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Supplementary Material

Mitochondrial DNA Copy Number and Genomic Variants in Recurrent Pregnancy Loss and Recurrent Implantation Failure: A Systematic Review

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SUPPLEMENTARY MATERIAL

Supplementary Table S1. Study-level data-verification matrix for the 29 included primary studies.

Study	Sample	Controls	Tissue	mtDNA Measure	Method	Outcome	Effect Estimate
Fragouli *et al.*, 2017 [22]	199 transferred euploid blastocysts from 174 couples	Normal mtDNA (n=190) *vs* elevated (n=9)	Trophectoderm	Relative mtDNA quantity	NGS-derived measure with qPCR/validation	Ongoing pregnancy / implantation	Ongoing pregnancy 64% with normal mtDNA *vs* 0% elevated; $p<0.0001$
Ravichandran *et al.*, 2017 [23]	1,505 euploid blastocysts from 490 couples; 282 transferred	Normal mtDNA (249 transferred) *vs* elevated (33)	Trophectoderm	Relative mtDNA level	qPCR after PGT-A/WGA	Implantation	185/249 (74.3%) normal-mtDNA blastocysts implanted *vs* 0/33 elevated; $p<0.0001$
Treff *et al.*, 2017 [24]	187 patients; 374 sibling euploid blastocyst biopsies	Within-patient sibling embryo comparison	Trophectoderm	Relative mtDNA level	qPCR	Reproductive potential / implantation	No systematic difference in mtDNA between sibling embryos with discordant reproductive outcomes
Victor *et al.*, 2017 [25]	1,396 blastocysts from 259 patients; qPCR validation in 150	Ploidy-, age- and implantation-defined comparisons	Trophectoderm	Adjusted relative mtDNA quantity	NGS + qPCR validation with correction factors	Implantation potential	After correction, mtDNA did not meaningfully differ by ploidy, maternal age or implantation potential
Lee *et al.*, 2019 [26]	1,617 embryos from 380 couples; 267 transferred blastocysts	Implanted (n=103) *vs* non-implanted (n=164)	Blastomere (n=99) / trophectoderm (n=1,518)	Adjusted mtDNA quantity	NGS with correction factors	Implantation	Median 0.00097 implanted *vs* 0.00088 non-implanted; $p=0.21$

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Zhou *et al.,* 2021 [27]	316 euploid single-transferred blastocysts from 233 couples	Live birth *vs* miscarriage *vs* non-implantation	Trophectoderm	mtDNA content	NGS/PGT-A-derived quantification	Live birth, miscarriage, implantation	No group difference p=0.999; implantation p=0.965; adjusted live-birth association p=0.567; elevated *vs* normal live birth 26.7% *vs* 33.6%, p=0.780
Kaare *et al.,* 2009 [28]	48 RM women	48 age-matched controls	Maternal blood; placental samples in subset	Heteroplasmic mtDNA variants	DHPLC + sequencing	Recurrent miscarriage	Heteroplasmic variants: 13/48 (27%) RM *vs* 9/48 (19%) controls; no apparent excess in RM
Seyedhassani *et al.,* 2010a [29] (D-loop)	96 idiopathic RPL women	96 women with ≥2 live births and no pregnancy loss	Peripheral blood; mtDNA D-loop mutations; mtDNA deletions; multiplex PCR + sequencing	Common deletions + D-loop variants	Multiplex PCR + direct sequencing	Repeated pregnancy loss	No common deletion; 129 variations in RPL and 22 mutations reported as significant (p<0.05)
Seyedhassani *et al.,* 2010b [30] (tRNA)	96 idiopathic RPL women	No concurrent control group	Peripheral blood; mt-tRNA Thr/Pro variants; sequencing	mt-tRNA Thr/Pro variants	PCR + automated sequencing	Repeated pregnancy loss	A15907G 2/96; A15924G 3/96; G15928A 10/96; G15930A 2/96 in full-text table; T15972C 1/96
Vanniarajan *et al.,* 2011 [31]	100 RPL women + 12 aborted fetal tissues	The study reported age- and ethnicity-matched fertile controls and a patient confirmation cohort.	Maternal blood + aborted fetal tissue	Whole mtDNA sequence variants/haplogroups	Complete mtDNA sequencing	Recurrent pregnancy loss	681 variants; T4216C present in 9% of RPL women; multiple pathogenic/novel variants reported
Colagar *et al.,* 2013 [32]	33 RPL women	100 controls	Peripheral blood	ND1 T4216C	PCR + direct sequencing	Recurrent pregnancy loss	T4216C: 10/33 (30%) *vs* 11/100 (11%); p<0.05
Azadi *et al.,* 2018 [33]	73 RPL women	100 healthy controls	Peripheral blood	Seven Complex-I mtDNA variants	PCR + direct sequencing	Recurrent pregnancy loss	RPL group had significantly higher proportions of seven specified variants than controls; exact per-variant estimates not reported in abstract
Ye *et al.,* 2020 [34]	75 EPL cases	75 healthy controls	Chorionic villi	mtDNA copy number + 4977-bp deletion rate	qPCR / CNV sequencing	Early pregnancy loss	Copy number OR 1.71 (p=0.005); deletion rate OR 1.07 (p=0.006)

Ji *et al.,* 2020 [35]	75 spontaneous-abortion villi	75 induced-abortion villi	Chorionic villi	mtDNA copy number + heteroplasmic/pathogenic variants	qPCR + NGS	Early embryonic loss	Copy number 566 (397–791) *vs* 614 (457–739), p=0.768; pathogenic mutations 70.7% *vs* 54.7%; ND2 26.67% *vs* 13.33%, p=0.041
Eker *et al.,* 2021 [36]	25 RIF women	25 fertile healthy controls	Endometrial biopsy (cycle days 20–24)	MT-ND1 and MT-CO2 copy number	ddPCR; GAPDH normalization	Recurrent implantation failure	Normalized MT-ND1/MT-CO2 copy number higher in RIF; Mann-Whitney and ROC analyses
Mojodi *et al.,* 2023 [37]	100 women with idiopathic recurrent miscarriage	100 women with no miscarriage history	Peripheral blood	mt-tRNA Cys/Tyr variants	PCR-SSCP + sequencing	Idiopathic recurrent miscarriage	Four variants identified in cases: 5824C>T, 5868T>A, 5849C>T, 5836T>C
Lledó *et al.,* 2018 [38]	159 euploid non-mosaic blastocysts from 142 couples	Low mtDNA (n=142) *vs* high mtDNA >0.003 (n=17)	Trophectoderm biopsy	mtDNA copy number + heteroplasmy	NGS; mtDNA/nDNA normalization; MitoSeek	Ongoing pregnancy, miscarriage, implantation	Ongoing pregnancy 42.96% *vs* 17.65% (p=0.048); miscarriage 6.15% *vs* 40.0% (p=0.011)
Diez-Juan *et al.,* 2015 [39]	270 single euploid transfers (205 day-3; 65 day-5) + 10 double transfers	MitoScore strata within euploid transfers	Blastomere / trophectoderm biopsy	Relative mtDNA content (MitoScore)	qPCR/quantification after PGT	Implantation	Day-3 Ms>160 (n=22) and day-5 Ms>60 (n=7): 0 implantations; implantation decreased across higher score strata
Fragouli *et al.,* 2015 [40]	379 embryos (39 cleavage-stage; 340 blastocysts)	Implanted *vs* non-implanted and euploid *vs* aneuploid comparisons	Blastomere / trophectoderm	Relative mtDNA quantity	aCGH/NGS with qPCR validation	Implantation potential; age; ploidy	Higher mtDNA associated with age/aneuploidy; implanted euploid blastocysts had lower mtDNA (p=0.007); threshold >0.003 associated with non-implantation in retrospective set
Stigliani *et al.,* 2014 [41]	699 day-3 spent-culture-medium samples	Outcome-defined comparison groups	Spent embryo culture medium	Extracellular mtDNA/gDNA ratio	qPCR-based non-invasive quantification	Blastulation and implantation	Higher ratio with blastocyst development (p=0.0251) and implantation of good-quality embryos (p=0.0452)
Klimczak *et al.,* 2018 [42]	259 patients; 1,510 blastocyst biopsies; 717 euploid in restricted analysis	Embryo grade and implantation groups	Trophectoderm	Embryonal mtDNA content	Relative mtDNA quantification	Embryo quality and transfer outcome	Poorer grade associated in full set (p=0.003), not euploid subset (p=0.834); implantation p=0.53

Łukaszuk *et al.,* 2022 [43]	716 embryos from 275 couples	Euploid *vs* aneuploid; day-5 *vs* day-6; implanted *vs* non-implanted	Trophectoderm	mtDNA/nDNA ratio	Low-pass NGS	Ploidy, development day, implantation	Ploidy difference p=0.02; day-5 *vs* day-6 p=0.001; implanted *vs* non-implanted 1.14±0.88 *vs* 1.21±1.16, p=0.39
Podolak *et al.,* 2022 [44]	314 cleavage-stage embryos from 69 couples	Euploid *vs* aneuploid; implanted *vs* non-implanted euploid embryos	Day-3 blastomere	mtDNA/gDNA ratio	Low-pass NGS	Ploidy, morphology, implantation/live birth	Euploid median 4.6 (2.8–7.2) *vs* aneuploid 5.9 (3.7–8.9), p=0.004; implanted *vs* non-implanted p=0.18
El-Damen *et al.,* 2021 [45]	355 SEFET cycles in 314 patients	Pregnant *vs* non-pregnant; clinical pregnancy *vs* miscarriage	Trophectoderm	mtDNA/nDNA ratio	NGS-derived ratio	Pregnancy and miscarriage	Pregnant *vs* non-pregnant 27.7±9.2 *vs* 29.4±8.6, p=0.095; clinical pregnancy *vs* miscarriage 30.5±9.3 *vs* 27.3±9.2, p=0.136
Wang *et al.,* 2021 [46]	769 euploid blastocysts in main analysis (day-5 n=355, day-6 n=388, day-7 n=26)	Developmental-day/quality groups and transferred-embryo outcomes	Trophectoderm	Relative mtDNA content	NGS	Embryo quality, implantation, live birth	Day-5 higher than day-6/7 ($p<0.001$); better TE quality associated with lower mtDNA (p=0.026); lower day-5 mtDNA associated with better outcomes but not an independent predictor
Chuang *et al.,* 2023 [47]	1,675 embryos from 1,305 individuals; 1,340 euploid single-transfer outcome cycles	Day-5 eSET *vs* day-6 eSET; success *vs* failure within day strata	Trophectoderm	mtDNA ratio	NGS algorithm with qPCR validation	Fetal heartbeat, ongoing pregnancy, live birth	Day-6 failure groups had lower mtDNA: fetal heartbeat p=0.004, ongoing pregnancy p=0.007, live birth p=0.01; no corresponding day-5 association
Brenner *et al.,* 2000 [48]	23 RIF patients undergoing ooplasmic transfer	No untreated parallel control	Embryos; amniocytes; placenta; cord blood in subsets	Donor-recipient mtDNA heteroplasmy	mtDNA fingerprinting / sequencing	Post-ooplasmic-transfer pregnancy and heteroplasmy	Donor mtDNA detected in 6/13 embryos, 1/4 amniocytes, 2/4 placentas and 2/4 cord-blood samples
Barritt *et al.,* 2001 [49]	2 offspring after ooplasmic transplantation	No parallel comparator group; follow-up report of two offspring after ooplasmic transplantation.	Offspring blood	Donor/recipient mtDNA heteroplasmy	mtDNA fingerprinting	Persistence/inheritance of donor mtDNA	Heteroplasmy demonstrated in both children studied
Victor *et al.,* 2019 [50]	109 transferred blastocysts in 100 women	Implanted (n=55) *vs* non-implanted (n=54)	Trophectoderm	Relative mtDNA content	PGT-associated mtDNA quantification	Implantation and live birth	No significant mtDNA difference; very high mtDNA did not preclude healthy birth

Supplementary Table S2. Summary of the included studies, their design or evidence type, appraisal methods, key sources of bias, overall risk of bias, and the resulting judgement regarding their use in the manuscript.

Study	Design / Evidence Type and Appraisal Tool	Key Bias Concerns	Overall Risk	Judgement and Manuscript Use
Fragouli *et al.,* 2017 [22]	Blinded prospective non-selection pregnancy outcome study Tool: JBI Cohort Checklist	Remaining platform and clinical heterogeneity	Low	Judgement: Prospective blinded non-selection design reduces selection and performance bias. Use: Use as higher-quality prospective evidence.
Ravichandran *et al.,* 2017 [23]	Retrospective multicentre single euploid blastocyst transfer study Tool: JBI Cohort Checklist	Retrospective design; embryo-selection and laboratory-platform effects	Low	Judgement: Large multicentre euploid dataset strengthens internal validity, although causality remains unproven. Use: Use as strong implantation-failure biomarker evidence.
Treff *et al.,* 2017 [24]	Sibling embryo comparative study Tool: JBI Cohort Checklist	Embryo-level rather than patient-level inference; platform-specific	Low	Judgement: Sibling design reduces patient-level confounding and is useful for negative evidence. Use: Use as important contradictory evidence.
Victor *et al.,* 2017 [25]	Method-focused blastocyst mtDNA quantification study Tool: JBI Cohort Checklist	Laboratory method dependence; clinical endpoint limitations	Low	Judgement: Strong methodological control and adjusted quantification reduce measurement bias. Use: Use to discuss assay heterogeneity and negative findings.
Lee *et al.,* 2019 [26]	Large double-blind prospective observational embryo study Tool: JBI Cohort Checklist	Infertility cohort not RIF/RPL-specific; platform-specific adjustment	Low	Judgement: Large dataset and blinded design reduce bias; negative finding is important. Use: Use as strong negative implantation-biomarker evidence.
Zhou *et al.,* 2021 [27]	Single euploid blastocyst transfer live-birth study Tool: JBI Cohort Checklist	Retrospective design; RIF/RM subgroup not isolated	Low	Judgement: Euploid embryo control and live-birth endpoint strengthen clinical relevance. Use: Use as strong negative live-birth biomarker evidence.
Kaare *et al.,* 2009 [28]	Case-control recurrent miscarriage mtDNA variant study Tool: JBI Case-Control Checklist	Small sample; maternal blood only; limited tissue specificity	Moderate	Judgement: Cases and controls were clearly compared, but sample size was modest and reproductive tissue was not assessed. Use: Use as direct but cautious evidence showing no strong association.
Seyedhassani *et al.,* 2010a [29]	Case-control repeated pregnancy loss; D-loop/deletion study Tool: JBI Case-Control Checklist	Population selection, limited confounder adjustment, D-loop focus only	Moderate	Judgement: Direct RPL comparison was present, but functional validation and broader mtDNA coverage were limited. Use: Use as supportive D-loop mutation evidence.
Seyedhassani *et al.,* 2010b [30]	Idiopathic repeated pregnancy loss; mt-tRNA mutation study Tool: JBI Case Series Checklist	No concurrent control group was included; interpretation is limited by absence of comparative analysis and lack of functional validation.	Moderate	Judgement: Relevant to RPL and mt-tRNA variants, but narrower genetic coverage and limited statistical detail. Use: Use in mt-tRNA subsection only.

Vanniarajan *et al.,* 2011 [31]	Case-control RPL whole-mtDNA/haplogroup study Tool: JBI Case-Control Checklist	Ethnicity-specific findings; sequencing status of confirmation/control cohorts incompletely detailed; multiple variant testing; functional validation absent	Moderate	Judgement: Larger control group and direct RPL design strengthen the study, but population-specific interpretation remains important. Use: Use as one of the strongest core RPL studies.
Colagar *et al.,* 2013 [32]	Case-control RPL single-mutation study Tool: JBI Case-Control Checklist	Small case group; single variant; possible population stratification	Moderate	Judgement: Clear case-control design and statistical comparison, but limited by small sample and single mutation focus. Use: Use as candidate ND1/T4216C evidence.
Azadi *et al.,* 2018 [33]	Case-control RPL complex-I variant study Tool: JBI Case-Control Checklist	Moderate sample; no functional validation; population-specific	Moderate	Judgement: Direct RPL relevance and variant sequencing are strengths, but causality is not established. Use: Use as coding-region variant evidence.
Ye *et al.,* 2020 [34]	Early pregnancy loss mtDNA copy number/deletion study Tool: JBI Case-Control Checklist	Early loss not necessarily recurrent; full confounder control unclear	Moderate	Judgement: Highly relevant to mtDNA copy number and pregnancy loss, but recurrence-specific inference is limited. Use: Use in mtDNA copy-number/deletion pregnancy-loss section.
Ji *et al.,* 2020 [35]	Spontaneous abortion *vs* induced abortion tissue study Tool: JBI Case-Control Checklist	Not strictly recurrent miscarriage; tissue differences possible	Moderate	Judgement: Strong tissue-level comparison, NGS/qPCR approach, and reported statistics reduce bias risk. Use: Use as strong miscarriage-tissue mutation evidence.
Eker *et al.,* 2021 [36]	RIF case-control endometrial mtDNA copy-number study Tool: JBI Case-Control Checklist	Small sample; endometrial timing and confounders may affect mtDNA	Moderate	Judgement: Most direct RIF evidence, but exploratory sample size limits certainty. Use: Use as core RIF mtDNA copy-number study.
Mojodi *et al.,* 2023 [37]	Idiopathic recurrent miscarriage mt-tRNA variant case-control study Tool: JBI Case-Control Checklist	Limited external replication; population-specific variants; functional validation absent	Moderate	Judgement: Direct recurrent miscarriage relevance, but interpretation depends on variant validation. Use: Use as recent supportive mt-tRNA evidence.
Lledó *et al.,* 2018 [38]	IVF outcome study with mtDNA analysis and RIF/RPL indications Tool: JBI Cohort Checklist	Mixed infertility indications; outcome heterogeneity	Moderate	Judgement: Clinically relevant, but not restricted to RPL/RIF patients. Use: Use as bridge evidence between RIF/RPL and IVF outcome.
Diez-Juan *et al.,* 2015 [39]	Euploid embryo mtDNA viability-score study Tool: JBI Cohort Checklist	Retrospective biomarker threshold; platform dependence	Moderate	Judgement: Strong embryo-biomarker relevance, but external validation and cut-off generalisability are concerns. Use: Use as early positive implantation biomarker evidence.
Fragouli *et al.,* 2015 [40]	Embryonic mtDNA, aneuploidy, age, implantation study Tool: JBI Cohort Checklist	Retrospective design; embryo selection bias; assay platform dependence	Moderate	Judgement: Statistically useful and clinically relevant, but biomarker interpretation may vary by platform. Use: Use as positive implantation-potential evidence.

Stigliani *et al.,* 2014 [41]	Spent embryo culture medium mtDNA biomarker study Tool: JBI Cohort Checklist	Culture contamination risk; indirect mtDNA source; non-invasive assay variability	Moderate	Judgement: Non-invasive design is innovative, but culture-medium mtDNA may not equal embryonic intracellular mtDNA. Use: Use in non-invasive biomarker subsection.
Klimczak *et al.,* 2018 [42]	Embryonal mtDNA and transfer outcome study Tool: JBI Cohort Checklist	Retrospective design; embryo quality and transfer confounding	Moderate	Judgement: Outcome relevance is clear, but confounding by embryo and patient characteristics may remain. Use: Use in embryo quality/transfer outcome section.
Łukaszuk *et al.,* 2022 [43]	PGT-A cohort including RIF/RM indications Tool: JBI Cohort Checklist	Mixed indications; outcome association partly non-significant; platform dependence	Moderate	Judgement: Large embryo number and RIF/RM indications are strengths, but clinical outcome signal was not robust. Use: Use as supportive evidence with caution.
Podolak *et al.,* 2022 [44]	Cleavage-stage embryo mtDNA copy-number outcome study Tool: JBI Cohort Checklist	Cleavage-stage focus; less direct to RIF/RPL; embryo-level confounding	Moderate	Judgement: Relevant to embryo developmental biology but indirect for recurrent reproductive failure. Use: Use only in embryo-developmental outcome subsection.
El-Damen *et al.,* 2021 [45]	Single euploid frozen embryo transfer miscarriage outcome study Tool: JBI Cohort Checklist	Retrospective/clinical confounding; single setting	Moderate	Judgement: Euploid single-transfer design improves outcome interpretation, but residual confounding remains. Use: Use in miscarriage-after-euploid-transfer section.
Wang *et al.,* 2021 [46]	Day-5 euploid blastocyst mtDNA transfer outcome study Tool: JBI Cohort Checklist	Day-5 only; residual patient/embryo confounding	Moderate	Judgement: Relevant to embryo quality and euploid transfer outcomes, but needs replication. Use: Use as supportive transfer-outcome evidence.
Chuang *et al.,* 2023 [47]	Day-6 single euploid embryo cryotransfer outcome study Tool: JBI Cohort Checklist	Retrospective; day-6 specific; clinical and laboratory confounders	Moderate	Judgement: Recent euploid cryotransfer evidence but limited generalisability to all embryos/RIF cases. Use: Use as recent supportive outcome evidence.
Brenner *et al.,* 2000 [48]	Non-randomized ooplasmic transplantation in RIF Tool: ROBINS-I	No randomized control; intervention selection bias; small sample	High	Judgement: Relevant to RIF heteroplasmy, but not a natural-history RIF biomarker study. Use: Use only as mechanistic heteroplasmy evidence, not core risk estimate.
Barritt *et al.,* 2001 [49]	Follow-up/brief communication after ooplasmic transplantation Tool: ROBINS-I	Case/follow-up nature; no comparator; small selected sample	High	Judgement: Important for mtDNA heteroplasmy safety but high selection and reporting limitations. Use: Use cautiously in heteroplasmy safety discussion.
Victor *et al.,* 2019 [50]	Clinical follow-up of embryos with elevated mtDNA Tool: JBI Cohort Checklist	Highly selected embryos; small clinical subgroup; no broad comparator	High	Judgement: Useful counterexample but not suitable for estimating predictive accuracy. Use: Use only to show high mtDNA does not always mean non-viability.

Supplementary Table S2A. Completed study-specific Joanna Briggs Institute (JBI) critical appraisal assessments.

Completed study-specific Joanna Briggs Institute (JBI) critical appraisal assessments.

A. JBI Case-Control Checklist Studies.

Study	Q1. Were the groups comparable other than the presence of disease in cases or the absence of disease in controls?	Q2. Were cases and controls matched appropriately?	Q3. Were the same criteria used for identification of cases and controls?	Q4. Was exposure measured in a standard, valid and reliable way?	Q5. Was exposure measured in the same way for cases and controls?	Q6. Were confounding factors identified?	Q7. Were strategies to deal with confounding factors stated?	Q8. Were outcomes assessed in a standard, valid and reliable way for cases and controls?	Q9. Was the exposure period of interest long enough to be meaningful?	Q10. Was appropriate statistical analysis used?	Overall Judgement
Kaare *et al.,* 2009 [28]	Yes	Yes	Yes	Yes	Unclear	No	Yes	Yes	Not applicable	Yes	Moderate
Seyedhassani *et al.,* 2010a [29]	Yes	Yes	Yes	Yes	Unclear	No	Yes	Yes	Not applicable	Yes	Moderate
Vanniarajan *et al.,* 2011 [31]	Unclear	Yes	Yes	Yes	Unclear	No	Yes	Unclear	Not applicable	Unclear	Moderate
Colagar *et al.,* 2013 [32]	Unclear	Yes	Yes	Yes	Unclear	No	Yes	Yes	Not applicable	Yes	Moderate
Azadi *et al.,* 2018 [33]	Unclear	Yes	Yes	Yes	Unclear	No	Yes	Yes	Not applicable	Yes	Moderate
Ye *et al.,* 2020 [34]	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes	Not applicable	Yes	Moderate
Ji *et al.,* 2020 [35]	Unclear	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes	Not applicable	Yes	Moderate
Eker *et al.,* 2021 [36]	Unclear	Yes	Yes	Yes	Unclear	No	Yes	Yes	Not applicable	Yes	Moderate
Mojodi *et al.,* 2023 [37]	Yes	Yes	Yes	Yes	Unclear	No	Yes	Yes	Not applicable	Yes	Moderate

B. JBI Case Series Checklist Studies.

Study	Q1. Were there clear criteria for inclusion in the case series?	Q2. Was the condition measured in a standard, reliable way for all participants included in the case series?	Q3. Were valid methods used for identification of the condition for all participants included in the case series?	Q4. Did the case series have consecutive inclusion of participants?	Q5. Did the case series have complete inclusion of participants?	Q6. Was there clear reporting of the demographics of the participants in the study?	Q7. Was there clear reporting of clinical information of the participants?	Q8. Were the outcomes or follow up results of cases clearly reported?	Q9. Was there clear reporting of the presenting site(s)/clinic(s) demographic information?	Q10. Was statistical analysis appropriate?	Overall Judgement
Seyedhassani *et al.,* 2010b [30]	Yes	Yes	Yes	Unclear	Yes	Yes	Unclear	Yes	Not applicable	Unclear	Moderate

C. JBI Cohort Checklist Studies (IVF embryo studies).

Study	Q1. Were the two groups similar and recruited from the same population ?	Q2. Were the exposures measured similarly to assign people to both exposed and unexposed groups?	Q3. Was the exposure measured in a valid and reliable way?	Q4. Were confounding factors identified?	Q5. Were strategies to deal with confounding factors stated?	Q6. Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)?	Q7. Were the outcomes measured in a valid and reliable way?	Q8. Was the follow up time reported and sufficient to be long enough for outcomes to occur?	Q9. Was follow up complete, and if not, were the reasons to loss to follow up described and explored ?	Q10. Were strategies to address incomplete follow up utilized?	Q11. Was appropriate statistical analysis used?	Overall Judgement
Fragouli *et al.,* 2017 [22]	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes	Yes	Not applicable	Yes	Low
Ravichandran *et al.,* 2017 [23]	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	Yes	Not applicable	Yes	Low
Treff *et al.,* 2017 [24]	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	Yes	Not applicable	Yes	Low

Victor *et al.*, 2017 [25]	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	Yes	Not applicable	Yes	Low
Lee *et al.*, 2019 [26]	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	Yes	Not applicable	Yes	Low
Zhou *et al.*, 2021 [27]	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	Yes	Not applicable	Yes	Low
Lledó *et al.*, 2018 [38]	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Not applicable	Yes	Moderate
Diez-Juan *et al.*, 2015 [39]	Yes	Yes	Yes	Unclear	No	Unclear	Yes	Yes	Yes	Not applicable	Yes	Moderate
Fragouli *et al.*, 2015 [40]	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Not applicable	Yes	Moderate
Stigliani *et al.*, 2014 [41]	Yes	Yes	Yes	Unclear	No	Unclear	Yes	Yes	Yes	Not applicable	Yes	Moderate
Klimczak *et al.*, 2018 [42]	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Not applicable	Yes	Moderate
Łukaszuk *et al.*, 2022 [43]	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Not applicable	Yes	Moderate
Podolak *et al.*, 2022 [44]	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Not applicable	Yes	Moderate
El-Damen *et al.*, 2021 [45]	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Not applicable	Yes	Moderate
Wang *et al.*, 2021 [46]	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Not applicable	Yes	Moderate
Chuang *et al.*, 2023 [47]	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Not applicable	Yes	Moderate
Victor *et al.*, 2019 [50]	No	Yes	Yes	Unclear	No	Unclear	Yes	Yes	Yes	Not applicable	Yes	High

Note: *JBI = Joanna Briggs Institute; IVF = in vitro fertilisation. Q = question. "Not applicable" indicates that the appraisal item was not applicable to the study design or reporting. Overall judgements are reproduced as provided.*

Supplementary Table S2B. Domain-level ROBINS-I assessment.

Study	Bias Due to Confounding	Bias in Selection of Participants	Bias in Classification of Intervention	Bias Due to Deviations from Intended Intervention	Bias Due to Missing Data	Bias in Measurement of Outcomes	Bias in Selection of Reported Results	Overall Risk of Bias
Brenner *et al.*, 2000 [48]	Serious	Serious	Low	Low	Low	Low	Moderate	High
Barritt *et al.*, 2001 [49]	Serious	Serious	Low	Low	Low	Low	Moderate	High

Brenner *et al.* (2000)

Confounding

Serious risk because the intervention was performed in a highly selected population with recurrent implantation failure and without a randomized comparator; underlying infertility factors, maternal age, embryo quality and previous reproductive history could influence outcomes independently of ooplasmic transfer.

Selection of participants

Serious risk because participants were selected from a highly specific clinical population with severe reproductive failure, creating substantial selection concerns and limiting generalisability.

Classification of intervention

Low risk because the ooplasmic transfer procedure was clearly described.

Deviations from intervention

Low risk because the intervention was performed according to the reported clinical protocol.

Missing data

Low risk because reported cases were followed and outcomes were described.

Outcome measurement

Low risk because reproductive outcomes and mtDNA heteroplasmy findings were laboratory-based and clearly assessed.

Selective reporting

Moderate risk because the study involved a small clinical series and reporting was limited by the early stage of the technology.

Barritt *et al.* (2001)

Confounding

Serious risk because offspring outcomes after ooplasmic transplantation may be influenced by multiple parental and embryo-related factors in the absence of a concurrent comparator.

Selection bias

Serious risk because the study represented a highly selected group undergoing an experimental reproductive intervention.

Classification of intervention

Low risk because donor and recipient mitochondrial contribution was assessed using molecular analysis.

Deviations from intervention

Low risk because the intervention and follow-up were reported according to the clinical procedure.

Missing data

Low risk because the available follow-up information was reported.

Outcome measurement

Low risk because mtDNA inheritance patterns were evaluated using molecular techniques.

Selective reporting

Moderate risk because the evidence came from a small number of reported cases.