

Integrated Biomarker System for Assessing Reproductive Potential in Mares: A Review

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Abstract: Complex interactions among structural, endocrine, and molecular factors determine mare reproductive potential. This narrative review summarizes current research data on clinical, hormonal, and molecular–biochemical biomarkers associated with mare fertility and evaluates their potential use in reproductive assessment. Clinical assessment remains the foundation of reproductive evaluation and includes age, reproductive history, ultrasonography, cytology, bacteriology, and endometrial biopsy. Hormonal biomarkers such as anti-Müllerian hormone, estradiol, progesterone, and gonadotropins provide additional information on ovarian reserve, follicular activity, ovulation, and luteal competence. Increasing attention has also focused on molecular and biochemical biomarkers related to inflammation, oxidative stress, extracellular matrix remodeling, and embryonic–maternal communication. Despite growing interest in advanced biomarker approaches, many molecular indicators are not yet sufficiently validated for routine clinical use in mares. Small sample sizes, methodological heterogeneity, and a lack of standardized reference ranges often limit available studies. Therefore, interpretation of reproductive biomarkers should remain cautious and clinically contextualized. Available data suggest that integrating structural, endocrine, and molecular information may improve reproductive evaluation and enable more individualized management strategies in mares. However, prospective studies evaluating the predictive value and clinical utility of integrated biomarker models remain limited.

Introduction

Reproductive efficiency in mares is influenced by several physiological factors, including seasonal reproductive activity, prolonged gestation, age-related fertility decline, and the production of a single offspring per pregnancy (Cuervo-Arango et al., 2010; Aurich, 2011; Scarlet et al., 2025). Fertility declines progressively with advancing age. This decline is associated with cumulative ovarian and uterine alterations, including depletion of the follicular reserve, impaired oocyte competence, chronic endometrial degeneration, and increased embryonic loss. Even though routine reproductive examinations allow identification of many clinically evident abnormalities, fertility outcomes in mares often remain difficult to predict accurately.

Traditional reproductive assessment relies primarily on clinical evaluation, ultrasonography, uterine cytology and bacteriology, and endometrial biopsy (Crabtree and Pycock, 2020; Gonçalves et al., 2020). These methods remain essential in equine reproductive practice because they provide direct structural and functional information about ovarian activity and uterine health. Nevertheless, some mares

with apparently normal clinical findings continue to exhibit subfertility or repeated pregnancy failure, suggesting that clinically undetectable endocrine or molecular disturbances may contribute to impaired reproductive performance.

Consequently, researchers have increasingly explored biomarkers reflecting ovarian reserve, endocrine activity, inflammatory status, oxidative balance, and embryo–maternal communication. Anti-Müllerian hormone has emerged as one of the most widely studied endocrine biomarkers of ovarian reserve and assisted reproductive performance (Ball et al., 2008; Canesin et al., 2023; Fonte et al., 2024), while progesterone and estradiol remain important indicators of luteal function and follicular activity (Satué et al., 2021; Goudet et al., 2022). However, endocrine markers alone cannot fully explain the complex cellular and molecular mechanisms underlying fertility.

Advances in transcriptomics, proteomics, lipidomics, and biochemical profiling have identified molecular signatures linked to uterine inflammation, oxidative stress, extracellular matrix remodeling, and early embryo–maternal signaling (Canisso et al., 2020; Ibrahim et al., 2021; Lawson et al., 2022; Silva-Álvarez et al., 2025). These approaches have the

potential to identify reproductive disturbances before overt clinical pathology becomes apparent. However, many proposed molecular biomarkers remain insufficiently validated for routine clinical use because small sample sizes, methodological heterogeneity, and lack of standardized reference ranges often limit available studies.

Because reproductive competence depends on interactions among multiple physiological systems, interpretation based on a single biomarker category may provide only partial information. Integration of clinical, endocrine, and molecular findings has therefore been proposed as a potential strategy to improve reproductive assessment in mares (Papaas et al., 2021; Fouché et al., 2022). Nevertheless, evidence supporting integrated biomarker-based assessment in mares is still limited and fragmented.

This review critically evaluates the current data on clinical, hormonal, and molecular-biochemical biomarkers related to the reproductive potential of mares. Even if these biomarker groups have been described separately in previous studies, their practical integration remains poorly defined. In addition to summarizing available data, this narrative review examines the relative relevance of individual biomarkers and proposes a clinically oriented model for their interpretation in reproductive decision-making.

Literature Search Strategy

This structured narrative review employed a transparent literature search strategy to identify relevant studies on biomarkers associated with reproductive potential in mares. Literature searches were performed in PubMed, Scopus, and Web of Science databases using combinations of the following keywords: *mare fertility, equine reproduction, biomarkers, anti-Müllerian hormone, endometritis, cytokines, oxidative stress, lipidomics, and reproductive biomarkers.*

A total of 312 records were identified across all databases. After removing 74 duplicate records, 238 publications remained for title and abstract screening. During the initial screening phase, 151 records were excluded because they were unrelated to reproductive biomarkers in mares, focused on other species, or did not address fertility assessment. The full texts of 87 articles were assessed for eligibility.

Studies were excluded if they: were conference abstracts, editorials, book chapters, or non-peer-reviewed publications; focused exclusively on other equine species or unrelated reproductive conditions; lacked methodological transparency; or did not provide direct evidence relevant to fertility assessment in mares.

After full-text review of 87 articles, 37 studies were rejected because they did not meet the eligibility criteria. Finally, 50 publications were selected for qualitative synthesis because they provided direct

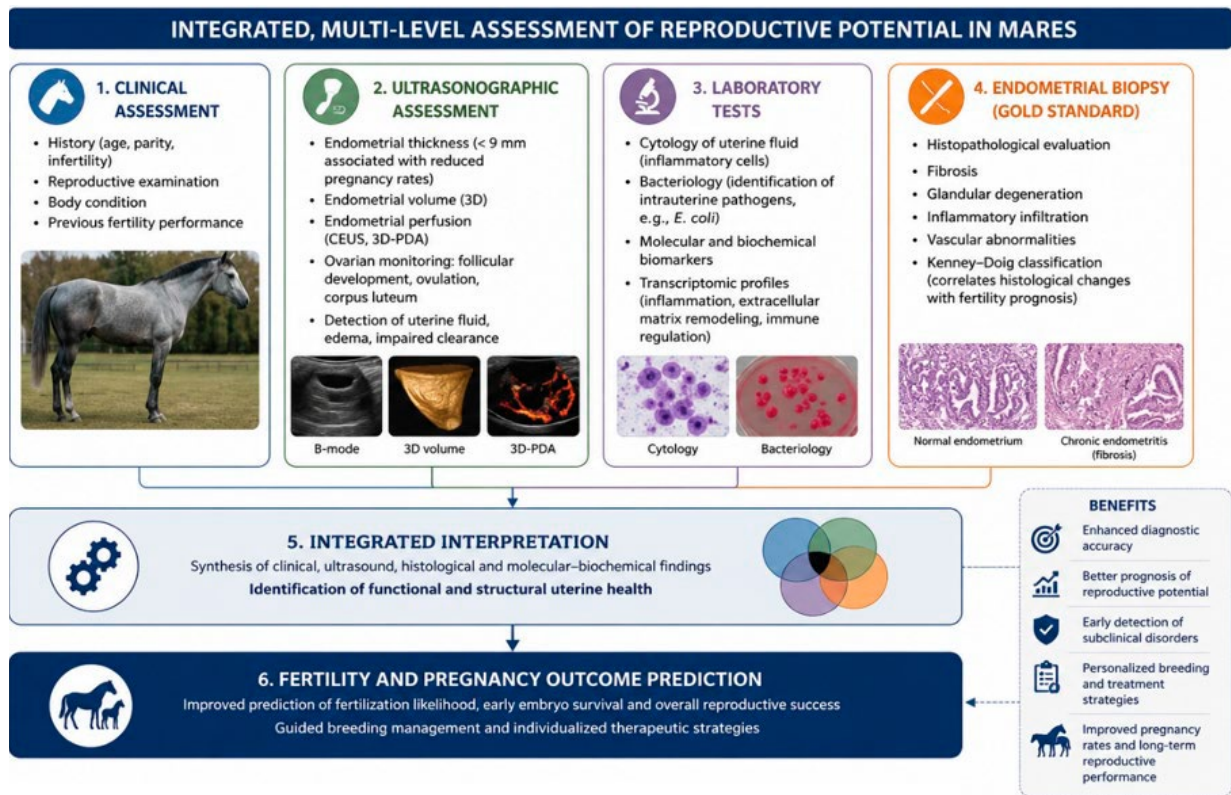


Fig. 1. Schematic representation of the integrated multi-level clinical assessment of reproductive potential in mares (adapted from Katila, 2016; Ferreira et al., 2015; Crabtree and Pycocok, 2020; Claes et al., 2017; Gonçalves et al., 2020; Fawcett et al., 2021; Morris et al., 2020; Ferreira-Dias et al., 2024; Ishak et al., 2019.) Abbreviations: CEUS, contrast-enhanced ultrasonography; 3D-PDA, three-dimensional power Doppler angiography.

evidence on the use of clinical, hormonal, molecular, and biochemical biomarkers to assess reproductive potential and fertility in mares. The final selection of studies was based on their relevance to the review objectives and the authors' critical appraisal of the available evidence.

Clinical Biomarkers

Clinical biomarkers remain the cornerstone of reproductive evaluation in mares, providing essential information about the structural and functional integrity and reproductive potential of the organism. These indicators – age, reproductive history, cytology of uterine fluid, bacteriology, endometrial biopsy, and ultrasound – capture macroscopic and physiological changes that directly affect fertility and remain irreplaceable in normal reproductive practices (Katila, 2016; Crabtree and Pycock, 2020; Cortés-Vidauri et al., 2018).

Mare age is one of the main determinants of reproductive potential, reflecting changes in the ovarian reserve, uterus, and endocrine system, because fertility gradually declines with age (Claes et al., 2017; Gonçalves et al., 2020; Fawcett et al., 2021). Older mares exhibit reduced antral follicle counts, slower follicular growth, prolonged interovulatory intervals, and decreased follicular maturation efficiency, along with reduced expression of epidermal growth factor receptor (EGFR), vascular endothelial growth factor

(VEGF), luteinizing hormone receptor (LHR), and the Ki67 marker in follicular cells. Ki67 is a classic marker of cell proliferation that indicates granulosa cell activity and follicular growth rate (Campos-Chillon et al., 2015). These changes, combined with age-related mitochondrial dysfunction and increased chromosomal abnormalities in follicles, reduce embryonic viability and increase the risk of early embryo loss (Hendriks et al., 2015; Rizzo et al., 2019). Age-related uterine degeneration, including fibrosis, glandular atrophy, and increased risk of endometriosis and collagen type III (COL3), further reduces the likelihood of fertilization (Ferreira et al., 2015; Sikora et al., 2016; Schöniger and Schoon, 2020; Morris et al., 2020).

This schematic in Fig. 2 illustrates the age-associated decline in reproductive potential in mares and the interconnected ovarian, uterine, and cellular mechanisms contributing to reduced fertility. An optimal ovarian reserve, high oocyte quality, and minimal fibrotic changes within ovarian and endometrial tissues characterize young mares. During the transition to middle age, declining anti-Müllerian hormone (AMH) concentrations and antral follicle count (AFC) are accompanied by increasing oxidative stress, resulting in reduced embryo competence and lower developmental potential. In aged mares, ovarian reserve is markedly diminished, while chronic inflammation, fibrosis, endometriosis, and

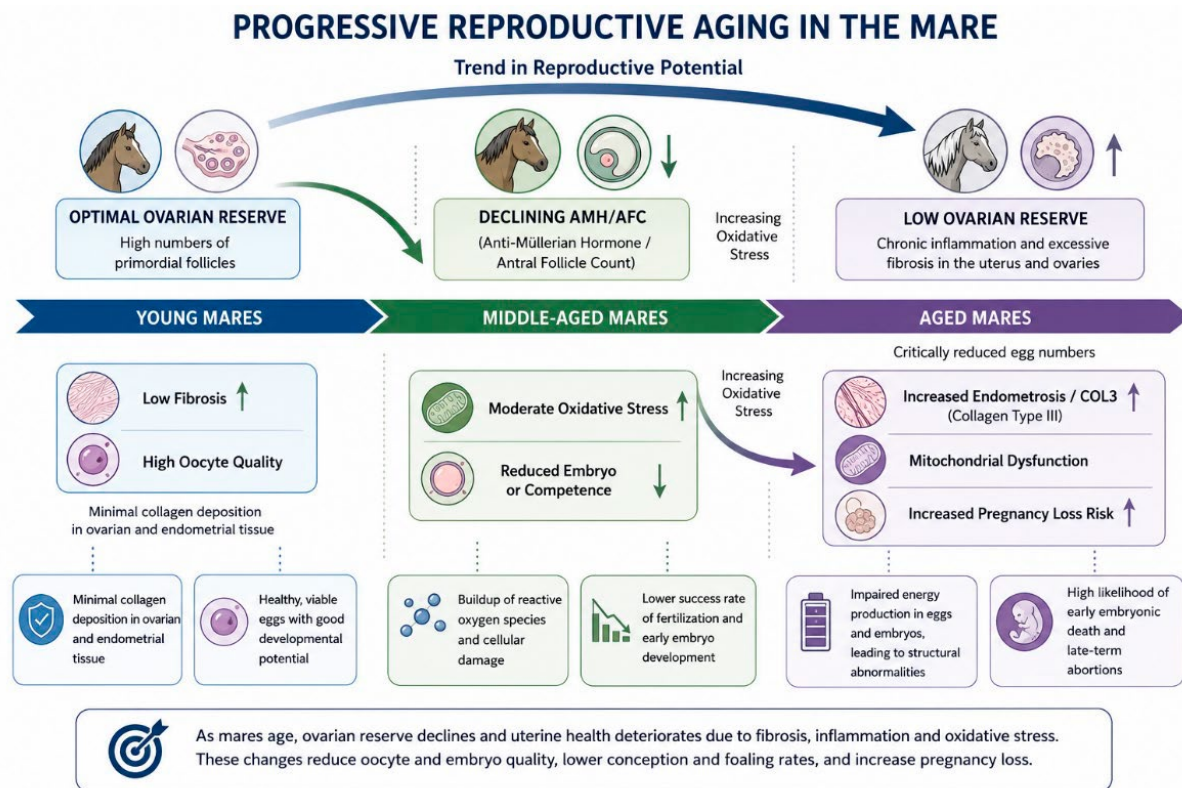


Fig. 2. Progressive reproductive aging and its impact on fertility outcomes in mares (adapted from Claes et al., 2017; Gonçalves et al., 2020; Fawcett et al., 2021; Campos-Chillon et al., 2015; Hendriks et al., 2015; Rizzo et al., 2019; Morris et al., 2020; Cortés-Vidauri et al., 2018). Abbreviations: AMH, anti-Müllerian hormone; AFC, antral follicle count; COL3, collagen type III.

increased deposition of type III collagen (COL3) further compromise reproductive function. Age-related mitochondrial dysfunction and oxidative damage impair oocyte and embryo quality, increasing the likelihood of early embryonic loss and pregnancy failure. Collectively, these progressive alterations reduce fertilization success, embryo viability, and overall reproductive performance in mares.

Reproductive history is an important clinical indicator of reproductive performance, reflecting previous reproductive capacity and providing retrospective evidence of general health status and reproductive system competence. Mares with a history of successful foaling tend to have higher rates of fertilization and fetal delivery, whereas mares that have not been fertilized or have had previous problems often have underlying uterine pathology, impaired clearance mechanisms, or endocrine dysfunction (Katila, 2016; Cecchini et al., 2023). Recurrent endometritis, accumulation of intrauterine fluid, or early embryo loss are particularly informative indicators of uterine dysfunction. Subclinical endometritis is frequently observed in intensively managed breeding mares and is especially common in older mares with slowed natural uterine clearance (Ferreira et al., 2015; Morris et al., 2020; Ferreira-Dias et al., 2024).

In such cases, cytology and bacteriology of the uterine fluid are necessary for diagnosing endometritis, enabling identification of inflammatory cells and intrauterine pathogens. Recent research has advanced current knowledge and identified an important subset of mares with abundant *E. coli* growth, yet important knowledge gaps remain regarding the etiologies that lead to poorer fertility outcomes (Fehin et al., 2025). Endometrial biopsy remains the gold standard for structural assessment of uterine health, especially in older mares with chronic endometritis (Silva Rua et al., 2018). Histopathological evaluation of uterine mucosal samples allows detection of fibrosis, glandular degeneration, inflammatory infiltration, and vascular abnormalities that impair uterine receptivity and fertility (Buchovska et al., 2016; Schöniger and Schoon, 2020). Chronic inflammation and fibrosis of the endometrium are closely related to fertilization and increased early embryo loss. The Kenney-Doig classification system correlates the intensity of histological changes in the uterine mucosa with the likelihood of fertilization, so biopsy remains a powerful prognostic tool for reproductive potential. Recent transcriptomic studies also show that mares with endometrosis exhibit altered expression of genes involved in inflammation, extracellular matrix remodeling, and immune regulation (Drzewiecka et al., 2025).

The uterine endometrium provides the critical interface for embryo implantation and pregnancy establishment. Endometrial receptivity – the capacity of the endometrium to accept an embryo – depends on precisely timed endocrine signaling, vascular

development, and immune regulation. While subjective ultrasonographic morphology assessment has been the traditional approach to endometrial evaluation in mares, emerging evidence demonstrates that quantitative ultrasound parameters enhance predictive accuracy for pregnancy outcomes.

Endometrial thickness represents one of the most commonly assessed parameters, with studies demonstrating that thickness below 9 mm is significantly associated with reduced implantation rates. More sophisticated ultrasound assessments, including three-dimensional (3D) imaging to measure endometrial volume and subendometrial blood flow, provide additional predictive information. Advanced ultrasound technologies such as contrast-enhanced ultrasonography (CEUS) and three-dimensional power Doppler angiography (3D-PDA) enable assessment of endometrial perfusion and microvascular function (Ferreira-Dias et al., 2024).

Transrectal ultrasound is a valuable tool for monitoring follicular development, ovulation timing, and corpus luteum formation, all of which are essential for optimizing breeding management (Ortega-Ferresola et al., 2022; Panzani et al., 2024; Khan et al., 2025). Additionally, ultrasound can detect abnormalities such as uterine fluid, abnormal endometrial edema, or impaired uterine clearance, findings that can directly predict the chances of fertilization. Seasonal variations in ovarian activity and patterns of follicular waves further highlight the importance of ultrasound in mare breeding. It is the primary method for early pregnancy diagnosis and monitoring embryonic development (Ishak et al., 2019). Despite its established clinical relevance, the use of clinical biomarkers may be limited by the inability to detect early or subclinical dysfunction. Most available evidence is derived from observational and retrospective studies, which can introduce biases related to mare selection, management conditions, and diagnostic criteria. Furthermore, clinical data often reflect late-stage pathology rather than early functional disorders, underscoring the need for additional hormonal and molecular-biochemical biomarkers.

For older mares with reproductive issues, the diagnostic and prognostic value of clinical tests is enhanced when findings are interpreted alongside hormonal and molecular-biochemical biomarkers, according to an integrated, multi-level assessment algorithm for reproductive potential.

Hormonal Biomarkers

Hormonal biomarkers are valuable indicators of reproductive function because they reflect activity within the hypothalamic–pituitary–gonadal (HPG) axis and provide information on ovarian reserve, follicular development, ovulation, and luteal function. Because endocrine regulation governs both the estrous cycle and the establishment of early

pregnancy, deviations in hormonal profiles serve as sensitive indicators of reproductive dysfunction. Unlike structural evaluation alone, hormonal biomarkers reflect dynamic physiological processes that vary throughout the reproductive cycle (Aurich, 2011; Ishak et al., 2019).

Anti-Müllerian hormone (AMH) is currently considered the most informative endocrine marker of ovarian reserve in mares. Circulating AMH concentrations correlate with antral follicle count, oocyte yield, and reproductive lifespan. Consequently, AMH is widely used to estimate the likelihood of successful oocyte recovery and embryo production in assisted reproductive programs (Ball et al., 2008; Fonte et al., 2024; Canesin et al., 2023). AMH is also used to select donor mares for OPU/ICSI programs, as higher AMH concentrations are associated with greater oocyte recovery and embryo production efficiency (Papaas et al., 2021). However, AMH does not directly predict pregnancy outcome and exhibits interindividual variability; it remains the strongest available indicator of granulosa cell activity and follicular pool size. Age-related reductions in AMH and altered antioxidant status further reflect declining ovarian reserve in older mares (Aboelmaaty et al., 2024).

Estradiol (E2), produced by granulosa cells of developing follicles, reflects follicular competence and readiness for ovulation. Increasing estradiol concentrations during the follicular phase indicate granulosa cell proliferation and aromatase activity, while reduced estradiol levels – particularly in aged or subfertile mares – are associated with impaired follicular development and decreased fertility (Goudet et al., 2022; Satué et al., 2021; Gonçalves et al., 2020). Estradiol also modulates uterine receptivity and immune responses, thereby influencing susceptibility to endometritis and potentially affecting embryo survival (Fumuso et al., 2000; Klein et al., 2010).

Progesterone (P4), secreted by the corpus luteum, is essential for establishing and maintaining pregnancy. It supports endometrial secretory transformation, suppresses uterine contractility, and promotes embryo development. Inadequate progesterone production has been linked to an increased risk of early embryonic loss, highlighting its clinical relevance in assessing luteal function (Satué et al., 2021; Goudet et al., 2022). Progesterone metabolites, including allopregnanolone, offer additional insight into gestational endocrine activity, although their use remains primarily research-based (Satué et al., 2023; Veronesi et al., 2025).

Gonadotropins, including folliclestimulating hormone (FSH) and luteinizing hormone (LH), regulate follicular recruitment, maturation, and ovulation. Their pulsatile secretion reflects hypothalamic and pituitary function and is influenced by photoperiod, metabolic status, and environmental

cues (Aurich, 2011; Donadeu et al., 2014; CortésVidauri et al., 2018). Seasonal reproductive transitions are accompanied by significant changes in gonadotropin secretion and ovarian responsiveness (Ishak et al., 2019). Interpretation is complicated by their pulsatile secretion patterns and the limited diagnostic value of individual measurements.

Additional steroid and stress-related hormones may offer complementary information about endocrine aging, metabolic status, and systemic stress responses. Although changes in these hormones have been linked to reproductive dysfunction, their diagnostic value is less established than that of AMH, progesterone, or estradiol (Falomo et al., 2020; Satué et al., 2023; Veronesi et al., 2025). Lipidomic and steroidomic approaches may identify additional metabolic indicators linked to reproductive performance (Lawson et al., 2022).

Interpretation of hormonal biomarkers is complicated by temporal variability, as most endocrine parameters strongly depend on cycle stage, season, and sampling time. Many studies rely on single-time-point measurements, which may not accurately reflect dynamic endocrine function. Additionally, interindividual variability and the lack of standardized reference ranges limit their predictive value across different mare populations.

Endocrine biomarkers provide information that structural assessment alone cannot offer. When integrated with clinical and molecular–biochemical biomarkers, hormonal profiles enable more precise assessment of reproductive potential and improved reproductive management strategies.

A summary of hormonal biomarkers associated with reproductive potential in mares is provided in Table 1.

Hormonal biomarkers reflect functional endocrine activity of the hypothalamic–pituitary–gonadal axis and provide essential information on ovarian reserve, follicular competence, ovulatory function, and luteal activity. Anti-Müllerian hormone is the most reliable indicator of ovarian reserve, while progesterone and estradiol reflect luteal competence and follicular activity. Gonadotropins and additional steroid hormones provide complementary insight into endocrine regulation and reproductive potential.

The integration of hormonal biomarkers within the hypothalamic–pituitary–gonadal axis and their relevance for reproductive potential is illustrated in Fig. 3.

Hormonal biomarkers reflect endocrine regulation of follicular development, ovulation, luteal function, and uterine receptivity. Anti-Müllerian hormone reflects ovarian reserve, estradiol indicates follicular competence, and progesterone reflects luteal function and pregnancy support. Gonadotropins regulate ovarian activity, while additional steroid hormones provide complementary information on systemic endocrine regulation and reproductive status.

Table 1. Hormonal biomarkers associated with reproductive potential in mares

Hormone	Clinical Interpretation	Prognostic Value	Limitations	Clinical Application Level	Key References
AntiMüllerian hormone (AMH)	Indicates ovarian reserve, predicts oocyte yield and response to ART (OPU-ICSI)	High	Does not directly predict pregnancy outcome; inter-individual variability	Widely used, validated	Ball et al., 2008; Fonte et al., 2024; Papaas et al., 2021
Progesterone (P4)	Assesses luteal function and pregnancy maintenance	High	Single measurements may not reflect dynamic luteal function	Routine clinical use	Ginther, 2008; Goudet et al., 2022; Satué et al., 2021
Estradiol (E2)	Indicates follicular competence and ovulatory readiness	Moderate-High	Strong cyclestage dependency	Routine clinical use	Goudet et al., 2022; Aboelmaaty et al., 2024; Satué et al., 2021
Folliclestimulating hormone (FSH)	Reflects ovarian responsiveness and follicular recruitment	Moderate	Pulsatile secretion complicates interpretation	Limited clinical use	Aurich, 2011; Cortés-Vidauri et al., 2018
Luteinizing hormone (LH)	Indicates ovulatory function and reproductive cyclicity	Moderate	Short surge duration limits the diagnostic window	Limited clinical use	Aurich, 2011; Cortés-Vidauri et al., 2018
Testosterone	Reflects ovarian steroidogenic activity	Moderate	Age and cycle-dependent variation	Emerging clinical relevance	Donadeu et al., 2014; Satué et al., 2023
Dehydroepiandrosterone (DHEA)	Indicates endocrine aging and reproductive endocrine status	Emerging	Limited clinical reference ranges	Research stage	Satué et al., 2023; Veronesi et al., 2025
Cortisol	Indicates stress-related reproductive suppression	Emerging	Influenced by environmental and physiological stressors	Research stage	Veronesi et al., 2025
Progesterone metabolites (e.g., allopregnanolone)	Indicate pregnancy progression and luteal function	Emerging	Primarily research application	Research stage	Veronesi et al., 2025

Molecular and Biochemical Biomarkers

Molecular and biochemical biomarkers enable the assessment of cellular and molecular processes that routine clinical tests cannot directly measure. These biomarkers can detect early cellular changes before clinical signs of reproductive disease appear. However, their clinical application remains limited. Many published studies involve relatively small populations, different analytical methods, and non-standardized sampling procedures, making direct comparison of results difficult. For this reason, most molecular biomarkers should currently be considered promising research tools rather than established diagnostic markers (Morris et al., 2020; Falomo et al., 2020; Lawson et al., 2022; Silva-Álvarez et al., 2025).

Even though most molecular biomarkers are still in the research stage, they may eventually complement routine reproductive assessment by improving our understanding of the biological mechanisms involved

in fertility. The main groups of molecular and biochemical biomarkers relevant to the reproductive potential of mares are summarized in Table 2.

Inflammatory biomarkers are among the most extensively studied molecular indicators of uterine dysfunction in mares. Subclinical endometritis is associated with increased production of inflammatory mediators like prostaglandin E₂ (PGE₂), 6ketoPGF₁α, and leukotriene C₄, which disrupt immune homeostasis and impair uterine function (Siemieniuch et al., 2017). Older mares exhibit heightened inflammatory responses, contributing to increased susceptibility to persistent breeding-induced endometritis (Morris et al., 2020; FerreiraDias et al., 2024). Altered cytokine expression has been linked to differences in uterine immune responsiveness between resistant and susceptible mares. This suggests potential value in identifying mares predisposed to reproductive dysfunction (Fumuso et al., 2000; Klein

et al., 2010). Neutrophil extracellular traps (NETs) further contribute to endometrial inflammation and tissue damage, exacerbating reproductive dysfunction (Rebordão et al., 2018; Rebordão et al., 2021).

Oxidative stress has received increasing attention because excessive levels of reactive oxygen species can impair oocyte quality, embryo development, and uterine receptivity. Several markers of oxidative stress have been associated with impaired reproductive

function and reduced uterine function (Agarwal et al., 2014; Boni et al., 2022). Oxidative dysregulation is particularly relevant in older mares, where reduced antioxidant capacity and increased levels of oxidative metabolites correlate with reduced ovarian reserve and altered AMH dynamics (Aboelmaaty et al., 2024). Oxidative stress has also been associated with reproductive disorders and impaired uterine function in mares (Falomo et al., 2020).

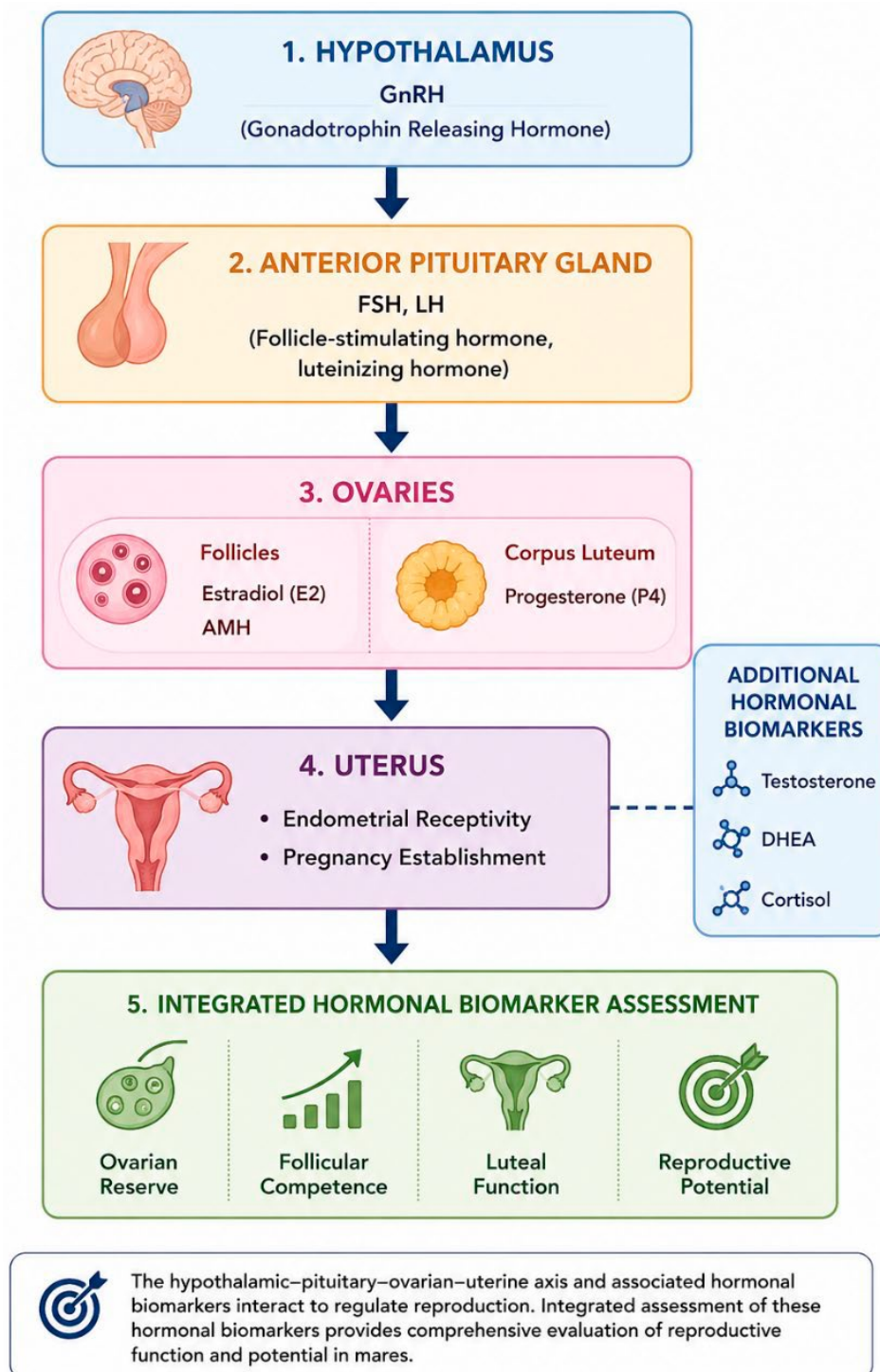


Fig. 3. Integration of hormonal biomarkers within the hypothalamic–pituitary–gonadal (HPG) axis and their role in determining reproductive potential in mares (adapted from Ball et al., 2008; Ginther, 2008; Aurich, 2011; Veronesi et al., 2025).

Table 2. Molecular and biochemical biomarkers associated with reproductive potential in mares

Biomarker Group	Clinical Interpretation Level	Clinical Application Level	Key References
Cytokines	Indicators of uterine inflammation and immune competence	Emerging and transitioning to clinical use	Fumuso et al., 2000; Klein et al., 2010
Acute-phase proteins	Biomarkers of uterine inflammation and reproductive pathology	Limited clinical use	Falomo et al., 2020; Morris et al., 2020
Oxidative stress biomarkers	Indicators of endometrial dysfunction and reproductive stress	Research only	Agarwal et al., 2012; Boni et al., 2022
MicroRNAs	Biomarkers of endometritis and reproductive dysfunction	Research only	Ibrahim et al., 2021
Proteomic biomarkers	Biomarkers of early pregnancy and reproductive status	Research only	Ortega-Ferresola et al., 2022
Extracellular matrix biomarkers	Indicators of endometrial fibrosis and reduced fertility	Research-clinical transition	Ferreira Dias et al., 2024
Lipidomic biomarkers	Reflect uterine inflammation and reproductive function	Research only	Ibrahim et al., 2021; Lawson et al., 2022

Extracellular matrix (ECM) remodeling biomarkers capture structural and functional changes in the endometrium. Altered expression of extracellular matrix remodeling factors has been associated with fibrosis and decreased uterine receptors (Schöniger and Schoon, 2020; Ferreira-Dias et al., 2024). ECM remodeling is closely associated with chronic endometritis and degenerative uterine changes, including endometriosis, which is a major factor in reduced fertility in older mares. Proteolytic enzymes and NET-associated proteases further contribute to ECM degradation and dysregulated prostaglandin synthesis (Rebordão et al., 2018; Rebordão et al., 2021).

Transcriptomic and proteomic approaches have expanded current understanding of embryo-maternal communication and uterine function. High-throughput analyses have identified molecular signatures linked to embryo viability and uterine receptivity (Morris et al., 2020; Silva-Álvarez et al., 2025). Transcriptomic studies have also demonstrated age-related alterations in myometrial gene expression and pathways associated with inflammation, ECM remodeling, and immune regulation (Drzewiecka et al., 2025). Circulating microRNAs are being studied as potential non-invasive biomarkers for uterine dysfunction (Ibrahim et al., 2021).

Lipidomic and steroidomic analyses are a relatively new area of equine reproductive research. Lipid mediators, steroid metabolites, and bioactive lipids reflect inflammatory status, oxidative balance, and steroidogenic activity, offering additional mechanistic insight into reproductive competence (Falomo et al., 2020; Lawson et al., 2022). These biomarkers may help identify mares with subtle metabolic or inflammatory disturbances that affect fertility but remain undetected by conventional endocrine or clinical assessment.

Overall, molecular and biochemical biomarkers provide valuable information about the cellular mechanisms underlying reproductive function. Their

main value lies in identifying subtle inflammatory, oxidative, or fibrotic changes that may otherwise remain undetected. Nevertheless, broader clinical application will require larger validation studies, methodological harmonization, and clearer reference ranges.

Integrated Biomarker-Based Reproductive Potential Assessment System

Reproductive performance in mares is influenced by the interaction of structural, endocrine, immunological, and molecular processes. Each biomarker category reflects a distinct aspect of reproductive physiology and provides additional information for assessing fertility. The assessment system proposed in this review integrates clinical, hormonal, and molecular-biochemical indicators into a conceptual multi-level approach to reproductive assessment (Papaas et al., 2021; Fouché et al., 2022).

The proposed system is based on clinical indicators that provide basic information about the integrity of the reproductive system, ovarian activity, and reproductive function history. Age, reproductive history, ultrasonographic findings, and endometrial biopsy collectively establish the structural and functional context within which endocrine and molecular processes operate (Katila, 2016; Crabtree and Pycock, 2020).

Endocrine biomarkers play a functional component in the system, characterizing ovarian activity and reproductive cycles. Anti-Müllerian hormone reflects granulosa cell activity and follicular pool size (Ball et al., 2008; Fonte et al., 2024; Canesin et al., 2023), estradiol indicates follicular maturation, and progesterone represents luteal function and early pregnancy maintenance (Satué et al., 2023; Goudet et al., 2022). Gonadotropins and other steroid hormones further characterize hypothalamic-pituitary-gonadal axis activity and seasonal reproductive transitions (Aurich, 2011; CortésVidauri et al., 2018).

Molecular and biochemical markers provide mechanistic information about inflammatory, oxidative, and extracellular matrix processes that may affect fertility. Cytokines, oxidative stress indicators, ECM remodeling factors, lipid mediators, and transcriptomic signatures may help identify biological alterations associated with subclinical reproductive dysfunction (Morris et al., 2020; Ibrahim et al., 2021; Lawson et al., 2022; Silva-Álvarez et al., 2025). Integration of these biomarker categories may provide a more comprehensive assessment of reproductive potential than evaluation of individual markers alone.

However, not all biomarker categories contribute equally to clinical decision-making. The authors propose the following categorization based on the

current level of validation, reproducibility, and clinical applicability reported in the literature. Step 1 (high clinical relevance) includes age, reproductive history, ultrasonographic findings, endometrial biopsy, anti-Müllerian hormone, progesterone, and estradiol, which are supported by consistent evidence and widely used in clinical decision-making. Step 2 (moderate relevance) comprises gonadotropins, uterine cytology and bacteriology, and selected acute-phase proteins, which provide supportive but context-dependent information. Step 3 (emerging or research-stage biomarkers) includes cytokines, oxidative stress markers, microRNAs, and proteomic and lipidomic biomarkers, which offer mechanistic insight but currently lack standardized validation and

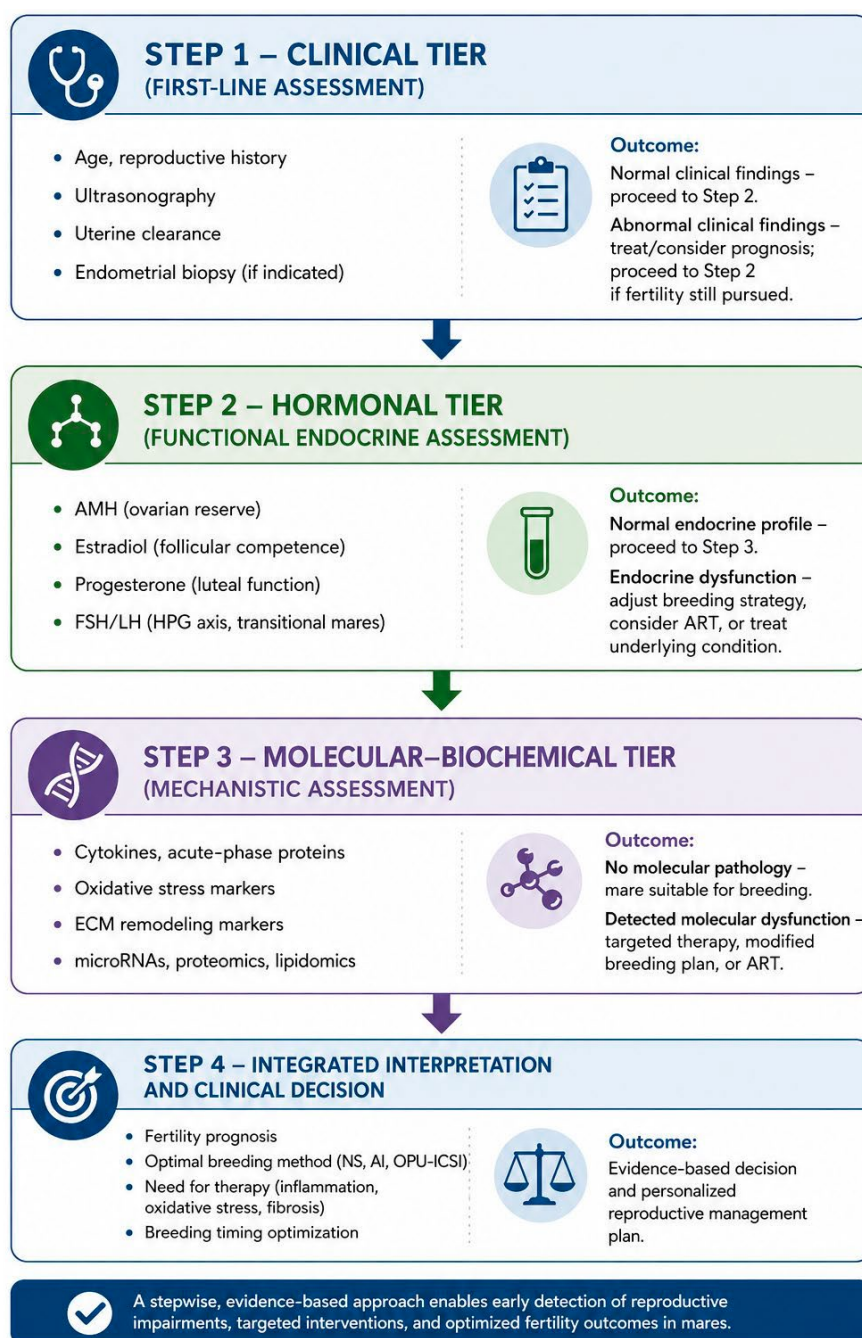


Fig. 4. Clinical decision algorithm for assessing reproductive potential in mares

routine clinical applicability.

This prioritization highlights that, despite increasing interest in molecular biomarkers, clinical and selected hormonal indicators currently provide the most robust and actionable information for fertility assessment in mares (Fig. 4).

This three-step decision algorithm guides clinicians through a series of assessments to identify structural abnormalities, endocrine dysfunction, and molecular pathology that may affect the reproductive potential of mares.

Evidence supporting integrated biomarker assessment is increasing; however, the relative contribution of individual biomarker groups to fertility prediction remains unclear. Clinical assessment and selected endocrine biomarkers provide the most reproducible and clinically applicable information, while many molecular biomarkers remain primarily research tools that require further validation before routine implementation can be recommended (Fouché et al., 2022).

Discussion

The assessment of reproductive potential in mares remains challenging because fertility is influenced by numerous interacting physiological, anatomical, endocrine, and environmental factors (Aurich, 2011; Gonçalves et al., 2020). Although individual biomarkers have been associated with specific reproductive outcomes, relatively few studies have evaluated how different biomarker categories perform when interpreted together (Papaas et al., 2021; Fouché

et al., 2022). Therefore, the framework proposed in this review should be viewed as a synthesis of currently available evidence rather than a validated predictive model.

Clinical examination continues to provide the foundation for reproductive evaluation. Parameters such as mare age, reproductive history, ultrasonographic findings, uterine cytology, bacteriology, and endometrial biopsy offer direct information regarding reproductive tract health and have established diagnostic relevance in equine practice. Also, understanding the normal events of foaling, causes of dystocia, and clinical outcomes is important for equine practitioners. (Katila, 2016; McCue and Ferris, 2011; Crabtree and Pycoc, 2020). Age-related reproductive decline, impaired uterine clearance, chronic inflammation, and endometrial fibrosis are consistently associated with reduced fertility and increased pregnancy loss (Ferreira et al., 2015; Claes et al., 2017; Morris et al., 2020; Ferreira-Dias et al., 2024). However, mares with apparently normal clinical findings may still experience subfertility, indicating that structural assessment alone does not always capture the biological mechanisms affecting reproductive success (Gonçalves et al., 2020; Fawcett et al., 2021).

Endocrine biomarkers provide complementary information by reflecting functional activity within the hypothalamic–pituitary–gonadal axis (Aurich, 2011; Ishak et al., 2019). Among currently available hormonal indicators, anti-Müllerian hormone appears to be the most informative marker of ovarian

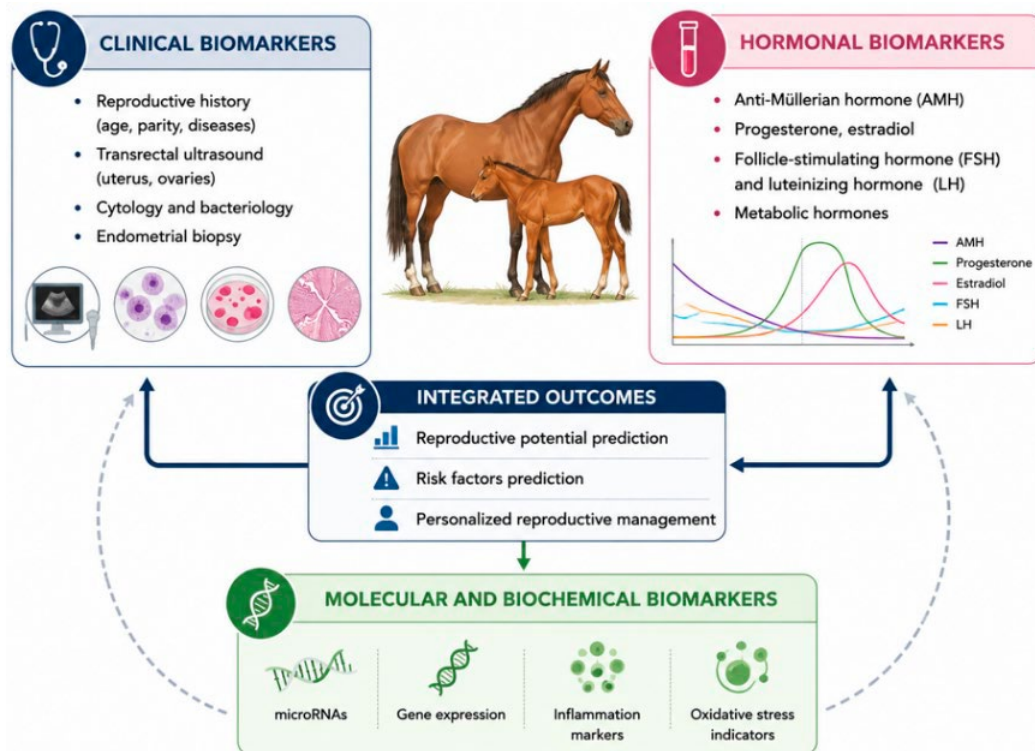


Fig. 5. Integrated biomarker-based assessment of reproductive potential in mares (adapted from Ball et al., 2008; Morris et al., 2020; Fouché et al., 2022; Fonte et al., 2024).

reserve and assisted reproduction performance (Ball et al., 2008; Canesin et al., 2023; Fonte et al., 2024). Nevertheless, interpretation of endocrine data requires consideration of cycle stage, season, age, and individual variability (Aurich, 2011; Cortés-Vidauri et al., 2018; Satué et al., 2021). Hormone concentrations represent dynamic physiological processes rather than static characteristics, which may partly explain the variability observed among studies evaluating their association with fertility outcomes (Goudet et al., 2022).

Recent advances in molecular biology have expanded understanding of reproductive processes beyond conventional clinical assessment. Cytokine profiles, oxidative stress indicators, extracellular matrix remodeling markers, transcriptomic signatures, and lipidomic profiles have all been linked to reproductive health and disease in mares (Fumuso et al., 2000; Ibrahim et al., 2021; Lawson et al., 2022; Silva-Álvarez et al., 2025). These approaches are particularly valuable because they may reveal biological alterations before overt clinical pathology becomes evident (Morris et al., 2020; Rebordão et al., 2021; Drzewiecka et al., 2025). At the same time, considerable methodological heterogeneity remains a major limitation. Differences in sampling procedures, analytical platforms, study populations, and outcome definitions complicate direct comparison of results and hinder the establishment of clinically meaningful reference values (Falomo et al., 2020; Lawson et al., 2022; Silva-Álvarez et al., 2025).

An important observation from the reviewed

literature is that biomarkers differ substantially in their level of validation and practical applicability. Clinical examination and selected hormonal markers are already integrated into routine reproductive management, whereas most molecular biomarkers remain primarily investigational (Katila, 2016; Ball et al., 2008; Fonte et al., 2024). This discrepancy does not diminish the biological importance of molecular findings; rather, it highlights the gap between mechanistic discovery and clinical implementation. Future studies should therefore focus not only on identifying new biomarkers but also on determining their reproducibility, diagnostic accuracy, and practical utility under field conditions (Fouché et al., 2022).

Integrating information from multiple biological levels may enhance reproductive decision-making, especially in mares with unexplained subfertility or inconsistent breeding outcomes (Papaas et al., 2021; Fouché et al., 2022). For example, assessing ovarian reserve using anti-Müllerian hormone may complement ultrasonographic evaluation (Ball et al., 2008; Fonte et al., 2024), while inflammatory and extracellular matrix biomarkers may provide additional insights into uterine health that are not apparent during routine examination (Schöniger and Schoon, 2020; Ferreira-Dias et al., 2024). Such combined approaches may enable more individualized breeding management and improve the selection of diagnostic or therapeutic interventions. However, evidence supporting specific biomarker combinations is limited, and prospective validation studies are needed before integrated biomarker panels can be

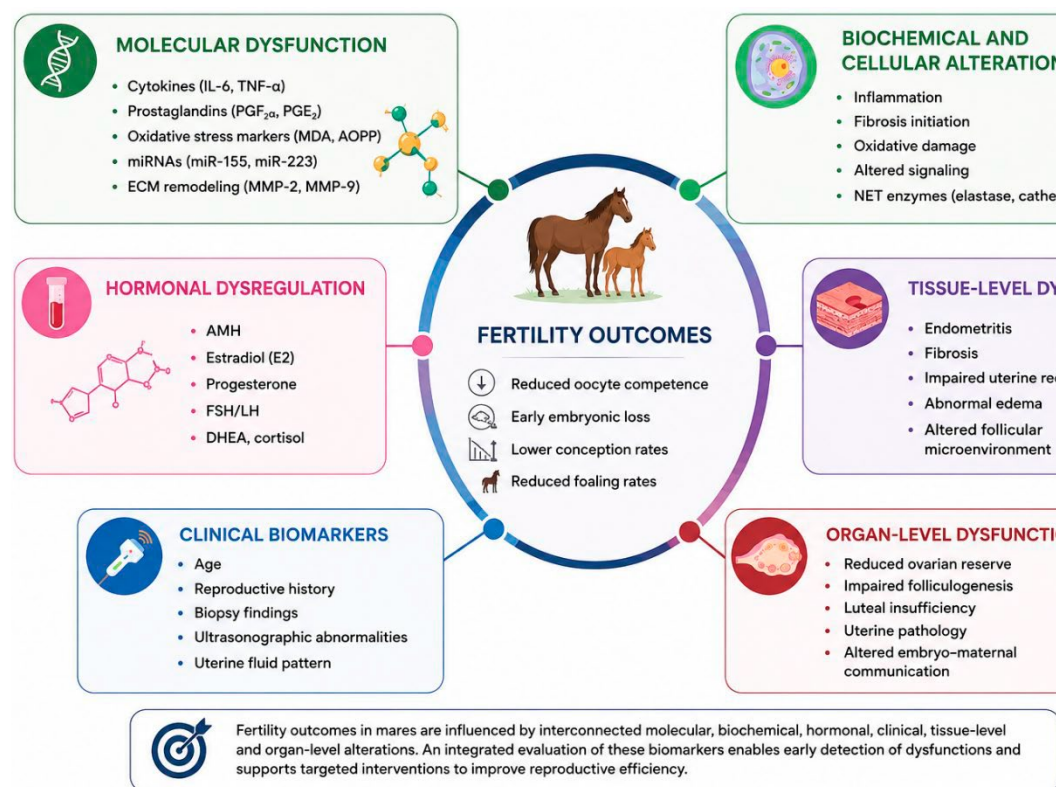


Fig. 6. Schematic representation of the biomarker cascade from molecular dysfunction to fertility outcomes in mares.

recommended for routine use (Fouché et al., 2022).

Future progress will require larger prospective studies, standardized sampling protocols, and greater methodological consistency across laboratories. Longitudinal investigations following mares through multiple breeding seasons may be particularly valuable, as they would allow evaluation of temporal biomarker dynamics and their relationship with reproductive outcomes (Ishak et al., 2019; Veronesi et al., 2025). Establishing robust reference ranges and validating promising molecular markers in clinically diverse populations should be considered essential priorities for future research (Lawson et al., 2022; Silva-Álvarez et al., 2025). The integration of clinical, hormonal, and molecular biomarkers and their collective contribution to reproductive potential assessment systems is illustrated in Fig. 5.

Fig. 5 summarizes the major biomarker categories currently investigated in mare reproductive assessment and illustrates their possible combined interpretation.

In Fig. 6, the central node is illustrated as the key fertility outcomes. Surrounding domains represent major contributors to reproductive dysfunction (Morris et al., 2020; Silva-Álvarez et al., 2025; Ferreira-Dias et al., 2024; Cecchini et al., 2023; Claes et al., 2017; Ishak et al., 2019; Rizzo et al., 2019; Hendriks et al., 2015; Canesin et al., 2023; Crabtree and Pycocck., 2020; Katila, 2016; Ginther et al., 2008; Aurich et al., 2011; Fonte et al., 2024; Satué et al., 2021; Veronesi et al., 2025).

Conclusions

Mare reproductive potential is influenced by multiple interacting structural, endocrine, inflammatory, and cellular mechanisms. No single biomarker can adequately capture this complexity. Clinical examination remains the foundation of reproductive evaluation, while hormonal biomarkers provide additional information about ovarian reserve, follicular development, and luteal competence. Molecular and biochemical biomarkers may further enhance understanding of inflammatory activity, oxidative balance, tissue remodeling, and embryo-

maternal communication.

Available evidence indicates that combining clinical, endocrine, and molecular information offers a broader perspective on factors influencing fertility in mares; however, the predictive value of most integrated biomarker models has yet to be established. Most molecular biomarkers are insufficiently validated for routine clinical use and should therefore be interpreted with caution.

Future Perspectives

Future research should prioritize direct comparisons of biomarker categories under field conditions to determine whether combined biomarker panels improve fertility prediction beyond conventional reproductive examinations. It is especially important to conduct studies that directly compare the prognostic value of different biomarkers in real farm or field conditions. Further advancements in this area will depend on the use of standardized sampling protocols, larger prospective studies, and validation across various mare populations.

Multi-omics approaches may aid in the discovery of new biomarkers associated with ovarian aging, uterine receptivity, and early embryonic survival. However, for these methods to be routinely applied, they must be sufficiently reliable, accessible, and cost-effective for clinical use.

In the future, researchers working in this field should prioritize prospective longitudinal studies that assess biomarker dynamics across different breeding seasons in mares and directly link biomarker profiles to fertility outcomes. By identifying a standardized panel of metabolic markers in follicular fluid, researchers can go beyond mere correlation and identify causal factors that regulate oocyte developmental potential.

Emphasis should be placed on validating molecular biomarkers in larger, more clinically diverse cohorts of mares, establishing standardized reference ranges, and evaluating cost-effectiveness under field conditions. Such evidence will be required before biomarker-based decision tools can be incorporated into routine reproductive management programs.

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