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# Does intracytoplasmic sperm injection improve live birth rate when compared with conventional in vitro fertilization in non-male factor infertility? A systematic review and meta-analysis

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## ► To cite this version:

Astrid de Bantel-Finet, Elisangela Arbo, Marina Colombani, Bernadette Darné, Vanessa Gallot, et al.. Does intracytoplasmic sperm injection improve live birth rate when compared with conventional in vitro fertilization in non-male factor infertility? A systematic review and meta-analysis. *F&S Reviews*, 2022, 3 (1), pp.57-68. 10.1016/j.xfnr.2021.09.004 . hal-04698786

**HAL Id: hal-04698786**

**<https://hal.science/hal-04698786v1>**

Submitted on 13 Nov 2024

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1 **Running title: ICSI use in non-male infertility**

2 **Does Intracytoplasmic sperm injection (ICSI) improve live birth**  
3 **rate when compared to conventional *in vitro* fertilisation (IVF) in**  
4 **non-male factor infertility? A systematic review and meta-analysis**

5

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## 25 **Support**

26 This work was sponsored by an unrestricted grant from Gedeon Richter France.

27 The funder provided support in the form of salaries for one of the authors (EA). EA  
28 participated in full-texts screening, quality assessment, data extraction, drafted and revised the  
29 manuscript. The funder did not have any additional role in the study design, analysis, and  
30 decision to submit the article for publication. The specific roles of the authors are articulated  
31 in the 'author contributions' section.

## 32 **Disclosure summary**

33 None

34 Having one author affiliated to the funder does not alter our adherence to F&S Reviews<sup>®</sup>  
35 policies on sharing data and materials.

36

## 37 **Abstract**

### 38 **Objective:**

39 Initially developed for sperm abnormalities, the indications of intracytoplasmic sperm  
40 injection (ICSI) have progressively expanded beyond male factor infertility, but the benefit of  
41 ICSI over conventional *in vitro* fertilisation (IVF) in terms of live birth rate (LBR) in non-  
42 male factor infertility has not been formally demonstrated in the literature. The aim of this  
43 systematic review and meta-analysis was to determine whether the use of ICSI improves LBR  
44 as compared to IVF in non-male factor infertility cases.

### 45 **Evidence review:**

46 This prospectively registered systematic review and meta-analysis was guided by PRISMA  
47 guidelines. PubMed, Embase, Cochrane Library were searched for relevant literature reported  
48 between January 01, 2004 and March 15, 2020. Only articles conducted in couples without  
49 obvious male factor infertility according to the authors were included. Live Birth Rate (LBR)  
50 per cycle and/or per transfer was used as main outcome. Fertilisation rate, cycles with total  
51 fertilisation failure, and clinical pregnancy rate were also recorded as secondary outcomes.  
52 Study selection, bias assessment and data extraction were performed by two independent  
53 reviewers. The methodological quality of the selected studies was assessed by two  
54 independent reviewers using the Cochrane Handbook methods and by the adapted Newcastle-  
55 Ottawa Quality Assessment Scale for cohort studies. The risks of bias were assessed using  
56 ROBIN-1 tools. Risk ratio and 95% confidence interval (CI) were estimated using random  
57 effect model.

### 58 **Results:**

59 Among the 1,760 potentially eligible studies, 61 were fully assessed and 21 were included in  
60 the meta-analysis, corresponding to almost 1,000,000 assisted reproductive technology cycles.  
61 Among the selected studies, 17 reported LBR per cycle, 11 reported LBR per transfer, and 7

62 among them reported both outcomes. One was a prospective randomised controlled trial,  
63 while others were either retrospective cohort (n=17) or case-control studies (n=4). Individual  
64 studies' sample size ranged from 60 to 569,605 cycles. Meta-analysis showed that IVF  
65 provided a significantly higher LBR per cycle when compared to ICSI (RR=1.10;  
66 95%CI=1.02-1.18). LBR per transfer tended to be higher when IVF was used as compared to  
67 ICSI, but this difference barely reached statistical significance (RR 1.18, 95% CI 1.00-1.38,  
68 p=.05). Subgroup analysis performed in poor responders showed no significant difference  
69 between IVF and ICSI concerning LBR per cycle or LBR per transfer. Secondary analyses  
70 demonstrated a lower fertilisation rate, but comparable clinical pregnancy and fertilisation  
71 failure rate with IVF as compared to ICSI. Sensitivity analyses led to similar results and  
72 conclusions.

73 **Conclusion:**

74 Despite its limitations, mainly related to the inclusion of a majority of retrospective studies,  
75 this meta-analysis synthesizes and reinforces the existing literature and provides further  
76 evidence that ICSI does not improve LBR per cycle / per transfer as compared to IVF in  
77 couples undergoing assisted reproductive technology for non-male infertility.

78 **Registration number:** CRD42019136383

79 **Key Words:** IVF; ICSI; infertility; live birth

## 80 **Essential points**

81 ► The use of ICSI has noticeably increased beyond male factor infertility and is now more  
82 frequently used than conventional IVF in most countries.

83 ► Numerous studies compared Live Birth Rate (LBR) after ICSI and IVF in various cases of  
84 non-male infertility, leading to conflicting results.

85 ► Our meta-analysis conducted in 21 studies, corresponding to almost 1,000,000 assisted  
86 reproductive technology cycles, demonstrates that ICSI does not improve LBR per cycle or  
87 per transfer as compared to IVF in couples undergoing assisted reproductive technology for  
88 non-male infertility.

89 ► Although most studies included in the analysis suffer from the inherent biases of  
90 retrospective studies, the relevance of our conclusion is supported and confirmed by the  
91 results of a recently published large randomised controlled trial.

## 92 **Introduction**

93 Intracytoplasmic sperm injection (ICSI) was first introduced in 1992 and revolutionized the  
94 treatment of infertile couples with poor semen quality, whose chances of pregnancy were up  
95 till then limited to conventional *in vitro* fertilisation (IVF) with disappointing results (1). Over  
96 recent decades, the use of ICSI has noticeably increased and is now more frequently used than  
97 conventional IVF in most countries, with significant variations among countries. Indeed, the  
98 last world report on assisted reproductive technology (ART), released by the International  
99 Committee for Monitoring ART (2), reported an overall ICSI use of 66.5% in 2011, ranging  
100 from ~50% in Scandinavian countries to >90% in Spain or Latin America, and even up to  
101 100% in Egypt or Lebanon. Along the same lines, the last publication of European data  
102 (cycles performed during year 2016) found an overall ICSI use of 72% (3). More strikingly,  
103 this increasing use of ICSI has been reported among couples with non-male factor infertility  
104 (4). Although the definition of non-male factor infertility can slightly vary across studies,  
105 generally referring to men with abnormal semen parameters according to WHO (5) but not  
106 only, this drift towards increasing ICSI use in non-male factor infertility can therefore be  
107 questioned.

108 Numerous observational, retrospective studies compared Live Birth Rate (LBR) after ICSI  
109 and IVF in various cases of non-male infertility, leading to conflicting results. Indeed, while  
110 some authors reported higher LBR with ICSI than with IVF in non-male factor infertility (6),  
111 some others concluded to the absence of superiority of ICSI over IVF (7,8) and few reported  
112 improved clinical outcome with ICSI as compared to IVF (9). However, the heterogeneity and  
113 limitations of these studies in terms of design and population provide poor quality evidence  
114 and prevent from drawing any firm conclusion. This debate was very recently fuelled by the  
115 results a large prospective randomised controlled trial (10), where no benefit of ICSI over IVF

116 was found in infertile couples in whom the male partner presented normal sperm count and  
117 motility according to WHO 2010 criteria (5).

118 Altogether, the steady increased use of ICSI in non-male factor infertility cases, along with  
119 the abundant available literature leading to conflicting results, advocates for a systematic  
120 review and meta-analysis on this topic in order to shed light on the relevance of ICSI use in  
121 non-male factor infertility, and ultimately improve medical practice and patients' counselling.

122 The aim of this systematic review of the literature and meta-analysis was to determine  
123 whether the use of ICSI improves live birth rate (LBR) as compared to IVF in non-male  
124 factor infertility cases.



## 125 **Methods**

### 126 **Search strategy and selection criteria**

127 The search strategy, selection criteria, data extraction, quality assessment, and statistical  
128 analyses described below were defined *a priori* (S1 File). The conduct and reporting of this  
129 systematic review and meta-analysis was guided by PRISMA guidelines (S2 File) and  
130 prospectively registered (PROSPERO CRD42019136383). All studies (including  
131 observational cohort studies and randomised clinical trials) reporting IVF outcome after the  
132 use of ICSI or IVF in non-male factor infertility cases were included in the initial screening.  
133 Only articles conducted in couples without obvious male factor infertility according to the  
134 authors were included. LBR per cycle and LBR per transfer were chosen as the main outcome  
135 of interest and differentiated so as to take into account the potential cycles in which no  
136 transfer was carried out, as in the cases of fertilisation failure. Studies included were required  
137 to report values of live birth (LB) in both ICSI and IVF groups. Secondary outcomes were  
138 clinical pregnancy rate, fertilisation rate and proportion of cycles resulting in fertilisation  
139 failure. Studies including preimplantation genetic testing or gamete donation cycles were  
140 systematically excluded, as well as studies reporting only other clinical outcomes than LBR.  
141 PubMed, Embase, Cochrane Central Register of Controlled Trials, and Cochrane Database of  
142 Systematic Reviews were searched for relevant literature. The search strategy was limited to  
143 articles published in English or French between January 01, 2004 and March 15, 2020, in  
144 order to limit the bias related to major modifications in lab procedures over a too long study  
145 period. The search strategy for electronic databases is described in detail in the S3 File. The  
146 literature search strategy was performed by an information specialist in association with the  
147 referral Inter-University Library of Medicine of Paris Descartes, Paris 5, France. Studies  
148 published in other languages than English or French were not retained.

## 149 **Study selection and data extraction**

150 Screening of titles and abstracts of all articles to determine which studies should be assessed  
151 further was carried out by two independent reviewers. This first screening was blinded to  
152 authors, institutions, journal titles and study results. Any disagreement or uncertainty was  
153 resolved by discussion with a third reviewer. Based on the pre-established inclusion criteria,  
154 full texts of potentially relevant articles were retrieved and assessed for inclusion by two  
155 reviewers independently. Methodological validity was also assessed prior to inclusion in the  
156 review. Any disagreement or uncertainty was resolved by discussion among reviewers to  
157 reach a consensus. Two independent reviewers carried out study selection, bias assessment  
158 and data extraction. Data were extracted from included articles using a data extraction form  
159 designed by the authors. The following study details were collected to characterize the  
160 included studies: country, study design, study type, indication of IVF/ICSI, split oocytes  
161 strategy or not, inclusion and exclusion criteria, study period; and for each group (IVF and  
162 ICSI): number of women, number of cycles, number of transfers, maternal age, number of  
163 oocytes injected/inseminated, fertilisation rate, cycles with total fertilisation failure, number  
164 of embryos transferred, clinical pregnancy per cycle (n, N, %), LBR per cycle, LBR per  
165 transfer (n, N, %). When only data reported by subgroups were available in the article (for  
166 example: age categories) data were pooled for overall meta-analysis.

## 167 **Data analysis**

168 All qualifying articles with quantitative data for LBR, with documented numbers for IVF and  
169 ICSI groups were included in the meta-analysis. No replacement of missing data was done.  
170 When only percentages were available, and when possible, the number of events was derived  
171 from the total number of cycles/transfers in each population group. LBR was compared  
172 between IVF and ICSI groups by estimation of risk ratio (RR IVF/ICSI) with its estimated

173 two-sided 95% confidence interval (CI). Secondary outcomes (clinical pregnancy rate,  
174 fertilisation rate and proportion of cycles with no fertilisation) were analysed independently  
175 using the same method.

176 The methodological quality of the selected studies was assessed by two independent  
177 reviewers using the Cochrane Handbook methods and by the adapted Newcastle-Ottawa  
178 Quality Assessment Scale for cohort studies (11). The following risks of bias were assessed  
179 using ROBIN-1 tools (12): confounding, selection of participants, intervention classification,  
180 intervention deviations, missing data, outcome measurement, and selection of reported  
181 results. Each risk of bias criteria was judged as 'low', 'high' or 'unclear' risk (12).

182 Meta-analysis was conducted using the random-effect model with the Mantel-Haenszel (M-H)  
183 method. Pooled effect sizes were deemed statistically significant at  $p < 0.05$ . In addition to the  
184 estimation of between-study variance ( $\text{Tau}^2$ ), the statistical heterogeneity across the studies  
185 was tested by the Q chi-square test. Inconsistency across studies was quantified using the  $I^2$   
186 statistic and interpreted following the Cochrane Collaboration guide (13). Possible publication  
187 bias was assessed using funnel plots. The software Review Manager 5.3.5 (Copenhagen: The  
188 Nordic Cochrane Centre, The Cochrane Collaboration, 2014) was used to combine and  
189 analyse the aggregated data. The quality of evidence for each outcome was judged using the  
190 Grading of Recommendations Assessment, Development and Evaluation working group  
191 methodology (14).

192 Pre-specified subgroup analyses were performed based on the type of non-male infertility  
193 (unexplained, poor responders, and other). Additional subgroup analysis was performed in  
194 studies conducted before and after the release of the new WHO manual for semen analysis in  
195 2010 (5).

## 196 **Sensitivity analysis**

197 Several pre-specified sensitivity analyses planned on statistical analysis plan were performed  
198 on overall population and subgroups based on the type of non-male infertility (unexplained,  
199 poor responders, other). To explore statistical heterogeneity, meta-analysis using a fixed-  
200 effect model was performed to compare estimates of the intervention effect of fixed- and  
201 random-effect models. Furthermore, sensitivity analysis was performed by excluding the  
202 outliers identified in the funnel plot and followed by exploration of other potential outliers  
203 that were excluded one by one until heterogeneity was no longer considerable. Sensitivity  
204 analyses were also performed to assess the possible impact of study weight. This was first  
205 performed by visual inspection of the forest plot displayed in ascending order of study weight.  
206 Studies with a high number of patients also underwent weight reduction under 25% before re-  
207 analysis. Sensitivity analysis was also redone after removing small studies (i.e. <100 subjects  
208 in either IVF or ICSI groups). The potential impact of publication year, and of “old” data (i.e.  
209 cycles performed <2001) was also independently evaluated. To verify whether the conclusion  
210 would have been different if eligibility was restricted to studies with low risk of bias, another  
211 sensitivity analysis was performed omitting all studies with at least one category with high  
212 risk of bias.

## 213 **Meta regression**

214 A meta-regression analysis was performed on the main outcome to adjust for the main  
215 potential confounding factor: mean female age of the whole population in each study. Since a  
216 fixed effect meta-regression is likely to produce misleading results in the presence of  
217 heterogeneity, a random-effect meta-regression was performed with mean age as the fixed  
218 effect. The variance components of random-effects were estimated using the restricted  
219 (residual) maximum likelihood (REML) method. The Knapp and Hartung method was used to  
220 adjust test statistics and CI (15). R statistical package ‘meta’ V4.11-0 was used.

## 221 **Results**

### 222 **Main characteristics of the available literature**

223 Literature screening allowed identification of 1,760 potentially eligible studies, among which  
224 61 were fully assessed. Forty-five studies were qualitatively assessed and 21 studies were  
225 included in the meta-analysis (Fig 1) after excluding other studies for various reasons (out of  
226 topic, main outcome (LBR) missing or not differentiated, major allocation bias, review/meta-  
227 analysis). Among the selected studies, a total of 17 studies reported LBR per cycle, 11  
228 reported LBR per transfer, and 7 among them reported both outcomes. One of these was a  
229 randomised controlled trial (16), and one was published in another language than English (i.e.  
230 in French) (17). The main characteristics of these studies are summarized in table 1.  
231 Individual studies' sample size ranged from 60 to 569,605 cycles. Results in the text are only  
232 reported from random effect models due to underlying heterogeneity in the studies.

233

### 234 **Main analysis: Live Birth Rate (LBR) per cycle and per transfer** 235 **in ICSI vs conventional IVF cycles**

236 A significant increased probability of LBR per cycle was found in favour of IVF as compared  
237 to ICSI (RR 1.10, 95% CI 1.02-1.18) (n=17 studies: 409,429 IVF and 560,716 ICSI cycles)  
238 (Fig 2), with a considerable heterogeneity ( $I^2=97%$ ,  $p<0.00001$ ).

239 Heterogeneity was neither associated with studies' weight nor with the year of publication.  
240 Weight reduction was not applicable. Removing small studies (7 out of 17) did not change the  
241 overall conclusion (n=10 studies: 407,919 IVF and 560,350 ICSI cycles, RR 1.11, 95% CI  
242 1.03-1.20,  $I^2=98%$ ,  $p<0.00001$ ). Funnel plot identified 4 outliers (9,19,21,27) (S1A Fig). The  
243 main characteristics of these studies appearing as outliers are listed in S1 table. After  
244 exclusion of these 4 outliers, the probability of LB per cycle was no longer significantly

245 different between IVF and ICSI (n=13 studies: 393,451 IVF and 516,246 ICSI cycles,  
246 RR 1.04, 95% CI 0.96-1.13), and heterogeneity remained very high ( $I^2= 97%$ ,  $p<0.00001$ ). To  
247 determine the origin of this heterogeneity, we then excluded the studies with at least one type  
248 of bias classified as high risk (n=5). This did not change the overall conclusion (n=12 studies:  
249 126,083 IVF and 223,168 ICSI cycles, RR 1.11, 95% CI 1.05-1.18) and had a limited effect  
250 on heterogeneity ( $I^2= 57%$ ). The exclusion of studies including data prior to 2001 (6,16) did  
251 not change the results nor the heterogeneity (n=15 studies: 136,966 IVF and 263,514 ICSI  
252 cycles, RR 1.13, 95% CI 1.05-1.21,  $I^2= 85%$ ). This lack of explanation for heterogeneity  
253 prompted further performance of post-hoc sensitivity analyses based on a one-by-one  
254 exclusion strategy of the studies included in the analysis. This led to identification of one  
255 specific study (6) which was responsible for heterogeneity. The exclusion of this study and  
256 the 4 outliers did not modify the overall conclusion of the analysis conducted in the remaining  
257 12 studies (121,018 IVF and 219,074 ICSI cycles, RR 1.08, 95% CI 1.06-1.09) but led to the  
258 dramatic decrease of heterogeneity ( $I^2= 0%$ ).

259 The probability of LB per transfer tended to be higher when IVF was used as compared to  
260 ICSI, but this difference barely reached statistical significance (n=11 studies: 6,433 IVF and  
261 8,440 ICSI transfers, RR 1.18, 95% CI 1.00-1.38,  $p=.05$ ) (Fig 3). Heterogeneity was moderate  
262 ( $I^2= 37%$ ,  $p>.05$ ). The funnel plot did not identify any outlier and did not suggest evidence for  
263 publication bias. Therefore, exclusion of outliers was not applicable (S1B Fig). Exclusion of  
264 studies with high risk of bias did not change conclusions (n=8 studies, RR 1.07, 95% CI 0.91-  
265 1.26,  $I^2= 19%$ ), nor did the exclusion of small studies (n=3 studies, RR 1.09, 95% CI 0.94-  
266 1.27,  $I^2= 23%$ ).

### 267 **Subgroup analysis in poor responders**

268 Subgroup analysis performed in poor responders showed no significant difference between  
269 IVF and ICSI concerning LBR per cycle (n=5 studies: 34,287 IVF and 29,473 cycles,

270 RR 1.03, 95% CI 0.65-1.62,  $I^2= 37%$ ) or LBR per transfer (n=3 studies: 489 IVF and 134  
271 transfers, RR 1.26, 95% CI 0.35-4.51,  $I^2= 72%$ ).

272

### 273 **Subgroup analysis before and after WHO criteria normal sperm** 274 **parameter modification**

275 Subgroup analysis performed in studies including patients from 2000-2009 (before the 2010  
276 modification of WHO criteria) showed no significant difference between IVF and ICSI  
277 concerning LBR per cycle (n=4 studies: 1,219 IVF and 206 ICSI cycles, RR 0.86, 95% CI  
278 0.56-1.34,  $I^2= 68%$ ). Subgroup analysis performed in studies including patients after 2010  
279 (after WHO criteria modification) showed a significant higher LBR per cycle in IVF than in  
280 ICSI group (n=5 studies: 11,495 IVF and 40,551 ICSI cycles, RR 1.25, 95% CI 1.21-1.30,  
281  $I^2= 0%$ ). The same subgroup analysis on LB per transfer led to the same conclusion.

282

### 283 **Secondary analysis: clinical pregnancy rate, fertilisation rate and** 284 **proportion of cycles with fertilisation failure in ICSI vs** 285 **conventional IVF cycles**

286 Secondary analyses demonstrated that fertilisation rate was significantly decreased when IVF  
287 was used when compared to ICSI (RR 0.80, 95% CI 0.71-0.89) (S2 Fig), while clinical  
288 pregnancy rate (RR 1.07, 95% CI 0.98-1.16) (S3 Fig) and fertilisation failure (RR 1.06,  
289 95% CI 0.75-1.48) (S4 Fig) were not significantly associated with the technique used for  
290 fertilisation. Meta-regression showed no significant association between mean female age and  
291 the log RR for LBR per cycle ( $p=0.42$ ) (S5 Fig) or the log RR for LBR per transfer ( $p=0.58$ ).  
292 Risk of bias of all included studies is displayed in S6 Fig. The Grade approach used for

293 grading the quality of evidence showed moderate and low certainty of evidence for primary  
294 and secondary outcomes respectively (S2 table).



## 295 **Discussion**

296 In this meta-analysis, we show that ICSI does not provide any additional benefit over IVF in  
297 terms of live birth rate in non-male factor infertility cases.

### 298 **Main characteristics of the available literature**

299 The use of ICSI has progressively increased over the past last years with indications  
300 expanding beyond male factor infertility, without any proven benefit of ICSI over IVF  
301 concerning LB in non-male factor infertility. The latest 2020 guidelines published by the  
302 Practice Committees of the American Society for Reproductive Medicine and Society for  
303 Assisted Reproductive Technology (ASRM) do not recommend the routine use of ICSI in  
304 cases without male factor infertility or a history of prior fertilisation failure (Practice  
305 Committees of the American Society for Reproductive Medicine and Society for Assisted  
306 Reproductive Technology, 2020). Our results further consolidate these recommendations.  
307 Given the complex, invasive and costly nature of ICSI, its widespread use is questionable.  
308 This issue has already been raised before. A previous meta-analysis published in 2003 and  
309 updated in 2011 (32) aimed to evaluate ICSI versus IVF in couples with non-male infertility,  
310 including only randomised clinical trials (33). Only one study met the inclusion criteria and  
311 reported no significant difference in pregnancy rates between both techniques (33). However,  
312 the authors did not find any RCT reporting LBR. Our work focused on all of the studies  
313 published on this topic over the last 15 years. Studies published before 2004 were not  
314 included in order to avoid potential bias related to different embryology procedures or clinical  
315 strategies in the early days of ICSI. Our review found mainly retrospective and cohort studies,  
316 and only one RCT (16) reporting LBR. This RCT shows that fertilisation rate was  
317 significantly lower after IVF than after ICSI but failed to identify any significant difference  
318 between both techniques in terms of LBR. More recently, a meta-analysis investigating

319 clinical pregnancy and live birth rates in ICSI versus IVF in non-male factor infertility was  
320 published (34). Authors found no significant difference between ICSI and IVF regarding  
321 LBR. However, this work suffers from some methodological issues: only 4 articles included  
322 reported LBR, versus 21 in the present review, there was no apparent search date limitation  
323 with the inclusion of article publication dates spanning over 20 years, and no sensitivity  
324 analysis was carried out.

325 Interestingly, the results of a randomised, open-label, multi-centre controlled trial were  
326 reported very recently (10). The authors of this high-quality study, with precisely defined  
327 non-male factor infertility, came to the same conclusion, asserting the lack of clinical benefit  
328 of ICSI over IVF in non-male factor infertility, thus reinforcing the scope and generalisability  
329 of our work. Of note, time-related outcomes, recently emphasised as important considerations  
330 (35), were also assessed in this RCT (10), with a non-statistically significant difference found  
331 between ICSI and IVF in terms of time to ongoing pregnancy resulting in a live birth at 12  
332 months.

333 Concerning secondary outcomes, we found that fertilisation rate was significantly higher with  
334 ICSI than with IVF. This is in agreement with a previous meta-analysis reported in 2013 (36).  
335 Although improved fertilisation rate is a desirable objective in ART, it does not necessarily  
336 translate into improved clinical outcome such as LBR. The use of cumulative outcome such  
337 as cumulative LBR might be an even more relevant option in this context to address the  
338 overall benefit of a given procedure after all embryos from one complete cycle have been  
339 transferred, but studies reporting this outcome are very scarce. Contrary to our results,  
340 Johnson *et al.* 2013, found a significantly lower risk of fertilisation failure rate with ICSI as  
341 compared to IVF. It is hard to provide a relevant explanation for this discrepancy, but the  
342 higher number of cycles included in our analysis (285,403 IVF and 338,153 ICSI cycles)  
343 provides a more robust quality of evidence. Among other outcomes of interest, it should be

344 noted here that congenital anomalies and miscarriage rates are also relevant. Congenital  
345 anomalies have, unfortunately, not been evaluated by most of selected studies. The analyses  
346 of LBR as the main outcome allowed us to overcome potential miscarriages, as it is a more  
347 reliable outcome than clinical pregnancy rate.

### 348 **ICSI overuse in non-male factor infertility**

349 The scientific rationale for the increasing use of ICSI in non-male factor infertility is  
350 unclear, but might be explained by the following related arguments: First, the better  
351 fertilisation rate expected with ICSI than with IVF, although not reported in all studies,  
352 appears falsely reassuring and is translated into a belief that ICSI also leads to better clinical  
353 outcomes than IVF. Second, the expected lower risk of fertilisation failure with ICSI than  
354 with IVF also leads embryologist and clinical staffs to counsel patients to undergo ICSI,  
355 especially for those who had previously experienced fertilisation failure or arrested embryonic  
356 development. However, LBR should be considered as the most relevant final outcome for  
357 infertile couples, and our results do not support the use of ICSI rather than IVF in order to  
358 improve LBR in couples with non-male factor infertility. Furthermore, higher fertilisation rate  
359 obtained with ICSI does not seem to ultimately lead to higher cumulative LBR when all  
360 frozen embryos are transferred (24), but this remains to be further confirmed in the literature.

361 Additionally, the lower handling of oocytes and more physiological gamete interaction  
362 observed in IVF might advocate for its preferential use when ICSI is not mandatory. It could  
363 even be anticipated that this minimal disturbance of oocytes would be even more beneficial in  
364 cases where a low oocyte quality and/or high susceptibility to manipulation is observed, such  
365 as advanced maternal age or low ovarian reserve (25). Still, this was not confirmed by our  
366 results, as neither subgroup analysis in poor responders nor meta-regression based on female  
367 age identified any significant difference between IVF and ICSI in terms of LBR, when a male  
368 infertility is not identified.

## 369 **Study limitations**

370 An important limitation for this work lies within the availability of only one published  
371 randomised clinical trial at the time the review was started. Possible biases related to the  
372 inclusion of retrospective studies in this work and the identification of several confounding  
373 factors, required very strict bias assessment and advocate for cautious interpretation.  
374 Additionally, a high heterogeneity level was observed for the main outcome analysis, which  
375 was mainly due to one study (6), but also to outliers (6,9,19,21,27). The careful analysis of  
376 this study (6) did not allow us to identify some reasons explaining this surprisingly high  
377 heterogeneity. Although some numbers presented in the tables in the original article needed to  
378 be corrected (37), the update with the corrected numbers did not change the proportions and  
379 conclusions of the study (38). However, it can be noted that this study was evaluated as  
380 having a high risk of bias concerning measurement of outcome and included data from 1991.  
381 Finally, sensitivity analysis excluding this study along with other outliers showed similar  
382 results in favour of IVF, with no more heterogeneity.

383 A potential bias among studies lies within the lack of clear definition of non-male  
384 factor. While some refer to sperm investigations with specific thresholds (10), others do not  
385 provide explicitly the criteria used for patients' classification. Actually, other sperm factors  
386 like DNA integrity might play a role in male infertility and IVF/ICSI outcome (39–41).  
387 Furthermore, sperm parameters fluctuate and a couple labelled with “non-male factor  
388 infertility” might finally be oriented towards ICSI in case of poorer sperm yield following  
389 sperm preparation. Also, the WHO manual for semen analysis was updated in 2010 (5), i.e.  
390 during our study period. Our subgroup analysis of studies including patients before and after  
391 this change did not, however, show any modification in the overall conclusion of our work.

392 A further limitation of this meta-analysis is the presence of cycle multiplicity for  
393 outcomes in some of the retrospective studies. Two recent studies, however, included only

394 women undergoing their first IVF-ICSI cycle, thereby reducing this bias (7,9). Both  
395 demonstrated no superiority of ICSI over IVF with regards to LBR and one even reported  
396 significantly higher LBR in favour of IVF in non-male factor infertility. Studies reporting live  
397 birth rate per transfer should also be interpreted with care, as couples with fertilisation failure  
398 in IVF were excluded from these cohorts, thus exposing to a risk of overestimation in the IVF  
399 group.

400 Of note, subgroup analysis conducted in poor responders should be interpreted with  
401 care, as the definition of this category can vary among studies. The ongoing effort to reach a  
402 consensus definition of poor responders might help in the future improving the results  
403 obtained in this specific population with increasing prevalence.

404 Here, we showed higher fertilisation rates after ICSI. It could be speculated that the  
405 number of embryo transfers would be greater with ICSI, ultimately leading to improved  
406 cumulative live birth rates (CLBR). This was not, however, observed. Of the articles included  
407 in this meta-analysis, 4 analysed CLBR after fresh and frozen transfers (7,8,24,25). Three  
408 (7,8,24) showed no significant difference in terms of CLBR in IVF vs. ICSI, and one (25)  
409 reported significantly increased CLBR with IVF than with ICSI.

410 However, and despite the methodological limitations of these retrospective studies  
411 included in this meta-analysis, our conclusions are comforted by the very recently published  
412 results of a properly designed RCT, as well as the comprehensiveness of the research using  
413 strict search terms and a unique and robust outcome (LBR), the high number of studies and  
414 cycles included, the rigorous methodology applied, and the sensitivity analysis confirming the  
415 main findings. Finally, our results need to be further enhanced by randomised controlled trials  
416 still lacking in numbers.

## 417 **Conclusion**

418 In conclusion, the results of this meta-analysis do not support the clinical relevance of using  
419 ICSI instead of IVF in non-male factor infertility cases.

420

## 421 **Author contributions**

422 AF was responsible for study design and supervision, screening (titles, abstracts and full  
423 texts), quality assessment, data extraction, drafted and revised the manuscript.

424 EA was responsible for full-texts screening, quality assessment, data extraction, drafted and  
425 revised the manuscript.

426 MC was responsible for study design and supervision, screening (titles, abstracts and full  
427 texts), quality assessment, data extraction, drafted the manuscript.

428 BD provided methodological support and statistical expertise, analysed and interpreted the  
429 data.

430 VG and VGM completed the screening on full text, assessed the quality, extracted the data,  
431 and drafted the manuscript.

432 SL provided methodological support, developed the search strategy, performed the literature  
433 search, analysed the data, drafted and revised the manuscript.

434 TF was responsible for study design and supervision, screening (titles, abstracts and full  
435 texts), quality assessment, data extraction, drafted and revised the manuscript, and validated  
436 the final version of the manuscript.

437

## 438 **Acknowledgments**

439 We thank the Inter-university Library of Medicine (BIUM) of Paris for methodological  
440 support.

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563 **Figure legends**564 **Fig 1: PRISMA Flow diagram of study selection**

565 **Fig 2: Forest plot of estimates for live birth rate per cycle.** Risks of bias are (A),  
 566 confounding; (B), selection of participants; (C), classification of intervention; (D), deviations  
 567 from intervention; (E), missing data; (F), measurement of outcome; (G), selection of reported  
 568 results.

569 **Fig 3: Forest plot of estimates for live birth rate per transfer.** Risks of bias are (A),  
 570 confounding; (B), selection of participants; (C), classification of intervention; (D), deviations  
 571 from intervention; (E), missing data; (F), measurement of outcome; (G), selection of reported  
 572 results.

573 **Table 1: Characteristics of included studies**

	Study design and type	Inclusion criteria*	Exclusion criteria	Study period	Number of cycles or transfers		Mean female age (years)
					cIVF	ICSI	
Biliangady <i>et al.</i> (2019) (18)	Retrospective Cohort	Women aged 25-35, FSH<10IU/L, AMH>1ng/mL, AFC 5-15, endometrium thickness >7mm, good quality blastocyst available on transfer day.	BMI >35Kg/m2, surgically retrieved sperm, third party reproduction, PGD, history of recurrent implantation failure.	2012 - 2017	214	136	31.2
Boulet <i>et al.</i> (2015) (4)	Retrospective Cohort	All fresh cIVF and ICSI cycles from the National Assisted Reproductive Technology Surveillance	Frozen embryo cycles or cycles cancelled prior to oocyte retrieval.	2008 - 2012	112,877	205,119	-

		System (NASS) database, including sperm/oocytes from donors.					
Check <i>et al.</i> (2009) [16]	Retrospective Cohort	Positive oocyte retrievals of unexplained infertility couples.	<2 embryos transferred.	7 years period	94	110	-
Drakopoulos <i>et al.</i> (2019) (7)	Retrospective Cohort	Women aged 18-45 undergoing their first ovarian stimulation cycle, in a gonadotropin-releasing hormone antagonist protocol.	Cycles with split insemination of sibling oocytes (cIVF vs. ICSI), frozen sperm samples, PGD, PGS, oocyte donation, oocyte freezing, natural IVF/ICSI cycles, women with cycle cancelation or zero oocytes retrieved.	2009 - 2014	664	4,227	-
Foong <i>et al.</i> (2006) (16)	Prospective Randomised	≥3 previous IUI cycles with clomiphene citrate or gonadotropins, normal uterine cavity, fallopian tubes and presence of both ovaries, normal ultrasound.	Previous laparoscopy with stage III or IV endometriosis.	1997 - 2001	30	30	33.4
Gennarelli <i>et al.</i> (2019) (8)	Retrospective Cohort	Autologous cycles from women aged ≥ 40, unexplained infertility, undergoing either cIVF or ICSI, ovulatory cycles and at least patency of	Female BMI: > 32 kg/m <sup>2</sup> , early FSH > 20 UI/l and/or AMH < 0.1, PGD, and cycles with split insemination (cIVF/ICSI) of	2012 - 2018	297	307	41.0

		one fallopian tube at sonosalpingography.	sibling oocytes, women with no mature oocyte retrieved.				
Grimstad <i>et al.</i> (2016) (19)	Retrospective Cohort	From the USA Society for Assisted Reproductive Technology Clinic Outcome Reporting System (SART CORS) online database for fresh, autologous oocyte cycles, including cycles with only tubal ligation.	Cancelled cycles, cycles with split insemination (cIVF/ICSI) of sibling oocytes.	2004 - 2012	3,956	3,189	35.6
Grzegorzyc <i>et al.</i> (2011) (17)	Retrospective Case-control	Repeated IUI failure; cycles with at least 8 oocytes retrieved, cycles with split insemination (cIVF/ICSI) of sibling oocytes.		2001 - 2008	27	26	-
Guo <i>et al.</i> (2018) (20)	Retrospective Cohort	Cycles with <5 oocytes retrieved.	Previous IVF cycles with fertilisation failure or low fertilisation rates (<50%), donor or frozen oocytes, frozen sperm samples, patients with chromosome abnormality; cycles with split insemination (cIVF/ICSI) of sibling	2012 - 2015	160	80	36

oocytes.

Hodes-Wertz <i>et al.</i> (2012) (21)	Retrospective Cohort	Fresh IVF cycles, with previous ICSI failure performed elsewhere.	Donor oocytes.	2005 - 2008	96	15	36.8
Keegan <i>et al.</i> (2007) (22)	Retrospective Cohort	First or second IVF cycle.	Donor oocytes.	2002 - 2004	454	64	37.6
Kim <i>et al.</i> (2007) (23)	Retrospective Cohort	All cIVF or all ICSI cycles performed within the centre.	Donor or frozen oocytes, cycles with split insemination (cIVF/ICSI) of sibling oocytes.	2002 - 2003	597	99	37.8
Li <i>et al.</i> (2018) (24)	Retrospective Cohort	First autologous stimulated cycle with fertilisation performed for at least one oocyte by either cIVF or ICSI.	Cycles with split insemination (cIVF/ICSI) of sibling oocytes; mixed fresh-frozen cycles; PGD.	2009 - 2014	8,807	14,513	34.8
Liu <i>et al.</i> (2018) (25)	Retrospective Cohort	Women aged $\geq 40$ , first cycle, $\leq 5$ oocytes; short agonist protocol.	Non-specified.	2011 - 2016	534	110	41.3
Ou <i>et al.</i> (2010) (26)	Retrospective Case-control	<4 oocytes retrieved. For cIVF: 2 controls matched for age, ovarian reserve, oocyte number, and morphology score, FSH treatment and	Gamete donation cycle.	2003 - 2007	56	28	36.0

		protocol, infertility history and etiology.					
Schwarze <i>et al.</i> (2017) (27)	Retrospective Cohort	Fresh cycles from latin american registry.	Cryopreserved semen; all embryos frozen (freeze all).	2012 - 2014	10,24 9	39,564	36.8
Sfontouri <i>s et al.</i> (2015) (28)	Retrospective Cohort	Bologna poor responders with single oocyte retrieved and at least two of the following: advanced female age, a diminished ovarian reserve as shown by AFC $\leq$ 5 or basal FSH >12 IU/L, and one or more failed previous IVF/ICSI cycles with poor ovarian response in which $\leq$ 3 COCs had been retrieved using a high gonadotrophin dose.	>1 oocyte retrieved, minimal stimulation for personal reason, frozen thawed sperm, testicular biopsy.	2009 - 2012	101	50	40.9
Shveiky <i>et al.</i> (2006) (29)	Retrospective Case-control	Unexplained infertility, at least 3 cycles of ovarian stimulation or IIU, sibling oocytes.	Embryos of mixed origin.	1999 - 2002	25	93	-
Supramani <i>am et al.</i> (2020) (6)	Retrospective Cohort	All cIVF or all ICSI cycles performed within the UK and recorded in the HFEA database.	Donor oocyte or sperm, IUI, unstimulated cycles, PGT, frozen cycles, all cycles performed prior to 1999.	1991 - 2016	272,433	297,17 2	-

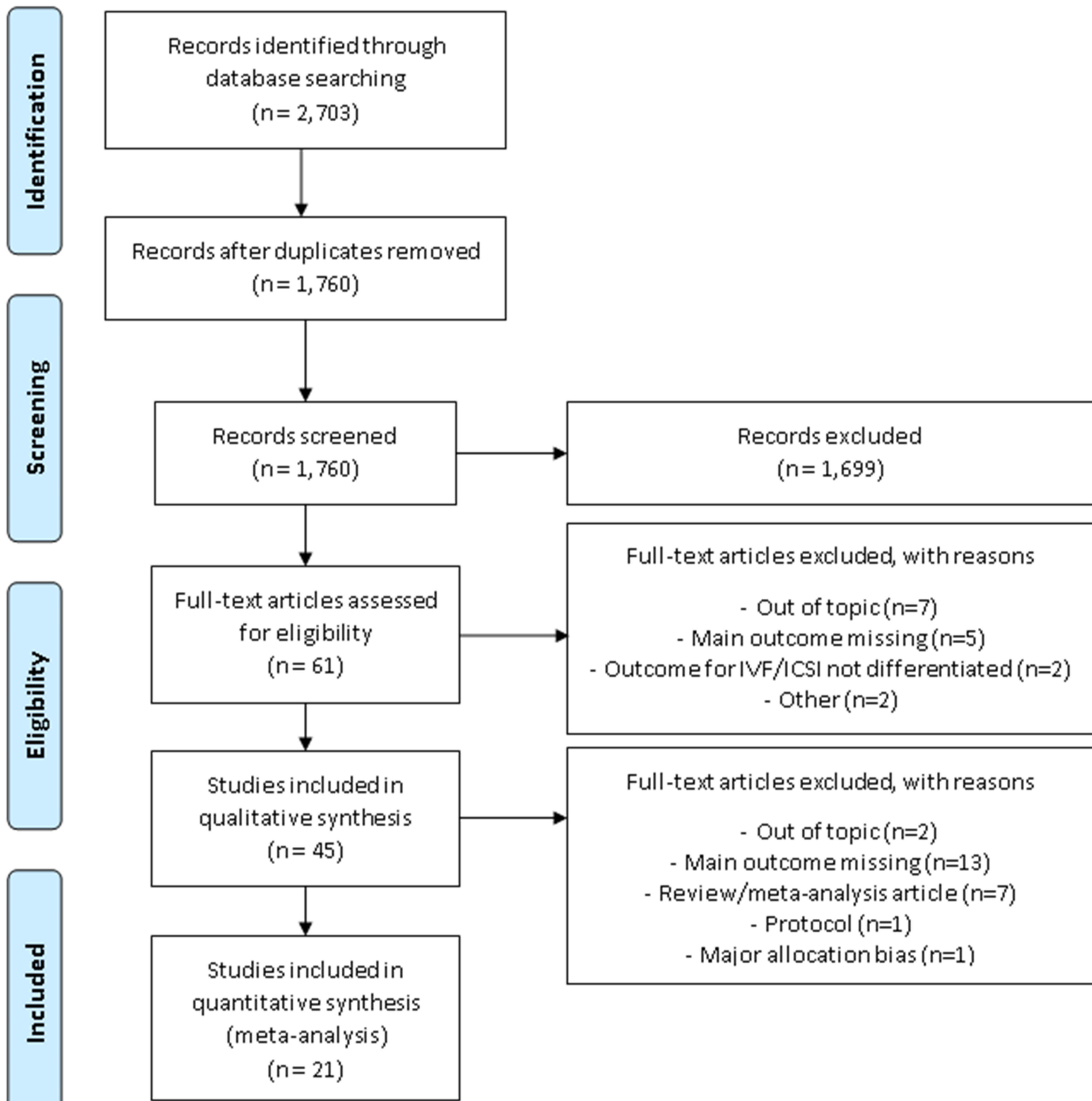
Sustar <i>et al.</i> (2019) (9)	Retrospective Cohort	First cycle of IVF in the centre.	Poor fertilisation in past IVF attempts and low oocyte yield ( $\leq 4$ oocytes); donor cycles; PGS.	2009 - 2015	1,661	1,702	37.5
Tannus <i>et al.</i> (2017) (30)	Retrospective Cohort	Women aged $\geq 40$ , only fresh cycles.	>3 previous IVF cycles; a history of fertilisation failure or low fertilisation (<50%); use of donor or frozen oocytes; use of donor or frozen sperm samples; PGD; cycles with split insemination (cIVF/ICSI) of sibling oocytes.	2012 - 2015	255	490	41.2

574 \*Absence of abnormal semen parameters as defined by WHO criteria or unexplained  
575 infertility.

576 AFC: Antral Follicle Count, AMH: Anti-Mullerian Hormone, BMI: Body Mass Index,

577 FSH: Follicle Stimulating Hormone, IUI: intrauterine insemination, PGD/PGS/PGT:

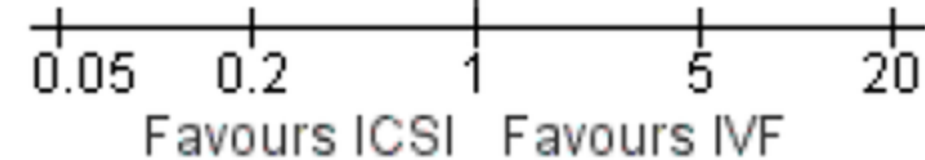
578 preimplantation genetic diagnosis/screening/testing.





	cIVF n/N	ICSI n/N	Weight	Risk ratio (95% CI)	Risk of Bias						
					A	B	C	D	E	F	G
Foong et al (2006)	14/30	15/30	1.6%	0.93 [0.55, 1.58]	+	+	+	?	+	+	+
Kim et al (2007)	124/597	16/99	1.9%	1.29 [0.80, 2.07]	?	+	+	+	+	+	+
Keegan et al (2007)	169/454	21/64	2.9%	1.13 [0.78, 1.64]	?	?	+	+	+	+	+
Ou et al (2010)	7/56	8/28	0.6%	0.44 [0.18, 1.08]	?	?	+	+	+	+	+
Hodes-Wertz et al (2012)	47/112	10/15	2.4%	0.63 [0.41, 0.96]	-	-	+	+	+	+	+
Boulet et al (2015)	40703/112877	68735/205119	14.1%	1.08 [1.07, 1.09]	+	+	+	+	+	+	+
Sfontouris et al (2015)	5/101	2/50	0.2%	1.24 [0.25, 6.16]	?	?	+	+	+	+	+
Grimstad et al (2016)	1566/3956	1051/3189	12.7%	1.20 [1.13, 1.28]	+	+	+	+	+	+	+
Tannus et al (2017)	27/255	41/490	2.0%	1.27 [0.80, 2.01]	-	?	+	+	+	+	+
Schwarze et al (2017)	2948/10249	9093/39564	13.6%	1.25 [1.21, 1.30]	-	+	+	+	+	+	+
Guo et al (2018)	5/160	1/80	0.1%	2.50 [0.30, 21.04]	+	?	+	+	+	+	?
Li et al (2018)	1123/4993	1840/8470	12.6%	1.04 [0.97, 1.11]	+	+	+	+	+	+	+
Liu et al (2018)	49/534	5/110	0.6%	2.02 [0.82, 4.95]	?	?	+	+	+	+	+
Gennarelli et al (2019)	38/297	40/307	2.5%	0.98 [0.65, 1.49]	-	?	+	+	+	+	+
Drakopoulos et al (2019)	195/664	1173/4227	9.7%	1.06 [0.93, 1.20]	?	?	+	+	+	+	+
Sustar et al (2019)	286/1661	225/1702	8.2%	1.30 [1.11, 1.53]	?	+	+	?	+	+	+
Supramaniam et al (2020)	84725/272433	96754/297172	14.1%	0.96 [0.95, 0.96]	+	+	+	+	+	-	+
<b>Total (95% CI)</b>	<b>132031/409429</b>	<b>179030/560716</b>		<b>1.10 [1.02, 1.18]</b>							

Heterogeneity: Tau<sup>2</sup> = 0.01; Chi<sup>2</sup> = 558.21, df = 16 (P < 0.00001); I<sup>2</sup> = 97%  
 Test for overall effect: Z = 2.49 (P = 0.01)



	cIVF n/N	ICSI n/N	Weight	Risk ratio (95% CI)		Risk of Bias						
						A	B	C	D	E	F	G
Shveiky et al (2006)	10/25	42/93	7.1%	0.89 [0.52, 1.50]		+	?	+	+	+	+	+
Keegan et al (2007)	169/454	21/64	11.8%	1.13 [0.78, 1.64]		?	?	+	+	+	+	+
Kim et al (2007)	124/562	16/95	8.4%	1.31 [0.82, 2.10]		?	+	+	+	+	+	+
Check et al (2009)	42/91	31/107	11.8%	1.59 [1.10, 2.31]		?	+	-	+	-	+	+
Ou et al (2010)	7/51	8/26	2.9%	0.45 [0.18, 1.09]		?	?	+	+	+	+	+
Grzegorzczak et al (2011)	8/27	6/26	2.8%	1.28 [0.52, 3.19]		+	+	+	+	+	+	+
Tannus et al (2017)	27/227	41/426	8.8%	1.24 [0.78, 1.95]		-	?	+	+	+	+	+
Guo et al (2018)	5/35	1/25	0.6%	3.57 [0.44, 28.72]		+	?	+	+	+	+	?
Li et al (2018)	1123/4344	1840/7369	30.3%	1.03 [0.97, 1.10]		+	+	+	+	+	+	+
Liu et al (2018)	49/403	5/83	2.9%	2.02 [0.83, 4.91]		?	?	+	+	+	+	+
Biliangady et al (2019)	70/214	33/136	12.5%	1.35 [0.95, 1.92]		?	-	+	+	+	+	+
<b>Total (95% CI)</b>	<b>1634/6433</b>	<b>2044/8440</b>		<b>1.18 [1.00, 1.38]</b>								

Heterogeneity: Tau<sup>2</sup> = 0.02; Chi<sup>2</sup> = 15.84, df = 10 (P = 0.110); I<sup>2</sup> = 37%  
 Test for overall effect: Z = 1.98 (P = 0.05)

0.05 0.2 1 5 20  
 Favours ICSI Favours IVF