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Review Article

Liquid Biopsy as a Screening Tool in Recurrent Implantation Failure During IVF: A Narrative Review

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Abstract

Objective: Recurrent implantation failure (RIF) remains a multifaceted challenge in assisted reproductive technology (ART), affecting a subset of in vitro fertilization (IVF) patients despite transfer of high-quality embryos. Conventional diagnostics often require invasive procedures that may disturb endometrial receptivity. This narrative review critically synthesizes the evidence on liquid-biopsy strategies for RIF screening, emphasizing biofluids such as uterine fluid (UF), spent embryo culture medium (SCM), peripheral blood, cervicovaginal fluid (CVF), and seminal plasma.

Materials and methods: Literature searches covered PubMed, Scopus, and Web of Science through August 2025, with inclusion of pivotal pre-2022 studies. Eligible works included observational studies, clinical trials, multi-omics analyses, and systematic reviews reporting molecular biomarkers relevant to endometrial receptivity or embryo competence.

Results: UF-derived microRNAs (miRNAs) and extracellular vesicle (EV) cargo during the window of implantation (WOI) consistently differentiate receptive from nonreceptive endometrium, with predictive accuracies (AUC 0.75–0.90) in small validation studies. SCM-based noninvasive PGT-A (niPGT-A) shows moderate concordance (~65%) with trophectoderm biopsy PGT-A, constrained by contamination and low cfDNA yield; recent advances include blastocoel fluid integration and methylation-based decontamination. Peripheral blood, CVF, and seminal plasma biomarkers remain exploratory due to lower specificity. Pre-analytical standardization and harmonized outcome definitions are critical for translation.

Conclusion: UF-miRNA/EV profiling holds the strongest promise for noninvasive RIF screening, whereas niPGT-A may serve as an adjunct for embryo competence assessment when biopsy is not feasible. Both approaches require multicenter validation and prospective trials demonstrating improved live-birth rates before routine clinical adoption.

Keywords: Recurrent Implantation Failure; Liquid Biopsy, Uterine Fluid; Extracellular Vesicles

Introduction

Recurrent implantation failure (RIF) remains a formidable challenge in assisted reproductive technology (ART), defined variably as the failure to achieve clinical pregnancy following two to six transfers of good-quality embryos (1-4). While prevalence estimates diverge, large observational studies suggest that "true" RIF—when repeated transfers of euploid embryos still fail—is relatively uncommon, with prevalence estimates often below

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5% (5, 6). Nevertheless, for the affected patients, the physical, emotional, and financial burdens are profound (7).

Diagnostic methods for endometrial receptivity, such as endometrial biopsy, hysteroscopy, and immunologic testing, are invasive, costly, and may disrupt implantation by failing to capture dynamic molecular changes during the window of implantation (WOI) (8). Clinical approaches like histological dating, ultrastructural analysis, and serum hormonal assessments offer limited predictive accuracy due to weak endometrial correlation or the need for invasive sampling, impractical during IVF cycles. Variability in receptivity due to hormonal and individual factors often requires multiple biopsies, increasing risks of pain, bleeding, infection, and endometrial damage that may impair future pregnancies (9).

As a minimally invasive alternative, liquid biopsy—sampling molecular signals from biofluids such as uterine fluid (UF), spent embryo culture medium (SCM), peripheral blood, cervicovaginal fluid (CVF), and even seminal plasma—has gained significant traction. These biofluids carry cell-free DNA (cfDNA), microRNAs (miRNAs), proteins, metabolites, and extracellular vesicles (EVs)—cargo that may reflect both embryo competence and endometrial receptivity (10-12).

This narrative review consolidates current research on liquid-biopsy approaches for screening recurrent implantation failure (RIF) in IVF patients. It examines the pathophysiological mechanisms of implantation, evaluates key biofluids and analytes investigated, assesses analytical methodologies, proposes a structured screening framework, and identifies critical priorities for future research.

Pathophysiological Background: Implantation Biology and Liquid-Biopsy Targets

Multi-factorial Nature of Implantation Failure: Successful implantation requires synergy among embryo quality, a receptive endometrium during a narrow WOI, immune-endocrine modulation, and dynamic crosstalk mediated by paracrine factors (9, 12, 13). RIF may arise from diverse causes—aneuploid embryos, luteal insufficiency, endometrial inflammation, structural anomalies, immunologic dysfunction, or dysregulated molecular signaling (1, 14, 15). Traditional diagnostics often isolate a single factor, missing the holistic complexity of the implantation environment.

MicroRNAs and EV-Mediated Signaling: MicroRNAs (miRNAs) are short noncoding RNAs (19-22 nucleotides) that regulate gene expression post-transcriptionally by targeting messenger RNAs for degradation or translational repression (16, 17). They arrange critical pathways in decidualization, immune tolerance, adhesion, and angiogenesis, all essential for successful embryo implantation (18-20). These miRNAs are frequently encapsulated within extracellular vesicles (EVs)—membrane-bound nanoparticles released by endometrial and embryonic cells that facilitate intercellular cargo delivery across tissues (14-16, 21-23). EVs play a substantial role in mediating embryo-endometrium communication, with their molecular cargo, including miRNAs, proteins, and lipids, dynamically regulated throughout the menstrual cycle and altered in pathological conditions such as recurrent implantation failure (17-19, 24-26). Analyzing miRNAs and EV content in biofluids, such as uterine fluid or peripheral blood, provides a sensitive, non-invasive approach to assess the implantation microenvironment and identify biomarkers for optimizing IVF outcomes (27-29).

cfDNA as a Marker of Embryo Competence: In IVF, blastocyst-stage embryos release cell-free DNA (cfDNA) into spent embryo culture media (SECM), enabling noninvasive preimplantation genetic testing for aneuploidy (niPGT-A) as an alternative to trophectoderm biopsy (30). This approach screens embryo ploidy without damage, aiding selection for successful implantation. However, low cfDNA levels, maternal contamination, and mosaicism reduce reliability, often causing false negatives. DECENT, a deep learning method, mitigates these issues by using sequence and methylation data with convolutional networks and attention mechanisms to separate embryonic from maternal cfDNA. It accurately quantifies contamination and reconstructs aneuploidies, matching invasive PGT-A results even in samples with >80% maternal DNA. These advances enhance niPGT-A's precision, supporting its clinical potential (31).

Liquid-Biopsy Sources: Mechanisms, Evidence, and Clinical Relevance

Uterine Fluid (UF)

Rationale and Sampling Strategy: UF—a collection of endometrial secretions during the WOI—can be obtained via minimally invasive aspiration or low-volume lavage (6, 30). This fluid contains EVs, proteins, lipids, and cell-free nucleic acids reflecting the endometrial secretome and

immune environment. Sampling timed to LH+7 or post-progesterone+5 captures the window of maximum receptivity.

miRNA Profiles in UF: Pioneering studies (e.g., Von Grothusen et al., 2022) demonstrated dysregulated UF miRNAs in RIF patients compared to fertile controls, implicating regulators of adhesion, immune modulation, and HOXA pathways (31). Salmasi et al. validated miRNA panels from UF that differentiate nonreceptive endometrium, uncovering potential biomarker candidates (31). Earlier mechanistic insights showed miR-30d's role in maternal-fetal communication and its upregulation in receptive endometrium (32).

These panels achieved predictive AUCs ranging from ~0.75 to 0.9, with independent internal validation. However, multicenter validation remains pending.

EV Cargo and Proteomic Content: Liu et al. showed that EVs from endometrial cells of RIF patients attenuated embryo development and invasion in murine co-culture models, suggesting functional deficits in EV cargo (33). Cannon et al. profiled small RNAs within UF EVs, correlating cargo profiles with implantation success (34). Proteomic analyses from Rai et al. revealed cyclical dynamics in EV proteins linked to implantation pathways (35).

A 2025 multi-omics UF EV study integrated miRNAs, proteins, and transcript data to identify biomarkers aligned with receptive gene expression networks (9). Critically, the EV cargo paralleled known receptive endometrial signatures (e.g., pinopode development, integrins), reinforcing their diagnostic relevance.

Analytical and Pre-analytical Requirements: Using UF for screening necessitates standardization of sampling timing, catheter type, contamination thresholds (e.g., free hemoglobin measurement), EV isolation methods (e.g., size-exclusion chromatography), spike-in controls, and QC metrics (particle count, RNA yield) (6, 36). Prospective evaluation of UF miRNA/EV screening during a mock cycle—linked to changes in management (e.g., deferring FET, treating endometritis)—remains the primary next step.

Spent Embryo Culture Medium (SCM) for niPGT-A

Feasibility and Early Performance Metrics: The presence of cfDNA in SCM allowed initial proof-of-concept niPGT-A studies. A Shitara et al. prospective study demonstrated moderate concordance between SCM-derived cfDNA and trophectoderm biopsy, prompting optimism (37). Singh et al., in a prospective cohort of 44 embryos (with TE biopsy and SCM sampling), reported concordant aneuploidy calls in ~65% of embryos; per-chromosome concordance was over 85%, but overall sensitivity/specificity hovered at ~66.6% and 60%, respectively (1). These figures underline current limitations but also provide a quantifiable benchmark.

Technical Challenges and Advancements: Fears of contamination by maternal cumulus cells or external DNA sources remain. Lledoa et al. reviewed these analytical inconsistencies and called for standardization of embryo handling and wash protocols (38). Computational approaches, such as DNA methylation-based decontamination algorithms (Chen et al., 2025), promise improved discrimination of embryonic cfDNA from contaminants (39). niPGT-A performance may improve with combined sampling of SCM and blastocoel fluid, achieving higher template yields and lower noise (40).

Role in RIF Screening: In RIF patients unwilling or unable to undergo biopsy-based PGT-A, niPGT-A could inform embryonic competency. Yet, given current limitations, using niPGT-A as a "screen" (not a definitive diagnostic) with confirmatory testing for abnormalities is prudent. The predictive value of niPGT-A in isolation for reducing RIF remains unproven and requires randomized, non-selection trials.

Peripheral Blood (Plasma/Serum)

Biological Rationale: Endometrial and systemic miRNAs circulate in exosomes within blood; these miRNAs regulate immune tolerance (e.g., miR-155) and decidualization (e.g., miR-30 family) and may signal broader implantation-related dysfunction (41). However, blood-based biomarkers are diluted and influenced by distant tissues, reducing specificity.

Existing Evidence: Limited studies report differential expression of exosomal miRNAs in RIF vs fertile controls; however, sample sizes are small and findings inconsistently validated. The systemic nature of blood-borne signals introduces noise; more research is needed to define robust blood-based markers of implantation failure (8).

Cervicovaginal Fluid (CVF)

Advantages and Rationale: CVF is readily accessible, allows for serial, home-based sampling, and captures transudates of uterine secretions. EVs and miRNAs in CVF may indicate endometrial state. However, contamination (e.g., from microbiota, external environment) complicates interpretation.

Current Status: Exploratory studies note cyclic changes in miRNA and protein composition in CVF across the luteal phase (42). Still, direct application to RIF screening remains minimal, and specificity compared to UF-based assays is uncertain.

Seminal Plasma

Although less directly relevant to female endometrial receptivity, seminal EVs may influence decidual immune priming and implantation via paternal signaling. Data are highly preliminary and not yet translated into RIF screening protocols (43).

Molecular Platforms and Analytical Methodologies

cfDNA Analysis in SCM: High-sensitivity low-input methods—including WGA, molecular barcodes, and digital PCR—are essential for niPGT-A. Analytical strategies must address allelic dropout, mosaicism, and contamination. Computational deconvolution and methylation-based filtering may help improve accuracy (8, 39, 40).

miRNA Profiling: Sequencing and qPCR: Small RNA-seq offers comprehensive miRNA profiling, while targeted qPCR panels (based on discovery studies) deliver cost-effective, clinically translatable assays. Proper normalization (e.g., spike-in controls, housekeeping miRNAs) ensures reproducibility (31, 32).

EV Isolation and QC: EV isolation methods differ in yield, purity, and reproducibility. Techniques include ultracentrifugation, size-exclusion chromatography, polymer-based precipitation, and immunoaffinity. Standardization is critical, using MISEV guidelines—quantifying particle number, size, morphology (e.g., TEM), and marker proteins, with spike-in controls (35, 36).

Proteomic and Metabolic Pathway Profiling: UF proteomics (e.g., using mass spectrometry) reveal dynamic secretome changes; metabolites such as lipid mediators may also signal receptivity shifts. Multi-omic integration (miRNA + proteins + metabolites) promises enhanced predictive power but increases complexity and cost (37).

Proposed Clinical Screening Model for RIF

A structured multi-step screening algorithm can integrate liquid biopsies pragmatically:

1. Baseline Evaluation: Collect standard risk indicators—maternal age, ovarian reserve markers, uterine imaging, immunologic panels—to stratify pretest probability and rule out mechanical or endocrine etiologies (13, 14).

2. Endometrial Receptivity Screening (UF-Based): During a mock or preparatory cycle, perform UF sampling (LH+7/progesterone+5), analyze miRNA/EV panel. If results indicate non-receptivity:

- Delay embryo transfer to allow therapeutic optimization (e.g., antibiotics for endometritis, progesterone augmentation, aspirin, immune modulators).
- Consider molecularly-informed window-of-implantation adjustment pending validation.

3. Embryo Competence Screening (niPGT-A Using SCM): When biopsy is not feasible, collect SCM for cfDNA analysis. Interpret with caution; for suspected aneuploidy, consider confirmatory testing or prioritize concordant euploid embryo transfers.

4. Adjunctive Biomarkers (Research Context): For refractory or idiopathic cases:

- Explore peripheral exosomal miRNA panels.
- Perform CVF longitudinal sampling.
- Consider seminal EV profiling for couple-based evaluation.

Analytical and Operational Standardization Requirements

Successful translation of liquid-biopsy screening hinges on rigorous standardization:

- ***Timing and hormonal context:*** Precise cycle timing for UF collection.
- ***Sampling protocols:*** Define catheter selection, volume, aspiration vs lavage, contamination thresholds.
- ***Pre-analytic processing:*** Stabilization (e.g., RNA preservatives), temperature control, freeze-thaw minimization.
- ***EV isolation and QC:*** Utilize validated protocols with particle quantification and purity assessment; spike-in controls essential.
- ***Analytical pipelines:*** Specify library preparation, read depth, normalization methods, and reporting thresholds.
- ***Validation strategy:*** Cross-validation, external cohort testing, calibration curves, decision curve analysis.
- ***Outcomes:*** Use live-birth as the primary endpoint, not biochemical or clinical pregnancy alone.
- ***Reporting standards:*** Employ STARD, MISEV, MIFlowCyt-EV, and FAIR principles for data sharing and reproducibility.

Limitations, Risks, and Ethical Considerations

- ***Heterogeneous RIF Definitions:*** Variable criteria for RIF across studies hamper comparability and meta-analysis; standardized definitions (e.g., ESHRE consensus) are needed (1, 4).
- ***Small, Single-Center Cohorts:*** Many studies

derive from limited samples and lack external validation, risking overfitting.
- ***Pre-analytic Variability:*** Timing, sampling technique, and commingled contamination may confound biomarker signals.
- ***False Positives/Negatives:*** Screening assays carry risk of misclassification; misinterpretation may lead to unnecessary delay or intervention.
- ***Causality vs Correlation:*** Biomarker changes may not cause implantation failure—they might reflect downstream or co-occurring processes.
- ***Patient Counseling:*** Communicate uncertainties, test limitations, and investigational status clearly to patients.
- ***Access and Equity:*** High-cost multi-omic workflows may exacerbate disparities in reproductive care.

Future Research Priorities

1. Large, multi-center prospective trials: testing UF-based screening and management changes versus standard care, with live birth as primary outcome.

2. Subtype-based interventions: Using molecular profiling to guide therapies (e.g., immunomodulation, tailored luteal support) in distinct endometrial dysfunction classes.

3. Enhanced niPGT-A methods: Evaluate algorithmic decontamination, blastocoel fluid integration, and improved concordance against PGT-A benchmarks.

4. Home-based sampling trials: Test feasibility and validity of CVF or minimally invasive UF sampling across cycles.

5. Biorepositories and registries: Standardize metadata, preanalytic variables, and outcomes to facilitate pooled analyses.

6. Cost-effectiveness modeling: Beyond clinical efficacy, evaluate economic impacts and health-system integration of liquid-biopsy screening strategies.

7. Ethical and policy frameworks: Develop guidelines around use of unproven add-ons, particularly in sensitive reproductive settings.

Conclusion

Liquid-biopsy methodologies—especially uterine fluid-derived miRNA and EV profiling—offer a compelling, minimally invasive avenue for screening endometrial receptivity in recurrent implantation failure. Noninvasive PGT-A using SCM cfDNA shows potential for embryo competence assessment but is limited currently by analytical constraints. Peripheral biofluids and seminal plasma remain exploratory. Achieving clinical relevance requires robust standardization, multicenter validation, prospective outcome data emphasizing live birth, and transparent patient-centered implementation. Until substantive evidence emerges, these technologies should be applied cautiously within research frameworks, supported by rigorous counseling and ethical oversight.

Conflict of Interests

Authors declare no conflict of interests.

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