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Review

Biomarkers of Female Infertility: An Updated Review

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Abstract

Female infertility affects approximately 10–15% of women of reproductive age worldwide and represents a major global health concern. Classical biomarkers such as anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH), and antral follicle count (AFC) remain central to infertility assessment; however, they have limited ability to predict oocyte competence, embryo quality, and implantation potential. Advances in molecular biology and omics technologies have expanded the range of biomarkers available for evaluating female reproductive function. This narrative review synthesizes current evidence on emerging biomarkers of female infertility, including molecular markers (microRNAs, cell-free DNA, exosomes, and extracellular vesicles), oxidative stress markers, inflammatory cytokines, metabolomic signatures, oocyte-derived growth factors, endometrial receptivity markers, microbiota profiles, and environmental exposure biomarkers. These biomarkers are discussed in relation to their biological relevance and potential clinical utility, with critical appraisal of evidence linking them to ovarian reserve, oocyte and embryo quality, implantation, and pregnancy outcomes. Their applicability to specific infertility phenotypes, such as polycystic ovary syndrome, endometriosis, recurrent implantation failure, and unexplained infertility, is also highlighted. Overall, emerging biomarkers show promise for improving diagnostic accuracy, prognostic evaluation, and personalized treatment strategies. Nevertheless, significant challenges remain, including assay standardization, limited validation in large and diverse populations, and uncertain clinical feasibility. Therefore, integrated biomarker panels, rather than single markers, are most likely to advance precision diagnostics and clinical decision-making in female infertility.

Keywords: Female infertility, biomarkers, microRNAs, cell-free DNA, extracellular vesicle

Introduction

Female infertility is a multifactorial reproductive disorder caused by endocrine, ovarian, and uterine as well as genetic or systemic reasons and affects about 10–15% of women of childbearing age worldwide [1]. Biomarkers are at the core of the assessment of infertility as objective indicators of ovarian reserve, endocrine function and competence of reproductive tissues. Technological advances in molecular biology have led to a new generation of minimally invasive biomarkers on the cellular and genomic levels that reflect reproductive function. MicroRNAs (miRNAs) regulate gene expression relevant to folliculogenesis, steroidogenesis, implantation and endometrial receptivity, with dysregulated levels reported in polycystic ovarian syndrome (PCOS), endometriosis, and decreased ovarian reserve [2]. Recent studies suggest that circulating and follicular cell-free DNA (cfDNA) may be useful in predicting ovarian aging, follicular apoptosis, and embryo developmental competence [3]. Concomitantly, exosomes and extracellular vesicles from the ovary and endometrium harbour bioactive proteins and nucleic acids that are representative of the functional state of the reproductive microenvironment [4]. In addition, oocyte-derived growth factors, growth differentiation factor 9 (GDF-9) and bone morphogenetic protein 15 (BMP-15), and endometrial receptivity markers, and transcriptomic tools like the endometrial receptivity array (ERA), are modifying knowledge of implantation failure [5]. Together, these biomarkers represent the recent evolution of diagnostic markers of female infertility.

Anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH), and antral follicle count (AFC) are commonly used markers of ovarian reserve and

predictors of ART cycle ovarian response [6]. Other hormonal determinants, estradiol (E2), progesterone, LH, prolactin, thyroid-stimulating hormone (TSH), inhibin B, testosterone (T), and androgens, can help to define ovulatory dysfunction, endocrine causes, and hyperandrogenism states such as PCOS [7]. In addition, cancer antigen-125 (CA-125) is a clinically valuable biomarker of endometriosis-associated infertility despite its limited specificity [8].

Although significant progress has been achieved in assisted reproductive techniques, diagnostic tools for female infertility are still limited and unable to fully predict oocyte competence, embryo developmental potential, or implantation. Epidemiologically, infertility plays a significant role in healthcare consumption as well as the psychological burden and long-term economic cost, especially when childbearing is postponed. Current biomarkers mainly measure ovarian quantity over quality, which leaves a diagnostic gap that leads to repeated treatment failure and poor use of resources. This unmet clinical need has stimulated the pursuit of biomarkers that could reflect functional, molecular, and microenvironmental factors that determine female fertility potential. Therefore, the purpose of this review is not simply to accumulate potential biomarkers but also to summarize what we currently know, compare them against one another diagnostically, and assess their practicality in being translated to the clinic. We then focus on new biomarkers that could help improve current diagnostic paradigms and facilitate precision interventions in the context of infertility.

Materials and Methods

The aim of this narrative review was to gather evidence from published human studies on conventional and novel biomarkers for the evaluation of female infertility. A formal systematic search was not used; however, PubMed and Google Scholar databases were searched for research articles and reviews published between 2014 and 2025. Keywords included: female infertility, women, biomarkers, markers, emerging, established, serum, and genetics. Only articles in the English language were included. Such heterogeneity in study design, assay performance, sample origin, and outcome measurements hampers their quantitative comparison across studies. These restrictions are recognized in this review and clearly establish the necessity of standardized reporting schemes and prospective validation.

Emerging biomarkers of female infertility

MicroRNAs (miRNAs)

MicroRNAs are short non-coding RNA molecules that exert important post-transcriptional control of gene expression. These molecules have become potential candidates for diagnostic and prognostic markers because of their involvement in controlling cellular functions such as proliferation, differentiation, apoptosis, and steroidogenesis [9]. A large body of research has demonstrated associations from abnormal miRNA expressions with different types of infertility-related disorders (especially those related to ovarian function), such as decreased and poor ovarian reserve (DOR), poor ovarian response (POR), polycystic ovarian syndrome (PCOS), and primary ovarian insufficiency (POI) [10]. Circulating miRNAs in follicular fluid have particular promise as biomarkers, using well-defined miRNA signatures such as let-7b, let-7c, and miR-21 that are all strongly associated with granulosa cell and cumulus cell control of oocyte maturation (Figure 1) [9].

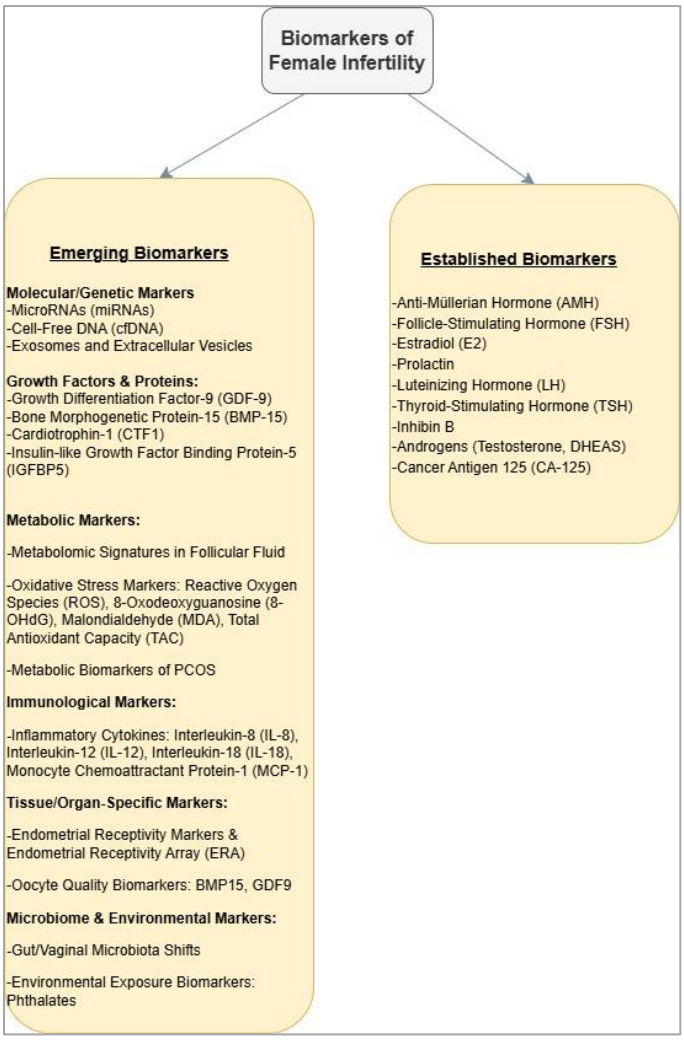

Figure 1: Established and emerging biomarkers of female infertility. The figure illustrates the conceptual integration of established and emerging biomarkers across key reproductive domains

Circulating miRNAs with abnormal levels in uterine diseases broaden the application prospects of these factors in clinical reproductive medicine. Several upregulated and downregulated miRNAs have been shown to be promising biomarkers for endometriosis, adenomyosis, reduced endometrial receptivity, and implantation failure [11]. The stability of miRNAs in extracellular vesicles (EVs) isolated from follicular fluid is a strength, as vesicular-encapsulated miRNAs are generally resistant to degradation relative to the circulating "free" molecules [12]. Oxidative stress-related miRNAs, including miR-132-3p, let-7, and miR-642a-5p, control mitochondrial activity and apoptosis via the redox-sensitive pathways whose dysregulation is associated with unfavourable reproductive outcomes, for instance PCOS, POI, and endometriosis [10]. Despite a body of literature in favour of miRNAs as biomarkers, there are still no standard protocols and precise diagnostic cut-off values, so more validation studies are needed to confirm their accuracy enough for introduction into everyday clinical practice.

Cell-free DNA (cfDNA)

Cell-free DNA (cfDNA) consists of nucleic acid fragments released into body fluids via physiological and pathological processes, such as apoptosis and necrosis, and represents a helpful non-invasive tool for reproductive health evaluation [13]. Regarding female infertility, follicular fluid cfDNA levels correlate with the ovarian reserve status, embryo quality, and *in vitro* fertilization (IVF) outcomes. It has been reported that the levels of cfDNA are much higher in women who are over 35 old years as compared to younger reproductive-aged women, and these elevated concentrations are associated with certain female factor infertility conditions such as endometriosis, PCOS, and premature ovarian failure (POF) [14]. Increased follicular fluid cfDNA levels have been associated with increased fragmentation of embryos and lower IVF pregnancy rates, suggesting that this could be a useful independent predictor of treatment outcomes [15]. The assessment of cfDNA might serve as a non-invasive and simple tool to assess the quality of the microenvironment provided by the preovulatory follicle, as well as the ovarian response to gonadotropin stimulation.

Exosomes and extracellular vesicles

Exosomes and extracellular vesicles (EVs) are membrane-limited nanovesicles, essential mediators of intercellular communication in reproductive biology, and their size is usually between 30 and 1000 nm according to their biogenic origin [16]. These vesicles are a class of biological vehicles that transport a variety of molecular passengers, such as proteins, nucleic acids, and lipids between reproductive cells and tissues. In the female reproductive tract, exosomes in follicular fluid, oviduct fluid, and uterine luminal fluid play roles in such processes as follicular development, oocyte maturation, and embryo-maternal communication required for successful implantation and pregnancy establishment [17]. A recent proteomic study of menstrual blood serum-derived EVs from women with unexplained infertility identified proteins associated with dysregulation of cell adhesion, immune response, apoptosis, oxidative stress response, and lipid metabolism, which provide novel molecular endotypes for patient stratification and personalized treatment regimens [4]. The enrichment of various molecular factors in exosomes for some assays provides the ability to maneuver their genetic and proteomic cargo that could be exploited for diagnostic and therapeutic purposes [18].

Growth differentiation factor-9 (GDF-9) and bone morphogenetic protein-15 (BMP-15)

GDF-9 and BMP-15 are oocyte-secreted paracrine regulators that play a role in ovarian function. These factors are secreted only by oocytes at every stage of follicular development and play an important role in the regulation of granulosa and theca cell function, namely cell proliferation, differentiation, steroidogenesis, cumulus expansion, and luteinization [19]. Heterodimers of GDF9 with BMP15 show greater bioactivity than homodimers in granulosa cell function and impact the upregulation of cumulus expansion-related genes such as Has2, Ptgs2, and others, with these heterodimerized proteins needing a distinct signaling complex that includes BMPR2, ALK4/5/7, and an ALK6 type receptor [20]. Levels of GDF9 and BMP15 in serum are highly variable between women (up to 64-fold and 15-fold) compared with each other, with GDF9 correlating with oocyte yield in non-PCOS but not PCOS cases, whereas the reverse is true for susceptibility status associations between GDF9 and BMP15 challenging previous observations [21]. Clinically, they are involved in primary ovarian insufficiency, with mutations in GDF9 and BMP15 resulting in primary ovarian insufficiency described by the premature depletion of the pool of follicles and a heterozygous variant presented among affected populations [22].

Endometrial receptivity markers and endometrial receptivity array (ERA)

Endometrial receptivity describes a state in which the endometrium is ready to accept and sustain an embryo during the so-called implantation window,

a transient period lasting 4–5 days that is characterized by dramatic changes in gene expression and molecular remodeling [23]. Leukemia inhibitor factor, homeobox A10, integrin αvβ3, and its ligand osteopontin, and mucin-1 represent these molecular markers of endometrial receptivity whose progesterone-dependent expression mediates epithelial cell adhesion, immune tolerance, and vascularization. Luminal/glandular MUC1 is observed at markedly decreased levels in women with recurrent implantation failure versus women with fertile controls or idiopathic recurrent miscarriage, whereby MUC1 status can be used as an independent indicator of endometrial receptivity irrespective of demographic and clinical demographics [24]. HOXA10 expression is an important downstream transcriptional regulator mediating the regulation of integrin αvβ3 expression by estrogen and progesterone: reduced HOXA10 in the endometrium of women with recurrent implantation failures and recurrent miscarriage leads to poor endometrial receptivity, impairing synchronization with embryo development [23].

Oxidative stress markers: reactive oxygen species (ROS), 8-hydroxy-2'-deoxyguanosine (8-OHdG), malondialdehyde (MDA) and total antioxidant capacity (TAC)

Oxidative stress is a pathological status reflecting an imbalance between the production of reactive oxygen species (ROS) and endogenous antioxidant defenses, with the ability to determine assayed ROS levels within follicular fluid playing a significant predictive role for oocyte quality and developmental competence [25]. Follicular fluid is the microenvironment that is essential for oocyte development, and monitoring oxidative stress biomarkers, including reactive oxygen species, 8-oxodeoxyguanosine, malondialdehyde (MDA), and total antioxidant capacity (TAC), allows assessment of the quality of the follicular milieu that affects oocyte/embryo developmental competence [25]. 8-oxodeoxyguanosine is considered a marker of oxidative DNA damage caused by the hydroxyl radicals attacking deoxyguanine, the most susceptible base to damage, and high levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in granulosa cells are negatively correlated with fertilization rates and embryo quality among women undergoing *in vitro* fertilization [26]. Malondialdehyde is the most reliable marker of lipid peroxidation and oxidative damage in membranes, which are significantly higher in the follicular fluid of infertile women than fertile controls, whereas total antioxidant capacity, as an indicator for the antioxidant defense system, is decreased not enough to scavenge free radicals [27].

Metabolomic signatures in follicular fluid (FF)

Follicular fluid is a special microenvironment that reflects the metabolic status of developing oocytes and is emerging as an excellent source for infertility-related biomarkers. Recent metabolomic investigations have revealed unique metabolic profiles in FF that are associated with oocyte competence and fertility. Perturbation of amino acid metabolism with phenylalanine in whole blood and low arginine abundance in follicular fluid were correlated with poor oocyte quality in women undergoing ART [28]. The lipid content of FF has become a key regulator of reproductive outcome, and analysis of particular phospholipid profiles could accurately predict embryo developmental potential [29]. In addition, markers of carbohydrate metabolism in FF, such as lactate: pyruvate ratios, have been related to the mitochondrial function in oocytes and fertilization rates. Increased FF levels of reactive oxygen species (ROS) and decreased antioxidant capacity were strongly correlated with a decrease in ovarian reserve and poorer IVF performance [30]. More recently, high-resolution mass spectrometry for the analysis of the FF metabolome found that disrupted energy metabolism pathways, including tricarboxylic acid cycle (TCA) intermediates, were enriched in women with unexplained infertility [31]. These metabolomic signatures provide noninvasive predictive signs for judging oocyte competence and improving personalized fertility treatment.

Inflammatory cytokines (IL-8, IL-12, IL-18, and chemoattractant protein-1)

Inflammatory cytokines play an important role in reproductive physiology, and their imbalance has been reported to be involved in many causes of female infertility. Interleukin-8 (IL-8), a known highly effective chemokine, has been well studied in ovarian biology and endometrial receptivity. Intrafollicular IL-8, IL-12, and adrenomedullin are promising prognostic markers of oocyte and embryo quality in women with endometriosis [32]. Moreover, IL-12 and IL-18, which are major factors of T-helper cell differentiation, have been reported as having abnormal expression levels in recurrent implantation failure (RIF) patients. Associations of adipokines and cytokines in follicles are related to IVF outcomes [33]. Monocyte chemoattractant protein-1 (MCP-1) has been shown to be a crucial mediator in ovarian pathologies, and higher MCP-1 concentrations were identified in the peritoneal fluid of infertile women with endometriosis when compared to fertile counterparts [34]. The

balance between pro-inflammatory and anti-inflammatory cytokines seems to be central for successful reproduction. Serum cytokine profiles in women with PCOS and infertility are being characterized for a better understanding [35]. Moreover, TNF-α and IFN-γ are known as candidate biomarkers for immunological infertility. These cytokine profiles provide important information on the local inflammatory environment of the reproductive tract and may influence targeted immunomodulation for fertility improvement.

Plasma protein biomarkers: Cardiotrophin-1 (CTF1)

Circulating protein biomarkers are increasingly growing in popularity as fair and utilitarian serum markers of female reproductive competency. Cardiotrophin-1 (CTF1), a member of the interleukin-6 cytokine family, has been shown to be an interesting endometrial receptivity and implantation success marker. Plasma CTF1 levels fluctuated during the menstrual cycle, being highest at the mid-secretory phase, which denotes optimal endometrial receptivity in fertile women [36]. Women with RIF displayed a lower expression of CTF1, which may become a predictor factor to assess the implantation program.

Metabolic biomarkers of polycystic ovary syndrome (PCOS)

Polycystic ovary syndrome (PCOS) is among the most common endocrine diseases in premenopausal women and exhibits multi-level metabolic aberrations that significantly impact fertility. Altered amino acid metabolism in the follicular fluid of PCOS patients has links with oocyte quality [37]. Also, aromatic amino acids, particularly phenylalanine and tyrosine, were observed to be the independent predictors for metabolic syndrome development of PCOS in women. The diagnostic value of serum miRNAs for polycystic ovary syndrome has been explored through meta-analysis [38]. Changes in lipid metabolism, one of the primary PCOS metabolic abnormalities, are being investigated. These changes in lipids were associated with chronic low-grade inflammation, and oxidative stress, major factors that contribute to PCOS infertility. Systematic investigation of the effect of gut microbiota on metabolic pathways in hyperlipidaemia conditions related to PCOS [39]. Furthermore, HbA1c and fructosamine have been shown to be useful for the early detection of glucose dysregulation in PCOS women with normal fasting glucose. Very recently, the involvement of gut microbiota-derived compounds has been focused on. The gut microbiota-bile acid-interleukin-22 axis orchestrates polycystic ovary syndrome [40].

Biomarkers of oocyte quality: Bone morphogenetic protein 15 (BMP15) and growth differentiation factor 9 (GDF9)

Oocyte quality is a significant factor influencing female fertility, and the developmental competence of oocytes can be predicted based on molecular biomarkers, such as bone morphogenetic protein 15 (BMP15) and its related molecule growth differentiation factor 9 (GDF9). These oocyte-derived factors regulate granulosa cell proliferation, follicular growth, and cumulus expansion [41]. Age-related decline in expression of GDF9 and BMP15 genes in follicle fluid and granulosa cells from poor ovarian responders has been documented [41]. Growth differentiation factor-9 and bone morphogenic protein-15 are predictors of oocyte and embryo quality in sub-fertile women [42]. From both animal and human studies, it is evident that BMP15 and GDF9 control important signaling pathways, including SMAD-mediated transcription and steroidogenesis, underscoring their mechanistic impact on oocyte competence [41]. Determination of the levels of these factors in FF or serum before stimulation may facilitate clinicians` their ability to evaluate intrinsic oocyte quality. So that stimulation protocol can be personalized and ART success rates optimized. Despite encouraging associations, there is still a need for standardized quantification and large cohort validation to demonstrate clinical utility.

Gut and vaginal microbiota shifts

The gut and vaginal microbiota are now becoming the focus of attention, since they appear to have a major effect on female reproductive health, affecting ovulation, implantation, and pregnancy. Gut and vaginal microbiota in female infertility: a systematic review has been conducted [43]. Dysbiosis (perturbations in the microbial makeup) has been associated with infertility, endometriosis, and RIF. In PCOS, the alterations of gut microbiota include a lower rate of the Bacteroidetes/Firmicutes ratio and less abundant *Lactobacillus* species, which are associated with systemic inflammation, insulin resistance, and hyperandrogenism [40]. Vaginal dysbiosis, including a loss of dominance by *Lactobacillus* species and increased abundances of *Gardnerella* and *Atopobium* species, is associated with dysregulated endometrial receptivity as well as increased risk for miscarriage [44]. Investigations demonstrated associations between microbiota metabolites, immune modulation, and local hormone metabolism, indicating the existence of a potential mechanism translating from microbial species composition to reproductive function [43]. By modulating the composition of microbiota through

probiotics, prebiotics, or dietary interventions to alter the host-brain-behaviour axis, an opportunity to improve fertility outcomes also exists, rendering microbiota profiling a new non-invasive biomarker that can be utilized for assessing reproductive health.

Environmental exposure biomarkers (phthalates)

Phthalates are a class of widely distributed environmental endocrine-disrupting chemicals (EDCs) and are also the most frequently utilized plasticizers in consumer products such as food packaging, personal care products, and medical devices. Human exposure is mainly through the oral, inhalation, and dermal routes. Recently, studies have reported that phthalates perturb the female reproductive system through impairment of steroidogenesis, folliculogenesis, and oocyte maturation. Environmental phthalate exposure and reproductive outcomes in women have been extensively studied [45]. Elevated urinary phthalate metabolite concentrations are associated with decreased antral follicle count (AFC), lower serum anti-Müllerian hormone (AMH) levels, and poor oocyte yield during IVF, suggesting compromised ovarian reserve and impaired fertility potential [46].

Cancer antigen 125 (CA-125)

Cancer antigen 125 (CA-125) is a large molecular mass glycoprotein that is expressed by coelomic epithelium-derived tissues such as endometrium, fallopian tubes, and peritoneum. Although it was originally developed as a tumour marker for epithelial ovarian cancer, the importance of CA-125 has been increasing in reproductive medicine and even more specifically in infertility, for instance, in relation to endometriosis-related infertility. Endometriosis represents a significant clinical condition affecting reproductive potential [47]. High levels of CA-125 are commonly found in the serum of women with moderate-to-severe endometriosis and correlate with disease load and inflammation, as mentioned in endometriosis and risk of infertility, role of CA-125 and disease severity [48]. Biomarkers for endometriosis have been identified and reviewed [49]. CA-125 as part of multimarker panels (with inflammatory cytokines and imaging) has also been studied to enhance the diagnostic accuracy for endometriosis-associated subfertility. CA-125, however, is not specific for epithelial ovarian cancer (EOC), since increased CA-125 levels have also been reported in connection with menses and pregnancy and conditions such as pelvic inflammatory disease (PID) or other benign gynaecological diseases.

While each class of biomarkers provides a unique biological understanding, few have provided adequate standalone accuracy to supplant current clinical practices. Direct comparisons across studies are hampered by methodological variability, but emerging patterns demonstrate that molecular biomarkers like miRNAs and cfDNA may better reflect oocyte and embryo quality (vs. ovarian reserve) compared to traditional endocrine markers. Meanwhile, metabolomic and oxidative stress biomarkers seem to be very sensitive to variations of the microenvironment, while they cannot distinguish different pathologies. Exosome-based cargo has a distinct, integrative signal but still requires technical complexity and expense. Together, these results suggest a multimarker approach rather than the "search-for-one-biomarker" principle.

Established biomarkers of female infertility

Anti-Müllerian hormone (AMH)

Anti-Müllerian hormone (AMH) is one of the most stable biomarkers employed to assess ovarian reserve and predict reproductive potential. Granulosa cells of pre-antral and small antral follicles secrete AMH to reflect the extent of the residual follicle pool and have low intra-cycle variability. Clinical research shows that AMH strongly correlates with antral follicle count (AFC) and the response of ovaries to controlled ovarian stimulation; it is therefore used as a fundamental marker in infertility assessment as well as in planning for assisted reproductive technology [6]. In addition, in the context of polycystic ovary syndrome (PCOS), AMH has a unique role for the diagnosis and potential pathophysiological process: it marks excessively small follicles and presumably abnormal folliculogenesis [50]. Recent meta-analyses support its superiority to FSH in the prediction of poor ovarian response, but its use for the identification of natural fecundability is still limited (Figure 1)[51].

Follicle-stimulating hormone (FSH)

Basal serum FSH, which is generally measured on cycle day 2–3, continues to be a well-accepted marker of ovarian reserve and hypothalamic-pituitary-ovarian (HPO) axis function. High FSH concentrations result from loss of ovarian feedback, resulting from low inhibin B and estradiol secretion, usually an indicator of late reproductive aging [52]. However, FSH has high inter-cycle variation and is less sensitive than AMH or AFC at early ovarian decline. However, despite these limitations, FSH is still frequently utilised in resource-limited settings and is clinically informative when considered with other indicators. Recent evidence showed that combined FSH and AMH provides more accuracy

in prognostication of ovarian stimulation outcomes compared to FSH alone [53].

Antral follicle count (AFC)

AFC is determined as follicles measuring 2–10 mm in diameter using transvaginal ultrasonography and offers direct visualization of ovarian reserve. AFC is strongly associated with the AMH and can predict ovarian response in ART cycles [54]. As opposed to hormonal assessment, AFC provides immediate anatomical information being, however, operator-related and influenced by the ultrasound quality. This approach is further backed by the fact that AFC can be a robust predictor of live birth rates in IVF, especially if performed according to standardized scanning protocols [55]. However, AFC is not a reliable predictor of oocyte quality or spontaneous pregnancy potential, and combined biomarker approaches are warranted.

Estradiol (E2) and progesterone

Estradiol (E2) and progesterone are key indicators of follicular growth, ovulation, and luteal function. Early follicular phase E2 assists in the interpretation of basal FSH, as high E2 can suppress FSH artificially and obscure diminished ovarian reserve (DOR) [56]. Mid-luteal progesterone remains the biochemical gold standard for proof of ovulation and evaluation of the adequacy of the luteal phase. Recent reports suggest there might be a role for subclinical luteal phase progesterone functional deficit in implantation failure/recurrent miscarriage, especially in unexplained infertility [57]. But the fluctuation of progesterone secretion and difficulties in assay hindered it from serving as an efficient stand-alone indicator.

Prolactin, luteinising hormone (LH), and thyroid-stimulating hormone (TSH)

Prolactin, luteinizing hormone (LH), and thyroid-stimulating hormone (TSH) are the most common endocrine workups for ovulatory dysfunction. Hyperprolactinaemia impairs the release of gonadotropins and is a recognized, treatable cause of anovulatory infertility [58]. The abnormalities in LH secretion, including increased LH/FSH ratios observed in PCOS, are pathognomonic and reflect central (hypothalamic–pituitary) dysregulation [59]. Knowing that thyroid disease, even when subclinical, presents usefully as an explanation for menstrual irregularities as well as the etiology for implantation failure and adverse pregnancy outcome, regular TSH screening has been recommended for all infertile women [1].

Inhibin B

Inhibin B is secreted by early follicular granulosa cells and exerts negative feedback on FSH, as well as being an additional marker of ovarian reserve. Although inhibin B decreases before FSH as the ovary ages, test variability and lower predictive ability limit its clinical utility compared to AMH [60].

Androgens

Androgens, such as testosterone and dehydroepiandrosterone sulfate (DHEAS), have a dichotomous effect on female fertility. Physiologic levels of androgens are required for early follicular development, but in PCOS, hyperandrogenism is a hallmark feature leading to the arrest of follicle development and anovulation [61]. Recent evidence even seems to support that mild androgen deficiency likewise may compromise ovarian responsiveness, in particular of older patients undergoing ART [62].

Common biomarkers in PCOS

PCOS is associated with a spectrum of endocrine and metabolic abnormalities that manifest in the form of increased AMH, hyperandrogenemia, insulin resistance, and anovulatory and dysregulation of gonadotropin release [63-64]. Current international guidance highlights AMH as a supplementary diagnostic marker, but it has not yet supplanted the use of ultrasound criteria [7]. Metabolic factors have now emerged as key players in reproductive dysfunction and infertility risk amongst PCOS patients, and therefore, body metabolism, such as fasting insulin and HOMA-IR, is considered a powerful predictive marker for responses to treatment of PCOS patients [65-67]. This combination of reproductive and metabolic markers provides more complete insight into infertility in PCOS, thereby promoting personalized care [68-70].

There are various challenges that exist in the transfer of new biomarkers to clinical practice. These include the absence of assay standardization, interlaboratory variation, analytical costs, and a lack of clear evidence for cost-effectiveness compared with existing diagnostic pathways. In addition, studies are often performed in small, relatively homogeneous population samples, decreasing the generalizability. Further validation in a variety of patient populations and infertility phenotypes is critical. In the absence of standardized protocols and prospective results-based studies, premature clinical deployment might be a cause of inconsistency or misinterpretation.

Conclusions

The broader profile of biomarkers in female infertility indicates a move away from single-hormone biomarker assessment to an approach that considers the integrated function and molecular constitution in reproductive health. Classical biomarkers

AMH, FSH, and AFC, and the reproductive hormones E2, progesterone, LH, prolactin, TSH, and testosterone, as well as inhibin B, still remain a widely used tool for the primary infertility diagnostic of ovarian reserve assessment and endocrine diagnostics. Moreover, CA-125 still presents clinical relevance for the detection of endometriosis-associated infertility, especially when it is associated with imaging and clinician factors. However, these routine markers can tell us little about oocyte competency, embryo quality, or implantation potential.
From an integrative point of view, biomarkers can be conceptually divided into four main functional domains: ovarian reserve (AMH and AFC), oocyte competence (miRNAs, GDF9, BMP15, and metabolomics), endometrial receptivity (ERA, cytokines, and CTF1), and systemic or environmental modifiers (oxidative stress markers, microbiota, and phthalates). The most promising biomarkers for a near-term translation to clinical practice are the circulating miRNAs, follicular fluid (FF), cfDNA, and combined endocrine-molecular panels, especially in combination with an algorithm-based diagnostic model. These types of models can assist in better stratifying patients, minimizing treatment burden, and maximizing clinical outcomes.
With the development of novel biomarkers, such gaps are being addressed by enabling us to monitor reproduction with genetics, epigenetics, and intercellular communication. However, microRNAs (miRNAs) have recently emerged with robust non-invasive diagnostic utility, given their stability in biological fluids and regulatory involvement in ovarian and endometrial functioning, whilst disease-specific expression patterns have been observed across infertility phenotypes. Plasma and FF cell-free DNA (cfDNA) concentrations are associated with ovarian aging, follicular atresia, and ART outcomes, indicating a potential for embryo selection and cycle prognostication. Moreover, exosomes and extracellular vesicles offer a molecular imprint of the ovarian follicle and endometrial milieu, holding miRNAs, proteins, and growth factors that affect implantation and early embryogenesis. In the future, integration of existing and new biomarkers into diagnostic panels based on artificial intelligence, together with large-scale validation studies, offers potential to push forward precision diagnostics, prognostication, and personalized treatment in women with infertility.

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