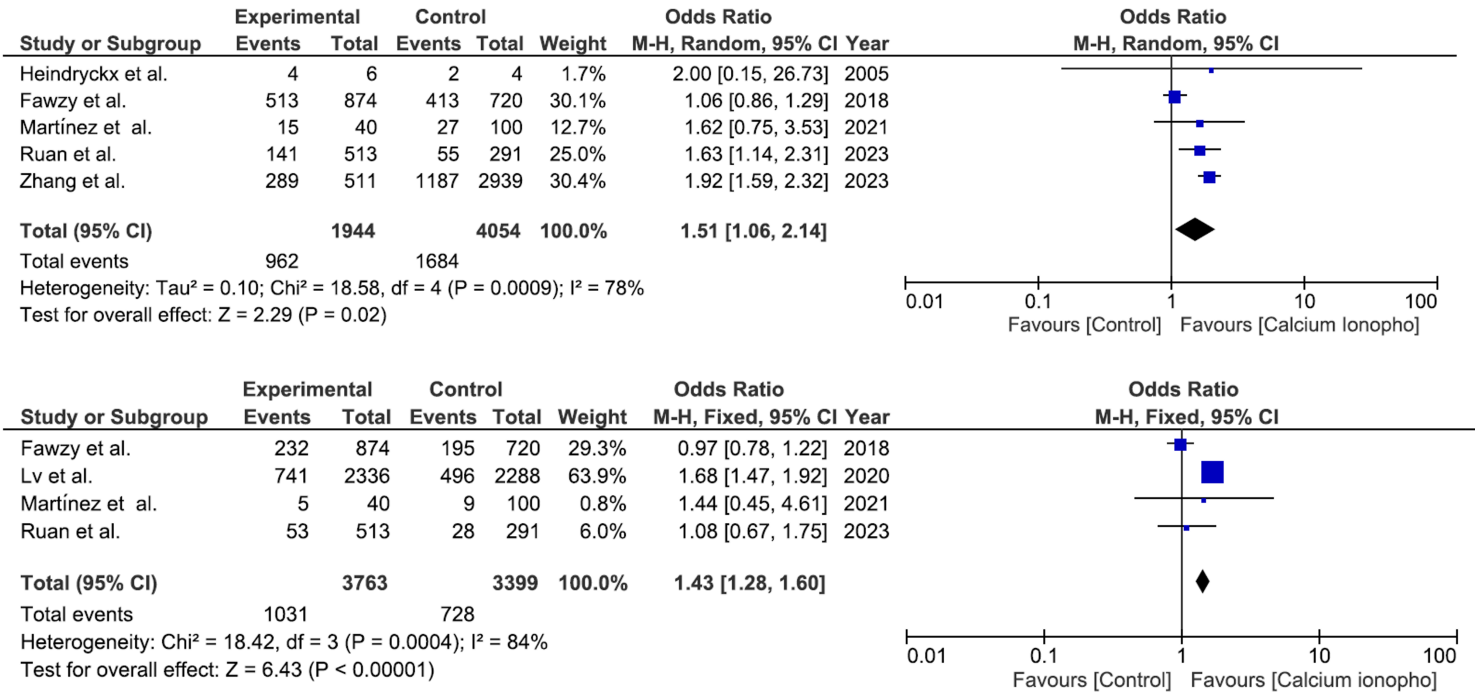


Figure 10

Embryo quality forest plots for D3 embryos (up) and blastocyst stage embryos (down).

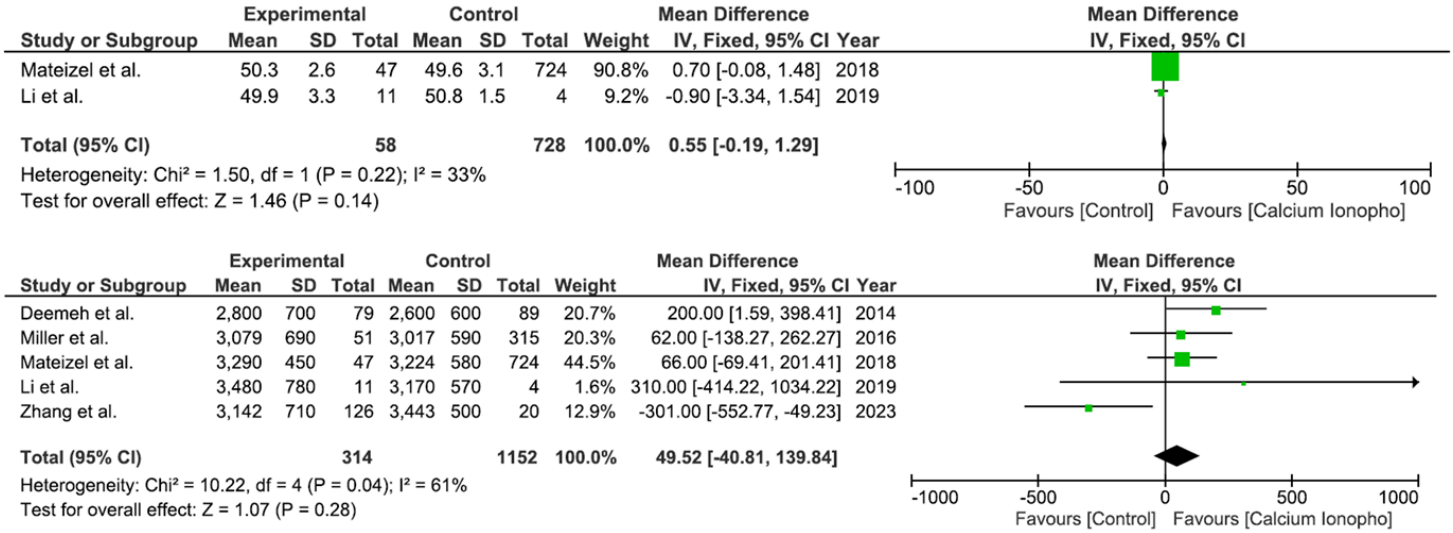


Figure 11

Perinatal characteristic forest plots for baby length (up) and weight (down).

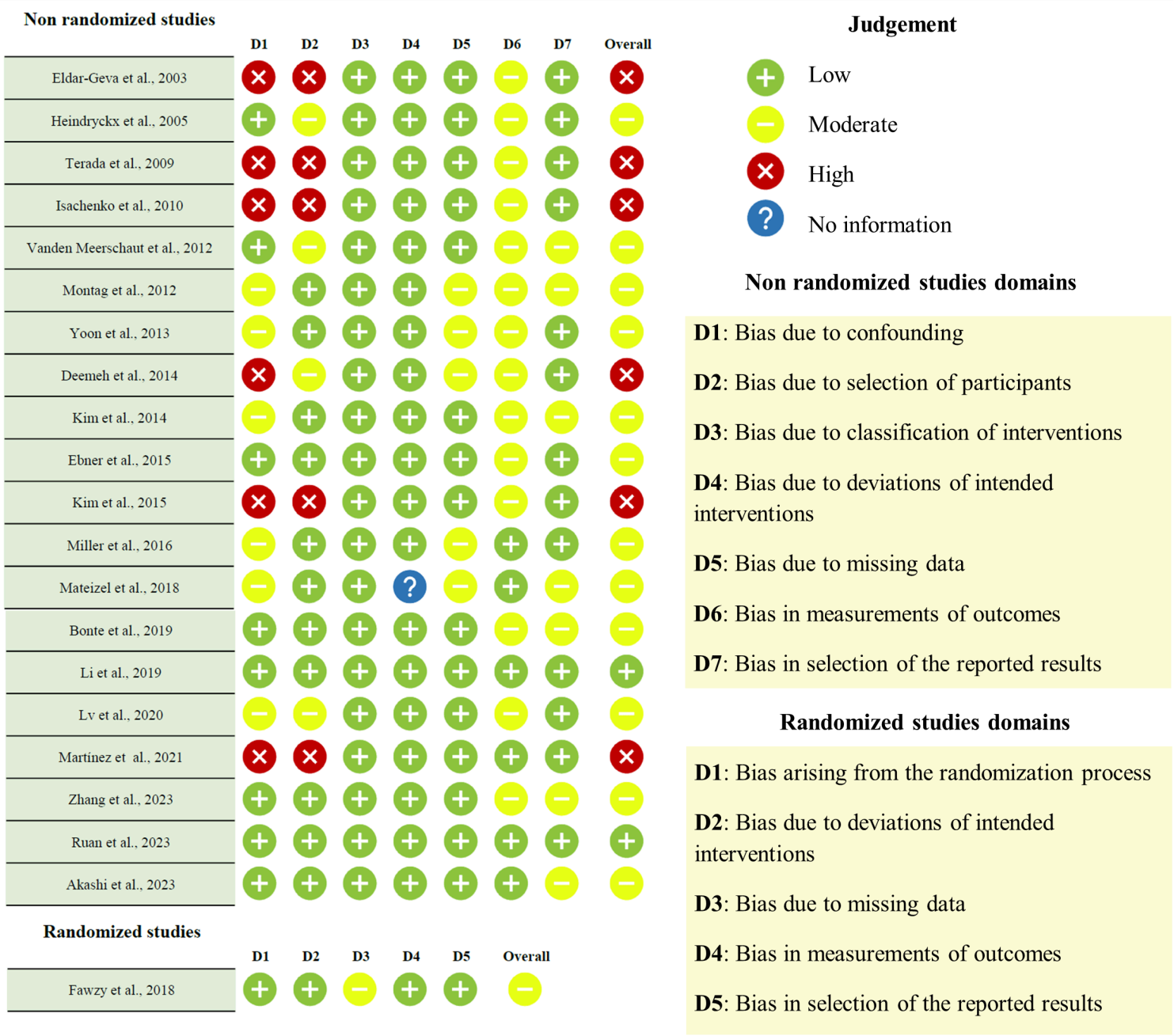


Figure 12

Risk of bias plot of all included studies.

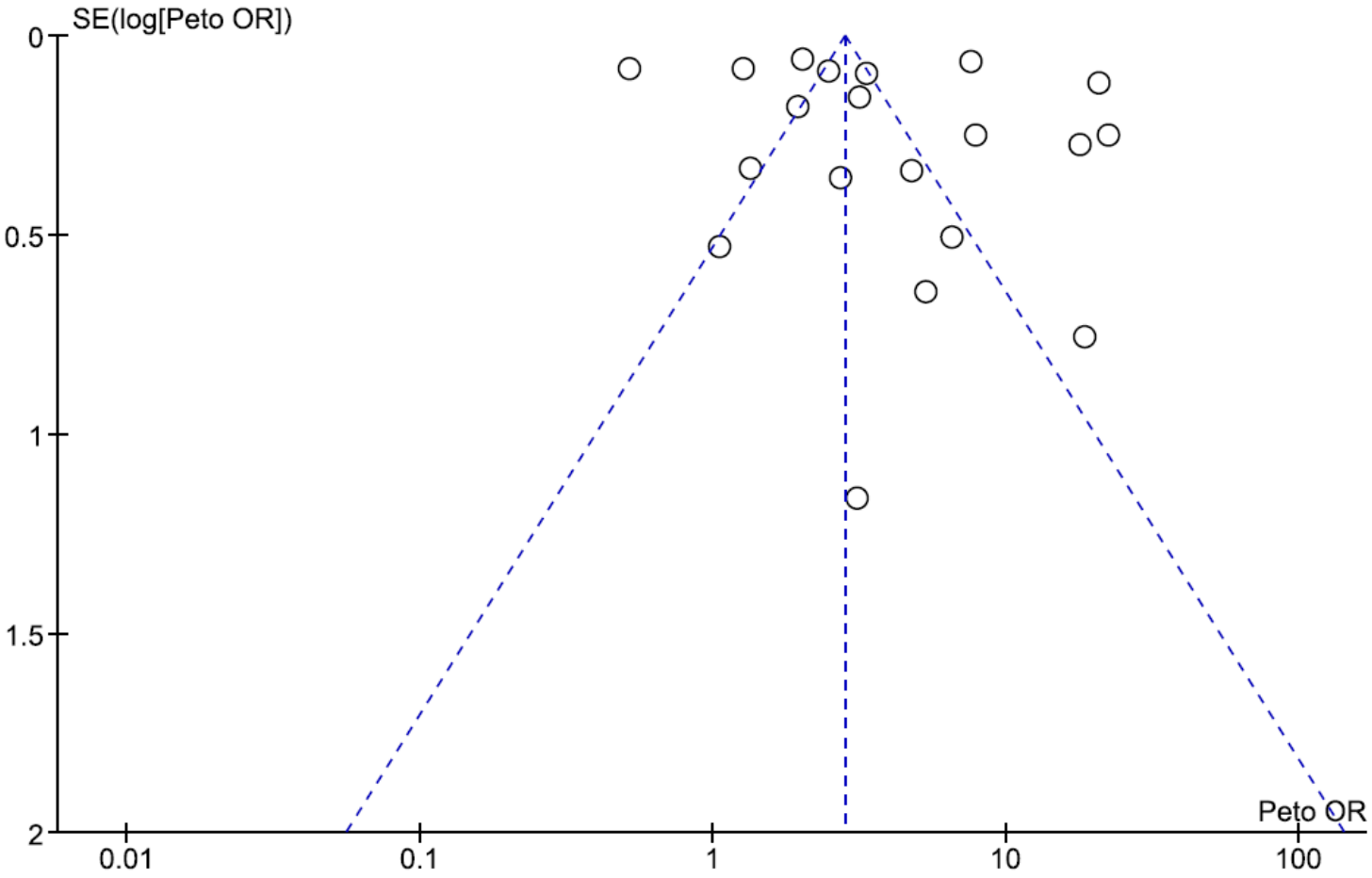


Figure 13

Funnel plot with all studies included in the meta-analysis.

Supplementary Files

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