

REVIEW

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Multi-omics biomarkers in female fertility: from oocyte quality to endometrial receptivity and clinical translation

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Abstract

High-throughput omics technologies have transformed our understanding of female reproductive biology, revealing molecular interactions governing oocyte maturation, ovarian reserve, endometrial receptivity, and embryo development, yet most findings remain fragmented and insufficiently translated into clinical practice. This review synthesizes multi-omics biomarker discoveries across the reproductive continuum, organizing evidence around three clinically accessible compartments: the follicular unit (follicular fluid, granulosa/cumulus cells), the endometrium (endometrial tissue, uterine fluid), and the embryo (spent culture media). For each compartment, we first characterize single-layer signatures: transcriptomic markers of oocyte developmental competence, metabolic profiles reflecting follicular health, immune and decidualization programs defining endometrial receptivity, and embryo secretomic footprints. We then examine how integrative analytical frameworks and computational modeling enhance prediction accuracy beyond individual modalities, enable mechanistic patient stratification, and reveal coordination failures invisible to single-omics approaches. Critical evaluation of receptivity testing illustrates that distinguishing timing misalignment from intrinsic endometrial dysfunction requires multi-layer endotyping beyond transcriptomics alone, while embryo selection benefits from combining metabolic efficiency readouts with morphokinetic parameters and stress response signatures. The review addresses translational challenges including workflow constraints, lack of standardization, limited external validation, and cost-effectiveness issues, positioning multi-omics biomarkers within clinically defensible scenarios, such as recurrent implantation failure with euploid embryos and embryo selection under discordant morphological signals, where integration may add actionable value. Future directions include precision-guided treatment strategies, proactive fertility monitoring, and AI-driven decision support, with multi-omics biomarkers representing the molecular foundation for transforming reproductive medicine from reactive infertility treatment to proactive fertility management.

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Keywords Multi-omics integration, Reproductive biomarkers, Oocyte developmental competence, Endometrial receptivity, Embryo assessment, Recurrent implantation failure, Precision reproductive medicine, Clinical translation

Introduction

Infertility affects approximately one in six people of reproductive age globally (lifetime prevalence 17.5%; period prevalence 12.6%), according to the World Health Organization's 2025 guideline [1]. Female reproductive biology spans oogenesis, folliculogenesis, ovarian function, and endometrial preparation for implantation, all of which present challenges for biomarker development [2–4]. Traditional approaches to evaluate female fertility rely primarily on hormonal measurements (anti-Müllerian hormone [AMH], follicle-stimulating hormone [FSH], luteinizing hormone [LH]), ultrasound-based assessments (antral follicle count [AFC], endometrial thickness), and limited molecular functional assays (e.g., endometrial receptivity array [ERA]-based receptivity testing) [2, 5]. However, these conventional biomarkers often fail to capture the intricate molecular processes that determine reproductive success.

The advent of high-throughput multi-omics technologies has changed our ability to study the molecular landscape of female reproduction at high resolution [2, 6]. Single-cell RNA sequencing (scRNA-seq) has revealed the cellular heterogeneity within ovarian follicles and endometrial tissue, while spatial transcriptomics has provided insights into tissue architecture and cell-cell communications [7–10]. Proteomics and metabolomics approaches have identified functional biomarkers in follicular fluid, endometrial tissue, and embryo culture media [11–14]. These advances have enabled discovery of biomarkers that reflect how genetic, epigenetic, and environmental factors jointly influence fertility.

Single-modality biomarkers often miss the biological complexity of fertility phenotypes because these phenotypes arise from coordinated molecular programs across multiple regulatory layers. Multi-omics integration helps identify cross-layer programs and reduces reliance on any single noisy measurement [15]. Multi-omics biomarkers hold significant potential for improving oocyte developmental competence assessment, personalized ovarian stimulation, endometrial receptivity mapping, and non-invasive embryo evaluation [5, 11–13, 16–18]. Translating these discoveries into clinical practice remains challenging because of gaps in protocol standardization, population-level validation, regulatory pathways, and cost-effectiveness [2]. Integrating multi-dimensional omics data also requires advanced computational approaches to extract clinically useful insights from high-dimensional datasets [19–22].

This review synthesizes multi-omics evidence across the follicular microenvironment, endometrial endotypes,

and embryo secretomics to frame a clinically oriented perspective on female fertility assessment. We distinguish between single-layer biomarker discovery and true multi-omics integration, emphasizing where integration adds clinical value beyond individual modalities. We critically evaluate standardization gaps, validation requirements, and translational bottlenecks that determine whether multi-omics biomarkers can transition from research tools to clinical decision-support systems. We organize evidence around three actionable dimensions: the capacity of integrated profiling to improve oocyte and embryo selection beyond morphology and ploidy testing; the ability of multi-layer endometrial assessment to differentiate timing misalignment from intrinsic dysfunction in recurrent implantation failure; and the feasibility of integration strategies given current assisted reproductive technology workflow constraints. (Fig. 1)

Methods

This article is a narrative review informed by targeted literature searches. We searched PubMed/MEDLINE, Web of Science, and Embase for English-language studies on female fertility biomarkers and multi-omics integration (last updated January 2026), using phenotype terms (oocyte competence, ovarian reserve, endometrial receptivity, RIF, RPL/recurrent miscarriage) combined with omics/integration keywords (transcriptomics, epigenomics, proteomics, metabolomics, microbiome, extracellular vesicles, single-cell/spatial profiling, machine learning, MOFA, DIABLO), supplemented by reference-list screening of key reviews and seminal studies. The scope was defined around three clinically accessible compartments: the follicular unit (follicular fluid, granulosa/cumulus cells), the endometrium (endometrial biopsy, uterine fluid), and the embryo (spent culture media); fallopian tube biomarkers were not a primary focus due to limited standardized minimally invasive sampling outside surgical settings. Evidence was synthesized thematically, prioritizing clinically annotated human cohorts and studies with external validation and/or prospective evaluation where available; mechanistic mammalian studies were cited selectively to support biological plausibility. Given substantial heterogeneity in designs, platforms, and endpoints, no quantitative meta-analysis was performed.

Multi-omics technologies for female fertility biomarkers

The following sections survey key omics platforms and computational approaches applied to female fertility biomarker research.

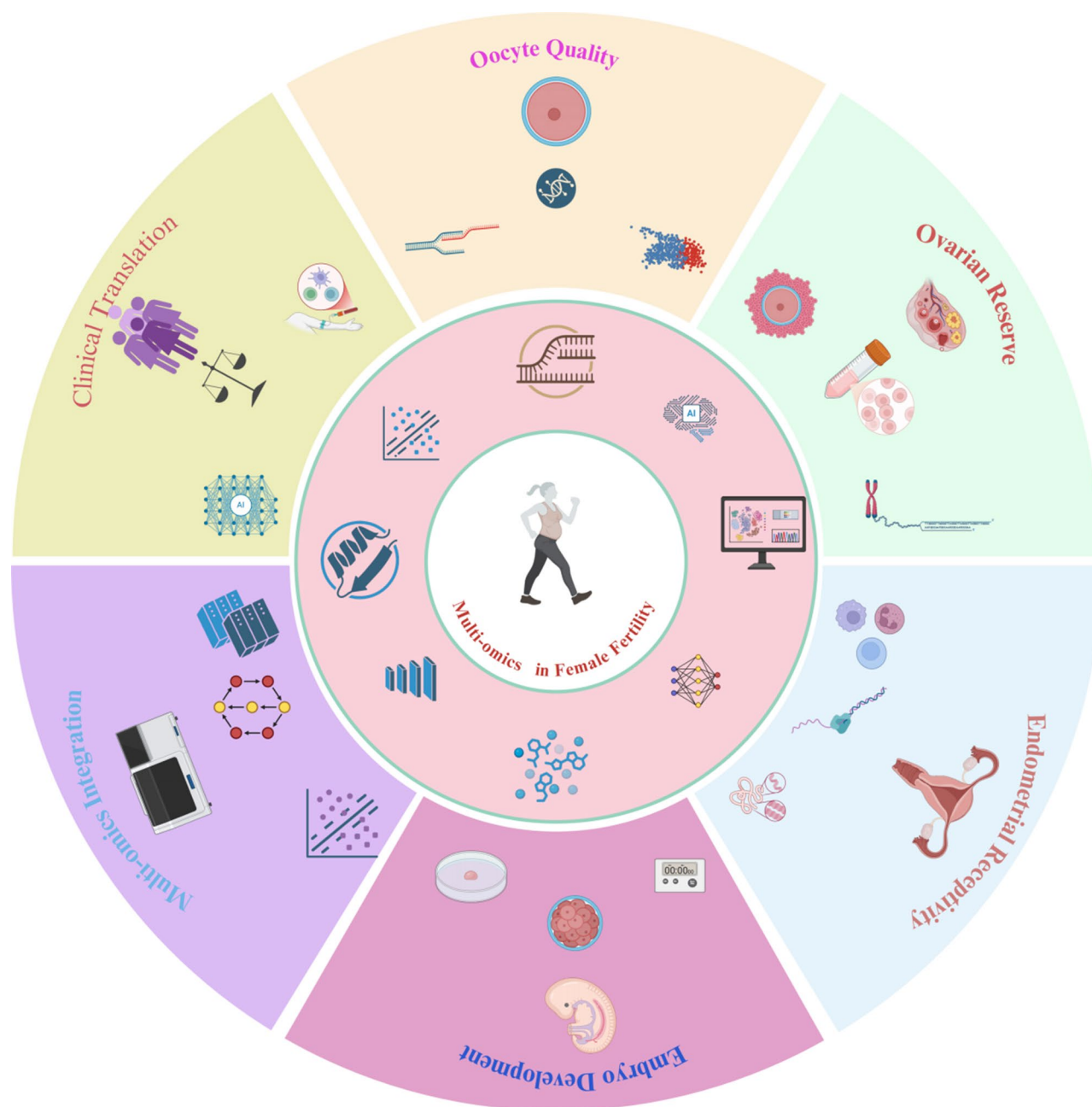


Fig. 1 Multi-omics biomarker landscape in female fertility. Comprehensive overview of multi-omics biomarker discovery and clinical translation across the female reproductive continuum. The figure integrates key stages from oocyte development, follicular maturation, and endometrial receptivity to embryo implantation, illustrating how complementary omics technologies (genomics, transcriptomics, proteomics, metabolomics, epigenomics) generate molecular profiles at each reproductive stage. Computational integration and machine learning algorithms identify robust biomarker signatures for clinical applications including oocyte quality assessment, ovarian reserve evaluation, endometrial receptivity testing, and embryo selection. The translational pathway from discovery through validation to clinical implementation enables precision reproductive medicine through individualized diagnosis, treatment optimization, and proactive fertility monitoring. (created by <https://BioRender.com>)

Single-cell omics technologies

Single-cell RNA sequencing (scRNA-seq) has become a key technology for studying female reproductive tissues, overcoming the fundamental limitation of bulk analysis that masks cellular heterogeneity [23, 24]. The cellular diversity within ovarian follicles, including oocytes,

granulosa cells, cumulus cells, and theca cells, makes single-cell approaches particularly valuable for identifying cell-type-specific biomarkers and understanding developmental trajectories [23, 25]. Recent technological advances have expanded beyond transcriptomics to include single-cell proteomics through technologies

such as CyTOF (Cytometry by Time-Of-Flight) and targeted antibody panels, enabling simultaneous measurement of dozens of proteins in individual cells [7, 26]. These approaches have proven particularly valuable for characterizing immune cell populations in reproductive tissues, including uterine natural killer cells and macrophages that are important for endometrial receptivity and implantation [27–30].

Beyond antibody-based single-cell proteomics (e.g., CyTOF), ultra-low-input mass spectrometry is beginning to approach true single-cell and even single-oocyte depth [31]. For example, a one-step vial-based pretreatment strategy (SOViP) enabled deep, label-free proteome profiling of individual mouse oocytes, quantifying > 6,500 protein groups on average and enabling high-resolution mapping of oocyte aging programs [32]. While clinical translation to human oocytes remains constrained by material scarcity and ethical considerations, these workflows provide a practical blueprint for single-oocyte proteomics and motivate multi-omics integration at the single-cell level in fertility research.

Single-cell epigenomics represents another frontier, encompassing single-cell assay for transposase-accessible chromatin using sequencing (scATAC-seq) for chromatin accessibility analysis and single-cell bisulfite sequencing for DNA methylation profiling [33–35]. These technologies have revealed epigenetic heterogeneity within follicular cell populations and identified regulatory elements controlling cell-type-specific gene expression programs [36, 37]. The integration of single-cell transcriptomic and epigenomic data enables reconstruction of gene regulatory networks underlying reproductive processes and gives insight into the molecular mechanisms controlling fertility.

Spatial omics technologies

Spatial transcriptomics preserves tissue architecture while providing transcriptional information at high resolution [8, 38]. This approach has been especially useful for studying endometrial receptivity, where spatial organization is critical for function. Spatial approaches have revealed complex cellular interactions between epithelial cells, stromal cells, and immune cells during the implantation window, identifying spatially restricted gene expression patterns that traditional bulk approaches cannot detect [8, 39]. Advanced spatial transcriptomics platforms achieve near-cellular resolution while maintaining spatial context, enabling mapping of gene expression gradients within tissues and identification of cellular communication patterns through ligand-receptor analysis [40–44].

The integration of spatial proteomics technologies, including imaging mass spectrometry and multiplexed immunofluorescence, complements spatial

transcriptomics by providing protein-level information with spatial context [45, 46]. These approaches have identified protein expression patterns within reproductive tissues that correlate with functional states and provide biomarkers for tissue health assessment [47, 48]. Combined analysis of spatial transcriptomics and proteomics data enables characterization of tissue microenvironments and identification of multi-modal biomarker signatures. [49].

Bulk omics technologies

Despite the advantages of single-cell and spatial approaches, bulk omics technologies continue to play essential roles in biomarker discovery and clinical validation [50, 51]. Bulk RNA sequencing provides cost-effective transcriptome-wide analysis and enables detection of lowly expressed genes that may be missed by single-cell approaches [52, 53]. Targeted gene-expression panels such as endometrial receptivity tests illustrate a translational pathway from discovery to assay development; however, evidence for routine add-on use remains mixed and appears highly dependent on patient selection and clinical context [54–56].

Proteomics approaches utilizing advanced mass spectrometry techniques enable simultaneous quantification of thousands of proteins, providing insights into biological pathways and molecular networks [57–60]. Targeted proteomics methods such as selected reaction monitoring (SRM) and parallel reaction monitoring (PRM) offer increased sensitivity and specificity for biomarker validation and clinical implementation [61, 62]. Metabolomics provides functional readouts of cellular processes and has proven particularly valuable for assessing oocyte and embryo metabolism through analysis of follicular fluid and culture media [11, 63–69].

Data integration and computational approaches

Advanced computational approaches, including machine learning algorithms and network-based analyses, are essential for extracting meaningful biological insights from high-dimensional multi-omics datasets [70–73].

Machine learning approaches, including random forests, support vector machines, and deep learning algorithms, have been successfully applied to multi-omics data for biomarker discovery and outcome prediction [74–76]. These methods can identify complex patterns and interactions that traditional statistical approaches might miss, particularly when dealing with non-linear relationships and high-dimensional data. Deep learning approaches have shown particular promise for analyzing spatial omics data and identifying tissue architecture patterns associated with fertility outcomes [77–81].

Network-based approaches enable reconstruction of molecular interaction networks from multi-omics data,

providing insights into biological pathways underlying reproductive processes [82]. These approaches can identify key regulatory nodes and pathways that serve as potential biomarkers or therapeutic targets. The integration of prior biological knowledge enhances the interpretability of multi-omics analyses and helps identify biologically meaningful patterns in complex datasets.

Quality control and standardization

Quality control and standardization determine the success of multi-omics biomarker development. Variations in sample collection, processing, and analysis protocols affect biomarker performance, so standardized operating procedures, reference materials, and proficiency testing programs are essential for reproducibility and clinical translation [83–86].

Sample quality assessment is particularly important for single-cell and spatial omics approaches, which are sensitive to tissue degradation and processing artifacts [87, 88]. Quality control metrics including cell viability, RNA integrity, and spatial resolution must be carefully monitored and reported. Batch effects arising from technical variations require appropriate correction methods to ensure reliable biomarker identification [89]. Data pre-processing and normalization procedures significantly impact downstream analyses, making standardized pipelines essential for generating reproducible results [89, 90].

Practical platform selection should match the clinical question, sample constraints, and intended output. Bulk transcriptomics/proteomics are efficient for cohort-scale signatures and assay development; single-cell and spatial methods are most valuable for resolving cellular heterogeneity (e.g., stromal-immune endotypes) and for generating mechanistic hypotheses that can later be distilled into targeted panels [91]. Metabolomics and proteomics are particularly attractive for non-invasive compartments (follicular fluid, uterine fluid, spent culture media), but require strict pre-analytics and contamination control [92]. For clinical translation, assays with standardized pipelines, interpretable outputs (risk scores or endotypes linked to interventions), and external validation are more likely to be adopted than discovery-oriented atlases that lack feasible clinical implementations [93]. With these technological foundations in place, the following sections examine clinically accessible biomarkers across the three compartments defined in our scope: the follicular unit, the endometrium, and the early embryo. For each compartment, we highlight where multi-omics evidence is most mature and where integration across layers may add clinical value. (Table 1, Fig. 2)

Oogenesis and oocyte competence biomarkers

Oocyte developmental competence is the main determinant of reproductive success and includes the molecular machinery required for fertilization, early embryonic development, and pregnancy establishment. The assessment of oocyte competence has traditionally relied on morphological criteria, but multi-omics approaches have revealed complex molecular signatures that provide far more accurate predictors of developmental potential. (Fig. 3)

Transcriptomic signatures of oocyte developmental competence

The oocyte transcriptome encodes both developmental history and future competence, reflecting the integrity of maternal programs that sustain early embryogenesis before zygotic genome activation [4, 94, 95]. A particularly coherent competence module is formed by maternal-effect genes that assemble into the subcortical maternal complex (SCMC), including NLRP5, OOEP, TLE6, and PADI6 [96, 97]. Beyond association, human genetic evidence supports mechanistic relevance: biallelic variants affecting SCMC components (notably OOEP and NLRP5) have been identified in infertile patients with recurrent preimplantation embryonic arrest, linking disruption of this cytoplasmic regulatory machinery to early developmental failure [98, 99]. In this context, SCMC-related signatures should be interpreted as coordinated programs governing oocyte-to-embryo continuity rather than as isolated markers.

Age-related transcriptomic remodeling intersects with pathways that preserve chromosomal fidelity during prolonged meiotic arrest. Comparative profiling of oocytes from younger versus advanced-age women has highlighted altered expression of genes involved in chromosome segregation, DNA repair, and oxidative stress response, alongside reduced expression of cohesin components (e.g., SMC1B, REC8) that maintain chromosome cohesion over time [100–103]. These changes converge with dysregulation of spindle assembly checkpoint and cell-cycle progression regulators, including CDC20, BUB1, and MAD2L1, which collectively buffer against segregation errors [4, 104–109]. The functional implication is a systems-level weakening of cohesion maintenance and checkpoint surveillance, providing a mechanistic bridge between transcriptomic shifts and the higher incidence of aneuploidy and reduced developmental competence observed with reproductive aging [110–112].

Metabolic biomarkers of oocyte competence

The metabolic profile of oocytes reflects their energetic status and developmental potential through the biochemical processes required to support fertilization and early

Table 1 Comparison of multi-omics technology platforms

Technology	Resolution	Sample Requirement	Key Advantages	Limitations	Recommended Reproductive Application
scRNA-seq	Single-cell	Fresh/frozen tissue, 1,000–10,000 cells	Cell-type-specific expression profiles; developmental trajectory reconstruction; rare cell detection	High cost per sample; low capture of rare transcripts; requires fresh tissue for optimal results	Follicular cell heterogeneity characterization; endometrial stromal–immune endotyping; discovery of cell-type-specific biomarkers
scATAC-seq	Single-cell	Fresh tissue, 1,000–5,000 cells	Maps chromatin accessibility landscape; identifies active regulatory elements and transcription factor motifs	High data sparsity; computational complexity; limited integration with protein-level data	Epigenetic regulation of oocyte maturation; chromatin remodeling during decidualization; regulatory network discovery
Single-cell proteomics (CyTOF/deep MS)	Single-cell	Fresh tissue; CyTOF: 0.5–1 M cells MS: single oocyte	CyTOF: simultaneous 40+ protein markers; Deep MS: ~7,000 proteins from single oocyte; unbiased discovery	CyTOF: limited to antibody panels, destructive; MS: requires specialized equipment, emerging technology	Immune cell phenotyping (uNK subsets, macrophage polarization); oocyte aging proteomics; cumulus cell signaling
Spatial transcriptomics	Near-cellular to cellular	Fresh-frozen tissue sections	Preserves tissue architecture; reveals spatial gene expression patterns and cell–cell communication	Resolution–throughput trade-offs; requires specialized equipment and computational pipelines	Endometrial receptivity mapping (epithelial–stromal crosstalk); implantation site characterization; follicular niche architecture
Imaging mass spectrometry	Subcellular to cellular	Fixed or frozen tissue sections	Label-free protein/lipid spatial detection; high spatial resolution; multiplexed molecular imaging	Expensive equipment; complex data analysis; limited molecular coverage vs. bulk approaches	Lipid metabolism in oocytes and follicular fluid; endometrial lipid profiling during implantation window
Multiplexed immuno-fluorescence	Subcellular	FFPE or frozen sections	Multiple markers simultaneously on standard microscopy platform; compatible with clinical archives	Limited by antibody availability and validation; signal crosstalk at high plex	Endometrial immune microenvironment; decidualization marker validation; clinical biomarker panel development
Bulk RNA-seq	Tissue-level	10–100 ng RNA	Cost-effective; comprehensive transcriptome coverage; well-established analytical pipelines	Averages across cell populations; loses cellular heterogeneity; less suitable for heterogeneous tissues	Cohort-scale receptivity signature development; cumulus cell gene panel validation; standardized clinical assay development
Proteomics (LC-MS/MS)	Tissue-level	1–50 µg protein	Direct functional readout; post-translational modification detection; dynamic range improvements	Variable protein stability; sample preparation complexity; dynamic range limitations in complex samples	Follicular fluid biomarker discovery; endometrial secretome profiling; embryo culture media protein analysis
Metabolomics (NMR/MS)	Tissue/fluid-level	50–200 µL fluid sample	Non-invasive sampling (FF, culture media, uterine fluid); functional metabolic readout; rapid turnaround potential	Metabolite identification challenges; matrix effects; batch variability; annotation databases incomplete	Non-invasive embryo viability assessment (spent media); follicular fluid metabolic profiling; oxidative stress monitoring
Targeted gene panels (e.g., ERA, beREADY, rsERT, Win-Test)	Gene-level	10–50 ng RNA	Cost-effective and rapid; clinically validated for specific applications; standardized protocols available	Limited scope (pre-defined gene sets); requires prior knowledge; may miss novel biomarkers	Endometrial receptivity timing (clinical WOI assessment); cumulus cell competence scoring; deployable clinical decision support

embryonic development [113, 114]. Glucose metabolism is central to oocyte energy production, and competent oocytes show efficient glucose uptake and utilization patterns [115–118]. The pentose phosphate pathway maintains NADPH levels and protects against oxidative stress, both of which are essential for oocyte health [119–121]. Biomarkers of glucose metabolism, including glucose uptake rates and lactate production, correlate strongly

with oocyte developmental competence and provide functional assessments of oocyte developmental competence [122–124]. Amino acid metabolism in oocytes involves both energy production and biosynthetic processes that are essential for protein synthesis and cellular function [125]. Specific amino acids serve distinct roles: glycine supports one-carbon metabolism and DNA synthesis, alanine

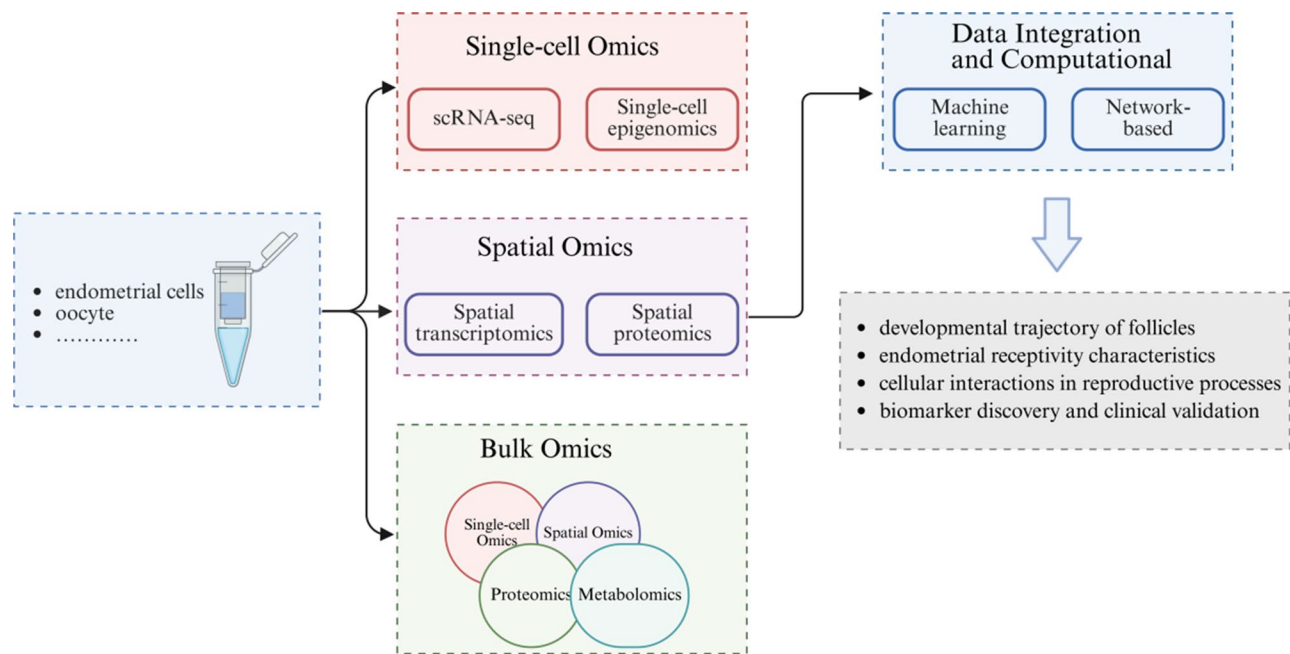


Fig. 2 Integrated multi-omics technology workflow for reproductive biomarker discovery. this figure illustrates the systematic integration of complementary omics approaches for fertility research. Three technology tiers are shown: single-cell omics (scRNA-seq, single-cell epigenomics), spatial omics (spatial transcriptomics, spatial proteomics), and bulk omics (proteomics, metabolomics). Reproductive samples including endometrial cells and oocytes undergo parallel profiling across these platforms. Data streams converge into computational integration using machine learning and network-based algorithms, yielding insights into developmental trajectories of follicles, endometrial receptivity characteristics, cellular interactions in reproductive processes, and robust biomarker signatures suitable for clinical validation. (created by <https://BioRender.com>)

provides metabolic substrates for energy production, and leucine regulates protein synthesis through mTOR signaling pathways [126–128]. The consumption patterns of amino acids during oocyte maturation reflect metabolic activity and developmental potential, with high-quality oocytes typically exhibiting balanced amino acid utilization patterns [129–131]. Poor-quality oocytes show altered consumption patterns and amino acid imbalances that correlate with reduced fertility outcomes [130–132].

Lipid metabolism contributes to oocyte energy storage, membrane function, and signaling processes needed for fertilization and early development [133, 134]. Competent oocytes accumulate lipid droplets containing triglycerides and cholesterol esters that serve as energy reserves during early embryonic development when external nutrient supply may be limited. Fatty acid composition, particularly the ratio of saturated to unsaturated fatty acids, influences oocyte membrane properties and developmental competence [135–137]. Biomarkers of lipid metabolism, including fatty acid profiles and lipid peroxidation products, correlate with oocyte developmental competence and pregnancy outcomes [138, 139].

Mitochondrial function is a key determinant of oocyte developmental competence because oocytes contain large numbers of mitochondria to support maturation, fertilization, and early development [140]. Mitochondrial DNA content, respiratory enzyme activities, and ATP

production capacity serve as biomarkers for oocyte energetic status [141–143]. Age-related mitochondrial dysfunction with decreased ATP production and increased reactive oxygen species contributes to oocyte competence decline [144–146].

Epigenetic biomarkers of oocyte developmental competence

Epigenetic modifications in oocytes are essential for embryonic development and genomic imprinting [147, 148]. DNA methylation patterns established during oocyte growth are essential for regulation of imprinted genes. Oocytes acquire methylation at key loci such as H19/IGF2, SNRPN, and KvDMR1 through DNMT3A and DNMT3L [149–152]. Age-related alterations at these loci serve as biomarkers for reproductive aging [153–156].

These DNA-level marks work together with histone modifications that undergo dynamic changes during maturation and fertilization [157–159]. Broad H3K4me3 domains mark developmentally important genes and reorganize during the maternal-to-zygotic transition, while H3K27me3 establishes repressive states maintained in early embryogenesis [160–163]. With aging, H3K4me3 breadth is lost and H3K27me3 patterns are altered, providing epigenetic biomarkers that complement DNA methylation signatures [164–166].

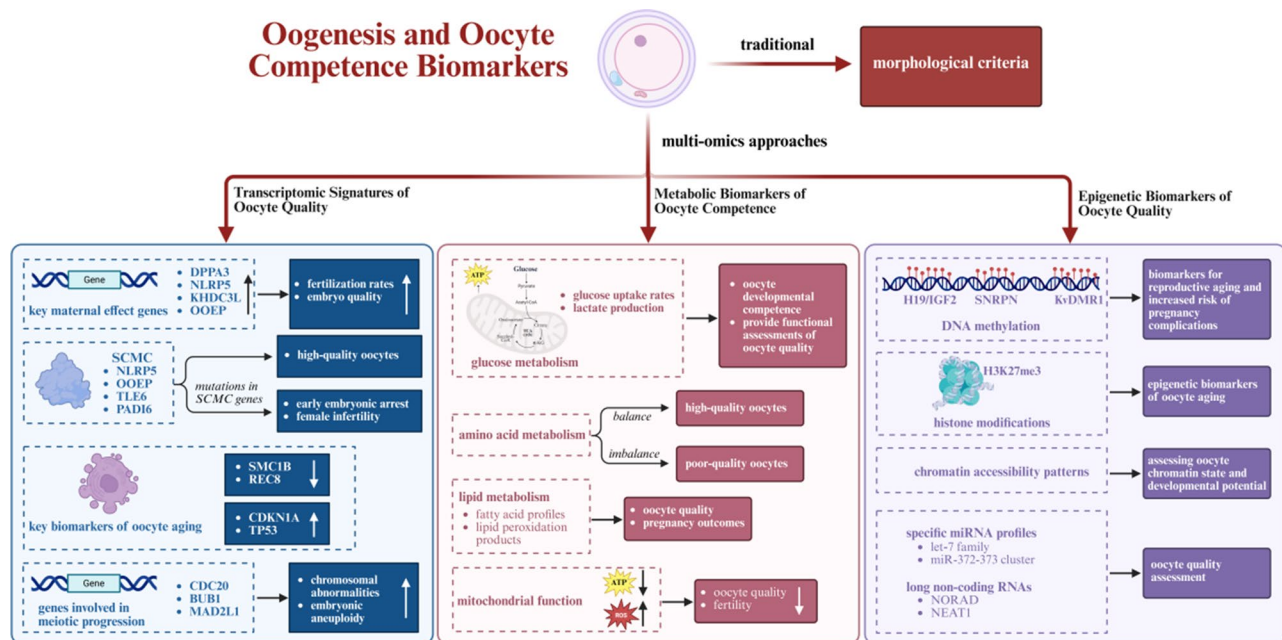


Fig. 3 Oogenesis and oocyte competence biomarkers: a multi-omics framework. this figure contrasts traditional morphological criteria with comprehensive multi-omics approaches for oocyte quality assessment across three molecular dimensions. Left panel: transcriptomic signatures include key maternal effect genes (DPPA3, NLRP5, OOEP), subcortical maternal complex components (PADI6, TLE6, FLOPED, NLRP5, NLRP2, KHDC3L, OOEP, TLE6), and meiotic progression markers (CDC20, BUB1, MAD2L1) that predict fertilization rates, high-quality oocytes, chromosomal abnormalities, and aneuploidy. Center panel: metabolic biomarkers encompass glucose metabolism (uptake rates, lactate production), amino acid metabolism (balance/imbalance), lipid metabolism (fatty acid profiles, peroxidation), and mitochondrial function, collectively determining oocyte quality and pregnancy outcomes. Right panel: epigenetic biomarkers include DNA methylation patterns linked to reproductive aging and pregnancy complications, histone modifications (H3K27ac, H3K4me3, H3K27me3), chromatin accessibility patterns reflecting developmental potential, and specific non-coding RNAs (miRNAs, NORAD, NEAT1) for quality assessment. (created by <https://BioRender.com>)

At a higher-order level, chromatin accessibility as assessed through ATAC-seq reflects the regulatory landscape of the oocyte-to-embryo transition [167–169]. Age-related changes in accessibility correlate with altered gene expression and reduced competence [37, 153]. Non-coding RNAs add further regulatory complexity: specific miRNA profiles (let-7 family, miR-372–373 cluster) correlate with oocyte competence and embryo potential [170–176], while long non-coding RNAs such as NORAD and NEAT1 regulate chromatin organization [177–180]. Dysregulation across any of these interconnected epigenetic layers, from DNA methylation through histone marks to chromatin architecture and non-coding RNA networks, serves as biomarkers for oocyte developmental competence, highlighting the value of multi-level epigenetic assessment over single-mark analysis [181–184] Table 2.

Follicular development and ovarian reserve biomarkers

The ovarian follicle is a complex biological system where multiple cell types interact to support oocyte development and determine reproductive potential. Understanding follicular development through multi-omics approaches has revealed complex molecular signatures

that reflect ovarian reserve, follicular health, and the aging process, providing clinically relevant biomarkers for fertility assessment and treatment optimization. (Fig. 4)

Granulosa cell heterogeneity and biomarkers

Single-cell analysis of human ovarian tissue has greatly expanded our understanding of follicular cell heterogeneity and development, revealing distinct cellular populations with specialized functions [185–187]. Granulosa cells, the somatic cells surrounding the oocyte, show wide transcriptional diversity depending on their spatial location within the follicle and developmental stage [188, 189]. This cellular heterogeneity has important implications for biomarker discovery and understanding of follicular function.

Mural granulosa cells, which line the follicular wall, demonstrate distinct molecular signatures compared to cumulus granulosa cells that directly surround the oocyte [188–190]. Mural granulosa cells express higher levels of steroidogenic enzymes including CYP11A1 and CYP19A1, as well as increased LH receptor (LHCGR) expression, reflecting their specialized role in hormone production and response to ovulatory signals [191–193]. In contrast, cumulus granulosa cells show elevated

Table 2 Key biomarkers of oocyte quality

Biomarker	Omics Type	Functional Role	Clinical Significance	Detection Method
DPPA3, ZAR1, KHDC3L, OOEP	Transcriptomics	Maternal effect genes essential for embryonic genome activation	Embryo developmental competence, fertilization success	scRNA-seq, RT-qPCR
SCMC genes (NLRP5, OOEP, TLE6, PADI6)	Transcriptomics	Subcortical maternal complex formation, early embryonic development	Female infertility, early embryonic arrest	RT-qPCR, IHC
CDKN1A, TP53, BRCA1, ATM	Transcriptomics	DNA damage response, cell cycle regulation	Oocyte aging biomarkers, reproductive lifespan	RNA-seq, qPCR
CDC20, BUB1, MAD2L1	Transcriptomics	Spindle assembly checkpoint, chromosome segregation	Chromosomal abnormalities, aneuploidy risk	scRNA-seq
Glucose uptake, pyruvate consumption	Metabolomics	Glycolysis and oxidative phosphorylation for energy production	Oocyte developmental competence, energy status	NIR spectroscopy, LC-MS
Amino acid turnover (asparagine, glutamine)	Metabolomics	Protein synthesis, energy metabolism, pH regulation	Oocyte quality assessment, developmental potential	HPLC, LC-MS
Lipid profiles, fatty acid oxidation	Metabolomics	Energy storage, membrane synthesis, signaling	Oocyte quality, pregnancy outcomes	LC-MS/MS
ATP content, mitochondrial DNA copy number	Metabolomics	Energy production, oxidative metabolism	Oocyte quality, fertility outcomes	Bioluminescence assay, qPCR
H19/IGF2 methylation, KvDMR1	Epigenetics	Genomic imprinting maintenance	Reproductive aging, pregnancy complications risk	Bisulfite-seq, Pyrosequencing
H3K27me3, H3K4me3, H3K9me3	Epigenetics	Chromatin remodeling during oocyte maturation	Epigenetic biomarkers of oocyte aging	ChIP-seq, CUT&RUN
Chromatin accessibility patterns	Epigenetics	Regulatory landscape controlling gene expression	Oocyte chromatin state, developmental potential	ATAC-seq, DNase-seq
miR-372/373, let-7 family, lncRNA NORAD	Epigenetics	Post-transcriptional regulation, maternal-zygotic transition	Oocyte quality assessment, implantation failure	Small RNA-seq, qRT-PCR

expression of genes involved in oocyte communication and matrix remodeling, including GJA1 (gap junction protein alpha 1), HAS2 (hyaluronan synthase 2), and PTGS2 (prostaglandin-endoperoxide synthase 2) [122, 194, 195]. These cell-type-specific differences serve as biomarkers for assessing follicular development and predicting treatment outcomes.

The balance between cell proliferation and apoptosis within the follicle is a key determinant of follicular fate and provides important biomarkers for follicular viability assessment [196–198]. Healthy follicles maintain a delicate balance of controlled cell proliferation with limited apoptotic activity, while atretic follicles show increased apoptotic signaling and reduced proliferation rates. Key biomarkers include proliferation markers such as PCNA and Ki67, anti-apoptotic genes including BCL2 and XIAP, and pro-apoptotic factors such as BAX and CASP3 [199–202]. The ratio between these opposing signals determines follicular survival versus atresia and serves as a powerful biomarker for follicular health assessment [203–205].

Granulosa cell responses to gonadotropin stimulation vary significantly with follicular development stage and oocyte developmental competence, providing valuable biomarkers for treatment optimization [206–209]. FSH receptor (FSHR) expression and downstream signaling pathways including cAMP and PKA show specific patterns in healthy versus atretic follicles. Granulosa cells

from high-quality follicles exhibit robust responses to FSH stimulation, while those from poor-quality follicles show blunted responses [210–213]. These hormone response signatures can help predict treatment outcomes and optimize stimulation protocols in assisted reproductive technologies.

Molecular signatures of ovarian aging

The concept of ovarian aging extends beyond chronological age to encompass molecular changes that directly affect follicular function and oocyte developmental competence [214, 215]. These age-related molecular alterations occur independently of chronological age and provide important biomarkers for assessing reproductive potential and predicting treatment success.

Granulosa cells from aged ovaries exhibit classic hallmarks of cellular senescence [185, 186, 216, 217]. Telomere length, a marker of replicative history, shows progressive shortening that correlates with reduced ovarian function, poor IVF outcomes, and increased pregnancy complications [187–191]. Telomerase activity, mediated by TERT expression, declines in an age-dependent manner, and the ratio of telomere length to telomerase activity provides a molecular measure of cellular aging that complements traditional ovarian reserve markers [192, 216].

This telomeric attrition does not happen alone. Aged granulosa cells simultaneously accumulate DNA damage,

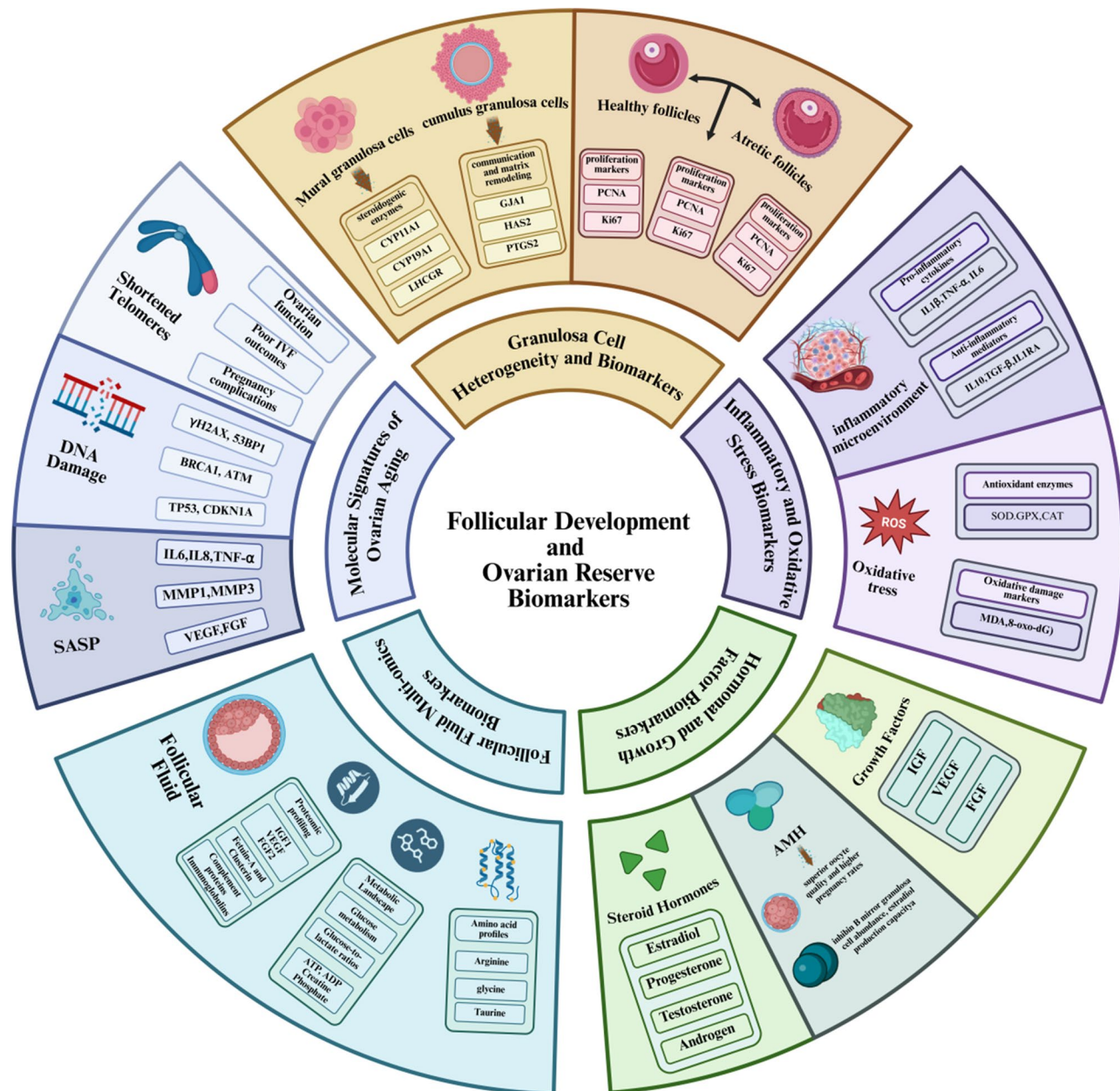


Fig. 4 Comprehensive biomarker landscape of follicular development and ovarian reserve. This circular diagram maps biomarkers across multiple dimensions of follicular biology and ovarian aging. The figure encompasses: (1) granulosa cell heterogeneity biomarkers distinguishing mural and cumulus granulosa cells in healthy versus atretic follicles, including proliferation markers (PCNA, Ki67, BAF), meiotic regulators (AURKA, AURKB, PLK1), steroidogenic enzymes (CYP11A1, CYP17A1, CYP19A1, HSD3B, HSD17B), and communication molecules (GJA1, PTGS2, HAS2); (2) steroidogenic and ovarian reserve markers featuring AMH, inhibin, and sex steroid hormones (estradiol, progesterone, testosterone, androgens); (3) oxidative stress indicators including anti-oxidant enzymes (SOD, GPX, CAT) and damage markers (MDA, 8-oxo-dG); (4) follicular fluid biomarkers spanning proteomic markers (albumin, transferrin, haptoglobin, complement proteins), metabolites (glucose, lactate, pyruvate, amino acids), and growth factors (VEGF, IGF, FGF); (5) molecular signatures of ovarian aging comprising DNA damage markers (TP53, CDKN1A, BRCA1, ATM), senescence-associated secretory phenotype (SASP) factors (IL6, IL8, TNF-α, MMPs), and telomere attrition indicators. (created by <https://BioRender.com>)

detectable through markers such as γ H2AX and 53BP1, while DNA repair capacity (reflected by BRCA1, ATM, and checkpoint proteins TP53 and CDKN1A) becomes progressively compromised [186, 193–199]. The convergence of shortened telomeres and unrepaired DNA lesions triggers the senescence-associated secretory

phenotype (SASP), through which granulosa cells secrete inflammatory cytokines (IL6, IL8, TNF-α), matrix metalloproteinases (MMP1, MMP3), and growth factors (VEGF, FGF2) [185, 200–204]. This creates a self-reinforcing pro-inflammatory microenvironment that further impairs follicular development and oocyte developmental

competence, linking cellular aging at the molecular level to clinically observable reproductive decline [185, 205].

Follicular fluid multi-omics biomarkers

Follicular fluid represents a unique and accessible source of biomarkers that reflects the complex interactions within the follicular microenvironment [11, 206, 207]. The specialized composition of follicular fluid results from selective transport across the blood-follicle barrier, local synthesis by follicular cells, and bidirectional exchange with the oocyte and surrounding cellular components [208–211]. This creates a rich repository of molecular information that can be analyzed using multiple omics approaches.

Proteomic profiling of follicular fluid has identified hundreds of proteins that correlate with follicular development stage and oocyte developmental competence [57, 207, 212]. Growth factors including IGF1, VEGF, and FGF2 promote follicular development and angiogenesis, with decreased levels associated with follicular atresia [213, 218–220]. Anti-inflammatory proteins such as fetuin-A and clusterin maintain follicular homeostasis and protect against oxidative stress [221, 222]. Complement proteins and immunoglobulins reflect immune surveillance mechanisms within the follicle [222, 223]. These proteomic signatures reflect the health status of the follicular environment and help predict oocyte developmental potential.

The metabolic landscape of follicular fluid provides functional insights into follicular physiology and oocyte-granulosa cell metabolic interactions [114, 224, 225]. Glucose metabolism within the follicle involves complex interactions between the oocyte, cumulus cells, and granulosa cells. Healthy follicles maintain optimal glucose-to-lactate ratios that support oocyte energy metabolism, while atretic follicles show altered metabolic profiles with reduced glucose consumption and increased lactate accumulation [226–228]. Energy metabolites including ATP, ADP, and creatine phosphate reflect the follicular energetic status and correlate with oocyte developmental competence [229, 230].

Amino acid profiles in follicular fluid reflect the complex interplay of protein synthesis, energy metabolism, and cellular signaling processes occurring within the follicle [231–234]. Specific amino acids serve distinct functional roles: arginine supports nitric oxide synthesis and vascular function, glycine acts as a neurotransmitter and osmolyte, and taurine provides antioxidant protection. Amino acid consumption and production patterns serve as biomarkers for follicular metabolic activity, and altered profiles correlate with poor oocyte developmental competence and reduced fertility outcomes. In practice, the translational utility of follicular-fluid profiling will hinge less on single-marker significance than on whether

cross-omic signatures remain stable under standardized pre-analytics and batch-aware normalization, enabling assay compression into reproducible panels compatible with routine ART workflows.

Hormonal and growth factor biomarkers

Follicular fluid contains a range of hormonal and growth factor biomarkers that provide localized insights into follicular physiology, complementing systemic endocrine assessments [235–238]. Classical steroid hormones including estradiol, progesterone, and testosterone display stage-dependent patterns reflecting steroidogenic activity within the follicle. The estradiol-to-progesterone ratio serves as a marker of granulosa cell luteinization status, while androgen levels indicate theca cell function and activity. Local hormone concentrations often show stronger correlations with oocyte competence and IVF outcomes compared to systemic hormone levels.

Anti-Müllerian hormone (AMH) has emerged as a key indicator of granulosa cell number and functional quality [239–242]. Elevated AMH levels in follicular fluid predict superior oocyte developmental competence and higher pregnancy rates, while low levels signal impaired follicular function. Similarly, inhibin B concentrations mirror granulosa cell abundance, estradiol production capacity, and oocyte developmental potential, providing complementary information about follicular health status [243–245].

Beyond hormonal regulation, a broad spectrum of growth factors and their binding proteins orchestrates follicular development through complex signaling networks [218, 246–248]. The insulin-like growth factor (IGF) system, including IGF1, IGF2, and IGF-binding proteins, facilitates oocyte maturation and granulosa cell function, with disruptions linked to reduced follicular competence. Vascular endothelial growth factor (VEGF) is important for angiogenesis and follicular vascularization, ensuring adequate nutrient and oxygen supply to the developing follicle. Fibroblast growth factors (FGFs) sustain granulosa cell proliferation and differentiation, integrating endocrine and paracrine signals into the precise regulation of follicular growth.

These locally regulated growth factors and hormones collectively underscore that the follicle functions as a partially autonomous endocrine microenvironment [249]. Local hormone concentrations in follicular fluid often correlate more strongly with oocyte competence and IVF outcomes than systemic endocrine measures, supporting the view that the follicular unit constitutes a partially autonomous endocrine microenvironment [206, 250, 251]. This observation aligns with intra-follicular endocrinology and intracrinology: the follicle is not a passive recipient of circulating signals, but actively shapes a compartmentalized steroid milieu through coordinated

theca-granulosa steroidogenesis and local enzymatic conversion [252–254]. As a result, follicular-fluid steroid concentrations and steroid ratios can diverge from serum values and may better approximate the hormonal context experienced by the oocyte-cumulus complex. From a multi-omics perspective, this “local endocrine state” can be captured at multiple levels, including (i) follicular-fluid steroidomics (e.g., estrogen/androgen balance as a proxy for aromatization capacity), (ii) granulosa/cumulus transcriptomic and proteomic signatures encoding steroidogenic and steroid-converting machinery, and (iii) metabolomic correlates of hormone-linked mitochondrial and redox programs associated with maturation competence [249, 252]. Of note, intra-follicular endocrine dysregulation is a plausible convergence layer for heterogeneous clinical phenotypes (e.g., endometriosis-, PCOS-, or age-associated follicular dysfunction), making it a rational target for integration rather than treating serum hormones as interchangeable proxies for the follicular milieu [255, 256].

Inflammatory and oxidative stress biomarkers

The inflammatory environment within follicles affects follicular development and oocyte developmental competence through complex immunomodulatory mechanisms [257, 258]. Healthy follicles maintain low-level inflammatory activity that supports normal physiological processes including ovulation, while excessive inflammation leads to follicular dysfunction and premature atresia [259–261].

Pro-inflammatory cytokines including IL1 β , TNF- α , and IL6 are elevated in follicles with poor oocyte developmental competence and reduced developmental potential [257, 262–264]. These cytokines activate inflammatory signaling pathways that can damage both oocytes and granulosa cells through oxidative stress and direct cytotoxic effects [265, 266]. The presence of high inflammatory cytokine levels correlates with poor IVF outcomes and reduced pregnancy rates [267]. On the other hand, anti-inflammatory mediators including IL10, TGF- β , and IL1RA balance out pro-inflammatory signals and maintain follicular homeostasis [268–270]. The ratio of pro-inflammatory to anti-inflammatory cytokines, particularly the IL6-to-IL10 ratio, serves as a biomarker for follicular inflammatory status [271, 272].

Oxidative stress is another factor that influences follicular development and ovarian aging [273]. Biomarkers of oxidative stress in follicular fluid include reactive oxygen species levels, antioxidant enzyme activities, and oxidative damage markers [274]. Antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT) reflect the follicular antioxidant defense capacity [275]. Oxidative damage markers such as malondialdehyde (MDA) and

8-oxo-deoxyguanosine (8-oxo-dG) indicate damage to lipids and DNA respectively [138]. The balance between pro-oxidant and antioxidant factors determines follicular fate and oocyte developmental competence, with excessive oxidative stress leading to lipid peroxidation, protein oxidation, and DNA damage in both oocytes and granulosa cells [276]. While follicular optimization shapes oocyte competence, successful reproduction equally depends on endometrial capacity to support implantation, a process governed by its own multi-layered molecular programs. (Table 3)

Endometrial receptivity and implantation biomarkers

Successful embryo implantation requires precise molecular coordination between a competent embryo and a receptive endometrium during a narrow temporal window. The endometrium undergoes profound, hormonally-regulated transformations to create the transient period of receptivity known as the “window of implantation,” during which complex molecular signatures determine the success or failure of reproductive outcomes (Fig. 5).

Molecular signatures of the implantation window

The molecular definition of the implantation window has been pioneered through comprehensive transcriptomic analysis of endometrial biopsies, leading to the development of clinically applicable diagnostic tools [55, 277, 278]. The endometrial receptivity array (ERA) was among the first clinically oriented transcriptomic tools designed to classify endometrial biopsies as pre-receptive, receptive, or post-receptive and to infer the window of implantation (WOI) [279]. The original microarray-era implementation is commonly described as a 238-gene signature, whereas later commercial iterations and annotation updates have reported slightly different gene counts (e.g., 248), reflecting platform updates and bioinformatics revisions rather than a fixed and immutable gene number. These differences in gene counts are primarily due to advancements in sequencing technologies, the incorporation of more comprehensive annotation systems, and optimization for clinical applicability in commercial platforms [277, 280].

Beyond gene-count differences and platform iterations, receptivity biology is shaped by intra-endometrial endocrinology (often conceptualized as intracrinology), which helps explain why “timing-correct” cycles can still fail. While systemic estrogen and progesterone provide cyclical cues, the endometrium modulates tissue-level hormone action through cell-type-specific receptor states, co-regulator programs, and local steroid-metabolizing/inactivating pathways that determine intracellular ligand exposure [281, 282]. This local processing creates

Table 3 Classification and clinical applications of follicular fluid biomarkers

Biomarker Category	Representative Biomarkers	Sample Source	Clinical Application	Predictive Value	Validation Status
Adhesion molecules	Integrin $\alpha v\beta 3$, $\alpha 4\beta 1$, VCAM1, ICAM1	Follicular fluid	Oocyte quality assessment, cumulus-oocyte interaction	Fertilization rate, embryo development	Research stage
Cytokines and growth factors	LIF, IL-11, CSF1, VEGF, FGF	Follicular fluid	Follicular development support, oocyte maturation	Embryo quality, IVF success	Research stage
Matrix metalloproteinases	MMP1, MMP3, MMP9, TIMP1	Follicular fluid	ECM remodeling during follicle rupture and ovulation	Ovulation success, oocyte release	Research stage
Anti-Müllerian hormone	AMH	Follicular fluid, serum	Granulosa cell number, functional quality indicator	Ovarian reserve, IVF response	Clinical use
Steroid hormones	Estradiol, Progesterone, Testosterone, Androgen	Follicular fluid	Follicular maturation status, oocyte competence	Oocyte quality, fertilization potential	Clinical use
Growth factors	IGF-1, IGF-2, IGFBP, TGF- β , BMP15, GDF9	Follicular fluid	Oocyte-granulosa cell communication, follicular development	Oocyte maturation, embryo quality	Research stage
Pro-inflammatory cytokines	IL-1 β , TNF- α , IL-6, IL-8, IL-18	Follicular fluid	Follicular inflammatory microenvironment assessment	Oocyte quality (negative correlation)	Research stage
Anti-inflammatory markers	IL-10, TGF- β , IL-4, M2 macrophage markers	Follicular fluid	Immunomodulation, tissue remodeling support	Favorable IVF outcomes	Research stage
Reactive oxygen species	ROS levels, oxidative damage markers	Follicular fluid	Oxidative stress status in follicular microenvironment	Oocyte quality (negative correlation)	Research stage
Antioxidant enzymes	SOD, GPX, CAT, Glutathione	Follicular fluid	Antioxidant defense capacity assessment	Oocyte quality (positive correlation)	Research stage
Oxidative damage markers	MDA, 8-oxo-dG, protein carbonyls, lipid peroxides	Follicular fluid	Cumulative oxidative damage indicators of aging	Ovarian aging, reduced fertility	Research stage
Amino acids	Glutamine, Glycine, Arginine, Taurine	Follicular fluid	Metabolic profiling of oocyte-granulosa interaction	Embryo developmental potential	Research stage
Lipids	Fatty acid profiles, Cholesterol, Lipid peroxidation	Follicular fluid	Energy metabolism, membrane synthesis assessment	Oocyte quality, pregnancy outcomes	Research stage
Energy metabolites	Glucose, Lactate, Pyruvate, ATP	Follicular fluid	Follicular energy status and metabolic activity	Oocyte maturation, embryo viability	Research stage

functionally meaningful heterogeneity across epithelial, stromal, and immune compartments and can yield endotypes characterized by progesterone-response attenuation, impaired decidualization trajectories, or persistent inflammatory signaling despite apparently appropriate cycle timing [8, 283, 284]. Methodologically, this layer can be interrogated by integrating hormone-response transcriptional programs, proteomic evidence of downstream effectors, and tissue or uterine-fluid metabolomic signatures capturing steroid-linked transformations. This framing of intracrinology shows that receptivity testing is not only a calendar problem, but also a local hormone-response and tissue-programming problem, and this is precisely the distinction where multi-omics endotyping may add value.

Interpretation of receptivity testing also benefits from distinguishing two biologically distinct causes of implantation failure: WOI displacement (timing) versus intrinsic endometrial dysfunction (disruption/quality) [55, 285, 286]. In a timing scenario, the endometrium may be capable of becoming receptive but is misaligned relative to progesterone exposure; adjusting progesterone duration or embryo transfer timing is a plausible intervention [287, 288]. In a disruption scenario, inflammatory activation, progesterone resistance, endometriosis/

adenomyosis-associated remodeling, chronic endometritis, fibrotic/vascular alterations, or aberrant stromal-immune crosstalk can prevent an appropriate receptive state even when timing is optimized; such dysfunction requires etiologic evaluation and targeted treatment rather than calendar-based timing adjustment [287, 289, 290].

Consistent with this distinction, clinical evidence for routine ERA-guided personalized embryo transfer is mixed. In a large multicenter, double-blind randomized clinical trial of single euploid frozen embryo transfer, live birth was 58.5% in the receptivity-timed group versus 61.9% with standard timing, a non-significant difference [5]. These findings argue against routine add-on use in unselected good-prognosis populations and highlight the importance of patient selection and pre-test probability of WOI displacement [5, 291]. By contrast, alternative transcriptomic receptivity approaches have been developed using different gene panels and analytical platforms, including RT-qPCR-based receptivity maps (e.g., ER Map/ER Grade), targeted allele counting by sequencing (e.g., beREADY), RNA-seq-based receptivity tests (e.g., rsERT), and transcriptomic Win-Test approaches [292–295].

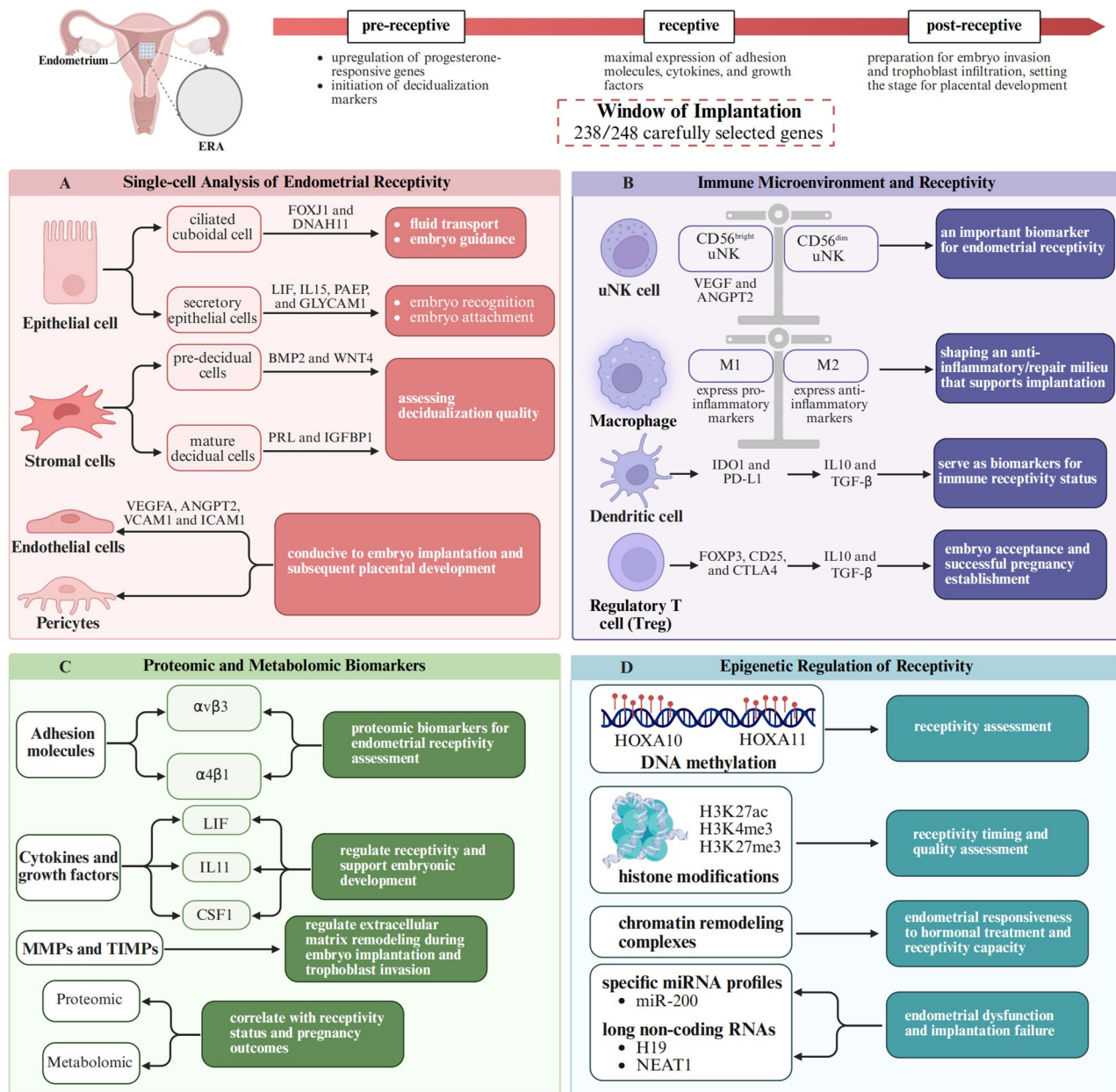


Fig. 5 Multi-layered molecular architecture of endometrial receptivity and implantation. this figure depicts the temporal and mechanistic complexity of endometrial receptivity across four regulatory dimensions. Top: timeline showing progression from pre-receptive (upregulation of proliferation-responsive genes, decidualization initiation) through receptive phase (window of implantation with 238/248 carefully selected genes, maximal expression of adhesion molecules, cytokines, growth factors) to post-receptive phase (preparation for embryo invasion and trophoblast infiltration). Panel A—Single-cell analysis reveals cellular heterogeneity: ciliated epithelial cells (FOXJ1, DNAH11) mediate fluid transport and embryo positioning; secretory epithelial cells (LIF, IL15, PAEP, GLYCAM1) enable embryo recognition and attachment; stromal cell subsets undergo decidualization (pre-decidual: BMP2, WNT4; mature decidual: PRL, IGFBP1); endothelial cells (VEGFA, ANGPT2, VCAM1, ICAM1) coordinate vascular remodeling; pericytes provide structural support. Panel B—immune microenvironment features: uNK cells (CD56^{bright}: VEGF, ANGPT2; CD56^{dim}: uNK) as key receptivity biomarkers; macrophage polarization (M1: pro-inflammatory vs. M2: anti-inflammatory) shaping implantation-permissive milieu; dendritic cells (IDO1, PD-L1, IL10, TGF-β) mediating immune tolerance; regulatory T cells (FOXP3, CD25, CTLA4) establishing embryo acceptance. Panel C—proteomic and metabolomic biomarkers include: adhesion molecules (αvβ3, α4β1) for receptivity assessment; cytokines and growth factors (LIF, IL11, CSF1) regulating embryonic development; matrix metalloproteinases and TIMPs controlling extracellular remodeling during trophoblast invasion; metabolomic profiles correlating with pregnancy outcomes. Panel D—epigenetic regulation encompasses: DNA methylation of HOXA10/HOXA11 for receptivity assessment; histone modifications (H3K27ac, H3K4me3, H3K27me3) determining receptivity timing and quality; chromatin remodeling complexes modulating hormonal responsiveness; endometrial responsiveness to hormonal treatment and receptivity capacity; specific miRNA profiles (miR-200) and long non-coding RNAs (H19, NEAT1) linked to endometrial dysfunction and implantation failure. (created by <https://BioRender.com>)

Table 4 Gene expression profiles of endometrial receptivity

Gene/Pathway	Expression Change	Function	Detection Method
PAEP, DPP4, APOD, IL2RB, IGFBP1	Upregulated	Secretory transformation markers defining receptive phase (ERA gene set)	RNA-seq, Microarray
LIF, IL-11, STAT3	Upregulated	Embryo adhesion, blastocyst attachment signaling	RT-qPCR, IHC
HOXA10, HOXA11	Upregulated	Endometrial receptivity master regulators	RT-qPCR, IHC
MMP2, MMP9, TIMP1, TIMP3	Dynamically regulated	Extracellular matrix remodeling, trophoblast invasion	ELISA, Zymography
Integrin $\alpha\beta 3$ (ITGAV, ITGB3)	Upregulated	Embryo attachment to endometrial epithelium	Flow cytometry, IHC
L-selectin ligands, Mucin-1	Regulated	Initial embryo recognition and tethering	IHC, Western blot
COX2 (PTGS2), Prostaglandins	Upregulated	Vascular permeability, decidualization signaling	ELISA, RT-qPCR
VEGFA, ANGPT2, FLT1	Upregulated	Angiogenesis supporting embryo implantation	ELISA, IHC
PRL, IGFBP1 (decidual markers)	Upregulated	Stromal decidualization, pregnancy maintenance	ELISA, IHC
Wnt signaling pathway	Activated	Endometrial proliferation and receptivity regulation	Pathway analysis

A clinically defensible pathway therefore requires standardizing pre-analytics (cycle type, progesterone route, biopsy timing/site), clarifying the clinical question (timing correction vs endotype diagnosis), and integrating receptivity readouts with complementary layers when disruption is suspected, such as immune profiling and endometrial/uterine fluid proteomics/metabolomics, so that non-receptive is interpreted within a multi-dimensional endometrial endotype framework rather than as a single timing label. These temporal patterns provide biomarkers for precise embryo transfer timing and optimization of treatment protocols (Table 4).

Single-cell analysis of endometrial receptivity

Single-cell RNA sequencing of human endometrium during the implantation window has revealed high-resolution cellular heterogeneity and identified distinct cell populations with specialized functional roles in supporting implantation [296, 297]. This technology has overcome the limitations of bulk tissue analysis and provided detailed insights into cell-type-specific contributions to endometrial receptivity, revealing rare cell populations and transitional states that were previously undetectable.

Epithelial cells in the receptive endometrium comprise luminal and glandular lineages with distinct roles in implantation [38]. The luminal epithelium mediates embryo apposition and adhesion and includes both ciliated and secretory states, whereas glandular epithelium supports receptivity by secreting histotroph and implantation-associated factors [38, 298]. Ciliated epithelial states express genes involved in ciliogenesis and fluid transport (e.g., FOXJ1, DNAH11), while secretory programs upregulate receptivity-associated mediators (e.g., LIF, IL15, PAEP, GLYCAM1) that support embryo recognition, immune modulation, and early attachment [298–301]. The abundance and state transitions of these epithelial populations vary across the menstrual cycle and can be perturbed in implantation failure [38, 298, 299].

Stromal cells undergo the most dramatic transformation during the transition to receptivity through the fundamental reprogramming process of decidualization [302]. Single-cell analysis has identified distinct decidual cell subtypes that represent different stages of this transformation process. Pre-decidual cells express early decidualization markers including BMP2 and WNT4, while mature decidual cells express classical markers such as PRL (prolactin) and IGFBP1 [8, 303–307]. The progression from pre-decidual to decidual states involves complex gene regulatory networks that can be disrupted in cases of implantation failure, providing biomarkers for assessing decidualization quality [308, 309].

Endothelial cells and pericytes contribute to the critical vascular changes necessary for successful implantation through coordinated angiogenesis and vascular remodeling [310, 311]. Single-cell analysis has revealed endothelial cell activation states characterized by expression of angiogenic factors including VEGFA and ANGPT2, as well as adhesion molecules such as VCAM1 and ICAM1 [8, 312–315]. Pericyte populations show heterogeneity in their contractile and secretory properties, with specific subtypes supporting vascular remodeling during implantation [316]. The coordination between endothelial cells and pericytes creates a vascular microenvironment that is conducive to embryo implantation and subsequent placental development.

Immune microenvironment and receptivity

The immune environment of the receptive endometrium must tolerate a semi-allogeneic embryo while maintaining protection against pathogens [317, 318]. This balance depends on coordinated changes across both innate and adaptive immune populations.

The first layer of immune regulation is established by innate immune cells. Uterine natural killer (uNK) cells are the most abundant immune cell type in the decidualized endometrium and contribute to vascular remodeling and immune regulation [319, 320]. Single-cell analysis has identified multiple uNK cell subsets: CD56^{bright} cells

exhibit strong cytokine production and support angiogenesis through VEGF and ANGPT2 secretion [321–325], while CD56^{dim} cells show enhanced cytotoxic potential but reduced cytokine production [326]. The balance between these subsets serves as a biomarker for endometrial receptivity, with altered ratios associated with recurrent implantation failure [327]. Complementing uNK activity, macrophages in the receptive endometrium display an overall M2 polarization skew, expressing anti-inflammatory markers (IL10, ARG1, MRC1) and promoting tissue repair and angiogenesis [328–331]. Dysregulated polarization, whether insufficient M2 skew or aberrant pro-inflammatory activation, is associated with recurrent implantation failure and pregnancy complications [332].

Beyond innate immunity, adaptive immune mechanisms also support tolerance. Dendritic cells adopt a tolerogenic phenotype, expressing IDO1 and PD-L1 and producing IL10 and TGF- β , which promotes regulatory T cell differentiation and suppresses effector T cell responses [333–335]. Regulatory T cells (Tregs), expressing FOXP3, CD25, and CTLA4, are essential for embryo acceptance; their expansion during the implantation window is critical, and reduced Treg frequencies are associated with recurrent pregnancy loss and implantation failure [336–338]. The layered coordination between innate remodeling (uNK/macrophage) and adaptive tolerance (DC/Treg) creates the immune permissiveness required for successful implantation. Disruption at any level can manifest as recurrent failure, and this underscores the value of comprehensive immune profiling rather than single-marker assessment.

Proteomic and metabolomic biomarkers

Proteomic analysis of endometrial tissue and fluid gives functional insights into receptivity mechanisms that complement transcriptomic signatures and reveal the molecular effectors of receptivity [339, 340]. The endometrial proteome undergoes dynamic changes throughout the menstrual cycle, with peak complexity during the receptive phase reflecting the coordinated expression of multiple functional pathways essential for implantation success [341].

Adhesion molecules represent a critical category of proteomic biomarkers for endometrial receptivity assessment [342]. Integrins, including $\alpha\beta3$ (ITGA5/ITGB3) and $\alpha4\beta1$ (ITGA4/ITGB1), mediate embryo-endometrial adhesion through specific interactions with extracellular matrix proteins and embryonic surface ligands [342, 343]. The temporal expression patterns of specific integrin subunits define the adhesion competence of the endometrium, with altered integrin expression patterns strongly associated with implantation failure and infertility [344].

Cytokines and growth factors in endometrial tissue provide essential paracrine and autocrine signals that regulate receptivity and support embryonic development [345]. Leukemia inhibitory factor (LIF) is absolutely essential for embryo implantation and shows peak expression during the receptive phase [346]. Interleukin -11 (IL11) supports decidualization processes and maintains pregnancy through anti-apoptotic effects on decidual cells. Colony-stimulating factor 1 (CSF1) regulates macrophage function and promotes the immune tolerance necessary for embryo acceptance. The coordinated expression of these signaling molecules creates a supportive microenvironment that facilitates successful embryo implantation [345, 347].

Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) regulate extracellular matrix remodeling during implantation and trophoblast invasion [348, 349]. MMP2 and MMP9 degrade collagen and other matrix proteins to allow trophoblast penetration, while TIMP1 and TIMP2 limit tissue damage during this process. The MMP/TIMP balance serves as a biomarker for implantation potential and pregnancy complication risk [350].

Endometrial fluid, collected non-invasively through aspiration or lavage techniques, contains proteins secreted by endometrial cells and provides a accessible window into endometrial function without requiring tissue biopsy [58, 60, 351]. Proteomic analysis of endometrial fluid has identified biomarkers that correlate with receptivity status and pregnancy outcomes. Key proteins include complement factors, immunoglobulins, and glycoproteins that reflect immune activity and secretory function, providing non-invasive biomarkers for clinical assessment.

Metabolomic profiling of endometrial tissue reveals characteristic patterns that support the high energy demands of implantation and tissue remodeling [352–354]. Glucose metabolism, amino acid turnover, and lipid synthesis show specific patterns during the receptive phase that serve as metabolic biomarkers of endometrial receptivity. These metabolic signatures reflect the functional capacity of the endometrium to support embryo implantation and early pregnancy development.

Epigenetic regulation of receptivity

Epigenetic modifications provide regulatory mechanisms that control gene expression in response to hormonal stimulation, translating cyclical hormone exposure into precise molecular changes that establish and maintain endometrial receptivity. These epigenetic mechanisms represent an additional layer of biomarkers that can provide insights into the regulatory status of the endometrium [355–357].

DNA methylation dynamics during the menstrual cycle regulate the expression of hormone-responsive genes and decidual markers through targeted modifications at specific gene promoters [358]. Progesterone signaling induces demethylation of specific gene promoters, including those controlling decidual markers such as PRL and IGFBP1, as well as receptivity genes including HOXA10 and HOXA11. Global DNA methylation patterns also undergo changes during decidualization, with genome-wide hypomethylation facilitating chromatin accessibility and gene activation [356, 359, 360]. Aberrant DNA methylation patterns are associated with endometrial pathologies and implantation disorders, making methylation status a valuable biomarker for receptivity assessment [360, 361].

Histone modifications provide dynamic regulatory mechanisms that control gene expression during endometrial cycling and decidualization processes [356, 362, 363]. H3K27ac and H3K4me3 modifications mark active enhancers and promoters in receptivity-related genes, creating permissive chromatin environments for gene expression. H3K27me3 modifications establish repressive chromatin states that are selectively removed during gene activation, providing precise temporal control of gene expression. The temporal dynamics of histone modifications can serve as epigenetic biomarkers for receptivity timing and quality assessment.

Chromatin remodeling complexes, including SWI/SNF and CHD family members, regulate chromatin accessibility and enable rapid gene expression changes in response to hormonal stimulation [364–367]. These complexes facilitate the binding of transcription factors to their target sites and enable the rapid gene expression changes required for receptivity establishment. The expression and activity of chromatin remodeling complexes serve as biomarkers for endometrial responsiveness to hormonal treatment and receptivity capacity.

Non-coding RNAs provide additional layers of gene regulation during endometrial receptivity establishment [368]. Specific miRNAs, including members of the miR-200 family, regulate epithelial-mesenchymal transition and decidualization processes that are essential for receptivity [368, 369]. Long non-coding RNAs, including H19 and NEAT1, regulate chromatin organization and gene expression in endometrial cells [370, 371]. Dysregulation of non-coding RNA expression serves as biomarkers for endometrial dysfunction and implantation failure, providing insights into the regulatory mechanisms controlling receptivity.

Recurrent implantation failure and recurrent pregnancy loss

Recurrent implantation failure (RIF) is defined as the absence of clinical pregnancy after three or more

transfers of euploid or morphologically graded embryos [372]. Recurrent pregnancy loss (RPL; clinically often termed recurrent miscarriage, RM) is defined as two or more pregnancy losses before 20 weeks of gestation [373]. Both represent clinically important phenotypes in which embryo competence alone often fails to explain outcomes. Omics-based studies have increasingly identified convergent endometrial and systemic pathways, including defective decidualization, immune dysregulation, abnormal extracellular matrix remodeling, and perturbed inflammatory signaling, that distinguish these conditions from isolated implantation or pregnancy failure and motivate a shift from single-marker interpretations toward multi-layer endotyping.

Although decidualization defects, immune perturbations, and matrix remodeling abnormalities have each been characterized in the context of normal implantation biology, their convergence and co-occurrence in RIF and RM patients reveals a qualitatively distinct pathological landscape. In RIF, single-cell transcriptomic studies have documented persistent activation of proliferative stromal programs and incomplete progression toward mature decidual states, even in biopsies collected in the mid-luteal phase [297, 299]. Proteomics of endometrial tissue and fluid has further highlighted pathway-level disturbances, including altered inflammatory mediator networks and dysregulated matrix turnover, that are not captured by timing-based receptivity labels alone [12, 374]. Critically, these decidualization and matrix remodeling defects do not occur in isolation: they frequently co-occur with immune dysregulation, suggesting that RIF may represent a syndrome of coordinated endometrial dysfunction rather than a single-pathway failure [287, 297].

In RM, a similar pattern of convergent dysfunction has been documented, with particular emphasis on the maternal–fetal interface [375]. Uterine immune dysregulation, including altered uNK abundance and functional imbalance, impaired macrophage-associated remodeling at the decidual–trophoblast boundary, and insufficient expansion of regulatory T cells, has been repeatedly implicated in early pregnancy loss [375–377]. Importantly, these immune alterations frequently coexist with decidualization and stromal niche defects identified by single-cell transcriptomic analyses, suggesting that risk arises from interacting biological programs rather than isolated abnormalities [378]. Together, these findings support an endotype-based interpretation of RM-associated endometrial dysfunction, in which immune and stromal defects are biologically intertwined rather than independent contributors [377, 378].

The reproductive tract microbiome has emerged as an additional investigative layer in both RIF and RM [379]. The endometrial and uterine fluid microbiome,

particularly the dominance or absence of *Lactobacillus*-dominant communities, has been linked to implantation outcomes and early pregnancy maintenance [380]. However, endometrial and uterine fluid samples represent low-biomass ecosystems, making them highly susceptible to contamination from environmental, reagent, or procedural sources [381, 382]. Rigorous negative controls, low-biomass-optimized library preparation protocols, and decontamination pipelines are therefore essential when interpreting microbiome data in this context. When contamination is adequately controlled, non-*Lactobacillus*-dominant profiles have been associated with increased risk of implantation failure and may contribute to the inflammatory endometrial phenotypes observed in a subset of RIF and RM patients [380].

Early embryonic development biomarker discovery

Early embryonic development encompasses critical molecular transitions that determine the developmental competence and implantation potential of preimplantation embryos. Multi-omics analysis has revealed biomarker signatures that reflect embryo quality, developmental trajectory, and implantation success, with particular emphasis on non-invasive assessment approaches that do not compromise embryo viability.

Maternal-to-zygotic transition biomarkers

During the maternal-to-zygotic transition (MZT), maternally deposited transcripts and proteins are progressively degraded while the embryonic genome becomes transcriptionally active. In humans this transition occurs from the zygote stage through the 8-cell stage, with major zygotic genome activation at the 4 to 8 cell stage [383, 384]. Because MZT events determine embryo viability and developmental competence, biomarker discovery in this area is especially relevant for assisted reproduction [385].

Maternal transcript degradation follows specific temporal patterns that serve as biomarkers for normal embryonic development. Key maternal effect genes show coordinated downregulation during early cleavage stages, with the kinetics of maternal transcript clearance correlating strongly with embryo developmental potential [386]. Delayed or incomplete degradation of maternal transcripts is associated with developmental arrest and provides early predictive biomarkers for embryo competence assessment [387]. The coordinated clearance of maternal factors including NLRP5, OOEP, and other SCMC components serves as a quality control checkpoint that must be successfully completed for normal development to proceed [98].

Zygotic genome activation involves the coordinated upregulation of embryonic genes that control fundamental processes including cell cycle progression, lineage

specification, and developmental patterning [388, 389]. Pioneer transcription factors, including OCT4, NANOG, and SOX2, initiate chromatin remodeling and activate embryonic gene expression programs that are essential for pluripotency maintenance and developmental progression. The timing and magnitude of zygotic genome activation gene expression correlate with embryo developmental potential, and robust activation is associated with improved implantation outcomes [390].

Chromatin remodeling during MZT involves global changes in histone modifications and chromatin accessibility that facilitate zygotic gene activation and establish the epigenetic landscape required for development [390, 391]. Maternal histones are systematically replaced by newly synthesized variants, and repressive chromatin marks are selectively removed from embryonic genes to allow transcriptional activation [390, 392]. The efficiency of chromatin remodeling correlates with developmental success and provides epigenetic biomarkers for embryo assessment that complement transcriptomic signatures [391, 393].

Cell cycle regulation during early embryonic development requires precise coordination of DNA replication, chromosome segregation, and cell division processes [394]. Cell cycle checkpoint genes, including ATM, CHEK1, and TP53, monitor genomic integrity and prevent the transmission of damaged DNA to daughter cells [394]. Aberrant cell cycle progression leads to chromosomal abnormalities and embryonic arrest, making cell cycle regulation biomarkers important predictors of embryo competence [395]. Cell cycle regulator expression patterns are linked to chromosomal stability and developmental competence [389].

Single-cell analysis of preimplantation development

Single-cell RNA sequencing of human preimplantation embryos has provided detailed insights into the molecular signatures associated with developmental competence at each stage from fertilization to blastocyst formation [95]. This technology has overcome the limitations of bulk embryo analysis and revealed cellular heterogeneity, developmental trajectories, and quality control mechanisms that were previously undetectable [389]. The identification of high-quality versus poor-quality developmental signatures has enabled the discovery of predictive biomarkers with clinical relevance.

Transcriptional noise represents an important concept in early embryonic development, with high-quality embryos showing coordinated gene expression patterns while poor-quality embryos exhibit increased transcriptional variability between cells [396, 397]. The measurement of transcriptional noise, quantified by cell-to-cell expression variance, has been proposed as a potentially informative biomarker dimension for embryo

developmental potential. Initial studies have reported associations between lower noise and improved developmental outcomes, but sample sizes have been modest and protocols heterogeneous. Whether lower transcriptional noise provides robust and generalizable predictive value for implantation and live birth across cohorts and laboratory protocols remains to be established [396, 397].

Cell fate specification begins during preimplantation development with the critical segregation of inner cell mass (ICM) and trophectoderm (TE) lineages, representing the first cell fate decision in mammalian development. The molecular markers of lineage commitment show specific expression patterns in high-quality blastocysts. OCT4 and NANOG expression marks ICM cells destined to form the fetus, while CDX2 and GATA3 expression characterizes TE cells that will form extra-embryonic structures [383, 398, 399]. The timing and efficiency of lineage specification provide biomarkers for blastocyst quality and implantation potential, with delayed or incomplete lineage segregation associated with developmental failure [399, 400].

Stress response pathways in early embryos reflect their ability to cope with suboptimal culture conditions and environmental stresses that are inherent in assisted reproductive technology procedures [401]. Heat shock proteins (HSPs), oxidative stress response genes including SOD1 and GPX1, and DNA repair pathways show activation patterns that correlate with embryo resilience and developmental success [402, 403]. Embryos with robust stress response capabilities demonstrate better survival rates and improved implantation potential, making stress response biomarkers valuable for embryo selection [401–403].

Metabolic reprogramming during preimplantation development involves complex transitions from oxidative metabolism in oocytes to glycolytic metabolism in cleavage-stage embryos, and back to oxidative metabolism in blastocysts [404]. The expression of metabolic enzymes and regulatory factors provides biomarkers for metabolic competence and developmental potential [405, 406]. Embryos that successfully complete these metabolic transitions show superior developmental outcomes and increased implantation success.

Metabolomic analysis of embryo culture media

Non-invasive biomarker assessment using embryo culture media has emerged as one of the most promising approaches for embryo selection without compromising developmental potential. During *in vitro* culture, embryos consume nutrients and release waste products and bioactive molecules into the culture medium, creating a molecular footprint that reflects embryo metabolism, secretory activity, and developmental status [407–409]. This approach enables repeated sampling and

continuous monitoring throughout embryo development without direct manipulation or biopsy.

Glucose metabolism represents a fundamental aspect of embryo energy production and biosynthesis, with high-quality embryos exhibiting specific consumption patterns that reflect their metabolic efficiency and developmental stage [409, 410]. The glucose uptake rate increases systematically during cleavage divisions and shows stage-specific patterns that correlate strongly with developmental competence [409, 410]. Abnormal glucose consumption patterns are associated with developmental arrest and poor implantation potential, making glucose metabolism a reliable biomarker for embryo assessment [409, 410].

Lactate production and pyruvate consumption provide additional metabolic biomarkers that reflect the balance between glycolytic and oxidative metabolism in developing embryos. The lactate-to-pyruvate ratio reflects metabolic efficiency and correlates with embryo developmental stage and quality [410]. High-quality embryos maintain optimal lactate-to-pyruvate ratios that support both energy production and biosynthetic processes, while poor-quality embryos show altered ratios indicating metabolic dysfunction and reduced developmental potential [405].

Amino acid consumption and production patterns reflect protein synthesis, energy metabolism, and cellular signaling processes occurring within developing embryos [411, 412]. Specific amino acids serve distinct metabolic and signaling roles during embryonic development [411, 413, 414]. The turnover patterns of amino acids provide sensitive biomarkers for embryo metabolic activity and developmental competence, with altered patterns indicating developmental problems [411, 412].

Clinical studies indicate that noninvasive multi-metabolite profiling of spent embryo culture medium can stratify implantation potential: spectroscopy-based approaches typically yield ~70–80% overall predictive, whereas targeted liquid chromatography-mass spectrometry (LC-MS) models built from a small set of metabolites have achieved an area under the receiver operating characteristic curve (AUC)≈0.88 [66, 415, 416]. Panels commonly include the metabolites discussed above (glucose, lactate, pyruvate) alongside selected amino acids (e.g., asparagine), but exact composition varies by platform and cohort. Compared with single-analyte readouts, integrated multi-metabolite models generally perform better and represent a promising noninvasive embryo-selection direction. However, reported performance is platform- and cohort-dependent and should be interpreted as supportive evidence for multimarker modelling rather than as a clinic-agnostic benchmark, pending calibration and external validation across laboratories and protocols.

Proteomic and secretomic analysis

Proteomic analysis of embryo culture media has revealed secreted proteins that correlate with embryo quality and developmental potential, providing functional biomarkers that complement metabolomic signatures [59, 417, 418]. The embryo secretome includes growth factors, cytokines, extracellular matrix proteins, and metabolic enzymes that reflect embryo physiology, developmental status, and communication with the external environment. These secreted proteins provide functional biomarkers that can be assessed non-invasively throughout development.

Growth factor secretion patterns correlate strongly with embryo developmental competence and implantation potential [59, 419]. Vascular endothelial growth factor (VEGF) promotes angiogenesis and vascular development, with higher secretion levels associated with successful implantation and pregnancy establishment [59, 420]. Platelet-derived growth factor (PDGF) supports cellular proliferation and differentiation processes essential for normal development. Transforming growth factor- β (TGF- β) regulates cell fate specification and tissue morphogenesis. The coordinated secretion of these growth factors indicates embryo developmental competence and provides biomarkers for implantation success.

Cytokine profiles in culture media reflect embryo immune signaling and inflammatory status, which can influence implantation success [421–423]. Interleukin-6 (IL6) and interleukin-8 (IL8) are commonly detected in embryo culture media and show patterns associated with developmental stage and quality. Low-level cytokine production typically indicates normal developmental processes, while excessive cytokine secretion suggests cellular stress or dysfunction. The balance of pro-inflammatory and anti-inflammatory cytokines provides biomarkers for embryo health assessment and implantation prediction.

Extracellular matrix proteins secreted by embryos contribute to cellular adhesion and tissue organization processes that are essential for implantation [59, 417]. Fibronectin, vitronectin, and laminin are commonly found in embryo culture media and support embryo attachment and development [417, 418]. The secretion patterns of these matrix proteins correlate with embryo morphology and developmental potential, with higher levels of specific matrix proteins associated with improved implantation rates and pregnancy outcomes [59, 418].

Extracellular vesicles (EVs) secreted by embryos carry microRNAs, proteins, and lipids that reflect embryo physiology and developmental status [418, 424]. These vesicles act as intercellular communication mediators and can be analyzed as stable biomarkers in culture media [418, 425]. MicroRNA content includes species

involved in cell cycle regulation, apoptosis, and developmental programming. The miR-302 family members are highly expressed in pluripotent cells and maintain stemness properties, with their presence in EVs correlating with embryo developmental competence [418, 425].

Morphokinetic and molecular integration

Time-lapse imaging systems enable continuous monitoring of embryo development and identification of optimal timing parameters for key developmental events [426, 427]. The integration of morphokinetic data with molecular biomarkers provides broad approaches for embryo assessment that combine morphological observations with functional molecular information, offering superior predictive accuracy compared to either approach alone [427, 428].

Critical morphokinetic parameters include the precise timing of pronuclear formation, first cleavage, compaction, and blastulation events [429, 430]. Embryos that develop within specific time windows consistently show higher implantation rates and live birth potential, while delayed or accelerated development kinetics are associated with chromosomal abnormalities and reduced developmental competence [429–431]. The identification of optimal timing windows provides standardized parameters for embryo selection that can be applied across different clinical settings.

The correlation between morphokinetic parameters and molecular signatures has revealed important mechanistic insights into embryo development [432, 433]. Embryos with optimal cleavage timing show coordinated expression of cell cycle genes and metabolic regulators, while delayed development correlates with altered gene expression patterns and metabolic dysfunction [433, 434]. These correlations provide validation for molecular biomarkers and enhance their predictive value by linking molecular signatures to observable developmental outcomes.

Artificial intelligence approaches have been successfully applied to integrate morphokinetic and molecular data for systematic embryo selection [435–437]. Deep learning algorithms can analyze time-lapse images and identify subtle morphological features that correlate with molecular signatures and predict developmental outcomes. The integration of imaging data with omics information provides multimodal biomarker approaches with superior predictive accuracy compared to individual modalities, representing the future direction of embryo assessment technology.

Non-invasive embryo assessment is increasingly moving toward multimodal integration rather than single-modality scoring [438]. Representative studies have combined proteomic signals with time-lapse imaging to improve implantation potential assessment and to

derive integrated decision-support outputs [432]. Related approaches have integrated time-lapse morphokinetics with biochemical or oxidative status features measured from spent embryo culture medium, illustrating how embryo physiology and morphodynamics can be fused within clinically feasible workflows [428]. More recently, machine learning models that combine metabolomic readouts with embryologic and imaging-derived variables have reported improved implantation prediction compared with single data streams, supporting late integration or stacked models under careful cross-validation and external replication [436].

Clinical translation requires caution because performance can vary across clinics and protocols [439–441]. Multimodal AI frameworks are best positioned as decision-support tools that complement clinical context, including maternal age, ovarian response, PGT status, and endometrial factors, rather than as standalone decision makers [439]. Prospective multicenter evaluation with explicit calibration and transparent reporting remains essential for clinical deployment.

Beyond conventional time-lapse imaging, hyperspectral microscopy has been investigated as a label-free metabolic imaging modality for embryo assessment by leveraging endogenous fluorophore spectra (notably NAD(P)H and FAD), providing an information axis distinct from morphokinetics [442]. Recent hyperspectral light-sheet implementations can capture volumetric metabolic maps across preimplantation development and have been discussed as a route toward noninvasive embryo viability readouts [443, 444]. However, current evidence remains largely proof-of-concept, and translation requires careful evaluation of phototoxicity, cross-platform calibration, and protocol-dependent domain shift (culture media, incubator conditions, optics), so hyperspectral features are best positioned within standardized, prospectively validated multimodal pipelines rather than as stand-alone add-ons [444, 445]. Biomarker candidates from each compartment have largely been characterized in isolation; linking them across regulatory layers requires formal integration strategies, which we address next [444].

Multi-omics integration for reproductive biomarkers

Female fertility emerges from tightly coupled molecular programs that span regulatory layers (transcriptome, proteome, metabolome, epigenome) and anatomical compartments (the follicular unit, endometrium, and early embryo) [446]. Although omics profiling has substantially expanded our understanding of these processes, much of the literature remains “single-layer,” making it difficult to distinguish causal biology from timing, protocol, and tissue-composition effects. However, robust

biomarkers for clinically relevant outcomes—implantation and live birth—must generalize across cycles, centers, and protocols, demanding integration that captures coordinated programs rather than isolated markers.

Biological and clinical rationale for integration

The rationale for integration in reproductive medicine rests on both biology and clinical reality. Biologically, fertility phenotypes are rarely driven by a single layer: decidualization requires coordinated transcriptional cascades, metabolic reprogramming, and epigenetic remodeling [302]; follicular competence reflects mitochondrial function, transcript stability, and chromatin accessibility within the follicular unit (oocyte-cumulus/granulosa cells-follicular fluid) [4, 167]; implantation depends on synchronized epithelial transformation, stromal differentiation, and immune modulation [302]. Consequently, single-modality biomarkers frequently fail to generalize because they are sensitive to context (cycle phase, progesterone exposure timing, stimulation protocols, and tissue/cell-type composition), so an apparent “signal” in one layer may reflect unmeasured confounding rather than functional competence [447]. Integration provides a principled way to reduce dependence on any single noisy layer, recover cross-layer programs that better reflect system function, and define biologically interpretable endotypes that can generate hypotheses for triage decisions or targeted interventions [70].

Clinically, integration is most compelling when standard work-up is non-diagnostic or repeated failures suggest heterogeneity beyond embryo morphology—especially euploid recurrent implantation failure (RIF) and recurrent pregnancy loss (RPL; clinically often termed recurrent miscarriage, RM) [373, 448]. In these scenarios, chromosomally normal embryos fail to implant or sustain pregnancy, while timing-focused assays or single-marker inflammatory panels may yield conflicting or inconclusive answers [5, 448]. Emerging evidence supports a syndromic view: RIF/RPL often reflect convergent endometrial dysfunction in which decidualization defects, immune dysregulation, and stromal-epithelial crosstalk abnormalities co-occur [297, 448]. Multi-layer integration (transcriptomic endotyping, immune phenotyping, and uterine fluid proteomics/metabolomics) is therefore valuable not because it “adds more markers,” but because it can help discriminate timing misalignment from intrinsic dysfunction and connect molecular programs to patient-relevant outcomes [448, 449].

Integration also amplifies the challenges of reproductive cohorts: high dimensionality under modest *n*, partially missing omics blocks (cycle cancellation, biopsy selection, embryo availability), center/protocol batch structure, and heterogeneous endpoints (biochemical pregnancy vs live birth vs cumulative live birth). Rigorous

integration therefore requires method selection aligned with study structure, transparent handling of batch effects and missingness mechanisms, and validation strategies anchored to patient-relevant outcomes rather than intermediate surrogates.

Strategic considerations for method selection

In reproductive cohorts, integration strategy is best treated as a design choice rather than an algorithmic preference [450]. What matters first is how modalities are paired in the cohort, whether they are measured within the same individuals, only partially paired because of workflow and specimen availability, or effectively unpaired as in cross-study collections [451]. What matters next is where integration is performed in the pipeline, at the level of features, learned representations, or calibrated fusion of modality-specific predictions [452].

When modalities are measured within the same individuals, integration can explicitly model cross-layer coordination and relate cellular programs to biochemical readouts, as in follicular-unit studies linking cumulus or granulosa transcriptomes with follicular-fluid metabolomics or proteomics [453]. In ART practice, however, even nominally matched cohorts are frequently incomplete. Cycle cancellation, limited material, selective sampling, and quality-control failures introduce structured missingness that is often prognosis-related. Methods that implicitly assume complete blocks can therefore produce biased estimates and over-optimistic performance. Strategy selection should, accordingly, prioritise approaches that tolerate missing blocks and avoid complete-case dependence [70, 454].

At the opposite extreme, endometrial single-cell and spatial resources often behave as cross-study datasets, where the principal barrier to generalisation is protocol and centre heterogeneity rather than within-individual pairing [8, 38]. In this setting, harmonisation and batch-aware mapping are prerequisites, and “integration” frequently refers to aligning representations across datasets so that cell-state programs and pathway signals are reproducible [89, 451]. Most clinical datasets lie between these extremes and exhibit mosaic pairing shaped by care pathways, including whether a biopsy is performed, whether spent media are retained, and whether cycles proceed to transfer [450, 451]. Under such structures, naïve concatenation is typically brittle. Greater stability is usually achieved by bridging modalities through shared biological representations, such as pathway activity scores, gene modules, latent factors, or calibrated model outputs, which also makes partial-data contribution feasible [450, 452, 455, 456].

Once cohort structure is established, the timing of integration within the pipeline becomes the central technical decision. Early integration combines normalised features

across modalities before modelling and can, in principle, capture cross-layer interactions [452]. In fertility cohorts, this approach is often fragile because sample sizes are modest relative to joint feature space, and the highest-dimensional or noisiest layer can dominate unless scaling, regularisation, and leakage control are stringent [450, 457, 458]. Intermediate integration first derives shared and modality-specific representations. For reproductive applications, this strategy is often well suited to mechanistic stratification because it yields stable axes that can be interpreted in terms of coherent programs, including progesterone-response attenuation, decidualisation trajectory deficits, inflammatory activation, or metabolic stress, while supporting subsequent assay compression into targeted panels [450]. Late integration trains modality-specific models and combines predictions through calibrated fusion. This approach is pragmatic when blocks are missing or modalities differ substantially in measurement properties, but it should not be taken as mechanistic evidence without transparent attribution within each modality and explicit evaluation of calibration and transportability across centres [459–461].

Method choice should remain anchored to the clinical question and the dominant sources of variability. Prediction tasks such as implantation or live birth can be addressed with calibrated late fusion under strong external validation, particularly when completeness differs across modalities. When the aim is mechanistic endotyping to support clinical reasoning, especially in euploid recurrent implantation failure or recurrent pregnancy loss, intermediate strategies that produce interpretable representations are typically more defensible. Regardless of approach, reproductive-specific confounding requires explicit documentation and control through adjustment or stratification, because cycle type, progesterone exposure, stimulation protocols, embryo ploidy status, and culture conditions can dominate variance and generate spurious associations if ignored.

Structural risks and reproducibility in reproductive multi-omics integration

Beyond method selection, reproductive integration is frequently limited by structural confounding embedded in how ART data are generated [462]. Assisted reproduction cohorts are intrinsically heterogeneous: cycle type, progesterone route and exposure duration, stimulation protocol, embryo ploidy/PGT status, culture media formulation, incubator conditions, and laboratory workflow can each introduce systematic variance that may be comparable to biological effects across centres and protocols, and can mask or mimic true reproductive signals [462–465]. If these factors are not explicitly documented and modeled, integration pipelines can learn protocol signatures rather than reproductive mechanisms [462, 463].

This risk is especially pronounced in endometrial and embryo datasets, where timing and laboratory context are tightly coupled to molecular readouts [296, 299, 462, 466]. In endometrial single-cell or spatial studies, centre-specific sampling windows and processing pipelines can shift apparent cell-state frequencies and pathway activity, yielding patterns that partially reflect sampling and handling rather than intrinsic dysfunction [296, 299]. In embryo metabolomics/secretomics, background signal and detection thresholds are sensitive to media composition, supplementation, handling time, oxygen tension, and incubator settings; consequently, components that appear predictive in one centre can weaken—or even invert—when transported [462, 466].

Informative missingness adds a second layer of distortion. In fertility cohorts, biopsy decisions, cycle cancellations, embryo availability, and QC exclusions can be prognosis-related [467]. Complete-case integration may therefore preferentially retain better-prognosis cases and inflate apparent performance. Robust practice requires transparent characterization of missingness patterns and sensitivity checks under alternative assumptions; where feasible, models should allow partial-block contributions rather than forcing fully paired datasets [454, 456].

High dimensionality under modest sample sizes further amplifies instability [468, 469]. If feature discovery, preprocessing, or hyperparameter tuning is not strictly separated from evaluation, leakage can yield over-optimistic estimates that do not transport. External validation across independent centres remains the most reliable test of generalizability in ART, where protocol drift and centre effects are common [459, 470, 471]. Finally, interpretability should accompany statistical robustness: integrated outputs should map onto recognizable biological axes; opaque composite signals disconnected from plausible reproductive programs undermine translational credibility even when internal metrics appear strong [459, 472].

Translational thresholds and implementation constraints

Even analytically robust integration does not translate automatically in assisted reproduction, because clinical adoption is constrained by workflow and standardisation as much as by model performance [84, 462]. ART decisions are time-locked to laboratory schedules including retrieval, fertilisation, culture, and transfer, so deep profiling is often incompatible with same-cycle decision-making unless assays are compressed, automated, and supported by standardised pre-analytics that fit routine practice [462]. At the same time, protocol drift across centres can generate signatures that appear biological in single-site studies but weaken when transported [462, 470]. Sources of drift include stimulation regimens, progesterone exposure timing, sampling windows, media

formulation, incubator conditions, and operator-dependent handling [462, 470]. Translation therefore depends on standardisation infrastructure, including consensus operating procedures, reference materials, and inter-laboratory proficiency testing, so that results remain comparable across time and sites [84, 462]. Feasibility and governance further define the translational threshold. Multi-omics panels must justify cost and turnaround with patient-relevant benefit and operate under transparent consent, data governance, and accountable reporting [84, 459]. These constraints shape how integration can be positioned in practice and why expansion beyond selected indications should follow demonstrated reproducibility, workflow compatibility, and outcome benefit.

Clinical positioning and interpretive considerations for integrated multi-omics biomarkers

Integrated multi-omics biomarkers are most credible when deployed to support decisions under defined clinical uncertainty, not as broad screening tools [5, 291]. In assisted reproduction, the central value proposition is not a marginal gain in discrimination metrics but a better separation of biological signal from cycle structure and laboratory context, both of which can dominate variance and undermine transportability when models are moved across centres [462, 470]. Integration is justified when it refines the question from whether a model predicts to which mechanism is most consistent with a given failure profile, and when it can be reported in a way that clinicians can act on without over-interpretation [291, 459, 471].

The clearest near-term indication is repeated failure after transfer of euploid embryos with a non-diagnostic standard evaluation, where the pre-test probability of an endometrial contribution is increased [291]. In this setting, integration is most useful when it adjudicates a clinically interpretable distinction. A timing-displacement phenotype implies that receptivity may be achievable but misaligned with progesterone exposure, making modification of progesterone duration or transfer timing a hypothesis to test [5, 297]. An intrinsic dysfunction phenotype implies failure to enter or sustain a receptive state despite apparently appropriate timing, often through immune activation, impaired decidualisation trajectories, or abnormal stromal–epithelial crosstalk, and therefore warrants etiologic investigation and targeted management rather than calendar-based adjustment [291, 297, 299]. Evidence supporting this framework is convergent across modalities. Single-cell studies in recurrent implantation failure report persistent proliferative stromal programmes and incomplete progression toward decidual states, including in mid-luteal biopsies [297, 299]. Proteomic analyses of endometrial tissue and uterine fluid highlight pathway-level perturbations, including

inflammatory mediator networks and dysregulated matrix turnover, that are poorly captured by timing labels alone [351, 473]. Endometrial immune profiling has been proposed as a pragmatic route to personalise evaluation in selected ART populations [474, 475]. What an integrated report can justify at present is stratification and trial-ready phenotyping with appropriate counselling, not a treatment prescription, because endotypes are not automatically therapeutically tractable without prospective evidence of benefit from biomarker-guided care [5, 291, 474].

A second practical niche is embryo selection when conventional signals are discordant [440, 476, 477]. When morphology is similar across embryos but time-lapse rankings diverge, or when morphokinetic patterns are difficult to interpret under a given laboratory protocol, non-invasive spent-media molecular profiles can contribute information on metabolic efficiency and stress response that is not redundant with imaging, enabling more transparent multimodal prioritisation within a cohort [476, 478]. Representative studies have combined time-lapse morphokinetics with proteomic signals to generate implantation decision-support outputs, integrated morphokinetics with oxidative-status features measured from spent culture medium, and fused metabolomic readouts with embryologic variables using machine-learning approaches for implantation prediction [436, 479, 480]. The clinical claim should remain proportionate. These tools may refine ranking within embryos generated under the same conditions, but they are sensitive to media formulation, incubator settings, and handling workflows; generalisability should be demonstrated through calibration and prospective multicentre evaluation rather than inferred from single-site retrospective performance [440, 462].

Across indications, interpretation should be disciplined by three safeguards. First, technical credibility must be explicit, including assessment and handling of batch and centre effects, treatment of missing blocks, and prevention of leakage through training-fold-restricted feature selection and ideally nested cross-validation [89, 456, 468]. Second, incremental value must be quantified against established predictors, including maternal age, ovarian response, embryo ploidy status, and standard endometrial assessment, because integration that does not change decision quality adds complexity without benefit [481]. Third, actionability should be treated as graded. Many current signatures support biological explanation, risk stratification, and trial enrolment more reliably than immediate intervention, and this should be communicated transparently [459]. Clinical expansion should therefore be contingent on prospective demonstration of utility, feasibility, and cost-effectiveness.

Limitations and bottlenecks

Several bottlenecks continue to constrain clinical adoption of integrated reproductive multi-omics. Cost and infrastructure remain limiting, especially for single-cell and spatial profiling and for deep proteomics, which require specialised equipment and expertise that are unevenly distributed across centres, restricting scalability and raising equity concerns [462]. Methodologically, most fertility cohorts combine modest sample sizes with high dimensionality, heterogeneous endpoints, and partially missing omics blocks. Without stringent leakage control, calibration, and external validation, these conditions favour overfitting and yield performance estimates that do not transport [456, 461, 468]. Variation in outcome definitions, including biochemical pregnancy, clinical pregnancy, live birth, and cumulative live birth, further complicates cross-study comparison and contributes to apparent inconsistency in reported performance [461].

Protocol heterogeneity is a pervasive confounder and a common reason for failed transportability. Differences in stimulation regimens, progesterone exposure timing, sampling windows, culture media formulation, incubator conditions, and operator-dependent handling can generate centre-specific signatures that mimic biology in single-site studies but weaken or invert in external cohorts [461, 462, 482, 483]. This makes cross-centre replication and calibration essential and strengthens the case for standardised reference materials, inter-laboratory proficiency testing, and consistent reporting of protocol variables that plausibly drive variance [462, 483]. Missingness is also rarely random in fertility cohorts. Cycle cancellation, biopsy decisions, embryo availability, and quality-control exclusions can correlate with prognosis and induce selection bias when complete-case analysis is used without sensitivity checks [482]. Transparent characterisation of missingness patterns and robustness analyses under alternative assumptions are therefore essential for interpretability [482, 483].

Ethical, governance, and regulatory considerations are increasingly central. Multi-omics profiling of embryos and reproductive tissues raises questions of consent, privacy, secondary use of genomic information, and model transparency [484, 485]. Regulatory approval and reimbursement require prospective evidence that biomarker-guided decisions improve patient-centred outcomes and demonstrate cost-effectiveness, and such evidence remains limited for most candidate signatures [483, 486]. Collectively, these constraints support a measured translational posture that prioritises selected high-need indications, simplified assays with standardised pipelines, and multi-centre prospective validation over premature deployment in unselected populations [483, 486].

Future perspectives and translational outlook

The next phase of reproductive multi-omics will be shaped less by additional data layers and more by convergence toward implementable clinical architectures. Contemporary fertility evaluation remains compartmentalised, with ovarian reserve, oocyte competence, embryo assessment, and endometrial receptivity typically considered in parallel rather than as coupled biological systems. A plausible trajectory is the emergence of integrated platforms that connect molecular information across the follicular unit, endometrium, and embryo within routine ART workflows. The dominant constraint is operational. Sampling, processing, and reporting must fit the retrieval-to-fertilisation interval, embryo culture timelines, and transfer scheduling [462, 476]. This will favour assay compression, automation, and standardised pre-analytics over bespoke deep profiling, with discovery-grade multi-omics serving primarily to identify stable programmes that can be distilled into targeted, deployable panels [61, 462].

If platform constraints can be met, integration could support precision-guided care at several decision points. In ovarian stimulation, baseline molecular signatures combined with early response signals could support protocol adaptation guided by predicted response patterns rather than empirical adjustment [487]. In embryo culture and selection, multimodal models that combine morphokinetics with non-invasive molecular readouts may add value when conventional assessment is uncertain or discordant [436, 479, 480]. In endometrial preparation, the most defensible direction is endotype-informed intervention, where the objective shifts from timing correction to addressing specific dysfunction programmes [3, 291]. Inflammatory endotypes, progesterone-response attenuation, and impaired decidualisation imply different mechanistic hypotheses and different standards of evidence for intervention design and evaluation [3, 462]. Translation will depend on whether these outputs can be operationalised within decision-support systems that integrate seamlessly into practice, and whether prospective trials demonstrate improved patient-relevant outcomes under biomarker-guided care [291, 483].

Longitudinal fertility monitoring is an additional frontier, but it requires careful framing. Wearable biosensors capable of tracking cycle characteristics, hormonal fluctuations, and physiological proxies could generate continuous data streams, while periodic minimally invasive profiling could provide intermittent molecular anchors to quantify ovarian reserve trajectories and systemic inflammatory or metabolic risk states [488–491]. In principle, such integration could enable earlier identification of accelerated reproductive ageing or modifiable risk profiles than current reactive paradigms [490, 491]. Its clinical legitimacy will be determined by whether monitored

signals can be mapped to interventions with demonstrated benefit and acceptable cost, and whether deployment can be equitable rather than limited to those able to pay for premium add-ons [492, 493].

Systematic translation requires coordinated efforts across realistic timescales. Near-term priorities include establishing consensus protocols and reference materials for quality control, achieving regulatory approval for biomarker products addressing well-defined clinical needs, and developing collaborative multi-centre validation networks [84, 483]. Mid-term goals involve completing prospective trials demonstrating that biomarker-guided care improves clinical and economic outcomes, developing platforms integrating multiple data types, and establishing practice guidelines [483, 494]. Long-term implementation includes deploying comprehensive monitoring platforms, demonstrating population health benefits, and incorporating fertility optimisation into standard gynaecological care. Achieving this vision will depend on collaboration among researchers generating evidence, clinicians participating in validation studies, technology developers building implementable platforms, regulators establishing proportionate pathways, payers recognising value propositions, and patient advocates ensuring equitable access [483, 492].

Conclusion

Female fertility reflects coordinated molecular programmes across regulatory layers and anatomical compartments. The evidence reviewed here indicates that multi-omics integration can recover biologically coherent structure that single modalities often miss, and that its highest value emerges when it clarifies clinically relevant heterogeneity rather than when it merely increases feature count. Across the follicular unit, endometrium, and early embryo, phenotypes such as oocyte competence, endometrial receptivity, and embryo viability appear to arise from systems-level coupling of transcriptional, epigenetic, metabolic, and immune programmes rather than isolated molecular events.

The translational opportunity lies in reframing clinical problems into mechanistically interpretable categories. Recurrent implantation failure can be approached as a differential diagnosis distinguishing timing displacement from intrinsic endometrial dysfunction, with each category implying different investigative and therapeutic pathways. Embryo assessment can incorporate metabolic efficiency and stress resilience alongside morphology and ploidy status, particularly when conventional signals are discordant. Ovarian reserve evaluation can extend beyond follicle counting toward molecular ageing signatures that better approximate functional capacity. These reframings create decision points where stratification can

guide targeted evaluation, enable rational trial design, and ultimately support more individualised care.

The gap between discovery and clinical implementation remains substantial. Most integration studies are limited by sample size, incomplete external validation, and insufficient control of protocol-dependent confounding. Progress will depend on prioritising reproducibility over novelty, prospective validation over retrospective association, and patient-relevant outcomes over intermediate surrogates. Only frameworks that demonstrate calibrated performance across centres and populations will justify the analytical complexity required for routine clinical deployment. The field has shown that multi-omics can generate candidate biomarkers; the remaining question is whether it can build the collaborative networks, consensus protocols, and health economic evidence required for translation, and whether these tools can improve outcomes in practice rather than only on paper.

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Author contribution

XL conceptualized the study, performed literature review, drafted the manuscript, and prepared visualizations. CB contributed to manuscript writing, provided resources, and prepared visualizations. QG and ML assisted with literature search and drafting. LX and ZZ contributed to reference management and manuscript organization. ML and XJ assisted with interpretation and manuscript revision. SW acquired funding, supervised the project, and critically reviewed and edited the manuscript.

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Data availability

All data analyzed in this review are included in the cited references.

Declarations

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Not applicable.

Consent for publication

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Conflicts of interest

We declare that we have no conflict of interest.

Competing interests

The authors declare no competing interests.

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