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SYSTEMATIC REVIEW AND META-ANALYSIS

Clinical Impact of Magnetic-Activated Cell Sorting Sperm Selection on Intracytoplasmic Sperm Injection Outcomes in Idiopathic Male Infertility: A Systematic Review and Meta-Analysis

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ABSTRACT

Background: Magnetic-activated cell sorting (MACS) removes apoptotic spermatozoa before intracytoplasmic sperm injection (ICSI), but its value in men whose infertility has no identified cause is uncertain.

Objective: To assess MACS and ICSI outcomes in idiopathic male infertility.

Methods: PubMed, Europe PMC and Scopus were searched for studies comparing annexin V MACS with conventional preparation before ICSI in unexplained infertility, idiopathic abnormal semen parameters or raised sperm DNA fragmentation. One reviewer screened and extracted data, verifying every value. Risk ratios (RRs) were pooled with random-effects models (Hartung-Knapp intervals). Risk of bias was assessed with RoB 2 and ROBINS-I, and certainty of evidence with GRADE.

Results: Twelve studies (2,091 couples; four randomized) were included. MACS was associated with more clinical pregnancies (RR 1.19, 95% CI 1.08 to 1.31; k = 9; I^2 = 0.0%), about 84 more per 1000 couples, and with more live births (RR 1.33, 95% CI 1.14 to 1.55; k = 6). Miscarriage per clinical pregnancy was lower (RR 0.66, 95% CI 0.46 to 0.94). Fertilization, implantation and embryo quality did not differ. Non-randomized cohorts at serious risk of confounding carried 83% of the weight, whereas the three randomized trials gave RR 1.41, 95% CI 0.64 to 3.10. Trim-and-fill suggested possible small-study bias. Certainty was low.

Conclusion: In idiopathic male infertility, MACS before ICSI was associated with higher pregnancy and live birth rates, while fertilization and embryo quality did not change. The evidence is of low certainty. MACS is best offered within trials or after transparent counseling.

1. Introduction

Male infertility is a growing global health problem. Global Burden of Disease analyses showed that the age-standardized prevalence rose over the past three decades and is projected to keep rising, with high age-standardized rates reported in Eastern Europe, East Asia and Western sub-Saharan Africa.[1,2] Couples often pay for assisted reproduction themselves in low- and middle-income countries, including in South-East Asia.[3] In a cohort of more than 130,000 patients that included 69,130 infertile men, more than half of the infertile men had abnormal semen parameters, and genital pathogens and antisperm antibodies were associated with poorer semen quality.[4] Once history, examination, hormones, karyotype and Y-chromosome testing are unremarkable, the infertility is labeled idiopathic. Recent work has linked this group to oxidative stress, sperm DNA damage, genetic variants in known and novel genes and even changes in the gut microbiome,[5–7] but none of these findings has so far produced a targeted treatment. Most of these couples reach a child through assisted reproduction. Intracytoplasmic sperm injection (ICSI) is used in most in vitro fertilization cycles in several national datasets, often beyond strict male-factor indications.[8–11]

ICSI bypasses the barriers of the female tract and the zona pellucida, which would normally exclude damaged spermatozoa. The embryologist picks one motile, morphologically normal spermatozoon. Neither motility nor morphology reveals the integrity of its DNA. Sperm DNA fragmentation has been linked to slower embryo development and, in some cohorts, to lower live birth rates and miscarriage, although the association is inconsistent and appears to depend on oocyte quality and maternal age.[12–18] Much of the fragmented DNA in ejaculates comes from spermatozoa that carry apoptotic markers. In native semen, the fraction of spermatozoa with fragmented DNA was related mainly to the fraction of non-viable cells and only weakly to early apoptotic cells,[19,20] and early sperm apoptosis measured by flow cytometry was related to fertilization and clinical pregnancy in IVF.[21] During apoptosis, phosphatidylserine moves to the outer leaflet of the plasma membrane. There it can be bound by annexin V (indexed in the Medical Subject Headings as annexin A5).

Magnetic-activated cell sorting (MACS) exploits this. Annexin V is conjugated to paramagnetic microbeads and labels spermatozoa that expose phosphatidylserine. These cells are retained in a column placed in a magnetic field, while the non-apoptotic fraction passes through and is used for injection. In laboratory studies, MACS lowered the proportion of spermatozoa with fragmented DNA and, in small studies, with abnormal chromosome content,[22,23] particularly when combined with density-gradient centrifugation.[24–27] Whether these laboratory gains translate into more pregnancies and births is a separate question. MACS is one of several sperm-selection add-ons marketed to couples, and many couples pay for such add-ons without good evidence of benefit, while the European Society of Human Reproduction and Embryology

classified MACS as currently not recommended for routine clinical use.[28–31]

Earlier syntheses have reached different conclusions. A recent meta-analysis of 41 studies found no benefit of MACS in intrauterine insemination, but in ICSI it reported higher clinical pregnancy and live birth per embryo transfer, especially with raised DNA fragmentation.[32] The latest Cochrane update was restricted to randomized trials comparing advanced sperm selection techniques with standard IVF or ICSI. It judged the evidence for advanced sperm selection to be of low or very low certainty.[33] A network meta-analysis found a higher clinical pregnancy rate with MACS combined with density-gradient centrifugation, yet rated the evidence as low certainty.[34] Several of these reviews pooled intrauterine insemination, conventional in vitro fertilization and ICSI, mixed donor and autologous oocytes, or combined unselected men with men who had a male factor. One population has not been examined on its own. These are men with infertility of unknown cause, in whom apoptosis and DNA damage are the leading candidate mechanisms and for whom the technique is biologically most likely to work.

The question is far from trivial for laboratories. MACS adds a preparation step and requires a minimum number of spermatozoa, because part of the sample is retained in the column, and it can reduce the number of total and rapidly progressive spermatozoa recovered.[35,36] The order in which it is combined with density-gradient centrifugation or swim-up, and whether serum-containing media are used, could change which spermatozoa are removed.[24,26,37] Clinics therefore need to know whether the technique works, in whom, and with what certainty before adding it to routine preparation. Couples with idiopathic male infertility have no other targeted option. An honest estimate of the benefit matters most for them, because they are the group most likely to be offered laboratory add-ons after an unexplained failed cycle.[28,30]

This review restricts the synthesis to ICSI in couples whose male partner had idiopathic infertility. That was defined as unexplained infertility, idiopathic abnormal semen parameters or raised sperm DNA fragmentation without a treatable cause. Cohorts of unselected men were excluded. Randomized and non-randomized evidence are combined using design-appropriate risk-of-bias tools, live birth and miscarriage are reported alongside laboratory outcomes, and the certainty of each estimate is graded. The aim of this study was to estimate the effect of annexin V MACS, compared with conventional sperm preparation, on clinical pregnancy and other ICSI outcomes in idiopathic male infertility.

2. Methods

2.1. Study Design

We conducted and reported this systematic review and meta-analysis in line with the PRISMA 2020 statement.[38] The question, eligibility criteria, outcomes and analysis plan were fixed before any search was run.

2.2. Search Strategy

We searched PubMed (MEDLINE), Europe PMC and Scopus from inception, with no language restrictions. Three concept blocks were combined: MACS, annexin V or non-apoptotic sperm selection; spermatozoa or semen; and ICSI, in vitro fertilization, assisted reproduction or reproductive outcomes. The PubMed string was ("magnetic activated cell sorting"[tiab] OR "magnetic-activated cell sorting"[tiab] OR MACS[tiab] OR "annexin V"[tiab] OR "annexin-V"[tiab] OR "non-apoptotic sperm*"[tiab] OR "nonapoptotic sperm*"[tiab] OR "apoptotic sperm*"[tiab]) AND (sperm*[tiab] OR Spermatozoa[mh] OR semen[tiab]) AND ("Sperm Injections, Intracytoplasmic"[mh] OR ICSI[tiab] OR "intracytoplasmic sperm injection"[tiab] OR "Fertilization in Vitro"[mh] OR IVF[tiab] OR "assisted reproduct*"[tiab] OR "Reproductive Techniques, Assisted"[mh] OR pregnan*[tiab] OR "live birth*"[tiab] OR fertili*[tiab] OR embryo*[tiab]); equivalent strings were used in Europe PMC and Scopus and are reproduced verbatim in Supplementary Table S1. Searching returned 344 PubMed, 173 Europe PMC and 459 Scopus records, with Scopus limited to articles. Reports describe the male phenotype in many different ways. For that reason the population was applied at screening and not built into the search string. We also screened the reference lists of nine previous syntheses and reviews, and citation indexes were used to find articles citing fifteen key comparative studies.

2.3. Eligibility Criteria

Table 1 details the eligibility criteria. Studies were eligible if they compared annexin V MACS with conventional preparation without MACS before ICSI. MACS could be used alone or combined with density-gradient centrifugation or swim-up, and the couples had a male partner with infertility of no identified cause. Three phenotypes met this definition: unexplained infertility with normal semen analysis, idiopathic abnormal semen parameters, and raised sperm DNA fragmentation without a treatable cause. We excluded studies of unselected men because they answer a different question. One example is a cohort in which every couple in an oocyte-donation program received the technique irrespective of male fertility. Some cohorts used conventional in vitro fertilization for part of the cycles. These were eligible when ICSI was used, since MACS acts on the prepared sperm suspension whatever the insemination method, and they were examined in a sensitivity analysis. Studies of men with a treatable cause were excluded. For each included study, we recorded the work-up used to rule out treatable or genetic causes.

Table 1. Eligibility Criteria.

Element	Inclusion	Exclusion
Population	Couples undergoing ICSI in whom the male partner had infertility without an identified cause: unexplained infertility with normal semen analysis, idiopathic abnormal semen parameters (oligo-, astheno- or teratozoospermia) or raised sperm DNA fragmentation without a treatable cause	Azoospermia or surgically retrieved sperm; known genetic cause (abnormal karyotype, Y microdeletion); varicocele, genital infection or leukocytospermia; cancer survivors; unselected men or couples without a male infertility criterion
Intervention	Annexin V magnetic-activated cell sorting of ejaculated spermatozoa, alone or combined with density-gradient centrifugation or swim-up, before ICSI	Other selection methods (hyaluronan binding, microfluidics, IMSI, zeta potential) as the only intervention
Comparator	Conventional preparation (density-gradient centrifugation and/or swim-up) without MACS, in parallel groups or as sibling-oocyte controls	Another advanced selection technique or testicular sperm as the comparator

Element	Inclusion	Exclusion
Outcomes	Clinical pregnancy (primary); live birth, ongoing pregnancy, miscarriage, implantation, fertilization, good-quality or usable embryos and blastocyst formation	Laboratory sperm measures only (DNA fragmentation, motility); neonatal outcomes only
Design	Randomized and quasi-randomized trials, prospective controlled studies, sibling-oocyte studies and retrospective cohorts; cycles using ICSI in all or part of the cohort	Reviews, case reports, conference abstracts, intrauterine insemination only, overlapping reports

Note: ICSI, intracytoplasmic sperm injection; IMSI, intracytoplasmic morphologically selected sperm injection; MACS, magnetic-activated cell sorting.

2.4. Study Selection and Data Extraction

Records were de-duplicated by PMID, DOI and normalized title. We screened titles and abstracts, then assessed potentially eligible reports in full. A single reviewer screened records, extracted data and assessed risk of bias on a piloted form. Every extracted value was checked in a second pass before analysis, and the risk-of-bias judgements were reviewed against the signalling questions of each tool. Extracted items were country, design, male phenotype, sperm DNA fragmentation threshold, oocyte source, MACS protocol, comparator, group sizes, female and male age, transfer strategy, and event counts for each outcome. Event counts were taken as reported. Where a study gave only a percentage and its denominator, events were computed and rounded. For oocyte- and embryo-level outcomes reported as group means per couple, we multiplied the mean by the number of couples to obtain totals. Some outcomes were defined per patient with embryo transfer while group sizes were reported as cycles. In that case the number of cycles served as the denominator, which leaves the risk ratio unbiased if the proportion of cycles reaching transfer was similar in both groups. Both kinds of computed counts are marked in the figures and were examined in sensitivity analyses. For studies with more than two arms, only the MACS arm and the conventional-preparation arm were used. Arms receiving hyaluronan-binding selection, testicular sperm or swim-up after centrifugation were not counted, and no arm was shared between comparisons. Missing values were recorded as not reported. No values were imputed.

2.5. Outcomes

Clinical pregnancy was the primary outcome. It was defined by an intrauterine gestational sac or fetal heartbeat on ultrasound, and we used the denominator each study reported: couples or cycles started (five studies), couples with embryo transfer (two studies), the first transfer (one study) or patients with transfer (one study). Secondary outcomes were live birth, ongoing pregnancy, miscarriage per clinical pregnancy, implantation per embryo transferred, fertilization per injected metaphase II oocyte, good-quality or usable embryos, and blastocyst formation. Counts of oocytes or embryos are clustered within couples. We pooled these laboratory outcomes as risk ratios so that they remain comparable with earlier syntheses, and their intervals are interpreted as approximate. Definitions followed each report. Fertilization was the proportion of injected metaphase II oocytes showing two pronuclei. Good-quality embryos were defined by blastomere number, symmetry and fragmentation on day 3, or by usable blastocysts for biopsy or transfer. Implantation was the number of gestational sacs or fetal heartbeats per embryo transferred. Miscarriage was pregnancy loss after a clinical pregnancy and before 20 weeks.

2.6. Risk of Bias

Risk of bias in randomized trials was assessed with the Cochrane RoB 2 tool across five domains: randomization process, deviations from intended interventions, missing outcome data, outcome measurement, and selection of the reported result. Non-randomized studies, which included sibling-oocyte and quasi-randomized designs, were assessed with ROBINS-I across seven domains.[39] Here the main concern was confounding by indication, meaning the clinician's or couple's choice of MACS.[40] We judged confounding serious when clinicians or couples chose MACS and baseline prognostic factors (previous failed cycles, female age, infertility duration) differed or were not reported. It was judged moderate in two situations. One was when allocation was not described but the reported imbalance would be expected to bias the estimate toward no effect. The other was when a sibling-oocyte design controlled couple-level confounding.

2.7. Statistical Analysis

For each study we calculated the risk ratio (RR) of MACS versus conventional preparation, together with the variance of its logarithm, as shown in Equation 1. Here a and n are the events and totals in the MACS (M) and control (C) groups. A continuity correction of 0.5 was added to the cells of a study only if that study had a zero cell.

$$y_i = \ln\left(\frac{a_M/n_M}{a_C/n_C}\right), \quad v_i = \frac{1}{a_M} - \frac{1}{n_M} + \frac{1}{a_C} - \frac{1}{n_C} \tag{1}$$

Log risk ratios were pooled with inverse-variance random-effects models. We estimated the between-study variance τ^2 with the DerSimonian-Laird method (Equation 2). Each study then received a weight equal to the inverse of its within-study variance plus τ^2 (Equation 3).

$$\tau^2 = \max\left\{0, \frac{Q-(k-1)}{\sum_i w_i - \frac{\sum_i w_i^2}{\sum_i w_i}}\right\}, \quad w_i = \frac{1}{v_i} \tag{2}$$

$$\hat{\mu} = \frac{\sum_i w_i^* y_i}{\sum_i w_i^*}, \quad w_i^* = \frac{1}{v_i + \tau^2} \tag{3}$$

Confidence intervals for the pooled estimates came from the Hartung-Knapp method, using a t distribution on k–1 degrees of freedom (Equation 4). This approach is recommended when studies are few.[41–43] The Hartung-Knapp interval can be narrower than the Wald interval when heterogeneity is close to zero. Wald intervals are therefore reported alongside, and an estimate was described as robust only if both intervals excluded no effect.

$$\hat{\mu} \pm t_{k-1,0.975}\sqrt{\frac{\sum_i w_i^*(y_i-\hat{\mu})^2}{(k-1)\sum_i w_i^*}} \tag{4}$$

We quantified heterogeneity with Cochran's Q, I^2 (Equation 5) and τ^2. The 95% prediction interval was derived from a t distribution on k–2 degrees of freedom (Equation 6).[44,45] The standard error in Equation 6 is that of the DerSimonian-Laird pooled estimate, and prediction intervals are unreliable when few studies are pooled.[44]

$$Q = \sum_i w_i (y_i - \hat{\mu}_{\mathrm{FE}})^2 , \quad I^2 = \max\left\{0, \frac{Q-(k-1)}{Q}\right\} \times 100\% \tag{5}$$

$$\hat{\mu} \pm t_{k-2,0.975}\sqrt{\tau^2 + \mathrm{SE}^2(\hat{\mu})} \tag{6}$$

Absolute effects were expressed as the difference in events per 1000 couples at the median control-group risk (Equation 7).

$$\mathrm{ARD} = 1000 \times p_{\mathrm{C}} \times (\mathrm{RR} - 1) \tag{7}$$

The prespecified subgroup analyses of the primary outcome compared study design (randomized versus non-randomized), male phenotype, oocyte source and overall risk of bias. Most subgroups held only two or three studies, so these analyses were treated as exploratory. Wald intervals were reported alongside Hartung-Knapp intervals, and tests for subgroup differences relied on the Wald random-effects fit. Sensitivity analyses covered several variations. They used restricted maximum-likelihood and Paule-Mandel estimators and a Mantel-Haenszel common-effect model. They also restricted the data to randomized trials, excluded studies at serious risk of bias, excluded counts computed from percentages, and excluded the mixed in vitro fertilization/ICSI trial. Further analyses restricted the data to studies whose denominator was couples or cycles started, used the autologous-oocyte strata of the two cohorts that also included donor oocytes, and added ongoing pregnancy from one trial that did not report clinical pregnancy. A leave-one-out analysis was also run. Sparse miscarriage data were examined with the methods discussed for rare events.[46] A funnel plot and trim-and-fill were used to explore small-study effects.[47,48] Egger's regression test was planned only when at least ten studies contributed.[49] Selection models and limit meta-analysis were not fitted, because their estimates are unstable with fewer than ten studies, although sensitivity analyses for publication bias in sparse data have been proposed.[50] All analyses were run in R version 4.5.3 with the meta (version 8.2.1) and metafor (version 4.8.0) packages. Two-sided $p < 0.05$ indicated statistical significance.

2.8. Certainty of Evidence

Certainty of evidence for each outcome was rated with GRADE. Ratings started at high for all designs because ROBINS-I was used for non-randomized studies, and were lowered for risk of bias, inconsistency, indirectness, imprecision and publication bias.[51–53] The thresholds that follow are review-specific rules. Risk of bias was rated down by two levels when at least half of the weight came from studies at serious risk, and by one level when 25% to 49% of the weight came from such studies or when the only contributing trial raised some concerns. Imprecision was rated down by one level when the confidence interval included no effect, and by two levels when it did so with two or fewer studies or fewer than 300 participants or units. The two-level rule for risk of bias follows the Core GRADE guidance. That guidance holds that serious risk of bias in studies contributing most of the information justifies rating down. It also treats confounding by indication in non-randomized studies as a very serious concern when adjustment cannot address it.[51] For every outcome we report the share of weight from studies at serious risk of bias.

3. Results

3.1. Study Selection

The searches identified 976 records (PubMed 344, Europe PMC 173, Scopus 459). Once 468 duplicates were removed, 508 records remained for screening, and 479 of these were excluded on title and abstract. Of the 29 reports sought for retrieval, 3 could not be obtained, so 26 were assessed in full. Fourteen reports were excluded. Seven studied unselected men or couples, four reported no clinical or embryological ICSI outcome, two compared MACS with another advanced technique or with testicular sperm, and one reported neonatal outcomes only. Citation searching (1,147 references and citing articles screened) yielded no further eligible reports. Twelve studies were included, as illustrated in Figure 1.

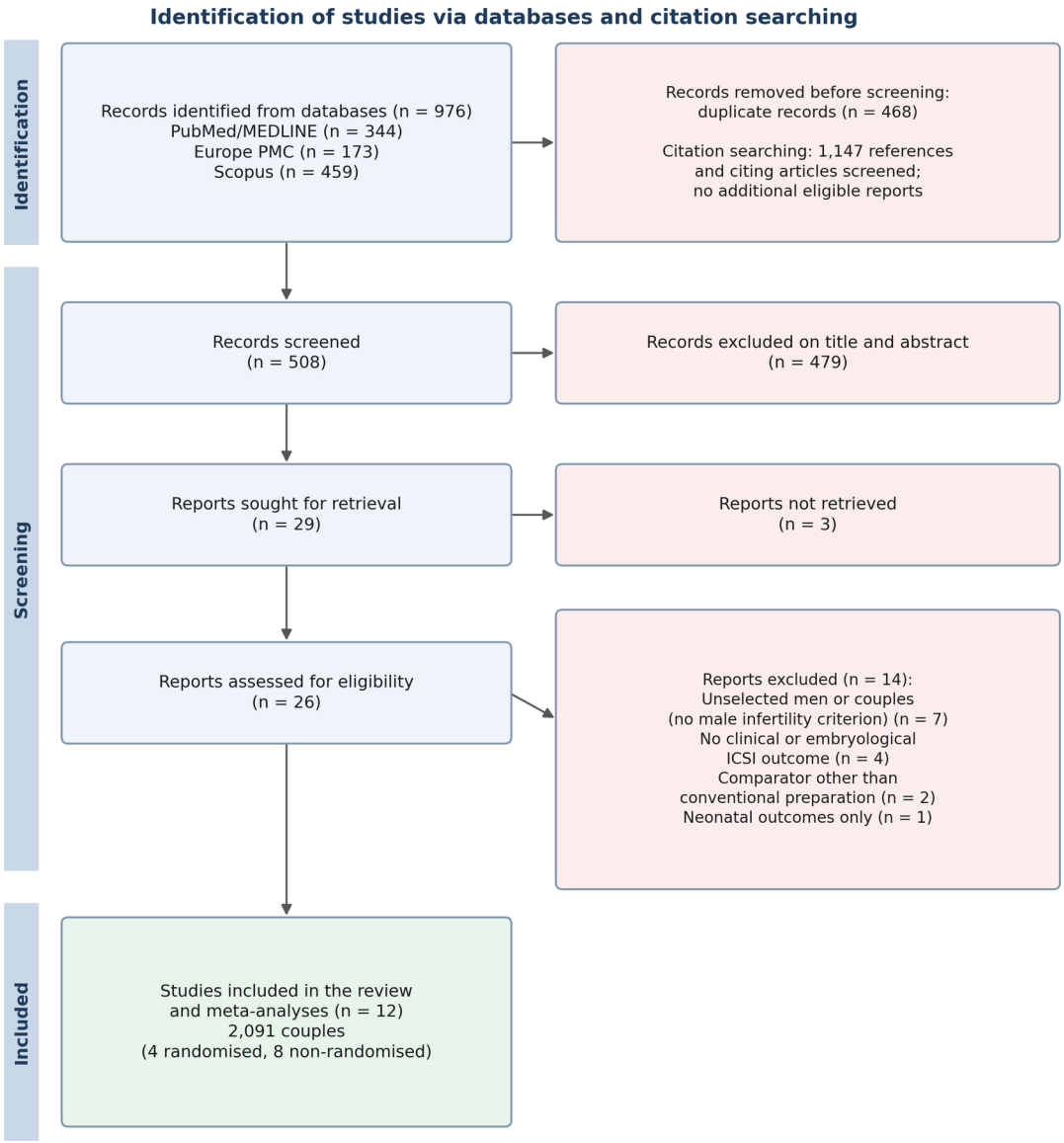


Figure 1. PRISMA 2020 flow diagram of the identification, screening and inclusion of studies.

3.2. Study Characteristics

The twelve studies enrolled 2,091 couples in 9 countries (China, Egypt, Iran, Mexico, Pakistan, Peru, Slovenia, Spain and Turkey), and Table 2 details their characteristics. Four were randomized trials,[54–57] four were prospective controlled or quasi-randomized studies,[58–61] two used a sibling-oocyte design,[62,63] and two were retrospective cohorts.[64,65] Couples with unexplained infertility or normozoospermic men were enrolled in three studies.[54,55,59] Five studies enrolled men with idiopathic abnormal semen parameters (oligo-, astheno- or teratozoospermia, or abnormal sperm aneuploidy testing),[58,60–63] and four enrolled men with raised sperm DNA fragmentation, using thresholds between 20% and 30%.[56,57,64,65] Ten studies combined MACS with density-gradient centrifugation, and one used swim-up alone. In two studies, MACS came before centrifugation. Donor-oocyte cycles were included in two cohorts, and one trial analyzed in vitro fertilization and ICSI cycles together.

The work-up used to define the male phenotype differed between studies. Three described the exclusion of treatable or genetic causes, such as varicocele, genital infection, cryptorchidism, Y-chromosome microdeletions or abnormal karyotype.[57,61,65] One study excluded severe male factor but gave no description of a urological work-up.[64] In the remaining studies, the phenotype was defined by semen analysis or by the absence of an explanation for the couple's infertility. Where reported, mean or median female age ranged from about 28 to 37 years, and two studies did not report it. One trial enrolled couples with tubal or pelvic female factors,[57] and in one cohort the donor-oocyte recipients were older than 40 years.[64]

The MACS protocols were broadly similar. All used commercial annexin V-conjugated microbeads and a miniature separation column, and the non-apoptotic fraction was injected. The preparation sequence differed. MACS followed density-gradient centrifugation or swim-up in nine studies, whereas in two it preceded centrifugation; in one of these, serum-free medium was used deliberately to avoid the phosphatidylserine exposure that accompanies capacitation.[58,60] The comparator was density-gradient centrifugation in seven studies, swim-up in one, and density-gradient centrifugation followed by swim-up in two. Two studies used conventional preparation that was not further specified. Fresh day-3 transfers predominated in the earlier studies, while the more recent ones used blastocyst culture and frozen transfers.

Table 2. Characteristics of the Included Studies.

Study	Country	Design	Male phenotype	MACS vs control	Oocytes	Couples or cycles (MACS / control)	Female age (years)	Outcomes	Risk of bias
Dirican 2008 [58]	Turkey	Prospective controlled study	Oligo-, astheno- and/or teratozoospermia	MACS before DGC vs DGC	Autologous	122 / 74	28.96 / 28.35	CP, IR, FR	Serious
Sheikhi 2013 [59]	Iran	Prospective controlled study	Unexplained infertility (normal semen analysis)	DGC followed by MACS vs DGC	Autologous	37 / 37	30.45 / 30.03	CP, LB, MC, EQ	Serious
Troya 2015 [54]	Peru	Randomized controlled trial (three arms)	Infertile men with normal semen parameters	MACS after DGC vs Conventional ICSI selection	Autologous	31 / 55	36.94 / 37.07	CP, MC, FR	Some concerns
Sánchez-Martín 2017 [64]	Spain	Retrospective cohort	Sperm DNA fragmentation ≥30% (non-severe semen parameters)	DGC followed by MACS vs DGC	Autologous and donor	87 / 218	NR	CP, LB, MC	Serious
Stimpfel 2018 [62]	Slovenia	Prospective sibling-oocyte study	Teratozoospermia	MACS after DGC (then swim-up) vs DGC then swim-up (sibling oocytes)	Autologous	26 couples (sibling oocytes)	31.3 (both)	FR, EQ, BL	Moderate
Barroso-Villa 2018 [55]	Mexico	Randomized controlled trial (pilot, triple-blind)	Unexplained infertility (normal semen analysis)	Swim-up followed by MACS vs Swim-up	Autologous	20 / 20	37 (both; median)	CP, LB	Some concerns
Pacheco 2020 [65]	Spain	Retrospective cohort	Sperm DNA fragmentation >20% after exclusion of treatable causes	DGC followed by MACS vs DGC	Autologous, donor and PGT-A	366 / 358	37.2 / 36.7	CP, LB, MC, FR	Serious
Hozyen 2022 [56]	Egypt	Randomized controlled trial (four arms)	Abnormal sperm DNA fragmentation	DGC followed by MACS vs DGC	Autologous	79 / 72	NR	OP	Some concerns
Mei 2022 [57]	China	Randomized controlled trial	Sperm DNA fragmentation index ≥30% (tubal or pelvic female factor)	DGC and swim-up followed by MACS vs DGC and swim-up	Autologous	39 / 41	30.1 / 30.4	CP, LB, MC, IR, FR, EQ, BL	Some concerns
Norozi-Hafshejani 2022 [60]	Iran	Prospective single-blind clinical trial	Teratozoospermia (≥96% abnormal forms)	MACS before DGC (serum-free) vs DGC	Autologous	106 / 100	32.02 / 31.55	CP, LB, MC, IR, FR	Moderate
Bibi 2023 [61]	Pakistan	Prospective comparative study (four arms)	Isolated teratozoospermia (varicocele and other causes excluded)	DGC followed by MACS vs DGC	Autologous	94 / 99	33.01 / 30.86	CP, FR	Serious
Tejero 2025 [63]	Spain	Quasi-randomized sibling-oocyte trial	Abnormal sperm FISH (male factor)	MACS added to conventional preparation vs Conventional preparation (sibling oocytes)	Autologous (PGT-A)	10 couples (sibling oocytes)	36.2 (overall)	FR, EQ	Moderate

Note: Group sizes are couples or cycles analyzed (MACS / control). Female age is the mean or median in years (MACS / control). Outcomes: CP, clinical pregnancy; LB, live birth; OP, ongoing pregnancy; MC, miscarriage; IR, implantation; FR, fertilization; EQ, good-quality or usable embryos; BL, blastocyst formation. Risk of bias: RoB 2 for randomized trials (low, some concerns, high) and ROBINS-I for non-randomized studies (low, moderate, serious). DGC, density-gradient centrifugation; FISH, fluorescence in situ hybridisation; MACS, magnetic-activated cell sorting; NR, not reported; PGT-A, preimplantation genetic testing for aneuploidy. Mei 2022: 39 / 41 are oocyte-retrieval cycles analyzed, and Figures 3 to 5 use the 38 / 39 first embryo transfers (live birth is first-transfer live birth). Norozi-Hafshejani 2022: all allocated cycles (106 / 100, including cancelled and freeze-all cycles) are used as denominators; per transfer the counts are 91 / 90. Bibi 2023: events were computed from the reported percentages.

3.3. Risk of Bias

Figure 2 presents the risk-of-bias judgements, and Table 3 gives the reasons. All four randomized trials raised some concerns, mainly because the randomization process or the selection of the reported result was incompletely described.[54–57] Of the eight non-randomized studies, five were at serious risk of bias, chiefly from confounding, since couples or clinicians chose MACS, often for couples with previous failed cycles.[58,59,61,64,65] The two sibling-oocyte studies and one prospective trial were at moderate risk.[60,62,63] In the prospective trial, couples who accepted MACS formed the MACS group and those who declined formed the control group. Its MACS group had more previous failed cycles (mean 1.60 versus 0.90), an imbalance expected to bias the estimate against MACS, so confounding was judged moderate rather than serious. In the sibling-oocyte studies, the design controlled couple-level confounding. However, embryos for transfer were chosen from either arm by morphology, and their pregnancy data were therefore not used.



Figure 2. Risk of bias of the included studies. (A) Randomized trials assessed with RoB 2. (B) Non-randomized studies assessed with ROBINS-I. Green, low risk; amber, some concerns or moderate risk; red, serious risk.

Table 3. Overall Risk-Of-Bias Judgements and Their Main Reasons.

Study	Tool	Overall judgement	Main reasons
Dirican 2008	ROBINS-I	Serious	Group membership chosen by couples after counseling; no adjustment for confounders.
Sheikhi 2013	ROBINS-I	Serious	Allocation method not described; infertility duration longer in the MACS group (4.59 vs 3.39 years).
Troya 2015	RoB 2	Some concerns	Randomisation method not described and arm sizes unequal; three MACS couples without transfer not analyzed.
Sánchez-Martín 2017	ROBINS-I	Serious	Retrospective; use of MACS not randomized; groups of unequal size.
Stimpfel 2018	ROBINS-I	Moderate	Split-oocyte design controls couple-level confounding; blinding of embryo grading not reported.
Barroso-Villa 2018	RoB 2	Some concerns	Sealed opaque envelopes and blinding of couple, clinician and statistician; pilot without a reported sample-size calculation or registration.
Pacheco 2020	ROBINS-I	Serious	Retrospective; MACS chosen by clinicians after counseling; rates are reported per cycle (366 / 358 cycles).
Hozyen 2022	RoB 2	Some concerns	Randomisation method, allocation concealment and attrition not reported.
Mei 2022	RoB 2	Some concerns	Computer-generated sequence; IVF and ICSI cycles analyzed together; several outcomes without a stated primary.
Norozi-Hafshejani 2022	ROBINS-I	Moderate	Couples who accepted MACS formed the MACS group and those who declined the control group; more previous ART cycles in the MACS group (1.60 vs 0.90).
Bibi 2023	ROBINS-I	Serious	MACS used mostly for couples with previous ART failure or raised fragmentation despite envelope allocation.
Tejero 2025	ROBINS-I	Moderate	Oocytes alternately allocated within couples; small sample (10 couples, 17 cycles).

Note: RoB 2, Cochrane risk-of-bias tool for randomized trials; ROBINS-I, risk of bias in non-randomized studies of interventions.

3.4. Primary Outcome: Clinical Pregnancy

Nine studies reported clinical pregnancy for 1,901 couples or cycles: 485 of 901 after MACS and 463 of 1000 after conventional preparation. Figure 3 shows that MACS was associated with a higher clinical pregnancy rate (RR 1.19, 95% CI 1.08 to 1.31; p = 0.004). The Wald interval also excluded no effect (95% CI 1.09 to 1.30). No heterogeneity was detected (I^2 = 0.0%, 95% CI 0.0 to 64.8; τ^2 = 0; Q = 7.26, df = 8, p = 0.51). The 95% prediction interval was 1.07 to 1.32. With a median control-group risk of 444 per 1000, the pooled estimate corresponds to 84 more clinical pregnancies per

1000 couples (95% CI 34 to 139). The cohort of Pacheco and colleagues contributed the largest weight (46.6%),[65] and the cohort of Sánchez-Martín and colleagues came next (18.4%).[64] These two retrospective cohorts carried almost two-thirds of the weight. All nine individual estimates lay above one, and two of them excluded no effect.

The individual studies contributed different information. The cohort of Pacheco and colleagues enrolled men with fragmentation above 20% after exclusion of treatable causes, and clinical pregnancy there was RR 1.18, 95% CI 1.04 to 1.34.[65] The cohort of Sánchez-Martín and colleagues was restricted to fragmentation of at least 30% and found RR 1.04, 95% CI 0.85 to 1.28; the autologous and donor strata pointed in the same direction.[64] Among the prospective studies, the comparison of Dirican and colleagues in oligoasthenozoospermic men gave RR 1.33, 95% CI 0.93 to 1.88.[58] The teratozoospermia studies gave RR 1.32, 95% CI 0.80 to 2.19 and RR 1.17, 95% CI 0.87 to 1.57.[60,61] For unexplained infertility, the estimates were RR 1.23, 95% CI 0.69 to 2.18, RR 1.33, 95% CI 0.88 to 2.03 and RR 2.13, 95% CI 1.26 to 3.60.[54,55,59]

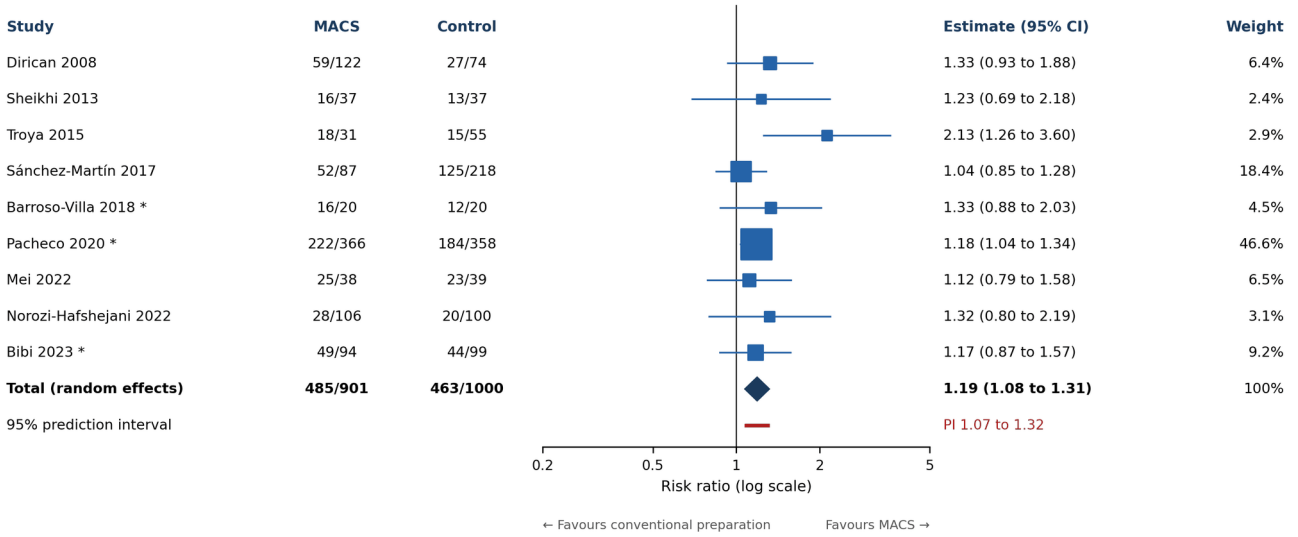
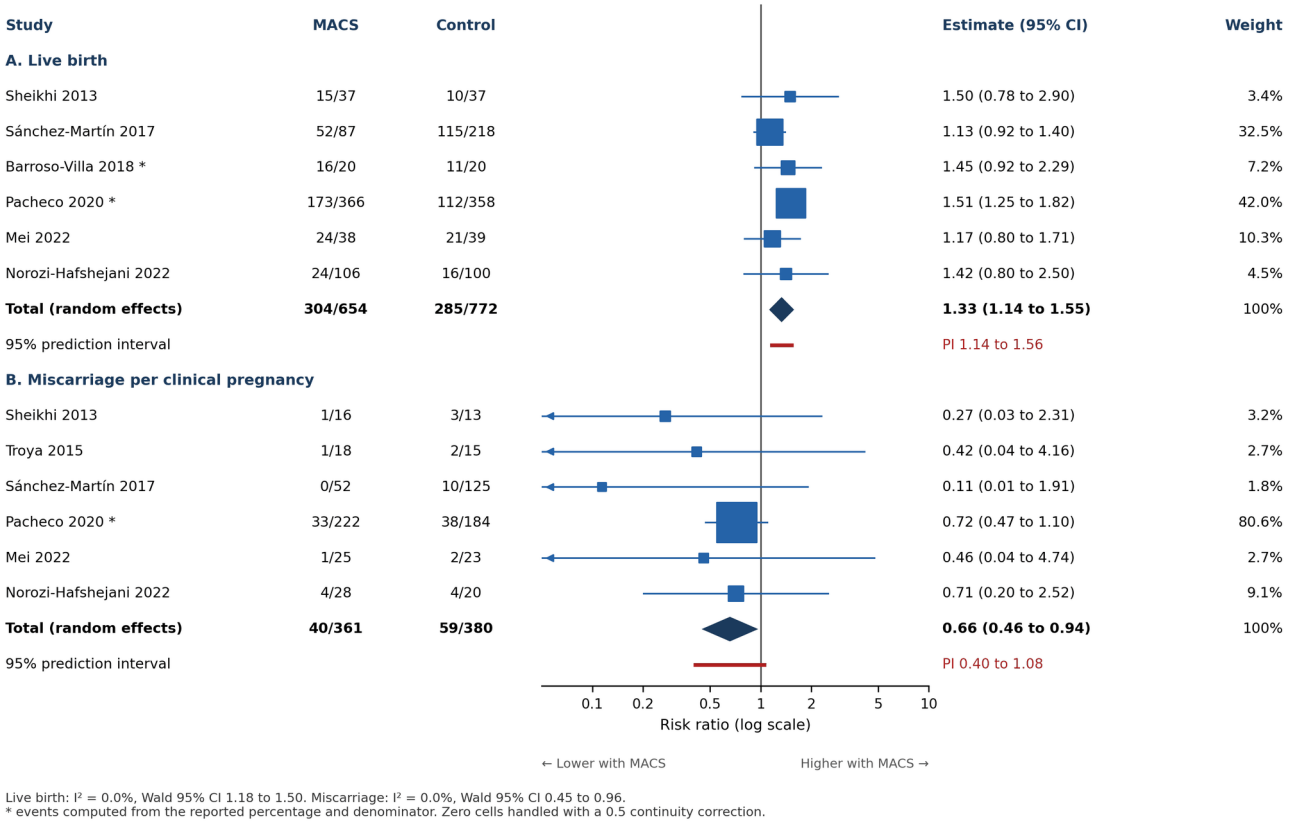


Figure 3. Forest plot of clinical pregnancy with MACS compared with conventional sperm preparation before ICSI. Squares are study risk ratios (size proportional to random-effects weight) with 95% confidence intervals; the diamond is the random-effects pooled estimate (DerSimonian-Laird, Hartung-Knapp) and the red bar the 95% prediction interval. Asterisks mark events computed from a reported percentage and denominator.

3.5. Secondary Outcomes

Live birth was reported by 6 studies (1,426 couples). MACS was associated with more live births (RR 1.33, 95% CI 1.14 to 1.55; I^2 = 0.0%; Wald 95% CI 1.18 to 1.50), equivalent to 140 more births per 1000 couples (95% CI 60 to 233), as shown in Figure 4A. Miscarriage per clinical pregnancy, reported by 6 studies (741 pregnancies), was lower after MACS (RR 0.66, 95% CI 0.46 to 0.94; I^2 = 0.0%; Wald 95% CI 0.45 to 0.96), as shown in Figure 4B. One cohort of 52 pregnancies recorded no miscarriage after MACS.[64] The estimate rests on 99 miscarriages. One retrospective cohort carried 80.6% of the weight, and the three studies not at serious risk of bias gave RR 0.60, 95% CI 0.29 to 1.23. Only one randomized trial reported ongoing pregnancy, which was higher with MACS, with the lower interval limit at 1.00 (RR 1.35, 95% CI 1.00 to 1.84; p = 0.05).[56]



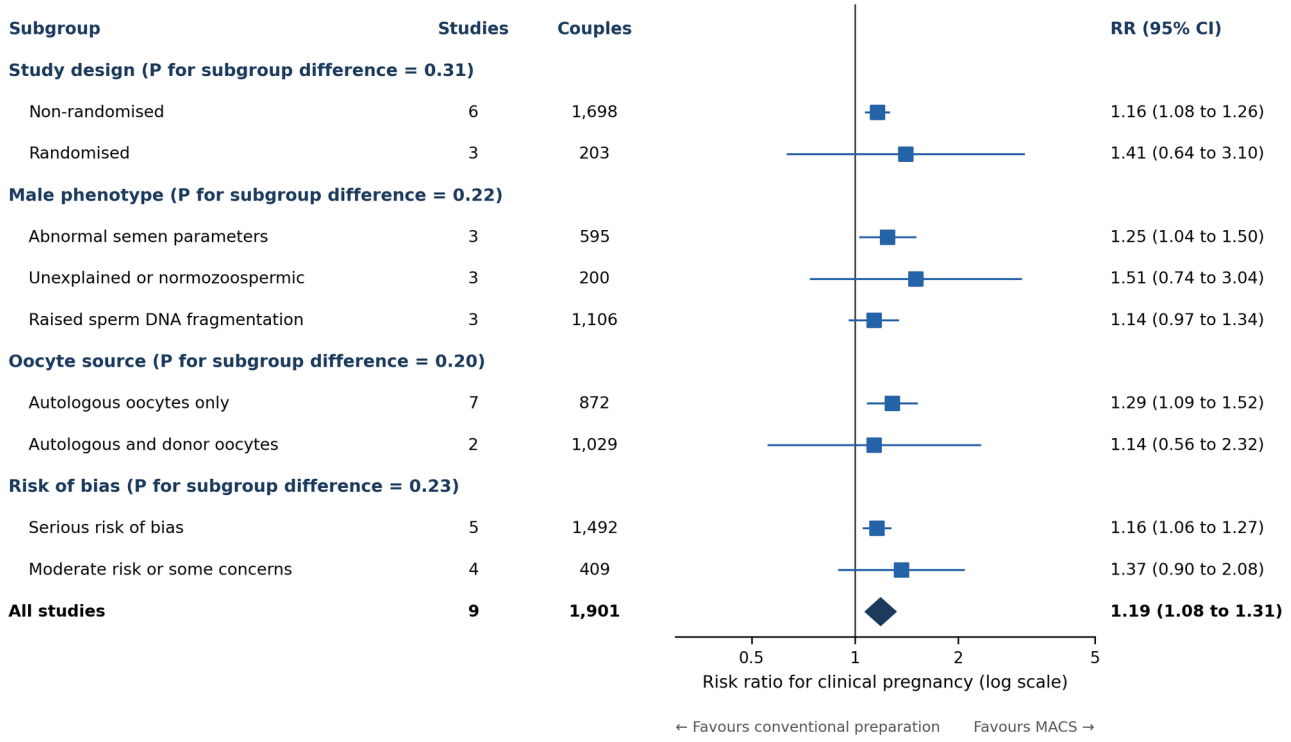
Live birth: I^2 = 0.0%, Wald 95% CI 1.18 to 1.50. Miscarriage: I^2 = 0.0%, Wald 95% CI 0.45 to 0.96.
* events computed from the reported percentage and denominator. Zero cells handled with a 0.5 continuity correction.

Figure 4. Forest plots of (A) live birth and (B) miscarriage per clinical pregnancy with MACS compared with conventional preparation. Layout as in Figure 3.

Laboratory outcomes did not differ. Fertilization per injected oocyte (8 studies, 14,336 oocytes) gave RR 1.02, 95% CI 0.99 to 1.05 (I^2 = 14.0%). The three studies with reported counts gave RR 0.97, 95% CI 0.84 to 1.13. Groups were similar for implantation per embryo transferred (RR 1.16, 95% CI 0.61 to 2.22; k = 3), good-quality or usable embryos (RR 1.06, 95% CI 0.93 to 1.20; k = 4) and blastocyst formation (RR 1.00, 95% CI 0.66 to 1.53; k = 2); see Supplementary Figures S1 to S5. Oocytes and embryos are clustered within couples. The intervals for these laboratory outcomes are therefore narrower than a couple-level analysis would give, and the absence of a difference is a more secure conclusion than the precise estimates.

3.6. Subgroup Analyses

Figure 5 presents the subgroup estimates. The pooled risk ratio was RR 1.16, 95% CI 1.08 to 1.26 in non-randomized studies and RR 1.41, 95% CI 0.64 to 3.10 in randomized trials (p for difference = 0.31). By male phenotype, the estimates were RR 1.51, 95% CI 0.74 to 3.04 for unexplained or normozoospermic infertility, RR 1.25, 95% CI 1.04 to 1.50 for abnormal semen parameters and RR 1.14, 95% CI 0.97 to 1.34 for raised DNA fragmentation (p = 0.22). Neither oocyte source (p = 0.20) nor risk of bias (p = 0.23) produced a difference in estimates. Most subgroups held three studies, so these comparisons were exploratory and had little power. Within subgroups of two or three studies, Hartung-Knapp intervals were often wider than any contributing study. The corresponding Wald intervals excluded no effect in every subgroup except the randomized trials (Supplementary Table S5).



Hartung-Knapp intervals within subgroups; tests for subgroup differences on the Wald random-effects fit.

Figure 5. Subgroup analyses of clinical pregnancy by study design, male phenotype, oocyte source and risk of bias. Squares are pooled risk ratios (95% confidence intervals) within each subgroup; the diamond is the overall estimate.

3.7. Sensitivity Analyses

Figure 6 illustrates the sensitivity analyses. Alternative estimators gave identical point estimates, and the Mantel-Haenszel common-effect model gave RR 1.20. Restricting the analysis to randomized trials gave RR 1.41, 95% CI 0.64 to 3.10 (k = 3; I^2 = 50.6%). Excluding studies at serious risk of bias gave RR 1.37, 95% CI 0.90 to 2.08 (k = 4). Both point estimates were larger than the primary estimate, but their intervals included no effect. Excluding computed counts gave RR 1.23, 95% CI 0.97 to 1.56 (k = 6). When the analysis was limited to the five studies whose denominator was couples or cycles started, it gave RR 1.15, 95% CI 1.00 to 1.31 (k = 5), with a Wald interval of 0.99 to 1.32, so the result was not stable under this restriction. The estimate was unchanged after excluding the mixed in vitro fertilization/ICSI trial (RR 1.20, 95% CI 1.07 to 1.34), after using the autologous-oocyte strata of the two mixed cohorts (RR 1.18, 95% CI 1.01 to 1.37) and after adding ongoing pregnancy from the remaining trial (RR 1.20, 95% CI 1.09 to 1.32; k = 10). Leave-one-out estimates ranged from 1.17 (without Troya 2015) to 1.22 (without Sánchez-Martín 2017). Every interval excluded no effect.

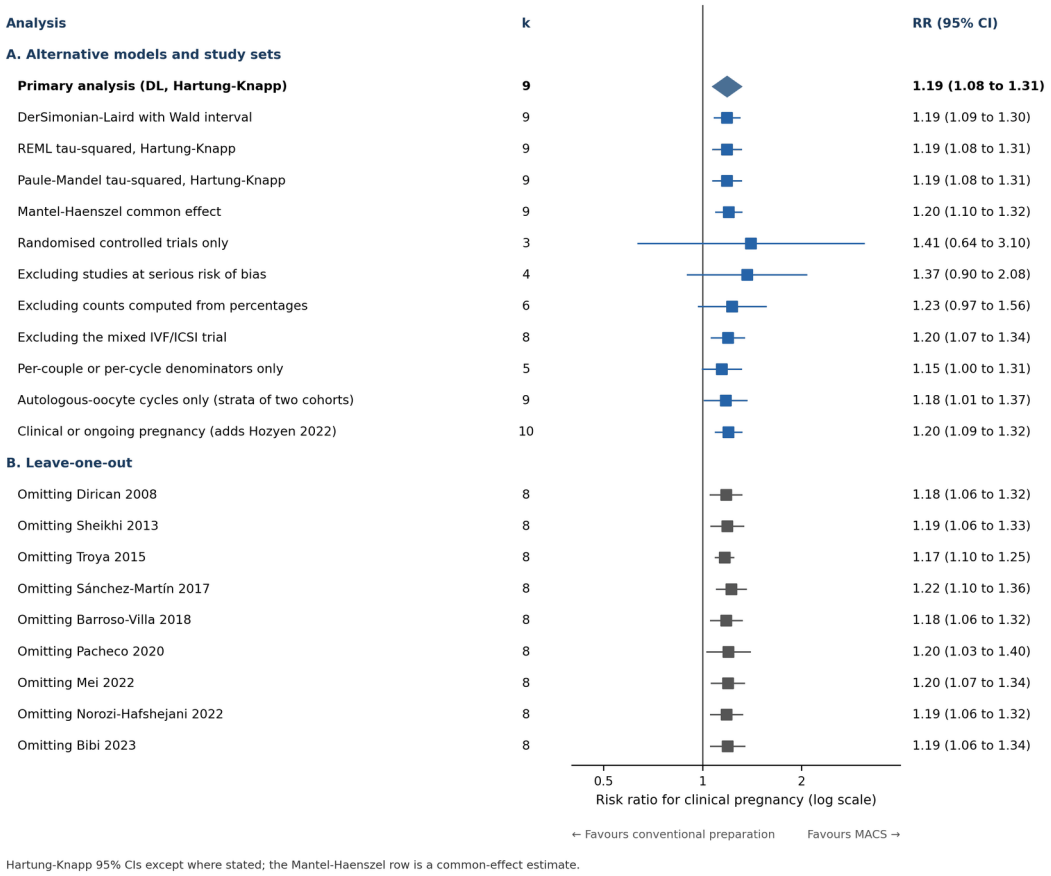


Figure 6. Sensitivity analyses of clinical pregnancy: (A) alternative estimators and study sets; (B) leave-one-out estimates. DL, DerSimonian-Laird; REML, restricted maximum likelihood.

3.8. Small-Study Effects

The funnel plot for clinical pregnancy (Figure 7) was asymmetrical. The smaller studies, mostly at the right of the plot, tended to report larger effects than the two large cohorts. Small-study bias, selective publication or genuine differences between small and large studies could all produce this pattern. Trim-and-fill imputed 3 studies and reduced the estimate to RR 1.15, 95% CI 1.01 to 1.30. Egger's test was not applicable to the nine studies of clinical pregnancy. In the ten-study analysis of clinical or ongoing pregnancy it gave p = 0.10, which, given the low power of the test, does not exclude asymmetry.

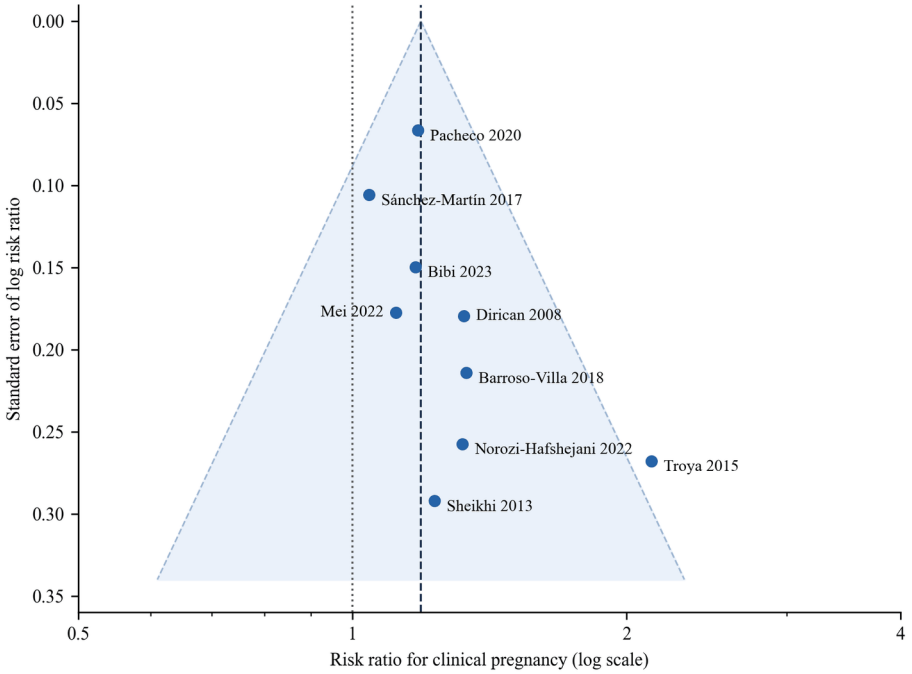
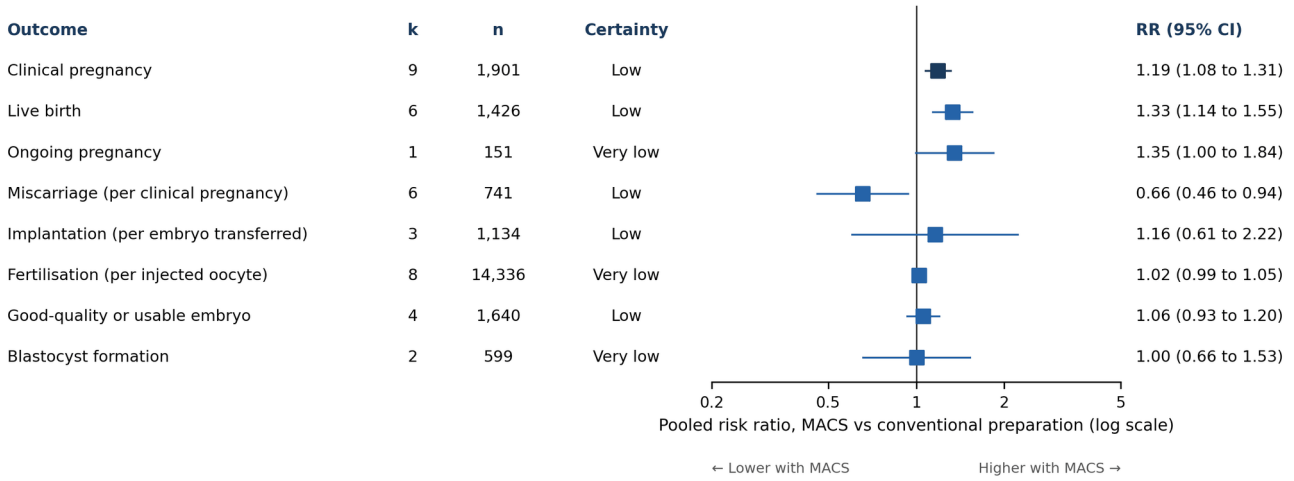


Figure 7. Funnel plot of study log risk ratios for clinical pregnancy against their standard errors. The dashed line marks the pooled estimate, the dotted line no effect and the shaded region the pseudo 95% confidence limits.

3.9. Certainty of Evidence

Figure 8 and Table 4 summarize the pooled estimates and their certainty. Certainty was low for clinical pregnancy, live birth and miscarriage. It was rated down by two levels for risk of bias, because 78% to 86% of the weight came from non-randomized studies at serious risk of confounding (83% for clinical pregnancy). For implantation and good-quality embryos the share was 43% and 33%, and for fertilization 63%. Certainty was low for implantation and good-quality embryos. It was very low for fertilization, blastocyst formation and ongoing pregnancy, because of risk of bias and imprecision.



n: couples (clinical outcomes), clinical pregnancies (miscarriage), embryos or oocytes (laboratory outcomes).
Hartung-Knapp 95% CIs; single-study estimate for ongoing pregnancy.

Figure 8. Pooled risk ratios (95% confidence intervals) for every outcome with the GRADE certainty of the evidence.

Table 4. Summary of Findings: MACS Compared with Conventional Sperm Preparation Before ICSI in Idiopathic Male Infertility.

Outcome	*k*	n	RR (95% CI)	Wald CI	I^2 (%)	Control risk per 1000	Absolute difference per 1000	Certainty
Clinical pregnancy (primary)	9	1,901	1.19 (1.08 to 1.31)	1.09 to 1.30	0.0	444	+84 (+34 to +139)	Low
Live birth	6	1,426	1.33 (1.14 to 1.55)	1.18 to 1.50	0.0	420	+140 (+60 to +233)	Low
Ongoing pregnancy	1	151	1.35 (1.00 to 1.84)	–	–	458	+162 (−1 to +383)	Very low
Miscarriage (per clinical pregnancy)	6	741	0.66 (0.46 to 0.94)	0.45 to 0.96	0.0	167	−57 (−90 to −10)	Low
Implantation (per embryo transferred)	3	1,134	1.16 (0.61 to 2.22)	0.88 to 1.53	37.3	–	–	Low
Fertilization (per injected oocyte)	8	14,336	1.02 (0.99 to 1.05)	1.00 to 1.05	14.0	–	–	Very low
Good-quality or usable embryo	4	1,640	1.06 (0.93 to 1.20)	0.98 to 1.15	11.9	–	–	Low
Blastocyst formation	2	599	1.00 (0.66 to 1.53)	0.88 to 1.14	0.0	–	–	Very low

Note: Risk ratios greater than 1 indicate more events with MACS. 95% CI, Hartung-Knapp confidence interval; Wald CI, DerSimonian-Laird interval without the Hartung-Knapp adjustment. n, couples or cycles for clinical outcomes, clinical pregnancies for miscarriage, embryos or oocytes for laboratory outcomes. Control risk is the median control-group risk per 1000; the absolute difference (per 1000, 95% CI) applies the pooled risk ratio to that risk (Equation 7). Certainty: GRADE, assessed with the reasons given in Section 3.9.

4. Discussion

4.1. Principal Findings

Twelve studies enrolled couples with idiopathic male infertility undergoing ICSI. Annexin V MACS was associated with a relative increase of about one-fifth in clinical pregnancy and one-third in live birth, along with a lower miscarriage rate. Fertilization, implantation and embryo quality showed no measurable difference. These are associations. The clinical pregnancy estimate stayed stable across studies and statistical models, yet it rested mainly on two Spanish retrospective cohorts in which MACS was offered by clinical choice, and it lost that stability once the analysis was restricted to studies that used couples or cycles started as the denominator (RR 1.15, 95% CI 1.00 to 1.31). The three randomized trials pointed the same way (RR 1.41, 95% CI 0.64 to 3.10). They were too small to confirm or exclude a benefit. The certainty of every estimate was low or very low.

The three trials differed in ways that limit what they can show together. In the Peruvian trial, couples with normozoospermic men were randomized to three arms after oocyte retrieval, with unequal arm sizes and three MACS couples excluded for lack of a transfer. It gave the largest estimate of all studies.[54] The Mexican trial was a triple-blinded pilot of 40 couples with unexplained infertility, described by its authors as a pilot trial.[55] The Chinese trial enrolled men with a DNA fragmentation index of at least 30% whose partners had tubal or pelvic factors, and it analyzed in vitro fertilization and ICSI together.[57] The combined interval ran from a one-third reduction to a threefold increase in clinical pregnancy. It fits no effect. It also fits an effect larger than that of the cohorts.

4.2. Comparison with Previous Syntheses

The direction of these findings agrees with the recent meta-analysis, which reported higher clinical pregnancy and live birth per embryo transfer with MACS in ICSI, mainly with

raised DNA fragmentation.[32] That review pooled 41 studies spanning intrauterine insemination, in vitro fertilization and ICSI. It also included cohorts of unselected men and expressed outcomes per transfer. The present review shares its larger cohorts but excludes unselected men, and it expresses outcomes with the denominator each study reported, which may explain the smaller relative effect. The network meta-analysis likewise found a higher clinical pregnancy rate when MACS was added to density-gradient centrifugation, and a lower miscarriage rate in pairwise analysis.[34] The Cochrane review, restricted to randomized trials of advanced sperm selection techniques, found no clear effect on live birth.[33] The difference is largely one of population. The largest cohorts of unselected men found little or no effect of MACS on pregnancy or live birth per transfer, with only a small increase in cumulative live birth per embryo transferred.[66,67] A technique that removes apoptotic spermatozoa is expected to help mainly where such spermatozoa are frequent. Diluting the population with men whose semen contains few of them would be expected to draw the pooled effect toward the null. The present review excluded such cohorts by design.

Other sperm-selection techniques have been tested against the same comparator, with mixed results. Microfluidic preparation gave embryo quality similar to density-gradient centrifugation, both in a randomized trial run under routine conditions and in a sibling-oocyte trial of men with abnormal semen parameters.[68,69] Selection through a cumulus-cell column improved DNA integrity and clinical outcomes in one trial,[70] while hyaluronan-based selection left embryo quality and euploidy unchanged, although one egg-donation trial reported a higher cumulative pregnancy rate.[71,72] An electrophoretic device was non-inferior to centrifugation.[73] When MACS was compared directly with testicular sperm in men with raised fragmentation, outcomes were similar.[74] In donor-sperm intrauterine insemination, MACS did not improve pregnancy rates.[75] No technique has shown a consistent effect on live birth in randomized trials.

Earlier reviews and this one also differ in how benefit is measured. Pregnancy and live birth per transfer capture the quality of the best embryo. Cumulative live birth per started cycle captures the whole cohort of embryos. In the Chinese trial, MACS did not change the first-transfer outcome significantly, but it reduced the number of transfers needed per retrieval (1.2 versus 1.6) and came with a numerically higher, but not statistically significant, cumulative live birth rate (79.5% versus 70.7%; $p = 0.37$).[57,66] In the large cohorts of unselected men, the only difference was in cumulative live birth per embryo transferred. If MACS acts by improving the average developmental competence of embryos, the clinically relevant benefit may be fewer transfers and less time to pregnancy, rather than a higher chance of pregnancy at the first transfer. Future trials should be designed to detect this.

4.3. Biological and Clinical Plausibility

The pattern of effects is compatible with the proposed mechanism. Fertilization after ICSI depends mainly on oocyte activation by the injected spermatozoon, which apoptotic markers do not prevent, so the absence of an effect on fertilization is expected. DNA damage, by contrast, is thought to act after activation of the embryonic genome, with consequences for implantation and early pregnancy loss.[13,17,18,76] The lower miscarriage rate after MACS fits this model, although it rests on few events. The oocyte can repair part of the paternal DNA damage, but this capacity declines with maternal age and, in a mouse model, after vitrification.[77,78] That decline may explain why a sibling-oocyte study found more good-quality blastocysts after MACS only in women older than 30 years (a post hoc subgroup).[62]

The prognostic weight of DNA fragmentation itself is disputed. Several large cohorts found no association with live birth after ICSI.[14,15,79] Others found lower cumulative live birth in couples with unexplained infertility and raised fragmentation,[80] whereas in men with raised fragmentation the choice between density-gradient centrifugation and swim-up did not change cumulative live birth.[81] A secondary analysis of a randomized trial found limited value of fragmentation testing for choosing between ICSI and conventional IVF, and whether testing can identify couples in whom the choice of sperm matters remains open.[82] At the cellular level, apoptotic markers in semen were associated with outcome after intrauterine insemination but not after ICSI.[83] Newer protocols that avoid centrifugation (MACS-Up[37] and a sperm separation device[84]) or that add MACS after gradient preparation before cryopreservation[85] preserved motility or post-thaw quality while lowering fragmentation, which supports the biological rationale without establishing a clinical benefit. MACS may also remove spermatozoa with abnormal chromosome content.[22] A quasi-randomized study of men with abnormal sperm aneuploidy testing, however, found no difference in fertilization or embryo euploidy.[63]

4.4. Interpretation of Heterogeneity

A lack of statistical heterogeneity should not be read as evidence that the effect is uniform. Nine studies were included, and two large cohorts dominated them. The confidence interval for I^2 reached 64.8%, and the subgroup analyses could not detect likely differences between male phenotypes. The point estimate was largest in unexplained or normozoospermic infertility and smallest with raised DNA fragmentation, the opposite of what the mechanism predicts. Several features of the studies may explain this. The unexplained-infertility subgroup consisted of three small studies, including the trial with the largest estimate, so its estimate is the one most exposed to small-study effects. The raised-fragmentation subgroup was dominated by the two retrospective cohorts. These used per-transfer or cycle denominators and included older donor-oocyte recipients, in whom oocyte quality may offset the paternal contribution. Fragmentation thresholds (20% to 30%) and assays (TUNEL, sperm chromatin dispersion and sperm chromatin structure assay) also differed.[20] The order of MACS and centrifugation, the serum content of the media and whether embryos were transferred fresh or frozen all varied between studies. None of these could be examined separately.[24,60]

4.5. Practical Considerations

Several practical points follow from the included studies. MACS was applied to fresh ejaculates with enough motile spermatozoa to tolerate the loss of cells in the column. Men with very low counts were excluded from some studies, so the findings do not apply to severe oligozoospermia or to surgically retrieved sperm. In most studies the technique was combined with density-gradient centrifugation, and the best sequence of MACS and centrifugation is unresolved,[24] although one laboratory study found higher progressive motility and normal morphology when MACS preceded centrifugation,[86] and one study performed MACS before centrifugation in serum-free medium to avoid the phosphatidylserine exposure that accompanies capacitation.[60] No included study reported that MACS prevented injection, and fertilization was unaffected. This suggests that the step did not compromise the cycle in these studies.

Selection of couples is the other practical issue. If MACS works by removing spermatozoa with apoptotic markers and DNA damage, it should be most useful when such cells are frequent. Testing for DNA fragmentation before treatment could identify these men. A recent secondary analysis of a randomized trial found limited value of fragmentation testing for choosing between ICSI and conventional IVF in non-severe male infertility, and its role in guiding sperm preparation has not been tested.[82] The present subgroup results do not yet support restricting MACS to men with raised fragmentation, because the subgroups were small and the pattern was the reverse of the expected one. A stratified trial would answer this question directly.

4.6. Strengths

This review addressed a clinically defined population. It applied RoB 2 and ROBINS-I according to design, and it reported Hartung-Knapp and Wald intervals together. Sensitivity analyses examined every source of computed data and every denominator type, and effects were expressed as absolute differences with GRADE certainty. The searches covered three databases, used citation searching and had no language restriction, which allowed a Spanish-language randomized trial to be included.[55]

4.7. Limitations

The evidence has several limitations. First, most weight came from non-randomized cohorts in which MACS was chosen for couples who differed in ways that were not adjusted for. Confounding by indication could bias the estimate in either direction, and when MACS was reserved for couples with previous failures it would bias against MACS. Second, three eligible reports were not retrieved. One trial reported only ongoing pregnancy, so the evidence base is incomplete. Third, the denominators for clinical pregnancy differed between studies, and the result did not hold up when restricted to couples or cycles started. Fourth, several counts were computed from reported percentages or group means. Excluding them widened the interval but did not change the direction of the estimate. Fifth, laboratory outcomes were analyzed at the oocyte or embryo level, ignoring clustering within couples, which overstates their precision. Sixth, the smaller studies reported larger effects, and trim-and-fill reduced the estimate, which raises the possibility of small-study bias. Seventh, the definition of idiopathic infertility varied. Only three studies described the exclusion of treatable or genetic causes, so a minority of men with treatable causes may have been included. Eighth, female factors such as tubal disease in one trial and advanced maternal age in donor-oocyte strata may have modified the effect in ways that study-level data cannot resolve. Finally, one reviewer performed screening and extraction, with second-pass verification of every value. This reduces but does not eliminate the risk of error.

4.8. Implications

Clinicians counseling couples with idiopathic male infertility can describe MACS as a laboratory step that was associated with higher pregnancy and live birth rates in studies of low certainty, with no effect on embryo quality and a possibility that the association reflects confounding or small-study bias. No included study reported neonatal outcomes. The only large safety data come from a multicenter cohort of unselected men, outside the eligibility criteria of this review, in which obstetric and perinatal outcomes were similar.[87] The evidence is of low certainty and add-ons carry costs for couples. The technique should therefore be offered within trials or with transparent information, not as routine practice.[29,31]

Researchers should conduct adequately powered randomized trials in defined male phenotypes, with live birth per randomized couple as the primary outcome and prespecified analyses by DNA fragmentation and female age. A trial powered to detect the pooled relative increase in live birth at a control rate of about 40% would need about 220 couples per arm (80% power, two-sided $\alpha = 0.05$, 40% versus 53%), and about 1,200 per arm if the lower interval limit (RR 1.14) is assumed, which is within reach of a multicenter collaboration. Selection techniques may act through the number of transferable embryos rather than through the first transfer, so trials should also report cumulative live birth per started cycle. Obstetric and neonatal outcomes belong in the report too.[16]

These considerations matter in settings where couples pay for assisted reproduction, as in much of Southeast Asia. A laboratory step that adds a modest cost per cycle may be reasonable if it raises the live birth rate by several percentage points. That holds only if the gain is real. If it reflects confounding, the cost is borne without benefit. Clinics that adopt MACS should therefore record the indication, the male phenotype, the DNA fragmentation result and the outcome of every cycle. Their own data could then inform future syntheses.

5. Conclusion

In couples with idiopathic male infertility undergoing ICSI, annexin V MACS was associated with higher clinical pregnancy (RR 1.19, 95% CI 1.08 to 1.31) and live birth (RR 1.33, 95% CI 1.14 to 1.55) rates. Fertilization, implantation and embryo quality did not change. The lower miscarriage rate rests on few events. It should be regarded as hypothesis-generating. The certainty of this evidence is low, because most of it comes from non-randomized cohorts at serious risk of confounding, the randomized trials are small, the result depended on the denominator used, and small-study bias cannot be excluded. Couples with idiopathic male infertility who are offered MACS should first be counseled about this uncertainty and the cost, preferably within a trial or with prospective recording of outcomes. Adequately powered randomized trials are needed before routine use can be recommended. Their outcome should be live birth per randomized couple, with stratification by DNA fragmentation and female age.

Declarations

Ethics Approval and Consent to Participate

Not applicable. This systematic review used published data and involved no new human participants. Consent for publication is not applicable.

Availability of Data and Materials

The de-identified dataset and the analysis scripts supporting the findings are available from the corresponding author on reasonable request.

Competing Interests

The authors declare no competing interests.

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Authors' Contributions

H.W.: Conceptualization, Methodology, Data curation, Formal analysis, Writing - original draft. R.C.P.: Validation,

Investigation, Writing - review and editing. Both authors approved the final manuscript.

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None.

Use of artificial intelligence

The authors declare that no generative artificial intelligence tools were used in the design, analysis, or writing of this manuscript.

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