International Platform of Registered Systematic Review and Meta-analysis Protocols

INPLASY

Cell-free maternal serum DNA (cfDNA) for the Diagnosis of Genetic Aneuploidy in Recurrent Pregnancy Loss (RPL): A Systematic Review

INPLASY202650129

doi: 10.37766/inplasy2026.5.0129

Received: 23 May 2026

Published: 23 May 2026

Corresponding author:
William Kutteh

wkutteh50@gmail.com

Author Affiliation:
University of Tennessee Health Sciences Center.

Esmail, J; Kutteh, WH.

ADMINISTRATIVE INFORMATION

Support - None.

Review Stage at time of this submission - Data extraction.

Conflicts of interest - None declared.

INPLASY registration number: INPLASY202650129

Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 23 May 2026 and was last updated on 23 May 2026.

INTRODUCTION

Review question / Objective Population (P): Studies must look at pregnant individuals experiencing spontaneous miscarriages, specifically focused on those meeting the criteria for recurrent pregnancy loss (typically defined as two or more failed pregnancies). Demographics must trace back to maternal serum drawn during or shortly following the loss.
Intervention (Index Test) (I): Studies must evaluate genome-wide or targeted cell-free DNA (cfDNA) technology to sequence floating fetal genetic material in the maternal bloodstream to identify numerical chromosome anomalies (monosomies, trisomies, and structural rearrangements).
Comparator (Reference Standard) (C): The index test must be compared directly against conventional tissue-based cytogenetics. This evaluates how well blood-based cfDNA performs when tissue extraction is either successful or limited by maternal cell contamination and cell culture failure.
Outcomes (O): Statistical verification of accuracy. The review will synthesize pooled data reflecting how reliably cfDNA matches the tissue array (concordance rates) and whether it effectively prevents unnecessary, invasive parental testing by immediately identifying a random fetal genetic cause.
Study Design (S): Inclusion is limited to primary, original human clinical studies evaluating diagnostic accuracy. Systematic reviews, case reports (n=1), expert opinions, and review commentaries will be excluded from the final data synthesis.

Rationale Recurrent pregnancy loss (RPL) is a profoundly challenging clinical and emotional disorder that impacts millions of families worldwide. For decades, establishing a definitive etiology has remained a cornerstone of clinical management, as it dictates future reproductive counseling and treatment strategies. The urgent need for definitive answers has driven a critical evolution in professional medical guidelines, prompting researchers to seek diagnostic methods

that maximize accuracy while minimizing patient burden.

Alignment with New Medical Guidelines

In May 2026, the American Society for Reproductive Medicine (ASRM) published an updated document titled "Recurrent Pregnancy Loss: A committee opinion". In this landmark update, the ASRM explicitly recommends chromosome evaluation of miscarriage tissue by microarray as a foundational first step in the clinical evaluation of a couple with RPL. Chromosomal Microarray Analysis (CMA) provides superior resolution over traditional karyotyping, helping clinicians identify numeric and structural genetic aneuploidies that cause spontaneous miscarriage without the risks of cell culture failure.

However, despite clear evidence favoring tissue-based microarray analysis, significant logistical and clinical hurdles prevent many patients from utilizing it. This study explores how cell-free maternal serum DNA (cfDNA) testing can serve as an innovative, highly accurate alternative or complementary screening mechanism to bridge these gaps.

Overcoming Logistical and Tissue Extraction Barriers

A primary challenge of adhering to the ASRM tissue microarray guideline is the frequent absence of available fetal tissue. Many individuals experience a spontaneous loss outside of a hospital setting, resulting in the accidental disposal or rapid degradation of the products of conception (POC).

Fortunately, fetal trophoblast cells release short fragments of cell-free fetal DNA directly into the maternal bloodstream. This study evaluates how cfDNA testing can successfully salvage genetic evaluation for the vast population of women who have had a spontaneous loss but possess no physical fetal tissue for CMA. By ensuring these patients can reach their physician for a simple blood draw within 24 hours of the loss, clinicians can capture viable circulating fetal DNA and effectively determine if a random aneuploidy triggered the event.

Addressing Shifting Trends in Miscarriage Management

Furthermore, this systematic review is critically timed to address a growing paradigm shift in obstetric care. An increasing number of women navigating pregnancy loss now opt for medical management (such as misoprostol regimens) or expectant management rather than invasive surgical treatments like dilation and curettage (D&C).

While medical management is safe and less invasive, it significantly limits the ability to collect intact, uncontaminated fetal tissue samples. By validating the use of blood-based cfDNA, these patients can completely avoid the physical collection of fetal tissue. Instead, they can undergo a non-invasive venipuncture to determine if the underlying cause of their loss was due to fetal aneuploidy.

Conclusion

By synthesizing current clinical data, this systematic review aims to establish the precise diagnostic accuracy and clinical utility of maternal serum cfDNA for genetic evaluation in RPL. This work ultimately seeks to provide clinicians with an evidence-based roadmap that aligns with ASRM's focus on genetic screening while expanding access to patients restricted by tissue availability or medical choices.

Condition being studied Clinical Profile: Recurrent pregnancy loss (RPL) is a complex, emotionally devastating reproductive disorder defined by the American Society for Reproductive Medicine (ASRM) as the spontaneous loss of two or more clinical pregnancies . Distinct from sporadic miscarriage, which occurs in 10% to 15% of all pregnancies, RPL affects approximately 2% to 3% of couples striving to conceive . It is categorized as a distinct disease of the reproductive system due to its recurrent nature, which indicates an underlying pathological, anatomical, or genetic vulnerability rather than an isolated, chance event .Pathophysiology and Genetic Etiology: The underlying causes of RPL are highly heterogeneous, spanning endocrine dysregulation (such as uncontrolled diabetes or thyroid disease), uterine anatomic anomalies, parental balanced chromosomal translocations, and autoimmune disorders like Antiphospholipid Syndrome (APS) . However, despite exhaustive clinical investigations, up to 50% of RPL cases remain classified as unexplained .Among identified causes, fetal chromosomal aneuploidy—the presence of an abnormal number of chromosomes in the developing embryo—is the single most common mechanism driving early pregnancy loss, accounting for 50% to 60% of all spontaneous miscarriages . These genetic anomalies typically arise from de novo errors during maternal or paternal gametogenesis (meiotic non-disjunction) rather than inherited traits. Consequently, identifying whether a miscarriage was euploid (chromosomally normal) or aneuploid (chromosomally abnormal) is clinically paramount. It distinguishes an unpreventable genetic accident from maternal or paternal structural factors that require targeted medical intervention.

Clinical Manifestations and Patient Burden: The physical presentation of pregnancy loss includes vaginal bleeding, pelvic cramping, and uterine

tissue passage, varying in intensity based on gestational age. Beyond physical symptoms, the psychological toll of RPL is severe. Patients face high rates of clinical depression, anxiety, and post-traumatic stress disorder (PTSD). The repeated cycle of anticipation and grief places an immense burden on couples and healthcare systems alike.
The Evolving Diagnostic Paradigm: Historically, investigating RPL began only after a third consecutive loss. Modern guidelines have shifted this threshold to two losses, accelerating the diagnostic timeline to mitigate patient trauma . Evaluating the chromosomal status of the lost fetus is the first critical fork in the diagnostic roadmap .If a loss is confirmed as aneuploid, couples are often reassured that the event was a sporadic genetic anomaly, sparing them from expensive, invasive, and unnecessary systemic testing. Conversely, confirming a euploid loss signals a high probability of maternal or paternal factors, prompting immediate, targeted clinical investigations. By establishing whether a loss is genetic or non-genetic, clinicians can provide accurate prognostic counseling and design evidence-based management plans for future pregnancies.

METHODS

Search strategy 1. PubMed Search Strategy
text
(("Abortion, Habitual"[Mesh] OR "Abortion, Spontaneous"[Mesh] OR "recurrent pregnancy loss"[Title/Abstract] OR "recurrent miscarriage"[Title/Abstract] OR "early pregnancy loss"[Title/Abstract] OR "miscarriage"[Title/Abstract] OR "spontaneous loss"[Title/Abstract]) AND ("Cell-Free Nucleic Acids"[Mesh] OR "Prenatal Diagnosis"[Mesh] OR "cell-free DNA"[Title/Abstract] OR "cfDNA"[Title/Abstract] OR "cffDNA"[Title/Abstract] OR "NIPT"[Title/Abstract] OR "NIPS"[Title/Abstract] OR "maternal serum DNA"[Title/Abstract] OR "liquid biopsy"[Title/Abstract]) AND ("Aneuploidy"[Mesh] OR "Chromosomal Aberrations"[Mesh] OR "Microarray Analysis"[Mesh] OR "aneuploidy"[Title/Abstract] OR "chromosomal abnormality"[Title/Abstract] OR "trisomy"[Title/Abstract] OR "monosomy"[Title/Abstract] OR "CMA"[Title/Abstract] OR "microarray"[Title/Abstract] OR "karyotype"[Title/Abstract]))

2. Embase Search Strategy
text
(('recurrent abortion'/exp OR 'spontaneous abortion'/exp OR 'recurrent pregnancy loss':ti,ab OR 'recurrent miscarriage':ti,ab OR 'early pregnancy loss':ti,ab OR 'miscarriage':ti,ab OR 'spontaneous loss':ti,ab) AND ('cell free DNA'/exp OR 'prenatal diagnosis'/exp OR 'cell-free dna':ti,ab OR 'cfdna':ti,ab OR 'cffdna':ti,ab OR 'nipt':ti,ab OR 'nips':ti,ab OR 'maternal serum dna':ti,ab OR 'liquid biopsy':ti,ab) AND ('aneuploidy'/exp OR 'chromosome aberration'/exp OR 'microarray analysis'/exp OR 'aneuploidy':ti,ab OR 'chromosomal abnormality':ti,ab OR 'trisomy':ti,ab OR 'monosomy':ti,ab OR 'cma':ti,ab OR 'microarray':ti,ab OR 'karyotype':ti,ab)).

Participant or population Pregnant women experiencing Recurrent Pregnancy Loss (RPL) (defined as 2 spontaneous miscarriages) or early pregnancy loss (EPL) undergoing genetic evaluation. Pregnancy losses will include biochemical losses. Excluded pregnancies are ectopic or molar pregnancies. Pregnancies without a history of loss, advanced gestational age (more than 20 weeks), or ongoing viable singleton/ multiple pregnancies will be excluded.

Intervention Non-invasive prenatal testing (NIPT) utilizing maternal cell-free DNA (cfDNA) sequencing (genome-wide or targeted) performed on blood drawn after the loss is diagnosed. Blood may be drawn during the loss, prior to any surgical or medical intervention, or after the loss. Excluded comparators will be Preimplantation Genetic Testing for Aneuploidy (PGT-A), amniocentesis, or chorionic villus sampling (CVS) used as the index screening tool rather than maternal blood.

Comparator Tissue cytogenetic analysis performed on products of conception (POC), specifically Chromosomal Microarray Analysis (CMA), Next-Generation Sequencing (NGS), or traditional karyotyping. Studies lacking an objective, tissue-based genetic confirmation (e.g., comparing cfDNA only to ultrasound findings or maternal phenotype will be excluded.

Study designs to be included Original, peer-reviewed primary diagnostic accuracy studies, including prospective cohort, retrospective cohort, or cross-sectional diagnostic studies.

Eligibility criteria Case reports (n < 5), review papers, systematic reviews, meta-analyses, consensus statements, and animal/in-vitro laboratory validation models will be excluded from this review. Other Inclusion and Exclusion criteria are listed about in the PICOS sections.

Information sources Electronic databases including PubMed/PMC, Google Scholar, BASE (Biefield Academic Search Engine). Scopus, Web of Science, and DOAJ (Directory of Open Access

Journals) are intended to be used. In addition, reference lists from selected articles, contact with authors, trial registries are intended to be used.

Main outcome(s) 1. Primary Outcomes: Diagnostic Accuracy
The primary outcomes focus on the statistical performance of cfDNA (the index test) compared directly against Chromosomal Microarray Analysis (CMA) or karyotyping of products of conception (the reference standard).
• Pooled Sensitivity: The ability of maternal cfDNA to correctly identify the presence of a fetal chromosomal aneuploidy when it exists.
• Pooled Specificity: The ability of maternal cfDNA to correctly confirm a normal, euploid fetus when no chromosomal abnormalities are present in the miscarriage tissue.
• Concordance Rate: The overall percentage of cases where the genetic diagnosis from the maternal blood sample matches the genetic diagnosis from the fetal tissue sample exactly.
• Area Under the Summary Receiver Operating Characteristic (SROC) Curve: A global metric grading the overall accuracy of cfDNA, with values approaching 1.0 indicating excellent diagnostic power.
2. Secondary Outcomes: Clinical Utility and Feasibility
The secondary outcomes evaluate the real-world performance, logistics, and clinical advantages of implementing blood-based cfDNA screening in standard obstetric care.
• Test Failure and "No-Call" Rates: The percentage of maternal blood samples that fail to yield a diagnostic result, primarily due to low fetal fraction (insufficient fetal DNA in the maternal bloodstream) or maternal cell contamination.
• Turnaround Time (TAT): The average number of days from the maternal blood draw to the final genetic report, evaluating if cfDNA provides faster answers to grieving families than tissue culture.
• Tissue-Salvage Rate: The proportion of patients who lacked physical fetal tissue for CMA (e.g., due to a spontaneous loss at home) but successfully obtained a definitive genetic diagnosis via a cfDNA blood draw within 24 hours.
• Utility in Medical Management: The diagnostic success rate of cfDNA specifically among women opting for medical miscarriage management (e.g., misoprostol) who wish to avoid physical fetal tissue collection.

Additional outcome(s) None.

Data management Data management for this systematic review will follow a secure, structured, and auditable pipeline to maintain data integrity and transparency from initial search to final synthesis.1. Data Software & Secure StoragePrimary Screening Platform: All citations retrieved from PubMed and Embase will be uploaded into Rayyan or Covidence for initial title, abstract, and full-text screening.
Data Extraction & Version Control: Confirmed studies will be managed using a centralized Microsoft Excel spreadsheet or an Access database hosted on a secure cloud network (e.g., institutional OneDrive or SharePoint).Security Protocol: The extraction sheet will be set to read-only for non-extracting team members, with password-encrypted access restricted to the primary investigators to prevent accidental alterations.
Extraction WorkflowDual Extraction: Two reviewers will independently extract data from each included study using the predefined data extraction form.Data Cleansing: Raw numbers for true positives (TP), false positives (FP), false negatives (FN), and true negatives (TN) will be cross-referenced with the paper's text. If data is presented only in percentages, the reviewers will manually calculate the absolute frequencies.
Handling Missing Data: If a study meets inclusion criteria but lacks critical data (e.g., exact failure rates or (2times2) diagnostic values), the corresponding author will be contacted via email. A maximum of three outreach attempts will be made over 4 weeks. If the data remains unavailable, the study will be excluded from the quantitative synthesis but retained in the qualitative narrative.
Conflict ResolutionDiscrepancy Identification: Rayyan/Covidence will flag conflicting screening decisions, while a statistical comparison tool will highlight data entry mismatches between the two Excel sheets.Arbitration: The two primary reviewers will meet to discuss discrepancies. If a consensus cannot be reached regarding a study's eligibility or extracted metrics, a third senior investigator will arbitrate and make the final decision.
Long-Term Archiving & Data SharingAudit Trail: A permanent master log will document all changes, screen dropouts, and reasons for full-text exclusion to feed directly into the final PRISMA flow diagram.

Quality assessment / Risk of bias analysis To assess the internal validity of the included studies, two reviewers will independently evaluate the risk of bias and clinical applicability using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool. This tool is specifically recommended by the Cochrane Collaboration for systematic reviews of diagnostic test accuracy.

The Four Domains of QUADAS-2. The assessment is structured around four core domains.
Domain 1: Patient SelectionRisk of Bias: Evaluates if the study enrolled a representative sample of patients. A random or consecutive enrollment scheme is required. Studies that selectively enrolled patients based on known tissue results or high fetal fractions will be rated as high risk.Applicability: Evaluates if the included patients match your target population (pregnant women with a history of recurrent pregnancy loss or early pregnancy loss).
Domain 2: Index Test (cfDNA)Risk of Bias: Evaluates if the cfDNA results were interpreted without knowledge of the reference standard (blinded interpretation). It also checks if a pre-specified threshold/cut-off for aneuploidy detection was established before analysis.Applicability: Ensures the cfDNA technology and timing (e.g., blood draw within 24 hours of loss) match your index test definitions.
Domain 3: Reference Standard (Tissue Cytogenetics)Risk of Bias: Evaluates if the reference standard (CMA, NGS, or karyotype) is highly accurate at detecting chromosomal aneuploidy and if it was interpreted without knowledge of the cfDNA results.Applicability: Ensures the tissue analysis correctly classifies target genetic abnormalities matching clinical diagnosis needs.
Domain 4: Flow and TimingRisk of Bias: Analyzes the flow of patients through the study. It evaluates:The time interval between the index test (maternal blood draw) and the reference standard (fetal tissue extraction).Whether all patients who received the index test also received the reference standard.Whether all patients were included in the final analysis (accounting for low fetal fraction or sample failure rates).

Strategy of data synthesis The statistical data analysis for this systematic review will employ a bivariate random-effects model to account for within-study and between-study heterogeneity. This approach models sensitivity and specificity simultaneously, preserving the inherent negative correlation between these two diagnostic metrics.

1. Descriptive and Diagnostic Metrics GenerationFor each included study, raw numbers from the reconstructed (2times2) contingency tables will be used to calculate point estimates and 95% confidence intervals (CI) for:
Sensitivity: The probability that cfDNA correctly identifies a fetal aneuploidy.
Specificity: The probability that cfDNA correctly identifies a normal (euploid) fetus.
Likelihood Ratios: Positive Likelihood Ratio (PLR) and Negative Likelihood Ratio (NLR).
Diagnostic Odds Ratio (DOR): A single indicator of diagnostic performance combining sensitivity and specificity.
These individual study metrics will be visually mapped using paired forest plots to contrast sensitivity and specificity side by side.

Subgroup analysis To explore identified sources of heterogeneity and address your study's specific clinical objectives, a priori subgroup analyses will be performed based on:Timing of Maternal Blood Draw: Comparing blood draws taken within 24 hours of loss versus those taken after 24 hours.Miscarriage Management Type: Comparing patients who underwent medical management (tissue uncollected) versus surgical management (tissue collected).Reference Standard Used: Comparing studies using Chromosomal Microarray Analysis (CMA) or NGS against those relying on traditional karyotyping.cfDNA Platform: Comparing genome-wide next-generation sequencing against targeted screening methods.

Sensitivity analysis The sensitivity analysis for this systematic review is a vital quality-control step designed to test the stability and robustness of the pooled diagnostic metrics (sensitivity, specificity, and Area Under the Curve). By systematically removing certain studies or altering core assumptions, it checks whether the final conclusions are heavily distorted by specific methodological choices or lower-quality data.
The following specific sensitivity analyses will be conducted a priori:
1. Methodological Quality and Risk of Bias
To ensure that flawed study designs do not skew the results, the data will be re-pooled after:
• Excluding studies classified as having a High Risk of Bias or "Unclear" status in any of the four primary domains of the QUADAS-2 assessment (especially Patient Selection or Flow and Timing).
• Excluding retrospective cohort studies to see if their reliance on historical medical charts significantly over- or under-estimates cfDNA accuracy compared to strictly monitored prospective cohort studies.
2. Clinical and Diagnostic Threshold Boundary Testing
To isolate and protect the exact clinical boundaries outlined in your study rationale, the data will be re-analyzed after:
• Excluding any studies where the maternal blood draw window was unknown, poorly documented, or extended beyond 24 hours from the time of loss confirmation.

• Excluding studies that included patients with less than 2 clinical pregnancy losses if the study mixed sporadic early pregnancy loss (EPL) data indistinguishably with strict recurrent pregnancy loss (RPL) cohorts.

3. Outlier and Sample Size Influence

To verify that a single massive trial or an anomalous data set is not driving the overall conclusion:

• Leave-One-Out Analysis: The meta-analysis will be re-run multiple times, sequentially omitting one study at a time, to evaluate if any single paper exerts an undue influence on the pooled summary estimates.

• Excluding small-sample studies (e.g., studies with fewer than 20 patients) to determine if small-study effects or high sampling variability are introducing noise into the final SROC curve.

4. Reference Standard Rigor

• Excluding older studies that utilized traditional tissue karyotyping as their reference standard, restricting the analysis exclusively to modern cohorts that used Chromosomal Microarray Analysis (CMA) or Next-Generation Sequencing (NGS) on the products of conception. This directly evaluates how cfDNA performs against the exact "gold standard" recommended by the May 2026 ASRM committee opinion.

Reporting the Results

If the pooled sensitivity, specificity, and diagnostic odds ratios remain stable (within narrow confidence intervals) after these exclusions, the primary meta-analysis conclusions will be declared highly robust. If significant changes occur, those specific factors (such as study design or blood draw timing) will be highlighted in the Discussion section as critical variables affecting clinical utility.

Language restriction English.

Country(ies) involved United States.

Other relevant information None.

Keywords recurrent pregnancy loss; abortion,habitual; abortion, spontaneous; cell free nucleic acids; cell free DNA; chromosomal microarray;maternal serum DNA; recurrent miscarriage.

Dissemination plans Publication in Fertility and Sterility or Journal of Clinical Medicine. Presentation at ASRM, ESHRE, PCFS and other meetings.

Contributions of each author

Author 1 - William Kutteh - Study design, identification of key articles, preparation of INPLASTY registration, review of articles, review and preparation of preliminary manuscript, approval of final manuscript.
Email: wkutteh50@gmail.com
Author 2 - Jihan Esmail - Identification of key articles from electronic data base, review of articles, analysis of data, preparation of manuscript, approval of final manuscript.
Email: jesmail@uthsc.edu